APPLICATION NOTE

Application of the PhysioMimix® Single-organ Higher Throughput System for DILI risk assessment

Emily Richardson, Alysha Bray, Dharaminder Singh, Tomasz Kostrzewski

CN Bio Innovations

visit **cn-bio.com**

CN-BIO

CN Bio's organ-on-chip systems, which include the PhysioMimix® Single- and Multi-organ lab-benchtop instruments, enable researchers to model human biology in the lab through rapid and predictive human tissue-based studies.

The technology bridges the gap between traditional cell culture and human studies, advancing towards the simulation of human biological conditions to support the accelerated development of new therapeutics in application areas including oncology, infectious diseases, metabolism and inflammation.

Learn more at **cn-bio.com**

Introduction

When developing drugs, the liver is a key organ. As well as being central to the metabolism of many molecules, it also represents one of the primary tissues affected by drug-induced injuries. Prediction of clinical safety is still a major hurdle in drug discovery and development, with many late-stage attrition events still occurring due to limited translatability of current *in vitro* or *in vivo* preclinical models. Additionally, the field is facing growing pressure from both societal and regulatory leaders, as well as economic and logistical barriers, to reduce unnecessary preclinical animal use through the use of new approach methodologies (NAMs).

Organ-on-a-chip (OOC), also known as microphysiological systems (MPS) have been developed to address these challenges. Several MPS platforms are available for assessing drug-induced liver injury (DILI), however, our PhysioMimix® OOC range of MPS is particularly well suited. The PhysioMimix Liver MPS (or Liver-on-a-chip) combines primary human hepatocyte (PHH) and Kupffer cells (KCs) to form microtissues within a perfused 3D collagen-coated scaffold (Figure 1)[1]. The model is used in the PhysioMimix DILI assay where it provides large amounts of recoverable material (media and tissue) for in-depth analysis to understand biotransformation and mechanisms of human toxicity. The assay delivers exceptional performance as exemplified in a prior study using 13 reference compounds from the IQ MPS Consortium DILI validation set which delivered 100% sensitivity, 85% accuracy, and 100% precision in the Liver-12 plate and has been recognized by the U.S. FDA CDER (Centre for Drug Evaluation and Research) group, who cited superior performance versus standard approaches in the first publication between OOC provider and regulator[2].

Until now, there has been an inverse relationship between the high human-relevance required to predict human outcomes and throughput capacity. To date, this has limited the application of MPS to smaller scale investigative toxicity studies. However, adopting Liver MPS earlier in the pipeline at the lead optimization phase provides significant advantages. Its data-rich analyses can be used to understand the root cause of DILI to facilitate early project termination or potentially enable flawed drugs to be recovered. By reducing the number of molecules passing through into

preclinical testing, the approach supports a reduction in the number of animals required, contributing to 3Rs efforts, and saving costs.

Here, we present a new higher throughput approach that can be applied within lead optimization – the PhysioMimix Single-organ Higher throughput (HT) System and Multi-chip Liver-48 plate. Although the 48 liver chips within the higher throughput plate are miniaturized versus the proven Liver-12 plate, its greater capacity enables additional drug concentrations to be explored within the same overall range to increase the assay's resolution and accuracy.

Delivering equivalent tissue formation and functionality (versus the original Liver-12 plate), plus data that the meets IQ MPS Consortium mandated functionality requirements for liver tissue in DILI assessment[6], the study results demonstrate the system's applicability for earlier DILI risk assessment bringing a new dimension of human relevance to this key drug discovery phase.

Two individual donors were used to understand the model's sensitivity and ability to elucidate donor-dependent responses to Chlorpromazine, a well-known cause of acute cholestatic liver injury in some patients. Different elevations in DILI markers were detected between donors, demonstrating the model's ability to differentiate patient sensitivities to the challenge. The Liver-48 plate was subsequently characterized using a pair of tool compounds recommended by the IQ MPS Consortium – Troglitazone (high DILI concern) and Pioglitazone (low DILI concern) – and demonstrated its ability to differentiate the compounds' DILI risk. The response of Liver-48 microtissues to Troglitazone was compared to Liver-12 plate microtissues, delivering similar results. Collectively, these data demonstrate the power of the new higher throughput MPS model to predict human DILI earlier in the drug development pipeline.

