Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting information

Enabling perfusion through multicellular tumor spheroid promoting lumenization in vascularized cancer model

*Joonha Park[1], Seunggyu Kim[1], Jiman Hong[1], Jessie Sungyun Jeon[1]**

[1] Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Daejeon, 34141, Republic of Korea

* Corresponding Author: Jessie Sungyun Jeon (jsjeon@kaist.ac.kr)

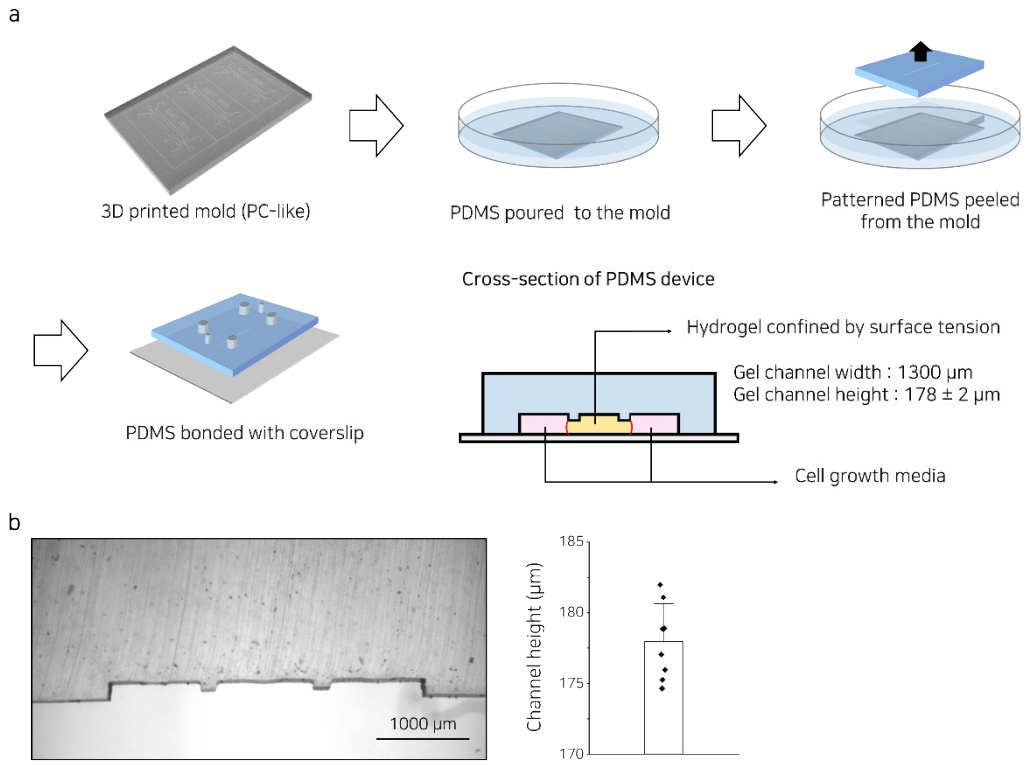
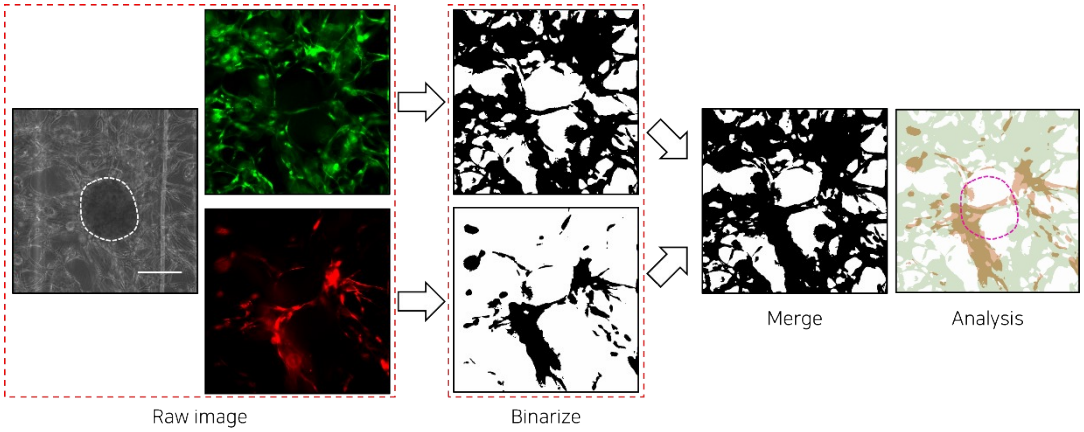


