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Supporting Information

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Microfiber-Templated Porogel Bioinks Enable Tubular Interfaces and Microvascularization Down to the Building Blocks for 3D Bioprinting

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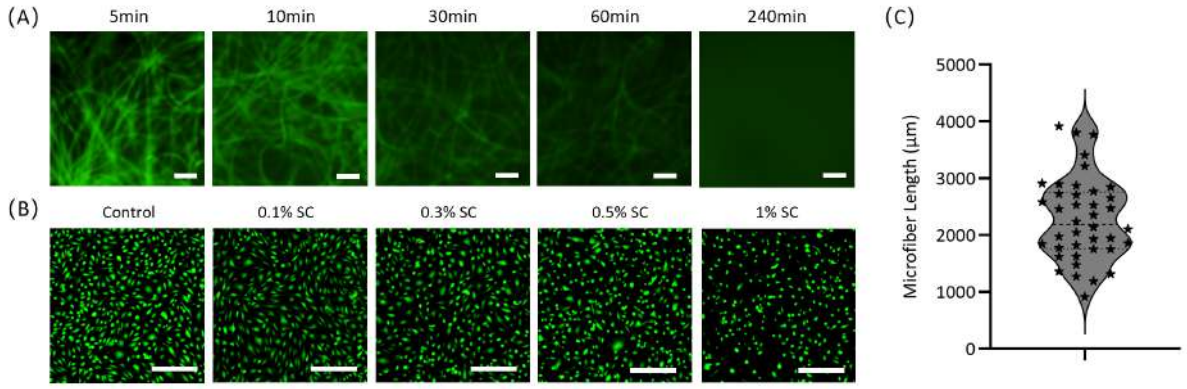

Figure S1. (A) Representative fluorescence images of alginate fibers treated with 0.3% sodium citrate, showing the microfiber's dissolution process. (B) Representative fluorescence images of live/dead stained HUVECs after treatment with sodium citrate at different concentrations for 12 hours. (C) Quantification of cut microfiber lengths. Scale bar: 500μm (A and B).

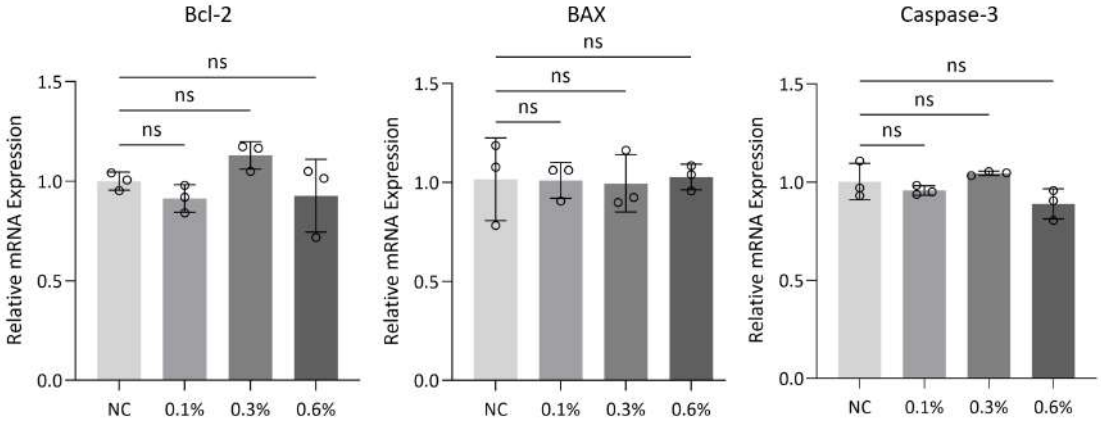

Figure S2. Apoptosis-related genes (Bcl-2, BAX, and Caspase-3) expression of HUVEC cells after being treated with different concentrations of sodium citrate solution. One-way ANOVA with a Tukey's multiple comparisons test, *P < 0.05; ns, not significant. The number of independent cell sample replicates N=3.