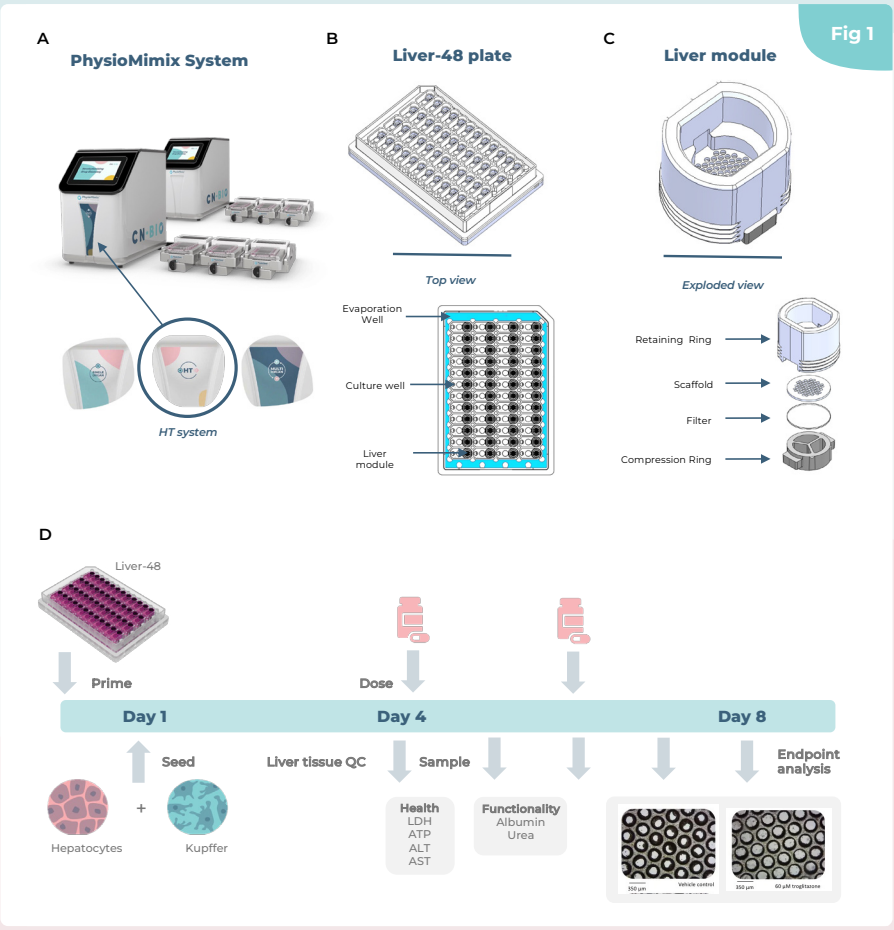


Figure 1. The PhysioMimix Single-organ HT System and workflow.

A. The PhysioMimix Single-organ HT System controller, docking station and associated drivers.

B. A schematic of the PhysioMimix Multi-chip Liver-48 plate, with top view shown below.

C. A schematic of the Liver Module within the Liver-48 plate, with an exploded view shown below with retaining ring, scaffold, filter and compression ring.

D. The PhysioMimix DILI assay workflow using the Liver-48 plate.

Materials and Methods

Cryopreserved primary human hepatocytes (PHH) and primary human Kupfer Cells (KC) were obtained from LifeNet Health. 0.6 x 10^6 PHH and 0.06 x 10^6 KC's were seeded into each well of a PhysioMimix Multi-chip Liver-12 plate (CN Bio) whilst 0.15 x 10^6 PHH and 0.015 x 10^6 KC's were seeded into each well of a PhysioMimix Multi-chip Liver-48 plate (CN Bio), both using Seeding Medium (Advanced DMEM, Cocktail A, FBS, Hydrocortisone). Media was changed at Day 1 to Maintenance Medium (Advanced DMEM, Cocktail B and Hydrocortisone). The microtissues were cultured in the PhysioMimix Single-organ Higher Throughput (HT) System for up to 8 days using the respective MPS driver for each plate type.

After quality control checks of viability and functionality at Day 4, compounds were dosed into triplicate wells at 7 concentrations in Maintenance Medium containing 0.1% DMSO, and then every 48 hours. Vehicle control consisted of maintenance medium containing 0.1% DMSO.

Production of LDH release was quantified using the Cyto-tox96 assay (Promega), albumin production was measured by ELISA (R&D systems), urea synthesis was quantified using QuantiChrom™ kit (VWR), CYP3A4 activity was measured using P450-Glo™ CYP3A4 Assay (Promega), ALT activity was measured with Alanine Transaminase Activity Assay kit (Abcam), and cell viability was assessed using the CellTiter-Glo® 3D Cell Viability assay (Promega).

