Supplementary Data

Integrated microfluidic system for cell co-culture and simulation of drug metabolism

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Materials and Chemicals

Caco-2, HepG2, and U251 cell lines were purchased (Cancer Institute & Hospital Chinese Academy of Medical Science, Beijing, China) and cultured in RPMI 1640 culture medium (Gibco, Grand Island, NY) at with 5% CO_2 at 37 °C. SU-8 2050 negative photoresist with the developer was purchased from MicroChem Corp (Newton, MA, USA). Poly dimethyl siloxane (PDMS) prepolymer and its curing agent were obtained from Dow Corning (Sylgard 184, Midland, MI, USA). Polycarbonate (PC) membrane (filter type: 3.0 μm) was purchased from Merck Millipore Ltd. Fetal bovine serum (FBS) and phosphate buffer saline solutions (PBS, 0.01 M, pH 7.4) were purchased from Gibco. Calcein AM and EthD-1, carbocyanine DiI, carbocyanine Dio and Hoechst (HOE) 33342 were purchased from Invitrogen (CA, USA). Dihydroethidium (DHE) and 2, 3-naphthalenedicarboxaldehyde (NDA) were bought from Beyotime Institute of Biotechnology (Shanghai, China). Methanol and acetonitrile of HPLC grade (JT Baker, Phillipsburg, NJ, USA) and purified water (>18 MΩ·cm) were used. All other reagents were of analytical reagent grade and used without further purification.

CCK-8 cytotoxicity assay

The anti-tumor activity of CPT-11 was evaluated by CCK-8 cytotoxicity assays through determining the inhibition effect on the proliferation of Caco-2, HepG2 and U251 cells *in vitro*. The experiment methods were as follows:

Caco-2, HepG2 and U251 cells were harvested and seeded in 96-well plates at the concentration of 7×10^3 cells per well. Aninitial stock solution of 100 mg/mL CPT-11 was prepared in DMSO. CPT-11 solutions were prepared with the concentration ranged from 5 to 375 μg/mL, and then incubated at 37 ºC in a 5% CO_2 incubator for 24, 48 and 72 h. Blank RPMI 1640 medium was used as a control. 10 μL CCK-8 solution (5 mg/mL in PBS) was then added to each well and incubated for 4 h at 37 ºC. The absorbance at 495 nm was determined using multilabel reader (EnVision, PerkinElmer, USA). CCK-8 cytotoxicity assay indicated that CPT-11 had obvious inhibition effects on the proliferation of cancer cells, suggesting the anti-tumor activities of the drug.

LC-MS/MS conditions

The experiments were carried out on a Shimadzu high-performance liquid chromatograph/mass spectrometer (LCMS-IT-TOF) and operated in the positive ion mode. The analytes were separated using an LaChrome C18 column (4.6 mm × 150 mm, 5 μm, HITACHI) equipped with a 5 μm guard column cartridge (4.0×10mm×2, GL Sciences). The samples were then transferred to an autosampler vial and a total volume of 5μL was injected into the LC-MS/MS system. Capillary interface voltage and current were set at 4.5 kV and 1.7 μA. Nebulizing gas flow was set at 1.5 L/min with an CDL temperature of 200 ºC. All mass spectrawere externally tuned by NaTFA solution in the positive ion mode with the mass range of *m/z* 300-650. For MS/MS analysis, the collision energy was set at 50%.

Fig. S1 Dynamic coculture Caco-2, HepG2, and U251 cells in microfluidic chip. (A) Photo of the fabricated microfluidic chip. (B) The integration of dynamic coculture microfluidic chip device.

