Supporting Information for

A novel multi-layer microfluidic device towards characterization of drug metabolism and cytotoxicity for drug screening†

Qin Wu,[†abc] Dan Gao,[†bc] Juntong Wei,[abc] Feng Jin,[b] Weiyi Xie,[abc] Yuyang Jiang,*[abd] Hongxia Liu*[bc]

[a]*Department of Chemistry, Tsinghua University, Beijing, 100084, China.*

[b]*State Key Laboratory Breeding Base-Shenzhen Key Laboratory of Chemical Biology, Graduate School at Shenzhen, Tsinghua University, Shenzhen, 518055, China.*

[c]*Key Laboratory of Metabolomics at Shenzhen, Shenzhen, 518055, China.*

[d]*Department of Pharmacology and Pharmaceutical Sciences, School of Medicine, Tsinghua University, Beijing, 100084, P. R. China.*

Reagents and Materials. Silicon wafers were obtained from Xilika Crystal polishing Material Co., Ltd. (Tianjin, China). Negative photoresist (SU-8 2050 and SU-8 2007) and the developer were purchased from Microchem Corp. (Newton, MA, USA). Poly(dimethylsiloxane) (PDMS) and the curing agent were obtained from Dow Corning (Midland, MI, USA). trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane, HLMs (20 mg mL^{-1}), magnesium chloride, D-saccharic acid-1,4-lactone, uridine diphosphate glucuronic acid (UDPGA) and Flavopiridol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). MCF-7, MCF-10A, HepG2 and QSG7701 were obtained from Cancer Institute & Hospital Chinese Academy of Medical Science (Beijing, China). Live/dead viability/cytotoxicity assay kit (Invitrogen, CA, USA) was used for viability tests of the cells. The packaging material for the drug pretreatment was obtained from the SPE C18 (macropore) column (Waters, Milford, MA, USA).

A syringe pump (PHD2000, Harvard Apparatus, MA, USA) was employed to deliver eluting solutions in accurate rates. A plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY, USA) was used for oxygen plasma treatment. A fluorescence microscope (Olympus IX5, Olympus America Inc., Center Valley, PA USA) was used to observe and to obtain images of the microfluidic devices and cells. ESI-Q-TOF detection was performed with a Waters Q-TOF mass spectrometer (Waters, Milford, MA, USA). All mass spectra were obtained in the positive mode. A 250 μL syringe was obtained from Hamilton (Bonaduz AG, Switzerland).

Microfluidic Device Design and Fabrication. The schematic of the multi-layer microfluidic device for simultaneous cytotoxicity assay and drug metabolite characterization is shown in Fig. S1. The device was composed of three different functional parts on two separated units. One unit was composed of five layers for drug metabolism, 3D cell culture, and a drug or metabolites cytotoxicity assay. The top layer containing a straight channel was aligned with the microwells for loading HLMs in the second PDMS layer with 2 mm thickness. The third layer was a PC membrane with 0.4 μm diameter pores sandwiched between the microwells and the inlet of the middle main channel in the forth PDMS layer. The forth PDMS layer was designed for 3D cell culture and drug stimulation. It was composed of a main channel (15 mm length × 0.6 mm width) with a microwell in the upstream, six cell culture chambers (12 mm length × 0.6 mm width) adjacent to the main channel, and corresponding six culture medium channels (12 mm length × 0.6 mm width) adjacent to cell culture chambers all with height of 100 μm which were connected by groups of stop-flow junctions with height of 10 μm (Fig. S1). A microwell in upstream of the main channel was used for inserting a PTFE cylinder to control the fluid in the microreactors flow or stop. The fifth layer was a glass substrate to seal with the forth PDMS layer to form microchannels. The cross section of these channels was shown in Fig. S1b. Another unit was straight channels (22 mm long × 2 mm wide × 80 μm deep) with micropillar arrays (30 μm wide intervals) at the end aimed to immobilize pretreatment materials for desalting and concentrating target molecules prior to MS detection which is shown in Fig. S4. The micropillar arrays was used to act as a weir

for immobilizing pretreatment materials with an average diameter larger than 30 μm. It was made from PDMS and glass as our previously reported.[1]

The PDMS layers were fabricated by replicate molding on silicon wafers. The microchannels with different heights in the PDMS layer was fabricated to use a two steps photolithography technology. The first layer patterns were fabricated by spin coating the negative photoresist SU-8 2007 to generate a 10 μm thick film on a silicon wafer. After patterned by photolithography, the wafer was coated by SU-8 2050 to obtain a 100 μm thick film for a higher structure. The wafer was loaded for 30 min before the second exposure, to avoid an uneven surface. After the exposure and development, a model of a desired microctructure was generated. The master was then silanized by trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane using a vapor method. PDMS base and curing agent were mixed thoroughly (10 : 1 by mass), degassed under vacuum, and poured onto the master. After curing at 80 °C for 2h, the solid transparent PDMS was easily peeled off from the masters. The inlets and outlets in the PDMS were punched with a blunt needle. The piece of PDMS was bonded to a glass slide irreversibly after oxygen plasma treatment for 180 s.