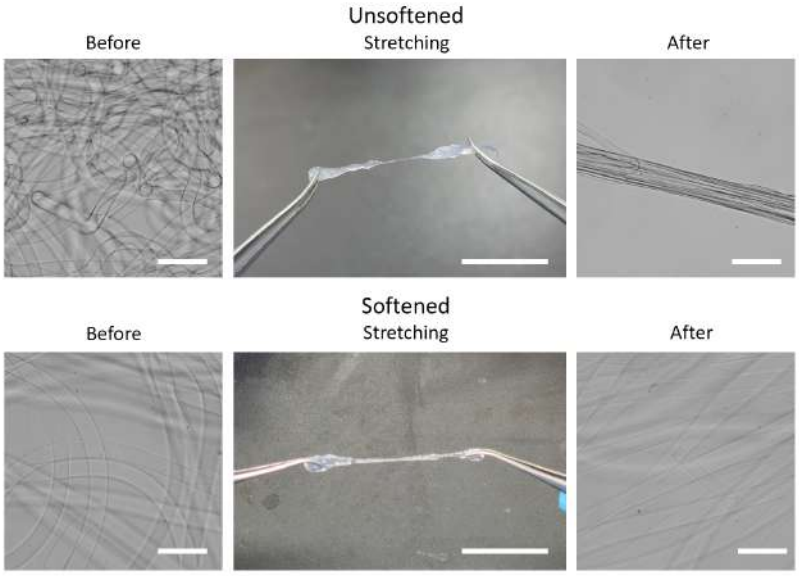

Figure S3. Representative microscopic images of unsoftened and softened alginate microfibers before and after stretching. Scale bars: 200 μm (before and after) and 10 mm (stretching).

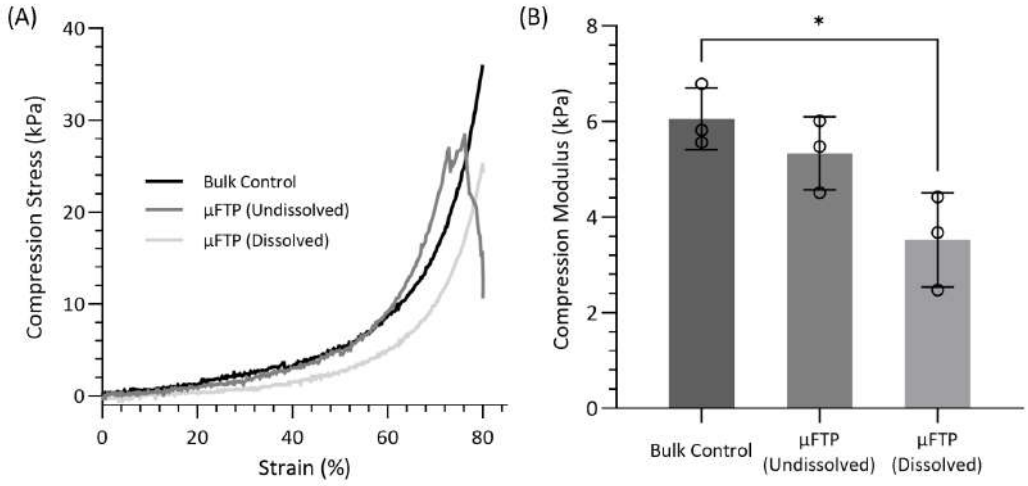

Figure S4. Compression test of bulk GelMA hydrogel and μFTP hydrogel samples before/after microfiber dissolution. (A) Compression stress-strain curve of bulk and μFTP hydrogel samples. (B) Measured compression modulus of bulk and μFTP hydrogel samples (N=3). One-way ANOVA with a Tukey's multiple comparisons test, *P < 0.05.

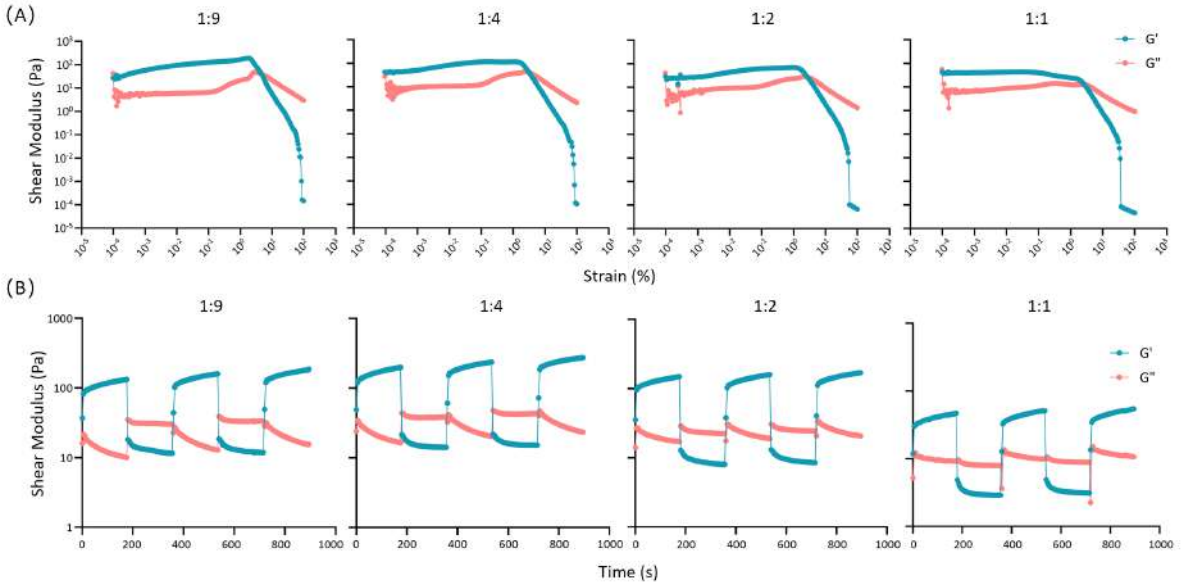

Figure S5. Rheological tests of microfiber-templated porogel bioink. (A) Strain sweeps and (B) high-low strain tests of μFTP bioinks at different component ratios (1:9, 1:4, 1:2 and 1:1), with 7.5% (w/v) GelMA as matrix.

