1 **Image-based evaluations of distribution and cytotoxicity of Irinotecan (CPT-11) in**
2 **a multi-compartment micro-cell coculture device**

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13 Short title: multi-compartment micro-cell coculture device

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15 coculture

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ABSTRACT

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2 We recently developed a polydimethylsiloxane (PDMS)-based three-compartment
3 microfluidic cocultivation device enabling realtime interactions of different cell
4 populations as an advanced physiologically-relevant cell-based assay. This device had
5 valves and small magnetic stirrer-based internal pumps for easy and flexible perfusion
6 operations. In this study, we applied this device for the evaluation of Irinotecan
7 (CPT-11) toxicity to the lung, because it is detoxified by the liver and accumulated in
8 the fat in humans. We successfully cultured representative three different tissue model
9 cells in each compartment under the individual culture conditions and also in entire
10 perfusion. Growth inhibition of rat lung epithelial cell line L-2, was measured when
11 administered with 50 μ M CPT-11 under various cocultivation conditions with respect to
12 the presences and absence of primary rat hepatocytes (liver tissue model) and
13 adipocyte-like cells (fat tissue model) induced from a mouse fibroblast cell line, 3T3-L1.
14 Although CPT-11 showed moderate toxicity to the pure culture of L2 cells in the device
15 after 72 hours of perfusion culture, this was lowered mainly in the presence of the liver
16 tissue. Inhibition of the L-2 cell growth agreed with the area under curve (AUC)
17 values obtained from fluorescent image-based analyses in each compartment. These
18 results demonstrate that developed simple and flexible microfluidic cocultivation device,

with appropriate image-based analyses, can be used in evaluating toxicokinetic behaviors of drug candidates in systemic levels.

Introduction

Integration of biological information obtained in cultured cell-based *in vitro* toxicological tests with whole-body numerical simulations such as physiologically-based toxicokinetic/toxicodynamic (PBTk/TD) model is proposed to be a future methodology alternative to animal experiments for toxicokinetic study in broad field such as pharmaceutical, food, industrial or environmental applications (1-4). However, unknown mechanisms such as those resulting from realtime organ-to-organ interactions are not considered in this method, although such multi-organ metabolic interactions led to increases in induced drug metabolism capacity or growth activity, which is keeping the homeostasis of human or animal body are important when we predict toxicity to a whole body (5-9).

Microfluidic technologies are suitable for making new *in vitro* toxicological tests where cells from different organ can be cocultured with real-time interactions by a perfusion of culture medium as mimicry of systemic blood circulation. Shuler and co-workers have been developing micro-cell culture analogs (μ CCA) that have several

1 compartments for individual organ derived cells and channels for mimicking the blood
2 circulation systems (5-9) where lung, liver, fat, gastrointestinal tissue models are
3 included. Although their studies are really pioneer works, there are still rooms that
4 should be improved such as device disposability or operation flexibilities of their device,
5 their reactors are not designed to be disposable or to have high flexibilities in terms of
6 the perfusion modes such as individual or whole perfusion modes. The latter is
7 particularly important because cells of each tissue model sometimes needs precultures
8 for induction of maturations before complete perfusion of the whole device for
9 evaluation of systemic level toxicities.

10 To better meet the demands of the applications in toxicological screening,
11 polydimethylsiloxane (PDMS)-based micro-bioreactors is promising because PDMS is
12 a biologically inert, transparent and a highly-oxygen-permeable material that can be
13 compatible with a mold-based microfabrication technology (10, 11). Recently, we
14 incorporated micro-stirrer bar-based pumping systems for complete on-chip perfusion in
15 a PDMS-based three compartment micro-cell culture device (12). This does not only
16 improve the device flexibility for multiple operating conditions as it enables
17 independent control of the flow rate of each compartment but also in enhancing the
18 disposability because the device material is cheap and because the pumping system is

1 completely put on-chip. We previously reported fabrication of such disposable
2 three-compartment devices and stable adipocytes culture using an internal pumping
3 system. However, evaluation of the toxicokinetic behavior of drugs was not achieved
4 in the previous study (13).