Fig. S1 Fabrication procedure and dimension of the microfluidic device. (a) Microfluidic device fabrication using 3D printing and soft-lithography. (b) Cross-section configuration and measured channel height from molds. The data are plotted as mean ± SD. Scale bar is 1000 microns.



- $GFP\ HUVEC\ area + RFP\ HUVEC\ area\ - (GFP \cap RFP\ HUVEC\ area) = total\ HUVEC\ area$
- $total\ RFP\ HUVEC\ area:\ total\ RFP\ HUVEC\ area\ in\ the\ FOV$
- $internal\ GFP\ or\ RFP\ HUVEC\ area: HUVEC\ area\ inside\ the\ tumor\ spheroid\ boundary$

Fig. S2 Endothelial cell dynamics quantification method. Fluorescent images were binarized with corresponding threshold conditions which were maintained throughout the experiment. Two binarized HUVEC images were merged and defined as 'total HUVEC area'. White (magenta right) dashed line indicates the boundary of the spheroid. Scale bar is 200 microns. Gray: Phase contrast, Red: HUVEC (RFP), Green: HUVEC (GFP).

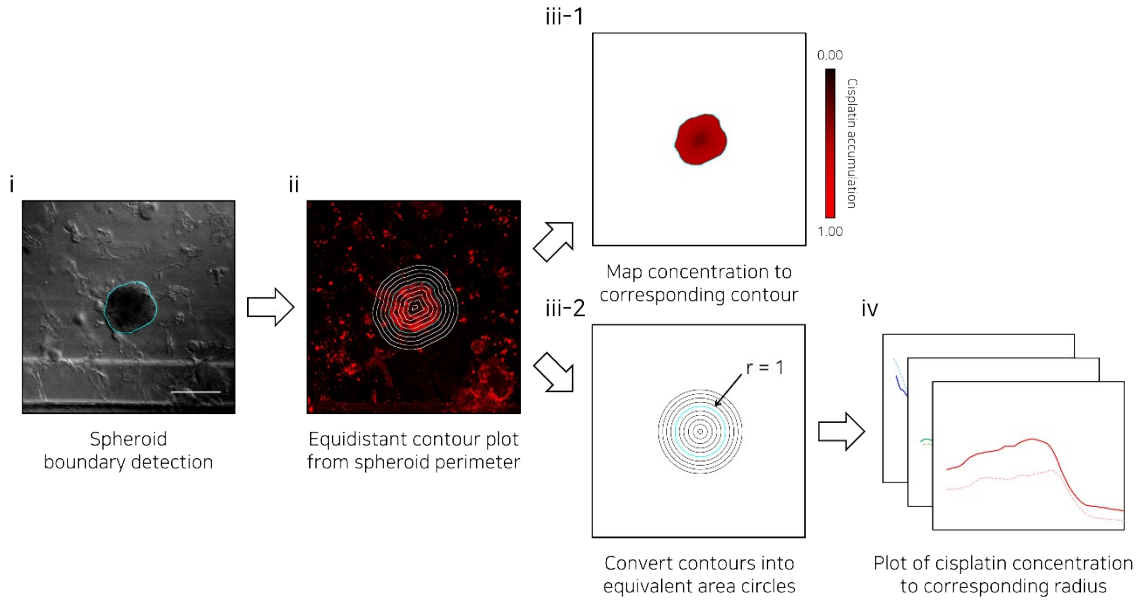


Fig. S3 Cisplatin concentration contour map and profile generation method. (i) Spheroid boundary was manually detected from the mid-plane of the phase contrast confocal stack