Supporting Information

Microstructured silk-fiber scaffolds with enhanced stretchability

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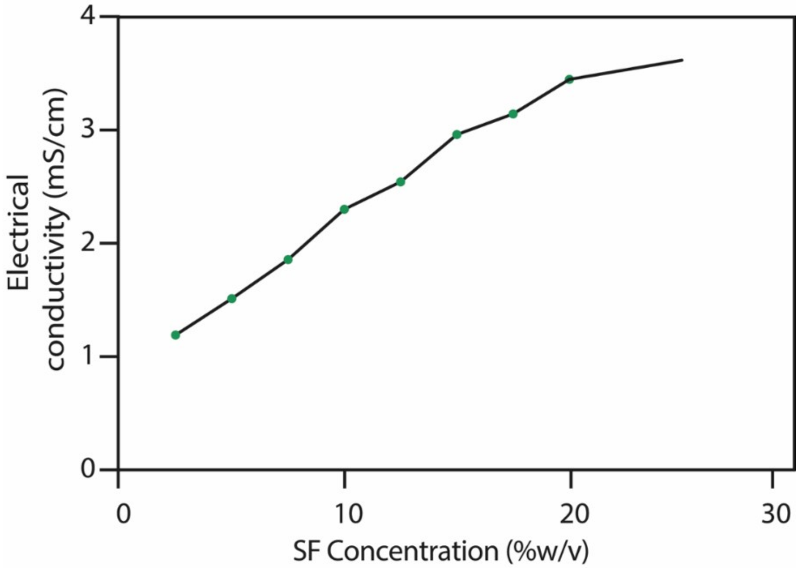

Figure S1. Electrical conductivity of SF5DT aqueous solutions at different concentrations.

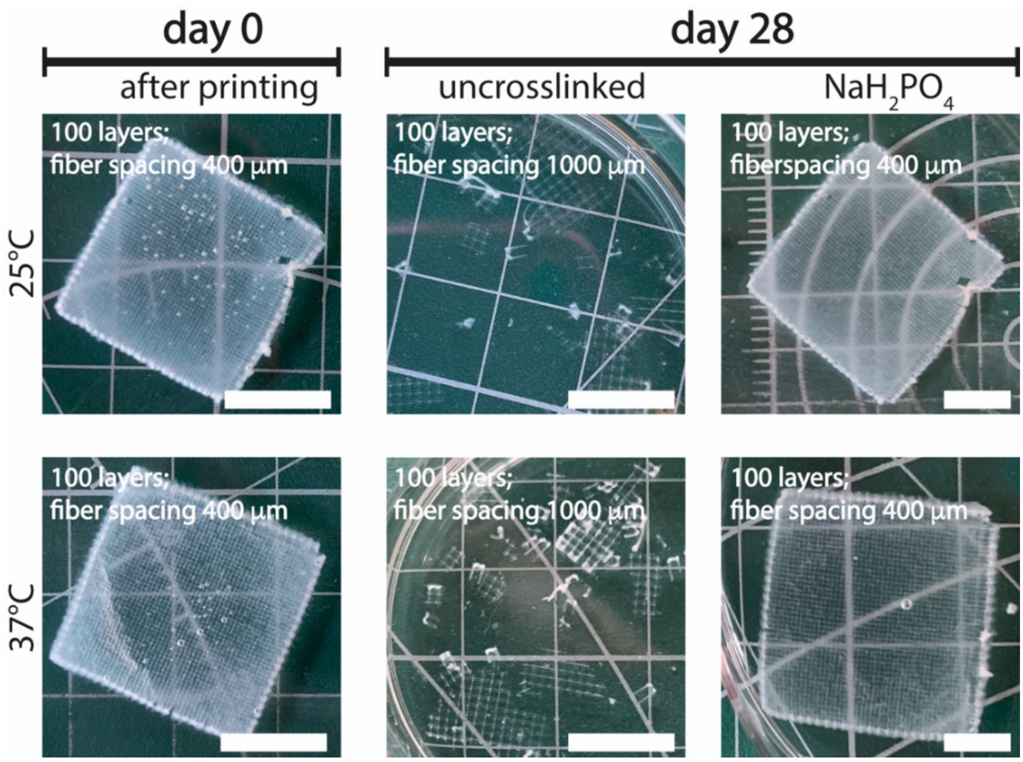

Figure S2. Stability of flat scaffolds at 25°C and 37°C in PBS over 28 days with and without NaH_2PO_4 treatment; scale bars: 1 cm.