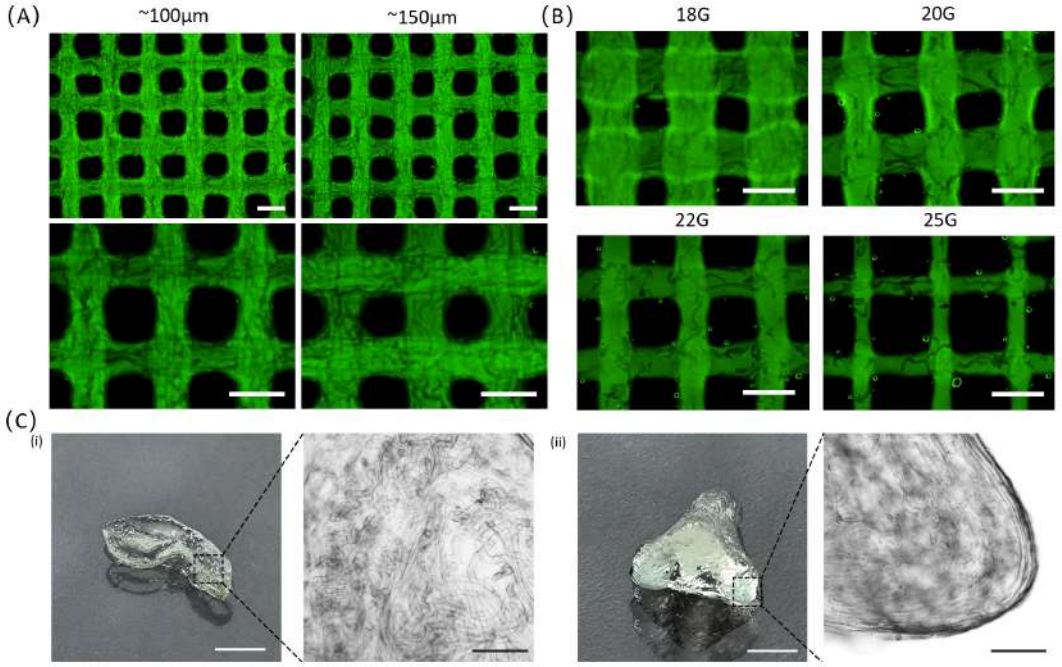

Figure S6. Representative microscopic images of printed multilayered lattice structures with (A) different pore sizes and (B) nozzle sizes. (C) Examples of large-scale samples (i.e., ear- and nose-like models) printed using μFTP bioinks at 1:4 component ratio. Scale bars: 1mm (A, B and microscopy in C), 5mm (photography in C).

