Electronic supplementary information

A multi-throughput multi-organ-on-a-chip on a plate-formatted pneumatic pressure-driven medium circulation platform

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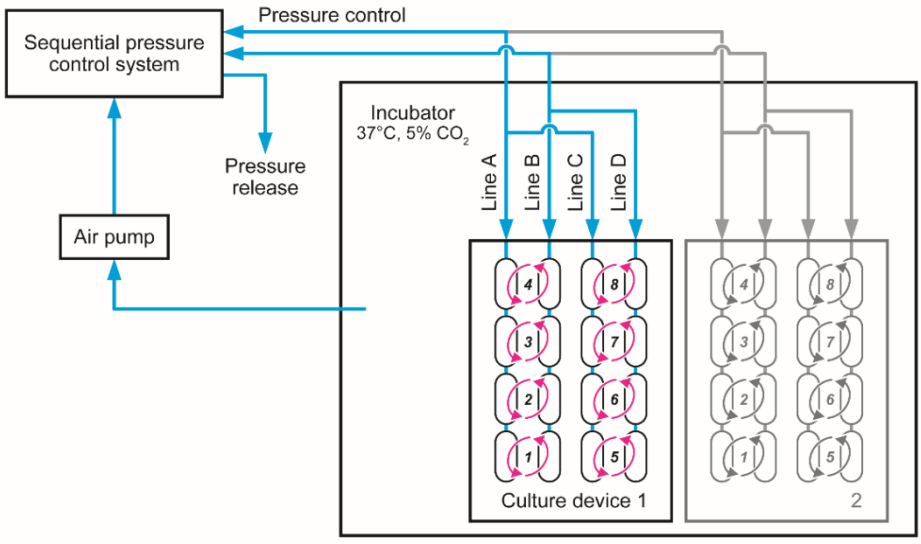

Figure S1 shows the layout of the pneumatic pressure lines for the pneumatic pressure-driven multi-throughput multi-organs-on-a-chip (MOC) system. Air containing 5% CO_2 was introduced by an air pump into a sequential pressure-control system from a CO_2 incubator. Sequential pressure was applied to culture device 1 in the incubator via four pressure lines. Blue arrows indicate the pressure lines and flow direction. Red arrows in the culture device indicate circulation of medium in each culture unit in the eight-throughput two-organ system, in which the two culture chambers were interconnected in each culture unit. Additional pressure lines (grey arrows) and culture devices can be added by branching the pressure lines for further parallelization, if necessary.