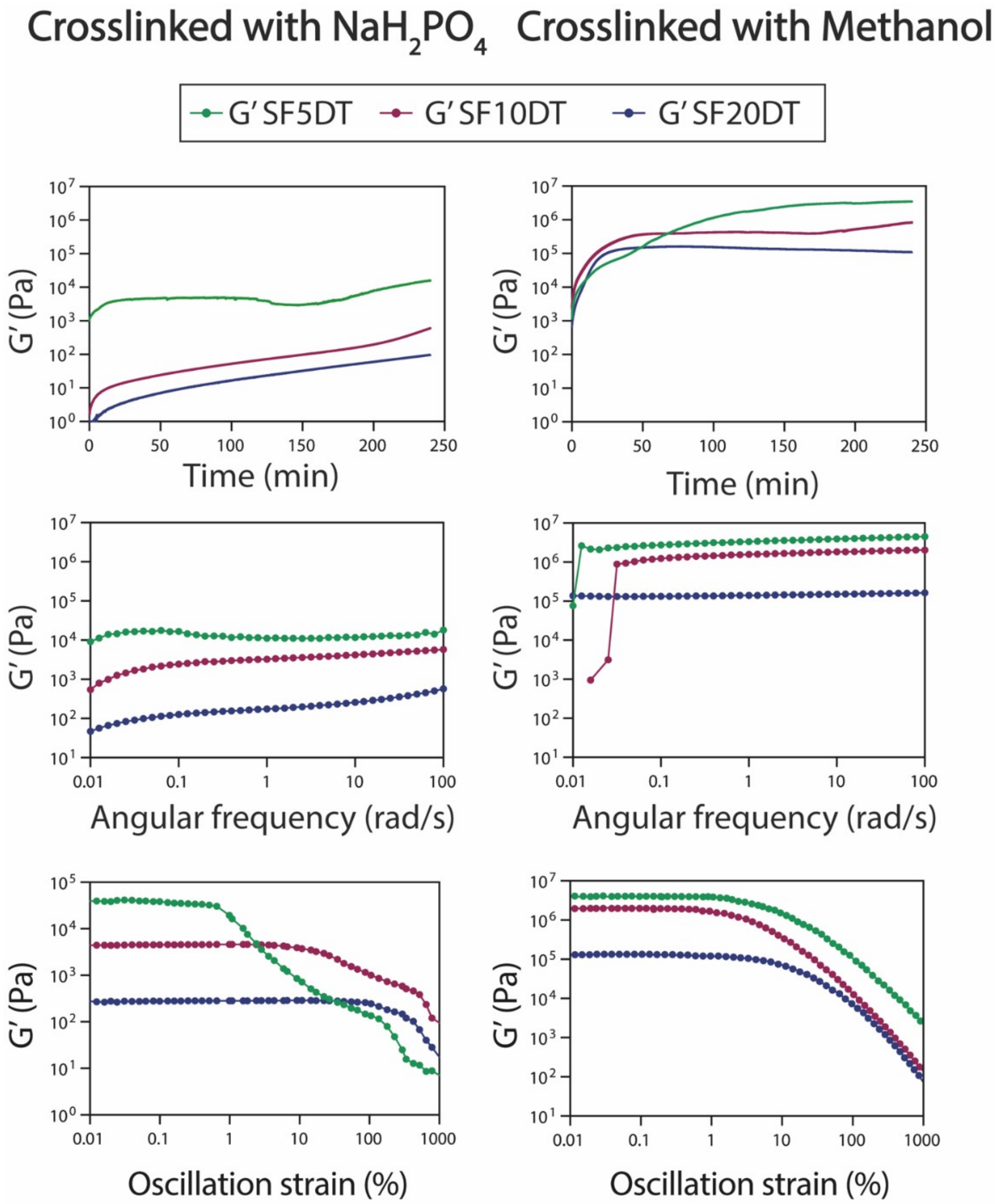

Figure S3. Rheological characterization of SF (at 20% w/v) with different degumming times, crosslinked with sodium dihydrogen phosphate (NaH_2PO_4; left column) and methanol (MeOH; right column).

