Supplementary Information

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1. Incorporation of hydrogel cell culture and assembly of a µCCA

The device assembly process begins by placing the silicone gasket and the polycarbonate base frame on top of the aluminum frame (Figure S1). Then the cell culture chamber layer was placed on top. To prevent wrinkling of a 0.25 mm thin cell culture chamber layer, a drop of 95% ethanol was placed on top of the polycarbonate base before placing the chamber layer, and was evaporated in a hot oven. The assembled aluminum bottom frame, silicone gasket, polycarbonate base, and the cell culture chamber layer were sterilized by autoclaving. Cells were prepared and resuspended in 2% (w/v) alginate solution at 4x10⁶ cells/ml. The alginate solution was made by dissolving alginate powder (10/60 LF sodium alginate, FMC biopolymer) in a HEPES buffer (15 mM HEPES, 92 mM sodium chloride), and filter-sterilizing with 0.2 µm pore size syringe filter (VWR scientific, West Chester, PA). Cell-alginate suspensions of three cell types were prepared (HepG2/C3A, HCT-116, Kasumi-1), and 10 µl of each cell suspension was placed in corresponding cell culture chamber. A porous polycarbonate membrane filter (8 µm pore size, VWR Scientific, West Chester, PA) was placed on top to flatten the alginate, and sterile-filtered 30 mM calcium chloride solution was applied onto each chamber to polymerize the hydrogel. After 30 minutes of incubation at room temperature, calcium chloride solution and the membrane filter

were removed. Then the top surface was flooded with 1 ml of DPBS, and the fluidic channel layer was placed on top, with channels facing down.



Figure S1. Hydrogel-encapsulated cells are seeded into corresponding holes on a cell culture chamber layer, and flattened with a porous polycarbonate membrane. Alginate gel is formed by wetting with calcium chloride solution for 30 minutes. After gel formation the membrane is removed and the channel layer is closed on top, and the top layer is closed for sealing.

Sealing of a multi-layered μCCA

To verify the sealing of the device in the presence of hydrogel, fluorescent beads were encapsulated in 2% alginate and inserted into the device. After device assembly, fluorescein solution was inserted into the device to visualize the channels, and the interface between the channels and hydrogel.



Figure S2. (a) A bright-field picture of the assembled μ CCA. A cell culture chamber and channels are shown on the left side of the picture (b) A fluorescein solution was inserted to visualize the channel and test sealing. Channels are shown in bright green. Fluorescein also diffuses into alginate, staining them in

light green. (c) A cell culture chamber is visualized with red fluorescent beads, which were mixed with alginate and inserted into a μ CCA.

2. Cell viability after long-term culture in a μCCA

To verify the long-term operation of a μ CCA, cells were cultured in the device for five days with a daily medium replenishment. Cells maintained high viability for five days, verifying that the nutrient depletion and waste accumulation in the cell culture medium is the main cause of cell death in case the medium was not replenished.



Figure S3. Live/dead staining result of cells cultured in a micro CCA for five days with daily medium change.

 Comparison of pharmacokinetic profile of a prodrug and its metabolite in two different modes of recirculation

Utilization a rocking platform and gravity-induced flow results in a different recirculation pattern from the recirculation using a peristaltic pump. The direction of the flow changes every three minutes, rather than forming a continuous, closed-loop recirculation. The pharmacokinetic profiles of a hypothetical prodrug (A), and its metabolite (B) were simulated with a mathematical model representing the two modes. Each compartment was segregated into five sub-compartments, since using well-mixed assumption for the compartment neglects any difference that may be caused the reciprocating recirculation. In the first model (Figure S4(a)), a continuous recirculation is shown, where the flow coming out of each compartment goes back into the reservoir for recirculation. In case of gravity-induced flow, the direction of the flow changes every time the rocking platform changes its angle, which is about every three minutes (Figure S4(b). A mathematical model describing each situation was constructed, and a conversion of A into B was simulated using same parameters (Table S1). In both cases, the rate of depletion of A and synthesis of B were almost identical. Additionally, the rocking platform continuously changes its angle during the three minutes, thus effectively changing the flow rate, until it changes the direction of the flow. However, this aspect was neglected in the model, and the constant flow rate was assumed during each three-minute period.



Figure S4. The effect of recirculation mode on the pharmacokinetic profile of a prodrug (A) and a drug (B), with a simple conversion reaction A->B. (a) Continuous, closed-loop circulation mode (b) Reciprocated circulation mode (c) Concentration of a prodrug (A) (d) Concentration of a drug (B), generated from a prodrug.