The PC membrane was aligned with a microwell on the PDMS layer using the stamping procedure[2, 3]. Briefly, PDMS prepolymer (10:1 weight mixture of PDMS prepolymer and curing agent) was mixed with toluene in equal ratio and spin-coated (500 rpm for 4s, 1500 rpm for 60 s) onto a glass slide to form a thin PDMS mortar film. A part of the third PDMS sheet containing the inlet was stamped onto the mortar film for 1 min and then positioned mortar side facing up. Membrane edges were

gently dipped onto mortar, and then covered the inlet of the third PDMS layer. The microwells for drug metabolism in the second PDMS layer was finally sealed on the third PDMS sheet with the microwell, membrane and inlet aligned. After assembly of the membrane and PDMS sheets, the device should be left at ambient conditions for 30-40 min before curing to eliminateair bubbles trapped along the edge of the membrane. The device was finally placed in an oven at 80 °C overnight to cure the mortar. The first layer which was used to introduce the drug metabolism system and the microwells was aligned with the second layer by oxygen plasma treatment. Another functional part for sample pretreatment was made from PDMS and glass as previously reported.

Fluorescein Sodium Dffusion in Agarose. In our experiment, because the drug solutions were diffused from the main channels into the 3D cultured cells through the minor connecting channels, the dimensions of the connecting channels had significant influence on the diffusion rate. Therefore, their dimensions were optimized to let the drug metabolites interact with the cells sufficiently. As shown in Fig. S5, three kinds of the minor connecting channels with different widths were designed and optimized. They were 2, 4 and 5 parallel connecting channels with widths of 300 μm (a), 200 μm (b) and 50 μm (c), separately. Fuorescein sodium with comparable weight of FLAP and [glu-FLAP] was used as a detectable indicator to investigate the diffusion rate. Firstly, a portion of 1.5% agarose was injected to the cell culture chamber and was allowed to gel under room tempreture for 10 min. The culture medium was then injected to the medium channel to imitate the drug diffusion microenvironment. 5 μM

fluorescein sodium was finally injected into the middle main channel, and the injection was immediately stopped after the channels were completely filled.

The microscopic image is shown in Fig. S5b. The connection part of main channel and cell culture was presented limited to the viewing field of the microscope. From the microscopic images, the fluorescence intensity of the main channel and cell culture chambers was similar when the diffusion balance reached, while the fluorescence intensity in the connecting channel area was significantly lower because of the lower height of the main channels. Afterwards the fluorescence intensity was evaluated by the software Image-Pro in the red dashed areas shown in Figure S5c. The results demonstrated a completely mass diffusion balance between the main channe and cell culture chambers within 60 min in the design of two parallel connecting channels, which was considered to be the chosen design of microfluidic chips in the following experiments.

Surface Tension Research in Cell Culture Chambers. When cell-agarose mixture was introduced into the chambers, the gas−liquid interface would form naturally and the flow could not go through the minor connecting channel. The vital factor was the surface tension of the mixture at the end positions of the minor channels where the gas−liquid interface existed. To ensure that the designed channels had the ability to prevent cell-agarose mixture from going through the minor channel, we made a theoretical analysis. As shown in Fig. S6, the mixture was assumed to flow uniformly and steadily, thus

$$P_2 = P_s + P_0 = \Delta P_f + P_1 = \Delta P_f + \Delta P_f' + P_0$$

$$P_s = \Delta P_f + \Delta P_f' \quad (1)$$

where P_0 is the atmospheric pressure, P_s is the surface tension induced pressure, and ΔP_f and $\Delta P_f'$ are the pressure drops in the cell culture chambers and the holes, respectively.

In accordance with the Fanning equation

$$\Delta P_f = \frac{\lambda l \rho \mu^2}{2d} \quad (2)$$

$$\Delta P_f' = \frac{\lambda l \rho \mu'^2}{2d} \quad (3)$$

where λ is the Friction coefficient, l is the length between the minor channel and the end of the chanbers (as shown in Fig. S6), d is the equivalent diameter, μ and μ′ are the line velocities in the chamber and outlet hole respectively. d is determined by eq 3:

$$\frac{\pi d^2}{4} = hw \quad (4)$$

h and w are the height and width of the chamber, respectively. The constants, friction coefficient and Reynold's number are

$$\lambda = \frac{64}{R_e} \quad (5)$$

$$R_e = \frac{d \mu \rho}{T} \quad (6)$$

where T is the viscosity coefficient of the solution. When combined eqs 2, 4, 5 and 6

$$\Delta P_f = \frac{8\pi T \mu l}{hw} \quad (7)$$

Although the cross-sectional area of the outlet hole is different from that of the chamber, the volumetric flow rate (v) is same. Thus

$$\mu hw = \frac{\mu' \pi D^2}{4} \quad (8)$$

When we combined eqs 3, 5, 6, and 8

$$\Delta P_f' = \left(\frac{8\pi T \mu H}{hw}\right)\left(\frac{4hw}{\pi D^2}\right)^2 \quad (9)$$

Then, we combined eqs 8, and 9 to get the surface tension induced pressure

$$P_s = \frac{8\pi vT\left[\left(\frac{4hw}{\pi D^2}\right)^2 H + l\right]}{h^2 w^2} \quad (10)$$

In the experiment, the constants are T = 6.95 × 10^{-4} Pa s (37 °C), w = 6 mm, h = 100 μm, l = 2.4 mm, H = 5.0 mm, and D = 0.40 mm. Because all the symbols in eq 10 are constants, the results showed that the surface tension induced pressure of the gas−liquid interface depends on just v. In our experiment, the flow rate (v) of introducing cell-agarose mixture is 10 μL min^{-1}, according to eq 10, the surface tension induced pressure is 0.91 Pa. The pressure is so small compared to the atmospheric pressure that the cell-agarose cannot leak into the main channel and culture medium channels.

