

## Supplementary information for

### **3D stamp-integrated open-top microfluidic organ-on-a-chip for high-fidelity and functional reconstruction of vascularized microtissue models**

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#### **This file includes:**

Figs. S1 to S8

Tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

Movie S1: Perfusion of fluorescent microbeads in the vascularized tumor model

Movie S2: Perfusion of 70 kDa FITC-dextran confined inside vessel lumen without non-physiological leakage

Movie S3 : Perfusion of fluorescent microbeads with the diameter of 5  $\mu\text{m}$  inside vessel lumen with the trajectory along vessel structure

Movie S4: Spontaneous simultaneous beating of aligned myocardial tissue in the vascularized myocardial model

Movie S5: Spontaneous simultaneous beating of non-aligned myocardial tissue in the vascularized myocardial model

Movie S6: Myocardial contraction and velocity calculated by OHW software in the vascularized myocardial model under control, noradrenaline and verapamil conditions

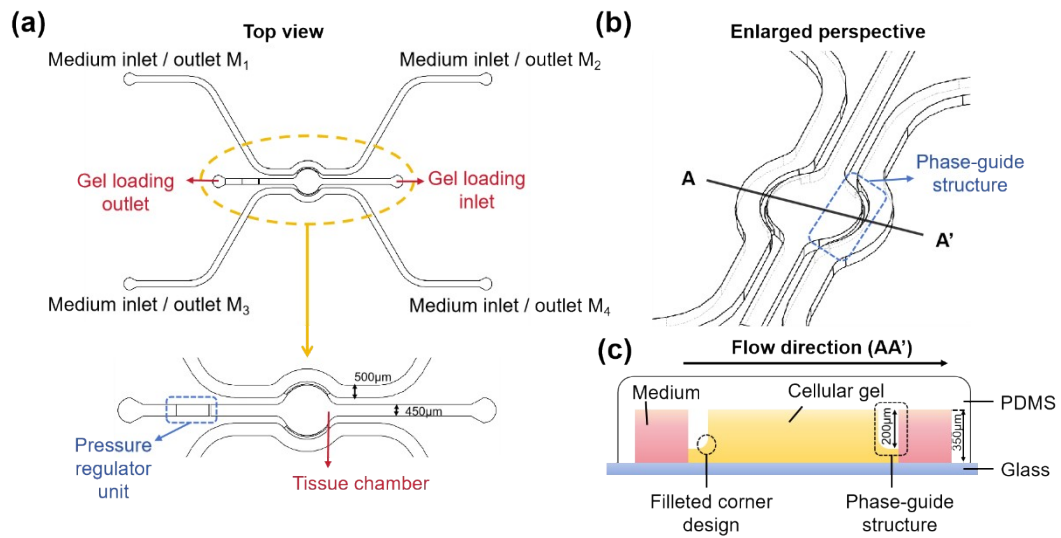


Fig. S1 Design of the stamp-integrated open-top microfluidic organ-on-a-chip. (a) Schematic diagram of the overall structure (b) Enlarged perspective view of the central chamber (c) Schematic diagram of medium flow along cross-section A-A'.

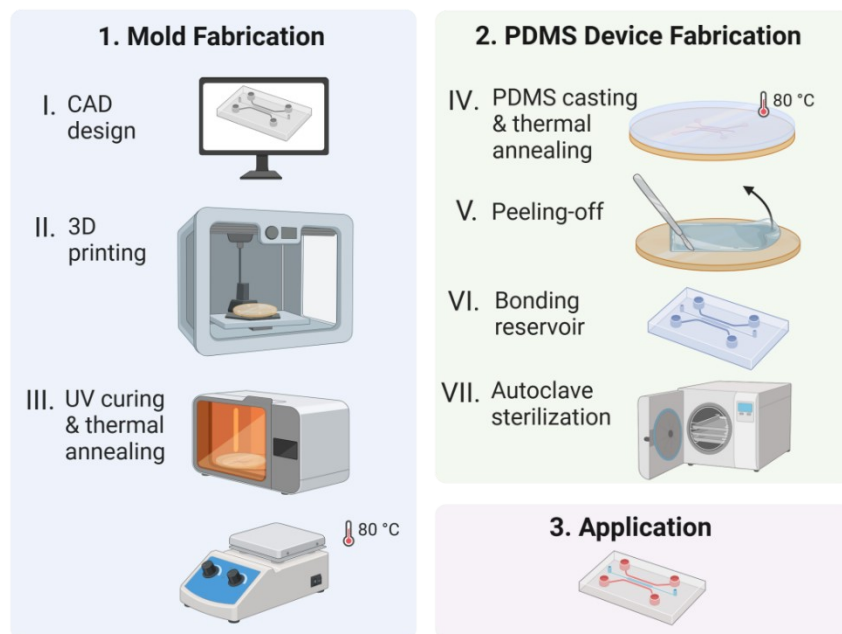


Fig. S2 Schematic diagram of the organ-on-a-chip platform fabricated using 3D-printed resin material