For immunofluorescence staining, briefly, microtissues were fixed using 4% paraformaldehyde and permeabilized using 0.3% Triton-X-100 before staining with anti-albumin (ab207327, Abcam), phalloidin-567 (P1951, Merck) and Hoechst-3334 (H3570, Invitrogen) in Blocking Buffer (BSA, FBS and GS in 0.1% PBS with Tween-20).

All compounds were procured from Tocris Bioscience.

Results

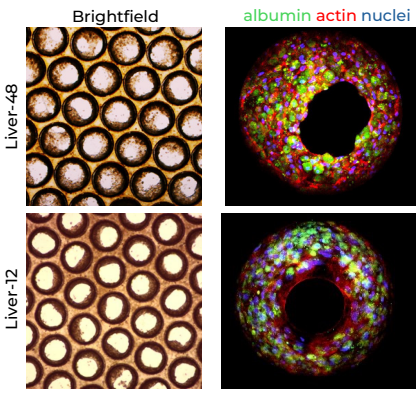
The Multi-chip Liver-48 plate delivers equivalent tissue formation and functionality to that of the Liver-12 plate.

The Liver-48 plate was first tested for its ability to produce viable and functional liver microtissues in an equivalent manner to the Liver-12 plate. The same donor combination of hepatocytes and Kupffer cells were seeded into each plate type and cultured for 8 days. Using brightfield and fluorescence microscopy, tissue formation and structure was compared between the Liver-12 and Liver-48 plates (Figure 2A). Liver microtissues, with robust "donut-shaped" structures forming within the scaffold pores, representing the liver sinusoidal structure, were created in each version. Visual observations were confirmed by immunofluorescence tagging of tissue actin cytoskeleton (morphology), as well as albumin (functionality, Figure 2A).

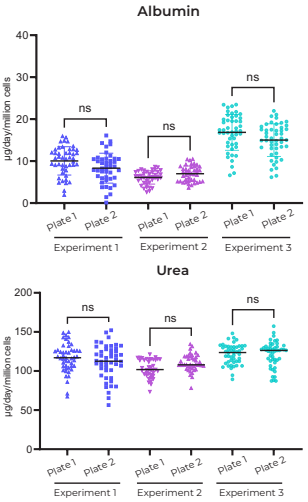
At day 4, quality control metrics were taken to understand the functionality of Liver-48 plate microtissues using albumin and urea as endpoints (Figure 1D). Three independent experiments were run using two separate plates, and the variation between plates and experiments was examined (Figure 2B). Over the three experiments, mean hepatocyte functionality ranged between 6 and 17 µg/min/million cells for albumin, and 97 to 122 µg/min/million cells for urea at day 4. To note, the initial PhysioMimix-validation of the hepatocyte donor used in this study demonstrated low albumin levels at day 4 which increased over time. The same trend was observed within this study.

The inter- and intra-experimental variation for the Liver-48 plate was examined and shown to be equivalent between experiments. No significant difference was seen between duplicate Liver-48 plate experiments, demonstrating little inter-experimental variation (Figure 2B). Variation was seen between experiments, however some of this can also be accounted for by batch-to-batch variation of hepatocytes and Kupffer cells. The coefficient of variance for albumin was higher than that of urea, urea had a range of coefficient variance values from 13-19% (Figure 2C). Overall, it was considered that the variance seen within and between experiments was within an acceptable range.

A



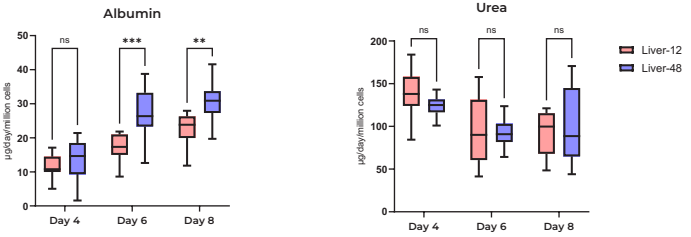
B



C

Exp	Plate	Albumin			Urea		
		Mean	Std Dev	% CV	Mean	Std Dev	% CV
1	1	10.06	3.38	33.6	116.6	18.5	15.5
	2	8.31	3.5	42.7	111.6	21.5	19.3
2	1	6.09	1.58	25.9	97.3	18.2	18.7
	2	7.51	2.23	29.7	112.7	12.9	11.5
3	1	16.8	4.27	25.3	121.6	12.8	10.5
	2	15.0	3.9	25.9	121.5	16.2	13.3

D



E

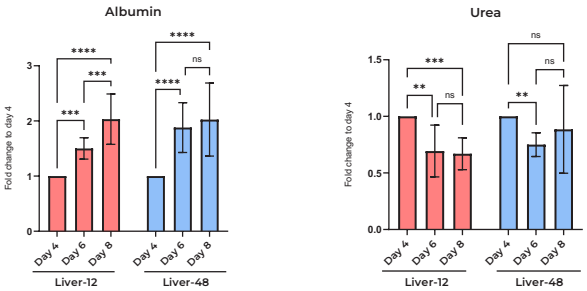


Figure 2 The Liver-48 plate PhysioMimix DILI assay format shows equivalent tissue formation and functionality to the Liver-12 plate.