5 In this article, as a demonstration of applicability of developed device, we
6 evaluated the distribution and resulting growth inhibition of rat lung epithelial cell line
7 L-2 by Irinotecan (CPT-11) used as a model chemical with or without primary
8 hepatocytes as a liver tissue model and adipocytes induced from mouse fibroblast
9 3T3-L1 cells as a fat-tissue model in 72 hours of real-time perfusion coculture. The
10 flexibility of the device with respect to the perfusion operation mode enables
11 independent precultures based on different protocols for each tissue model before
12 starting whole perfusion for toxicological experiment. CPT-11 is a model chemical
13 suitable for evaluating toxicokinetic behaviors because it is an anti-cancer reagent that
14 inhibits cell proliferation by its metabolite SN-38, which is produced by intracellular
15 carboxylesterase and specifically inhibits the binding topoisomerase to cellular DNA
16 (14, 15). These chemicals, CPT-11 and SN-38, are also useful for visualization of their
17 distributions in terms of their fluorescence. CPT-11 is also known to be metabolized in
18 the liver and accumulated in the fat-tissue due to its hydrophobic nature (16-19).

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Material and method

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Device fabrication, conditioning and operation

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Device design and photograph of the fabricated device is shown in Fig. 1. ← FIG. 1

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The device has three separated compartment as cell culture chamber for different tissue

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cell model. These compartments have two access ports for cell inoculation and

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medium exchange and connected by silicone tube based valves in perfusion circuit. In

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addition, each compartment has also internal pumping system based on rotating

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stirrer-bar consists of stainless steel (12). Cell culture chambers were designed to have

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1 cm in length and 1 cm in width. All cell culture chambers were connected by the 1

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mm width cannels. Silicone tube-based valves were 1 mm in diameter and 1 cm in

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length. This device was fabricated by the replica molding method following the

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previous report (12). Briefly, we prepared a master mold with negative photo resist

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SU-8 on the silicon wafer where designed channels and chambers were irradiated by

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UV light through the photo-mask. PDMS resin and cross linker were dipped on the

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master mold and then heated in the oven. Solidified PDMS having channels and

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chambers was peeled off from the master mold and bonded with flat PDMS plate after

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O₂ plasma treatment.

1 This device can select two operation modes in terms of perfusion using the
2 silicone tube valves and the internal pumping system. The first operation mode is the
3 individual perfusion mode for model cell attachment and induction of differentiation,
4 with silicon tube valves being closed. The second operation mode is the entire
5 perfusion mode for real-time perfusion coculture, with silicon tube valves being opened.
6 The device was sterilized using 70 % ethanol and filled with a 1 mM HCl (Wako Pure
7 Chemicals; Osaka, Japan) water solution including 0.3 mg mL⁻¹ collagen. Once
8 collagen was physically absorbed onto the PDMS surface, it was washed in 10 mM of
9 phosphate buffer (pH = 7.4). The device was filled with cell culture medium. L-2
10 cells and 3T3-L1 cells were purchased from JCRB cell bank (JCRB9053, IFO50416,
11 National Institute of Medical Innovation; Osaka, Japan).

12 **Fluorescent labeling and device based culture of L-2 cells**

13 L-2 cells used as target tissue cell model were cultured on the tissue culture
14 plastic in Dulbecco's modified eagle medium supplemented with 20 mM HEPES, 100
15 units-penicillin/mL, 100 µg streptomycin/mL, 1 mg amphotericin/mL, non-essential
16 amino acid (Wako Pure Chemicals; Osaka, Japan). Medium exchange was every other
17 day. For long-term monitoring of maintenance or growth of L-2 cells on the surfaces
18 of the lung tissue compartment, the cells were labeled as red with 10 µM fluorescent

dye, Cell tracker, CMTMR (Invitrogen; CA, USA). Then, the labeled cells were inoculated at 1×10^4 cells/cm² in the lung compartment of the device from the access port and the inoculated cells were cultured in the individual perfusion mode for 24 hours before chemical loading experiments. Number of the cells attached was detected at 540 nm excitation wavelength and 565 nm emission wavelength, respectively.

Culture and adipocyte induction of 3T3-L1 cells in the device

3T3-L1 cells used as fat-tissue cell model were cultured on the tissue culture plastic in Dulbecco's modified eagle medium supplemented with 20 mM HEPES, 100 units-penicillin/mL, 100 µg streptomycin/mL, 1 mg amphotericin/mL, non-essential amino acid (Wako Pure Chemicals; Osaka, Japan). Medium exchange was every other day. Harvested 3T3-L1 cells from confluent culture were inoculated at 1×10^5 cells/cm² in the fat-tissue compartment of the device by the access port. Inoculated cells were cultured in the individual perfusion mode for 24 hours. For the induction of differentiation to adipocytes, cultured 3T3-L1 cells were treated with 125 µM insulin (Takara Bio Inc.; Siga, Japan), 1 µM dexamethason and 1 µM Indomethasin (Wako Pure Chemicals; Osaka, Japan) in the individual perfusion mode for 2days (8).