image (marked with cyan). (ii) Modified built-in imageJ macro was used to generate equally spaced (2 pixel) contour inner and outward from the spheroid boundary. Repeated operation was performed until the radius of the largest contour was 1.5 times that of the initial spheroid. (iii-1) Fluorescence was measured between the adjacent contour and was normalized to the largest fluorescence value among the conditions. The individual concentrations were then mapped to the color code. (iii-2) Contours obtained from (ii) were converted into concentric circles with equal area. The radii were then normalized to the radius of the spheroid boundary. (iv) The plot was obtained between the radii and corresponding fluorescent intensity. Scale bar is 200 microns. Gray: Phase contrast, Red: TR-cisplatin (Texas Red).

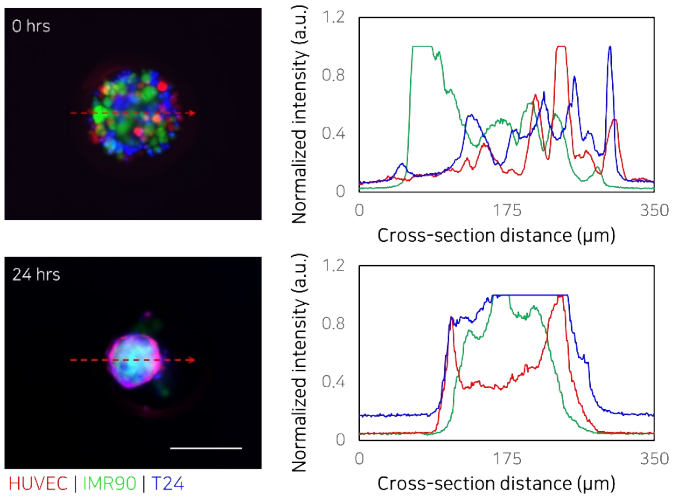
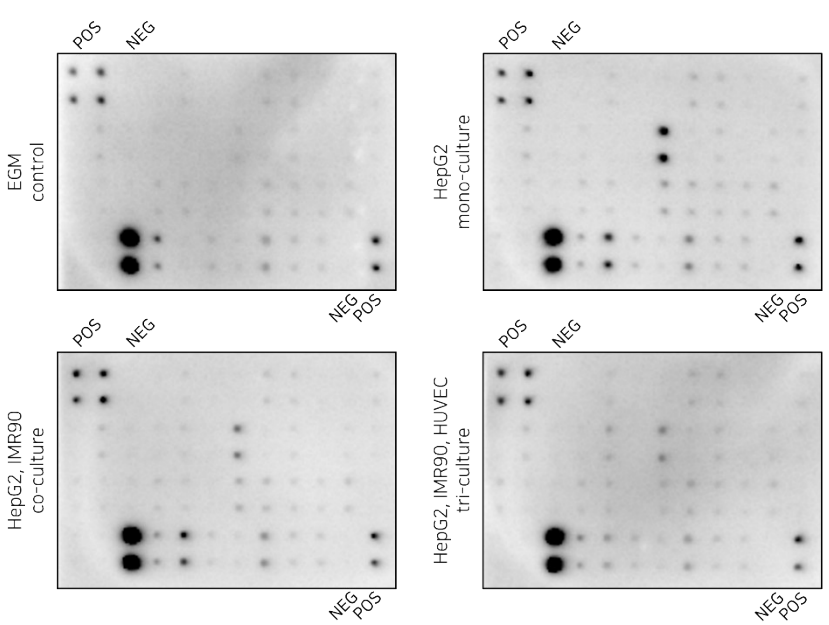


Fig. S4 Formation of multicellular tumor spheroid using bladder cancer cell line and normalized fluorescence intensity along the centroid passing line (red dotted arrow). Scale bar is 200 microns. Red: HUVEC (RFP), Green: IMR90 (CellTrackerTM CMFDA), Blue: T24 (Hoechst 33342).

