



**HAL**  
open science

## Versatile fiber-reinforced hydrogels to mimic the microstructure and mechanics of human vocal-fold upper layers

Daniel Ferri-Angulo, Hamid Yousefi-Mashouf, Margot Michel, Anne Mcleer, Laurent Orgéas, Lucie Bailly, Jérôme Sohier

### ► To cite this version:

Daniel Ferri-Angulo, Hamid Yousefi-Mashouf, Margot Michel, Anne Mcleer, Laurent Orgéas, et al.. Versatile fiber-reinforced hydrogels to mimic the microstructure and mechanics of human vocal-fold upper layers. *Acta Biomaterialia*, 2023, 172 (December), pp.92-105. 10.1016/j.actbio.2023.09.035 . hal-04236439

**HAL Id: hal-04236439**

**<https://hal.science/hal-04236439>**

Submitted on 10 Nov 2023

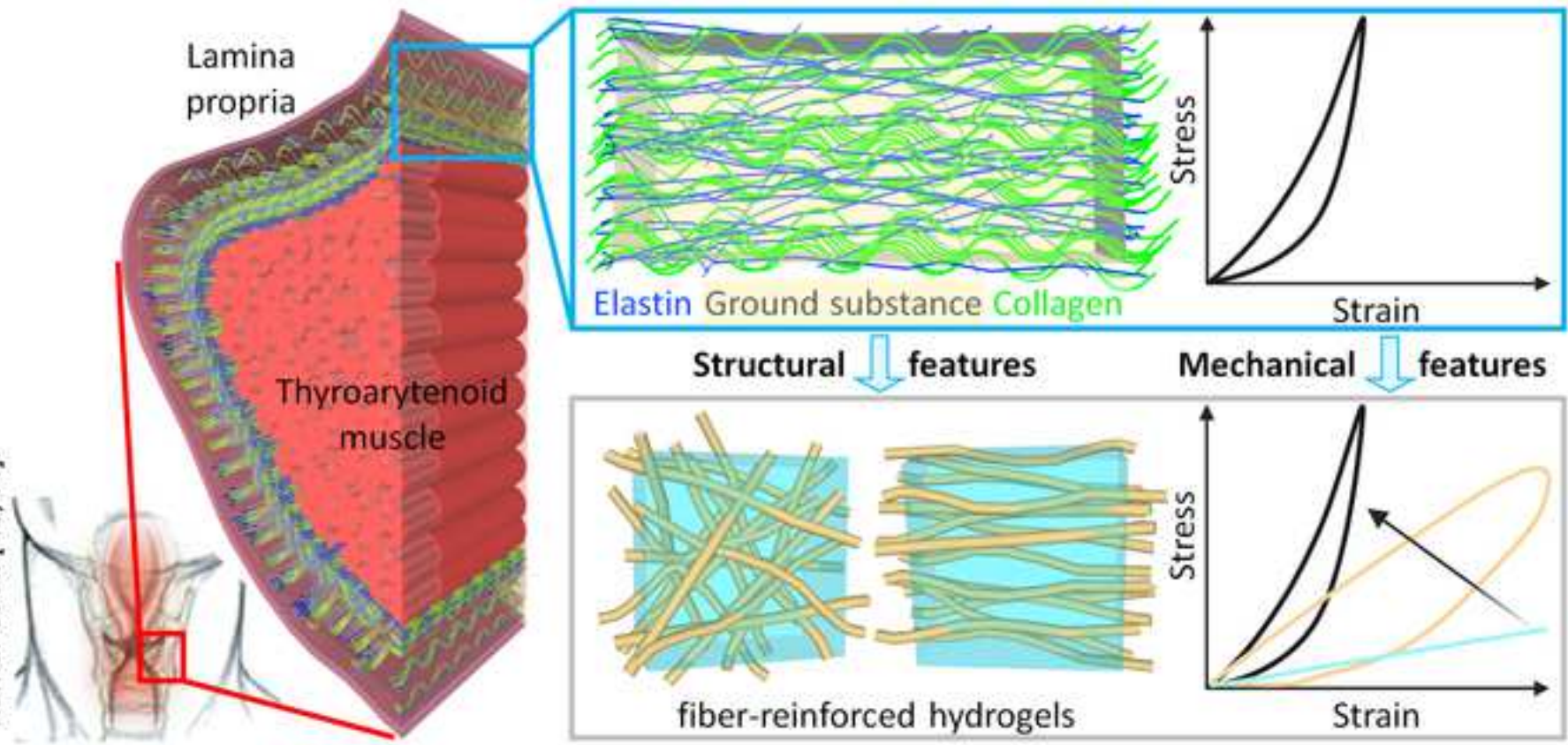
**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

### **Statement of significance**

Human vocal folds are outstanding vibrating soft living tissues allowing phonation. Simple physical models that take into account the histological structure of the vocal fold and recapitulate its mechanical features are scarce. As a result, the relations between tissue components, organisation and vibromechanical performances still remain an open question. We describe here the development and the characterization of fiber-reinforced hydrogels inspired from the vocal-fold microstructure. These systems are able to reproduce the mechanics of vocal-fold tissues upon realistic cyclic and large strains under various multi-axial loadings, thus providing a mimetic model to further understand the impact of the fibrous network microstructure in phonation.

Illustration adapted from Murthy et al. and Kumar et al. [43,44].



# Versatile fiber-reinforced hydrogels to mimic the microstructure and mechanics of human vocal-fold upper layers

Daniel Ferri-Angulo<sup>\*a</sup>, Hamid Yousefi-Mashouf<sup>bc</sup>, Margot Michel<sup>d</sup>, Anne McLeer<sup>e</sup>, Laurent Orgéas<sup>b</sup>, Lucie Bailly<sup>tb</sup>, Jérôme Sohier<sup>t§d</sup>

<sup>a</sup>CNRS UMR5510 MATEIS, 7 Avenue Jean Capelle, F-69621, Villeurbanne, France

<sup>b</sup>Univ. Grenoble Alpes, CNRS, Grenoble INP, 3SR, 38000 Grenoble, France

<sup>c</sup>Univ. Grenoble Alpes, CNRS, Grenoble INP, GIPSA-lab, 38000 Grenoble, France

<sup>d</sup>CNRS UMR5305 LBTI, Passage du Vercors, 69007, Lyon, France

<sup>e</sup>Univ. Grenoble Alpes, Service d'Anatomie et Cytologie Pathologiques CHU Grenoble Alpes, Institute for Advanced Biosciences UGA/INSERM U1209/CNRS 5309, 38000 Grenoble, France

\*Current affiliation: CNRS UPR8001 LAAS-TEAM, 7 Avenue Colonel Roche, 31031, Toulouse, France

<sup>†</sup>Contributed to this work equally

<sup>§</sup> Corresponding author: [jerome.sohier@ibcp.fr](mailto:jerome.sohier@ibcp.fr)

## Keywords:

Vocal folds, Fiber-reinforced hydrogels, Materials by design, Two-photon excitation microscopy, Multiaxial mechanical characterization, Anisotropy, Viscoelasticity.

## Abstract

Human vocal folds are remarkable soft laryngeal structures that enable phonation due to their unique vibro-mechanical performances. These properties are tied to their specific fibrous architecture, especially in the upper layers, which comprise a gel-like composite called *lamina propria*. The *lamina propria* can withstand large and reversible deformations under various multiaxial loadings. Despite their importance, the relationships between the microstructure of vocal folds and their resulting macroscopic properties remain poorly understood. There is a need for versatile models that encompass their structural complexity while mimicking their mechanical features. In this study, we present a candidate model inspired by histological measurements of the upper layers of human vocal folds. Bi-photon observations were used to quantify the distribution, orientation, width, and volume fraction of collagen and elastin fibers between histological layers. Using established biomaterials, polymer fiber-reinforced hydrogels were developed to replicate the fibrillar network and ground substance of native



1 vocal fold tissue. To achieve this, jet-sprayed poly( $\epsilon$ -caprolactone) fibrillar mats were  
2 successfully impregnated with poly(L-lysine) dendrimers/polyethylene glycol hydrogels. The  
3 resulting composites exhibited versatile structural, physical and mechanical properties that  
4 could be customized through variations in the chemical formulation of their hydrogel matrix,  
5 the microstructural architecture of their fibrous networks (*i.e.*, fiber diameter, orientation and  
6 volume fraction) and their assembly process. By mimicking the collagen network of the *lamina*  
7 *propria* with polymer fibers and the elastin/ground substance with the hydrogel composition,  
8 we successfully replicated the non-linear, anisotropic, and viscoelastic mechanical behavior of  
9 the vocal-fold upper layers, accounting for inter/intra-individual variations. The development  
10 of this mimetic model offers promising avenues for a better understanding of the complex  
11 mechanisms involved in voice production.  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## 1. Introduction

Human vocal folds are soft multi-layered laryngeal structures displaying remarkable mechanical performances [1,2]. These performances are primarily attributed to the unique properties of vocal-fold tissues, which allow them to endure large and reversible deformation under various multiaxial loading modes, including tension, compression and shear [3–5]. Furthermore, vocal folds exhibit the ability to adapt their vibro-mechanical behavior in response to environmental changes. These properties are related to the fibrous microstructures of the vocal folds and their surrounding gel-like matrices. In particular, the vocal-fold upper layers comprise a very thin *epithelium* surface and an underlying composite structure known as *lamina propria* (LP, thickness  $\approx$  1–2.5 mm; Fig. 1). The stratified squamous *epithelium* is secured to the LP through a basement membrane and serves as a protective covering [6]. The LP plays a crucial role in vocal-fold vibrations [7–9]. The LP is a loose connective tissue composed of cells and an extracellular matrix (ECM). This matrix includes an amorphous ground substance containing hyaluronic acid and is reinforced by entangled fibrous networks of collagen (mainly Type I and III) and elastin. As a whole, the LP significantly contributes to the mechanical properties of the upper layers, including non-linear viscoelasticity and anisotropy. More specifically, as schematized in Fig. 1, the mechanical strength and anisotropy of the LP are often ascribed to its collagen network, while its damping and elastic properties are more related to other tissue components (cells, elastic fibers, ground substances) [10,11].

Due to its physiological functions in voicing but also breathing and swallowing, the vocal-fold tissue has to accommodate many traumas (*e.g.*, vocal misuse or chemical aggression) [12,13]. Though characterized by an enhanced healing capacity of micro-lesions, the vocal-fold tissue can be damaged past a critical threshold of injury, resulting in benign lesions (*e.g.*, polyps, nodules [14]), or cancerous lesions (*carcinomas* [15]). Whatever the lesion, the LP microstructure is quasi-systematically altered, which induces dysfunctions in the vocal-fold vibro-mechanical performances. Current surgical procedures involve excision of the lesion or resection of the damaged tissue. However, in some complex cases, operative interventions can result in scarring lesions with partial disappearance of the LP layers and/or undesirable fibrous rearrangement, which may lead to the loss of phonatory abilities [16,17].

1  
2 To treat these destructive lesions, several options are explored, including growth factor  
3 therapies, cell therapies and biomaterials, with the aim of rebuilding or replacing the layered  
4 structure of the *lamina propria* [13,16,18–24].  
5

6  
7 Among the relevant biomaterial candidates, hydrogels are attractive due to their tunable  
8 physical properties [25,26] and tissue-like water content [27–29], which provides cells with an  
9 environment comparable to the extracellular matrix of native tissues [30]. For instance, it has  
10 been shown that the implantation of hyaluronic acid (HA)-based hydrogels in the surgical  
11 wound maintains the superficial sublayer of the *lamina propria* and prevents the *epithelium*  
12 from adhering to the deeper sublayers during the healing process [31]. Similarly, injectable  
13 poly-ethylene glycol (PEG) polymers have also yielded positive vocal outcomes [32], albeit with  
14 limited effectiveness over time due to gradual material degradation. However, due to their  
15 isotropy and relatively low mechanical strength, homogeneous hydrogels may be limited in  
16 guiding tissue remodeling processes towards a native-like microstructure. The role of oriented  
17 fibrous microstructures and their anisotropic mechanics is increasingly recognized as a key  
18 factor influencing matrix synthesis, particularly in the case of elastin [33].  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30

31  
32 The mechanical features of native tissues are linked to their complex architecture, where  
33 fibrous protein networks support the hydrated ground substance [34]. Mimicking such a  
34 composite architecture through the reinforcement of hydrogels with fibrillar structures, as  
35 shown for cartilage, therefore appears as a promising concept to match target mechanical  
36 properties [35]. Many different models of various complexities have been proposed to better  
37 understand the phonatory ability of the vocal folds by reproducing their shape and multi-  
38 layered organization. However, these models are mostly made up of homogeneous  
39 elastomers with isotropic microstructures, resulting in quasi-linear stress-strain responses  
40 that differ significantly from those experienced by the vocal folds [36,37]. Over the last decade,  
41 a few promising *in vitro* developments have emerged to create composite elastomers aimed  
42 at mimicking the key microstructural features and anisotropic mechanical behavior of native  
43 vocal folds. For instance, Shaw *et al.* demonstrated that incorporating bundles of wavy  
44 polymeric fibers into isotropic self-oscillating silicone replicas could lead to a much more  
45 relevant fundamental frequency response, especially during anterior-posterior stretching  
46 [38]. Regarding biocompatible materials, a few examples able to mimic certain structural  
47 properties of the *lamina propria* have also been reported. Multi-layered hydrogels were  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 designed to promote cell viability and flow-induced vibrations [39], yet without taking into  
2 account the fibrous component of the native tissue. Other hydrogel scaffolds were developed  
3 from networks of collagen nanofibrils (Types I and III) in a glycol-chitosan matrix for vocal-fold  
4 tissue engineering [40]. However, their fibrous architecture, including diameter, orientation  
5 and density, was not tailored. Furthermore, the matching of their mechanical properties with  
6 those of the native tissue is rarely sought, which might render any potential mechanism of  
7 vibration difficult to translate to the native situation. Matching the mechanics of vocal fold  
8 models with those of native vocal folds has often been limited to a single loading mode, mainly  
9 shear, and to the linear regime at very small strains. These conditions are far from the cyclic  
10 and large-strain multiaxial loadings experienced by vocal folds during phonation [5]. The  
11 possibility of mimicking both the microstructural and quasi-static mechanical properties of the  
12 vocal fold under representative multi-axial loadings appears as a crucial first step towards a  
13 deeper investigation of the link between vocal-fold architecture and their mechanical  
14 vibration properties at higher strain rates.

15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27 The general objective of this work is twofold: (i) to develop and characterize hydrogels  
28 reinforced with tunable fibrous microstructures, inspired by quantitative histological data, and  
29 exhibiting versatile mechanical properties under both cyclic and finite strains; (ii) to identify  
30 among the proposed hydrogels those capable of replicating the major structural and  
31 mechanical specificities of the human vocal-fold upper layers, including longitudinal tension  
32 and transverse compression.

33  
34  
35  
36  
37  
38 To achieve this, poly( $\epsilon$ -caprolactone) microfibers with adjustable densities and orientations  
39 [41,42] were embedded within a mimetic hydrogel. This hydrogel is composed of poly-lysine  
40 dendrimers and polyethylene glycol, offering highly modular mechanical properties [43]. This  
41 setup is schematically illustrated in Fig. 1. The structural and mechanical behavior of the  
42 resulting composites were characterized and compared to the histo-mechanical properties of  
43 native tissues.

## 44 45 46 47 48 49 50 51 52 **2. Materials and method**

### 53 54 55 *2.1. Materials and sample preparation*

#### 56 57 *2.1.1. Vocal folds*

58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
1000

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Procedures were conducted following the French ethical and safety laws related to Body Donation. Right and left vocal folds were dissected along the longitudinal (anterior-posterior) direction  $e_{ap}$  (Fig. 1) using a dissection technique outlined by Bailly *et al.* [44]. The vocal folds were separated from their cartilaginous ends, resulting in four tissue samples, approximated as parallelepiped beams with dimensions of 10 mm × 3 mm × 3 mm.