Isolation and culture of rat hepatocytes

A male Wistar rat aged 5–8 weeks was anaesthetized and its hepatocytes were

1 isolated by the collagenase perfusion method (20). Viability was determined by the
2 trypan blue exclusion method and hepatocytes with over 85% viability were used as
3 liver tissue model cell. Cells were inoculated and cultured at a cell density of 1.2×10^5
4 viable-cells/cm² in the collagen-coated liver compartment by the access port in the
5 device and cultured in individual perfusion mode for 24 hours before chemical loading
6 experiments. Dulbecco's modified Eagle high glucose medium (Invitrogen; CA,USA)
7 was chosen as a base and supplemented with 20 mM HEPES (Dojindo Laboratories;
8 Kumamoto, Japan), 100 units-penicillin/ml (Wako Pure chemicals; Osaka, Japan), 100
9 μ g streptomycin/ml (Wako Pure Chemicals; Osaka, Japan), 1 μ g amphotericin/ml
10 (Wako Pure Chemicals; Osaka, Japan), non-essential amino acid (Gibco; USA), 10
11 ng/ml mouse epidermal growth factor (EGF) (Takara Bio Inc.; Siga, Japan), 0.1 μ M
12 insulin (Takara Bio Inc.; Siga, Japan), 0.1 μ M dexamethasone (Wako Pure Chemicals;
13 Osaka, Japan), 0.8 μ M copper sulfate (CuSO₄, 5H₂O) (Wako Pure Chemicals; Osaka,
14 Japan), 20 nM selenious acid (H₂SeO₃) (Wako Pure Chemicals; Osaka, Japan), 2.6 μ M
15 zinc sulfate (ZnSO₄, 7H₂O) (Wako Pure Chemicals; Osaka, Japan), and 0.3 μ M
16 manganese sulfate (MnSO₄, 5H₂O) (Wako Pure Chemicals; Osaka, Japan) (21, 22).
17 Morphologies of cells were observed by phase-contrast microscopy DMIRB (Leica
18 Microsystems; Tokyo, Japan).

1 **Toxicokinetic experiment of CPT-11 in the device**

2 CPT-11 is known as hydrophobic anti-cancer reagent having fluorescence. Its
3 metabolic pathway is shown in Fig. 2. Its cytotoxic effects are exerted through ← FIG. 2
4 interaction with the topoisomerase I-DNA complex, eventually leading cell death (14,
5 15). SN-38 (7-ethyl-10-hydroxy-camptotecin), one of CPT-11 metabolic derivatives,
6 has considerable cytotoxicity and is thought as a predominant toxicity derivative
7 produced by carboxylestrase in the cells (16, 17). In the liver, CPT-11 is also
8 metabolized to non-toxic derivatives; APC
9 (7-ethyl-10-[4-N-(5-aminopentanoicacid)-1-piperidino]carbonyloxycamptotecin), NPC
10 ([7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptotecin]) etc., by the cytochrome
11 3A4. In addition, SN-38 is metabolized to glucronized non-toxic metabolite; SN-38G
12 in the liver by (UGT) (18,19). Therefore, we thought L-2 cells as a target tissue model
13 and tried to evaluate effects of cocultivation with rat primary hepatocytes (metabolism
14 process) and induced adipocytes (distribution process) by CPT-11 loading experiment.

15 Before starting entire perfusion, culture mediums for each cell preculture were
16 exchanged with fresh culture medium used for L-2 cell culture but containing 50 μ M
17 CPT-11. We measured distribution of CPT-11 over the device at every 24 hours using
18 a fluorescent image analyzer (LAS-3000, FUJIFILM Corporation; Tokyo, Japan).

Excitation and emission wavelengths are 365 nm and 440 nm, respectively. At least five pictures of randomly-chosen positions in each tissue compartment were taken, and the fluorescence intensity per unit area was calculated and used as an index for distribution and accumulation in each compartment. Cell viability was analyzed in terms of CMTMR fluorescence image observed by confocal microscope (Olympus; Tokyo, Japan) at every 24 hours.