a

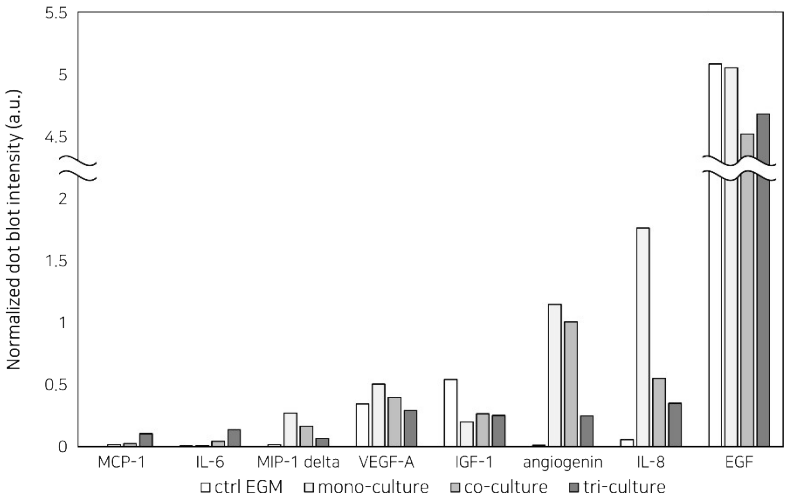


b

	c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11	c12
r1	POS	POS	NEG	NEG	ENA-78 (CXCL5)	G-CSF	GM-CSF	GRO a/b/g	GRO alpha (CXCL1)	I-309 (TCA-3/CCL1)	IL-1 alpha (IL-1 F1)	IL-1 beta (IL-1 F2)
r2												
r3	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8 (CXCL8)	IL-10	IL-12 p40,p70	IL-13	IL-15	IFN-gamma
r4												
r5	MCP-1 (CCL2)	MCP-2 (CCL8)	MCP-3 (CCL7)	M-CSF	MDC (CCL22)	MIG (CXCL9)	MIP-1 delta	RANTES (CCL5)	SCF	SDF-1 (CXCL1 2 alpha)	TARC (CCL17)	TGF beta1
r6												
r7	TNF-alpha	TNF-beta	EGF	IGF-1	Angiogenin	OSM	TPO	VEGF-A	PDGF-BB	Leptin	NEG	POS
r8												

Fig. S5 Analysis of cytokines using human cytokine array. (a) Array result incubated with EGM, mono-culture, co-culture, and tri-culture. EGM was used as a control. (b) Whole cytokine list of the array.

Abbreviations are listed as follows. POS: positive control spots, NEG: negative control spots, CXCL: chemokine (C-X-C motif) ligand, G-CSF: granulocyte-colony stimulating factor, GM-CSF: granulocyte-macrophage colony-stimulating factor, CCL: chemokine (C-C motif) ligand, IL: interleukin, IFN: interferon, M-CSF: macrophage colony-stimulating factor, MIP: macrophage inflammatory protein, SCF: stem cell factor, TGF: transforming growth factor, TNF: tumor necrosis factor, EGF: epidermal growth factor, IGF: insulin-like growth factor, OSM: oncostatin M, TPO: thrombopoietin, VEGF: vascular endothelial growth factor, PDGF: platelet-derived growth factor.



cytokine	coefficient of variation (%)
IL-8	62.06
Angiogenin	38.62
MIP-1 delta	7.37
IL-6	5.76
IGF-1	5.62
MCP-1	4.44
VEGF-A	1.60
EGF	1.21

Fig. S6 Plot of dot blot intensity of top 8 cytokines, showing the greatest deviation between the conditions. Coefficient of variation was calculated to quantitatively express the deviation.