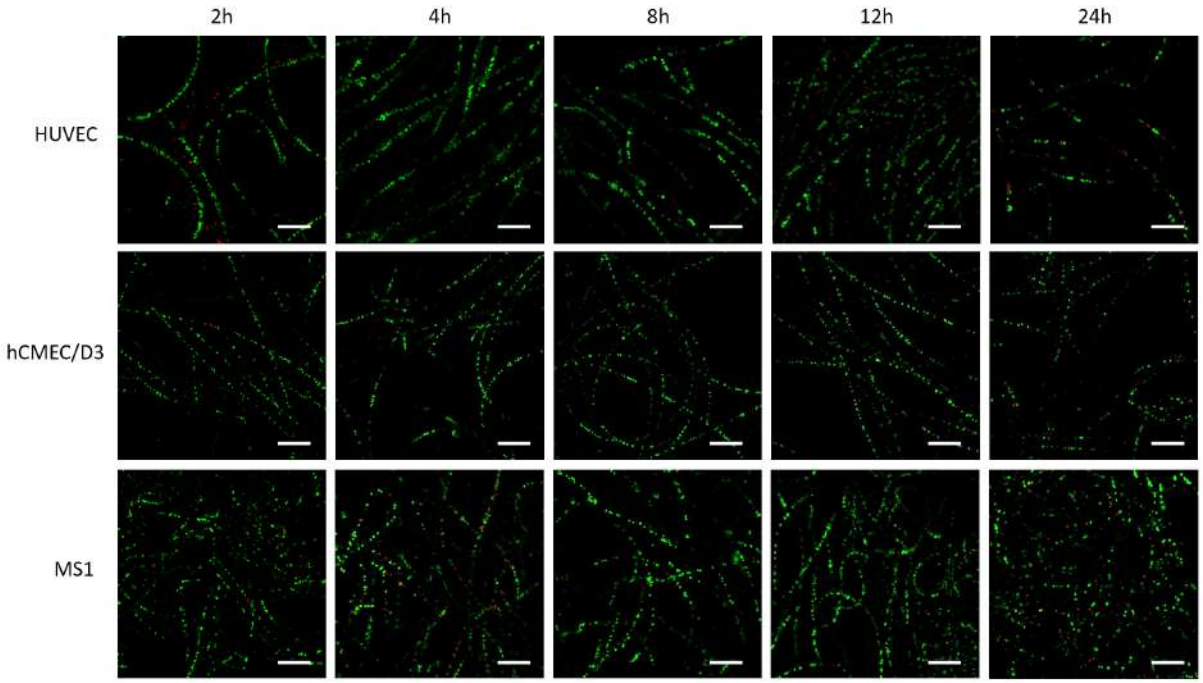

Figure S7. Live/Dead™ staining fluorescence images of HUVECs, hCMEC/D3, and MS1 cells encapsulated in microfibers with time. Scale bar: 500 μm.

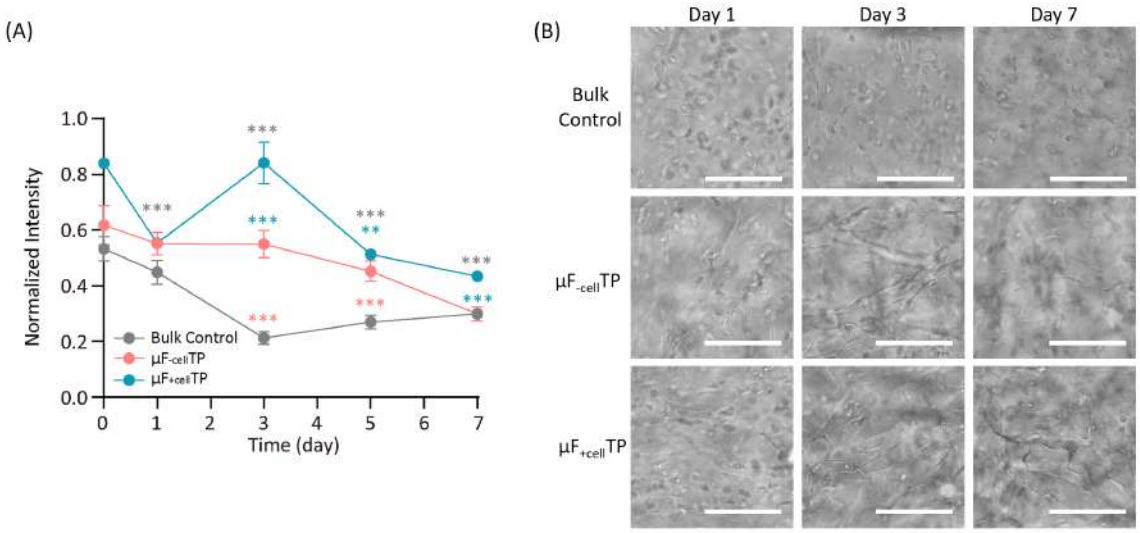

Figure S8. (A) Cell proliferation of HUVECs in bioprinted structures under different conditions: cells are encapsulated in microfiber-free GelMA (bulk control), cells are encapsulated in the GelMA matrix in the presence of acellular microfibers ($\mu F_{-cell}TP$), and cells are encapsulated in both GelMA matrix and microfibers ($\mu F_{+cell}TP$). One-way ANOVA with a Tukey's multiple comparisons test, *P < 0.05; **P < 0.01; ***P < 0.001. The number of cellularized sample replicates N=3. (B) Representative brightfield images of HUVEC-laden bioprinted multilayered lattice structures using bulk control, $\mu F_{-cell}TP$ and $\mu F_{+cell}TP$ bioinks. Scale bars: 200 μm.

