Electronic Supplementary Information for

Imitation of Drug Metabolism in Human Liver and Cytotoxicity Assay using a Microfluidic Device Coupled to Mass Spectrometric Detection

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Mass Transfer to HLMs in PEG Hydrogel Microstructures

Human liver microsomes (HLMs) were stained with Vybrant DiO cell-labeling solution (10 μ M). After UV photopolymerization, PEG hydrogels containing stained HLMs were monitored for 100 min to insure that no HLMs leached into the surrounding fluid when the channel was washed by PBS at a flow rate of 20 μ L min⁻¹. Fluorescence images of PEG hydrogel microstructures were captured by coupling the microscope with a cooled CCD camera and then analyzed by image processing and analysis software (Image-Pro 6.0, Media Cybernetics, USA). As shown in Figure S1a, no substantial decrease in fluorescent intensity was observed. After analysis by Image-Pro 6.0, the fluorescence change with washing time is shown in Figure S1b.



Figure S1 Fluorescence change of a single PEG hydrogel microstructure over washing time. (A) Fluorescence images of the microstructure at 0, 5, 60 min after PBS washing. (B) Relationship between fluorescence intensity and washing time. Scale bar, 100 µm.

Drug cytotoxicity on HepG2 cells

HepG2 cells were cultured in 96 well plates and microchannels, and then they were stimulated with a series of drug solutions in cell culture medium. After maintained in a humidified atmosphere of 95% air, 5% CO₂ at 37 °C for 12 h, they were stained with Hoechst 33342 (5 μ g mL⁻¹) at 37 °C for 30 min and then washed by PBS. Fluorescence images were captured by the microscope with a cooled CCD camera and then analyzed by Image-Pro 6.0.

AP cytotoxicity on HepG2 cells is shown in Figure S2. Cell variability decreased sharply, following an increase in AP concentration; whereas APG showed no cytotoxicity on HepG2 cells (Figure S3).



Figure S2 AP cytotoxicity on HepG2 in 96 well plates and microchannels. <u>Fluorescence images of HepG2 cells stained with Hoechst 33342</u>, after incubation with a series of AP concentrations, (a) 0mM; (b) 8mM; (c) 30 mM. (d) The relationship between cell variability and AP concentration. The standard error bars mean the variation of three individual experiments.



Figure S3 APG cytotoxicity on HepG2 in 96 well plates and microchannels. <u>Fluorescence images of HepG2 cells stained with Hoechst 33342</u>, after incubation with a series of APG concentrations, (a) 0 mM; (b) 6 mM; (c) 10 mM. (d) The relationship between cell variability and APG concentration. The standard error bars mean the variation of three individual experiments.