Evaluation of the Activity of Major Drug Metabolizing Enzymes in a Novel All Human Hepatic Cell-Based Tri-Culture System

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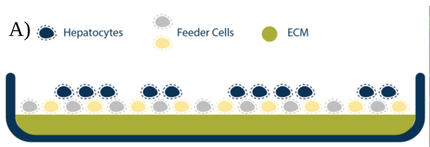
Background

Assessing *in vitro* hepatic clearance and identifying enzymes involved in metabolism is an important part of characterizing drug candidates in development. However, challenges exist for current *in vitro* models and can include enzyme levels and ratios of expression that are not physiologically relevant, as well as suspension culture durations limited to a 4–6-hour incubation, which is not long enough to accurately evaluate the clearance of slowly metabolized compounds.



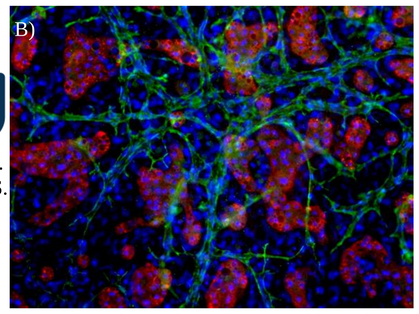


Figure 1. **A)** A model representation of the TCS. **B)** Confocal laser microscopy image of the TCS. *Blue*: nuclei (DAPI), *green*: endothelial cells (CD31), *red*: hepatocytes (albumin). Images provided by LifeNet Health LifeSciences (https://lnhlifesciences.org/tri-culture).

There have been advancements with novel hepatic models such as spheroids, organoids, and microfluidic systems that help address these current limitations, but these systems also require a high level of expertise to develop, operate, and maintain. The human hepatocyte tri-culture system (TCS), developed by LifeNet Health LifeSciences, may be a useful tool to help overcome some of these challenges. The TCS is comprised of three types of entirely human cells (primary hepatocytes and primary human "feeder" cells, including hepatic endothelial and stromal cells). The TCS is designed to extend culture duration while maintaining cell viability, functionality, and metabolic capability. The overall goal of this study was to characterize functionality and enzyme activity of major CYPs and UGTs in the TCS over several weeks.

B)

	24-well format	96-well format
Number of hepatocytes/ well seeded	300,000	20,000
Number of hepatocytes/ well that attach	124,000	9,837
Number of feeder cells/well seeded	100,000	10,000

Figure 2. **A)** 5-(6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) staining of TCS highlights bile canaliculi at day 22. **B)** Table of 24- and 96-well formats of TCS with number of cells seeded and correct number of cells attached determined by cytokeratin-18 staining in conjunction with 4′,6-diamidino-2-phenylindole (DAPI)

Experimental Methods

- Assess basic functionality of the TCS and culture establishment
- Compare 24-well and 96-well formats
- Monitor hepatic health by measuring albumin and lactate dehydrogenase (LDH) production for 24 hrs at each timepoint over 31 days
  - Albumin was detected using Abcam Human Albumin Simple Step Elisa® Kit
  - LDH was detected using Promega LDH-Glo Cytotoxicity® Kit
- Assess CYP and UGT activity by administering test compound and monitoring metabolite formation over a 60-min incubation over 31-days
  - Metabolites were quantified using LC-MS/MS
  - Activity rates were compared to that of suspended hepatocyte (same donor lot) incubated at 0.5 million cells/mL

Enzyme	Test Compound	Concentration	Metabolite Monitored
CYP2D6	dextromethorphan	70 µM	dextrorphan
CYP1A2	phenacetin	80 µM	acetaminophen
CYP2C9	diclofenac	20 µM	4′-hydroxydiclofenac
CYP3A4	midazolam	15 µM	1′-hydroxymidazolam
UGT1A1	estradiol	50 µM	estradiol 3-glucuronide
UGT2B7	azidothymidine	50 µM	3′-azido-3′-deoxythymidine β-D-glucuronide

Table 1. CYP and UGT enzymes tested with specific probe substrates indicated. Reactions were quantified by monitoring metabolite formation over 30 minutes in suspended hepatocytes, 24-well, and 96-well TCS formats.

Hepatic Functionality

Figure 4. **A-F)** Microscopy images of TCS 96-well format days 1-24 at 10X. **G-L)** Microscopy images of TCS 24-well format days 1-24 at 10X.

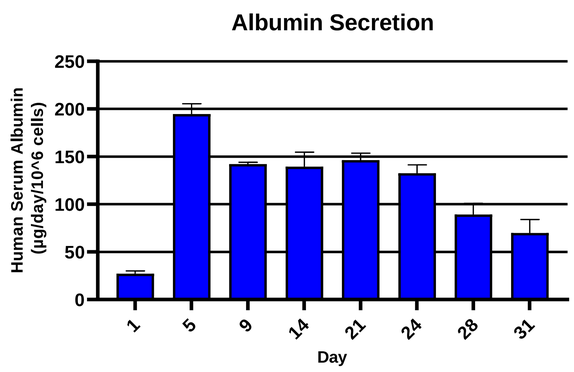


Figure 5. Albumin secretion over a 24 h period for each time point for the 96-well format TCS.