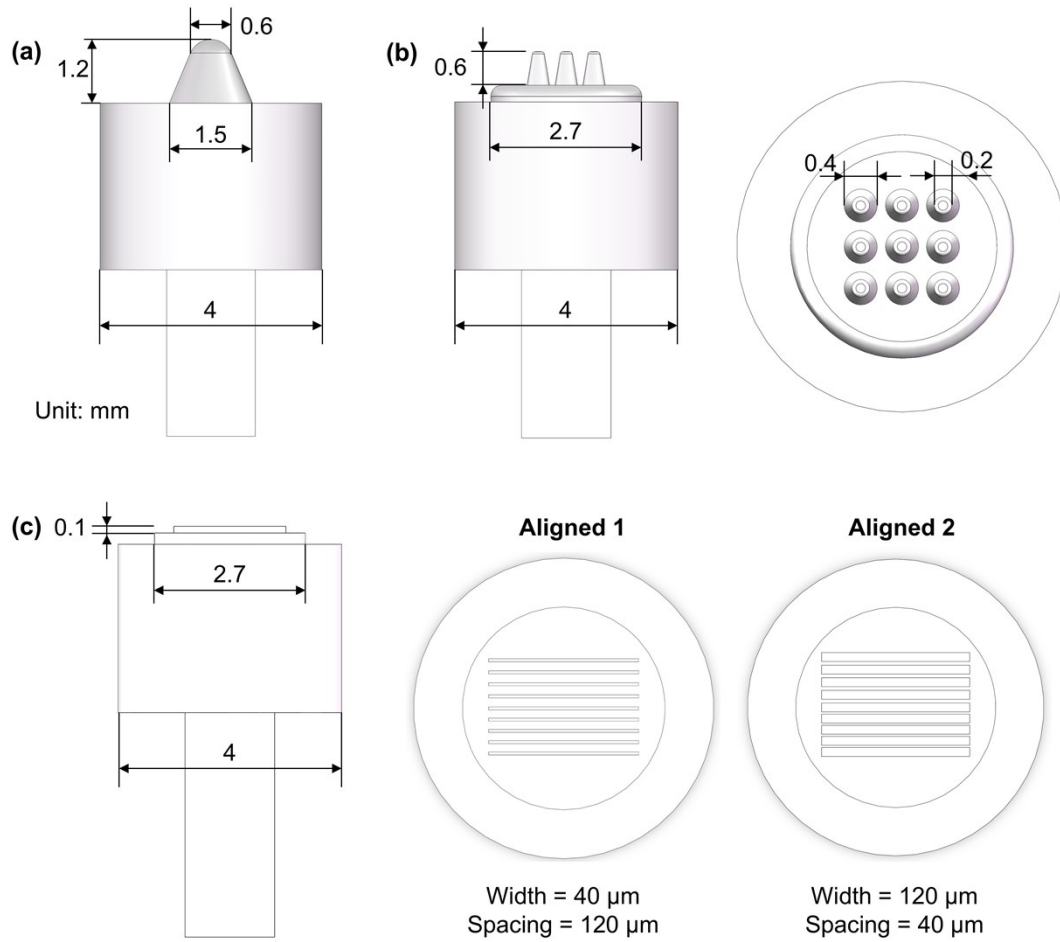


Fig. S3 Structure dimensions of the (a) tumor stamp, (b) colonic stamp, and the (c) myocardial stamp

