Supplementary Information (SI) for Biomaterials Science. This journal is © The Royal Society of Chemistry 2024

1 Supporting Information

2 Microstructured silk-fiber scaffolds with enhanced stretchability

- 3 Martina Viola^{1, 2}, Gerardo Cedillo-Servin^{1,3}, Anne Metje van Genderen⁴, Isabelle Imhof¹, Paula
- 4 Vena^{1,3,5}, Marko Mihajlovic², Susanna Piluso⁷, Jos Malda^{1,6}, Tina Vermonden² and Miguel
- 5 Castilho^{1,3,5*}
- 6¹ Department of Orthopaedics, University Medical Centre Utrecht, Utrecht, the Netherlands.
- 7² Division of Pharmaceutics, Department of Pharmaceutical Sciences (UIPS), Faculty of
- 8 Science, Utrecht University, Utrecht, the Netherlands.
- 9³ Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, the
- 10 Netherlands
- 11⁴ Division of Pharmacology, Department of Pharmaceutical Sciences (UIPS), Faculty of
- 12 Science, Utrecht University, Utrecht, the Netherlands.
- 13 ⁵ Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven,
- 14 the Netherlands
- 15 ⁶ Department of Clinical Sciences, Faculty of Veterinary Sciences, Utrecht University, Utrecht,
- 16 the Netherlands
- 17 ⁷ SentryX B.V., Utrecht, the Netherlands.
- 18 *E-mail: m.dias.castilho@tue.nl
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SF Concentration (%w/v)
 Figure S1. Electrical conductivity of SF5DT aqueous solutions at different concentrations.





24 Figure S2. Stability of flat scaffolds at 25°C and 37°C in PBS over 28 days with and without

25 NaH₂PO₄ treatment; scale bars: 1 cm.



Crosslinked with NaH₂PO₄ Crosslinked with Methanol



28 Figure S3. Rheological characterization of SF (at 20% w/v) with different degumming times,

- 29 crosslinked with sodium dihydrogen phosphate (NaH₂PO₄; left column) and methanol (MeOH; 30 right column).
- 31



- Figure S4. Stability of bulk SF gels treated with NaH₂PO₄ or methanol over 28 days at 25°C
 and 37 °C.
- 36



- 38 Figure S5. AFM 3D visualization of topography of single SF fibers before crosslinking (SF
- 39 only) and after crosslinking (SF MeOH and SF NaH₂PO₄); scale bars: 200 nm.
- 40
- 41





43 Figure S6. μ CT scan reconstruction of a SF tubular scaffold with NaH₂PO₄ post-processing

44 treatment.



46 **Figure S7.** A) Cyclic tensile testing of flat scaffolds with inter-fiber spacing of 1000 μ m: i) 47 Representative curves, and ii) decrease in maximum tensile force of scaffolds after 10 cycles 48 with 5% maximum strain (cycles 1–10), followed by 10 cycles with 10% maximum strain 49 (cycles 11–20), and 10 cycles with 20% maximum strain (cycles 21–30) (n = 3–5; two-way 50 ANOVA with Tukey's multiple comparisons test). B) Variation of tubular scaffold diameter 51 with the swelling and deswelling of a balloon catheter.

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55 Figure S8. Comparison of SF elastic modulus, and fiber diameter, and patternability obtained

56 through the methods listed in Table S1, with respect to the dimensions of cells or native features 57 relevant in vascular and kidney tissue engineering. Electrowriting followed NaH_2PO_4 post-

58 processing provides elastic modulus values in the range of native vascular and kidney tissues,

59 while coming closest to the native tissue scales. *Sourced from van Genderen *et al.*¹ #Sourced 60 from Camasão *et al.*² PT: kidney proximal tubule; BM: basement membrane; MeOH: methanol;

61 NaAc: sodium acetate.

i. ciPTEC



ii. ciGEnC



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- 63 Figure S9. Human conditionally immortalized proximal tubular epithelial cells (ciPTEC) and
- 64 glomerular endothelial cells (ciGEnC) cultured on non-coated flat scaffolds and stained for live
- 65~ (green) and dead (red) cells; scale bars: $100~\mu m.$



- 68 Figure S10. Tissue-specific staining of cell-seeded SF tubular scaffolds (60°, 100 layers). A)
- 69 Human conditionally immortalized proximal tubular epithelial cells (ciPTEC) with expression
- 70 of α -tubulin (green), marker for primary cilia; nuclei in blue (DAPI): i) staining and ii)
- 71 orthogonal section. White arrows indicate localization of α -tubulin. iii) Graphical overview of
- 72 polarized monolayer of epithelial cells within the pore of a tubular scaffold. B) Human
- 73 conditionally immortalized glomerular endothelial cells (ciGEnC) with expression of
- 74 CD31/PECAM1 (green); nuclei in blue (DAPI). Scale bars: 100 μ m.
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77 Table S1. Notable examples of SF spinning and post-processing methods and their obtained

78 patterning and mechanical properties.