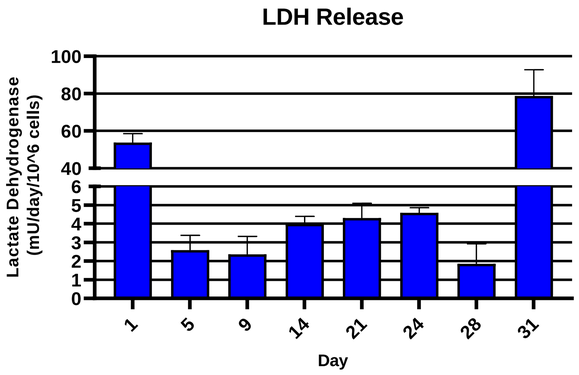


Figure 6. LDH secretion over a 24 h period for each time point for the 96-well format TCS.

Assessment of CYP and UGT enzyme activity over 31 days

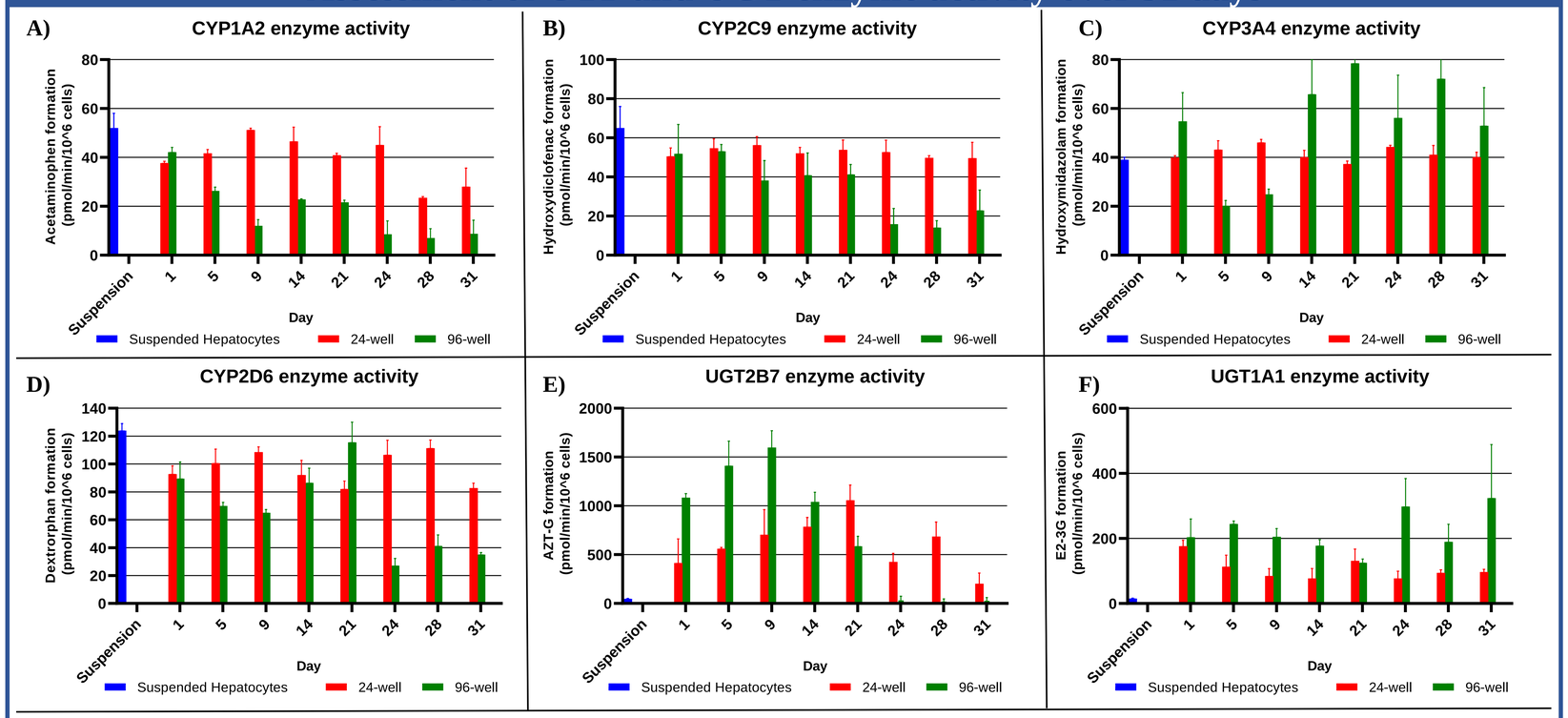


Figure 7. Assessment of activity of major CYP and UGT enzymes in the TCS plated in both 24- and 96-well formats over 31 days. Activity rates were compared to that of suspended hepatocytes utilizing the same lot of hepatocytes over a 30 min incubation. Media was changed every 48 hours, and on specified day media was removed and replaced with media containing probe substrate. Metabolite formation was monitored at 0, 15, 30 and 60 min. Control plates containing only feeder cells showed no metabolite formation (data not shown). A) phenacetin at 80 µM, B) diclofenac at 20 µM, C) midazolam at 15 µM, D) dextromethorphan at 70 µM, E) azidothymidine at 50 µM, F) estradiol at 50 µM.

Conclusions

- TCS hepatocytes generally appeared healthy and exhibited high levels of albumin secretion and low levels of LDH production for ~4 weeks.
- Hepatocytes in both 24-well and 96-well formats maintained CYP activity that was generally comparable to that of suspended hepatocytes for as long as 31 days in culture.
- CYP activity was consistent over 31 days in the 24-well format. In the 96-well format, CYP activity was stable over 21 days but dropped by day 28 and 31. Feeder cells showed no metabolism of tested CYP and UGT probes indicating that metabolism was due to hepatocyte metabolism.
- UGT activity was higher in TCS when compared to suspension hepatocytes, but also more variable when compared to CYP activity.