Published in final edited form as:

Technology (Singap World Sci). 2015 December ; 3(4): 155–162. doi:10.1142/S2339547815200034.

A novel low-volume two-chamber microfabricated platform for evaluating drug metabolism and toxicity

Shyam Sundhar Bale¹, Gautham Vivek Sridharan¹, Inna Golberg¹, Ljupcho Prodanov¹, William J. McCarty¹, Osman Berk Usta¹, Rohit Jindal^{1,★}, and Martin L. Yarmush^{1,2,★}

¹Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School and Shriners Hospital for Children, Boston, MA 02114, USA

²Department of Biomedical Engineering, Rutgers University, Piscataway, NJ 08854, USA

Abstract

To evaluate drug and metabolite efficacy on a target organ, it is essential to include metabolic function of hepatocytes, and to evaluate metabolite influence on both hepatocytes and the target of interest. Herein, we have developed a two-chamber microfabricated device separated by a membrane enabling communication between hepatocytes and cancer cells. The microscale environment created enables cell co-culture in a low media-to-cell ratio leading to higher metabolite formation and rapid accumulation, which is lost in traditional plate cultures or other interconnected models due to higher culture volumes. We demonstrate the efficacy of this system by metabolism of tegafur by hepatocytes resulting in cancer cell toxicity.

Innovation

This work demonstrates the fabrication and use of a two-chamber microdevice for inter-tissue drug toxicity testing and evaluating metabolite effects. The microscale environment created enables cell culture in a low media-to-cell ratio leading to higher metabolite formation and rapid accumulation, which is lost in traditional plate cultures or other interconnected models due to higher culture volumes. By building a two-chamber microfabricated device that allows for direct interaction through a permeable membrane, we have eliminated the need for any fluidic flow — simplifying the model to a great extent. In this report, we demonstrate the applicability of this device using a chemotherapeutic pro-drug tegafur-uracil, which when metabolized by primary hepatocytes produces 5-Fluorouracil (5-FU), a metabolite toxic to cancer cells. The metabolite conversion and its resultant toxicity are measureable in the microscale model within 24 hours. Overall, the two-chamber device provides a novel, easy-to-use platform to evaluate drug metabolism, toxicity and interactions between multi-tissue systems.

Correspondence should be addressed to R.J. (RJINDAL@mgh.harvard.edu) or M.L.Y. (ireis@sbi.org).

Author Contributions: SSB, RJ, OBU and MLY designed the experiments, analyzed the data, and co-wrote the manuscript. SSB and IG performed the experiments. GS performed mass spectrometry and analysed the data. LP carried out laser-cutting operations and contributed to manufacture of the devices. All authors participated in discussions and commented on the manuscript.

Competing Interests Statement: The authors declare competing interests statement. Invention Disclosure filed “Methods and devices to study metabolism” — Application No. 62067239.

Narrative

The liver is the primary organ where drugs are metabolized. While drug induced liver injury remains an important reason for withdrawal of drugs, metabolites generated by the liver can also be responsible for toxicity in other organs and tissues^{1,2}. Significant progress has been made in the development of platforms for evaluating drug hepatotoxicity³⁻⁵. They include incorporation of hepatic cell lines or primary hepatocytes in various monoculture and co-culture configuration both in flow and static conditions⁴. However, development of systems that facilitate evaluation of drug toxicity in other organ or tissues as a result of metabolite(s) generated by liver has lagged somewhat. Typically, these systems are comprised of interconnected chambers in which each chamber is seeded with a tissue or organ model and communication is achieved by fluid flowing across and in between the chambers⁶⁻⁸. In many cases, the liver chamber is seeded with a hepatocyte cell line^{6,7}, which generally lack metabolic function in comparison to primary hepatocytes⁹. While these models may provide an initial assessment for drug toxicity and multi-cell interactions, most of these *in vitro* models incorporate high media volumes (including tubing and reservoir volumes) creating an artificially high media-to-cell ratio, leading to loss of sensitivity in detecting product interactions, particularly, in case of highly unstable metabolites^{6,7}. There is a critical need for the development of simpler, more effective, multi-tissue systems that can facilitate screening of drug toxicity as a result of primary hepatocytes communicating with the cells in other organs through the metabolic conversion of drugs.