A. Hepatocyte and Kupffer cells were cocultured in Liver-48 and Liver-12 plates for 8 days. The scaffolds were removed, imaged in brightfield (images on left) and subsequently fixed and stained with anti-albumin antibodies (**green**), phalloidin (actin, **red**) and Hoechst-33342 (nuclei, blue) (images on right – zoom of single liver microtissue).

B. Three independent experiments were carried out with two Liver-48 plates run per experiment. At day 4, media samples were taken and analysed for production of albumin (top) and urea (bottom).

C. Descriptive statistics of B., indicating the mean, standard deviation (Std Dev) and coefficient of variance (% CV) of albumin and urea.

D. Hepatocyte and Kupffer cells were cocultured in the Liver-48 and Liver-12 plates for 8 days. At days 4, 6 and 8 media samples were taken and analyzed for production of albumin and urea. Functionality was compared between Liver-12 (pink) and Liver-48 (blue) at each timepoint. Whiskers indicate min to max and central solid line the median. A one-way ANOVA was used to determine statistical differences, indicated as **** (<0.0001), *** (0.0002), ** (0.0021), * (0.0332) and ns (0.1234).

E. Hepatocyte and Kupffer cells were cocultured in the Liver-48 and Liver-12 plates for 8 days. At days 4, 6 and 8 media samples were taken and analyzed for production of albumin and urea. Error bars indicate standard deviation. Functionality between days was compared in each plate. A one-way ANOVA was used to determine statistical differences, indicated as **** (<0.0001), *** (0.0002), ** (0.0021), * (0.0332) and ns (0.1234).

Hepatocyte functionality in Liver-12 and Liver-48 plates was subsequently compared over 8 days of culture (Figure 2D). No significant decrease in functionality was observed between the two multi-chip plates, however a significant increase in albumin was detected at days 6 and 8 in the Liver-48 plate compared to Liver-12. The pattern of functionality from day 4 to 8 was observed to be similar for both plates. Albumin levels increased from day 4 to 8, whereas urea decreased (Figure 2E). The decrease in urea from day 6 to 8 was not significant in either plate. Over time, the overall change from day 4 to 8 remained within 0.5-log change, whereas albumin's fold change remained within a 2-log increase.

Overall, it was considered that tissue viability and functionality was equivalent to the Liver-12 plate, providing proof that microtissue quality is retained within the miniaturized format. The next phase of the study explored the plate's ability to deliver equivalent results when challenged with drugs in the PhysioMimix DILI assay so that it can be applied with the same degree of trust to earlier phases of the drug discovery process where there is a change of changing flawed drug chemistry to facilitate recovery.

The Liver-48 plate can be relied upon to deliver high sensitivity and equivalent drug-induced liver injury predictions compared to the proven Liver-12 plate.

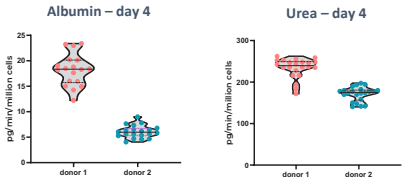
To test the Liver-48 plate's utility in assessing hepatotoxicity, two different hepatocyte donors were seeded into the plate and cocultured with the same Kupffer cell donor for 8 days. The donors were chosen based on their differing characteristics and lifestyles to understand whether these factors influence functionality and/or response (Figure 3A). At day 4, functionality metrics were taken to determine the health of liver microtissues (Figure 3B).

Higher albumin production was observed for donor 1 (mean donor 1, 18.2; versus donor 2, 6.1 µg/min/million cells), as was the level of well-to-well variation (standard deviation. Figure 3B). Despite the difference in the means reported by the two donors, the coefficient of variance (% CV) was similar at 17.2 and 20.3% for donor 1 and 2, respectively. A similar trend between the two donors was seen in urea production, with donor 1's production being marginally higher than donor 2's (233.8 and 171.2 µg/min/million cells, respectively). The variance was also similar between the donor pair, with a low CV of 10.3% and 10.1% respectively.

