Lonza
Specialized Modalities

Validating a high-throughput co-culture of hepatocytes and non-parenchymal cells

Bringing physiological relevance to screening applications

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Summary

1. A high-throughput co-culture model comprising hepatocytes and non-parenchymal cells was developed and validated.
2. The model supports hepatocyte and NPC health and functionality for up to 5 days.
3. This versatile and physiologically-relevant model is suited to a range of drug discovery and ADME-Tox screening applications where understanding inflammatory responses and crosstalk between hepatocytes and NPCs is critical.

Introduction

Researchers in ADME-Tox and drug discovery and development are increasingly seeking models that combine high physiological relevance with high-throughput formats to accurately and efficiently represent the complexity of interactions between hepatocytes and various non-parenchymal cells (NPCs) in the human liver.

This Technical Note demonstrates the development and validation of a co-culture model that pairs primary human hepatocytes with Kupffer cells (KCs), stellate cells (SCs), and liver endothelial cells (LECs). Developed in a 96-well, high-throughput format, the model supports cultures for up to five days while maintaining the health and functionality of both hepatocytes and NPCs.

Designed for versatility, this model is ideal for applications such as immuno-toxicology, drug-induced liver functionality studies, drug-drug interaction (DDI) assessments, and other ADME-Tox research needs.

Methods

The materials used in the final validated model are listed in Table 1, while hepatocyte and NPC donor combinations are detailed in Table 2.

The co-cultures were prepared according to protocol instructions available on the Lonza website.[1] In brief, we began by preparing Lonza HCM™ Medium (part no. MP250) and Lonza Hepatocyte Culture Medium (HCM™ Medium BulletKit®; part no. CC-3198) as per the instructions supplied in the media kits. We also prepared a basement membrane overlay working solution (Corning® Matrigel® Matrix) by diluting the stock solution with HCM™ Medium to achieve a final concentration of 0.3 mg/mL.

After preparing all media, we thawed the hepatocytes (part no. HUCPI) at 37°C in a water bath until only a sliver of ice remained. The cells were then transferred to a pre-warmed 50 mL tube of Lonza Hepatocyte Thawing Medium (part no. MCHT50). After gently suspending cells by rocking, the tubes were centrifuged for 8 min at 100xg. The supernatant was removed and the cells were gently resuspended in 3 – 4 mL of pre-warmed Hepatocyte Plating Medium.

We assessed cell viability and density using trypan blue and a hemocytometer. The cells were then diluted to 500,000 cells/mL using Hepatocyte Plating Medium and gently suspended by inverting the tube. 100 µL of the resulting cell suspension was then added to each inner well of a collagen-coated 96-well plate (which can be bought pre-coated; if preparing your own, do so prior to cell thawing). Unused outer wells were filled with 100 µL PBS to minimize edge effects. We then placed the plates in the incubator for 1 hour at 37°C and 5% CO_2.

After 1 hour of incubation, all three types of NPCs (KCs, SCs, and LECs; part nos. HLKC-500K, HUCLS-200K and HLECP1, respectively) were thawed at 37°C until only a sliver of ice remained. Each cell type was transferred to its own 15 mL centrifuge tube containing cold (4°C) Hepatocyte Plating Medium and centrifuged at 300xg for 10 minutes at 4°C.

The supernatant was then removed, and the KCs and SCs were resuspended in 500 µL cold Hepatocyte Plating Medium while the LECs were resuspended in 1 mL of the same medium. For each cell type, we assessed cell viability and density using trypan blue and a hemocytometer.

To create the NPC mixture, aliquots from each cell type suspension were combined into a single tube and diluted with Hepatocyte Plating Medium. Figure 1 provides an example of this workflow for clarity. The volume of each aliquot and the added medium were calculated such that adding 100 µL of combined NPC cell suspension to each hepatocyte well would achieve the following ratio: 1 hepatocyte : 0.25 KCs : 0.13 SCs : 0.29 LECs.

Next, the hepatocyte plate was removed from the incubator. Medium was aspirated from each co-culture well, and 100 µL of the combined NPC cell suspension was added to each well. The plate was then returned to the incubator for 4–5 hours to allow cells to attach.

Following this incubation, the medium and any nonattached cells were aspirated from the co-culture wells. Each well was then fed with 100 µL of pre-chilled basement membrane working solution. The plates were returned to the incubator overnight.