Microfluidic Cell Culture. MCF-7, MCF-10A, HepG2 and QSG7701 (Cancer Institute & Hospital Chinese Academy of Medical Science, Beijing, China) were chosen to perform the cytotoxity assays. MCF-7 and HepG2 cells were grown in pH 7.4 growth medium consisting of DMEM supplemented with 10% heat-inactivated fetal calf serum, 100 mg mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin, while the MCF-10A and QSG7701 cells required RP1640 culture medium. All cell types were cultured at 37 °C and 5% CO_2 in a humidified incubator. After being cultured in flasks flasks at 80% confluence, the cells were released from tissue culture flasks by trypsinization with 0.25% Trypsin EDTA. For the 3D cell culture, 100 μL 3% (w/v) low gelling temperature agarose (type VII-A, Sigma-Aldrich, St. Louis, MO) solution in phosphate buffer saline (PBS) which was melted and sterilized by autoclaving, was

used as a support for the cells.[4,5] When cells reached confluence, cells were trypsinized, centrifuged, and resuspended to be 10^6 cells mL^{-1} using the cell culture medium with 20% FBS. Prior to the cell seeding, the whole microfluidic device was sterilized with ultraviolet radiation on a super clean bench for at least 30 min. Same volume of cell suspension and matrix were mixed and then infused into cell culture chambers. The device then was kept at room temperature for 10 min to accelerate gelling of agarose. Cell culture medium was finally filled into the culture medium channels and a thin layer of medium was coated on the surface of device to invade the evaporation of medium in channels. After that, the device was put in a 10 cm cell culture dish and incubated inside a 37 °C incubator with 5% CO_2 humidified air atmosphere for cell culture. The medium was refreshed each day to supply enough nutrients and to wash away the cellular debris and waste. The viabilities of these cultured cells were evaluated using the Live/Dead assay kit after three days cultivation, and the results were shown in the Fig. S7. From the results, it could be obviously seen that the viability of 3D cultured cells was not affected.

Metabolism of Flavopridol in the Microfluidic Device. HLMs (20 mg mL^{-1}, Sigma-Aldrich, St. Louis, MO) were preincubated on ice for 30 min. Afterward, HLMs (1 mg mL^{-1}), magnesium chloride ($MgCl_2$ 10 mM), D-saccharic acid-1,4-lactone (5 mM), and FLAP (final concentration, 5, 10 and 20 μM) in DMSO were added to a 100 mM potassium phosphate buffer (pH 7.4). The reaction was started by the addition of 4 mM uridine diphosphate glucuronic acid (UDPGA).[6] Then 3 μL of the mixture was injected through the inlets in the upper channels and flowed

into the microwells one by one, rapidly. Meanwhile, the PTFE cylinders were firstly inserted in the microwells in the main channels to stop fluid flow to the downstream. After incubation at 37 °C for a certain time, the PTFE cylinder was pulled up and the drug metabolites were injected to downstream of the main channel (Fig. S2). Then a trace amount of metabolites diffused into the cell culture channel for the cytotoxicity assay, while the majority of the metabolites still retained in the main channel for following ESI-Q-TOF MS detection.

Drug Pretreatment by Micro-SPE Column and Analysis by ESI-Q-TOF MS. C18 silica particles were selected as the packing material to desalt and extract FLAP prior to MS detection.[6] Although the C18 packing material had a wide particle size distribution, the designed micropillar arrays with the width of 30 μm at the end of the channels could trap all the materials. As shown in Fig. S3, C18 packing material could be firmly filled inside micro-SPE column, but it also induced a high column back pressure. Moreover, an unexpected sample leakage from microwells through PC membrane to the downstream channel might happen if a higher drug injection flow rate was used. Thus, slow drug injection flow rate of 1 μL min^{-1} was used in all experiments. The channels were conditioned with methanol and water at a flow rate of 10 μL min^{-1} for 5 min respectively. Then 3 μL sample was injected into the micro-SPE column with 1 μL min^{-1}, and the micro-SPE column was washed with 5% (v/v) methanol in water at a flow rate of 10 μL min^{-1} for 10 min to remove any salts. After that, the column was directly connected to the ESI-Q-TOF MS by a fused-silica capillary (i.d., 50 μm; o.d., 365 μm) with poly(tetrafluoroethylene) (PTFE) cannula.

The target, FLAP and its metabolite were eluted by methanol containing 2% formic acid and directly to the ESI-Q-TOF MS for online detection.

Mass Spectrometry. The experiments were carried out on a ESI-Q-TOF MS and operated in the ion spray mode at 3.8 kV in the positive ion mode. The heated inlet capillary was maintained at 120 °C. The sample flow rate for direct infusion was 5 μL min^{-1}. The voltage of the sample cone is 48 V. A coaxial nebulizer N_2 gas flow around the ESI emitter was used to assist generation of ions. All mass spectra were externally calibrated by sodium formate in the negative ion mode with the mass range of *m/z* 50-1000. MS/MS analysis was carried out using argon as a collision gas to fragment precursor ions via collision-induced dissociation prior to mass analysis. Suitable collision energy for small-molecule fragmentation was found by adjusting the intensity of the parent ion and fragmen ion.

Characterization of the Vitality of HLMs. In our experiment, HLMs were immobilization on the membrane, this physical immobilization method could maintain maintain the activity of HLMs without any damage. To test the vitality of HLMs before before and after immobilization, we investigate the metabolism of 10 μM FLAP for 0, 60 and 180 min in and out of the microfluidic device. The same reaction system was prepared in duplicate and one was introduced into the microfluidic device, the other was reacted in centrifuge tube directly to test the vitality. As shown in Table S1, the metabolism status of 10 μM FLAP was nearly the same both inside and outside the microfluidic device. And this demonstrated that, the vitality of HLMs was not damaged damaged after assembly in the microfkuidic device.