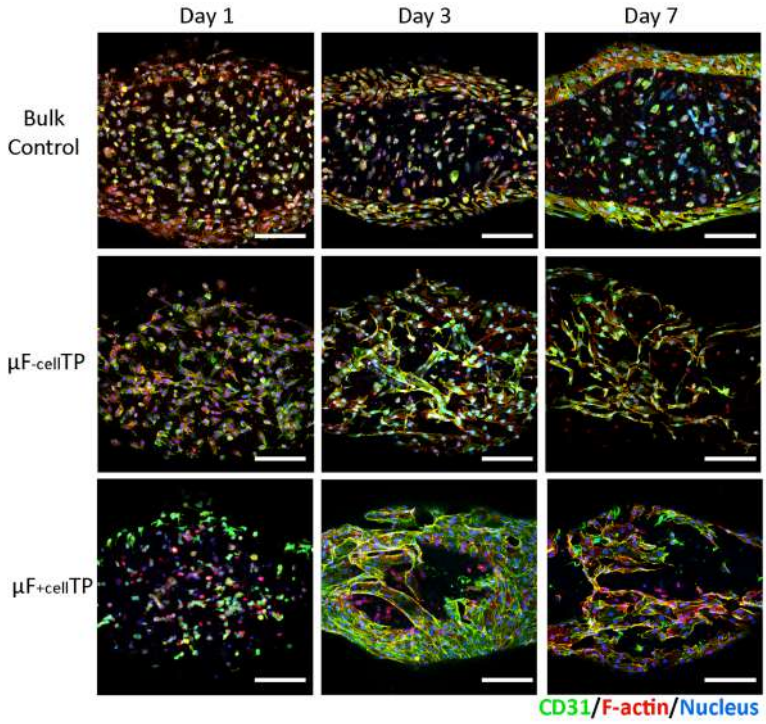

Figure S9. Z-stack fluorescent images of 3D printed lattice structures indicate the behavior and morphology of HUVECs. CD31, F-actin, and DAPI are stained with green, red, and blue, respectively. Scale bars: 200 μm.

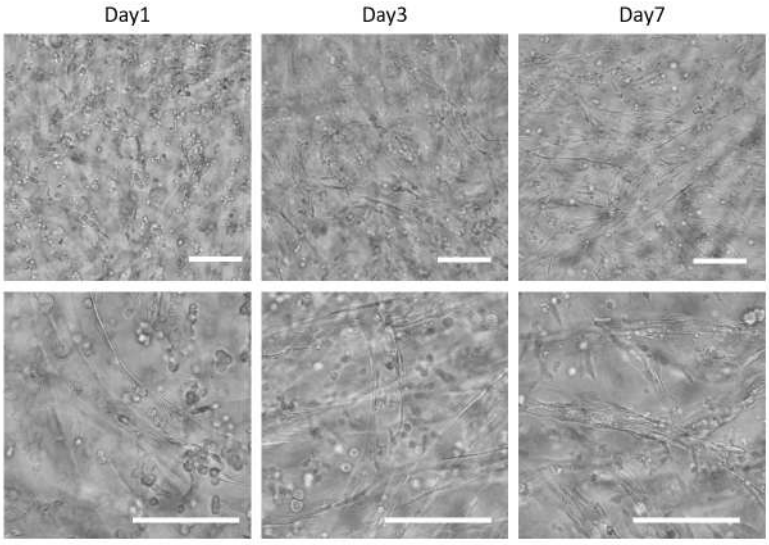

Figure S10. Representative brightfield images of hCMEC/D3-laden cast disk structures using $\mu F_{+cell}TP$ bioinks at different time points. Scale bar: 200 μm.

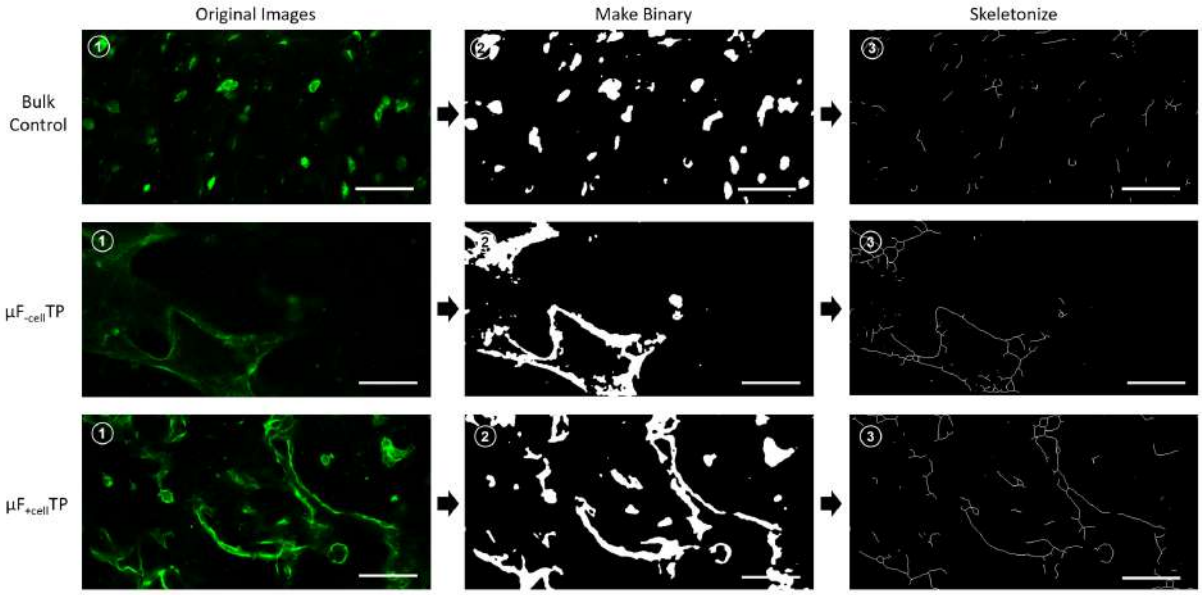

Figure S11. The standard protocol for image processing to quantify the length of connected endothelial cells using ImageJ software. Scale bars: 100 μm.

