Characterization of the effect of primary human hepatocyte (PHH) lots on function in CN Bio Innovations Liver-Chip using acetaminophen.

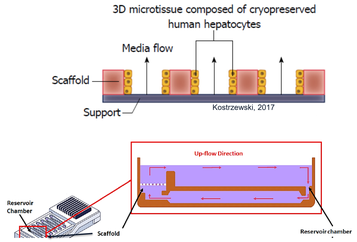
Kirsten Eckstrum, Kyra Headrick, Anneliese Striz, and Robert Sprando

Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD 20708

Introduction

Microphysiological systems (MPS), including organ-on-a-chip, are being evaluated for their ability to model human physiology, human and animal disease, and for their use in regulatory testing. Organ-chip systems use microfluidics to model physiological microenvironments experienced *in vivo*, such as shear flow and oxygenation levels experienced by cells that may impact the toxicological response. Traditional toxicity testing uses animal models to predict human responses. However, there can be inconsistencies with the accuracy of these predictions due to species differences. Organ-chips, using human cells, may be able to minimize these differences.

CN Bio Innovation's Liver MPS platform consists of an LC12 plate which is connected to a MPS Driver and loaded onto a Docking Station. This is connected to the PhysioMimix Controller which allows for regulation of flow and pre-set parameters.



The LC12 plate consists of a scaffold upon which primary human hepatocytes are seeded and form 3D microtissues inside the microchannels. Media circulates through the scaffold and reservoir to continually provide flow.

Primary human hepatocytes (PHH) are known to exhibit interindividual variability including drug metabolism and drug induction. To this end, our objective was to determine the individual, averaged individual, and pooled donor/lot variability in both baseline expression and toxic response to a known hepatotoxin (acetaminophen, APAP).

Preliminary studies have indicated that there is variability in all baseline endpoints between donors as well as sensitivity in response to the toxic effects of APAP. This was observed in the variability, sensitivity and specificity, and power analysis within this study. These findings suggest that more studies need to be conducted to elucidate how cell populations and combinations utilized in these studies should be selected to accurately represent the population of interest.

Methods

Four single donor lots and one 5 donor pooled lot were exposed to acetaminophen (APAP) at a Low (1-2mM), Medium (3-5mM), or High (10-13mM) dose for up to 96 hrs.

Cell Lot	Single donor/Pooled	Sex/Age Range	Qualification	Thaw Viability	Thaw Yield (cells/ml)
1	Single	Female 43	Human Plateable Metabolism Qualified (ThermoFisher)	97%	8.7 X 10^6
2	Single	Male 54	Human Plateable Induction Qualified (Lonza)	79%	6.5 x 10^6
3	Single	Male 59	Human Plateable Transporter Qualified (ThermoFisher)	91%	11.5 x 10^6
4	Single	Male 31	Human Plateable Induction Qualified (Lonza)	85%	9.6 x 10^6
5	Pooled (5 donor)	1 Males 4 Females Ages: 52-69	Human Plateable Hepatocytes, 5-Donor (ThermoFisher)	88%	8.4 X 10^6

- Liver MPS were seeded with primary human hepatocytes (PHH) at 0.6x10^6 cells/well and dosing concentrations were assigned randomly to minimize edge effects from evaporation
- Liver MPS were exposed to APAP for 4 consecutive days and media was collected for analysis at Day 4 (prior to exposure to APAP), Day 5 (24 hrs after exposure), Day 6 (48 hrs after exposure), and Day 8 (96 hrs after exposure).
- Media was analyzed for Albumin, Urea, LDH, ALT, and AST.
- Variability was determined by the average standard deviation per lot.
- Sensitivity/specificity was calculated based on the number of positives. A positive result was determined to be a change +/- 50% of the averaged control depending on the endpoint.
- Power and sample size were calculated based on the ability to detect a difference 50% greater or lesser than control, depending on the endpoint.

Results

Variability in baseline albumin secretion as well as response to APAP may impact interpretation of results between lots.