4. Equations for the PBPK model of a micro CCA

5-FU in blood (reservoir)

$$V_{B} \cdot \frac{dC_{FU,B}}{dt} = Q_{L} \cdot C_{FU,L} + Q_{T} \cdot C_{FU,T} + Q_{M} \cdot C_{FU,M} - Q_{B} \cdot C_{FU,B}$$

5-FU in liver

$$\mathbf{V}_{L} \cdot \frac{\mathbf{d}\mathbf{C}_{FU,L}}{\mathbf{d}\mathbf{t}} = \mathbf{Q}_{L} \cdot \mathbf{C}_{FU,B} - \mathbf{Q}_{L} \cdot \mathbf{C}_{FU,L} - \frac{\mathbf{V}_{m,FU} \cdot \mathbf{C}_{FU,L} \cdot \mathbf{V}_{L}}{\mathbf{K}_{m,FU} \cdot \left(\mathbf{1} + \frac{\mathbf{C}_{U,L}}{\mathbf{K}_{L,U}}\right) + \mathbf{C}_{FU,L}}$$

5-FU in tumor

$$\mathbf{V}_{\mathrm{T}} \cdot \frac{\mathbf{d}\mathbf{C}_{\mathrm{FU,T}}}{\mathbf{d}\mathbf{t}} = \mathbf{Q}_{\mathrm{T}} \cdot \mathbf{C}_{\mathrm{FU,B}} - \mathbf{Q}_{\mathrm{T}} \cdot \mathbf{C}_{\mathrm{FU,T}}$$

5-FU in marrow

$$\mathbf{V}_{\mathbf{M}} \cdot \frac{\mathbf{d}\mathbf{C}_{\mathbf{FU},\mathbf{M}}}{\mathbf{d}\mathbf{t}} = \mathbf{Q}_{\mathbf{M}} \cdot \mathbf{C}_{\mathbf{FU},\mathbf{B}} - \mathbf{Q}_{\mathbf{M}} \cdot \mathbf{C}_{\mathbf{FU},\mathbf{M}}$$

Uracil in blood (reservoir)

$$\mathbf{V}_{\mathbf{B}} \cdot \frac{\mathbf{d}\mathbf{C}_{\mathbf{U},\mathbf{B}}}{\mathbf{d}\mathbf{t}} = \mathbf{Q}_{\mathbf{L}} \cdot \mathbf{C}_{\mathbf{U},\mathbf{L}} + \mathbf{Q}_{\mathbf{T}} \cdot \mathbf{C}_{\mathbf{U},\mathbf{T}} + \mathbf{Q}_{\mathbf{M}} \cdot \mathbf{C}_{\mathbf{U},\mathbf{M}} - \mathbf{Q}_{\mathbf{B}} \cdot \mathbf{C}_{\mathbf{U},\mathbf{B}}$$

Uracil in liver

$$\mathbf{V}_{L} \cdot \frac{\mathbf{d}\mathbf{C}_{\mathbf{U},L}}{\mathbf{d}\mathbf{t}} = \mathbf{Q}_{L} \cdot \mathbf{C}_{\mathbf{U},B} - \mathbf{Q}_{L} \cdot \mathbf{C}_{\mathbf{U},L} - \frac{\mathbf{V}_{\mathbf{m},U} \cdot \mathbf{C}_{\mathbf{U},L} \cdot \mathbf{V}_{L}}{\mathbf{K}_{\mathbf{m},U} \cdot \left(\mathbf{1} + \frac{\mathbf{C}_{\mathbf{U},L}}{\mathbf{K}_{\mathbf{I},FU}}\right) + \mathbf{C}_{\mathbf{U},L}}$$

Uracil in tumor

 $\mathbf{V}_{\mathrm{T}} \cdot \frac{\mathbf{d}\mathbf{C}_{\mathrm{U},\mathrm{T}}}{\mathbf{d}\mathbf{t}} = \mathbf{Q}_{\mathrm{T}} \cdot \mathbf{C}_{\mathrm{U},\mathrm{B}} - \mathbf{Q}_{\mathrm{T}} \cdot \mathbf{C}_{\mathrm{U},\mathrm{T}}$

Uracil in marrow

 $V_{M} \cdot \frac{dC_{U,M}}{dt} = Q_{M} \cdot C_{U,B} - Q_{M} \cdot C_{U,M}$

- FU: 5-FU, U: uracil, B: blood, L: liver, T: tumor, M: marrow
- $C_{a,b}$: Concentration of a in compartment b.
- V_a: Volume of compartment a
- Q_a: Flow rate into compartment a

5. Equations for a PD model



 C_n : Number of cells in the n_{th} transit compartment.

K_{max}: Maximum rate of cell death progression

KC₅₀: Saturation constant

FU: 5-FU

tau: Time constant for cell death progression

 k_d : Natural cell death rate

C_{ss}: Maximum cell number that can be reached

K_g: Cell proliferation rate

Name	Value	Unit
V _{liver}	11.3x10 ⁻³	ml
V _{tumor}	11.3x10 ⁻³	ml
V _{marrow}	11.3x10 ⁻³	ml
V _{blood}	1.5	ml
Q _{liver}	87.6	μl/min
Q _{tumor}	26.7	μl/min
Q _{marrow}	35.6	μl/min
Q _{blood}	149.9	μl/min
V _{m1}	80	nmol/min
K _{m1}	2700	nmol/ml
V _{m2}	0.5	nmol/min
K _{m2}	40	nmol/ml

Table S1 Parameters for a PK model used in the supplementary information