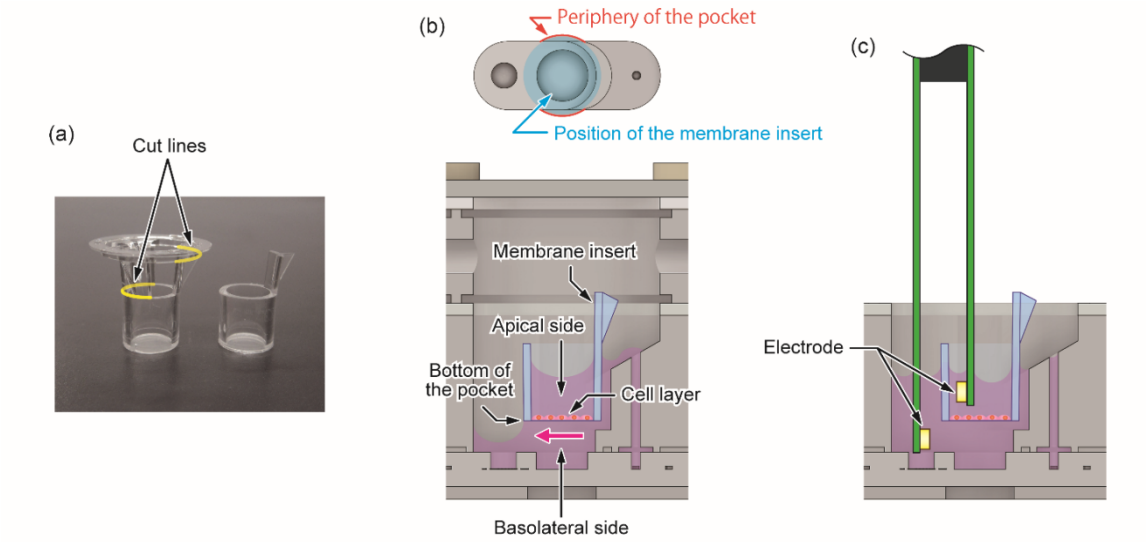

Fig. S2 Membrane insert for barrier-type organs. (a) Membrane inserts (Corning Transwell® permeable supports) before and after that the top half was removed. Two arms of the original insert were cut at the lower side, and one left was cut at the upper side by using a heat nipper. (b) Top and cross-sectional illustrations of the culture chamber equipped with the modified membrane insert. The insert was fitted in a pocket-like structure with 9.5-mm-diamter. Red arrow indicates flow direction of medium during the circulation culture. (c) TEER measurement in the culture device equipped with the membrane insert.

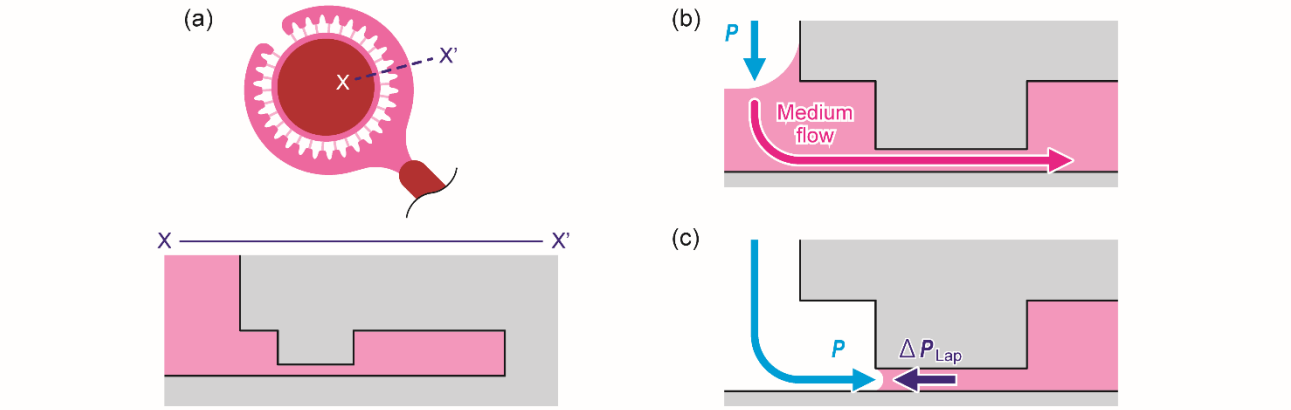

Fig. S3 Schematic of the structure and function of a "Laplace valve." (a) Top and cross-sectional illustrations of the Laplace valves placed around the outlet of the culture chamber. (b) Medium passes through the thin microchannels when pneumatic pressure (P) is applied to the culture chamber. (c) When all of the medium has passed through the thin microchannels, gas flow into these microchannels is prevented if P is lower than the Laplace pressure (ΔP_{Lap}). The theoretical value of ΔP_{Lap} is calculated from the Young–Laplace equation: $\Delta P_{Lap} = 2\gamma(1/w_L + 1/h_L)$, where γ is the interfacial tension of the medium and w_L and h_L are the width and height, respectively, of the shallow microchannels in the Laplace valve.