We preliminarily checked fluorescent intensity of CPT-11 and SN-38. Briefly, pure reagents of CPT-11 and SN-38 were dissolved and diluted in cell culture medium at 6.25, 12.5, 25, 50 μ M in micro-vials. Then, fluorescent intensities were measured by the fluorescent image analyzer. The results showed that fluorescent intensities were commensurable with concentrations of CPT-11. We obtained calibration curves of pure CPT-11 and SN-38 in the device and the slope of these curves showed that SN-38 was about nine times higher than that of CPT-11 (data not shown).

We controlled the flow rates for both individual and entire perfusion modes based on the rotating speeds of the stirring bar. The relationship between the flow rates and the rotating speed was previously determined in microdevices having similar dimensions (12). The low rate of each compartment in the individual perfusion mode was set at 6 μ L/min in order to avoid cell detachment which might be caused after the

inoculation. In the entire perfusion mode, however, we differently increased the flow rates in each compartment to reflect the physiological flow rates for each tissue; the rates for lung, liver and fat tissue compartments in the CPT-11 loading experiments were set at 10 $\mu\text{L}/\text{min}$ (1040 rpm), 60 $\mu\text{L}/\text{min}$ (2340 rpm), 6 $\mu\text{L}/\text{min}$ (680 rpm), respectively (23).

Results and Discussion

Cell morphologies of the device

To realize physiologically-relevant coculture systems in microscale, it is ideal to allow preculture of each tissue model cells for their preconditioning such as induction of differentiation, maturation or forming tissue-like structures before starting entire perfusion of the device, where each tissue cell is put in realtime interactions with a relevant chemical. In addition, it is also ideal that we can change operation conditions such as flow rates of each tissue compartment in order to examine various scenarios to determine physiological parameters. To meet these demands, we designed the device so that on the same device we can culture different tissue model cells in respective compartments and cultured simultaneously but under different culture conditions with respect to the flow rates and compositions of medium before starting chemical loading

1 experiment (13).

2 Different culture conditions on the same device (Fig. 3 (A)) were well achieved ← FIG. 3
3 by the developed device having pumps based on small stirrers and valves based on
4 silicone tubes and surgical clips (Fig. 1). L-2 cells were attached well and able to be
5 cultured in the lung compartment (Fig. 3 (B)). L-2 cells proliferated approximately
6 twice in 72 hours perfusion culture without a toxicant (data not shown). Rat
7 hepatocytes were also attached well and formed a continuous monolayer in the liver
8 compartment (Fig. 3 (C)). 3T3-L1 cells were also attached well (Fig. 3 (D)) and on the
9 second day after the induction, they successfully differentiated to adipocytes-like cells
10 exhibiting fat droplets in the cytosol stained by the oil red O assay (Fig. 3 (E)).

11 Shuler's μ CCA cannot realize different culture conditions for each tissue
12 compartment by independent perfusion on the same device because there are neither
13 valves nor micro-pumping systems in their device: the entire circuit was connected to
14 one external syringe pump and thus the flow rate of each compartment was implicitly
15 adjusted according to the differences in the pressure drops of each compartment. Our
16 device has really has an advantage in terms of the operation flexibility over such
17 existing μ CCA devices because it enables parallel employment of different preculture
18 conditions for each tissue cells on the same device.

Image-based analyses of CPT-11 distribution in the device

We initially checked chemical fluorescence relevant to CPT-11 and its derivatives SN-38 by measuring the fluorescent intensities of the two pure chemicals. Measured values were well explained by the fluorescence of CPT-11 and SN-38, although specific molar fluorescence was different (see Materials and Methods). In addition, in actual perfusion experiments using the devices, we checked background fluorescent intensities resulting from possible auto-fluorescence of cells in each compartment and adsorption of CPT-11 in the surface of the compartments.

Fig. 4 shows auto-fluorescence of each tissue model cell (Fig. 4 (A)) and fluorescent intensity of adsorbed chemicals in the liver and fat tissue compartments (Fig. 4 (B)). Auto-fluorescent intensity of L-2 cells and Induced adipocyte-like cells from 3T3-L1 was remarkably lower than that of rat hepatocytes (Fig. 4 (A)). This is because rat hepatocytes are larger than other tissue model cells and because they contain well developed intracellular organelles and granules. Adsorptions of chemical in liver and fat tissue compartment are also shown in Figure 4 (B). This result showed that chemical adsorption surely occurred in the current PDMS-based device and that the adsorption amount was particularly higher in the liver compartment presumably due to its high flow rate: the flow rates were 60 $\mu\text{L}/\text{min}$ in the Liver compartment whereas 10

1 $\mu\text{L}/\text{min}$ in the Lung compartment and $6 \mu\text{L}/\text{min}$ in Fat tissue compartment. According
2 to these results, we decided to consider the cellular auto-fluorescences and the
3 fluorescences caused by the chemical adsorptions onto the inner PDMS surfaces when
4 we evaluate the chemical distribution based on fluorescent images.