A

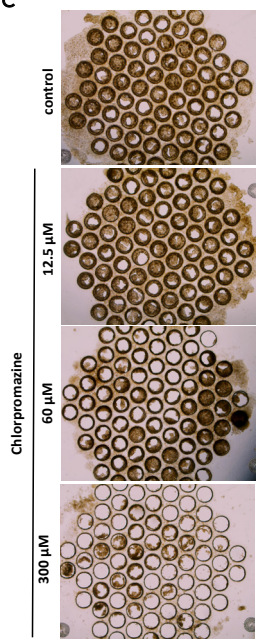
Donor	1	2
Characteristics	Male, 36, African American	Female, 44, Caucasian
Alcohol/drug use	Alcohol and cocaine user	Smoked and vaped
Disease state	High blood pressure	ALS

B

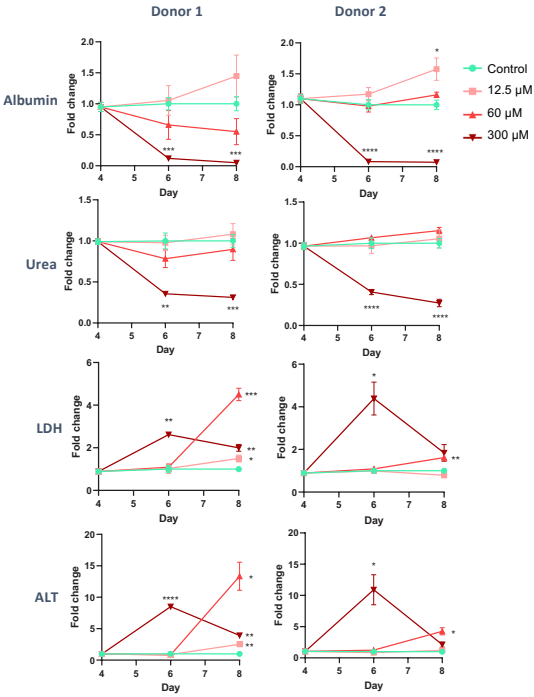


Donor	Albumin			Urea		
	Mean	Std Dev	% CV	Mean	Std Dev	% CV
Donor 1	18.2	3.1	17.2	233.8	24.1	10.3
Donor 2	6.1	1.2	20.3	171.2	17.4	10.1

C



D



E

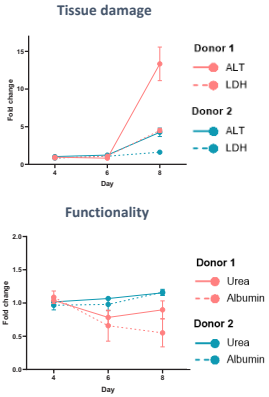


Figure 3. The PhysioMimix Liver-48 plate enables donor to donor variability in microtissue function and sensitivity to drugs to be explored.

A. The two hepatocyte donors used for this study are shown with their characteristics, alcohol/drug use and disease states.

B. Two hepatocyte donors (**Donor 1** and **Donor 2**) were cultured with the same Kupffer cell donor over 8 days. At day 4, albumin and urea production were measured (n=24). The mean, standard deviation (Std Dev) and coefficient of variance (% CV) are indicated for both metrics.

C. Images of representative scaffolds from the Liver-48 plate are shown for the control and chlorpromazine treated wells with concentrations 12.5, 60 and 300 µM.

D. The fold change in tissue damage (LDH and ALT) and functionality (albumin and urea) markers from day 4 to day 8 are indicated for donors 1 and 2 when treated with Chlorpromazine. Control (**green**), 12.5 µM (**pink**), 60 µM (**red**) and 300 µM Chlorpromazine (**dark red**). A two-way ANOVA was used to determine statistical differences, indicated as **** (<0.0001), *** (0.0002), ** (0.0021), * (0.0332) for significant differences.

E. A summary of tissue damage (top – LDH and ALT) and functionality (bottom – urea and albumin) fold changes at 60 µM Chlorpromazine from day 4 to 8 are indicated. Donor 1 (**pink**) and donor 2 (**blue**).

Following the functional assessment on day 4, liver microtissues were dosed with 3 concentrations of Chlorpromazine – 12.5 µM, 60 µM and 300 µM– in triplicate for 4 days, with repeat dosing 48 hrs after the initial dose (Figure 1D). Media samples were taken at days 4, 6 and 8 to determine tissue health and functionality post dosing. At the end of the experiment, microtissues were taken from the Liver-48 plate and imaged to analyze tissue integrity. As Chlorpromazine concentration increased, the integrity of microtissues was shown to decrease, particularly at the highest concentration (Figure 3C).