Quantify FLAP in the Metabolites. Under the optimum conditions, FLAP with the serially diluted concentrations (1, 5, 10, 25, 50 μM) was used to evaluate the relationship between mass spectrometry signal intensity and drug concentrations. The solutions were then concentrated and detected according to the above described procedures. Because the sample volume in the microchannel was about 3 μL, and 3 μL sample solutions was injected each time and it took about 3 min for sample loading. In order to ensure reproducibility, the sum of the intensity of *m/z* 402.1 was used to generate a calibration line. As shown in Fig. S8, the monitor ion peak intensity (Y) increased linearly with FLAP concentration (X) in the range 1 to 50 μM, and the linear equation was $Y \times 10^4 = 6.662\ X + 6.913$ with a R^2 of 0.991. The assays were performed three times in triplicate to assess the precision.

Evaluation of the Semiquantitative Analysis of FLAP Metabolism. In our study, after FLAP metabolization, the metabolites were injected into the middle channel in the forth layer to permeate into cell culture chambers for cytotoxicity assay. When the balance between middle main channel and cell culture chambers was obtained after diffusion for 60 min, metabolites in the middle channel then were injected into the micro-SPE columns for enrichment. To determining the loss of the metabolites for MS, FLAP metabolite system was directly injected into the micro-SPE columns and then eluted into ESI-Q-TOF MS detection. As shown in Fig. S10, the percentage of drug metabolites which permeated into cell chambers was too little so the loss of metabolites for MS can be ignored.

Cytotoxicity Assay. When the PTFE cylinder in the main channel was pulled up, FLAP and its metabolites were injected into the middle channel and diffused into the cell culture chambers. MCF-7, MCF-10A, HepG2, and QSG7701 cells encapsulated inside agarose gel were cultured within independent culture chambers for drug and its metabolites induced cytotoxicity assay. After 12 h drug exposure, their viabilities were evaluated using a Live/Dead assay kit (Invitrogen, Carlsbad, CA, USA, Ex = 450 − 490 nm, Em = 515 − 550 nm) containing calcein and (Ethd-1). Briefly, the Live/Dead assay kit containing 1 μM calcein and 2 μM ethidium homodimer-1(Ethd-1), prepared according to the manufacturer's instruction, was introduced into the channels and incubated at 37 °C for 60 min to allow the dye penetrate into agarose gel completely. The cells were finally rinsed with PBS and imaged under a fluorescence microscop for image capture. Cell viability was then quantified by counting the live (green) and dead (red) cells using image processing and analysis software (Image-Pro, Media Cybernetics, USA).

MTT Cytotoxicity Assay. All the four cells were harvested and seeded in 96-well plates at the concentration of 7000 cells (MCF-7, HepG2 and MCF-10A) and 10000 cells (QSG7701) per well. FLAP with an final concentration of 5 μM metabolized with the HLMs for 0, 3, 60, 120 and 180 min, respectively. When the reaction was stopped, the solutions were dried by N_2 and redissolved using methanol. Then the samples were diluted to the initial volume by cell culture medium (the final concentration of methanol was less than 0.1%). Cells were cultured in the prepared medium containing FLAP and its metabolites and incubated at 37°C in a 5% CO_2

incubator for 12 h. Medium containing the same concentration of methanol was used as a control. 10 μL MTT solution (5 mg mL^{-1} in PBS) was then added to each well and incubated for 4 h at 37 ºC. 100 μL DMSO was added to dissolve the formazan precipitate and the absorbance at 495 nm was determined using Multimode Detector DTX880 (Beckman Coulter). Compared with the cytotoxicity assays performed on microfluidic device, as shown in Table S2-S5, the obtained cell viabilities of 3D cultured MCF-7 cell in microfluidic device was a little higher than 2D culture mode and the differences of other cells are not obviously, which is in accordance with the previously reported studies.