In the context of drug metabolism, hepatocytes communicate with the cells in other organ via secreted metabolites. Traditionally, co-culture interactions mediated by secreted factors have been evaluated in transwell systems where different cell types are separated by porous membrane that enables soluble factor communication. One limitation of these systems is the dilution of secreted factors due to exposure of the cells to high media volume^{10,11}. This becomes especially limiting in the settings where the secreted factor such as metabolite is cleared by other mechanisms, preventing it from reaching effective or toxic levels.

While plate cultures have inherently high media-to-cell ratio, micro-fluidic interconnected chamber systems rely on fluidic connections and reservoirs which eventually result in higher media-to-cell ratio. For drug screening and toxicity related studies, it is essential to develop *in vitro* models that incorporate both hepatocytes and target-organ of interest, while reducing the culture volume to increase metabolite production and interrogate resulting metabolite toxicity. Herein, we have developed a simple microscale device with a two-chamber design separated by a tissue-culture membrane allowing the culture of two different cells within the same device, while addressing cells within each chamber (Fig. 1). This device design is similar to our recently published work wherein rat primary hepatocytes were cultured in a collagen gel for 14 days under flow conditions¹². The use of a two chamber device allows for the culture of primary hepatocytes in collagen gel (in the bottom chamber), while media circulates in the top chamber. In this work, we have created a hepatocyte — cancer co-culture model by culturing hepatocytes (liver) and cancer cells (MCF-7, breast cancer) in the bottom and top chamber, respectively. We have tested the metabolic conversion of tegafur, a pro-drug which is converted into 5-FU by hepatocytes, and is then taken up by dividing tumor cells (MCF-7), leading to their cell death. The two-chamber device is fabricated with

a) top PDMS chamber (100 μm height), b) a tissue culture membrane, c) bottom chamber cut from a PDMS sheet (250 μm height), and d) a glass slide. The top and bottom chambers accommodate $\sim 10,000$ cells each (cultured on tissue culture membrane and glass slide respectively, 10 mm^2 area), which are seeded separately from ports for each chamber. In addition, single chamber devices were prepared by bonding a PDMS chamber with similar dimensions of the top chamber (100 μm height, 10 mm^2 area, 10,000 cells) onto a glass slide. Media volumes and other pertinent parameter for the well and device configurations are given in Table 1. Within the human body, there are ~ 60 hepatocytes per 1 nL blood¹³, and in the case of our microfabricated co-culture model, we are able to accommodate ~ 3 hepatocytes per 1 nL of media. While the media-to-cell ratio is an order of magnitude higher than the *in vivo* conditions, microfabrication technologies enable the culture of cells in much lesser media volumes when compared with traditional plate cultures (~ 3 hepatocytes vs. 0.3 hepatocytes per 1 nL media).

Our hypothesis is that the media-to-cell volume ratios in culture is a key parameter in metabolite synthesis and will affect reaction dynamics within the volume, enabling higher concentrations of secreted factor or drug metabolite to be achieved in micro-scale dimensions in comparison to macroscale plate cultures. To confirm this hypothesis, we evaluated the enzyme kinetics within single chamber microfabricated devices and compared with a 12-well plate assay using primary rat hepatocytes. While in a single chamber microfabricated device, hepatocytes are cultured in 0.1 nL/hepatocyte volume, similar culture in a 12-well plate requires 1 nL media (Table 1). We have evaluated the kinetics of CYP1A1/2 and CYP3A4, and the reaction rate (normalised to volume) for microfabricated device and 12-well plate is shown in Fig. 2. Our results indicate a 3–4 times higher product accumulation within devices when compared with plate cultures. The reduction in the media volume leads to an increase in product accumulation within the device, which in comparison gets diluted in a higher volume for well cultures.

We examined the co-culture of rat primary hepatocytes and human breast cancer cells (MCF-7) to create a hepatocyte-cancer model for drug screening. In our two-chamber device, intercellular communication between hepatocytes and cancer cells is achieved without any flow within the devices^{6,7}. In the two-chamber device, both cells were independently seeded into their respective chambers in the device using inlet ports on fibronectin-coated devices (see Materials and Methods). Rat primary hepatocytes and MCF-7 cells were seeded into the bottom and top chamber respectively. Control monocultures were seeded in a single chamber device. The co-culture model created provides a microenvironment with cells cultured in close proximity and relatively small volume. We compared co-culture of hepatocytes and MCF-7 cells in 12-well plates against a two-chamber device. A two-chamber device co-culture requires 0.35 nL/hepatocyte volume, while similar culture in a 12-well plate requires 3 nL/hepatocyte (Table 1).