The pattern of decreasing microtissue integrity with increasing Chlorpromazine concentration correlated with the functional readouts. Both albumin and urea significantly decreased at days 6 and 8 after treatment with 300 µM Chlorpromazine compared to day 4 in both donors (Figure 3D). LDH and ALT peaked at day 6 in both donors and decreased at day 8. Together, the functional, viability and microscopy data indicate that the 300 µM Chlorpromazine was highly hepatotoxic in both donors, with cellular stress observed at day 6 and subsequent tissue destruction occurring by day 8.

Interestingly, at 60 µM Chlorpromazine, differences between the donor response were detected in the Liver-48 plate. The functionality of donor 1 began to decrease at day 6, as indicated by albumin and urea readouts (Figure 3D). Increases in LDH and ALT were detected at day 8 in both donors, however more significant fold changes were observed for donor 1 than donor 2. Through direct comparison, it was evident that donor 1 was more susceptible to hepatotoxicity from Chlorpromazine, with albumin and ALT being the most sensitive readouts (Figure 3E).

To determine the Liver-48 microtissue's ability to predict DILI risk, a pair of tool compounds recommended by the IQ MPS Consortium were applied to the model[6]. Troglitazone is a well-recognized cause of DILI (DILI rank 8) and Pioglitazone is of low DILI concern (DILI rank 3). Seven concentrations (each in triplicate) of each compound, ranging from 0.4 to 300 µM, were added to Liver-48 plate microtissues over a 4-day period, and liver microtissue viability and functionality were measured (Figure 4A). The approach enabled the differing DILI risk of the two compounds to be distinguished. Microtissues treated with increasing concentrations of Pioglitazone retained viability and functionality (Figure 4B), whereas microtissues treated with Troglitazone displayed decreased functionality

(albumin, urea), increased cellular stress markers (ALT, AST) and ATP depletion (Figure 4B).

The Liver-12 plate PhysioMimix DILI assay was prior qualified using a range IQ MPS Consortium recommended tool compounds by Novac *et al* in 2020[1]. To determine whether the Liver-48 plate delivers equivalent predictivity, the toxicity profile of Troglitazone was evaluated using both plate types and the results compared. Of note, an additional concentration (80 µM), in triplicate, was tested in the Liver-48 plate. The higher throughput capacity the Liver-48 plate enables additional drug concentrations to be explored within the same overall range to increase the assay's resolution/accuracy. Functionality and viability IC50 curves demonstrated that the two plates provided similar toxicity profiles (Figure 4C). Slight shifts in IC50 curves were observed, for example a decrease in urea was detected at 60 µM in the Liver-12 plate compared to 80 µM in the Liver-48 plate. Additional work is required to understand these minor differences in response, however overall, the two plates predicted a similar DILI outcome for Troglitazone.