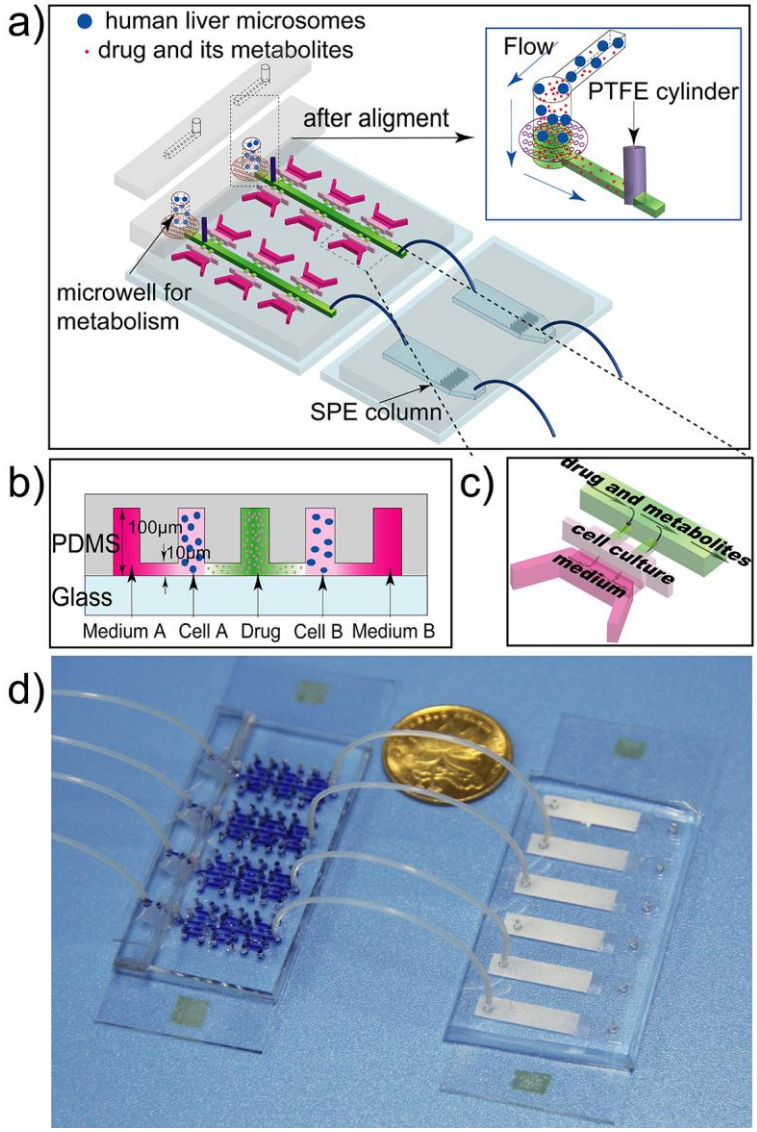

Fig. S1 Microfluidic device for cell culture, cytotoxicity assay and metabolite analysis. a) A schematic drawing shows the integrated microfluidic device containing bioreactor for drug metabolism, 3D cell cultivation modules, and sample pretreatment module prior to ESI-Q-TOF MS detection. The enlarged inset shows the layer-by-layer aligned microstructures. b) Sectional view of the cell culture channel. c) Partial enlarged detail of the cell cultivation modules. d) An image of the microfluidic device filled with blue dyes in the channels of the metabolic and cytotoxicity layers.

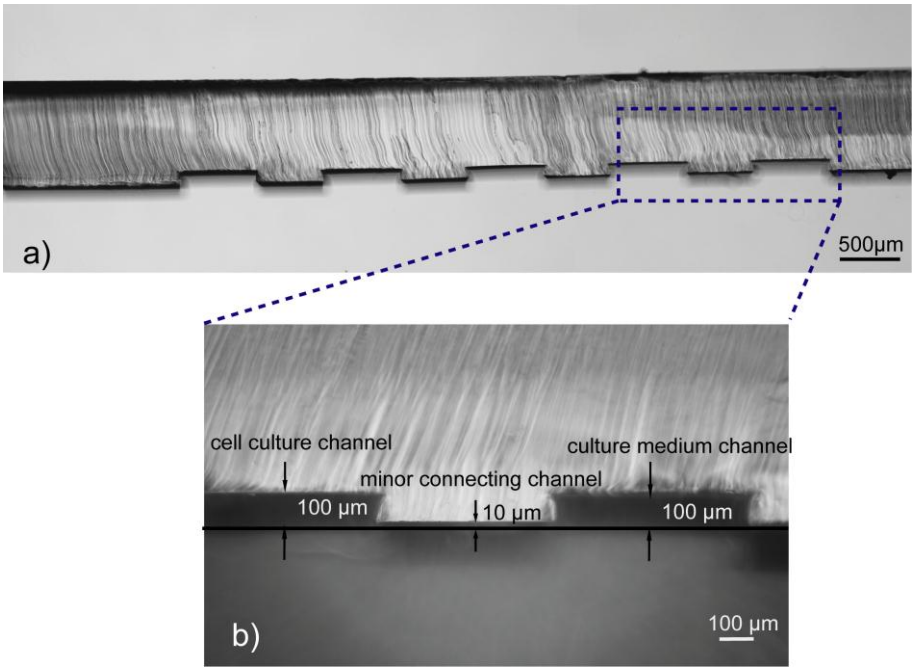

Fig. S2 a) Sectional photo of the cytotoxicity assay channel. b) The depth of the chambers and the minor brige lower channel of the cytotoxicity assay device.

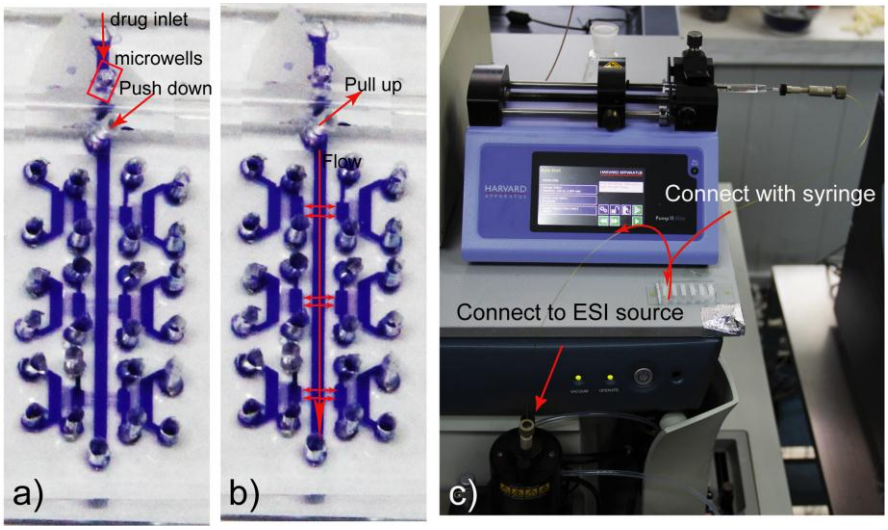

Fig. S3 The operations on the microfluidic chip. a) Operation for FLAP metabolism with the HLMs in the microwells. b) Operations for metabolites cytotoxicity assaya and the final metabolites detection. c) Home-made chip-to-MS. The channels were filled with a blue dye.

