

RESEARCH ARTICLE

Swelling compensation of engineered vasculature fabricated by additive manufacturing and sacrifice-based technique using thermoresponsive hydrogel

Supplementary File

Table S1. The composition of P/G_1 , P/G_3 , and P/G_5 hydrogel.

	PNIPAM	GelMA
P/G ₁	10%	1%
P/G ₃	10%	3%
P/G ₅	10%	5%



Figure S1. FTIR spectra of P/G hydrogel and Pluronic F-127 (PF-127).



Figure S2. Measurement of contact angle of the P/G hydrogel scaffolds with different concentrations.



Figure S3. Images of P/G₂ hydrogel scaffolds with different vasculature densities at different shrinking time points.



Figure S4. Images of P/G₄ hydrogel scaffolds with different vasculature densities at different shrinking time points.



Figure S5. Fabrication of vasculatures with six layers using P/G_1 and P/G_2 hydrogels.



Figure S6. Fabrication of curved vessels with P/G hydrogels.



Figure S7. Microscopy images of HUVECs attached on the vasculatures fabricated by using PF-127 and PF-127 + gelatin as sacrificial materials.



Figure S8. SEM images of the vasculatures fabricated by using PF-127 and PF-127 + gelatin as sacrificial materials.



Figure S9. CCK-8 diagram of P/G hydrogel extract for (A) HUVECs and (B) MG63.



Figure S10. Confocal images of HUVECs–OCs interactions. Scale bar = 100 μ m; scale bar in the magnified images = 75 μ m.



Figure S11. Calculation of (A) number of vascular branches and (B) vascular length around the P/G hydrogel scaffolds after subcutaneous implantation for 4 weeks. (C) The compression stress–strain curves of the control group (no vascular scaffold), group I (1×1 scaffold), group II (4×4 scaffold), and group III (8×8 scaffold).



Figure S12. Implantation of scaffolds with engineered vasculature in ischemia model for 8 weeks. (A) H&E staining for the implanted scaffolds and surrounding tissues. Scale bar = 1 mm. (B) Masson's trichrome staining for the implanted scaffolds and surrounding tissues. Scale bar = 1 mm. (C) Immunofluorescence staining of CD31 and α -SMA for the implanted scaffolds and surrounding tissues. Scale bar = 1 mm. (D) H&E staining of the vasculature lumens within the (i) 1 × 1, (ii) 4 × 4, and (iii) 8 × 8 scaffolds. Scale bar = 50 µm; scale bar in the magnified images = 20 µm. (E) Immunofluorescence staining of CD31 and α -SMA for the vasculature lumens within the (i) 1 × 1, (ii) 4 × 4, and (iii) 8 × 8 scaffolds. Scale bar = 50 µm; scale bar in the magnified images = 20 µm; scale bar in the magnified images = 20 µm.



Figure S13. (A) Schematic diagram of arterial connections in rats. Created with BioRender.com. (B) Comparison of blood supply to the left and right plantar skin of rats after dissection of the left limb artery. The number of α -SMA formed by each group of channel species for (C) 4 weeks and (D) 8 weeks.