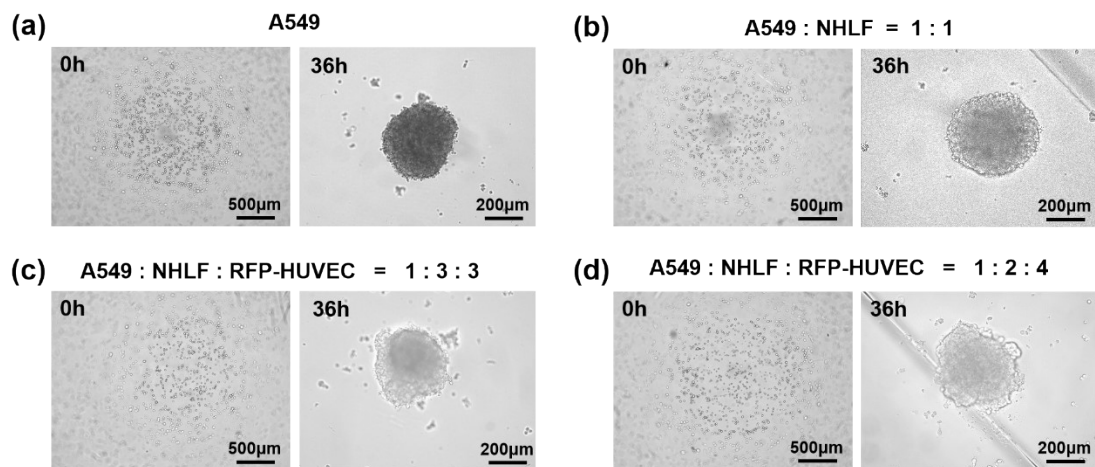


Fig. S4 Bright images of spheroid formation with different cell ratios: (a) A549 (b) A549 and NHLF (c) A549, NHLF and RFP-HUVEC (1:3:3) (d) A549, NHLF and RFP-HUVEC (1:2:4)

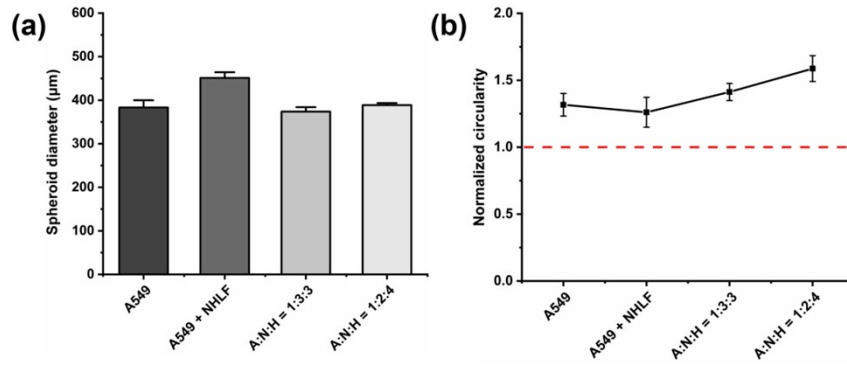


Fig. S5 Quantification of the tumor spheroids' (a) diameter and (b) normalized circularity. n=5, all data were obtained from independent experiments.

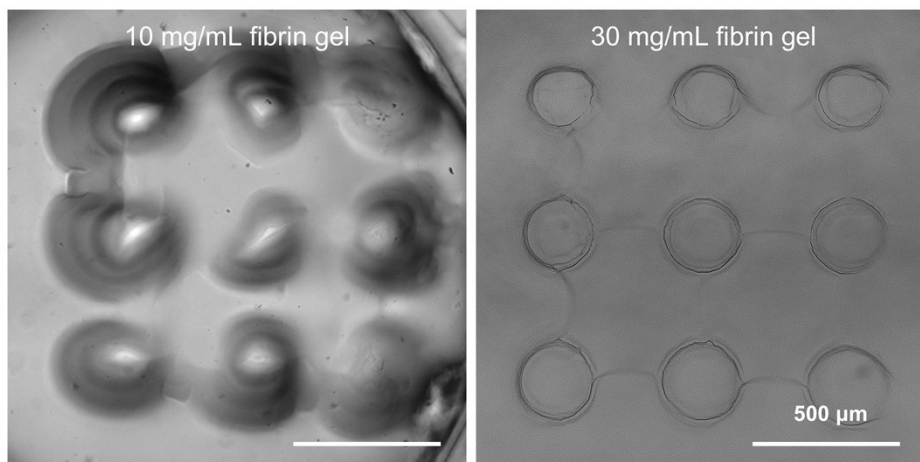


Fig. S6 Stamping characteristics of colonic crypts in fibrin gel at concentrations of 10 and 30 mg/mL

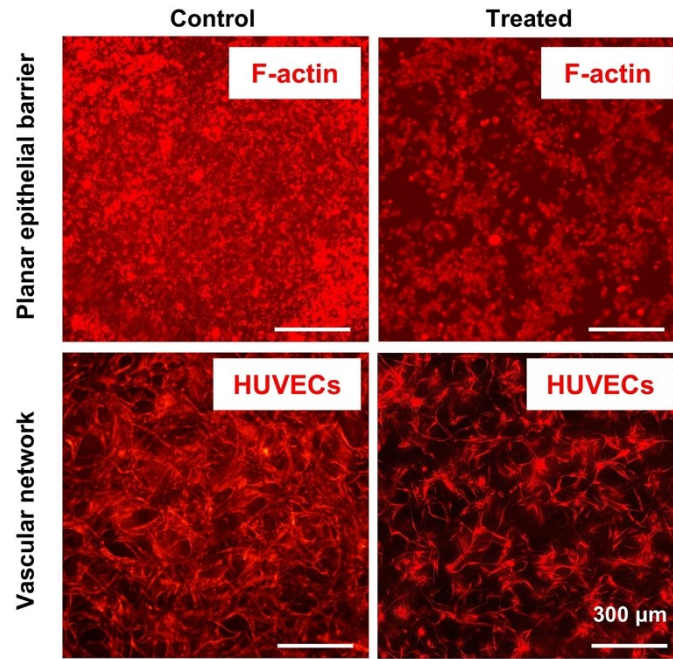


Fig. S7 Immunofluorescence images of the colonic epithelial barrier and vascular network before and after LPS treatment, scale bar=300 μm