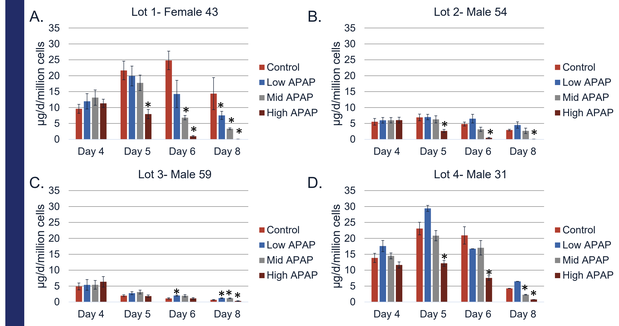


Figure 1: ***Different lots have different baseline albumin expression and exhibit variable sensitivity to APAP exposure***. *Albumin starting concentration was higher in 1-A and 1-D and lower for 1-B and 1-C. Lot 1 (1-A) was the most sensitive to APAP exposure because the low dose eventually became toxic, whereas Lot 3 (1-C) had the most difficultly surviving the duration of the experiment. 2-3 experiments per lot N=1-5 per condition per experiment. (*)= P<0.05*

Donor specific assay results may impact the interpretation of the effects of the toxic response

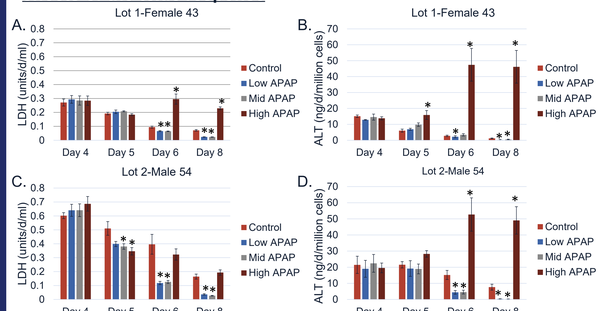


Figure 2: ***ALT, but not LDH, was consistently increased across donors***. *Lot 1 shows significant cell death with both LDH (2-A) and ALT (2-B). However, Lot 2 did not show significant cell death with LDH (2-C), but it did with ALT (2-D). Overall, starting cell death did appear higher in Lot 2 (2-C&D) than in Lot 1 (2-A&B) demonstrating lot differences with cell viability. 2-3 experiments per lot N=1-5 per condition per experiment. (*)= P<0.05*

Averaged individual donor results may capture large effects, while smaller changes can go undetected.

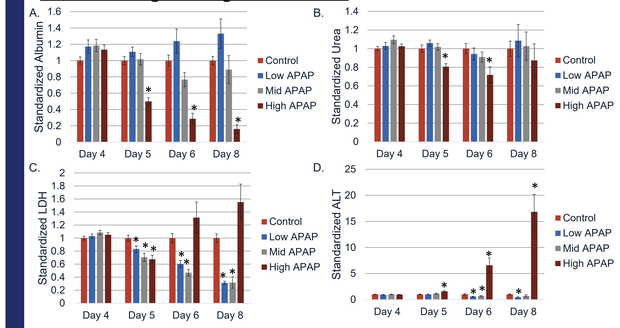


Figure 3: ***Averaged data from four single donors showed toxicity at high doses, but lost the sensitivity seen by some lots at lower doses***. *Standardized albumin of all individual donors showed toxicity with albumin at the highest concentration (3-A), but missed the toxicity demonstrated with Lot 1(1-A) at lower doses. Urea (3-B) showed a decrease with the highest APAP dose, but not on the final day due to lot effect differences. LDH (3-C) did not show an increase with the highest APAP dose, due to lot differences with LDH release. ALT (3-D) was strongly increased in combined lots, despite variability between lots. (Combined 4 single donor lots, 2-3 experiments per lot, 1-5 chips per condition per experiment. N=23-29.) (*)=p<0.05*

Pooled hepatocytes did not express high albumin levels

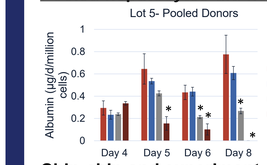


Figure 4: ***The pooled donors demonstrated very low albumin production at less than 1 µg/d/million cells***. *A dose response was still observed. (2 experiments, 1-2 chips per condition per experiment N=3-4 total.) (*)= p<0.05*

Chip-chip and experiment-experiment variability were similar within a lot, but different between lots

Assay	Lot 1- Female 43		Lot 2- Male 54		Lot 3- Male 59		Lot 4- Male 31		Lot 5- Pooled	
	Chip-Chip	Exp-Exp	Chip-Chip	Exp-Exp	Chip-Chip	Exp-Exp	Chip-Chip	Exp-Exp	Chip-Chip	Exp-Exp
LDH	0.14	0.10	0.30	0.31	0.28	0.28	0.18	0.18	0.15	0.15
Albumin	0.16	0.14	0.31	0.29	0.30	0.28	0.15	0.15	0.33	0.29
Urea	0.07	0.06	0.30	0.30	0.59	0.48	0.05	0.05	0.83	0.71
ALT	0.30	0.20	0.30	0.29	0.30	0.30	0.20	0.17	0.18	0.16
AST	0.25	0.21	0.21	0.20	0.38	0.37	0.27	0.25	0.18	0.18

Table 1: Variability was calculated by the average standard deviation of the controls across all days. Chip-Chip indicates the variation between chips within an experiment and experiment-experiment indicates the variation between the chips between experiments.