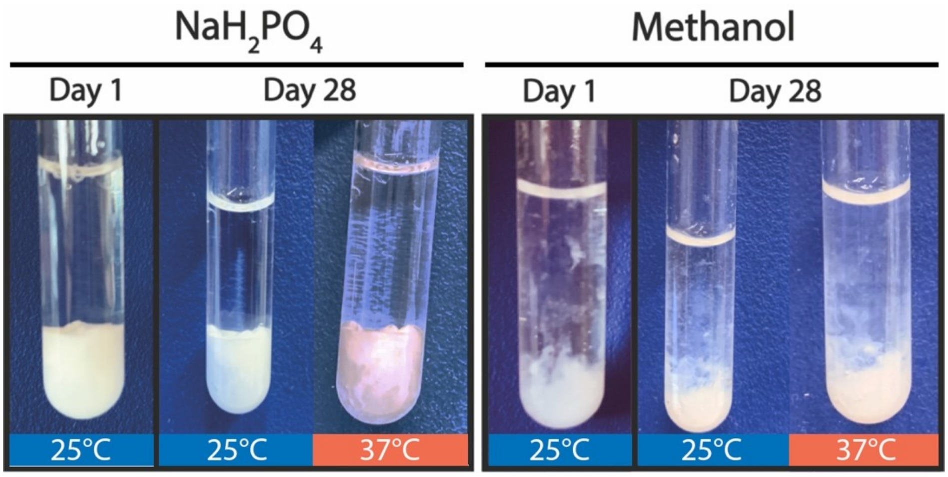

Figure S4. Stability of bulk SF gels treated with NaH_2PO_4 or methanol over 28 days at 25°C and 37 °C.

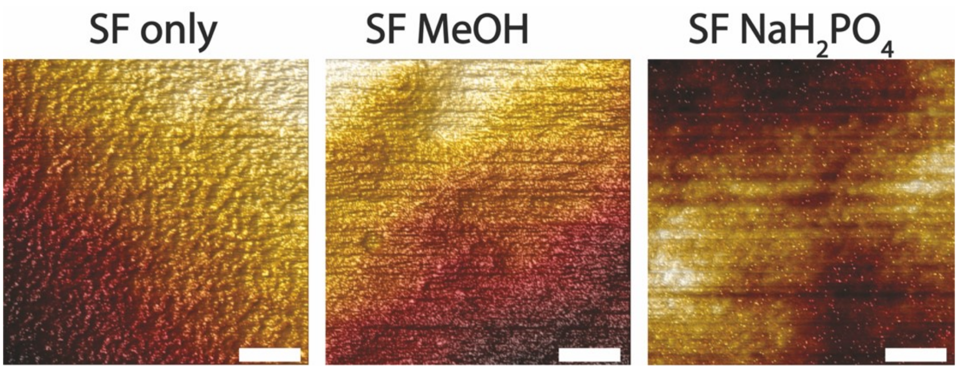

Figure S5. AFM 3D visualization of topography of single SF fibers before crosslinking (SF only) and after crosslinking (SF MeOH and SF NaH_2PO_4); scale bars: 200 nm.