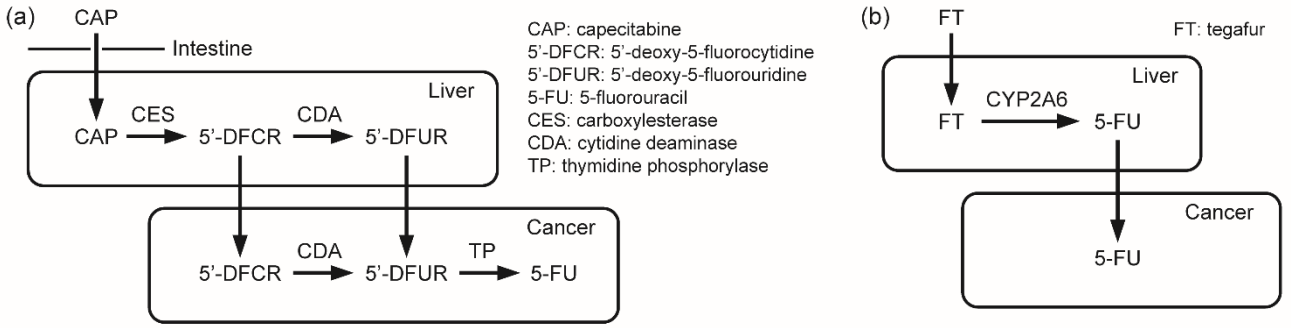

Figure S4 Metabolic pathways of two prodrugs of 5-FU, capecitabine (CAP) and tegafur (FT), *in vivo*. (a) Orally administered CAP is adsorbed from the intestine and then metabolized to 5'-deoxyfluorocytidine (5'-DFCR) by carboxylesterase (CES) in liver. Conversion from 5'-DFCR to 5'-deoxyfluorouridine (5'-DFUR) is catalyzed by cytidine deaminase (CDA) in both liver and cancer tissue. Finally, 5-fluorouracil (5-FU) is formed from 5'-DFUR by the action of thymidine phosphorylase (TP) in cancer tissue. Therefore, the inhibitory effect of 5-FU on cell growth appears mainly in cancer tissue. (b) FT is metabolized to 5-FU by CYP2A6 in the liver.

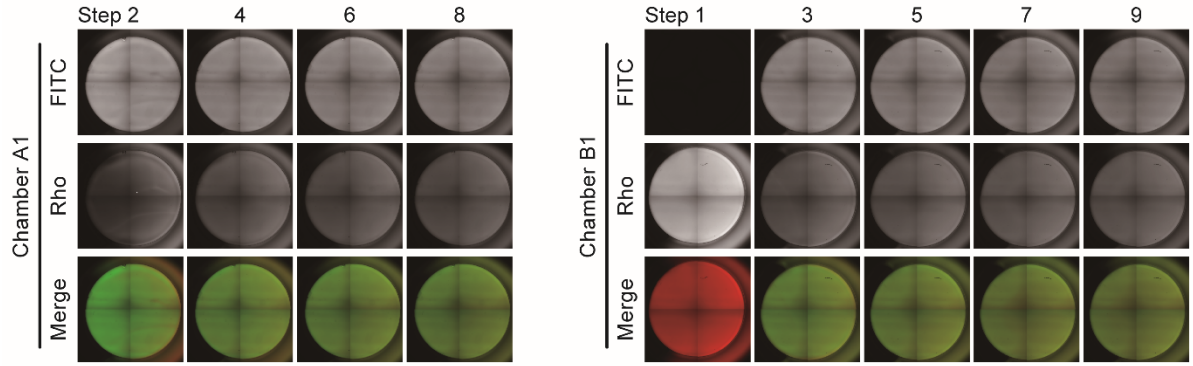

Fig. S5 Composite images from overhead views of wells A1 and B1 after completion of medium transfer at each step during medium circulation in the two-organ system.