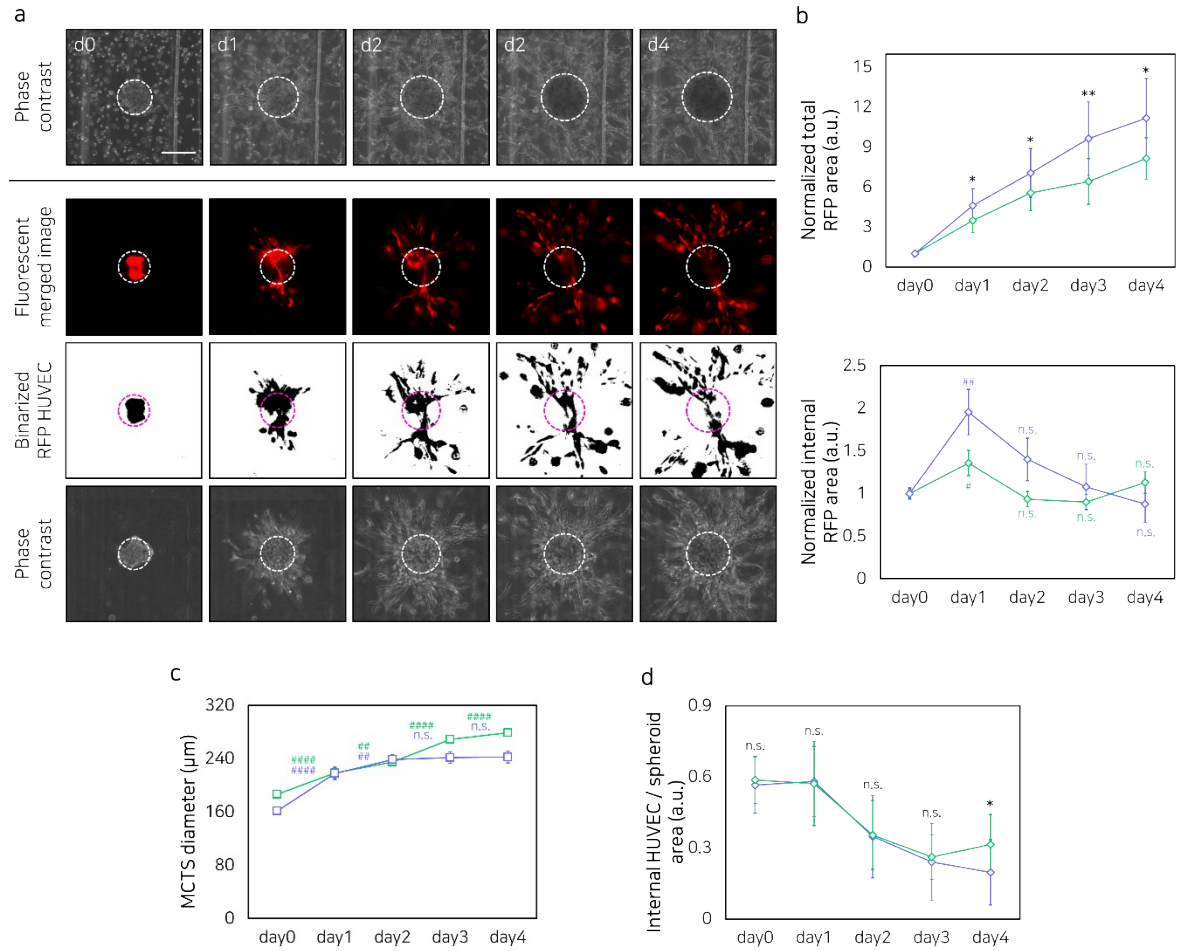


Fig. S7 Endothelial cell dynamics in MCTS perfusion and sprouting. (a) Times series phase contrast image corresponding to the fluorescent images in Figure 5a (top). Times series image of endothelial cell sprouting from identical MCTS condition without external endothelial cells (bottom). White (magenta in binarized image) dashed line approximately indicates the boundary of the spheroid. Scale bar is 200 microns. (b) Total RFP area and internal RFP area are normalized to that of the first day in both cases; with and without external vasculature. The significance was measured between those two conditions on the same day. (c) MCTS diameter was measured daily using the previously established method. The significance of the spheroid growth was independently measured. (d) Total internal HUVEC area was normalized to the corresponding spheroid area. The significance was measured between two conditions. Green and blue lines indicate endothelial dynamics analysis with and without external vasculature respectively. Total 10 MCTSs were analyzed from 6 independent devices. Black significance mark (*) is a comparison between conditions, and the data are plotted as mean ± SD; colored significance marks (#; green and blue) are the comparison within the condition, and the data are plotted as mean ± SEM.