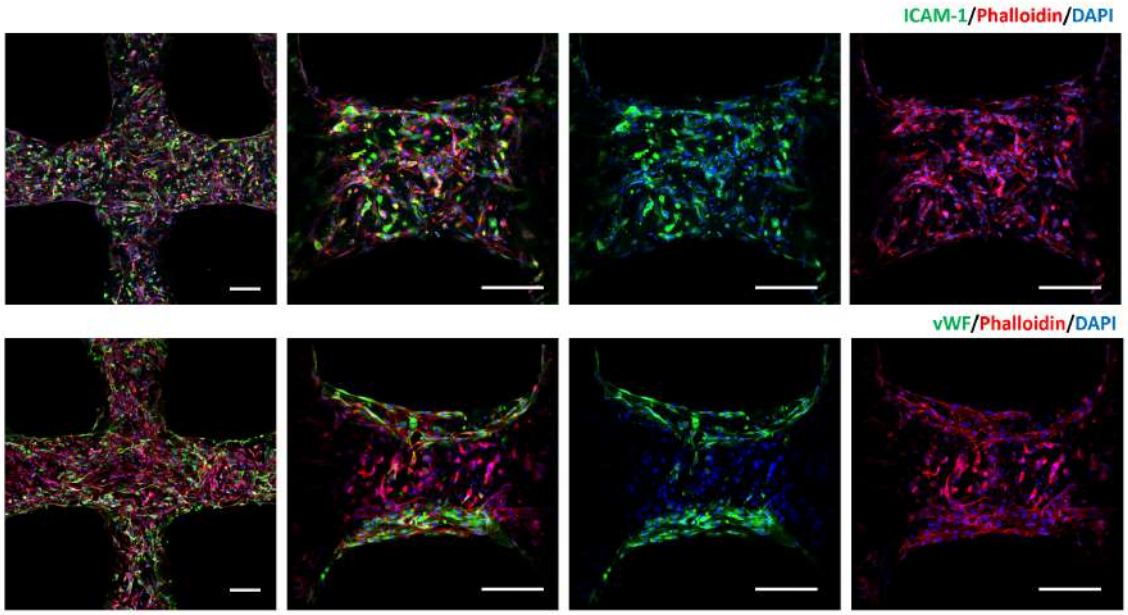

Figure S12. Z-stack immunofluorescence images of co-culture 3D bioprinted structures on day 3. ICAM-1, F-actin, DAPI are stained with green, red and blue (top panel) and vWF, F-actin, DAPI are stained with green, red and blue (bottom panel), respectively. Scale bars: 200 μm.

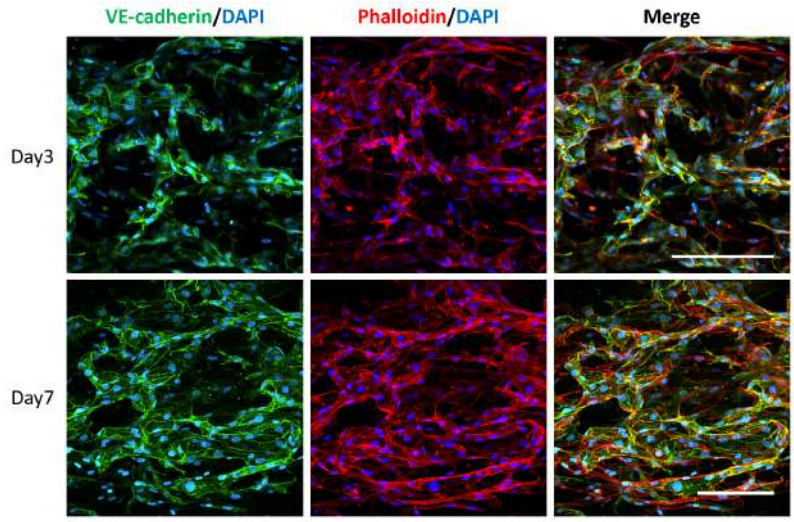

Figure S13. Higher magnification channel split images of bioprinted filament of co-culture μF+cellTP group on day 3 and day 7. VE-cadherin, F-actin, and DAPI are stained with green, red, and blue, respectively. Scale bars: 200 μm.