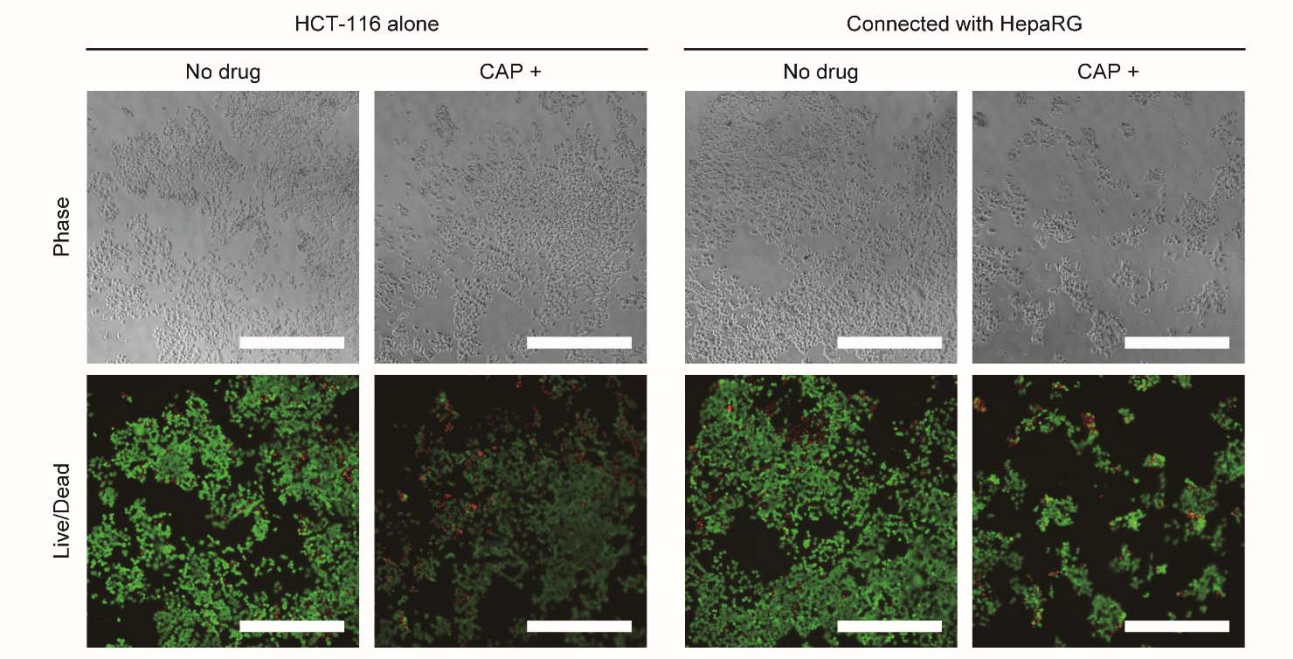

Fig. S6 Enlarged images of Fig. 4b. Scale bars: 500 µm.