Spinning solution	Spinning method	Post- processing method	Control over patterning	Mechanical properties after post-processing	Ref.
SF in HFIP	Wet-spinning	Coagulation in MeOH	No patterning. Fibers only (20–100 μm)	Tensile strength = 50– 200 MPa	3
SF in HFA- hydrate	Wet-spinning	Coagulation in MeOH, steam annealing	No patterning. Fiber only	Young's modulus = 54 cN/dtex (compared to 61 cN/dtex of native silk fiber)	4
SF in formic acid	Casting	MeOH treatment	No patterning.	NS	5
SF in water or HFIP	Casting	NaCl addition; MeOH treatment	No patterning. Tunable pore size. Porosity = 85– 97%.	Compressive modulus = 70–3330 kPa. Compressive stress = 11–320 kPa.	6
Aqueous SF	Casting	Treatment with Mg ²⁺ , Cu ²⁺ , Zn ²⁺ , K ⁺ , Na ⁺ , or Ca ²⁺	No patterning.	NS	7
SF in formic acid	Electro- spinning	MeOH or EtOH treatment	No patterning. Nanofibers (diameter = 80–210 nm) with random alignment	Elastic modulus = 140– 610 MPa. Breaking strain = 8–19%.	8
Raw non- degummed silk yarn	Yarn spinning	PEG coating, annealing	No patterning. Yarn fibers (100 μm diameter).	Tensile strength = 250– 540 MPa. Young's modulus = 5–13 GPa.	9
Aqueous SF	Casting with dityrosine photo- crosslinking	MeOH treatment	No patterning.	Compressive modulus = 1.5 MPa (at 20% strain) and 11 MPa (at 40% strain)	10
Aqueous SF/PVA	Casting	Horseradish peroxidase crosslinking	No patterning. Tunable pore size (= 32–68%) and porosity (77–90%).	Compressive modulus = 0.6–3 kPa.	11
Aqueous SF	Extrusion 3D printing	Treatment in aqueous NaCl and K ₂ HPO ₄	High patternability. Medium porosity. Printed filaments (diameter = 100 μm).	Young's modulus = 25 MPa. Ultimate tensile toughness = 40 MJ ³ /m ³ .	12
Aqueous SF/ tyramine- modified hyaluronan	Casting	Horseradish peroxidase crosslinking, sonication, long-time incubation	No patterning. Tunable porosity.	Highly time dependent properties. Compressive modulus = $300-700$ kPa (day 0) and $10-1000$ kPa (day 5). Stress at fracture = $100-200$ kPa (day 0) and $150-1000$ kPa (day 5).	13
Aqueous SF	Wet-spinning	Coagulation in aqueous NaAc/NaCl	No patterning. Microfiber (diameter 10– 20 μm)	Young's modulus = 1.5-4 GPa. Toughness = 2-40 MJ ³ /m ³ .	14
Methacryla ted SF	Digital light processing with UV crosslinking	None	High patternability.	Bending properties higher than native cartilage. No modulus specified. Stability over 14 days in medium.	15
Pre-	Microfluidic	None	No patterning.	Elastic modulus* =	16

assembled SF nanofibers in formic	spinning		Microfibers (diameter = 100 μm) with hierarchical surface nanotopography.	350–1200 MPa. Tensile strength = 12–46 MPa.	
Aqueous SF	Electrowriting	None	High patternability. High porosity. Printed microfibers (diameter = 7–13 µm)	NS. No stability reported.	17
Aqueous SF	Electrowriting	Treatment in aqueous NaH ₂ PO ₄	High patternability. High porosity (>95%). Printed microfibers (diameter = 5–20 μm). Directional striations resembling self-assembled silk fibrils.	Elastic modulus = 0.7 – 12 MPa. High stability in PBS over 28 days.	This work

80 SF: silk fibroin, NS: not specified, PBS: phosphate buffered saline solution, PEG: polyethylene

81 glycol, HFIP: hexafluoroisopropanol, HF: hexafluoroacetone, MeOH: methanol, EtOH:

82 ethanol, PVA: polyvinyl alcohol, UV: ultraviolet light, NaAc: sodium acetate. *Estimated from

- 83 stress-strain curves.
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86 **Table S2.** List of antibodies and fluorophores used for immunocytochemistry.

87

Primary antibody or fluorophore	Secondary antibody	
Live/Dead TM viability/cytotoxicity kit	NA	
(L3224, Invitrogen)		
AlexaFluor 488 phalloidin (A22283,	NA	
ThermoFisher Scientific) 1:1000 dilution		
Rabbit polyclonal anti-collagen I (Ab34710,	AlexaFluor 488 donkey anti-rabbit	
Abcam) 1:100 dilution	(Invitrogen) 1:200 dilution	
Goat monoclonal anti-collagen IV (1340-01,	AlexaFluor 568 donkey anti-goat or	
Southern Biotech) 1:50 dilution	AlexaFluor 546 donkey anti-goat	
	(Invitrogen) 1:200 dilution	
Mouse monoclonal anti-α-tubulin (T6793,	AlexaFluor 488 donkey anti-mouse	
Sigma Aldrich) 1:200 dilution	(Invitrogen) 1:200 dilution	
Mouse anti-CD31/PECAM1 (BBA7, R&D	AlexaFluor 488 donkey anti-mouse	
Systems) 1:150 dilution	(Invitrogen) 1:200 dilution	
DAPI (D9542, Sigma Aldrich) 1:1000	NA	
dilution		

88 NA: not applicable.

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