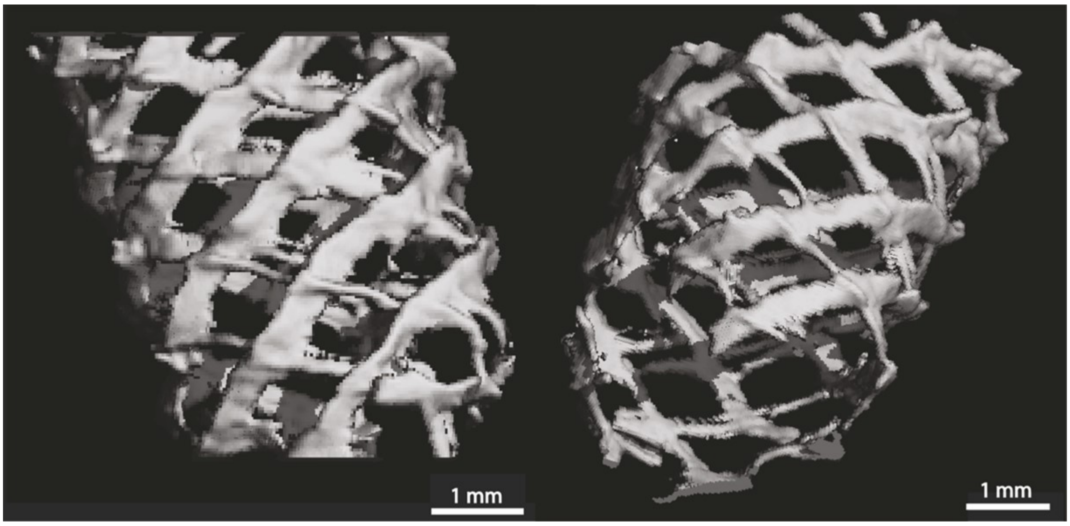

Figure S6. μCT scan reconstruction of a SF tubular scaffold with NaH_2PO_4 post-processing treatment.

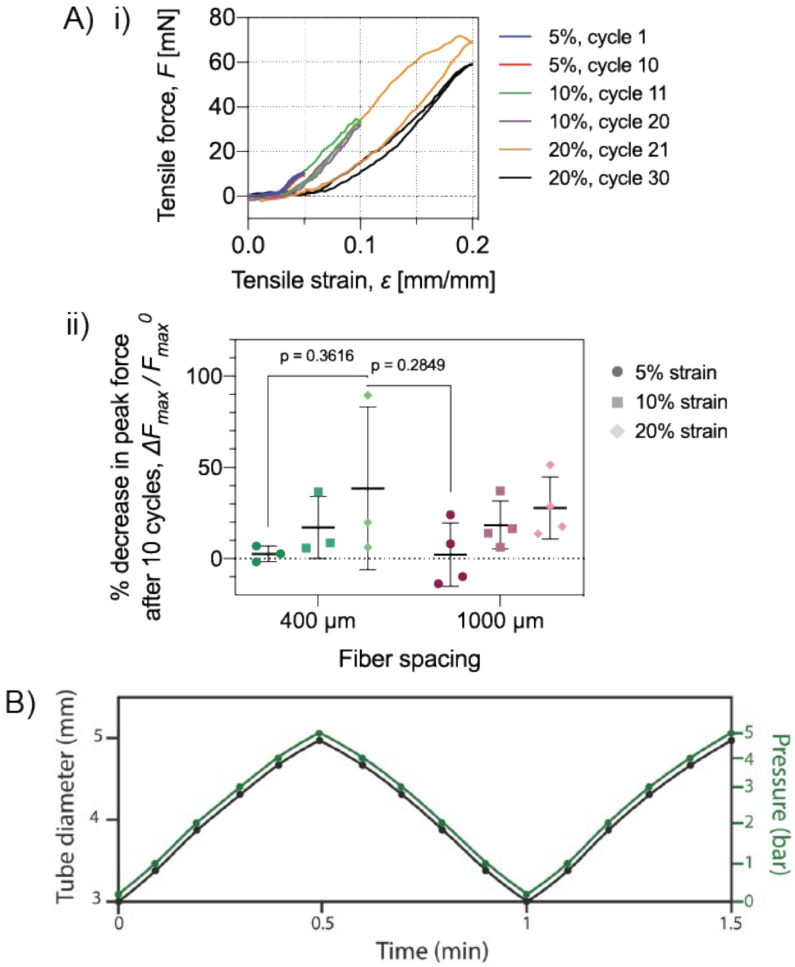

Figure S7. A) Cyclic tensile testing of flat scaffolds with inter-fiber spacing of 1000 µm: i) Representative curves, and ii) decrease in maximum tensile force of scaffolds after 10 cycles with 5% maximum strain (cycles 1–10), followed by 10 cycles with 10% maximum strain (cycles 11–20), and 10 cycles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