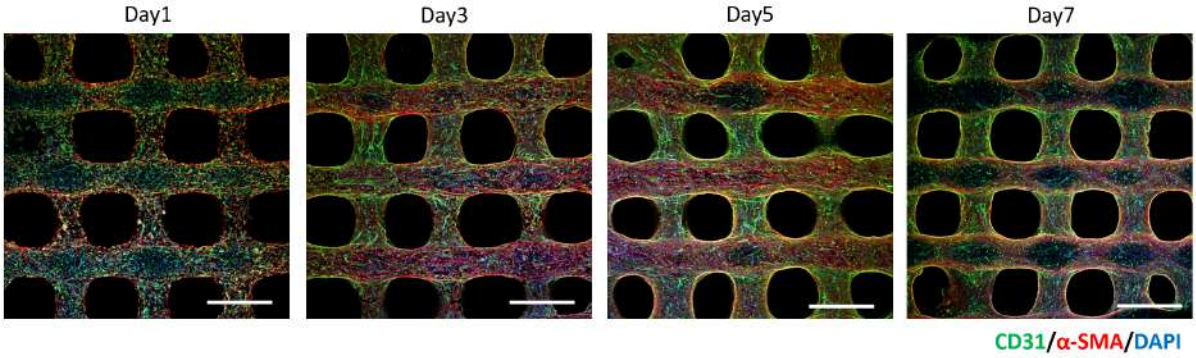

Figure S14. Immunofluorescence images of co-cultured bioprinted multilayered lattice structures using $\mu F_{+cell}TP$ bioinks with inclusion of Laminin and VEGF on day 1, day 3, day 5 and day 7. CD31, α-SMA, and DAPI are stained with green, red, and blue, respectively. Scale bars: 1mm.

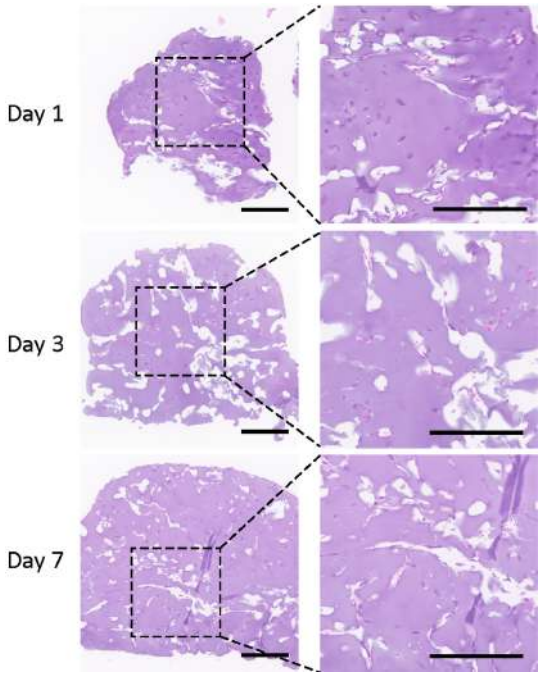

Figure S15. Representative microscopic images of H&E staining of $\mu F_{+cell}TP$ groups bioprinted scaffolds. Scale bars: 200 μm.

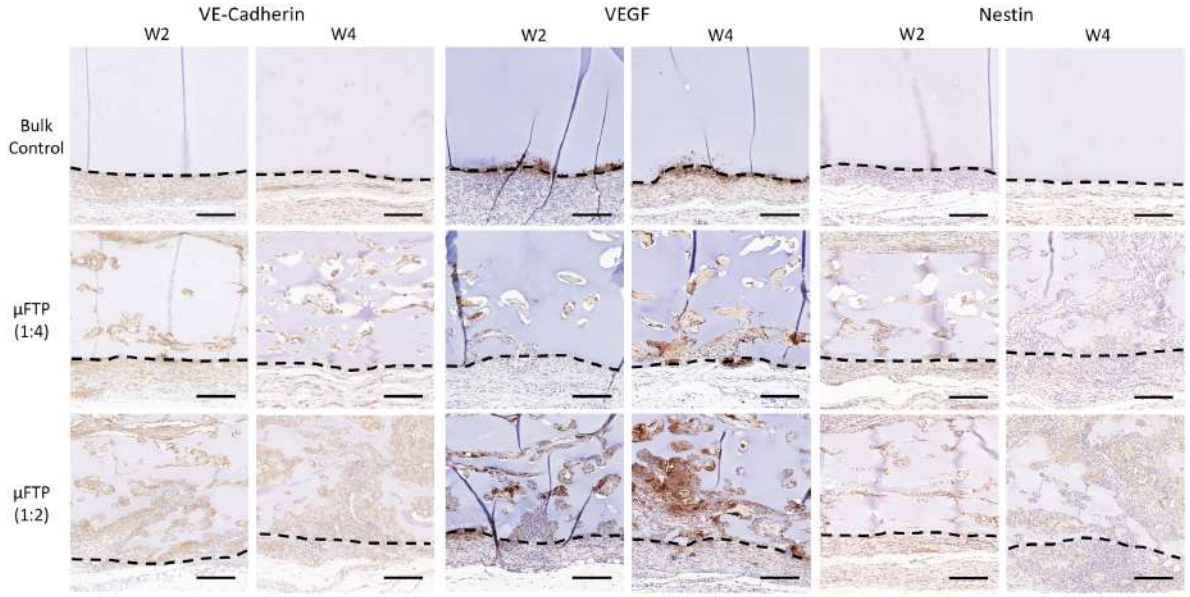

Figure S16. Immunofluorescence staining of VE-cadherin, VEGF, and Nestin of 3D printed structures at week 2 and week 4 post-implantation. Scale bars: 200 μm.

