Supplementary Information (SI) for Lab on a Chip.
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Methods and materials

1.1Cell resource and culture

The human colon adenocarcinoma cell line Caco-2 at passage 30, kindly provided by the Stem Cell Bank of the Chinese Academy of Sciences (Shanghai, China), were grown in dulbecco's modified eagle medium (DMEM, with sodium pyruvate, VivaCell, cat No:C3113-0500) supplemented with 20% fetal bonus serum (FBS, Gibco), 1% non-essential amino acids (NEAA, Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (Solarbio, China) at 37 ℃ in a humidified atmosphere containing 5% CO2. Cells were passaged at a confluence of 80–90%. Caco-2 cells with lower passage number (37–47) were used.

The human colon adenocarcinoma cell line HT29-MTX-E12, purchased from YUDER Technology (Shanghai) Co., Ltd. (Shanghai, China), were grown in DMEM supplemented with 10% FBS, 1% NEAA, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 ℃ in a humidified atmosphere containing 5% CO2. Cells were passaged at a confluence of 80–90%. In this study, we used HT29-MTX-E12 cells with a lower passage number from 7 to 20.

The human hepatocellular carcinoma cell line HepG2, provided by the Stem Cell Bank of the Chinese Academy of Sciences (Shanghai, China), were grown in DMEM supplemented with 10% FBS, 1% NEAA, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 ℃ in a humidified atmosphere containing 5% CO2. Cells were passaged every 2–4 days at a confluence of 80–90%.

The human umbilical vein endothelial cell line (HUVEC-T1), obtained from the Cell Resource Center, Peking Union Medical College (Beijing, China), were cultured in DMEM supplemented with 10% FBS, 1% NEAA, 1% Endothelial Cell Growth Supplement (ScienCell), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 ℃ in a humidified atmosphere containing 5% CO2. Cells were passaged until they confluence of about 80%.

The human monocyte leukemia cell line (THP-1) was obtained from National Institutes for Food and Drug Control (Beijing, China) and cultured in 1640 medium (VivaCell) supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 ℃ in a humidified atmosphere containing 5% CO2. The cells were passaged every 2–3 days.

Human hepatic stellate cells (HHSC), purchased from ScienCell Research Laboratories (San Diego, California), were grown in Stellate Cell Medium (ScienCell) at 37 ℃ in a humidified atmosphere containing 5% CO2. Cells were passaged at a confluence of 80–90%. The cells were

cryopreserved before being used for spheroid establishment.

1.2 Intestinal equivalents' integrity

Transendothelial electrical resistance (TEER) was measured at different time points using a resistance meter (Beijing Daxiang Biotech Co., Ltd.). Electrical resistance measurements were performed after the electrode pairs were immersed in the medium of the two chambers. TEER was calculated using equation (1-1):

$$\mathrm{TEER} = (\mathrm{R} - \mathrm{R_0}) \times \mathrm{A} \quad (1\text{-}1)$$

where R (Ω) is the resistance across the cell layer, R0 (Ω) is the resistance of the transparent membrane, and A is the effective area (cm^2).

Intestinal membrane permeability was determined using the small molecular compound fluorescein sodium. Fluorescein sodium was administered from the apical chamber, and D-Hanks solution was administered to the basal chamber. The solution was collected from the basal chamber, and the fluorescence signal was detected at Ex/Em = 498/517 nm using a fluorescent microplate reader (PerkinElmer, VICTOR X5).

The transferred concentration was determined using standard curves in the appropriate treatment solutions, and the intestinal apparent permeability coefficient (Papp) was calculated using the following equation (1-2):

$$\mathrm{Papp} = \triangle \mathrm{Q}/(\triangle \mathrm{t} \cdot \mathrm{A} \cdot \mathrm{C0})(^{\mathrm{cm}}/_{\mathrm{s}}) \quad (1\text{-}2)$$

where dQ/△t, A (cm^2), and C0 are the amount of permeated compound per unit of time, the surface area of the transport membrane, and the initial compound concentration in the donor chamber, respectively.

1.3 Drug Toxicity and Viability Assay

According to the recommended kit protocol, ALB (Solarbio), AST and ALT (Nanjing Jiancheng Bioengineering Institute) was detected during cultivation and at the present of APAP for hepatocyte injury detection. Cell mitochondrial membrane potential was determined using tetramethylrhodamine, ethyl ester (TMRE) (Beyotime) or stained with rhodamine 123 (Rho123). Mito-tracker (Solarbio) was used for trace the amount of mitochondria at Ex/Em 550/575 nm. For

oxidative stress detection, CellROX oxidative stress reagent (ThermoFisher, C10444) was selected at the present of APAP. For apoptosis assay and DNA damage detection, cleaved caspase-3 and H2AFX (Phospho-Ser139) was detected *in situ* by immunofluorescence staining.