These samples represent sandwich lamellar structures, encompassing all vocal-fold sublayers from the *epithelium* to the *vocalis* muscle (see Fig. 1). When possible, markers were sewn onto the samples, using Samco® surgical nylon monofilaments (10/0) and suture knots, to aid in anatomical identification during imaging experiments at the anterior end and within sublayers.

To enable high-resolution 3D imaging of their ECM fibers at the micrometer scale, a portion of the vocal-fold samples was immersed sequentially in ethanol (EtOH, Sigma-Aldrich) solutions with increasing alcohol concentration (30 %, 50 % and 70 %) for 24 h each. These samples were then stored in a 70 % EtOH solution at 4 °C until observation using two-photon microscopy. The other portion of the samples was fixed, embedded in paraffin and sectioned into 5 µm thick slices. These slices were either observed using two-photon microscopy or stained using conventional Hematoxylin-eosin-saffron (HES, in-house protocol) and Verhoeff Van Giesen (Labo Moderne, France). These stains were used to reveal both Type I and Type III collagen, as well as elastin fibers (see Supplementary Fig. S1). Stained sections were then imaged using an Aperio AT2 scanner (Leica, France).

### 2.1.2. Synthetic samples

*PCL fibrous mats* – In a first step, several mats made of poly( $\epsilon$ -caprolactone) (PCL, molecular weight 80,000 g/mol, Solvay Caprolactones, United Kingdom) microfibers with tunable orientation and volume fraction were prepared using a previously reported method, with modifications [41,42]. To make the PCL fibers hydrophilic, tween® 80 (245 mg, 187 mmol, Sigma-Aldrich, France) was stirred in chloroform (140 mL, Sigma-Aldrich, France) at room temperature (approximately 25 °C) for 10 min. Subsequently, PCL (9.8 g, 0.122 mmol) was added to the solution, and it was stirred for 3 h until complete dissolution occurred. The resulting solution was placed in a reservoir and projected onto different targets through the spraying device (standard class, Revell, Germany). Due to a Venturi effect, an airflow drove the polymer solution from the reservoir to an adjustable nozzle, where it was diffracted on a needle and sprayed. Solid polymer fibers were formed during transit due to chloroform evaporation. Two types of

1 targets were selected to create optional preferred orientations within the fibrous network,  
2 thereby allowing control over their induced mechanical properties. These targets included a  
3 static grid, which was used to produce mats with a planar random fiber orientation, and a  
4 rotating cylinder for fabricating mats with a planar fiber orientation featuring a preferred in-  
5 plane direction [41].  
6  
7  
8  
9

10 The thickness of the fibrous mats (was controlled by spraying time, and measured using a  
11 Keyence laser sensor. Typical values ranged within 0.5 to 3 mm. To increase the volume  
12 fraction of fibers, a compaction system was developed. Various levels of compaction were  
13 achieved by depositing one or multiple layers of fibrous mats of known thickness between  
14 layers of glass slats (each 0.14 mm thick). These layers were then covered by a glass plate and  
15 a 500 g weight was placed on top of the covering glass plate. Compaction was applied for 1 h  
16 at room temperature before release, new thickness measurement, and further use. Levels of  
17 compaction ranged from approximately 25 % up to 80 %, defined as the ratio of the relative  
18 change in thickness of the mat compared to its initial thickness.  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29

30 *DGL/PEG hydrogels* – In a second step, hydrogels were obtained by mixing poly(L-lysine)  
31 dendrigrafts (DGL<sup>®</sup>, Colcom, France) and O,O'-Bis[2-(N-succinimidyl-succinylamino)ethyl]  
32 poly-ethylene glycol (PEG-NHS, Sigma-Aldrich, France) in aqueous solutions with various  
33 DGL/PEG ratios (1/1, 1/2, 1/2.5 or 1/3 w/w) and PEG molecular weights, referred to as PEG<sub>MW</sub>  
34 (2 or 10 kDa). In a typical experiment, PEG-NHS in anhydrous dimethylformamide (DMF,  
35 Sigma-Aldrich, France) solution at different concentrations, noted  $C_{PEG}$  (5, 8, 10, 12 or 16 w/v  
36 %) was added to DGL in phosphate buffer saline (1x PBS, Euromedex, France) in eppendorf  
37 tubes at 4 °C. This mixture was swiftly vortexed and transferred to 3D-printed molds, where it  
38 cross-linked between 15 to 30 min at room temperature. After cross-linking, the hydrogels  
39 were removed from the mold and stocked in PBS solution at 4 °C until use. The resulting  
40 samples were typically 22 mm in length, 5 mm in width, and from 1 to 5 mm in thickness. In  
41 the following, for the sake of clarity, the DGL/PEG hydrogels characterized by the triplet of  
42 manufacturing parameters ( $C_{PEG}$  in %, PEG<sub>MW</sub> in kDa, DGL/PEG ratio) will be denoted as  
43 Hyd( $C_{PEG}$  %, PEG<sub>MW</sub>k, DGL/PEG ratio).  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

*Fiber-reinforced hydrogels* – PCL fibrous mats, which had planar random or aligned orientations and were either compacted or not, were used along with DGL/PEG hydrogels to produce fiber-reinforced hydrogels. Each processed PCL mat was placed on a hydrophobic glass slide, and a fresh mix of PEG-NHS and DGL was rapidly poured onto it, allowing impregnation through the fibrous mats before the cross-linking reaction. Once the cross-linking was complete, the resulting composite samples were cut into parallelepiped beams measuring 22 mm in length, 5 mm in width and 0.5–2 mm in thickness. These composite materials were labelled FHC( $C_{PEG}$  %,  $PEG_{MWk}$ , DGL/PEG ratio, fibrous orientation) and stored in PBS solution at 4 °C until use.

## 2.2. Structural, physical and mechanical characterization

### 2.2.1. Histological analyses of biological samples

The fibrous microstructure of the *lamina propria* samples was analyzed using two-photon excitation microscopy (Zeiss LSM 780) at the Cell Imaging Platform of the Cancer Research Center of Lyon (CRCL, UMR Inserm 1052 CNRS 5286 – Centre Léon Bérard, France). Samples conserved in a 70 %-ethanol solution were placed on a microscopy glass slide, moistened with PBS and covered by a glass coverslip, which was then sealed with patafix (UHU, Bühl, Germany) to prevent sample drying during observation. Collagen fibers were visualized using second harmony generation with an excitation at 900 nm and emission at 412–464 nm. For elastin, auto-fluorescence was utilized with an excitation at 900 nm and emission at 499–534 nm. When working with paraffin-embedded and sectioned samples, collagen fibers were observed via second harmony generation with an excitation at 900 nm and emission between 412 and 456 nm, while elastin autofluorescence was captured using an excitation at 700 nm and emission at 473–674 nm. Combining optics with CCD sensor features, regions of interest were sequentially scanned at multiple resolutions with effective voxel sizes of ( $0.69 \mu\text{m} \times 0.69 \mu\text{m} \times 6.17 \mu\text{m}$ ), ( $0.35 \mu\text{m} \times 0.35 \mu\text{m} \times 0.91 \mu\text{m}$ ) and ( $0.17 \mu\text{m} \times 0.17 \mu\text{m} \times 0.75 \mu\text{m}$ ) in reference to the anatomical frame defined as ( $\mathbf{e}_{ap}$ ,  $\mathbf{e}_{ml}$ ,  $\mathbf{e}_{is}$ ), where  $\mathbf{e}_{ap}$  coincides with the anterior-posterior direction,  $\mathbf{e}_{ml}$ , with the medial-lateral direction, and  $\mathbf{e}_{is}$  with the inferior-superior direction (see Fig. 1). The entire vocal-fold sample was examined in the ( $\mathbf{e}_{ap}$ ,  $\mathbf{e}_{ml}$ ) plane, with a maximum depth of 377  $\mu\text{m}$  along the  $\mathbf{e}_{is}$  direction. Stacks of images with distances of 6.17  $\mu\text{m}$ , 0.91  $\mu\text{m}$  and 0.75  $\mu\text{m}$  were acquired along  $\mathbf{e}_{is}$ , resulting in thicknesses of 58.8  $\mu\text{m}$ , 46.2  $\mu\text{m}$  and 30.75  $\mu\text{m}$ , respectively.



1 Distributions of diameter and 2D orientation of collagen and elastin fibers (parallel to the  
2 sample midplane) were calculated using the DiameterJ and OrientationJ plug-ins available in  
3 Fiji® [45,46]. For a acquisition stack, one  $e_{is}$ -position was randomly selected at a depth of 22  
4  $\mu\text{m}$ , and six close-up areas of interest were analyzed to determine diameter and orientation  
5 populations, resulting in more than 6,000 values for each image. Additionally, to determine  
6 the diameter distribution of fiber bundles, manual quantification was performed at three  
7 random  $e_{is}$ -positions within a given stack (depths of 15.3, 22 and 29.5  $\mu\text{m}$ ). Along the primary  
8 axis of each fiber bundle, a minimum of 10 orthogonal thickness measurements were  
9 recorded, resulting in more than 100 values for each image. Finally, to determine the volume  
10 fraction of collagen and elastin fibers in the tissue, the BoneJ plug-in available in Fiji® was  
11 utilized [47]. This involved manually thresholding three areas of interest from an acquisition  
12 stack for collagen and elastin signals, followed by the calculation of the volume ratio using the  
13 area/volume fraction algorithm.

### 2.2.2. Structural and physical characterization of synthetic samples

28 *PCL fibrous mats* – Jet-sprayed networks were observed by scanning electron microscopy  
29 (SEM, FEI XL30 ESEM-FEG, Philips, CLYM-INSA-Lyon, France) operated at a 2 kV accelerating  
30 voltage. Prior to observation, the fibers were coated with gold using a sputter coater (BAL-TEC  
31 SCD 005). For each sample, six SEM images were captured at random positions and different  
32 magnifications ( $\times 250$ ,  $\times 500$  and  $\times 2,000$ ). Quantification of fiber diameters (more than 25,000  
33 values for a given image) and orientations (more than 1,000 values for a given image) was  
34 performed in a manner similar to that employed for native tissue.

42 The porosity of the fibrillar mats ( $\Phi_{PCLmats}$ ) was determined, using a liquid displacement  
43 method with a pycnometer [48]. Ethanol served as the non-solvent working liquid, with a  
44 known density ( $\rho_{EtOH} = 0.7892 \text{ g/cm}^3$ ) [49]. The measurements involved weighing the fibrillary  
45 matrices ( $m_s$ ) of  $0.035 \text{ cm}^3 (V_s)$ , weighing the pycnometer filled with ethanol ( $m_{py-EtOH}$ ) and  
46 the pycnometer containing both ethanol and the fibrillar sample ( $m_T$ ). The porosity was  
47 calculated as follows:

$$48 \Phi_{PCLmats} = 1 - \frac{(m_s + m_{py-EtOH}) - m_T}{\rho_{EtOH} V_s} \quad (1)$$

59 All measurements were conducted in quintuplicate at 25 °C.

Hydrogels and fiber-reinforced composites – The morphology and structure of hydrogels and fiber-reinforced composites (FHC) were examined using a binocular microscope (Olympus, France).

Swelling ratio assays were performed to assess the water uptake and swelling properties of both materials. For hydrogels, the desired concentrations of DGL/PEG were mixed and the mixture promptly deposited between a hydrophobic glass slide and a hydrophobic round coverslip (10 mm in diameter) to create hydrogel disks after cross-linking. For FHC, the desired mixture of DGL/PEG was directly poured over fibrous mats cut as 10 mm-diameter disks. After cross-linking, the samples were immersed in PBS solution for 24 h at room temperature. Prior to freezing in liquid nitrogen, samples were washed with deionized water multiple times (8 × 1 h) and then freeze-dried, to measure their dry weight ( $w_d$ ). Subsequently, the samples were immersed in PBS solution, and their swollen weight ( $w_s$ ) was measured at various time intervals (ranging from 10 min to 48 h) after removing excess surface water. The swelling ratio ( $Q$ ) was calculated for each sample using the formula:

$$Q = (w_s - w_d)/w_d \quad (2)$$

The volume fraction of fibers in FHC samples ( $\phi_{PCL}$ ) was determined using a pycnometer [48], with PBS as working liquid. Composites were prepared with aligned or planar random fibrous mats of controlled volumes ( $V_s = 3.17$  and  $9.06$  mm<sup>3</sup>, respectively). The volume of the FHC samples,  $V_{FHC}$ , was calculated based on the weight of the composites ( $m_{FHC}$ ), the weight of the pycnometer filled with both PBS and FHC samples ( $m_t$ ), the PBS density ( $\rho_{PBS} = 1.002938$  g/cm<sup>3</sup>) and the known properties of the empty pycnometer (volume  $V_{py}$ , mass  $m_{py}$ ) using the equation:

$$\phi_{PCL} = \frac{V_s}{V_{FHC}} = \frac{V_s \rho_{PBS}}{V_{py} \rho_{PBS} - (m_t - m_{FHC} - m_{py})} \quad (3)$$

For each of these data, four measurements were carried at 25 °C.

To determine the hydrogel mesh size ( $\zeta$ ), the rubber elastic theory was employed [50], where  $\zeta = (G'/k_b T)^{-1/3}$ , with  $G'$  as the shear storage modulus of the gel,  $k_b$  as the Boltzmann constant, and  $T$  as the temperature. Given the isotropic homogeneous cross-linking of PEG hydrogels, we set  $G' = E/2(1 + \nu)$ , where  $E$  is the tensile elastic modulus, and  $\nu$  the Poisson ratio [51,52]. Assuming quasi-incompressibility of the gel ( $\nu \approx 0.5$ ), the mesh size

1  $\zeta$  was deduced from the tensile tests described below. Three measurements were used for  
2 mesh size determination.  
3

### 4 2.2.3. Mechanical characterization 5

6 The mechanical response of PCL fibrous mats, DGL/PEG hydrogels and fiber-reinforced  
7 hydrogels was investigated using an electromechanical tension-compression testing machine  
8 (Instron® 5944) equipped with a load cell of  $\pm 10$  N (measurement accuracy  $\pm 0.5$  % of reading),  
9 following a protocol similar to that developed in Yousefi-Mashouf *et al.* [53] to characterize the  
10 mechanics of gelatin-based hydrogels. In particular, all tests were conducted in a thermo-  
11 regulated atmosphere ( $T \approx 25$  °C) and at proper hygrometric conditions to prevent samples from  
12 air drying: a dedicated hygro-mechanical set-up was used to maintain hydration of the gels in a  
13 saturated air atmosphere ( $\approx 98$ – $100$  % RH) throughout testing. Tests were repeated at least 5  
14 times for each condition (Supplementary Fig. S2A showing the typical level of scatter in the  
15 measurements).  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25

26 All materials were first characterized in tension along their main fiber orientation, which is the  
27 standard loading mode used to characterize the mechanical behavior of native tissues [54–57].  
28 To do so, materials were cut in parallelepiped beams for an effective gauge length ( $\ell_0$ ) of 10  
29 mm and width ( $w_0$ ) of 5 mm. The initial thickness ( $t_0$ ) of the samples could vary from 0.5 to 5  
30 mm, depending on the sample nature and processing route. The maximum relative error  
31 obtained on the initial cross-section of the samples was estimated optically from their width  
32 and thickness profiles before loading using a high-resolution CCD camera (JAI® BM-500GE, 15  
33 Hz), yielding to an error of  $\pm 0.02$ %. The cell force signal ( $f$ ) and displacement of the machine  
34 cross-head ( $\delta$ ) were used to calculate the nominal stress ( $P = f/w_0 t_0$ ) and the natural tensile  
35 strain ( $\varepsilon = \ln(1 + \delta/\ell_0)$ ). Each sample was subjected to successive load/unload cycles with an  
36 increasing strain amplitude ( $\varepsilon^{max}$ ) and a very low force at each unload phase for the inversion  
37 condition ( $f > 5 \cdot 10^{-3}$  N), with an initial strain rate  $|\dot{\delta}/\ell_0| \approx 10^{-3} s^{-1}$ .  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

51 To quantify the influence of cyclic loading, various mechanical descriptors were derived from  
52 the stress-strain curves [53], including the peak stress ( $P^{max}$ ) achieved at every peak strain  
53 ( $\varepsilon^{max}$ ); the residual strain ( $\varepsilon_r$ ) recorded at the end of each cycle; the volumetric energy stored  
54 during the load of each cycle ( $W^{abs}$ ) and the one dissipated after the unloading phase ( $W^{diss}$ );  
55 the corresponding energy dissipation coefficient ( $\eta \approx W^{diss}/W^{abs}$ ) at the end of each cycle  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

[58]; and the tangent modulus ( $E_t = dP/d\varepsilon$ ) assessed at the early stage of each unloading phase to capture the instantaneous stiffness of the material (with  $E = E_t$  at small strains).