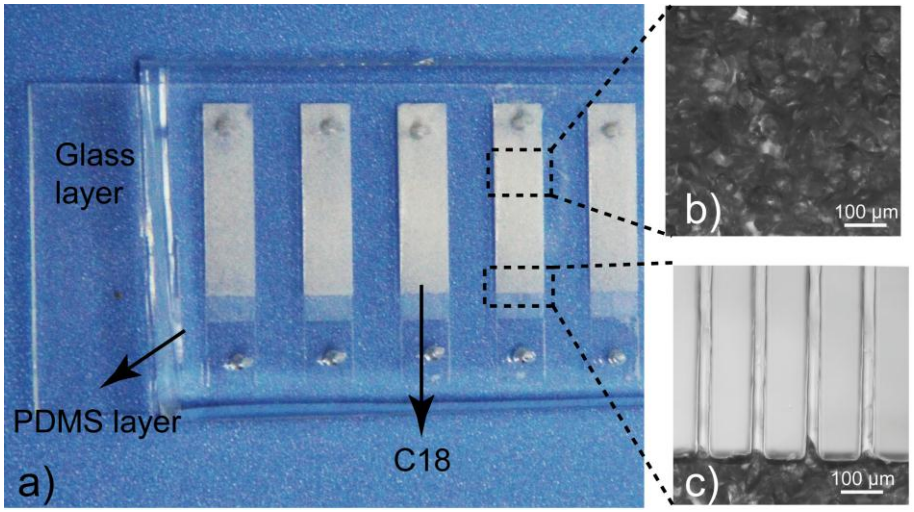

Fig. S4 Integrated on-chip micro-SPE columns. a) Photograph of the on-chip micro-SPE column. b) The triangular end of a micro-SPE column. c) Particles tightly accumulated in the channel.

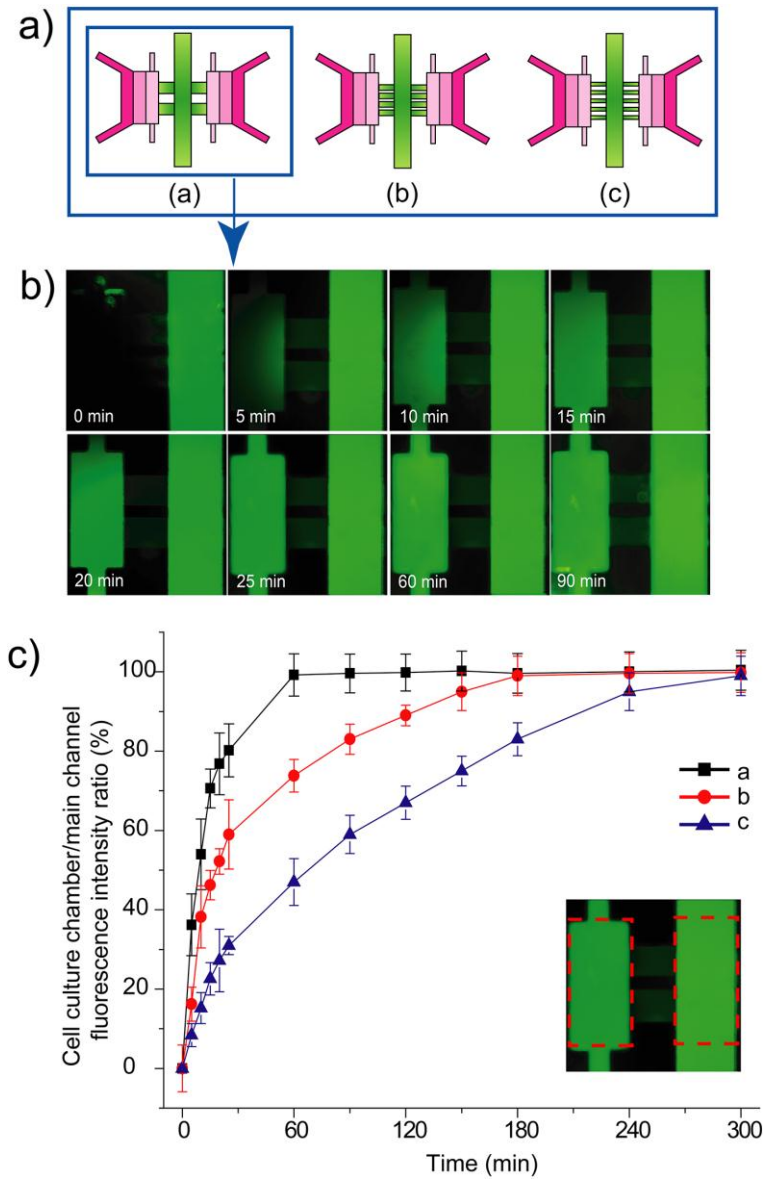

Fig. S5 Optimization of the minor connecting channels by evaluation of the mass diffusion rate between the middle main channel and the cell culture chambers. a) Three designed microchannels with 2, 4 and 5 parallel connecting channels. b) Microscope fluorescence images obtained from 0-90 min after fluorescein sodium was injected into the middle channel. c) The fluorescence intensity in the red dashed areas of fluorescein sodium solutions in the cell culture chambers and the middle main channel along with permeating time were compared and their ratios were quantified.