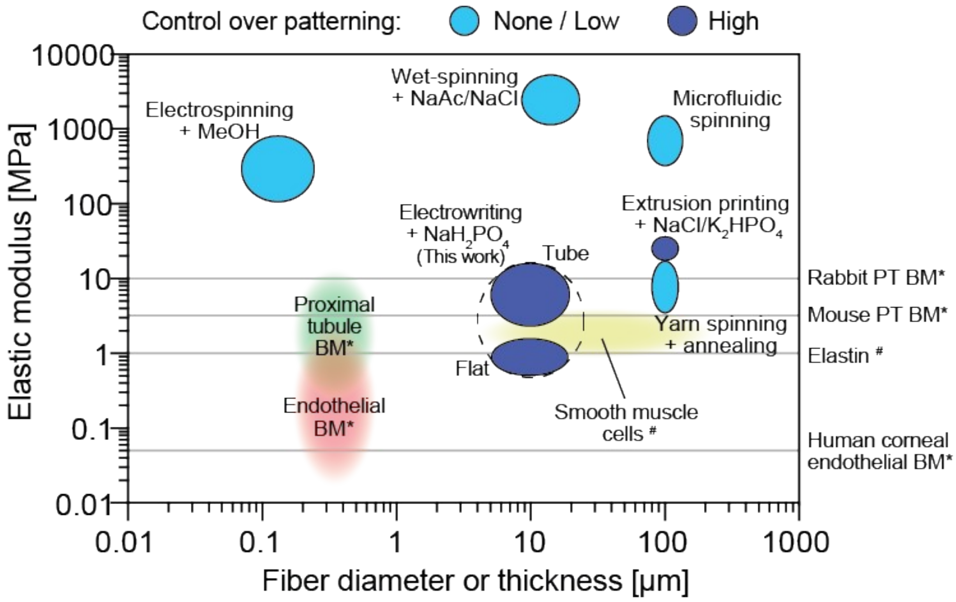

Figure S8. Comparison of SF elastic modulus, and fiber diameter, and patternability obtained through the methods listed in Table S1, with respect to the dimensions of cells or native features relevant in vascular and kidney tissue engineering. Electrowriting followed NaH_2PO_4 post-processing provides elastic modulus values in the range of native vascular and kidney tissues, while coming closest to the native tissue scales. *Sourced from van Genderen *et al.*[43] #Sourced from Camasão *et al.*[55] PT: kidney proximal tubule; BM: basement membrane; MeOH: methanol; NaAc: sodium acetate.