Theoretical calculation of surface tension at air-liquid interface

When solution was introduced into the channels, the air-liquid interface would form naturally and the surface tension plug (STP) would stay "off", and the flow could not go through the narrow channel. The STP was defined as a channel with the air-liquid interface in it, which stops the flow going through the channel. The vital factor of the STP was the surface tension of the solution at the end positions of the narrow

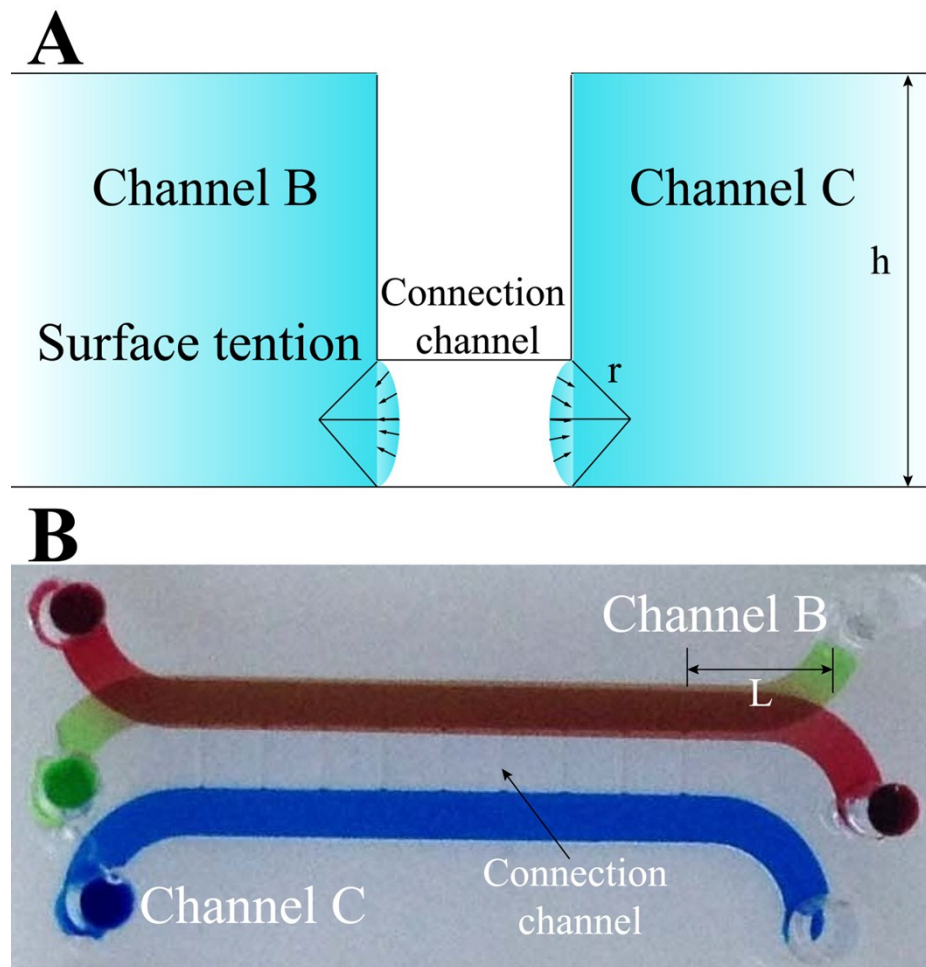channels where the air-liquid interface existed.

Fig. S2. Design of the junction of the connection channel and the main channel. (A) Sectional view of the junction. (B) Image of the whole connection channel.

Here we carried out a simple theoretical analysis [1]:

At the interface of flowing liquid and air of STP, a balance existed:

$$P_s = P_f$$

P_s is the surface tension while P_f is the lateral pressure of the flowing fluid.

According to the Young − Laplace equation:

$$P_s = \frac{2\gamma}{r}$$

And according to Poiseuille law: For incompressible constant laminar flow with the coefficient of viscosity η, flowing in a tube with the radius of R, if the pressure drop between distance of L is (P_1-P_2), then the flow velocity distribution is:

$$v_r = \frac{p_1 - p_2}{4\eta L}(R^2 - r^2)$$

For the similar flow between two parallel plates, the flow velocity distribution is:

$$v_y = \frac{p_1 - p_2}{2\eta L}(hy - y^2)$$

$$v_{max} = \frac{p_1 - p_2}{8\eta L}h^2$$

Here h is the height of main channel, y is the vertical length.