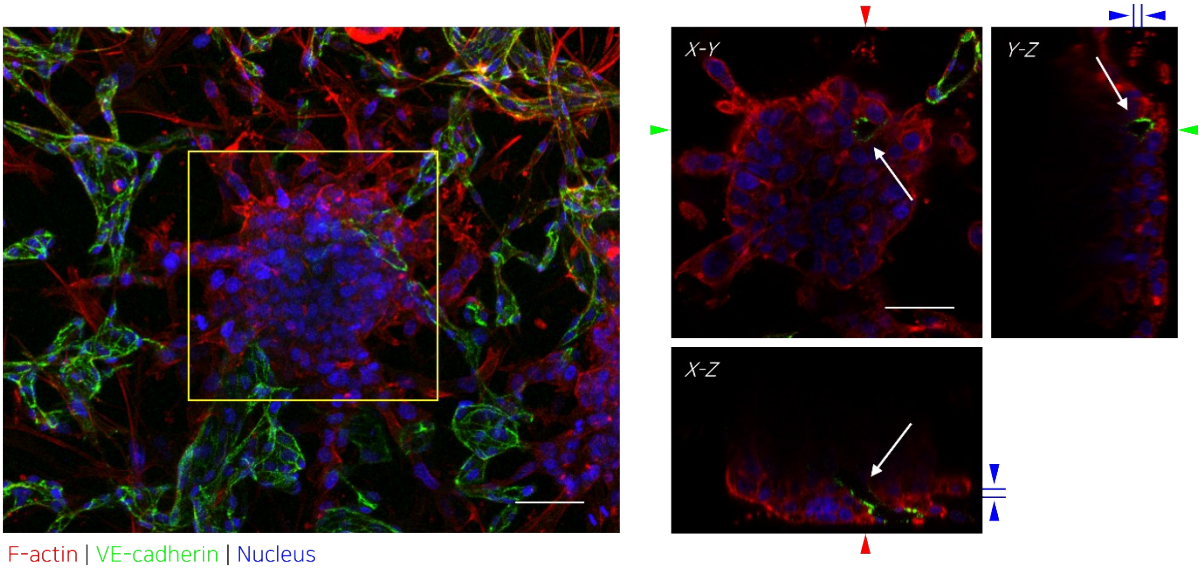


Fig. S8 Confocal projection and magnified cross-sectional view of the constructed perfusable tri-culture spheroid. 10 slices (corresponding region is marked as blue arrowhead) were stacked for *X-Y* cross-section to enhance the visibility of the lumen, denoted as white arrow. Red and green arrowhead denotes the location of *Y-Z* and *X-Z* cross-section respectively. Scale bar is 50 microns. Red: F-actin (TRITC), Green: VE-cadherin (Alexa Fluor 488), Blue: Nucleus (DAPI).