Tegafur-uracil is a pro-drug that is widely used in chemotherapeutic applications for colorectal and breast cancer¹⁴. Briefly, orally administered tegafur is metabolized in the liver to form 5-Fluorouracil (5-FU), which gets incorporated into fast-dividing cells and cancerous cells. 5-FU is an analogue of uracil, which is an essential component during cell division and integrates into cellular DNA, inhibiting cell division¹⁴. However, 5-FU in the

human body is degraded by a) conversion into secondary and tertiary metabolites and b) degradation by dihydropyrimidine dehydrogenase (DPD)^{14,15}. Due to these processes, the half-life of 5-FU in circulation in human body is very short (~ 30 minutes), while DPD activity clears ~80% of 5-FU produced^{14,16}. Uracil is added in combination with tegafur to reduce pyrimidine catabolism and increase the longevity of 5-FU circulation. The low bioavailability of 5-FU and faster clearance makes understanding tegafur metabolism *in vitro* very challenging.

We tested metabolism of tegafur to 5-FU and the resulting toxicity, in both 12-well transwell and two-chamber microfabricated device with co-cultures exposed to 100 μM tegafur + 100 μM uracil (Fig. 3a,b). The dose of 100 μM for tegafur was chosen based on the concentration that is achieved by oral uptake of tegafur-uracil (UFT), $C_{\text{max}} = 31.159 \mu\text{M}$, Area Under Curve (AUC) = $121 \mu\text{M} \times \text{min}^{15}$. Furthermore, IC-50 values (Table 2, Supplementary Fig. 1) indicate that at the concentration of 100 μM tegafur is not toxic to either hepatocytes or MCF-7 cells. Cells in both co-culture formats were incubated with tegafur-uracil for 24 hours and media was collected and evaluated for LDH content. Single cell controls were prepared with single chamber device or 12-well plate. LDH release from drug-exposed samples was normalized with respective Configurations exposed to vehicle only controls (see “Material and Methods”). Our results indicate that there is an increase in the LDH release (~3.5 times) in the case of microfluidic co-cultures, while there was no such increase in case of plate cultures or single cell controls (Fig. 3c). The images in Supplementary Fig. 2 indicate that in microfabricated co-culture MCF-7 cells show somewhat reduced cell density for tegafur treatment in comparison to control, while hepatocytes monolayer is similar in both conditions. This is not surprising given the dramatically higher susceptibility of MCF-7 cells to 5-FU in comparison to hepatocytes (Supplementary Fig. 1). Thus, the increase in LDH release for tegafur treatment of microfabricated co-culture was primarily due to toxicity of 5-FU towards MCF-7 cells. This is a clear demonstration of the advantages of a microscale cell culture model, which overcomes the challenges (dilution effects) of traditional plate techniques.

To further understand tegafur metabolism and kinetics, we used mass spectrometry to measure the concentrations of tegafur, uracil and 5-FU within the media in the device. First, we measured the kinetics of tegafur, uracil metabolism; 5-FU production in a single chamber device with hepatocytes alone. Tegafur and uracil concentrations within the media shows a decrease ~75% over a period of 24 hours and 5-FU shows an increase in production upto 8 hours and a subsequent decrease (Fig. 4a,b). While tegafur and uracil are actively metabolized by hepatocytes, 5-FU is actively produced and subsequently converted by hepatocytes into secondary metabolites¹⁴. Further, the bioavailability of 5-FU is significantly lower ($C_{\text{max}} = 0.847 \mu\text{M}$) when compared with tegafur ($C_{\text{max}} = 31.159 \mu\text{M}$) due to its rapid clearance^{14,15}. In co-culture drug exposure experiments within two-chamber microfabricated device, measurements were made at the end of incubation (24 hours), and are shown in Supplementary Fig. 3. A similar trend is noticed in these cultures, showing a decrease in tegafur and uracil in hepatocyte and co-culture samples (Supplementary Fig. 3). Within the co-culture microfabricated device there is $\sim 0.53 \pm 0.11 \mu\text{M}$ 5-FU (at $t = 8$ hours) produced, which is similar to the concentrations observed *in vivo* ($C_{\text{max}} = 0.847 \mu\text{M}$, Area

Under Curve (AUC) = $0.79 \mu\text{M} \times \text{min}$), albeit the differences in the systems¹⁵. Further, absorption studies with empty devices exposed to these drugs showed no appreciable decrease upto 24 hours, excluding any artifacts due to PDMS (data not shown).