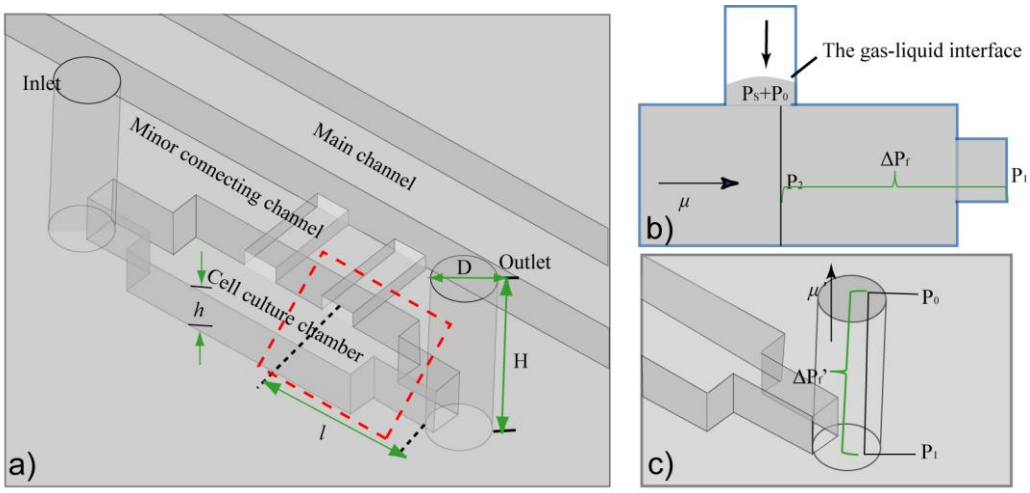

Fig. S6 Design and mathmodeling for the surface tension research. a) Three-dimensional (3D) structure of the main channel and cell cultire chamber connecting with minor channels. b) The horizontal section of the area in the rectangle with a red line in Figure a) are the pressures at different positions of the main cell cultire chamber and the gas−liquid interface when the chamber was filled with agarose. c) The pressures at the bottom and the top of the outlet hole when filled with agarose. P_2, P_1, P_0, and (P_s + P_0) are the pressures of the corresponding positions; ΔP_f and $\Delta P_{f'}$ are the pressure drops in the cell culture chamber and the holes, respectively. The line velocities in the main channel and outlet hole are μ and μ′, respectively.

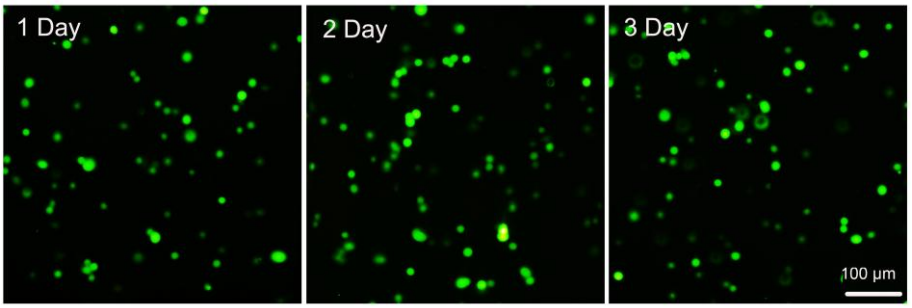

Fig. S7 Analysis of cell viability in the microfluidic device after 3 days cultivation. Fluorescence images show 3D cultured cells stained by Live/Dead assay kit.

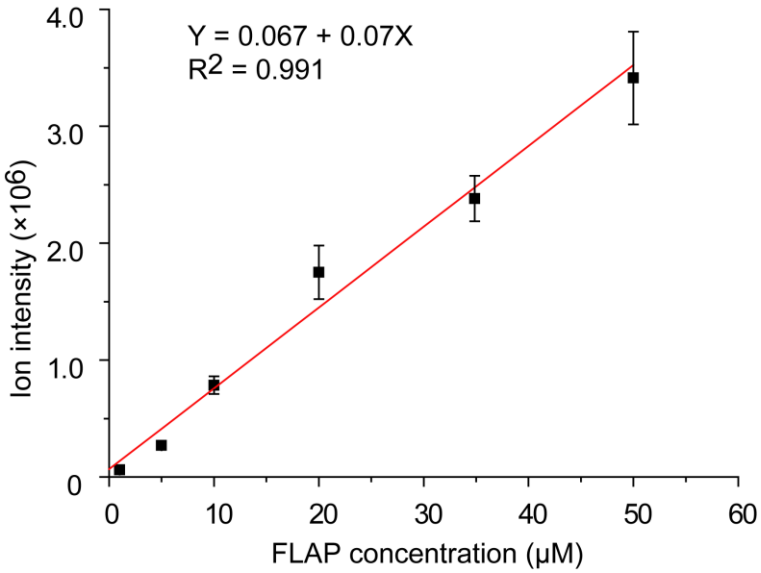

Fig. S8 Relationship between molecular ion intensity and FLAP concentration. The standard error bars are the variation of three individual experiments.