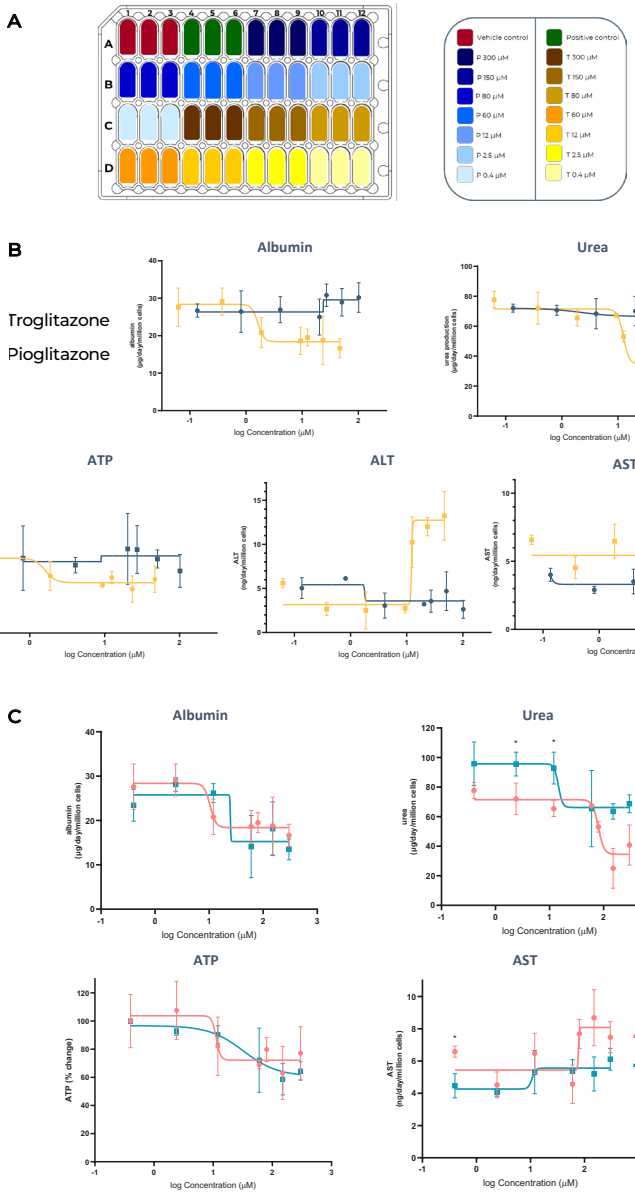

A
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
Vehicle control
P 300 µM
P 150 µM
P 80 µM
P 60 µM
P 12 µM
P 2.5 µM
P 0.4 µM
Positive control
T 300 µM
T 150 µM
T 80 µM
T 60 µM
T 12 µM
T 2.5 µM
T 0.4 µM
B
Troglitazone
Pioglitazone
Albumin
Urea
ATP
ALT
AST
log Concentration (µM)
C
Albumin
Urea
ATP
AST
log Concentration (µM)
Liver-48
Liver-12

Figure 4. The Liver-48 plate assay predicts the DILI risk of IQ MPS Consortium pair Troglitazone and Pioglitazone.

A. An example plate map for experiments used to perform studies. Actual plate maps may have different individual well placement.

B. Liver microtissues cultured for 4 days in the Liver-48 plate were exposed to Troglitazone (**yellow**) and Pioglitazone (**cyan**) for a further 4 days. Functionality (top panel – albumin and urea) and microtissue health (bottom panel – ATP, ALT and AST) were examined against concentration of the drugs. The dose-response curves shown were generated from a 7-dose concentration gradient. N=3 for all conditions. Error bars indicate standard deviation.

C. The dose-response curves of liver microtissues cultured in either Liver-48 (**pink**) or Liver-12 (**cyan**) plates and treated with Troglitazone for 4 days are indicated. Functionality (top panel – albumin and urea) and microtissue health (ATP, AST) were compared. Liver-48 microtissues were treated with 7-dose concentration gradient, and Liver-12 with 6-dose concentration gradient, respectively. N=3 for all conditions. Error bars indicate standard deviation. Multiple unpaired t-tests were undertaken to determine significant differences, with all comparisons having a P value of >0.0332 (ns).

Discussion

Pre-IND enabling studies, safety risk assessments in drug development rely heavily on *in vitro* cell-based assays, as well as animal testing. These *in vitro* assays are often high throughput, which is useful to rule out any obvious intrinsic DILI risks, however, they lack the human-relevance and complexity to decipher indirect or idiosyncratic DILI events, which are often linked to human-specific interactions with drugs. MPS technology is designed to address these limitations, however until now, there has been an inverse relationship between high human-relevance and throughput capacity.

The PhysioMimix Single-organ HT System and its associated Multi-chip Liver-48 plate were designed to deliver a higher throughput PhysioMimix DILI assay without significantly compromising microtissue complexity, assay robustness or reliability versus the original Liver-12 plate format. Experiments performed here evaluated 2 drugs, with a 7-point dose response curve, in triplicate, with positive and negative controls within one plate - allowing for better control of inter- and intra- experiment variability (Figure 4A). The study demonstrated that the viability and function of microtissues grown in the Liver-48 plate for 8 days were directly comparable to those in the Liver-12 plate. Subsequent studies will further characterize microtissue functionality over increasing periods of time (e.g., up to 28 days), to confirm equivalence versus the Liver-12 plate[2]. This will enable confidence in the use of the miniaturized format for chronic dosing studies to understand latent toxic events, providing researchers with a choice of plate formats to suit their throughput requirements.

The application of the Liver-48 plate for evaluating DILI was successfully tested. Firstly, two hepatocyte donors with differing characteristics were cultured in the Liver-48 plate and challenged with Chlorpromazine, a well-known cause of acute cholestatic liver injury in some patients. Subtle differences in response were detected using the Liver-48 plate, highlighting the sensitivity of the miniaturized model. Donor 1 exhibited a more significant and sensitive response to Chlorpromazine compared to donor 2. Although the specific mechanisms are still unknown, literature suggests that higher sensitivity to Chlorpromazine may be elicited by either hypersensitivity or production of toxic metabolites through idiosyncratic metabolic pathways[7]. Intriguingly, donor 1 was a high alcohol

and cocaine user, and so it may be hypothesized that these environmental factors may have led to increased liver damage and therefore hepatocyte sensitivity. These results demonstrate that the Liver-48 plate can be used to investigate differing idiosyncratic DILI risks due to population or environmental factors. Future studies will explore the responses derived from a broader range of donors with differing phenotypes to further characterize the approach.