In a second step, after comparison with the reference mechanical database on native tissues [5], a suitable biomimetic fiber-reinforced hydrogel candidate was selected from the previous campaign. It was further tested in compression transversely to the planar fiber orientation, such a loading being representative of vocal-fold loading during *in vivo* periodic collision [59,60]. Briefly, following the procedure developed in Yousefi-Mashouf *et al.* [53] in such a case, samples were cut for an effective gauge length  $\ell_0 = 2$  mm, a width and thickness  $w_0 = t_0 = 10$  mm at rest. They were then subjected to successive load/unload cycles down to various strain levels ( $\varepsilon^{min}$ ), with a strain rate ( $|\dot{\varepsilon}|$ ) of approximately  $10^{-3} s^{-1}$  as applied in the reference database. Compression stress ( $P$ ) and strain ( $\varepsilon$ ) were recorded during the test, as previously mentioned.

#### 2.2.4. Statistical analysis

Statistical analyses were performed with OriginPro (OriginLab). After a Kolmogorov-Smirnov or Shapiro-Wilk normality test, parametric t-tests, variance analysis (ANOVA) or non-parametric Mann-Whitney U-tests were performed. P-values of 0.05 and below were considered significant.

### 3. Results and discussion

#### 3.1. Structure of human vocal folds and lamina propria

Using two-photon microscopy, significant variations of collagen and elastin distributions across different histological layers of the human vocal folds were observed (Supplementary Fig. S1). Few collagen and elastin fibers were apparent in the *vocalis* muscle layer and arytenoid cartilage, while the *lamina propria* was rich in both types of fibers. In the *lamina propria*, both elastin and collagen fibers formed wavy structures, entangled in a network. As anticipated, the covering *epithelium* lacked these fibers (Fig. 2A). The predominant 2D orientation of the fibrillar proteins was parallel to the anterior-posterior direction ( $\mathbf{e}_{ap}$ ), with density and orientation variations from the superficial to deep layers along the medial-lateral direction ( $\mathbf{e}_{ml}$ ) (Fig. 2B and Supplementary Video S3).

Fig. 3 displays typical in-plane fiber orientation distributions and the width of ECM fibers measured at various depths of the *lamina propria*. In these in-plane analyses, both elastin and

collagen fibers intertwined networks showed a similar preferred orientation parallel to the anterior-posterior direction ( $\mathbf{e}_{ap}$ ) (Fig. 3A). Close to the vocal fold's edge, beneath the *epithelium*, the angular distribution of fibers appeared broad, with the majority aligned parallel to the epithelial layer ( $\theta = 0^\circ$ ). At deeper focal points within the *lamina propria*, the distribution of fibrillar orientation was narrower and predominantly followed the epithelial surface. When quantified through image analysis (Fig. 3B), collagen fibers were found to be significantly thicker, with broader dispersions (mean  $2.1 \pm 1.1 \mu\text{m}$ , median  $1.7 \mu\text{m}$ , values ranging from  $0.4$  to  $7.3 \mu\text{m}$ ) compared to elastin ones (mean  $1.4 \pm 0.5 \mu\text{m}$ , median  $1.4 \mu\text{m}$  and values ranging from  $0.4$  to  $3.1 \mu\text{m}$ ). Both fiber types formed bundles, which exhibited a similar pattern and reached widths of up to  $94.1$  and  $62.9 \mu\text{m}$ . The mean values were  $12.9 \pm 12.1 \mu\text{m}$  (median  $8.8 \mu\text{m}$ ) for collagen and  $9.9 \pm 7.7 \mu\text{m}$  (median  $7.8 \mu\text{m}$ ) for elastin. Concerning the volume fractions of collagen and elastin fibers within the *lamina propria*, no significant different values were observed ( $9.7 \pm 3.7 \%$  and  $10.9 \pm 3.5 \%$ , respectively; t-test  $p = 0.67$ ). These findings are in good agreement with and complement the limited quantitative microstructural analyses of the human vocal fold reported in the existing literature [43,62,63]. Furthermore, our study indicated that using a larynx from a 76-year-old male did not result in significant differences in collagen and elastin fiber diameters and orientations when compared to those obtained from a 50-year-old female vocal fold using two-photon microscopy [62].

### 3.2. Tunable fiber-reinforced hydrogels

#### 3.2.1. Structural and physical versatility

To approximate the observed microstructure of the native vocal-fold upper layers, several fiber-reinforced hydrogels (FHC) were developed. In this initial approach, the complex multi-layered structure of the upper layers was simplified into a one-layered composite structure.

The fibrillar component of the *lamina propria* can readily be emulated by polymeric materials, such as PCL, as they can be formulated in fibrous mats with controlled diameters, densities and orientations through various methods, including jet-spraying or electrospinning [42,63]). However, since the width of *lamina propria* ECM fibers is polydisperse, jet-spraying appeared to be a more suitable option to mimic this feature. Furthermore, this fiber production method results in highly porous structures, with a typical porosity around 97 %, which is a requisite to allow for the impregnation of a hydrogel phase for the creation of a composite.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

By selecting jet-spraying parameters carefully, it was possible to emulate some of the target microstructural features of the *lamina propria* at the scale of fibers and fiber bundles. As presented in Fig. 4A, the in-plane orientation of the PCL fibers within the processed fibrous mats could be modulated to mimic the orientations of the fibrillar component observed in the *lamina propria* (Fig. 3). The resulting fibers diameters were in the micron range and significantly thicker when aligned, with averages of  $2.9 \pm 2.9 \mu\text{m}$  and  $1.6 \pm 1.4 \mu\text{m}$ , and medians of  $1.9 \mu\text{m}$  and  $1.2 \mu\text{m}$  for aligned and random mats, respectively. Similarly, the mean and median width of the fiber bundles produced during spraying were significantly increased, with means of  $6 \pm 5.6 \mu\text{m}$  compared to  $3.2 \pm 2.4 \mu\text{m}$ , and medians of  $3.9 \mu\text{m}$  against  $2.3 \mu\text{m}$ . In comparison to the measured collagen fibers of the *lamina propria*, mean diameters of both random and aligned fibrillar PCL mats were similar, but with a wider distribution. PCL fiber bundles diameters were also in the same order of magnitude as bundles of native fibrillar proteins, albeit with a lower mean and distribution range. It is worth noting that the polymer fibers obtained by jet-spraying did not reproduce the wavy pattern of collagen fibers of the *lamina propria* at the microscopic scale; instead, they appeared rather straight (see Fig. 4).

To replicate the remaining tissue components of the vocal-fold upper layers (*lamina propria* and *epithelium*), and to allow for control over the overall mechanical properties of the synthetic composites, a hydrogel of versatile properties was evaluated. In recent studies, we have demonstrated that poly(L-lysine) dendrigrafts/polyethylene glycol (DGL/PEG) hydrogels offer several advantages. These hydrogels can be easily prepared by mixing two aqueous solutions, and their elastic properties can be adjusted extensively by varying the component ratios and concentrations [43,64,65]. Importantly, unlike other PEG-based hydrogels, they exhibit inherent cytocompatibility, allowing for attachment and proliferation of multiple cell types *in vitro*, through the presence of DGL [43,65]. When implanted *in vivo*, these hydrogels are biocompatible, biodegradable and induce a mild inflammatory response, accompanied by extensive cellularization, tissue formation and vascularization [43]. The aqueous nature and low viscosity of PEG/DGL mixtures before cross-linking, along with the hydrophilicity imparted by the addition of tween® 80 to the PCL fibers, facilitated the impregnation of the hydrogel within the fibrous polymer mats through a simple pouring process. This resulted in a homogeneous impregnation throughout the thickness of the mats (see Fig. 5A). Moreover, due to the extremely high initial porosity of the mats ( $\Phi_{PCLmats}$  of 97 and 96 % for 2D aligned and 2D random mats, respectively), neither the orientation of the PCL fibers nor the

1 composition of the DGL/PEG hydrogel posed any obstacles to the preparation of the  
2 composites (FHC), as summarized in Table 1.  
3

4 The efficient incorporation and embedding of fibrous mats within the hydrogels were  
5 evidenced in the modulation of their swelling properties. As presented in Fig. 5B, the increased  
6 swelling of DGL/PEG hydrogels under physiological conditions aligns with previously reported  
7 findings [43,65]. Increasing the PEG molecular weight ( $PEG_{MW}$ ) from 2 to 10 kDa led to an  
8 increase in the swelling ratio from 15 to 22, whereas higher PEG concentration resulted in  
9 reduced water uptake by the hydrogels. Similarly to other PEG-based hydrogels, these effects  
10 can be attributed to the increased proximity of the amine (DGL) and NHS (PEG) reactive groups  
11 at higher reactants concentrations, leading to increased cross-linking, smaller mesh sizes and  
12 restricted network expansion [66]. In contrast, longer PEG chains resulted in larger mesh sizes  
13 and higher swelling, as indicated by the estimation of hydrogel mesh sizes (Table 1). However,  
14 for any given DGL/PEG composition and PEG molecular weight, the presence of PCL fibers  
15 significantly decreased the resulting FHC water uptake up to 3 times. This restriction in swelling  
16 can be attributed to the non-swelling nature of PCL fibers, as reported in previous studies on  
17 PEG-diglycidylether/polyoxyalkyleneamines hydrogels composites with polyurethanes fibers  
18 [67]. Additionally, this effect is influenced by the entangled organization of fibers in the non-  
19 woven jet-sprayed mats [42]. Accordingly, the swelling decrease was largely dependent on PCL  
20 fibers orientations and volume fractions in the final composite. In particular, the lower volume  
21 of hydrogel in the composites with random fibrous mats ( $\approx 91\%$ , compared to  $\approx 96\%$  for  
22 aligned ones) induced a lower swelling ratio (Table 1).  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42

43 Aside from the variation of fibers orientations and hydrogel composition, it was also  
44 possible to control the fiber content in the formed composites in a straightforward fashion.  
45 The elevated porosity of the fibrous mats permitted their relative compaction up to 80 %  
46 through compression, while not hampering further impregnation with the hydrogel. As a result,  
47 the volume fraction of fibers  $\varphi_{PCL}$  within the resulting composites could be readily adjusted,  
48 as demonstrated in Fig. 5C for FHC(5%, 2k, 1/1, Aligned), where it increased from  
49 approximately 4 % up to about 16 %.  
50  
51  
52  
53  
54  
55  
56

### 57 3.2.2. Tunable mechanical properties

58  
59 *Typical stress-strain responses* – The cyclic tensile responses of the various elaborated  
60 materials are reported in Fig. 6, highlighting the differences in mechanical behavior between  
61  
62  
63  
64  
65



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

each isolated component (DGL/PEG hydrogels and PCL fibrous mats) and the fiber-reinforced hydrogels, in the illustrative case of aligned fibers (see the case of 2D random fibers in Supplementary Fig. S2B).

In Fig. 6A, stress-strain curves for DGL/PEG hydrogels display quasilinear reversible behavior with minimal residual stress hysteresis. As anticipated [68,69], an increase in the PEG/DGL ratio led to higher stiffness (*e.g.*, tangent modulus  $E_t$  varying from 10 to 75 kPa for hydrogels with  $PEG_{MW} = 2$  kDa at 5 % strain), while an increase in  $PEG_{MW}$  resulted in greater strains at break (from approximately 30 % to over 65 %). Accordingly, the softer hydrogel (*i.e.*, Hyd(10%, 10k, 1/2.5)) could hardly be manipulated and tested with the present protocol. In contrast, as shown in Fig. 6B, the processed fibrous mats exhibit elasto-plastic behavior, consistent with findings for raw PCL polymer and single PCL electrospun microfibers, which show irreversible deformation and necking past 5 % strain [70–72].

In the case of the composites (Fig. 6C–D and Supplementary Fig. S2), the incorporation of PCL fibers into the hydrogels consistently produced non-linear stress-strain responses characterized by J-shaped curves, likely resulting from the gradual reorientation of fibers along the tensile direction [70,73]. However, this strain hardening remains far less pronounced than that observed on isolated fibrous mats, due to the presence of the matrix, which constrains their kinematics to isochoric ones. Compared to non-reinforced hydrogels, stress levels achieved in the composites could be enhanced by up to two orders of magnitude in finite strains, in line with previous works on fiber-reinforced hydrogels [67,74] or other fiber-reinforced gel-like systems [75]. A typical stress hysteresis was also observed after each unloading sequence, resulting in non-zero residual strains. However, these residual strains were much less pronounced than those observed with fibers alone (see Fig. 6B and Supplementary Fig. S2B). This is ascribed to the elastic properties of the DGL/PEG matrix, which presumably limit the microstructural rearrangements (*e.g.*, fibers rotations and interactions) [11] and the large plastic deformation of the stretched PCL fibers. Finally, as anticipated, the strong anisotropy of the aligned fibrous mats was also reflected in the behavior of the composite (see Supplementary Fig. S2C).