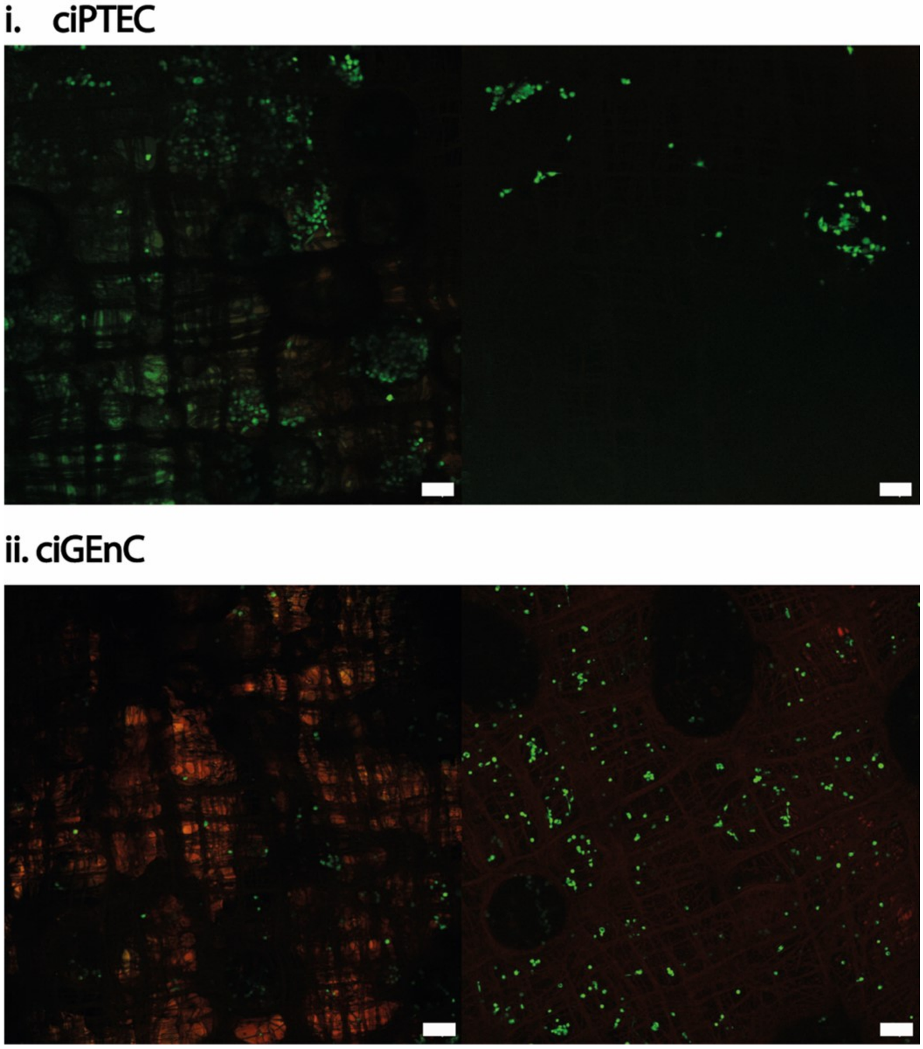

Figure S9. Human conditionally immortalized proximal tubular epithelial cells (ciPTEC) and glomerular endothelial cells (ciGEnC) cultured on non-coated flat scaffolds and stained for live (green) and dead (red) cells; scale bars: 100 µm.