Here in the microchip we focus on the area between the STP near the outlet and the outlet, we estimate the pressure at the outlet as 0, and we use v_{max} for an approximate calculation, then we can get:

$$P_f = \frac{8\eta L v_{max}}{h^2}$$

For $P_s = P_f$

$$\frac{8\eta L v_{max}}{h^2} = \frac{2\gamma}{r}$$

$$v_{max} = \frac{\gamma h^2}{4\eta L r}$$

Here γ=6.95*10-4 Pa s (37 °C), h=400 μm, η=0.07 N/m (37 °C), L of 2.0 mm is the vertical length between the STP and the end of the channel, r is the radius of curvature. We assume when r is half the height of the connection channel, that is 25μm, liquid will flow into the connection channel (as shown in Fig. S2).

Then v_{max}≈ 0.13 m/s, which is quite fast and can't be reached in real manipulations. So this theoretical analysis proved that the STP was very stable and reliable.
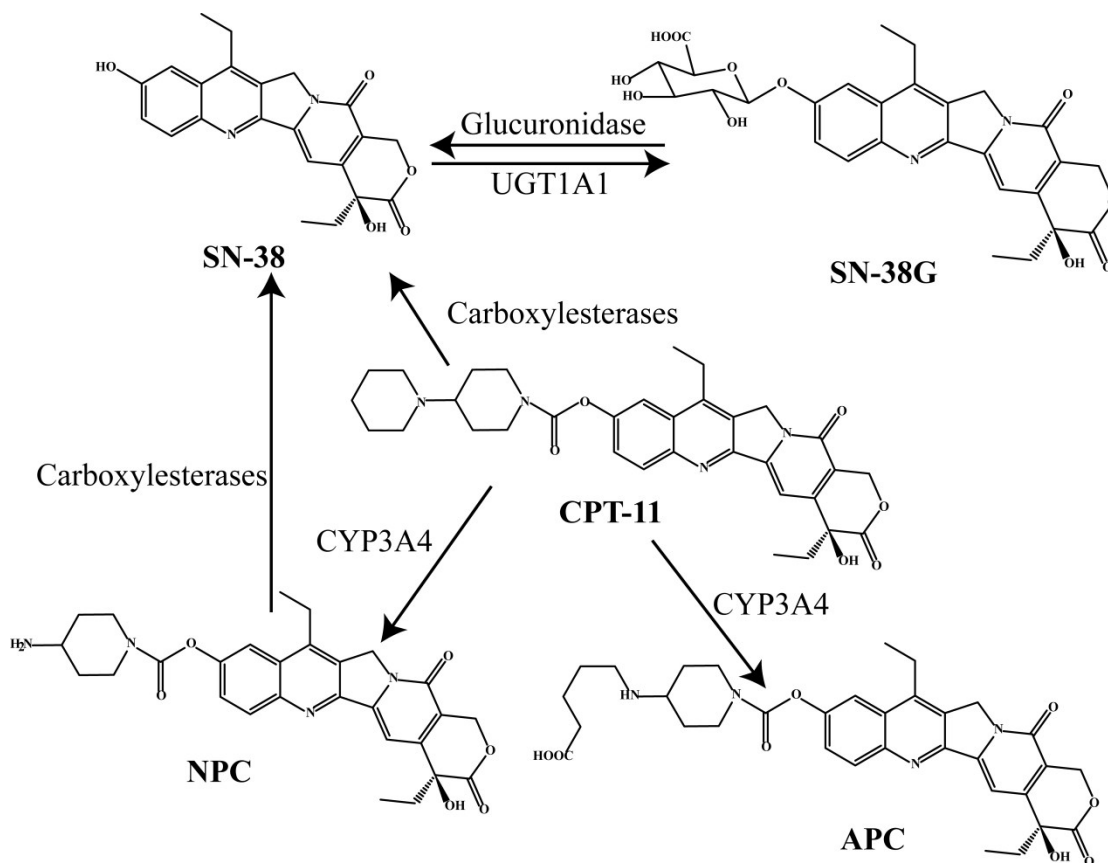
Fig. S3 Metabolic tranforms and pathways of CPT-11 *in vivo*. There are two main metabolic pathways of CPT-11: 1) CPT-11 cleaved by carboxylesterases (CEs) to form the primarily active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) which is further metabolized by UDP glucuronosyltransferase isoform (UGT1A1) to an inactive β-glucuronic acid conjugate of 7-ethyl-10-O-glucuronyl-camptothecin (SN-38G); 2) CPT-11 inactivated by CYP3A4 to transform 7-ethyl-10- [4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin (NPC), respectively.