Therefore, these global trends point out that the overall response of fibrous composites combines the high tensile strength, non-linear and anisotropic behavior of the fibrous mat upon finite strains (as ensured by collagen networks in native tissues), together with the elastic

1 recovery due to the cured homogeneous hydrogels (as induced by surrounding elastin and  
2 ground substance *in vivo*; Fig. 1).  
3

4 *Influence of the composite's formulation* – Fig. 7 presents the strain-induced evolution of  
5  $E_t$ ,  $P^{max}$ ,  $W^{diss}$  and  $\varepsilon_r$  related to FHC composites listed in Table 1.  
6  
7

8  
9 Firstly, figures 6 and 7 highlights the effect of PEG molecular weight. Throughout the entire  
10 strain range studied (up to 40 %), composites with the lowest  $PEG_{MW} = 2$  kDa showed higher  
11 tensile stiffness and peak strength (Fig. 7 A–B), in agreement with observations made for  
12 matrices alone (see Fig. 6A). These results align with the anticipated trends for hydrogels with  
13 shorter chains between chemical cross-links, as indicated by the corresponding shorter mesh  
14 sizes in Table 1. This suggests a higher cross-linker concentration [69]. Furthermore, beyond  
15 the early stage of deformation (for  $\varepsilon^{max} > 5$  %), cyclic events with successive strain increments  
16 resulted in higher dissipated energy and lower residual strains in composites with the lowest  
17  $PEG_{MW}$  (Fig. 7 C–D). Notably, this trend was not observed with neat hydrogels alone (see Fig.  
18 6A). Hence, the observed changes in  $\varepsilon_r$  and  $W^{diss}$  with varying  $PEG_{MW}$  may be linked to  
19 structural rearrangements of PCL fibers within the composite (*e.g.*, rotations) and/or  
20 variations in fiber interfaces decohesion, likely influenced by matrix properties.  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31

32  
33 Secondly, Figures 6 and 7 also highlight the influence of the fiber content, a well-known  
34 key factor in the mechanics of fibrous materials [76,77]. This is particularly evidenced in the  
35 stress-strain responses of composites reported in Fig. 6D, the volume fraction of fibers  $\varphi_{PCL}$   
36 of which from 0 % (neat gel) to 11 %, while keeping constant all other parameters (data shown  
37 here only for  $\varepsilon^{max} = 10$  % for the sake of clarity). Furthermore, Fig. 8 clearly demonstrates that  
38 all the derived mechanical descriptors ( $E_t$ ,  $P^{max}$ ,  $\eta$ ,  $\varepsilon_r$ ) increased with  $\varphi_{PCL}$ , except for the  
39 residual strain  $\varepsilon_r$  which remained nearly constant for a given peak strain  $\varepsilon^{max}$ .  
40  
41  
42  
43  
44  
45

### 46 3.3. Towards the mechanical biomimetism of the vocal-fold upper layers 47 48

49 The structural properties of jet-sprayed PCL mats can be tuned to partially mimic collagen  
50 networks in the *lamina propria*. Additionally, the elastic moduli of PCL and collagen single  
51 fibrils fall within the same range (approximately from 500 MPa to 1 GPa [11,71,78]). These  
52 factors enabled us to propose a mechanical biomimicry of the human vocal-fold upper layers.  
53 However, it is important to note that, in this initial approach, fiber-reinforced hydrogels were  
54 developed to replicate the overall bulk mechanical properties of the vocal-fold upper layers,  
55 without distinguishing between the *epithelium* and the *lamina propria*. The mechanical  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 contribution of the micrometer-thick *epithelium* in the native sandwich composite structure  
2 was deemed negligible compared to the millimeter-thick, fiber-reinforced *lamina propria*.  
3

4 Initially, from the various prepared composites, we selected a series of suitable candidates  
5 to replicate the visco-hyperelastic response of native tissues in longitudinal tension, aligning  
6 with the primary orientation of their collagen fibers. Specifically, in Fig. 9A, we illustrate the  
7 relevance of four composite hydrogels tuned to replicate samples of human vocal-fold upper  
8 layers (noted LP<sub>1</sub>, LP<sub>2</sub>, LP<sub>3</sub> to simplify labelling), which were subjected to comparable large-  
9 strains and cyclic uniaxial loads up to  $\varepsilon^{max} \approx 10\%$  [5]. Such reference targets were extracted  
10 to define the typical range of mechanical responses observed *ex vivo* and to demonstrate the  
11 variability related to intra/inter-individual. Notably, LP<sub>1</sub> and LP<sub>2</sub> were obtained from left and  
12 right vocal folds of the same larynx. Previous studies have assessed the mechanical  
13 characteristics of human vocal fold tissue using various measurement techniques [79–81].  
14 Here, our study endeavors to harmonize with data acquired from the same excised upper  
15 layers of human vocal fold subjected to a spectrum of physiological loading conditions [5]. This  
16 deliberate approach aimed at mitigating inherent variability arising from both inter/intra-  
17 individual differences and disparate measurement techniques. The mechanical descriptors  
18 ( $E_t, P^{max}, \eta, \varepsilon_r$ ) relative to these target stress-strain behaviors are reported in Fig. 8.  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33

34 This first selection was made among FHC composites with 2D preferred orientation. This  
35 choice aimed to induce structural and mechanical anisotropy close to those observed in living  
36 tissues. It also allowed for a wide distribution of orientations, allowing fiber rotation toward the  
37 load direction during tension. This micro-mechanism enable us to replicate the gradual  
38 recruitment of wavy collagen fibers during stretching of the *lamina propria* in the anterior-  
39 posterior direction [11]. Furthermore, composites with the lowest  $PEG_{MW}$  (2 kDa) were selected  
40 due to their increased stiffness and dissipative properties from small to finite strains, along with  
41 minimal residual strains after cycling. Specifically, as demonstrated in Fig. 8, the candidate  
42 FHC(5%, 2k, 1/1, Aligned) proved suitable for approximating the desired range represented by  
43 targets LP<sub>*i*</sub>. This was achieved by adjusting  $\varphi_{PCL}$  values within the range of 3 % to 11 %,  
44 corresponding to the measured volume fraction of collagen fibers in the superficial *lamina*  
45 *propria* in our study ( $9.7 \pm 3.7\%$ ). Nonetheless, to match the softest target mechanical response  
46 (LP<sub>1</sub>), a higher  $PEG_{MW}$  was necessary. For this specific case, the candidate FHC(12%, 10k, 1/3,  
47 Aligned) composed of the softest elaborated matrix (see Fig. 6A) was chosen.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 In a second step, we evaluated the candidate FHC(5%, 2k, 1/1, Aligned) for suitability in  
3 transverse compression, *i.e.*, perpendicularly to the main fiber orientation. This loading mode  
4 is also very important during phonation, keeping in mind that the quality of contact between  
5 vocal folds is a key factor in voice quality, and that high contact pressure are observed in  
6 common lesions of the *lamina propria* [14,82]. In the end, as shown in Figs. 9A–B, the choice  
7 of  $\varphi_{PCL} \approx 3\%$  (in *green*) allowed the macroscale mechanics of the upper layers to be  
8 reproduced fairly well both in longitudinal tension and in transverse compression. In contrast  
9 to the trends observed in longitudinal tension, it is noteworthy that the response of the fibrous  
10 FHC composite under compression closely resembled that of that of the sole hydrogel matrix  
11 at low strains (see Fig. 9B), aligning with the characteristics of native vocal-fold tissue [11].  
12 Additionally, it was observed that the mechanical contribution of the matrix in this mode  
13 decreased significantly for  $\varepsilon < -0.05$ , in line with predictions for biological tissue due to steric  
14 interactions likely occurring at the fiber scale [11].  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27

#### 28 **4. Conclusion**

29  
30 In this study, we propose a set of versatile biocompatible fiber-reinforced hydrogels with  
31 tunable mechanical properties when subjected to cyclic and large-strains tensile and  
32 compressive loadings, such as those experienced by the *lamina propria* during human voice  
33 production. These composite hydrogels consist of jet-sprayed PCL fibrous mats embedded in  
34 a DGL/PEG matrix. The chemical formulation of their hydrogel matrix (*i.e.*, PEG molecular  
35 weight, concentration and DGL/PEG ratio), the microstructural architecture of their fibrous  
36 networks (*i.e.*, diameter, orientation and volume fraction of fibers) and their assembly process  
37 were tailored to ensure that the composites mimicked the anisotropic structure of the human  
38 upper layers (*i.e.*, the *lamina propria*, together with the very thin superficial *epithelium*), and  
39 its non-linear viscoelastic mechanical behavior. To do so, collagen and elastin fibrillar networks  
40 of deceased patients were characterized by two-photon excitation microscopy to quantify  
41 fiber and bundles diameters, preferential orientations and volume fractions, which, aside from  
42 providing concrete targets to match, completed the rare histological descriptors of the vocal  
43 fold. Using a recent mechanical database on native vocal-fold tissues subjected to longitudinal  
44 tension and transverse compression, the processed biomimetic composites were validated  
45 with respect to their non-linear strain hardening, mechanical strength, residual strain and  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 strain energy density dissipated during cycling. The versatility of the selected architected  
2 materials was also highlighted, showing their ability to capture the dispersion of stress-strain  
3 responses related to inter or intra-individual variability. Overall, together with their potential  
4 to support cellularization and tissue formation *in vitro* and *in vivo*, the produced model  
5 materials allow to envision a better understanding of the role of the *lamina propria*'s structural  
6 characteristics in phonation under physiological aero-mechanical and acoustical loadings.  
7  
8  
9

## 10 **Acknowledgements**

11  
12  
13 This work was supported by the French National Research Agency (ANR MicroVoice grant  
14 n° ANR-17-CE19-0015-01). The 3SR Lab is part of the LabEx Tec 21 (Investissements d'Avenir -  
15 grant agreement n° ANR-11-LABX-0030) and the Carnot PolyNat Institute (Investissements  
16 d'Avenir - grant agreement n° ANR-16-CARN-0025-01). We would like to thank Philippe Masson  
17 and Yohann Robert (Univ. Grenoble Alpes, CHU Grenoble Alpes, LADAF) for their helpful  
18 assistance in the excision of the biological samples. We also acknowledge Christophe Vanbelle  
19 (Centre Léon Bérard, CRCL) and Baptiste Robbiani (UMR 5510, MATEIS) for their technical  
20 support to the two-photon excitation microscopy and the estimation of hydrogel mesh sizes,  
21 respectively.  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

## 33 **Conflict of interest statement**

34  
35  
36 The authors declare no conflict of interest.  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## References

- 1  
2  
3 [1] M. Hirano, Morphological Structure of the Vocal Cord as a Vibrator and its Variations, *Folia*  
4 *Phoniatrica et Logopaedica*. 26 (2009) 89–94. <https://doi.org/10.1159/000263771>.  
5  
6  
7 [2] I.R. Titze, *Principles of voice production*, National Center for Voice and Speech, Iowa City,  
8 Ia, 2000. <http://books.google.com/books?id=ytAeAQAAMAAJ>.  
9  
10  
11 [3] A.K. Miri, Mechanical Characterization of Vocal Fold Tissue: A Review Study, *Journal of*  
12 *Voice*. 28 (2014) 657–667. <https://doi.org/10.1016/j.jvoice.2014.03.001>.  
13  
14  
15 [4] T. Vampola, J. Horáček, I. Klepáček, Computer simulation of mucosal waves on vibrating  
16 human vocal folds, *Biocybernetics and Biomedical Engineering*. 36 (2016) 451–465.  
17 <https://doi.org/10.1016/j.bbe.2016.03.004>.  
18  
19  
20 [5] T. Cochereau, L. Bailly, L. Orgéas, N. Henrich Bernardoni, Y. Robert, M. Terrien, Mechanics  
21 of human vocal folds layers during finite strains in tension, compression and shear, *Journal*  
22 *of Biomechanics*. 110 (2020) 109956. <https://doi.org/10.1016/j.jbiomech.2020.109956>.  
23  
24  
25 [6] S.D. Gray, CELLULAR PHYSIOLOGY OF THE VOCAL FOLDS, *Otolaryngologic Clinics of North*  
26 *America*. 33 (2000) 679–697. [https://doi.org/10.1016/S0030-6665\(05\)70237-1](https://doi.org/10.1016/S0030-6665(05)70237-1).  
27  
28  
29 [7] K. Zhang, T. Siegmund, R.W. Chan, A two-layer composite model of the vocal fold lamina  
30 propria for fundamental frequency regulation, *Journal of the Acoustical Society of America*.  
31 122 (2007) 1090–1101. <https://doi.org/10.1121/1.2749460>.  
32  
33  
34 [8] Z. Zhang, J. Neubauer, D.A. Berry, Aerodynamically and acoustically driven modes of  
35 vibration in a physical model of the vocal folds, *J Acoust Soc Am*. 120 (2006) 2841–2849.  
36 <https://doi.org/10.1121/1.2354025>.  
37  
38  
39 [9] W. Jiang, X. Zheng, Q. Xue, Influence of vocal fold cover layer thickness on its vibratory  
40 dynamics during voice production, *J Acoust Soc Am*. 146 (2019) 369–380.  
41 <https://doi.org/10.1121/1.5116567>.  
42  
43  
44 [10] C. Finck, Implantation d'acide hyaluronique estérifié lors de la microchirurgie des lésions  
45 cordales bénignes, (2008). <https://orbi.uliege.be/handle/2268/142631>.  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- [11] A. Terzolo, L. Bailly, L. Orgéas, T. Cochereau, N. Henrich Bernardoni, A micro-mechanical model for the fibrous tissues of vocal folds, *Journal of the Mechanical Behavior of Biomedical Materials*. 128 (2022) 105118. <https://doi.org/10.1016/j.jmbbm.2022.105118>.
- [12] R.C. Branski, K. Verdolini, V. Sandulache, C.A. Rosen, P.A. Hebda, Vocal Fold Wound Healing: A Review for Clinicians, *Journal of Voice*. 20 (2006) 432–442. <https://doi.org/10.1016/j.jvoice.2005.08.005>.
- [13] J.K. Kutty, K. Webb, Tissue engineering therapies for the vocal fold lamina propria, *Tissue Eng Part B Rev*. 15 (2009) 249–262. <https://doi.org/10.1089/ten.TEB.2008.0588>.
- [14] A. Hantzakos, M. Remacle, F.G. Dikkers, J.-C. Degols, M. Delos, G. Friedrich, A. Giovanni, N. Rasmussen, Exudative lesions of Reinke’s space: a terminology proposal, *Eur Arch Otorhinolaryngol*. 266 (2009) 869–878. <https://doi.org/10.1007/s00405-008-0863-x>.
- [15] P. Schultz, Vocal fold cancer, *European Annals of Otorhinolaryngology, Head and Neck Diseases*. 128 (2011) 301–308. <https://doi.org/10.1016/j.anorl.2011.04.004>.
- [16] L. Li, J.M. Stiadle, H.K. Lau, A.B. Zerdoum, X. Jia, S.L. Thibeault, K.L. Kiick, Tissue engineering-based therapeutic strategies for vocal fold repair and regeneration, *Biomaterials*. 108 (2016) 91–110. <https://doi.org/10.1016/j.biomaterials.2016.08.054>.
- [17] P. Woo, J. Casper, R. Colton, D. Brewer, Diagnosis and treatment of persistent dysphonia after laryngeal surgery: a retrospective analysis of 62 patients, *Laryngoscope*. 104 (1994) 1084–1091. <https://doi.org/10.1288/00005537-199409000-00007>.
- [18] M. Imaizumi, R. Nakamura, Y. Nakaegawa, B.T. Dirja, Y. Tada, A. Tani, T. Sugino, Y. Tabata, K. Omori, Regenerative potential of basic fibroblast growth factor contained in biodegradable gelatin hydrogel microspheres applied following vocal fold injury: Early effect on tissue repair in a rabbit model, *Brazilian Journal of Otorhinolaryngology*. 87 (2021) 274–282. <https://doi.org/10.1016/j.bjorl.2019.09.003>.
- [19] R.S. Bartlett, S.L. Thibeault, R.S. Bartlett, S.L. Thibeault, Bioengineering the Vocal Fold: A Review of Mesenchymal Stem Cell Applications, in: *Advances in Biomimetics*, IntechOpen, 2011. <https://doi.org/10.5772/13803>.



- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- [20] J.L. Long, D.K. Chhetri, Restoring voice, *Science*. 350 (2015) 908–909.  
<https://doi.org/10.1126/science.aad7695>.
- [21] C. Ling, Q. Li, M.E. Brown, Y. Kishimoto, Y. Toya, E.E. Devine, K.-O. Choi, K. Nishimoto, I.G. Norman, T. Tsegay, J.J. Jiang, W.J. Burlingham, S. Gunasekaran, L.M. Smith, B.L. Frey, N.V. Welham, Bioengineered vocal fold mucosa for voice restoration, *Sci Transl Med*. 7 (2015) 314ra187. <https://doi.org/10.1126/scitranslmed.aab4014>.
- [22] M. Gugatschka, S. Ohno, A. Saxena, S. Hirano, Regenerative Medicine of the Larynx. Where are we Today? A Review, *Journal of Voice*. 26 (2012) 670.e7-670.e13.  
<https://doi.org/10.1016/j.jvoice.2012.03.009>.
- [23] N. Wan-Chiew, M.M. Baki, M.B. Fauzi, Y. Lokanathan, M. Azman, In Vitro Evaluation of Biomaterials for Vocal Fold Injection: A Systematic Review, *Polymers*. 13 (2021) 2619.  
<https://doi.org/10.3390/polym13162619>.
- [24] J. Cedervall, L. Ahrlund-Richter, B. Svensson, K. Forsgren, F.H.J. Maurer, D. Vidovska, S. Hertegård, Injection of embryonic stem cells into scarred rabbit vocal folds enhances healing and improves viscoelasticity: short-term results, *Laryngoscope*. 117 (2007) 2075–2081. <https://doi.org/10.1097/MLG.0b013e3181379c7c>.
- [25] S. Bashir, M. Hina, J. Iqbal, A.H. Rajpar, M.A. Mujtaba, N.A. Alghamdi, S. Wageh, K. Ramesh, S. Ramesh, Fundamental Concepts of Hydrogels: Synthesis, Properties, and Their Applications, *Polymers*. 12 (2020) 2702. <https://doi.org/10.3390/polym12112702>.
- [26] Q. Chai, Y. Jiao, X. Yu, Hydrogels for Biomedical Applications: Their Characteristics and the Mechanisms behind Them, *Gels*. 3 (2017) 6. <https://doi.org/10.3390/gels3010006>.
- [27] K. Deligkaris, T.S. Tadele, W. Olthuis, A. van den Berg, Hydrogel-based devices for biomedical applications, *Sensors and Actuators B: Chemical*. 147 (2010) 765–774.  
<https://doi.org/10.1016/j.snb.2010.03.083>.
- [28] K.Y. Lee, D.J. Mooney, Hydrogels for Tissue Engineering, *Chem. Rev*. 101 (2001) 1869–1880.  
<https://doi.org/10.1021/cr000108x>.
- [29] A.S. Hoffman, Hydrogels for biomedical applications, *Advanced Drug Delivery Reviews*. 64 (2012) 18–23. <https://doi.org/10.1016/j.addr.2012.09.010>.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- [30] C.A. DeForest, K.S. Anseth, *Advances in bioactive hydrogels to probe and direct cell fate*, *Annu Rev Chem Biomol Eng.* 3 (2012) 421–444. <https://doi.org/10.1146/annurev-chembioeng-062011-080945>.
- [31] C.L. Finck, B. Harmegnies, A. Remacle, P. Lefebvre, *Implantation of esterified hyaluronic acid in microdissected Reinke’s space after vocal fold microsurgery: short- and long-term results*, *J Voice.* 24 (2010) 626–635. <https://doi.org/10.1016/j.jvoice.2008.12.015>.
- [32] S.S. Karajanagi, G. Lopez-Guerra, H. Park, J.B. Kobler, M. Galindo, J. Aanestad, D.D. Mehta, Y. Kumai, N. Giordano, A. d’Almeida, J.T. Heaton, R. Langer, V.L.M. Herrera, W. Faquin, R.E. Hillman, S.M. Zeitels, *Assessment of canine vocal fold function after injection of a new biomaterial designed to treat phonatory mucosal scarring*, *Ann Otol Rhinol Laryngol.* 120 (2011) 175–184. <https://doi.org/10.1177/000348941112000306>.
- [33] L.A. Hughes, J. Gaston, K. McAlindon, K.A. Woodhouse, S.L. Thibeault, *Electrospun fiber constructs for vocal fold tissue engineering: effects of alignment and elastomeric polypeptide coating*, *Acta Biomater.* 13 (2015) 111–120. <https://doi.org/10.1016/j.actbio.2014.10.039>.
- [34] A. Abbott, *Cell culture: biology’s new dimension*, *Nature.* 424 (2003) 870–872. <https://doi.org/10.1038/424870a>.
- [35] J. Visser, F.P.W. Melchels, J.E. Jeon, E.M. van Bussel, L.S. Kimpton, H.M. Byrne, W.J.A. Dhert, P.D. Dalton, D.W. Hutmacher, J. Malda, *Reinforcement of hydrogels using three-dimensionally printed microfibrils*, *Nat Commun.* 6 (2015) 6933. <https://doi.org/10.1038/ncomms7933>.
- [36] P.R. Murray, S.L. Thomson, *Vibratory responses of synthetic, self-oscillating vocal fold models*, *J Acoust Soc Am.* 132 (2012) 3428–3438. <https://doi.org/10.1121/1.4754551>.
- [37] R.G.T. Romero, M.B. Colton, S.L. Thomson, *3D-Printed Synthetic Vocal Fold Models*, *Journal of Voice.* 35 (2021) 685–694. <https://doi.org/10.1016/j.jvoice.2020.01.030>.
- [38] S.M. Shaw, S.L. Thomson, C. Dromey, S. Smith, *Frequency response of synthetic vocal fold models with linear and nonlinear material properties*, *J Speech Lang Hear Res.* 55 (2012) 1395–1406. [https://doi.org/10.1044/1092-4388\(2012/11-0153\)](https://doi.org/10.1044/1092-4388(2012/11-0153)).

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- [39] R.K. Tindell, M.J. McPhail, C.E. Myers, J. Neubauer, J.M. Hintze, D.G. Lott, J.L. Holloway, Trilayered Hydrogel Scaffold for Vocal Fold Tissue Engineering, *Biomacromolecules*. 23 (2022) 4469–4480. <https://doi.org/10.1021/acs.biomac.1c01149>.
- [40] N. Latifi, M. Asgari, H. Vali, L. Mongeau, A tissue-mimetic nano-fibrillar hybrid injectable hydrogel for potential soft tissue engineering applications, *Sci Rep*. 8 (2018) 1047. <https://doi.org/10.1038/s41598-017-18523-3>.
- [41] J. Sohier, I. Carubelli, P. Sarathchandra, N. Latif, A.H. Chester, M.H. Yacoub, The potential of anisotropic matrices as substrate for heart valve engineering, *Biomaterials*. 35 (2014) 1833–1844. <https://doi.org/10.1016/j.biomaterials.2013.10.061>.
- [42] J. Sohier, P. Corre, C. Perret, P. Pilet, P. Weiss, Novel and simple alternative to create nanofibrillar matrices of interest for tissue engineering, *Tissue Eng Part C Methods*. 20 (2014) 285–296. <https://doi.org/10.1089/ten.TEC.2013.0147>.
- [43] M. Carrancá, L. Griveau, N. Remoué, C. Lorion, P. Weiss, V. Orea, D. Sigaudou-Roussel, C. Faye, D. Ferri-Angulo, R. Debret, J. Sohier, Versatile lysine dendrigrafts and polyethylene glycol hydrogels with inherent biological properties: in vitro cell behavior modulation and in vivo biocompatibility, *J Biomed Mater Res A*. 109 (2021) 926–937. <https://doi.org/10.1002/jbm.a.37083>.
- [44] L. Bailly, T. Cochereau, L. Orgéas, N. Henrich Bernardoni, S. Rolland du Roscoat, A. McLeer-Florin, Y. Robert, X. Laval, T. Laurencin, P. Chaffanjon, B. Fayard, E. Boller, 3D multiscale imaging of human vocal folds using synchrotron X-ray microtomography in phase retrieval mode, *Sci Rep*. 8 (2018) 14003. <https://doi.org/10.1038/s41598-018-31849-w>.
- [45] N.A. Hotaling, K. Bharti, H. Kriel, C.G. Simon, DiameterJ: A validated open source nanofiber diameter measurement tool, *Biomaterials*. 61 (2015) 327–338. <https://doi.org/10.1016/j.biomaterials.2015.05.015>.
- [46] Z. Püspöki, M. Storath, D. Sage, M. Unser, Transforms and Operators for Directional Bioimage Analysis: A Survey, *Adv Anat Embryol Cell Biol*. 219 (2016) 69–93. [https://doi.org/10.1007/978-3-319-28549-8\\_3](https://doi.org/10.1007/978-3-319-28549-8_3).

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- [47] M. Doube, M.M. Kłosowski, I. Arganda-Carreras, F.P. Cordelières, R.P. Dougherty, J.S. Jackson, B. Schmid, J.R. Hutchinson, S.J. Shefelbine, BoneJ: Free and extensible bone image analysis in ImageJ, *Bone*. 47 (2010) 1076–1079. <https://doi.org/10.1016/j.bone.2010.08.023>.
- [48] C.C. Xu, R.W. Chan, Pore architecture of a bovine acellular vocal fold scaffold, *Tissue Eng Part A*. 14 (2008) 1893–1903. <https://doi.org/10.1089/ten.tea.2007.0243>.
- [49] A. Kiziltay, A. Marcos-Fernandez, J. San Roman, R.A. Sousa, R.L. Reis, V. Hasirci, N. Hasirci, Poly(ester-urethane) scaffolds: effect of structure on properties and osteogenic activity of stem cells, *J Tissue Eng Regen Med*. 9 (2015) 930–942. <https://doi.org/10.1002/term.1848>.
- [50] M. Rubinstein, R.H. Colby, *Polymer Physics*, 1st edition, Oxford University Press, Oxford ; New York, 2003.
- [51] P.X. Ma, J. Elisseeff, *Scaffolding In Tissue Engineering*, CRC Press, Boca Raton, 2005.
- [52] M.S.M. Alger, *Polymer science dictionary*, 2nd ed, Chapman & Hall, London, 1997.
- [53] H. Yousefi-Mashouf, L. Bailly, L. Orgéas, N. Henrich Bernardoni, Mechanics of gelatin-based hydrogels during finite strain tension, compression and shear, *Frontiers in Bioengineering and Biotechnology*. 10 (2023). <https://www.frontiersin.org/articles/10.3389/fbioe.2022.1094197>.
- [54] J.E. Kelleher, K. Zhang, T. Siegmund, R.W. Chan, Spatially varying properties of the vocal ligament contribute to its eigenfrequency response, *Journal of the Mechanical Behavior of Biomedical Materials*. 3 (2010) 600–609. <https://doi.org/10.1016/j.jmbbm.2010.07.009>.
- [55] J.E. Kelleher, T. Siegmund, M. Du, E. Naseri, R.W. Chan, The anisotropic hyperelastic biomechanical response of the vocal ligament and implications for frequency regulation: A case study, *J Acoust Soc Am*. 133 (2013) 1625–1636. <https://doi.org/10.1121/1.4776204>.
- [56] R.W. Chan, M. Fu, L. Young, N. Tirunagari, Relative contributions of collagen and elastin to elasticity of the vocal fold under tension, *Ann Biomed Eng*. 35 (2007) 1471–1483. <https://doi.org/10.1007/s10439-007-9314-x>.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- [57] E. Goodyer, J.J. Jiang, E. Devine, A. Sutor, S. Rupitsch, S. Zorner, M. Stingl, B. Schmidt, Devices and Methods on Analysis of Biomechanical Properties of Laryngeal Tissue and Substitute Materials, *Current Bioinformatics*. 6 (n.d.) 344–361.
- [58] B. Gadot, O. Riu Martinez, S. Rolland du Roscoat, D. Bouvard, D. Rodney, L. Orgéas, Entangled single-wire NiTi material: A porous metal with tunable superelastic and shape memory properties, *Acta Materialia*. 96 (2015) 311–323. <https://doi.org/10.1016/j.actamat.2015.06.018>.
- [59] H.E. Gunter, Modeling mechanical stresses as a factor in the etiology of benign vocal fold lesions, *J Biomech*. 37 (2004) 1119–1124. <https://doi.org/10.1016/j.jbiomech.2003.11.007>.
- [60] H. Bakhshae, J. Young, J.C.W. Yang, L. Mongeau, A.K. Miri, Determination of strain field on the superior surface of excised larynx vocal folds using DIC, *J Voice*. 27 (2013) 659–667. <https://doi.org/10.1016/j.jvoice.2013.05.009>.
- [61] A.K. Miri, U. Tripathy, L. Mongeau, P.W. Wiseman, Nonlinear Laser Scanning Microscopy of Human Vocal Folds, *Laryngoscope*. 122 (2012) 356–363. <https://doi.org/10.1002/lary.22460>.
- [62] F. Benboujja, C. Hartnick, Quantitative evaluation of the human vocal fold extracellular matrix using multiphoton microscopy and optical coherence tomography, *Sci Rep*. 11 (2021) 2440. <https://doi.org/10.1038/s41598-021-82157-9>.
- [63] M.J. Mochane, T.S. Motsoeneng, E.R. Sadiku, T.C. Mokhena, J.S. Sefadi, Morphology and Properties of Electrospun PCL and Its Composites for Medical Applications: A Mini Review, *Applied Sciences*. 9 (2019) 2205. <https://doi.org/10.3390/app9112205>.
- [64] R. Debret, C. Faye, J. Sohier, P. Sommer, Polypeptide Derived from Tropoelastin and Biocompatible Material Comprising Same, 2017. <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2017194761>.
- [65] L. Griveau, M. Lafont, H. le Goff, C. Drouglazet, B. Robbiani, A. Berthier, D. Sigaud-Roussel, N. Latif, C.L. Visage, V. Gache, R. Debret, P. Weiss, J. Sohier, Design and characterization of an in vivo injectable hydrogel with effervescently generated porosity for regenerative

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

medicine applications, *Acta Biomaterialia*. 140 (2022) 324–337.  
<https://doi.org/10.1016/j.actbio.2021.11.036>.

[66] B. Unal, R.C. Hedden, Gelation and swelling behavior of end-linked hydrogels prepared from linear poly(ethylene glycol) and poly(amidoamine) dendrimers, *Polymer*. 47 (2006) 8173–8182. <https://doi.org/10.1016/j.polymer.2006.09.048>.

[67] A. Agrawal, N. Rahbar, P.D. Calvert, Strong fiber-reinforced hydrogel, *Acta Biomaterialia*. 9 (2013) 5313–5318. <https://doi.org/10.1016/j.actbio.2012.10.011>.

[68] J.S. Temenoff, K.A. Athanasiou, R.G. LeBaron, A.G. Mikos, Effect of poly(ethylene glycol) molecular weight on tensile and swelling properties of oligo(poly(ethylene glycol) fumarate) hydrogels for cartilage tissue engineering, *J Biomed Mater Res*. 59 (2002) 429–437. <https://doi.org/10.1002/jbm.1259>.

[69] M.L. Oyen, Mechanical characterisation of hydrogel materials, *International Materials Reviews*. 59 (2014) 44–59. <https://doi.org/10.1179/1743280413Y.0000000022>.

[70] A. Delp, A. Becker, D. Hülsbusch, R. Scholz, M. Müller, B. Glasmacher, F. Walther, In Situ Characterization of Polycaprolactone Fiber Response to Quasi-Static Tensile Loading in Scanning Electron Microscopy, *Polymers (Basel)*. 13 (2021) 2090. <https://doi.org/10.3390/polym13132090>.

[71] D. Alexeev, N. Goedecke, J. Snedeker, S. Ferguson, Mechanical evaluation of electrospun poly( $\epsilon$ -caprolactone) single fibers, *Materials Today Communications*. 24 (2020) 101211. <https://doi.org/10.1016/j.mtcomm.2020.101211>.

[72] H. Mondésert, Anisotropic PCL electrospun scaffolds for soft tissue engineering: Elaboration, morphological and mechanical properties, 2020.

[73] L. Bailly, M. Toungara, L. Orgéas, E. Bertrand, V. Deplano, C. Geindreau, In-plane mechanics of soft architected fibre-reinforced silicone rubber membranes, *Journal of the Mechanical Behavior of Biomedical Materials*. 40 (2014) 339–353. <https://doi.org/10.1016/j.jmbbm.2014.09.012>.