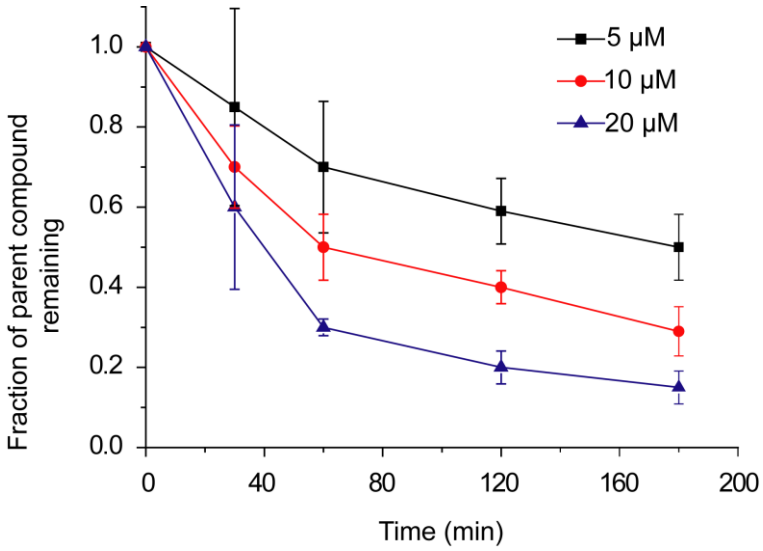

Fig. S9 Metabolic profiles of FLAP by HLMs. Fraction of remaining parent compound with initial concentration of 5 μM (■, low concentration), 10 μM (●, medium concentration), and 20 μM (▲, high concentration) was plotted as a function of metabolic time. The data were presented as a mean ± standard deviation with at least three replicates.

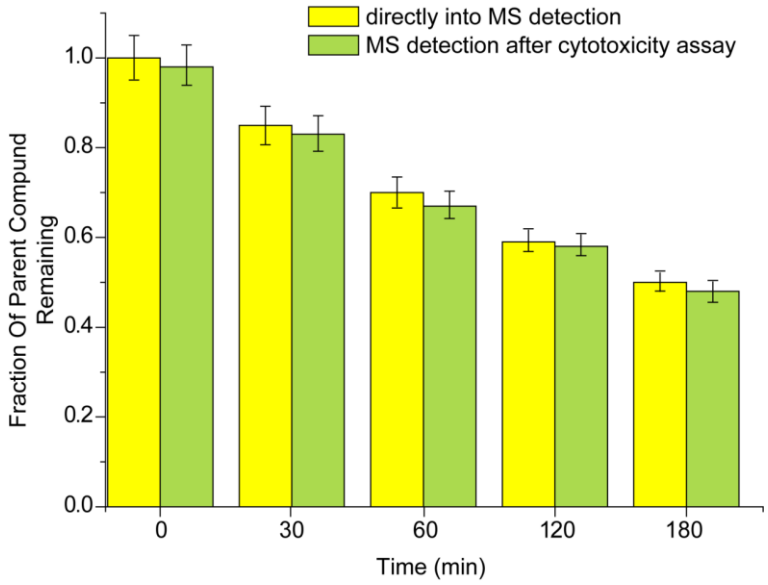

Fig. S10 Fraction of FLAP remaining in metabolites by MS detection directly and after cytotoxicity assay.

Table S1. The sum of peak intensity of *m/z* 402.1 (FLAP) after 10 μM FLAP metabolized by HLMs for 0, 60 and 180 min inside and outside microfluidic chip.

Metabolic time (min)	0	60	180
Ion intensity of remaining FLAP outside chip ($\times 10^4$)	77.5 ± 1.5	43 ± 1.7	25.5 ± 0.9
Ion intensity of remaining FLAP inside chip ($\times 10^4$)	78.5 ± 1.9	41 ± 1.4	27 ± 0.7
RSD (%)	1.2	4.8	5.6

Table S2 The MCF7 cell viabilities of MTT assay on 96-well plates and the metabolite cytotoxicity on the microdevice after UGT metabolism of FLAP

Metabolic time (min)	0	30	60	120	180
96-well plate	0.275 ± 0.011	0.302 ± 0.009	0.361 ± 0.015	0.412 ± 0.007	0.447 ± 0.012
on-chip	0.286 ± 0.014	0.340 ± 0.017	0.381 ± 0.019	0.462 ± 0.023	0.493 ± 0.025
SD	3.9%	12.5%	5.5%	12.2%	10.4%

Table S3 The MCF10A cell viabilities of MTT assay on 96-well plates and the metabolite cytotoxicity on the microdevice after UGT metabolism of FLAP

Metabolic time (min)	0	30	60	120	180
96-well plate	0.863 ± 0.031	0.869 ± 0.028	0.883 ± 0.013	0.910 ± 0.046	0.923 ± 0.032
on-chip	0.857 ± 0.044	0.867 ± 0.037	0.880 ± 0.049	0.920 ± 0.053	0.921 ± 0.025
SD	0.7%	0.3%	0.4%	1.0%	0.2%

Table S4 The HepG2 cell viabilities of MTT assay on 96-well plates and the metabolite cytotoxicity on the microdevice after UGT metabolism of FLAP

Metabolic time (min)	0	30	60	120	180
96-well plate	0.422 ± 0.014	0.594 ± 0.011	0.746 ± 0.025	0.758 ± 0.021	0.767 ± 0.017
on-chip	0.475 ± 0.024	0.631 ± 0.032	0.738 ± 0.037	0.777 ± 0.040	0.794 ± 0.045
SD	12.6%	6.3%	1.1%	2.5%	3.5%

Table S5 The QSG7701 cell viabilities of MTT assay on 96-well plates and the metabolite cytotoxicity on the microdevice after UGT metabolism of FLAP

Metabolic time (min)	0	30	60	120	180
96-well plate	0.926 ± 0.014	0.935 ± 0.008	0.933 ± 0.012	0.963 ± 0.017	0.957 ± 0.012
on-chip	0.929 ± 0.004	0.938 ± 0.012	0.938 ± 0.015	0.956 ± 0.022	0.952 ± 0.015
SD	0.3%	0.4%	0.5%	0.7%	0.5%

Reference

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