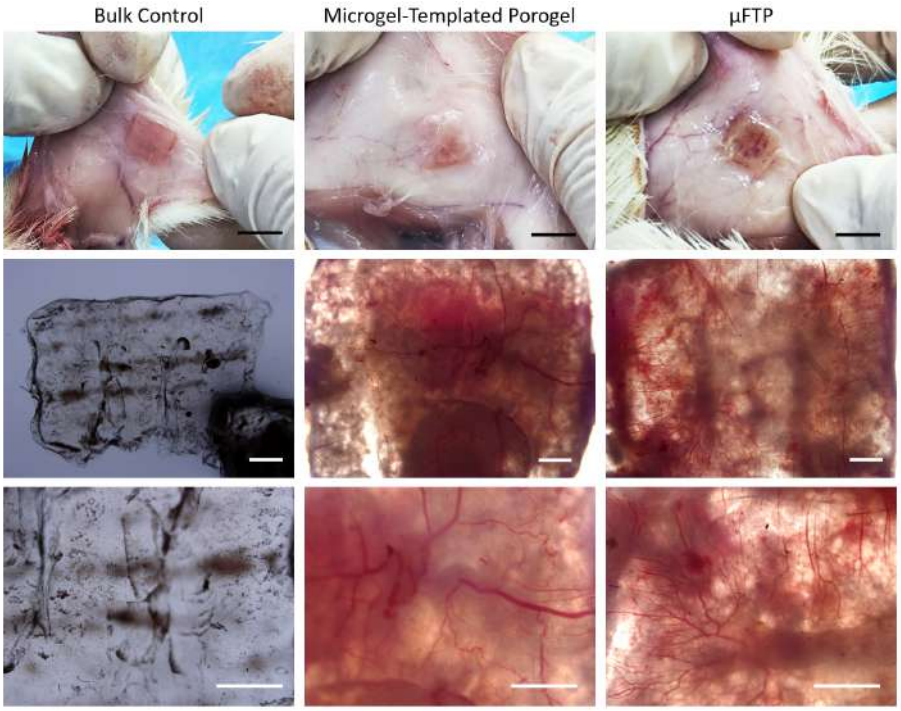

Figure S17. Representative optical macroscopic and microscopic images of implanted samples at week 3. Scale bars: 10 mm (top panel) and 1mm (middle and bottom panels).

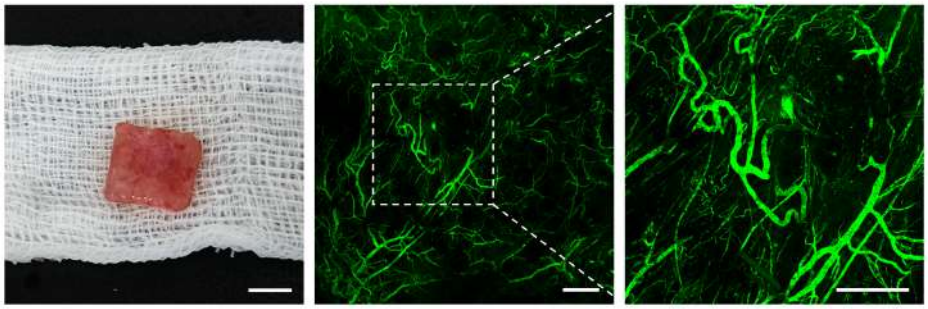

Figure S18. The appearance of subcutaneously implanted μFTP hydrogel after removing host tissue and representative fluorescent images of vasculature inside after perfused with FITC-dextran via the tail vein at week 3. Scale bars: 5 mm (left) and 300 μm (middle and right).

Table S1. Sequences of primers used for qRT-PCR. The forward and reverse primer sequences for Bcl-2, BAX, and Caspase-3 genes are listed in the 5' to 3' direction.

Primer	Sequencing (5'-3')
Bcl-2	Forward: GGCCGGCGACGACTTCTCCC
	Reverse: CCCCAGTTCACCCCGTCCCT
BAX	Forward:GGTTGTCGCCCTTTTCTACT
	Reverse: CCAATGTCCAGCCCATGATG
Caspase-3	Forward:ACGTGAAGAAATTGTGGAAT
	Reverse: TTTTTCAGGTCAACAGGTCC