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- [74] K. Tonsomboon, A.L. Butcher, M.L. Oyen, Strong and tough nanofibrous hydrogel composites based on biomimetic principles, *Mater Sci Eng C Mater Biol Appl.* 72 (2017) 220–227. <https://doi.org/10.1016/j.msec.2016.11.025>.
- [75] T. Laurencin, P.J.J. Dumont, L. Orgéas, S.L. Corre, F. Martoia, S. Rolland du Roscoat, P. Laure, 3D real time and in situ observation of the fibre orientation during the plane strain flow of concentrated fibre suspensions, *Journal of Non-Newtonian Fluid Mechanics.* 312 (2023) 104978. <https://doi.org/10.1016/j.jnnfm.2022.104978>.
- [76] J. Jang, J. Lee, Y.-J. Seol, Y.H. Jeong, D.-W. Cho, Improving mechanical properties of alginate hydrogel by reinforcement with ethanol treated polycaprolactone nanofibers, *Composites Part B: Engineering.* 45 (2013) 1216–1221. <https://doi.org/10.1016/j.compositesb.2012.09.059>.
- [77] F. Martoia, P.J.J. Dumont, L. Orgéas, M.N. Belgacem, J.-L. Putaux, On the origins of the elasticity of cellulose nanofiber nanocomposites and nanopapers: a micromechanical approach, *RSC Adv.* 6 (2016) 47258–47271. <https://doi.org/10.1039/C6RA07176G>.
- [78] P. Dutov, O. Antipova, S. Varma, J.P.R.O. Orgel, J.D. Schieber, Measurement of Elastic Modulus of Collagen Type I Single Fiber, *PLoS One.* 11 (2016) e0145711. <https://doi.org/10.1371/journal.pone.0145711>.
- [79] D.K. Chhetri, Z. Zhang, J. Neubauer, Measurement of Young’s modulus of vocal folds by indentation, *J Voice.* 25 (2011) 1–7. <https://doi.org/10.1016/j.jvoice.2009.09.005>.
- [80] J.E. Kelleher, T. Siegmund, M. Du, E. Naseri, R.W. Chan, Empirical measurements of biomechanical anisotropy of the human vocal fold lamina propria, *Biomech Model Mechanobiol.* 12 (2013) 555–567. <https://doi.org/10.1007/s10237-012-0425-4>.
- [81] F. Alipour, S. Vigmostad, Measurement of vocal folds elastic properties for continuum modeling, *J Voice.* 26 (2012) 816.e21–29. <https://doi.org/10.1016/j.jvoice.2012.04.010>.
- [82] F. Silva, T. Legou, P. Champsaur, A. Giovanni, A. Lagier, Contact Pressure Between the Vocal Folds in Reinke’s Edema: Experimental Observations on an Excised Human Larynx, *J Voice.* 35 (2021) 931.e15-931.e20. <https://doi.org/10.1016/j.jvoice.2020.02.020>.



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Versatile fiber-reinforced hydrogels to mimic the microstructure and mechanics of human vocal-fold upper layers

Daniel Ferri-Angulo<sup>\*a</sup>, Hamid Yousefi-Mashouf<sup>bc</sup>, Margot Michel<sup>d</sup>, Anne McLeer<sup>e</sup>, Laurent Org as<sup>b</sup>, Lucie Bailly<sup>tb</sup>, J r me Sohier<sup>tsd</sup>

<sup>a</sup>CNRS UMR5510 MATEIS, 7 Avenue Jean Capelle, F-69621, Villeurbanne, France

<sup>b</sup>Univ. Grenoble Alpes, CNRS, Grenoble INP, 3SR, 38000 Grenoble, France

<sup>c</sup>Univ. Grenoble Alpes, CNRS, Grenoble INP, GIPSA-lab, 38000 Grenoble, France

<sup>d</sup>CNRS UMR5305 LBTI, Passage du Vercors, 69007, Lyon, France

<sup>e</sup>Univ. Grenoble Alpes, Service d'Anatomie et Cytologie Pathologiques CHU Grenoble Alpes, Institute for Advanced Biosciences UGA/INSERM U1209/CNRS 5309, 38000 Grenoble, France

\*Current affiliation: CNRS UPR8001 LAAS-TEAM, 7 Avenue Colonel Roche, 31031, Toulouse, France

<sup>†</sup>Contributed to this work equally

<sup> </sup> Corresponding author: [jerome.sohier@ibcp.fr](mailto:jerome.sohier@ibcp.fr)

**Figure 1:** Synthetic approach followed to mimic the major structural and mechanical specificities of human vocal-fold *lamina propria* through the design of poly-lysine dendrimers and polyethylene glycol (DGL/PEG) hydrogels reinforced with tunable jet-sprayed polymer fibrous architectures. Top center illustration adapted from Murthy et al. and Kumar *et al.* [43, 44].

**Figure 2:** Structural analysis of human *lamina propria*. **(A)** Close view of the *epithelium* and *lamina propria* layers of a human vocal-fold cross-section where collagen (blue) and elastin (yellow) are visualized through second harmonic generation and autofluorescence, respectively (pixel size 0.17  $\mu\text{m} \times 0.17 \mu\text{m}$ ). **(B)** Examples of the organization of collagen at various depths of the same *lamina propria* sample (top panel: voxel size 0.69  $\mu\text{m} \times 0.69 \mu\text{m} \times 6.17 \mu\text{m}$ , thickness 58.8  $\mu\text{m}$ ; bottom panel: voxel size 0.17  $\mu\text{m} \times 0.17 \mu\text{m} \times 0.75 \mu\text{m}$ , thickness 30.8  $\mu\text{m}$ ).

**Figure 3:** Quantitative analysis of the fibrous architecture in the human *lamina propria*. **(A)** Representative 2D micrograph at several depths of the *lamina propria* (collagen appears blue and elastin yellow, voxel size 0.17  $\mu\text{m} \times 0.17 \mu\text{m} \times 0.75 \mu\text{m}$ ) and in-plane angular distribution of the collagen and elastin fibers, defined relative to the *epithelium*. **(B)** Quantification of the diameters of collagen and elastin fibers and fiber bundles. Mann-Whitney U-test for fibers:  $U = 308$ ,  $n_1 = 6047$ ,  $n_2 = 1496$ , \*\*\*\*  $p < 0.0001$  two-tailed. Mann-Whitney U-test for bundles:  $U = 80951$ ,  $n_1 = 461$ ,  $n_2 = 308$ , \*\*\*\*  $p < 0.0001$  two-tailed.

**Figure 4:** Mats of random and aligned PCL fibers. **(A)** Representative SEM pictures and quantification of their 2D orientations. **(B)** Quantification of the diameters of random and aligned fibers and fiber bundles. Mann-Whitney U-test for fibers:  $U = 3.1E10$ ,  $n_1 = 165168$ ,  $n_2 = 168364$ , \*\*\*\*  $p < 0.0001$  two-tailed. Mann-Whitney U-test for bundles:  $U = 3.6E10$ ,  $n_1 = 165168$ ,  $n_2 = 221722$ , \*\*\*\*  $p < 0.0001$  two-tailed.

**Figure 5:** Characterization of 2D random and 2D aligned fiber-reinforced hydrogels (FHC). **(A)** Representative pictures of both composite types. **(B)** Swelling ratios of hydrogels prepared with 2 (top) and 10 kDa PEG (bottom) at different concentrations ( $C_{PEG} = 5$  and 10 %) and their homogeneous composites obtained with 2D random and 2D aligned fibrous PCL mats. **(C)** Modulation of FHC volume fraction through pre-compaction of aligned fibrous mats. Data are mean  $\pm$  SD (n=5).

**Figure 6:** Mechanical characterization of hydrogels, PCL mats and FHC samples. Typical cyclic tensile stress-strain response of **(A)** various DGL/PEG homogeneous hydrogels, **(B)** aligned PCL fibrous mats under longitudinal loading, and **(C-D)** FHC composites reinforced by aligned fibers: illustrative cases of **(C)** FHC(10%, 2k, 1/2, Aligned) as listed in Table 1, and **(D)** FHC(5%, 2k, 1/1, Aligned) with a volume fraction of fibers  $\varphi_{PCL}$  varying from 0 % to 11 %. Error bars represent the measurement uncertainty, including the dispersion of the data over 5 repeated tests (min-max values) and the inherent accuracy errors. Grey areas illustrate hysteresis loops leading to a dissipation of mechanical energy.

**Figure 7:** Evolution of the mechanical descriptors of the composite hydrogels in function of the applied peak strains  $\varepsilon^{max}$ : **(A)** tangent modulus,  $E_t$ ; **(B)** peak stress level,  $P^{max}$ ; **(C)** dissipated energy density per cycle,  $W^{diss}$ ; **(D)** residual strain,  $\varepsilon_r$ .

**Figure 8:** Modulation of fiber volume fraction  $\varphi_{PCL}$  in aligned FHC through pre-compaction of fibrous mats to mimic human vocal-fold upper layers (*lamina propria + epithelium*). Tensile mechanical descriptors as a function of the applied peak strain,  $\varepsilon^{max}$ , set at 10, 20 or 30 % (*from smallest to largest symbol*): **(A)** tangent modulus,  $E_t$ ; **(B)** peak stress level,  $P^{max}$ ; **(C)** energy dissipation coefficient,  $\eta$ ; **(D)** residual strain,  $\varepsilon_r$ . Data acquired on composites FHC(5%, 2k, 1/1, Aligned). Colored areas (in orange) represent the range of target values as derived from Fig. 9, owning identical matrix and fibrous orientation, but tailored values of  $\varphi_{PCL}$ .

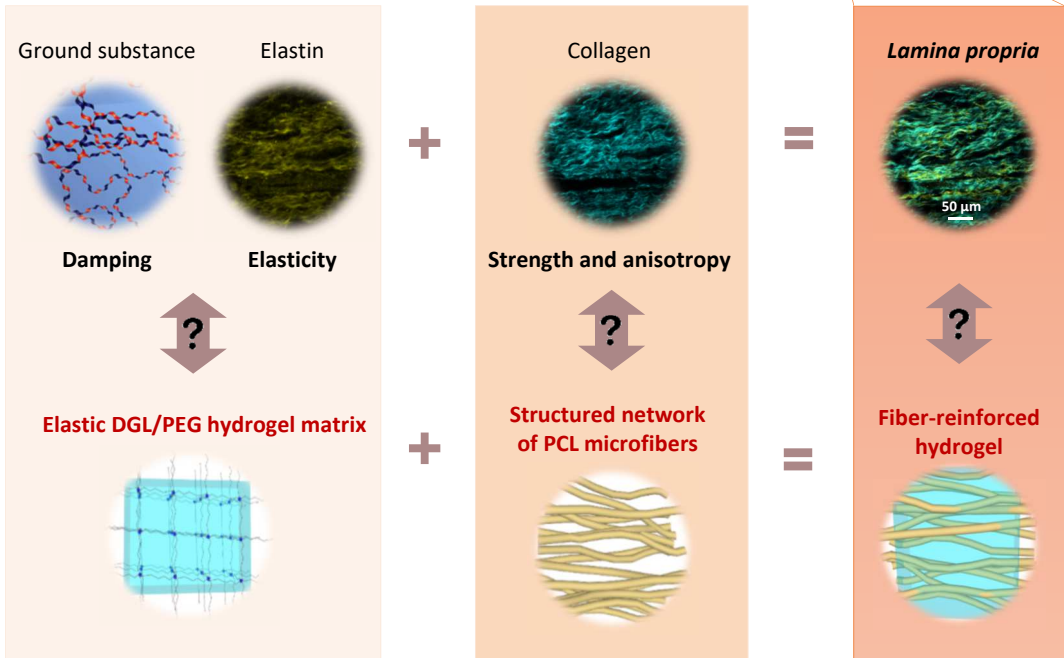
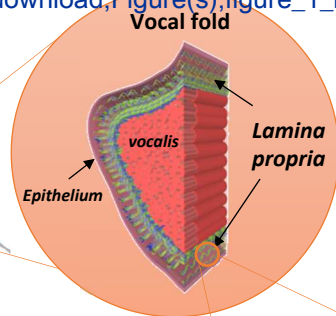
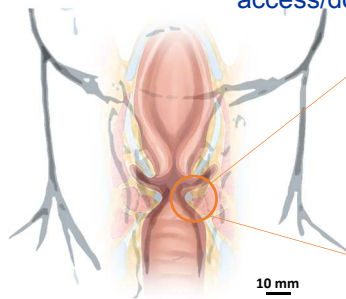
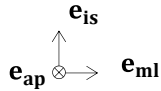
**Figure 9:** Comparison with reference data acquired on human vocal folds. **(A)** Stress-strain responses of tailored FHC composite candidates and upper layers samples (*lamina propria + epithelium*) subjected to cyclic and finite-strains in longitudinal tension [5]. **(B)** Typical stress-strain response of

the composite FHC(5%, 2k, 1/1, Aligned) under transverse compression, as previously selected from the tension curves in **(A)** (curve in green). Comparison with the corresponding homogenous hydrogel without fibers, and reference data on vocal-fold upper layers (*lamina propria + epithelium*) [5].

**Table 1.** Average mesh size ( $\zeta$ ) of PEG hydrogels and volume fraction of fibers ( $\varphi_{\text{PCL}}$ ) in the fiber-reinforced hydrogels. One-way Anova with Tukey's multiple comparison test Hyd(x%, 2k, x/x) versus (x%, 10k, x/x): \*\*\*\*:  $p < 0.0001$ ,  $n = 3$  per condition. T-test random versus aligned FHC composites: \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ ,  $n = 4$  per condition.

