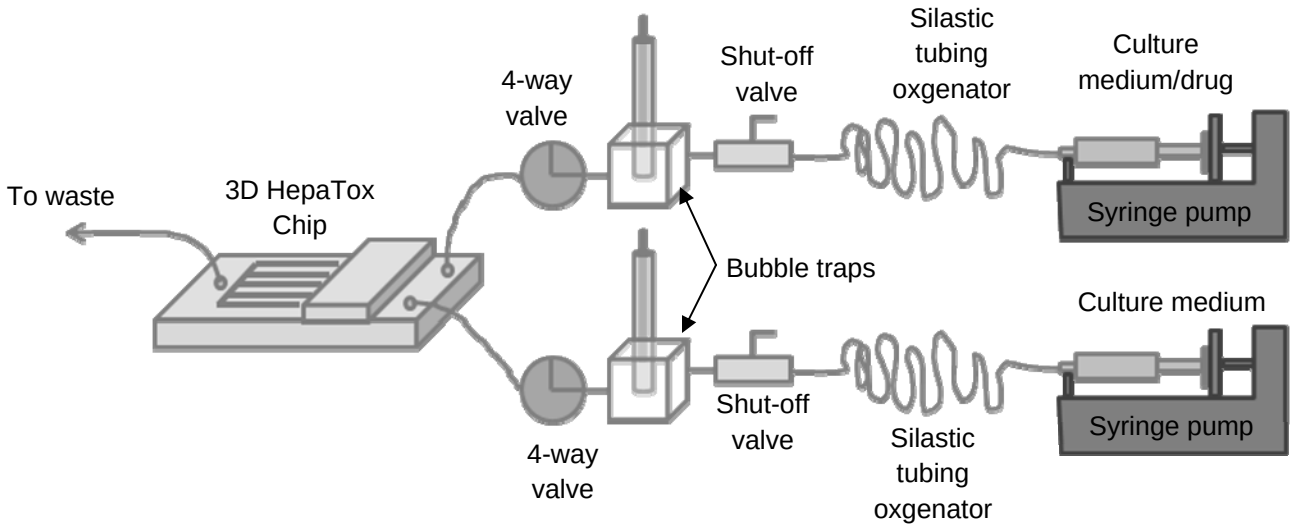
A microfluidic 3D hepatocyte chip for drug toxicity testing

Toh *et al*., 2009

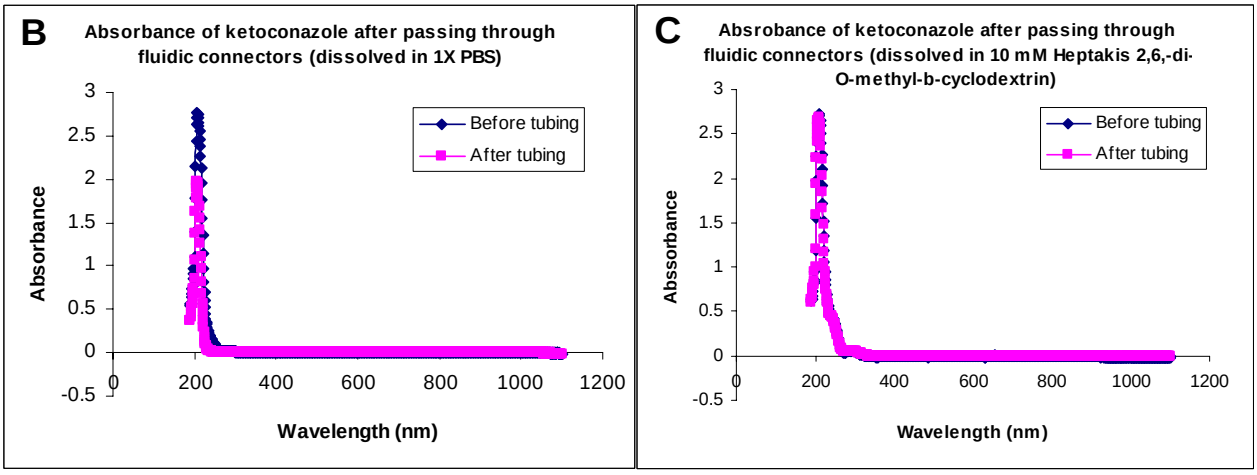
Supplementary Figures



Supplementary Figure 1. Schematic of one-pass perfusion system used during perfusion culture and drug testing with the 3D HepaTox Chip. During perfusion culture, both inputs were filled with culture medium. One of the inputs was replaced with drug solution during drug testing.