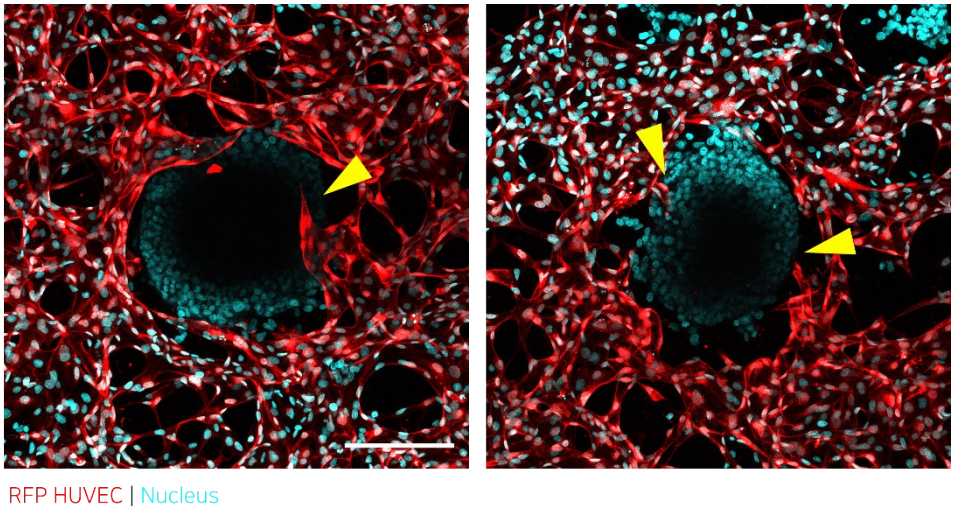


Fig. S9 Confocal projection image of endothelial sprouting motion in mono-culture spheroid. Yellow arrow head denotes the endothelial sprouts. Scale bar is 200 microns. Red: RFP-HUVEC (RFP), Turquoise: Nucleus (DAPI)

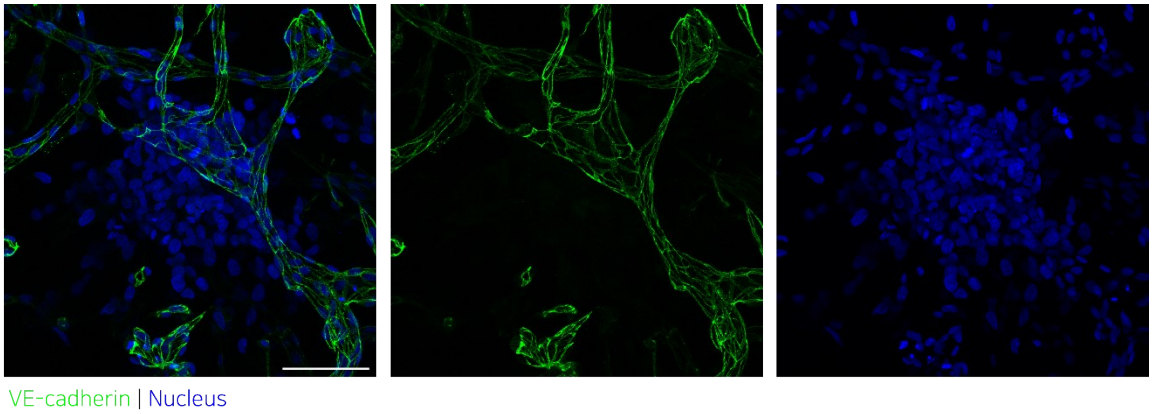


Fig. S10 Magnified confocal projection image of VE-cadherin. Scale bar is 100 microns. Green: VE-cadherin (Alexa Fluor 488), Blue: Nucleus (DAPI)

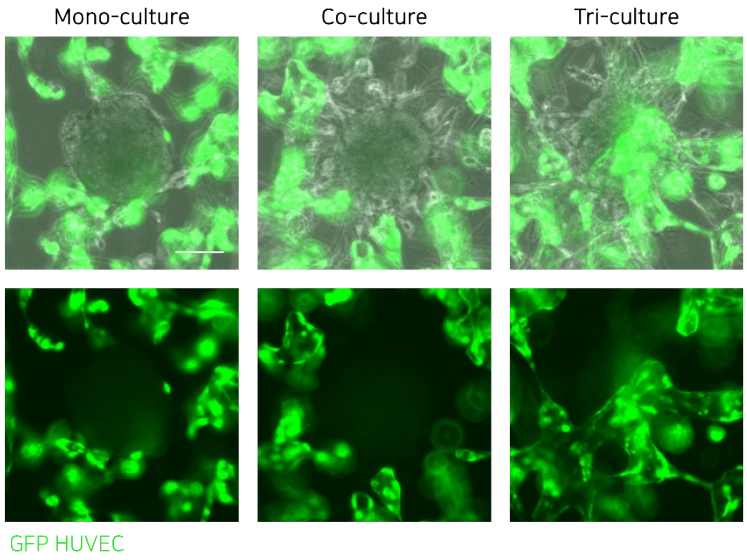


Fig. S11 Phase contrast and fluorescence image of the spheroid before TR-cisplatin administration on day 4. Scale bar is 100 microns. Green: GFP HUVEC (GFP)