The temporal profile (Fig. 4b) of 5-FU suggest that relatively quick turn over rate of 5-FU may prevent it from reaching toxic level in macroscale cultures with high media volume. This is supported by our results whereby MCF-7 toxicity was observed in microscale cultures but not in macroscale cultures. Furthermore, while the process of metabolism of 5-FU into subsequent secondary and tertiary metabolites is kinetic, the use of microfabricated models provides a unique opportunity to understand the mechanisms of the process, which has not been shown so far.

In summary, we have developed a simple and versatile two-chamber microfabricated device that captures metabolic functions of hepatocytes, suggesting the importance of media-to-cell ratio in drug metabolism studies. The proposed device demonstrates that microscale architecture recapitulates the metabolism of hepatocytes for drug screening. While several studies have evaluated the use of microfluidic devices for drug metabolism and toxicity applications, our model provides a simple alternative for pro-drug metabolism studies where microscale technology is utilized for creating static co-cultures in low media-to-cell ratio microenvironment.

Materials and Methods

Materials

Fibronectin (Cat No. F1141), thiazolyl blue tetrazolium bromide (MTT reagent, Cat No. M5655), tegafur (Cat No. T7205), 5-fluorouracil (5-FU, Cat No. F6627), and uracil (Cat No. U1128) were purchased from Sigma. DMEM (Cat No. 31600083), 0.05% trypsin-EDTA (Cat No. 25300062), Williams E media (Cat No. A1217601), epidermal growth factor (EGF, Cat No. E3476), penicillin-streptomycin (Cat No. 15140122), and glutamine (Cat No. 21051040) were purchased from Life Technologies. LDH assay kit was purchased from Promega (Cat No. G1780). Fetal bovine serum (FBS, Hyclone Cat No. SH30071.03), glucagon (Bedford Laboratories, Cat No. 55390-004-01), hydrocortisone (SOLU-CORTEF[®] hydrocortisone sodium succinate for injection, Pharmacia Corporation), and insulin (Eli Lilly, Cat No. HI-213) were purchased and used as per manufacturer's directions. All other chemical reagents were purchased from Sigma.

Media formulations

1. MCF-7 cell culture media was prepared with high glucose (4.5 g/L) DMEM supplemented with 10% FBS, 2 mM glutamine, and 2% penicillin-streptomycin.
2. Hepatocyte maintenance media was prepared with high glucose (4.5 g/L) DMEM supplemented with 10% FBS, 20 $\mu\text{g/L}$ EGF, 14.28 $\mu\text{g/L}$ glucagon, 7.5 mg/L hydrocortisone, 500 U/L insulin, 2 mM glutamine, and 2% penicillin-streptomycin.
3. Williams E medium was supplemented with, 20 $\mu\text{g/L}$ EGF, 14.28 $\mu\text{g/L}$ glucagon, 7.5 mg/L hydrocortisone, 0.05 U/L insulin, and 2% penicillin-streptomycin.

Rat hepatocyte isolation

Hepatocytes were obtained from female lewis rat using two-step col-lagenase protocol. Two to three month old female Lewis rats (Charles River Laboratories, Wilmington, MA) weighing 180 to 200 g were used as a hepatocyte source and were maintained in accordance with National Research Council guidelines. Experimental protocols were approved by the Subcommittee on Research Animal Care, Massachusetts General Hospital. Using a modification on the two-step collagenase perfusion method^{17,18}, which involves purification of cell suspension by means of centrifugation over percoll, we routinely isolated approximately 200 million hepatocytes per rat liver with viability between 85% and 98% as evaluated by trypan blue exclusion.

MCF-7 cell culture

MCF-7 cells were maintained in DMEM at 37 °C, 5% CO₂. Cells were grown till 80% confluency and trypsinized using trypsin-EDTA and passaged at 1:10 dilution.

Toxicity experiments

Toxicity experiments were performed in 96-well plates. Briefly, 96-well plates were coated with 50 µg/mL fibronectin for 1 hour at 37 °C. Freshly isolated rat hepatocytes and MCF-7 cells were seeded at 50,000 cells/well in 100 µL of media and incubated overnight at 37 °C, 10% CO₂. Hepatocytes were seeded in hepatocyte maintenance media while MCF-7 cells were seeded in DMEM. Media was replaced and cells were exposed to tegafur or 5-FU + uracil in Williams E media at 37 °C, 10% CO₂. Uracil concentration was maintained at 100 µM, while 5-FU concentration varied. After 24 hour exposure, media was removed and cells were incubated with 0.5 mg/mL MTT reagent for 2 hours. Media was removed from the wells and 100 µL DMSO was added to each well and mixed on a shaker for 10 minutes. The absorbance was measured at 570 nm and IC-50 values were obtained using Sigmaplot software with a sigmoidal 4-parameter fit.