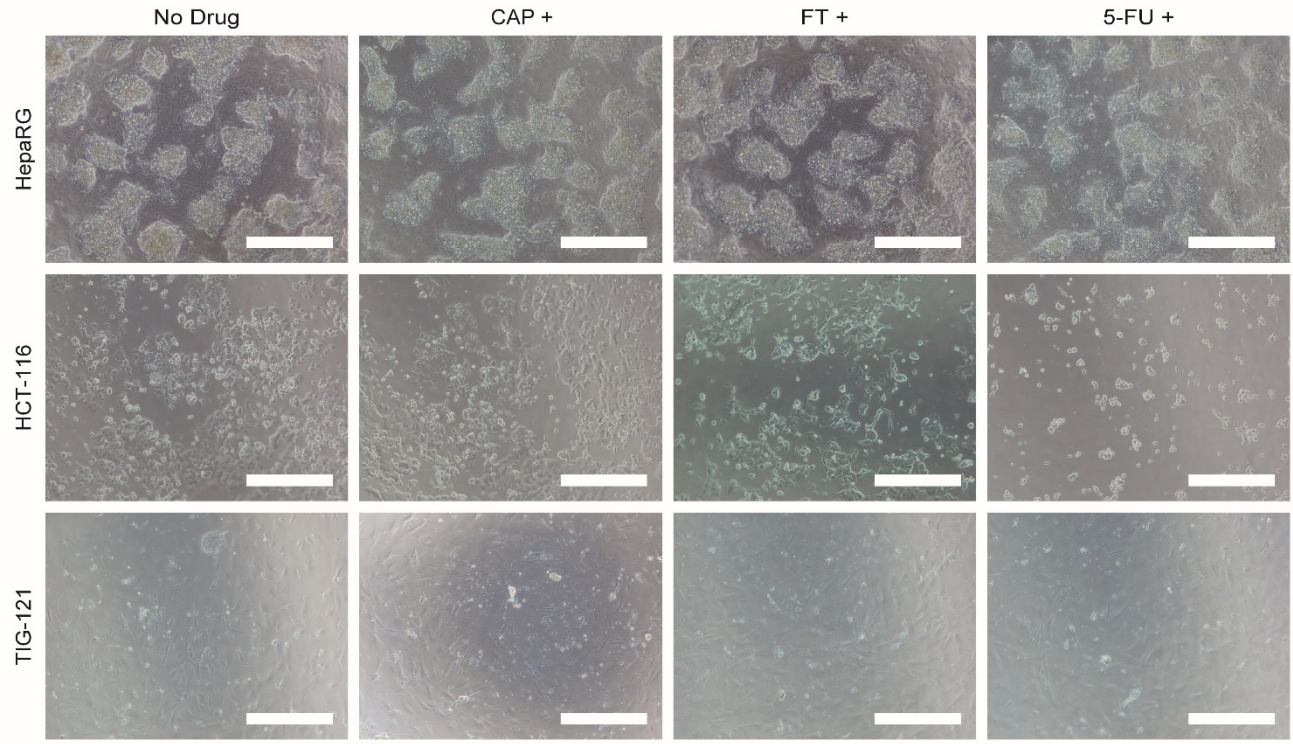

Fig. S7 Enlarged images of Fig. 5b. Scale bars: 500 µm.

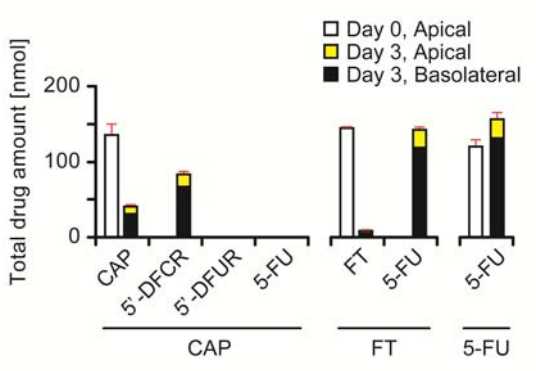

Fig. S8 Degradation and absorption of CAP, FT, and 5-FU on the PDMS microfluidic plates for 3 days of circulation without cells. Total drug amount was calculated from results of LC-MS analysis considering the volume of the medium in the apical side (200 μL in apical side of chamber A) and basolateral side (1,200 μL in basolateral side of chamber A and chamber B, C, and D) (n = 2, ± S. D.). Two thirds of CAP were converted into 5'-DFCR during the circulation without cells. Most of the FT was converted into 5-FU. Most of the 5-FU was detected as it was. Total mass balance of the drugs suggests that absorption of those on the PDMS microfluidic plates was not major.

Table S1 List of primers used in the RT-PCR

Gene	Primer	Catalog number
carboxylesterase 1 (*CES1*)	Hs_CES1_1_SG QuantiTect Primer Assay	QT00998501
carboxylesterase 2 (*CES2*)	Hs_CES2_1_SG QuantiTect Primer Assay	QT00037443
cytidine deaminase (*CDA*)	Hs_CDA_1_SG QuantiTect Primer Assay	QT00227150
albumin (*ALB*)	Hs_ALB_1_SG QuantiTect Primer Assay	QT00063693
glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)	Hs_GAPDH_1_SG QuantiTect Primer Assay	QT00079247