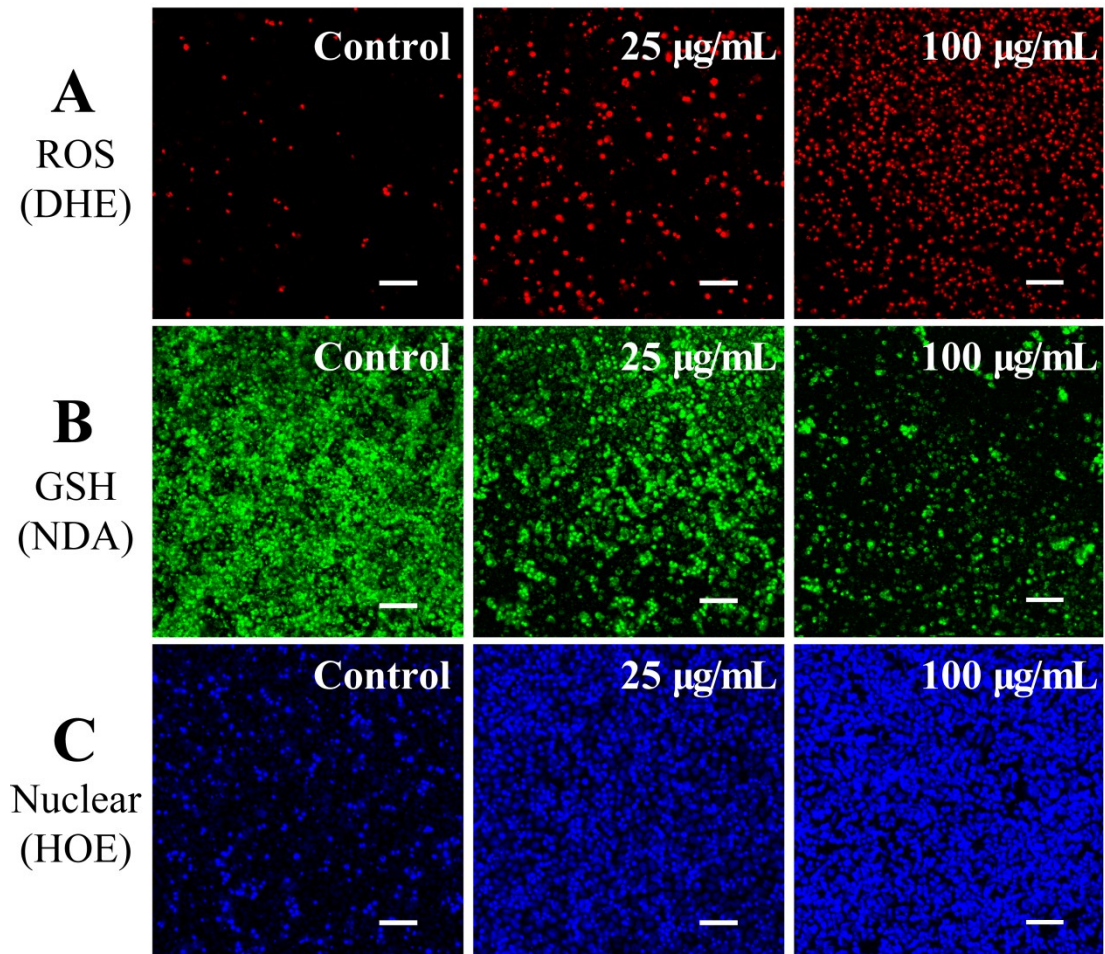
Fig. S4 CPT-11 influences on U251 cells apoptosis (scale bar: 100 μm). Comparison of fluorescent images of U251 intracellular ROS generation (A) and GSH reduction (B) without and with 25 or 100 μg/mL CPT-11 treatment. (C) Fluorescent images of U251 cells stained by Hoechst 33342 without and with 25 or 100 μg/mL CPT-11 treatment (n = 3, *$P < 0.05$).
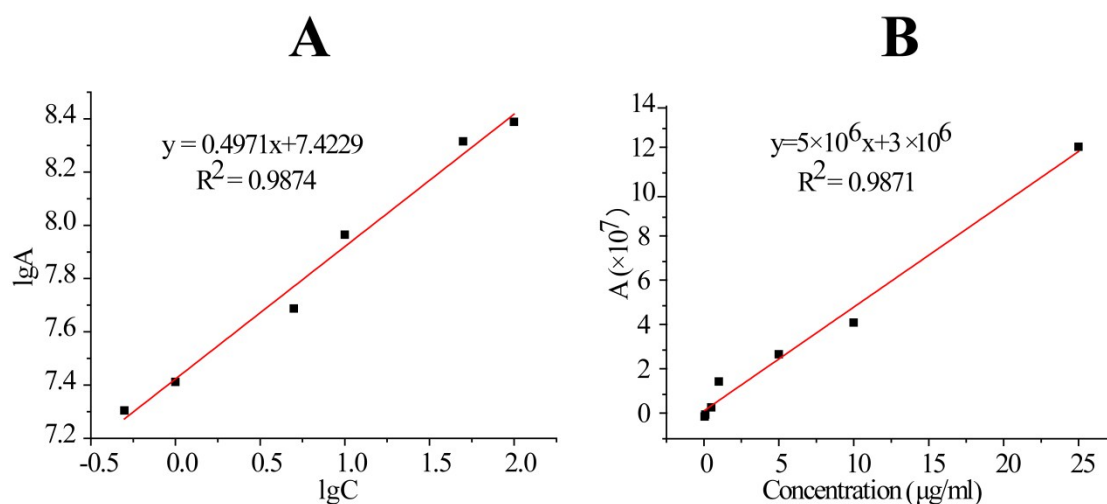
Fig. S5 Calibration curves of CPT-11 and SN-38 for quantitation. (A) Calibration curve of CPT-11 in the range of 0.5 to 100 µg/mL. (B) Calibration curve of SN-38 in the range of 0.05 to 25 µg/mL (n = 3, *P < 0.05).