5 Fluorescent intensity in each compartment during the chemical loading
6 experiment is shown in their net values in Figure 5. We subtracted autofluorescence ← FIG. 5
7 background from the observed fluorescence only when each model cells were attached
8 in respective compartments. Otherwise, we subtracted adsorption background
9 whenever respective compartments were empty. Because the background values
10 determined as in Figure 4 were higher than those obtained in the empty compartments,
11 net fluorescent intensities in each compartment took sometimes minus values after
12 subtracting the fluorescent caused by the adsorption (Fig. 5 (B) and (C)). We
13 unavoidably applied collagen coating to enhance cellular attachment to the PDMS
14 surfaces, but it might enhance the non-specific adsorption of the chemicals. Anyway,
15 when lung tissue model cells were cultured alone, fluorescent intensity in the lung
16 compartment was higher than that of incorporation with liver or fat tissue model cells
17 (Fig. 5 (A)). Fluorescent intensity of lung compartment in the L-2 cells cocultured
18 with induced adipocyte-like cells exhibited behaviors similar to that in L-2 cell culture

1 alone during 72 hours of perfusion culture. In contrast, fluorescent intensity of lung
2 compartment in the L-2 cells cocultured with hepatocytes and/or adipocytes exhibited
3 lower than that in L-2 cells culture alone (Fig. 5 (A)), indicating the deeper involvement
4 of the liver tissue compartment in the CPT-11 metabolism. However, further details of
5 real-time distribution of CPT-11 and its metabolites need to be measured by
6 conventional analysis systems such as high performance liquid chromatography (HPLC)
7 for additional information as a future issue.

8 Fluorescence-based area under the curve (AUC) values in the lung tissue
9 compartment as a target tissue in this study under each culture condition were calculated
10 from the data presented in Fig. 4 (A), (B) and Fig. 5 (A), and are shown in Figure 5 (D).
11 AUC in L-2 cell pure culture was significantly higher than that of liver tissue model
12 cells existing in the device, although AUC in L-2 cells cocultured with induced
13 adipocyte-like cells had no significant differences compared with that in L-2 cell culture
14 alone (Fig. 5 (D)). AUC values varied in each cell combinations and coculture
15 condition having hepatocytes in the liver tissue compartment showed significantly
16 lower values. This clearly showed that hepatocytes sufficiently decreased CPT-11
17 disposition in other compartments: hepatocytes presumably metabolized CPT-11 to its
18 non-fluorescent metabolic derivatives, although fluorescent derivative SN-38 was also

1 metabolized in the liver compartment. Interestingly, the AUC in the lung compartment
2 cocultured with hepatocytes was lower than that of cocultured with both hepatocytes
3 and adipocyte-like cells. These results indicated that detoxification in the liver was
4 critical phenomena in the coculture condition and that accumulation of CPT-11 in the fat
5 tissue compartment did not contribute so seriously in the current perfusion experiments.

6 **Image-based analyses of CPT-11 cytotoxicity in the device**

7 The changes in the viability of L-2 cells in the lung compartment showed in
8 Figure 6. The viability in L-2 cell culture alone showed a decreased tendency in the
9 first run (Figure 6 (A)) and a significant decrease in the second run when compared with
10 other culture conditions having either liver or fat tissues (Figure. 6 (B)) after 72 hours
11 perfusion with CPT-11-containing medium. This is reasonable because L-2 cells have
12 carboxylesterase in cytosol and not have enzyme which can metabolize CPT-11 to
13 non-toxic derivatives. Therefore, CPT-11 was metabolized to SN-38 in L-2 cells and
14 led to the cell growth inhibition in the L-2 cell pure culture. In contrast, no toxicity
15 was observed in the L-2 cells cocultured with hepatocytes and induced adipocyte-like
16 cells. This is also reasonable because the fluorescence-based AUC value was lower
17 than that of L-2 cell culture alone (Figure 5 (D)). Accumulation of CPT-11 in
18 adipocyte-like cells did not influence the L2 cell viability as expected from the AUC

values (Figure 5 (D)). Although further improvements of device design enabling such as higher cell density and/or 3D structure like human organs are necessary to evaluate more remarkable drug effect to cell growth, these results well matched with the fact that the liver specific enzyme plays important role of detoxification of CPT-11 compared to the *in vivo* studies (16-19).