Figure 1

Click here to  
access/download;Figure(s);figure\_1\_revise



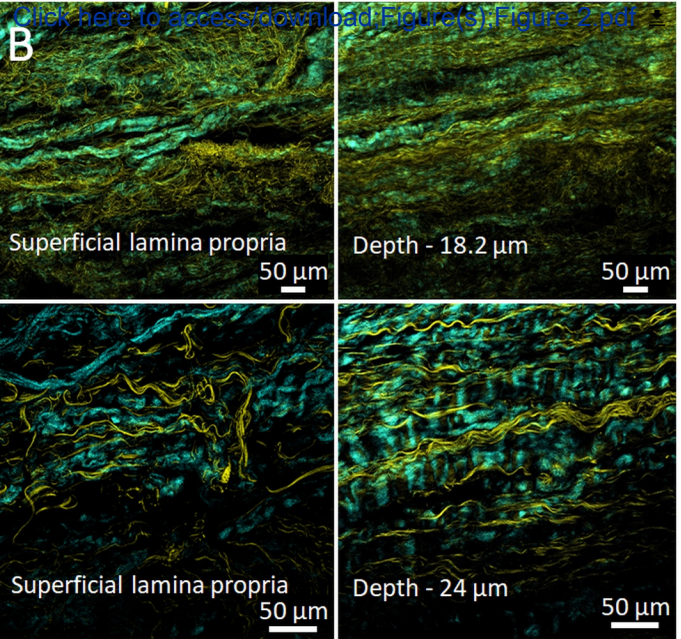
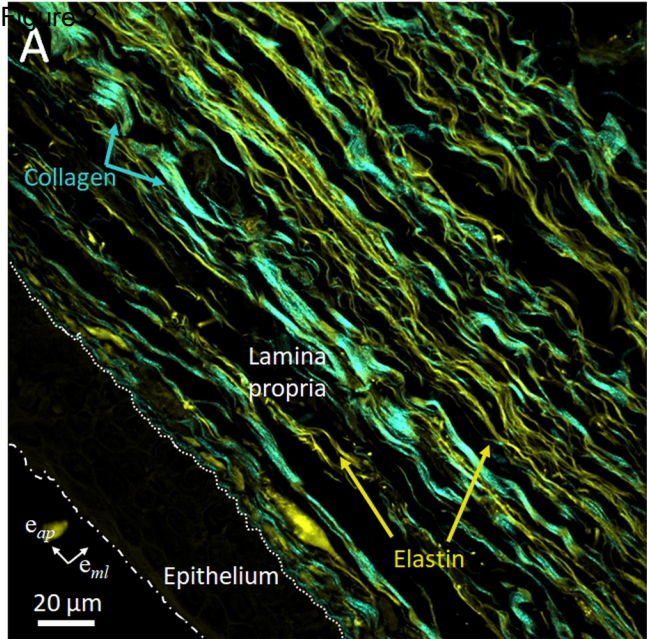
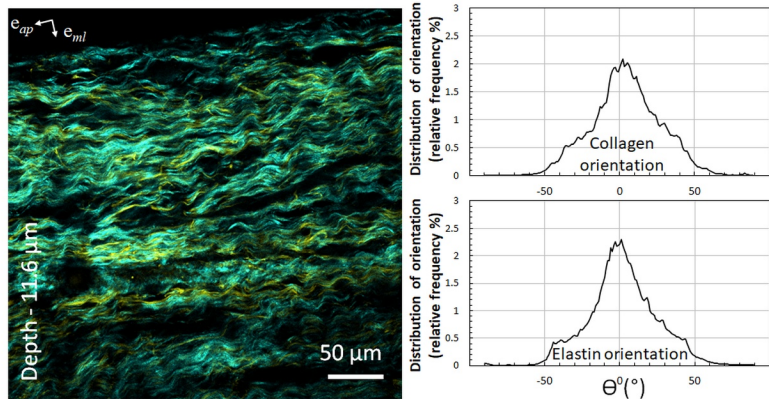
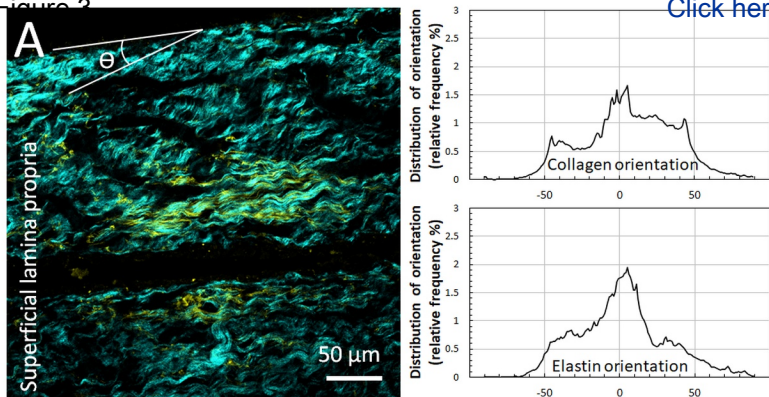


Figure 2



[Click here to access/download;Figure\(s\);Figure 3.pdf](#)

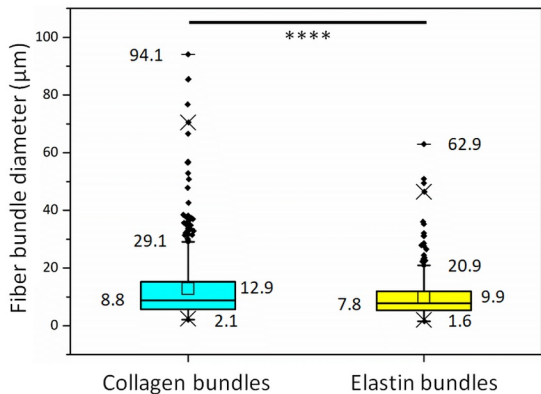
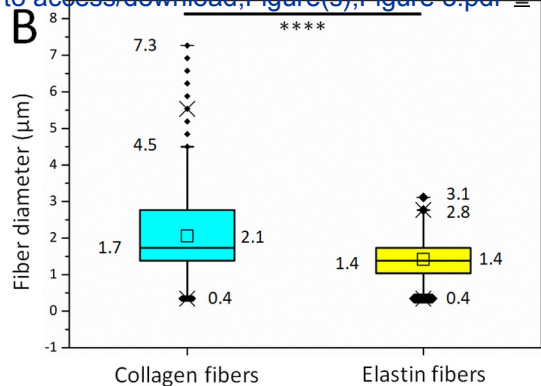
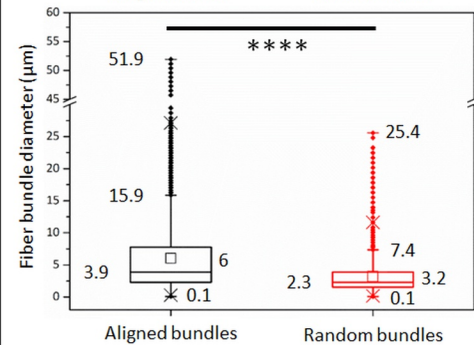
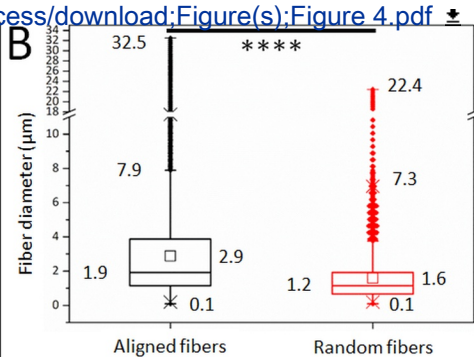
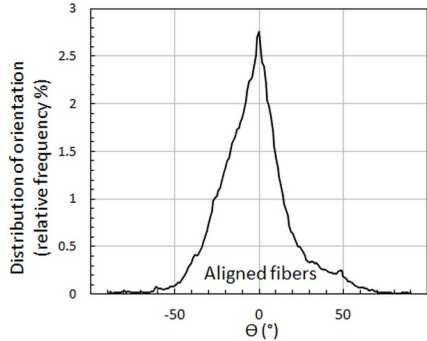
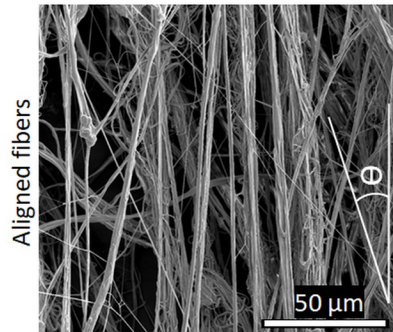
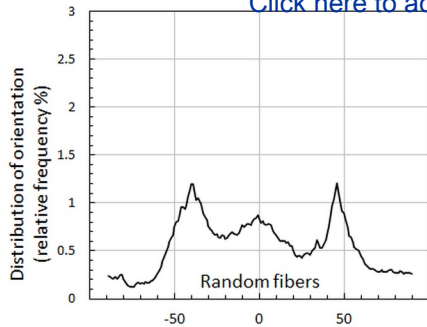
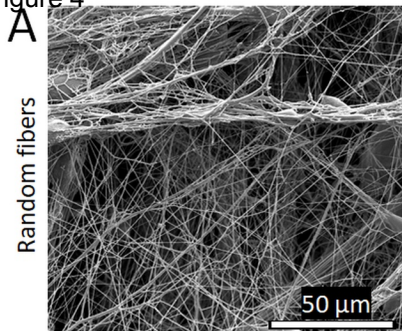




Figure 4





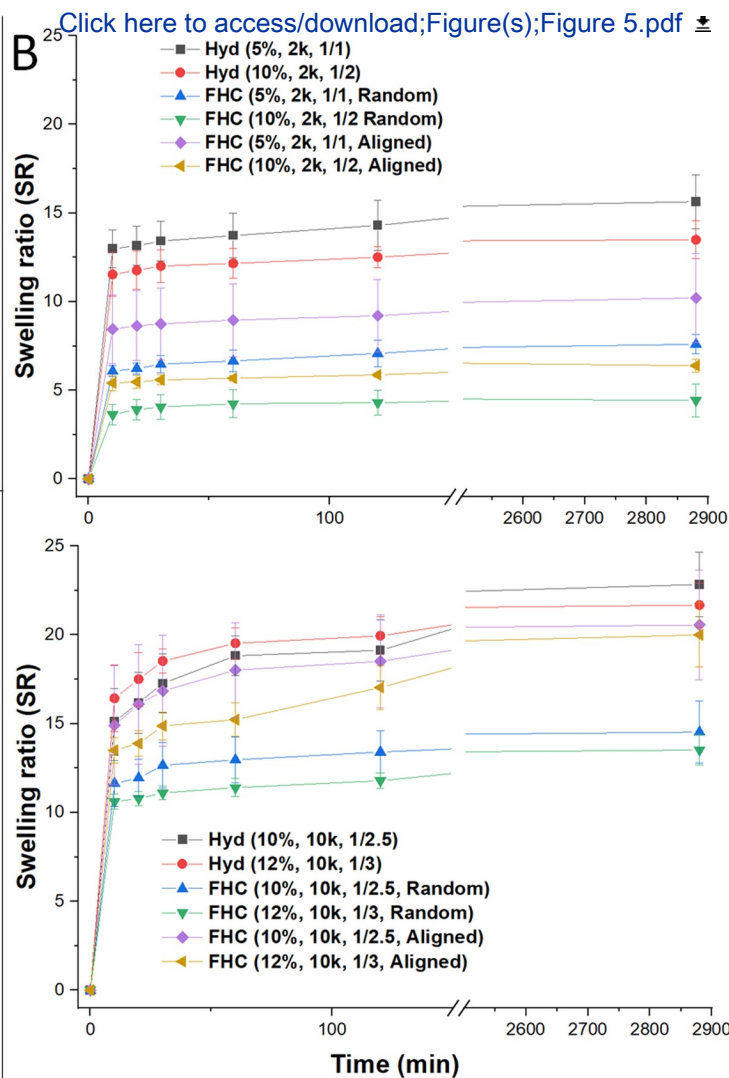
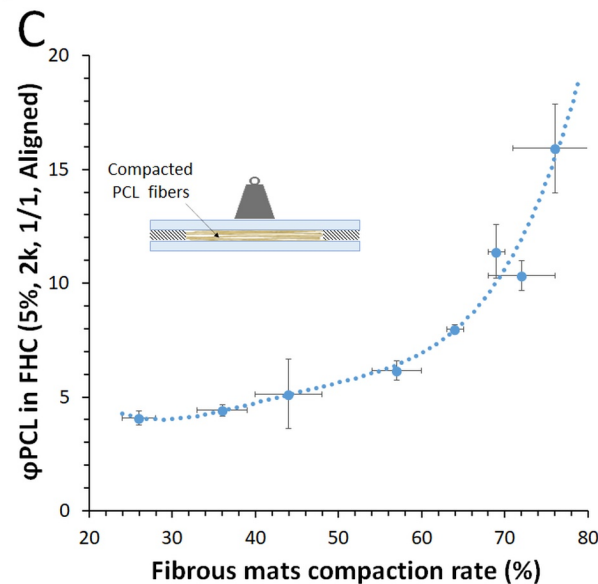
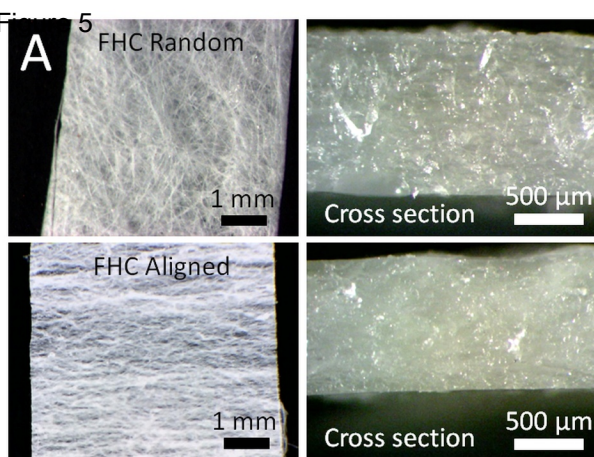
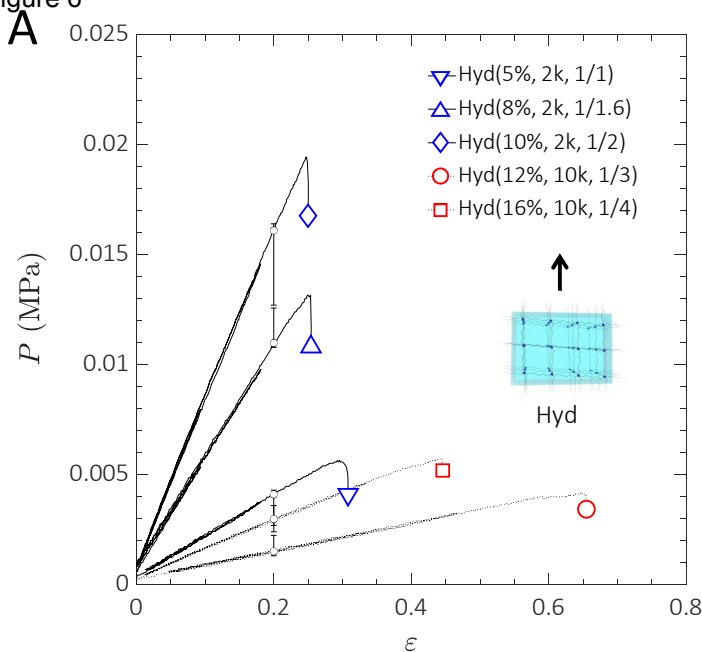