Microfluidic device fabrication and cell culture

Fabrication of microfluidic device—A two-chamber, membrane-based microfluidic device was fabricated at Massachusetts General Hospital's BioMEMS Research facility and assembled in the lab. Briefly, silicon-wafer templates served as negative molds to generate the top layer of the device in poly(dimethyl)siloxane (PDMS, Sylgard 184, Dow Corning), using standard soft-lithography protocols¹⁹. Appropriate dimension of channel for the bottom layer was laser-cut on a thin PDMS sheet (250 µm, HT-6240-.010, Rogers Corporation) and bonded to a 50 × 22 mm glass slide using oxygen plasma treatment followed by incubation at 70 °C for 10 minutes. A 3.0 µm pore sized PET-based transwell membrane insert (FisherSci, Cat No. 07-200-171) was cut to dimensions using a laser cutter. The top chamber of the device was first bonded to the membrane. Briefly, a 10 µm layer of PDMS pre-polymer was spin-coated onto a clean glass coverslip and a clean top layer was placed onto it for the PDMS to spread on the surface around the channel. A clean laser-cut membrane was then applied to the PDMS pre-polymer coated surface and bonded carefully while ensuring the channels remained free of PDMS pre-polymer and covered the ports for the top chamber only. The top layer with the membrane was cured at RT for 48 hours until

the PDMS cured and held the membrane tightly. Once the top and bottom layers are assembled individually, they are then bonded to each other using oxygen plasma treatment, with the membrane being in the center of the device. The assembled final device is then heated at 70 °C overnight to strengthen the bonds and stored in a dry, dark place till use.

Seeding cells into the microfluidic device culture—Microfluidic devices were wiped clean with 70% isopropanol and sterilized under UV in a hood for 20–30 minutes. Both the top and bottom chambers of the device are then filled with 50 µg/mL fibronectin and incubated for at least 1 hour at 37 °C. In the bottom chamber, 10 µL of primary rat hepatocytes (5 million/mL), and in the top chamber 10 µL of MCF-7 (10 million/mL) were introduced and incubated at 37 °C, 10% CO₂ overnight. Media in the device was replaced with Williams E media for toxicity experiments.

Transwell culture systems

Transwell experiments were performed in 12-well transwell culture systems with a 3.0 µm pore size. Briefly, well and transwell were coated with 50 µg/mL fibronectin and incubated for 1 hour at 37 °C. To the well, 0.5 M freshly isolated rat hepatocytes were added and to the transwell, 0.15 M MCF-7 cells were added and incubated overnight at 37 °C, 10% CO₂. Media was replaced with Williams E media for toxicity experiments.

CYP 450 assay

CYP450 1A1/2 activity was evaluated using 7-ethoxyresorufin. For hepatocytes in wells, 500 µL of substrate (10 µM 7-ethoxyresorufin + 80 µM dicumarol) in Williams E media was added and incubated at 37 °C; 100 µL of the reagent was withdrawn at 15, 30, 45 and 60 minute intervals. For hepatocytes in devices, multiple devices ($n = 2$ per time point) were used and reagent was collected at 15, 30, 45 and 60 minute intervals in 20 µL Williams E media, and diluted to 100 µL. Rate of resorufin production was calculated by diluting resorufin standard in Williams E media. Fluorescence from the collected sample was measured at $\lambda_{\text{ex}} = 525 \pm 10$ nm and $\lambda_{\text{em}} = 580 \pm 10$ nm.

CYP450 3A4 activity was evaluated using CYP3A4 kit from Promega (Cat No. V9001) with setup similar to CYP450 1A1/2 assay. Hepatocytes in both wells and transwells were exposed to substrate solution (3 µM Luciferin-IPA) and media collected at 15, 30, 45 and 60 minute intervals. Media in the devices was collected in 20 µL Williams E media, and diluted to 100 µL. To 50 µL sample, 50 µL detection reagent was added and luminescence from the sample was measured with a 1 second integration time. Rate of luciferin production in the samples was calculated using beetle luciferin (Promega, Cat No. E1601) as standards.