Sensitivity and Specificity depended on the lot as well as the assay

Assay	Lot 1- Female 43				Lot 2- Male 54				Lot 3- Male 59				Lot 4-Male 31				Lot 5-Pooled				Averaged single			
	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H
LDH	1	0	0	1	1	0	0	0.1	0.9	0	0	0	1	0	1	1	1	0	0	0	1	0	0.1	0.4
ALB	1	0.4	1	1	1	0	0.4	1	1	0	0	0.6	1	0	0.5	1	1	0.3	1	1	1	0.1	0.5	0.9
Urea	1	0	0	1	1	0.3	0.3	0.4	0.9	0.1	0	0	1	0	1	1	0.5	0.7	1	0	1	0.1	0.2	0.5
ALT	1	0	0	1	1	0	0	1	1	0.1	0.4	1	1	0	1	1	1	0	0	0.3	1	0	0.2	1
AST	1	0	0	1	1	0	0	0.6	0.7	0	0	0.7	1	0	0.5	1	1	0	0	0.3	0.9	0	0	0.8

Table 2: Sensitivity is the percentage of treated chips that showed a positive effect (+/- 50% changed from control), and Specificity is the percentage of control chips within 50% of the average control. Some lots and assays were more sensitive than others. C=Control, L=low APAP, M=Mid APAP, H=High APAP

Power and sample size varied based on lot

Assay	Lot 1- Female 43		Lot 2- Male 54		Lot 3- Male 59		Lot 4- Male 31		Lot 5- Pooled		Combined Single	
	Power	Sample size	Power	Sample size	Power	Sample size	Power	Sample size	Power	Sample size	Power	Sample size
LDH	100	1	69.4	4	75.5	3	96.3	2	94.6	1	100	2
Albumin	99.8	1	72.7	3	77.5	3	99.2	1	54.0	3	99.8	2
Urea	100	1	95.5	2	55.9	5	100	1	17.4	18	99.9	2
ALT	94.7	2	72.2	4	39.0	9	96.9	2	92.1	1	96.3	5
AST	93.1	2	74.2	3	71.4	4	77.9	3	87.2	2	99.6	3

Table 3. Power analysis and sample size were conducted based on the average standard deviation of the control in order to detect a +/- 50% change from control with 90% power and 5% Type I error rate. Therefore, the power indicates the strength of the data generated whereas the sample size is the number of chips that would be needed to detect a +/- 50% change from the control value.

CONCLUSIONS

- Variability in endpoint baseline levels as well as sensitivity to a toxic agent may make it difficult to combine and interpret results.
  - Lot 1 had decreased albumin with low APAP, which is missed if the data are combined.
  - Is Lot 1 more sensitive to APAP and would combining lots lead to potential susceptibility to sensitive groups?
- Some assays are more consistent across lots than others.
  - ALT was a more reliable marker across lots than LDH, which seemed to work well in some lots, but not in others.
  - Urea was a less sensitive marker than albumin for cell function.
- Variability, sensitivity, specificity, and power analysis all appeared to vary as a consequence of individual donor cell characteristics (ability to attach, response to toxic components, baseline biomarker levels).
- Ultimately, it is unknown why some lots/donors performed better or worse than others, however, age, sex, ethnicity, and life exposures all should be considered when designing toxicity studies in MPS systems in order to represent a large portion of the population.
- Further work needs to be performed to explore whether individual, averaged individual, or pooled donors more accurately predict toxicity for large populations.

FDA MISSION RELEVANCE

The project is in line with OARSA Division of Toxicology's Strategic Plan to use *in vitro* predictive toxicology methods to evaluate chemicals of interest to CFSAN in order to promote public health.

DISCLAIMER:

The mention of trade names or commercial products does not constitute endorsement or recommendation for use. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Food and Drug Administration.

References

Kostrzewski T, Cornforth T, Snow S. et al. Three-dimensional perfused human in vitro model of non-alcoholic fatty liver disease. World J Gastroenterol. Jan 2017.