Figure 6



Click here to access/download:Figure(s):figure\_6\_revised\_PDF.pdf

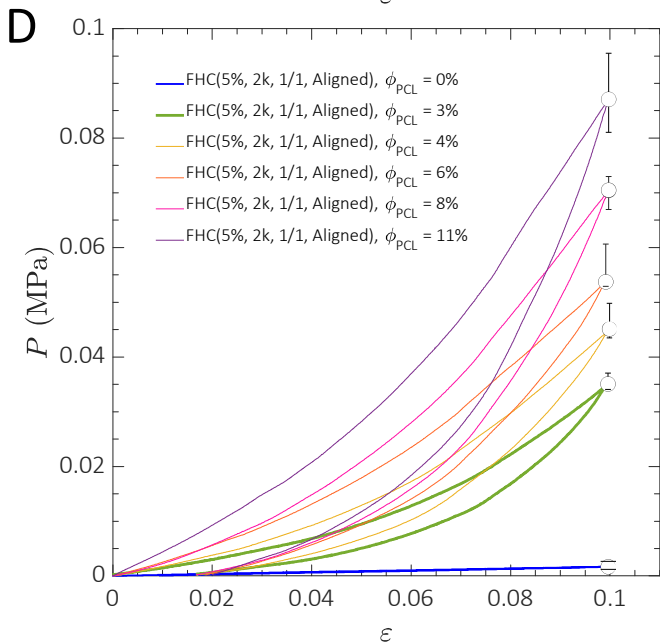
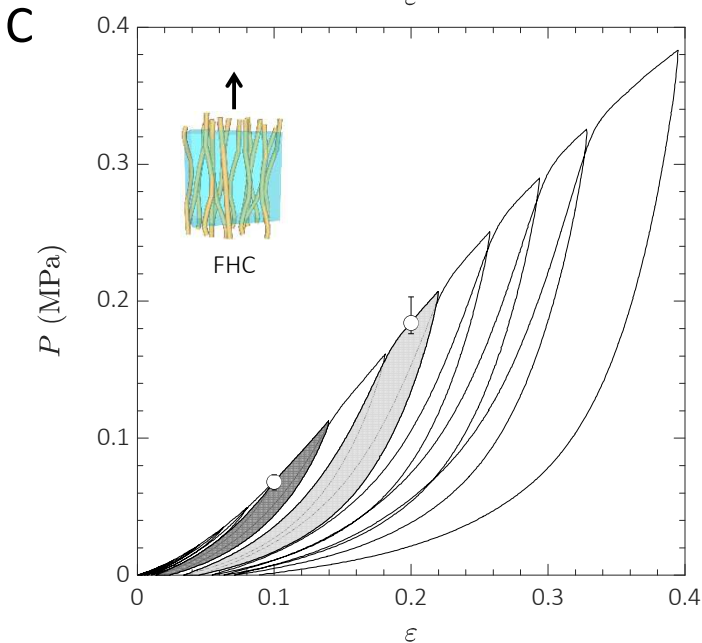
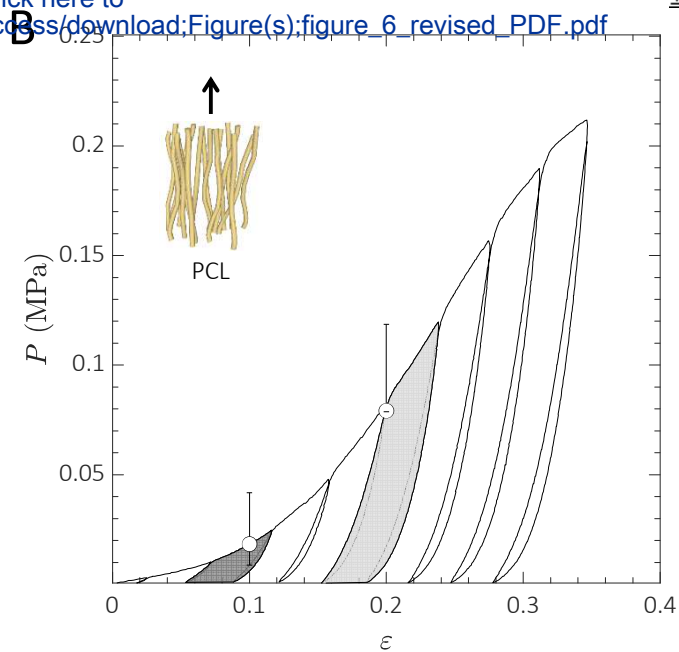
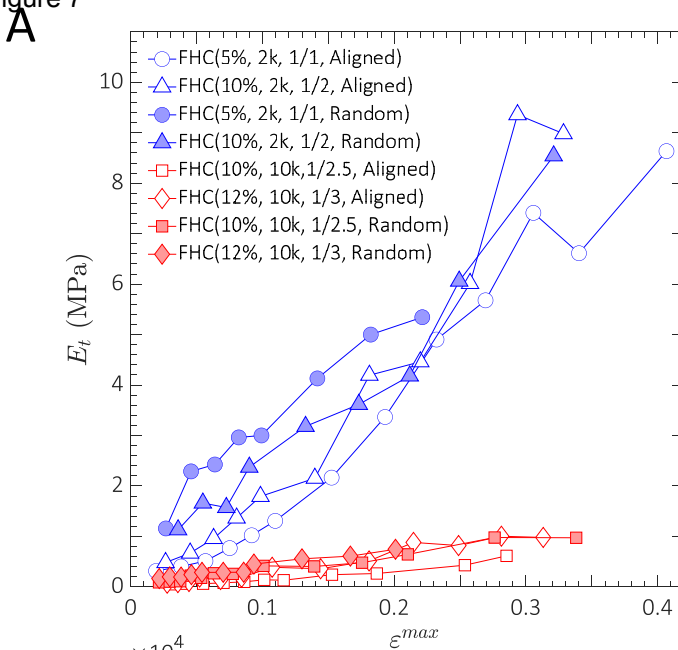
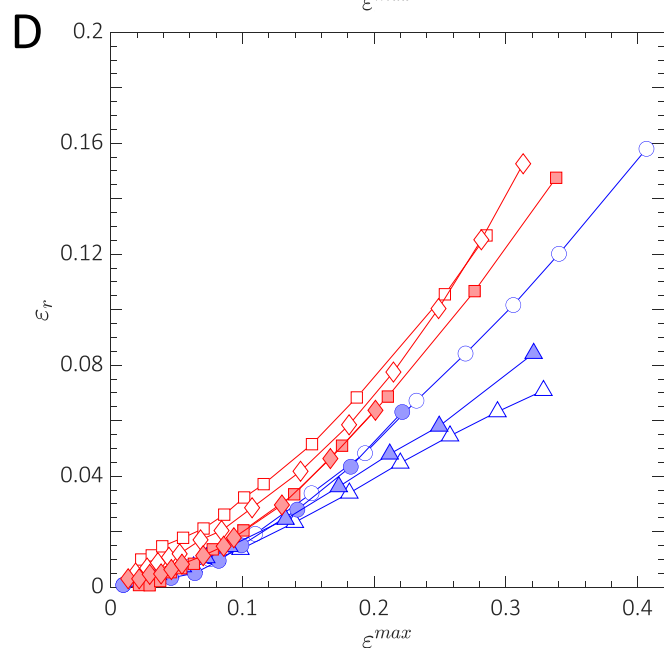
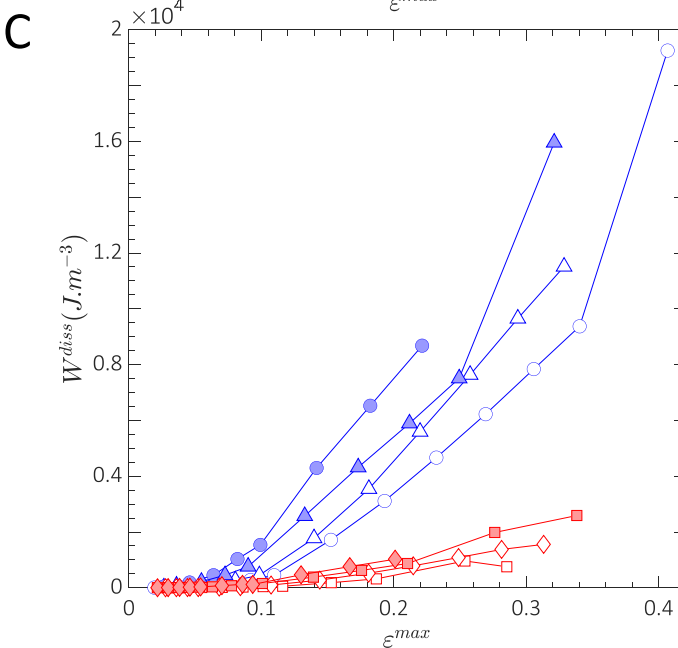
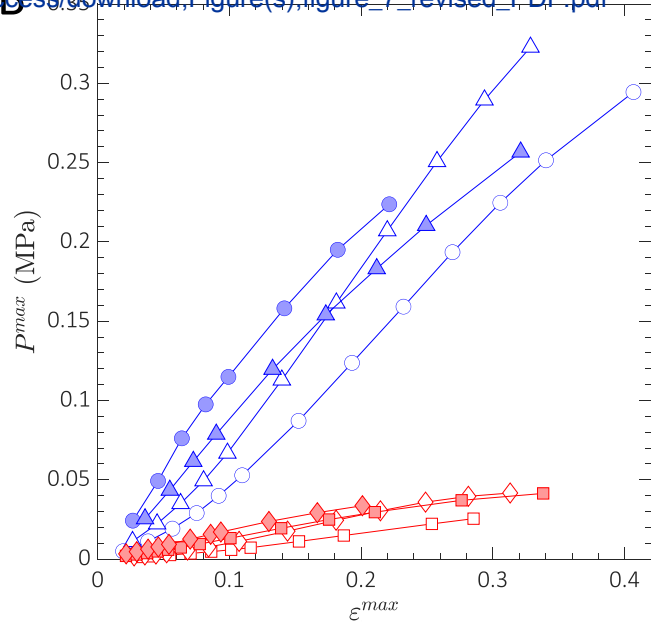


Figure 7



Click here to access/download;Figure(s);figure\_7\_revised\_PDF.pdf



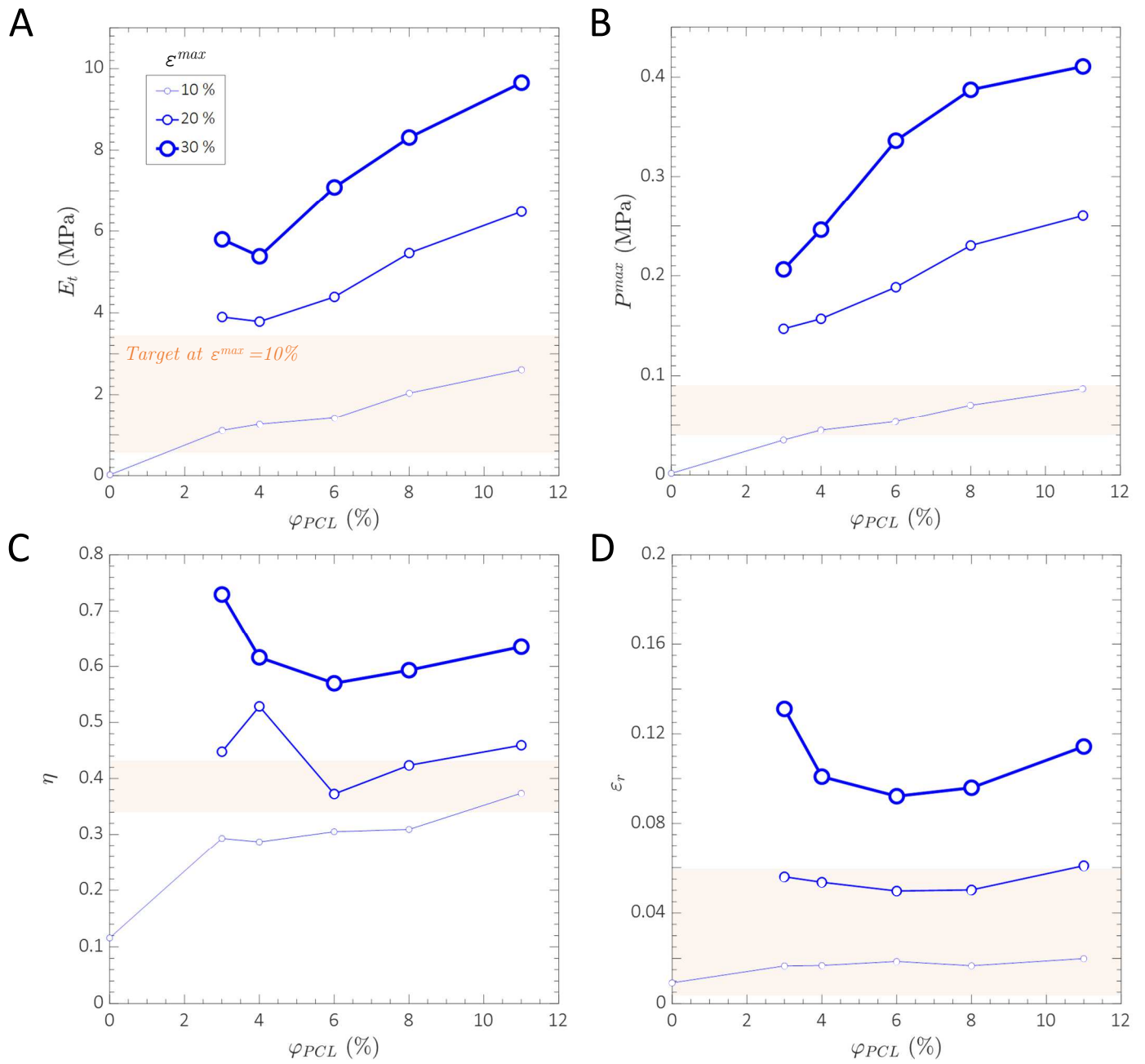
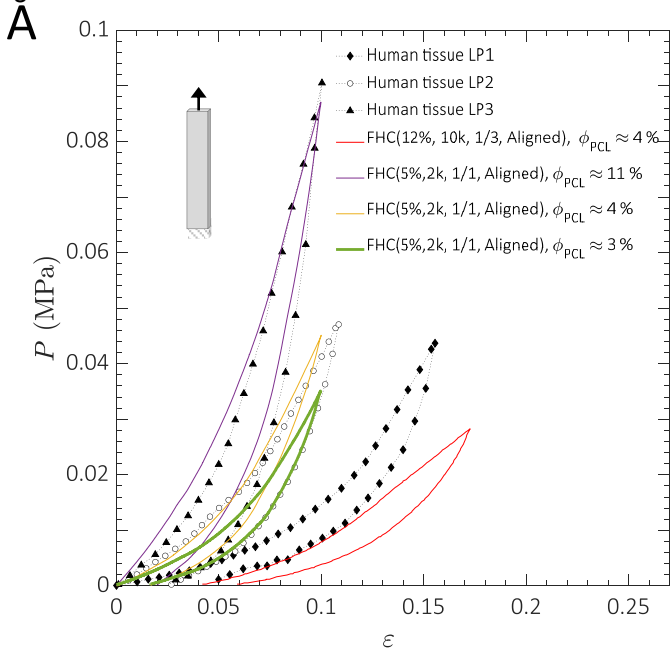
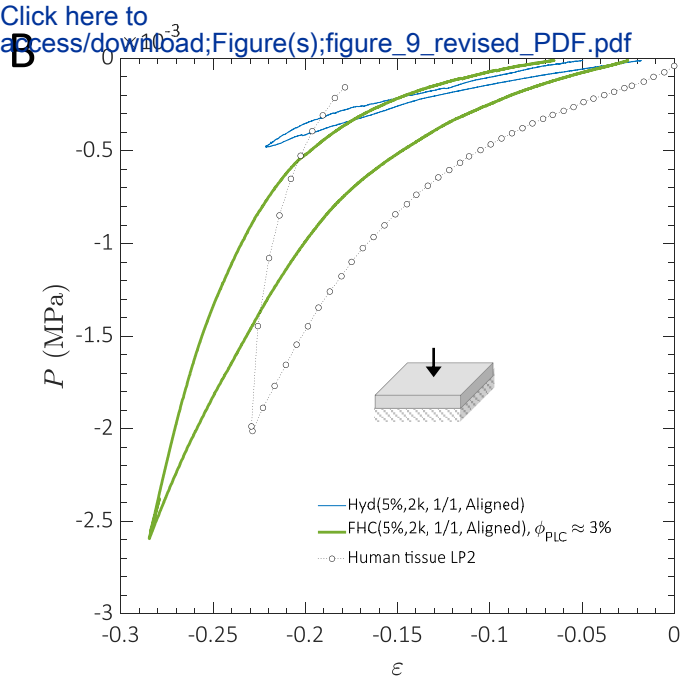


Figure 9



[Click here to access/download;Figure\(s\);figure\\_9\\_revised\\_PDF.pdf](#)



**Table 1**

DGL/PEG hydrogels	$\zeta \pm SD$ (nm)	FHC composites	$\varphi^{PCL} \pm SD$ (%)
Hyd(5%, 2k, 1/1)	6.1 $\pm$ 0.2 ****	FHC(5%, 2k, 1/1, Random)	10.3 $\pm$ 0.7
		FHC(5%, 2k, 1/1, Aligned)	4.6 $\pm$ 0.1 ***
Hyd(10%, 2k, 1/2)	5.6 $\pm$ 0.3 ****	FHC(10%, 2k, 1/2, Random)	11.3 $\pm$ 0.5
		FHC(10%, 2k, 1/2, Aligned)	5.2 $\pm$ 0.5 ***
Hyd(10%, 10k, 1/2.5)	10.1 $\pm$ 0.4	FHC(10%, 10k, 1/2.5, Random)	7.4 $\pm$ 0.9
		FHC(10%, 10k, 1/2.5, Aligned)	3.4 $\pm$ 0.2 **
Hyd(12%, 10k, 1/3)	9.5 $\pm$ 0.6	FHC(12%, 10k, 1/3, Random)	8.1 $\pm$ 1
		FHC(12%, 10k, 1/3, Aligned)	3.7 $\pm$ 0.6 ***



Click here to access/download  
**Supplementary Material**  
supplementary data.pdf