Data normalization and statistics—Concentration of product formed in microfluidic devices was normalized to 1 µL of culture media within the device. Data was averaged from $n = 2$ experiments with $n = 2$ wells, $n = 8$ devices per experiment.

Lactose dehydrogenase (LDH) assay

LDH in the supernatant was evaluated using CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega Cat No. G1780). For plate assays, 50 µL of media was mixed with 50 µL

reagent and incubated for 30 minutes at RT in dark. For microfluidic devices, media in the device was collected in 20 μ L of fresh Williams E media. To 5 μ L of the media, 45 μ L of Williams E media was added and mixed with 50 μ L reagent and incubated for 30 minutes at RT in dark. At the end of incubation, 50 μ L stop solution was added and absorbance measured at 490 nm.

Data normalization and statistics—LDH from culture supernatant is normalized as LDH from cultures exposed to 100 μ M tegafur + 100 μ M uracil to LDH from cultures exposed to vehicle control. Data is averaged from $n = 2$ experiments for plate with $n = 3$ samples per experiment, and $n = 5$ experiments for devices with $n = 2$ or 3 per experiment. Data is plotted as fold change over controls.

Mass spectrometry

Tegafur, 5-FU, and uracil concentrations in media were quantified using an LC/MS-MS 3200 QTRAP Hybrid Triple Quadrupole Linear Ion Trap mass spectrometer (AB SCIEX, Foster City, CA) coupled to a 1200 Series Binary LC System (Agilent Technologies, Santa Clara, CA). A standard solution of each analyte was directly infused into the mass spectrometer, which was operating under negative mode with the following settings: Curtain gas (CUR) at 30.0, collision gas (CAD) at 5, IonSpray Voltage (IS) at -4500.0 , the temperature (TEM) of the turbo gas in the TurboIonSpray at 400°C and both Ion Source Gases (GS1 and GS2) at 60.0. Using Analyst software (Version 1.5, AB Sciex), a 'Compound Optimization' routine was performed to identify multiple reaction monitoring (MRM) precursor/product ion transition pairs for each analyte that maximizes peak intensity. The tunable MS-specific parameters for the optimization were the declustering potential (DP), the entrance potential (EP), the collision energy (CE), as well as the collision cell exit potential (CXP). The optimized parameters for each MRM transition are summarized in Supplementary Table 1.

MS data acquisition for each sample was achieved by injecting 10 μ L of media through a liquid chromatographic (LC) separation phase followed by simultaneous detection of all three MRM transitions, each with a dwell time of 500 ms. The LC method utilized a Synergy Hydro-RP (reverse phase) column (150 mm $\times$ 2 mm inner 4 μ m 80 $\text{\AA}$ particles; Phenomenex, Torrance, CA), which was kept at ambient temperature. The aqueous mobile phase A was HPLC grade water with 0.1% formic acid and the organic phase B was HPLC grade methanol with 0.1% formic acid. The elution gradient was set as: 0 minute — 3% B, 3 minute — 3% B, 12 minute — 95% B, 15 minute — 95% B, 18 minute — 3% B, 25 minute — 3% B. A sample chromatogram for all three analytes is shown in Supplementary Fig. 4.

Data normalization and statistics—Data was averaged from $n = 3$ samples per time point. The concentration of each analyte in the media was determined by comparing the Area Under Curve (AUC) for each peak with the corresponding standard curve obtained from serial dilutions of each pure chemical dissolved in methanol. Tegafur, uracil and 5-FU was measured from atleast $n = 3$ samples and averaged.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from the National Institutes of Health NIH-5UH2TR000503, NIH-F32DK098905 for WJM and NIH-F32DK103500 for GS. We acknowledge support from the MGH BioMEMS resource center in fabricating microdevices.