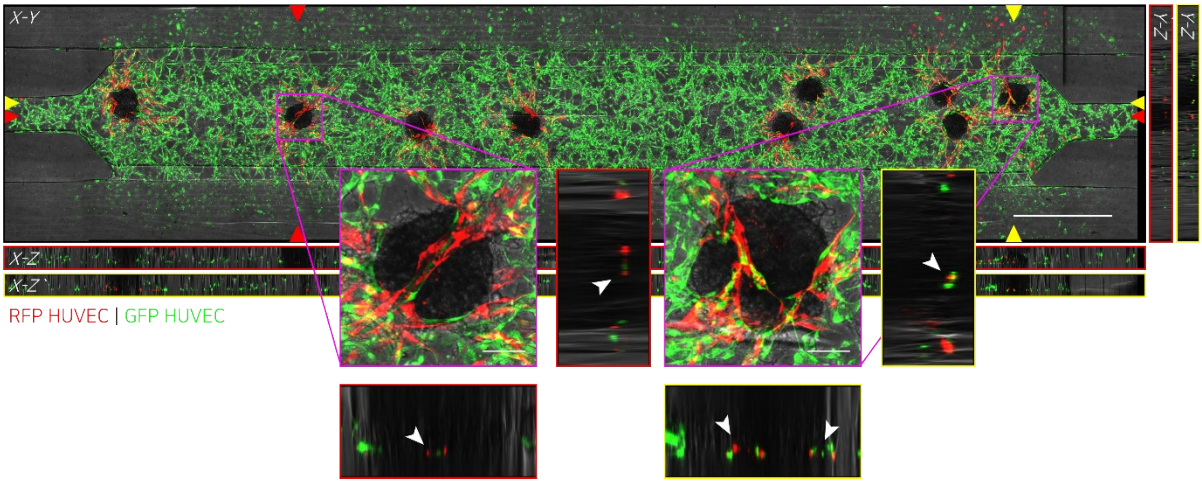


Fig. S12 Confocal projection image and magnified cross-section image of the whole channel. Location of the cross-sections are marked with red and yellow (designated with ') arrow head on the X-Y projection image. Magnified spheroid and corresponding cross-sections are drawn in the inset. White arrow head denotes the anastomosed GFP HUVEC and RFP HUVEC in the spheroid. Scale bar is 1000 microns for the whole channel image and 100 microns for the inset. Red: RFP-HUVEC (RFP), Green: GFP-HUVEC (GFP)

	HepG2	IMR90	HUVEC	Spheroid formation medium
HepG2 mono-culture	7	-	-	DMEM (10% Heat inactivated FBS, 1% A/A)
HepG2, IMR90 co-culture	6	1	-	DMEM (10% Heat inactivated FBS, 1% A/A)
HepG2, IMR90, HUVEC tri-culture	3	1	3	EGM2 (1% A/A)

Table S1 Cellular composition of MCTS and corresponding growth medium.

Antibody / Probe	Species	Volume ratio / Concentration	Product information
Collagen IV	Rabbit	1:100	Abcam
VE-cadherin	Rabbit	1:400	Abcam
CD31	Mouse [JC/70A]	1:100	Invitrogen
CD34	Rabbit [EP373Y]	1:100	Abcam
Hoechst 33342		5 μg/mL	Invitrogen
4′, 6-diamidino-2-phenylindole		1:750	Invitrogen
Goat anti-rabbit Alexa-488	Goat	1:100	Invitrogen
Goat anti-mouse Alexa-488	Goat	1:100	Abcam
CellTracker™ Red CMTPX		10 mM	Invitrogen
CellTracker™ Green CMFDA		10 mM	Invitrogen

Table S2 List of antibodies and immunofluorescent dyes used in the experiment.