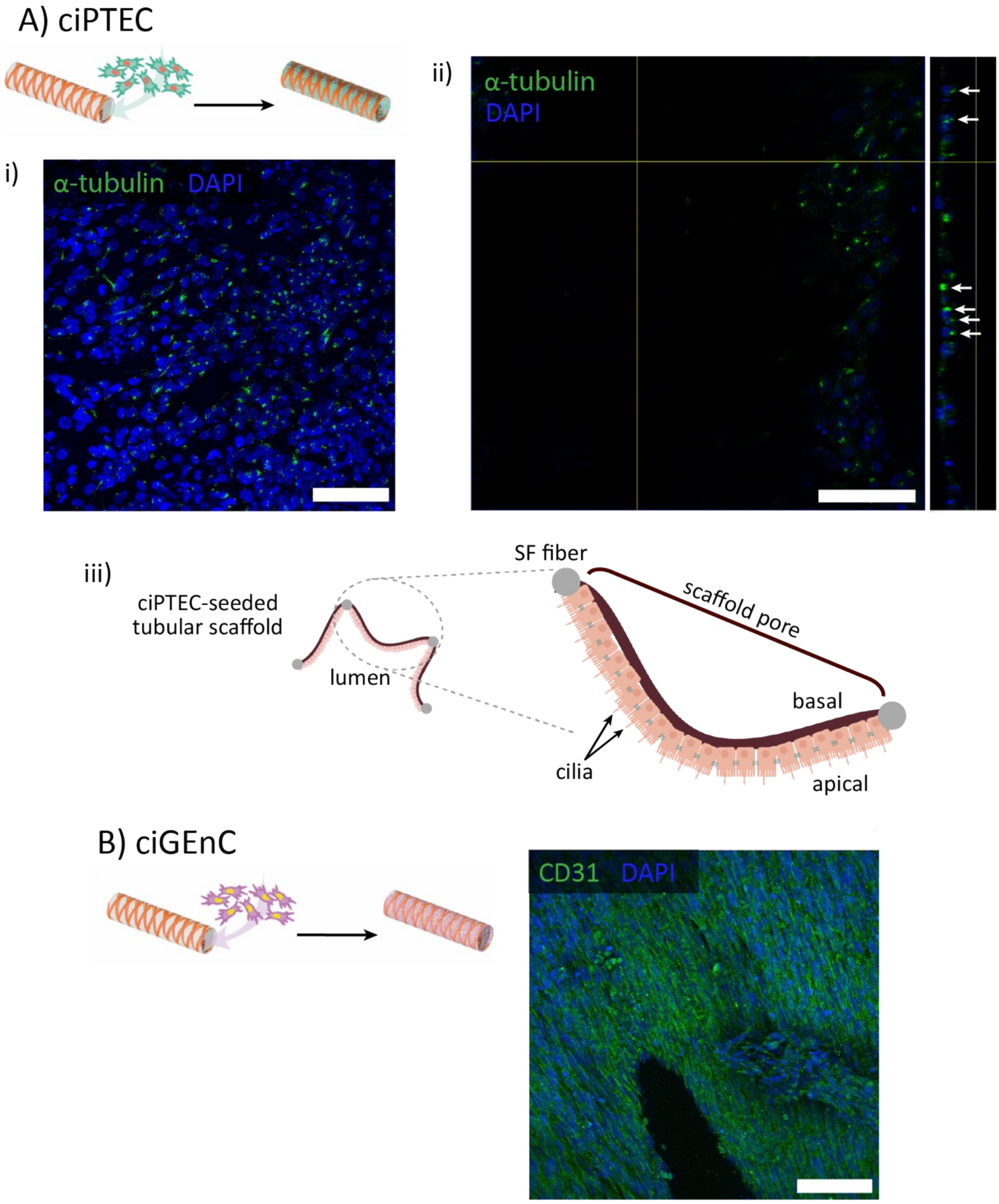

Figure S10. Tissue-specific staining of cell-seeded SF tubular scaffolds (60°, 100 layers). A) Human conditionally immortalized proximal tubular epithelial cells (ciPTEC) with expression of α-tubulin (green), marker for primary cilia; nuclei in blue (DAPI): i) staining and ii) orthogonal section. White arrows indicate localization of α-tubulin. iii) Graphical overview of polarized monolayer of epithelial cells within the pore of a tubular scaffold. B) Human conditionally immortalized glomerular endothelial cells (ciGEnC) with expression of CD31/PECAM1 (green); nuclei in blue (DAPI). Scale bars: 100 µm.

Table S1. Notable examples of SF spinning and post-processing methods and their obtained 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	15
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)	16
SF in formic acid	Casting	MeOH treatment	No patterning.	NS	17
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.	19
Aqueous SF	Casting	Treatment with Mg^{2+}, Cu^{2+}, Zn^{2+}, K^{+}, Na^{+}, or Ca^{2+}	No patterning.	NS	20
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%.	12
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.	56
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)	57
Aqueous SF/PVA	Casting	Horseradish peroxidase crosslinking	No patterning. Tunable pore size (= 32–68%) and porosity (77–90%).	Compressive modulus = 0.6–3 kPa.	25
Aqueous SF	Extrusion 3D printing	Treatment in aqueous NaCl and K_2HPO_4	High patternability. Medium porosity. Printed filaments (diameter = 100 µm).	Young's modulus = 25 MPa. Ultimate tensile toughness = 40 MJ^3/m^3.	39
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).	24
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.	58
Methacrylated 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.	59
Pre-	Microfluidic	None	No patterning.	Elastic modulus* =	60

assembled SF nanofibers in formic acid	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.	21
Aqueous SF	Electrowriting	Treatment in aqueous NaH_2PO_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

SF: silk fibroin, NS: not specified, PBS: phosphate buffered saline solution, PEG: polyethylene glycol, HFIP: hexafluoroisopropanol, HF: hexafluoroacetone, MeOH: methanol, EtOH: ethanol, PVA: polyvinyl alcohol, UV: ultraviolet light, NaAc: sodium acetate. *Estimated from stress-strain curves.

Table S2. List of antibodies used for immunocytochemistry.

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

NA: not applicable.