References

1. Kaplowitz, N. Drug-induced liver injury: Introduction and overview. In: Kaplowitz, N.; DeLeve, LD., editors. *Drug Induced Liver Disease — Third Edition*. Elsevier; 2013.
2. Schuster D, Laggner C, Langer T. Why drugs fail — A study on side effects in new chemical entities. *Curr Pharm Des*. 2005; 11(27):3545–3559. [PubMed: 16248807]
3. Bale SS, Vernetti L, Senutovitch N, Jindal R, Hegde M, Gough A, McCarty WJ, Bakan A, Bhushan A, Shun TY, Golberg I, Debiasio R, Usta BO, Taylor DL, Yarmush ML. *In vitro* platforms for evaluating liver toxicity. *Exp Biol Med* (Maywood). 2014; 239(9):1180–1191. [PubMed: 24764241]
4. Godoy P, Hewitt N, Albrecht U, Andersen M, Ansari N, Bhattacharya S, Bode J, Bolleyn J, Borner C, Böttger J, Braeuning A, Budinsky R, Burkhardt B, Cameron N, Camussi G, Cho CS, Choi YJ, Craig Rowlands J, Dahmen U, Damm G, Dirsch O, Donato M, Dong J, Dooley S, Drasdo D, Eakins R, Ferreira K, Fonsato V, Fraczek J, Gebhardt R, Gibson A, Glanemann M, Goldring CP, Gómez-Lechón M, Groothuis GM, Gustavsson L, Guyot C, Hallifax D, Hammad S, Hayward A, Häussinger D, Hellerbrand C, Hewitt P, Hoehme S, Holzhütter HG, Houston JB, Hrach J, Ito K, Jaeschke H, Keitel V, Kelm J, Kevin Park B, Kordes C, Kullak-Ublick G, LeCluyse E, Lu P, Luebke-Wheeler J, Lutz A, Maltman D, Matz-Soja M, McMullen P, Merfort I, Messner S, Meyer C, Mwinyi J, Naisbitt D, Nussler A, Olinga P, Pampaloni F, Pi J, Pluta L, Przyborski S, Ramachandran A, Rogiers V, Rowe C, Schelcher C, Schmich K, Schwarz M, Singh B, Stelzer EK, Stieger B, Stöber R, Sugiyama Y, Tetta C, Thasler W, Vanhaecke T, Vinken M, Weiss T, Widera A, Woods C, Xu J, Yarborough K, Hengstler J. Recent advances in 2D and 3D *in vitro* systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. *Arch Toxicol*. 2013; 87(8):1315–1530. [PubMed: 23974980]
5. Dash A, Inman W, Hoffmaster K, Sevidal S, Kelly J, Obach RS, Griffith LG, Tannenbaum SR. Liver tissue engineering in the evaluation of drug safety. *Expert Opin Drug Metab Toxicol*. 2009; 5:1159–1174. [PubMed: 19637986]
6. Sung JH, Shuler ML. A micro cell culture analog (MicroCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. *Lab Chip*. 2009; 9:1385–1394. [PubMed: 19417905]
7. Ma L, Barker J, Zhou C, Li W, Zhang J, Lin B, Foltz G, Kublbeck J, Honkakoski P. Towards personalized medicine with a three-dimensional micro-scale perfusion-based two-chamber tissue model system. *Biomaterials*. 2012; 33(17):4353–4361. [PubMed: 22429982]
8. Chao P, Maguire T, Novik E, Cheng KC, Yarmush ML. Evaluation of a micro-fluidic based cell culture platform with primary human hepatocytes for the prediction of hepatic clearance in human. *Biochem Pharmacol*. 2009; 78:625–632. [PubMed: 19463793]
9. Szabo M, Veres Z, Baranyai Z, Jakab F, Jemnitz K. Comparison of human hepatoma HepaRG cells with human and rat hepatocytes in uptake transport assays in order to predict a risk of drug induced hepatotoxicity. *PLoS One*. 2013; 8(3):e59432. [PubMed: 23516635]
10. Wikswo JP. The relevance and potential roles of microphysiological systems in biology and medicine. *Exp Biol Med*. 2014; 239(9):1061–1072.
11. Mehling M, Tay S. Microfluidic cell culture. *Curr Opin Biotechnol*. 2014; 25:95–102. [PubMed: 24484886]