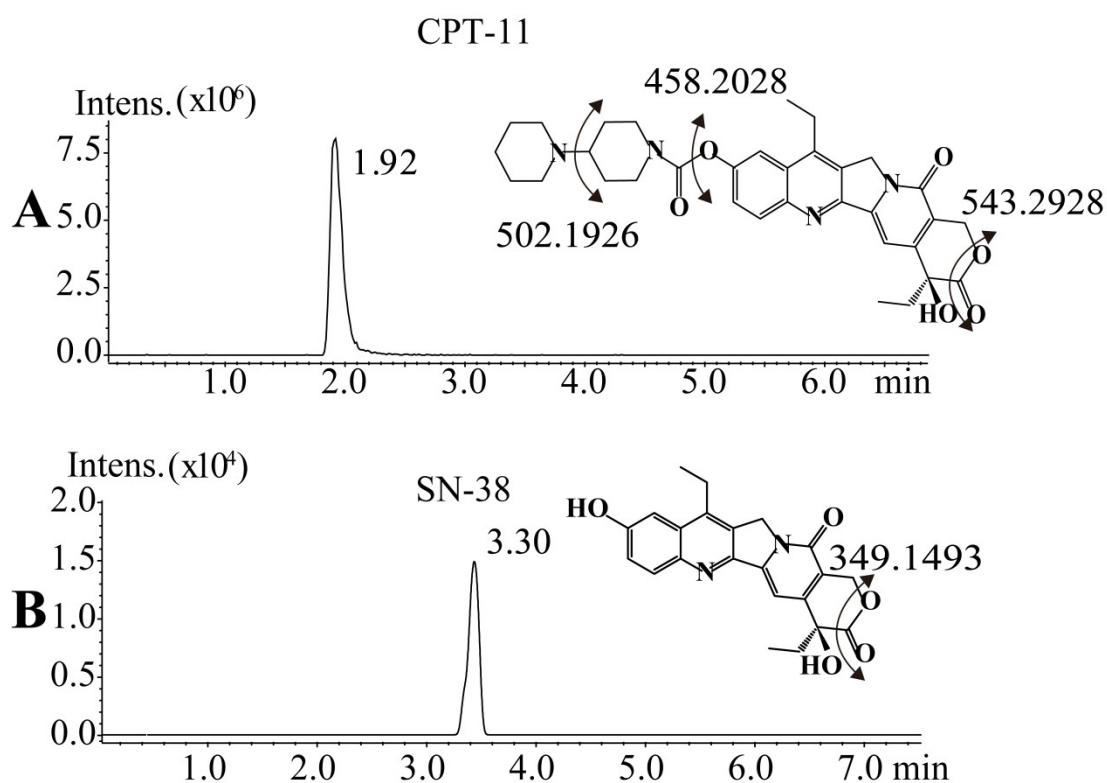**Fig. S6** LC-MS analysis of intracellular CPT-11 and SN-38 in HepG2 cells. The concentrations are 15.1 µg/mL and 55.5 ng/ml for CPT-11 (A) and SN-38 (B) in HepG2 cells, respectively.
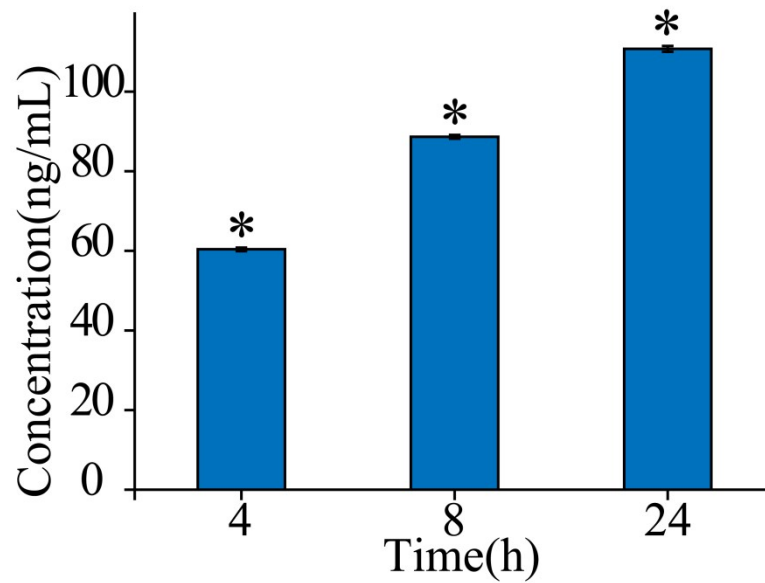
Fig. S7 LC-MS analysis of extracellular SN-38 in HepG2 medium. The concentrations are 60.4 ng/mL, 88.7 ng/mL and 110.8 ng/mL for 4, 8 and 24 h of 25 μg/mL CPT-11 stimulation, respectively. The standard error bars mean the variation of three individual experiments (n = 3, *P < 0.05).

Table 1 The population percentage of cells distributed in different cell cycle phases based on flow cytometer detection.

Cell cycle	Control cells (%)	25 μg/mL CPT-11 treated cells (%)	100 μg/mL CPT-11 treated cells (%)
G0/G1	69.96	35.05	27.79
S	23.92	54.75	67.45
G2/M	6.12	10.19	4.76