In this study, we demonstrated practical application of the developed device to toxicological evaluation incorporated with fluorescent image-based analysis. The developed device enables easy controls of several cell culture condition based on on-chip pumps and valves. This point is strong advantage to evaluate many kinds of chemical exposure scenarios in toxicological study. The obtained results overall agreed with knowledge obtained from *in vivo* studies (16-19), although contribution of adipocytes to the distribution was not clear in this study. Chemical distribution and metabolism in the systemic levels will possibly be mimicked by such multi-compartment micro-coculture devices immobilizing respective organ-derived cells with target tissue cells. Such devices also may help development of numerical simulation models such as physiologically based toxicokinetic or toxicodynamic models to better predict chemical effects in whole body levels. This is because such numerical simulation cannot find unknown biological mechanisms but coculture device can do it

(5). One not well-recognized but serious issue of such micro-coculture devices, which is originated from its micro-scale nature, is its unsuitability in performing extensive or time-course analysis of an originally-loaded chemical and its metabolites. Micro-analyses technologies are expected to be developed so that they can work cooperatively with micro-cell culture devices.

In conclusion, we determined CPT-11 toxicity to L-2 cells cultured with hepatocytes and induced adipocytes as a demonstration of pharmacological and toxicological application of the developed device. The developed device equipped with on-chip pumps and valves enabled highly-flexible control of different cell culture conditions simultaneously on the same device before chemical loading in entire perfusion of the device. Integrations of the device experiments with appropriate image-based analyses indicated that overall trend of the results corresponded to the results obtained from *in vivo* pharmacokinetic studies. These results demonstrate that developed simple and flexible microfluidic cocultivation device can be used in evaluating toxicokinetic behaviors of drug candidates in systemic levels.

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Figure legends

FIG. 1. Fabricated three-compartment coculture device. (A) Illustration of the device structure. (B) Representative photograph of the device. (C) Stirrer bar for perfusion culture. Open arrows (A) indicate motors that rotate the magnetic stirrers on the device (C). The channel width and the inner diameter of silicone tube-based valves were all 1 mm. The length and width of each cell culture chamber were 1 mm. Closed arrows (A) indicate the schematic fluid network. Open arrows (B) indicate stirrer-based pumps. Closed arrows (B) indicate cell culture chambers. Dashed circles (B) indicate silicone tube based valves. Scale bar is 1 cm for (A) and (B), and 500 μm for (C).

FIG. 2. Possible metabolic pathway of CPT-11 (18).

FIG. 3. Experimental schedules of preculture (A) and morphologies of the three kinds of cells before chemical loading experiments (B, C, D and E). (B) Rat lung epithelial L-2 cells, (C) primary cultured rat hepatocytes, (D) adipocyte-like cells induced from mouse fibroblast 3T3-L1 cells and (E) oil red O staining of the induced adipocyte-like cells. Scale bar is 100 μm

1 FIG. 4. Background fluorescences of the culture device. (A) Auto-fluorescence of
 2 the tissue model cells without CPT-11 loadings and (B) blank fluorescences derived
 3 from the adsorption of CPT-11 (without cells) onto the bottom surfaces of the cell-free
 4 liver and fat tissue compartments. Each point represents mean $\pm$ SD obtained from at
 5 least 10 images of each compartment in two independent experiments.

6

7 FIG. 5. Changes in the fluorescent intensities in the three-compartments of the
 8 coculture device (A, B and C) and AUC of CPT-11 in the lung-tissue compartment as
 9 the target tissue (D). (A) Lung-tissue compartment, (B) liver-tissue compartment and
 10 (C) fat-tissue compartment. E Each point represents mean $\pm$ SD obtained from at least
 11 ten images of each compartment in two independent experiments. Student's t-test was
 12 performed with only respect to the comparisons with the lung compartment, with $P <$
 13 0.01 (**).

14

15 FIG. 6. Changes in relative L-2 cell viability in CPT-11 loading experiments. (A)
 16 Run 1 and (B) Run2. Relative cell viability calculated compared to negative control
 17 experiment (perfusion experiments without drug). Each point represents mean $\pm$ SD
 18 obtained from five fluorescent images. Student's t-test was performed with only

1 respect to the comparisons with the lung compartment, with $P < 0.01$ (**).

2

A

B

C

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5a

Fig. 5b

Fig. 5c

Fig. 5d

Fig. 6