Next, a pair of tool compounds (Troglitazone and Pioglitazone) recommended by the IQ MPS Consortium were used to challenge the model[6]. Troglitazone was the first and Pioglitazone the third marketed thiazolidinedione for type II diabetes. Three years post approval, Troglitazone was withdrawn from the market following reported deaths due to liver failure, whereas Pioglitazone is still used as a third-line therapy and is rarely associated with DILI. Liver-48 plate microtissues were able to successfully differentiate the DILI risks of the compounds, using liver functionality (albumin and urea) and viability (ATP, ALT and AST) markers. The DILI profile of Troglitazone was found to match that predicted by Liver-12 plate microtissues, demonstrating result equivalence between the two plate types. The results of Pioglitazone testing accurately predicted this compound to be safe.

The Liver-12 plate has previously been qualified for DILI assessment performance using a range of tool compounds, as well as several new modality drugs[1]. It has also been applied to the understanding of DILI mechanisms through analysis of the liver microtissues and secretome in the surrounding media[3–5]. Further DILI assay evaluations will commence using the Liver-48 plate to determine its performance versus Liver-12 across the DILI landscape to elucidate mechanism of toxicity for more informed stop/go decision making, however, the results of this study indicate that the miniaturized Liver-48 plate format is ideally placed for lead optimization and investigative toxicology applications.

Incorporating this approach within the earlier lead optimization phase of drug discovery, will enable the design of more refined pre-clinical studies through the human relevant insights generated. By justifying the progression of only the most promising drugs into *in vivo* studies, the PhysioMimix Liver-48 DILI assay can reduce unnecessary animal use and potentially prevent the misclassification of drugs as toxic due to inter-species differences. Collectively, the results of the study have indicated that

the higher capacity format will facilitate the design and development of safer and more cost-effective drugs that can be brought to patients more rapidly than ever before.

More about the PhysioMimix Single-organ HT system and Multi-chip Liver-48 plate

An advantage of the PhysioMimix approach is its multi-chip plate consumable design. This enables the consumable to be purpose built to match the needs of each individual organ and permits increased throughput through miniaturization.

By miniaturizing each Liver chip, 48 individual tissues can now be cultured per plate. Each well in the Liver-48 plate is roughly equivalent to a quarter of a Liver-12 well. The number of pores (or microchannels) within its 3D scaffold was reduced to approximately a quarter but importantly, the size of the individual pores within the scaffold remains the same across both plates. Up to three plates can currently be controlled per PhysioMimix® Single-organ HT System, enabling a total of 144 chips per run. By increasing the throughput of the Liver plate, more drug candidates, replicates and controls can be simultaneously tested over a broader range of concentrations, conditions, or cell donors. The format thereby delivers a fast, robust and cost-effective understanding of human drug safety profiles earlier in the pipeline.

References

1. Novac, O. *et al*. Human Liver Microphysiological System for Assessing Drug-Induced Liver Toxicity In Vitro. JoVE (Journal Vis. Exp. 2022, e63389 (2022).
2. Rubiano, A. *et al*. Characterizing the reproducibility in using a liver microphysiological system for assaying drug toxicity, metabolism, and accumulation. Clin. Transl. Sci. 14, 1049–1061 (2021).
3. Gallagher, E. M. *et al*. Normalization of organ-on-a-Chip samples for mass spectrometry based proteomics and metabolomics via Dansylation-based assay. Toxicol. Vitr. 88, 105540 (2023).
4. Rowe, C. *et al*. Perfused human hepatocyte microtissues identify reactive metabolite-forming and mitochondria-perturbing hepatotoxins. Toxicol. Vitr. 46, 29–38 (2018).
5. Sarkar, U. *et al*. Integrated assessment of diclofenac biotransformation, pharmacokinetics, and omics-based toxicity in a three-dimensional human liver-immunocompetent coculture systems. Drug Metab. Dispos. 45, 855–866 (2017).
6. Baudy, A. R. *et al*. Liver microphysiological systems development guidelines for safety risk assessment in the pharmaceutical industry. Lab Chip 20, 215–225 (2020).
7. Telles-Correia, D. *et al*. Psychotropic drugs and liver disease: A critical review of pharmacokinetics and liver toxicity. World J. Gastrointest. Pharmacol. Ther. 8, 26 (2017).

CN-BIO

332 Cambridge Science Park
Milton Road, Cambridge, CB4 0WN

+44 (0) 1223 737941 | **enquiries@cn-bio.com**

visit **cn-bio.com**