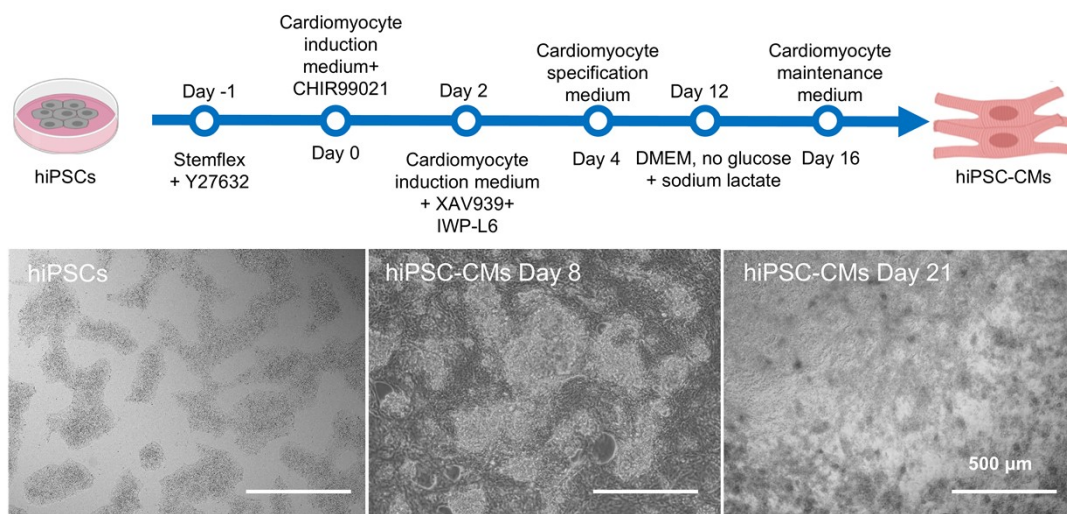


Fig. S8 Schematic diagram of hiPSCs differentiation to hiPSC-CMs, and bright-field images of the cells at days 0, 8, and 21. By day 8, locally beating hiPSC-CMs were observed

Table S1 Cardiomyocyte induction medium

Component	Quantity for 100 mL
IMDM	47.25 mL
F12	47.25 mL
BSA (10% wt/vol in IMDM)	2.5 mL
$\alpha$ -Monothioglycerol (13 $\mu$ L in 1 mL IMDM)	0.3 mL
L-ascorbic acid 2-phosphate (5 mg/mL in distilled water)	1 mL
Glutamax	1 mL
Trace elements B	0.01 mL
Trace elements C	0.1 mL
Penicillin-streptomycin	0.5 mL

Table S2 Cardiomyocyte specification medium

Component	Quantity for 100 mL
IMDM	47.25 mL
F12	47.25 mL
BSA (10% wt/vol in IMDM)	2.5 mL
$\alpha$ -Monothioglycerol (13 $\mu$ L in 1 mL IMDM)	0.3 mL
L-ascorbic acid 2-phosphate (5 mg/mL in distilled water)	1 mL
Glutamax	1 mL
Trace elements B	0.01 mL
Trace elements C	0.1 mL
Penicillin-streptomycin	0.5 mL
Insulin-Transferrin-Selenium-Ethanolamine (ITS-X) 100X	0.1 ml

Table S3 Cardiomyocyte maintenance medium

Component	Quantity for 100 mL
IMDM	47.25 mL
F12	47.25 mL
BSA (10% wt/vol in IMDM)	2.5 mL
$\alpha$ -Monothioglycerol (13 $\mu$ L in 1 mL IMDM)	0.3 mL
L-ascorbic acid 2-phosphate (5 mg/mL in distilled water)	1 mL
Glutamax	1 mL
Trace elements B	0.01 mL
Trace elements C	0.1 mL
Penicillin-streptomycin	0.5 mL
Insulin-Transferrin-Selenium-Ethanolamine (ITS-X) 100X	0.1 ml
Chemically Defined Lipid Concentrate (CDLC)	1 mL