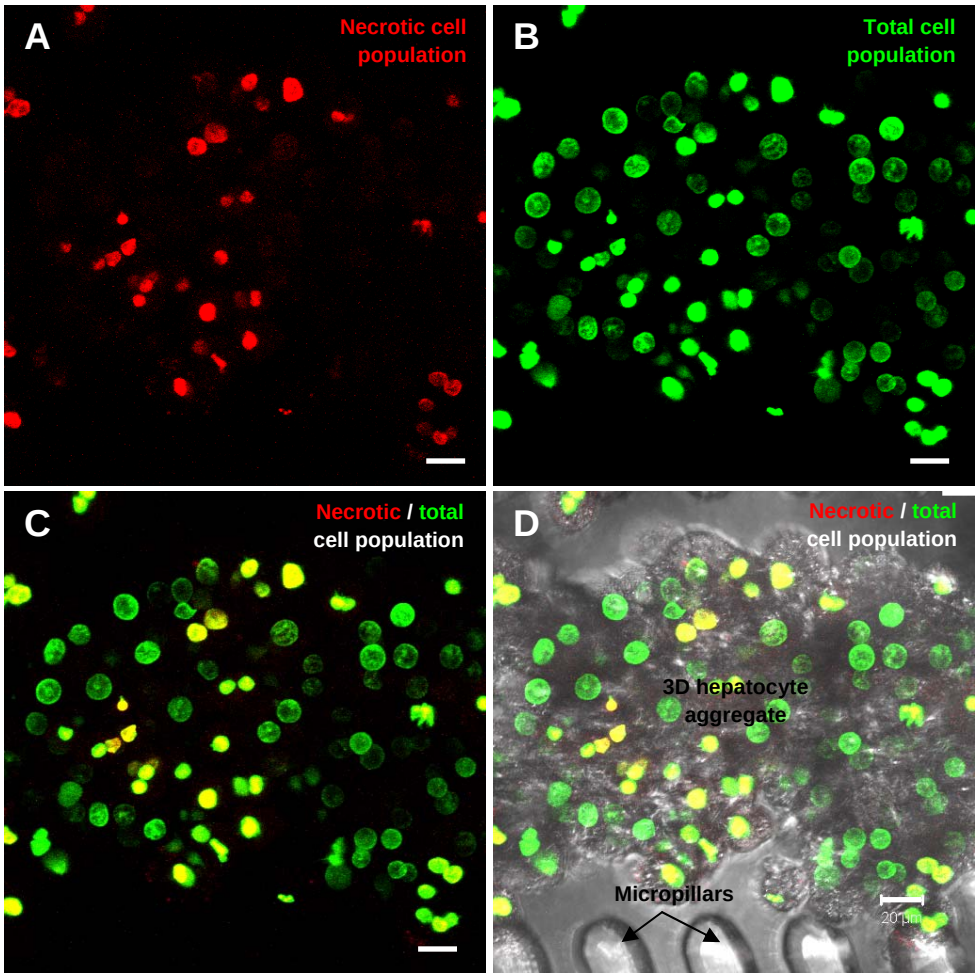
A

Drug / Solubilizing agent	1X PBS		5 % Bovine serum albumin (BSA)		10 mM heptakis (2,6,-di-O-methyl)-β-cyclodextrin	
	Peak Abs (initial)	Peak Abs (after tubing)	Peak Abs (initial)	Peak Abs (after tubing)	Peak Abs (initial)	Peak Abs (after tubing)
210 µM ketoconazole	2.76	1.96	Not detected		2.72	2.66
1 mM quinidine	3.84	3.86	2.65	2.43		
2 mM rifampin	Not dissolved		2.54	2.31		



Supplementary Figure 2. The use of solubilizing agents can increase the solubility of hydrophobic drugs (ketoconazole and rifampin), which had a tendency to be adsorbed onto the tubing used to deliver the drug solutions into the 3D HepaTox Chip, resulting in a lower effective drug concentration. Quinidine was aqueous soluble and used here as a comparison. Drugs were solubilized in either 1X PBS, 5 % bovine serum albumin (BSA) or 10 mM heptakis (2,6,-di-O-methyl)-β-cyclodextrin and perfused through fluidic connectors and tubing used for delivering drug solutions at 1 ml hr^{-1} for 30 minutes. The absorbance of the perfusates and fresh drug solutions were measured at the respective peak wavelengths of the drug solutions using a UV-Vis spectrophotometer (Agilent, USA). The peak absorbance was measured at 208 nm for ketoconazole, 208 nm (in 1X PBS) or 331 nm (in 5 % BSA) for quinidine and 473 nm for rifampin. Absorbance of drug solutions at 208 nm cannot be measured when solubilized in 5 % BSA because BSA is a protein which absorbs strongly in the UV range. We assessed 10 mM heptakis (2,6,-di-O-methyl)-β-cyclodextrin as an alternative solubilizing agent for ketoconazole. (B)-(C) are absorbance spectra of ketoconazole solubilized in 1X PBS and 10 mM heptakis (2,6,-di-O-methyl)-β-cyclodextrin respectively before and after perfusing through fluidic connectors. However, heptakis (2,6,-di-O-methyl)-β-cyclodextrin was found to be more cytotoxic than 5 %

BSA (data not shown), hence we used 5 % BSA as the solubilzing agent in all drug testing experiments.



Supplementary Figure 3. Confocal images of nuclei staining for necrotic and total cell population in the 3D HepaTox Chip after 24 hours of treatment with 14.29 mM acetaminophen (APAP). (A) The necrotic cell population was stained with 25 μg ml^{-1} Propidium iodide and (B) the total cell population was stained with 250 nM Sytox Green. (C) Overlay of red and green channels where green nuclei indicate live cells and co-stained nuclei indicate necrotic cells. (D) Overlay of fluorescence images with transmission images showing the 3D hepatocyte aggregate in the center cell compartment of the microfluidic channel. Images are projection of a 100 μm optical stack at 1 μm interval. Scale bars = 20 μm.