12. Hegde M, Jindal R, Bhushan A, Bale SS, McCarty WJ, Golberg I, Usta OB, Yarmush ML. Dynamic interplay of flow and collagen stabilizes primary hepatocytes culture in a microfluidic platform. *Lab Chip*. 2014; 14(12):2033–2039. [PubMed: 24770663]
13. Wikswo JP, Curtis EL, Eagleton ZE, Evans BC, Kole A, Hofmeister LH, Matloff WJ. Scaling and systems biology for integrating multiple organs-on-a-chip. *Lab Chip*. 2013; 13(18):3496–3511. [PubMed: 23828456]
14. Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: Mechanisms of action and clinical strategies. *Nat Rev Cancer*. 2003; 3(5):330–338. [PubMed: 12724731]
15. Meropol NJ, Sonnichsen DS, Birkhofer MJ, Ferreira I, Noel D. Bioavailability and Phase II Study of Oral Uft Plus Leucovorin in Patients with Relapsed or Refractory Colorectal Cancer. *Cancer Chemotherapy and Pharmacology*. 1999; 43(3):221–6. [PubMed: 9923552]
16. Boisdron-Celle M, Remaud G, Traore S, Poirier AL, Gamelin L, Morel A, Gamelin E. 5-Fluorouracil-Related Severe Toxicity: A Comparison of Different Methods for the Pretherapeutic Detection of Dihydropyrimidine Dehydrogenase Deficiency. *Cancer Letters*. 2007; 249(2):271–82. [PubMed: 17064846]
17. Seglen PO. Preparation of isolated rat liver cells. *Methods Cell Biol*. 1976; 13:29–83. [PubMed: 177845]
18. Dunn JC, Yarmush ML, Koebe HG, Tompkins RG. Hepatocyte function and extracellular matrix geometry: Long-term culture in a sandwich configuration. *FASEB J*. 1989; 3(2):174–177. [PubMed: 2914628]
19. McDonald JC, Chabinyc ML, Metallo SJ, Anderson JR, Stroock AD, Whitesides GM. Prototyping of microfluidic devices in poly(dimethylsiloxane) using solid-object printing. *Anal Chem*. 2002; 74(7):1537–1545. [PubMed: 12033242]

Figure 1.

Schematic showing the preparation of a two-chamber microfabricated device. **(a)** Device components include a top chamber (PDMS cast), a laser cut tissue-culture membrane, a bottom chamber (PDMS sheet) and a glass slide. The top chamber has ports for access to both top and bottom chambers. **(b)** The tissue culture membrane is attached to the top chamber (spin-coated PDMS, 10 μm) and the bottom chamber is bonded by plasma treatment onto the glass slide. **(c)** The top chamber with membrane and bottom chamber on glass slide are aligned and bonded using plasma treatment. **(d)** Top view of the assembled two-chamber device showing inlet and outlet ports for top chamber and bottom chamber. **(e)** Cross-section of the two-chamber device showing MCF-7 cells seeded in the top chamber (100 μm height) and hepatocytes seeded in the bottom chamber (250 μm height).

Figure 2. Comparison of enzyme activity and product accumulation in 12-well plate and single chamber microfabricated device. Rate of product formation of (a) Resorufin (CYP1A1/2) and (b) Luciferin (CYP 3A4) in both culture formats.

Figure 3.

Tegafur metabolism and 5-Fluorouracil (5-FU) toxicity comparison in 12-well plate and microfabricated devices. **(a)** Metabolic conversion of tegafur into 5-FU by CYP present in hepatocytes. Tegafur is a non-toxic pro-drug, whereas 5-FU is toxic to dividing, cancerous cells. **(b)** Cell placement within the two-chamber device. Hepatocytes in the lower chamber convert tegafur into 5-FU, which is uptaken by MCF-7 cells in the top chamber resulting in cell death and is measured by LDH release. **(c)** Comparison of LDH release of hepatocyte, MCF-7 and co-culture exposure to 100 μ M tegafur + 100 μ M uracil in 12-well plate and microfabricated device formats. Single cell controls were performed in single chamber devices.

Figure 4. Mass spectrometry analysis of tegafur and uracil consumption, and 5-FU production in microfabri-cated devices. Kinetics showing of (a) tegafur, uracil consumption and (b) 5-FU production in single chamber device seeded with rat primary hepatocytes.

Table 1

Table listing culture parameters of device and plate set-up used in this study.

	Single chamber device		Two-chamber device		12-well plate	12-well plate with transwell	
			Top	Bottom		Transwell	Well
Culture area	10 mm ²		10 mm ²	10 mm ²	3.8 cm ²	1.12 cm ²	3.8 cm ²
# Cells	10,000		10,000	10,000	500,000	150,000	500,000
Volume	1 µL		1 µL	2.5 µL	500 µL	500 µL	1000 µL
Media volume/hepatocyte	0.1 nL		0.35 nL		1 nL	3 nL	

Table 2
IC-50 values of tegafur and (5-Fluorouracil + uracil)

Cell type	Tegafur	5-Fluorouracil + uracil
Rat hepatocytes	> 1200 μ M	> 200 μ M
MCF-7	340 μ M	13.5 $\pm$ 0.5 μ M