The following day, medium was aspirated from all co-culture wells and replaced with fresh HCM™ Medium. From this point onwards, medium was changed daily using fresh HCM™ Medium for up to five days.

Several endpoints were tested to validate the final co-culture:

- Hepatocyte morphology was assessed using phase-contrast microscopy.
- Presence of all four cell types in culture was visualized though immunocytochemistry (ICC) staining and fluorescent microscopy. Specific ICC stains included albumin (hepatocytes), CD68 (KCs), vimentin (SCs), and CD31 (LECs).
- Hepatocyte functionality was evaluated by measuring albumin release via ELISA.
- Cellular release of cytokines/chemokines from NPCs, including TNF-α, IL-1β, IL-10, TIMP-1, and CCL2, was measured via ELISA following LPS stimulation. These measurements served as indicators of NPC health and functionality.

To further validate the protocol, the performance of Lonza's HCM™ Medium in supporting this high-throughput model was compared against a similar protocol in which DMEM was substituted for HCM™ Medium.

Vendor	Catalog no.	Description	Size
Lonza	HUCPI	Cryopreserved Human Hepatocytes	≥ 5 million cells
	HLKC-200K	Cryopreserved Human Kupffer Cells	≥ 0.2 million cells
	HLKC-500K		≥ 0.5 million cells
	HUCLS-200K	Cryopreserved Human Stellate Cells	≥ 0.2 million cells
	HUCLS-1M		≥ 1 million cells
	HLECP1	Cryopreserved Human Liver-derived Endothelial Cells (LECs)	≥ 1 million cells
	MCHT50	Hepatocyte Thawing Medium	50 mL
	MP250	Hepatocyte Plating Medium with Supplement	250 mL + supplement
	CC-3199	HBM Basal Medium	500 mL
	CC-4182	HCM™ Medium SingleQuots® Supplements	1 kit
	CC-3198	HCM™ Medium Hepatocyte Culture Medium BulletKit®	1 kit
Corning®	354234	Corning® Matrigel® Matrix	10 mL vial
Any		Collagen-coated 96-well Plates	
		Trypan Blue, 0.4%	
		PBS	

Table 1.
Materials used in the final validated high-throughput hepatocyte and NPC co-culture protocol.

Donor combination ("Donor")	Hepatocyte donor (HUCPI)	KC donor (HLKC-500K)	SC donor (HUCLS-200K)	LEC donor (HLEP-1)
1	HUM182351	HKC223011	HSC212641	HLE231781
2	HUM182531	23TL253295	HSC222051	HLE222411
3	HUM200691	HKC212641	HSC212641	HLE231781

Table 2.
Donor numbers for each combination of hepatocytes and NPCs used in this study. Note that each hepatocyte donor is unique, as is each combination of NPCs used with each hepatocyte donor.

Results

Like with previous co-culture models developed by Lonza[2,3], this study showed that, overall, hepatocytes displayed better longevity and morphology (tight, polygonal "cobblestone" morphology) when co-cultured with NPCs (Figure 2).

No qualitative differences were observed between co-cultures grown in DMEM versus HCM™ Medium. After five days of co-culture in HCM™ Medium, all four types of cells were clearly present and easily visualized using ICC staining and fluorescent microscopy (Figure 3), indicating a healthy, functional co-culture model.

ELISA results for hepatocyte albumin production (a measure of both health and functionality) revealed that HCM™ Medium supported significantly higher albumin production after 5 days of co-culture compared to DMEM (Figure 4A).

Inflammatory cytokine release from NPCs (TNF-α and IL-1β) was much higher on average in DMEM than in HCM™ Medium (Figure 4B and 4C). However, this difference was not significant due to high donor-to-donor variability in cytokine release. Notably, cytokine release in HCM™ Medium remained within the typical range observed for Kupffer cells in monoculture. This difference was likely due to the presence of hydrocortisone in HCM™ Medium, which we have found to moderate inflammatory responses in KCs in co-culture. DMEM does not contain hydrocortisone.

Hydrocortisone is beneficial for supporting healthy hepatocyte function but can be omitted from the completed HCM™ Medium formulation if a strong inflammatory response by KCs is desired. Other cytokine concentrations released by NPCs did not vary by media type and were indicative of healthy, functional NPCs (Figure 4D – 4F).

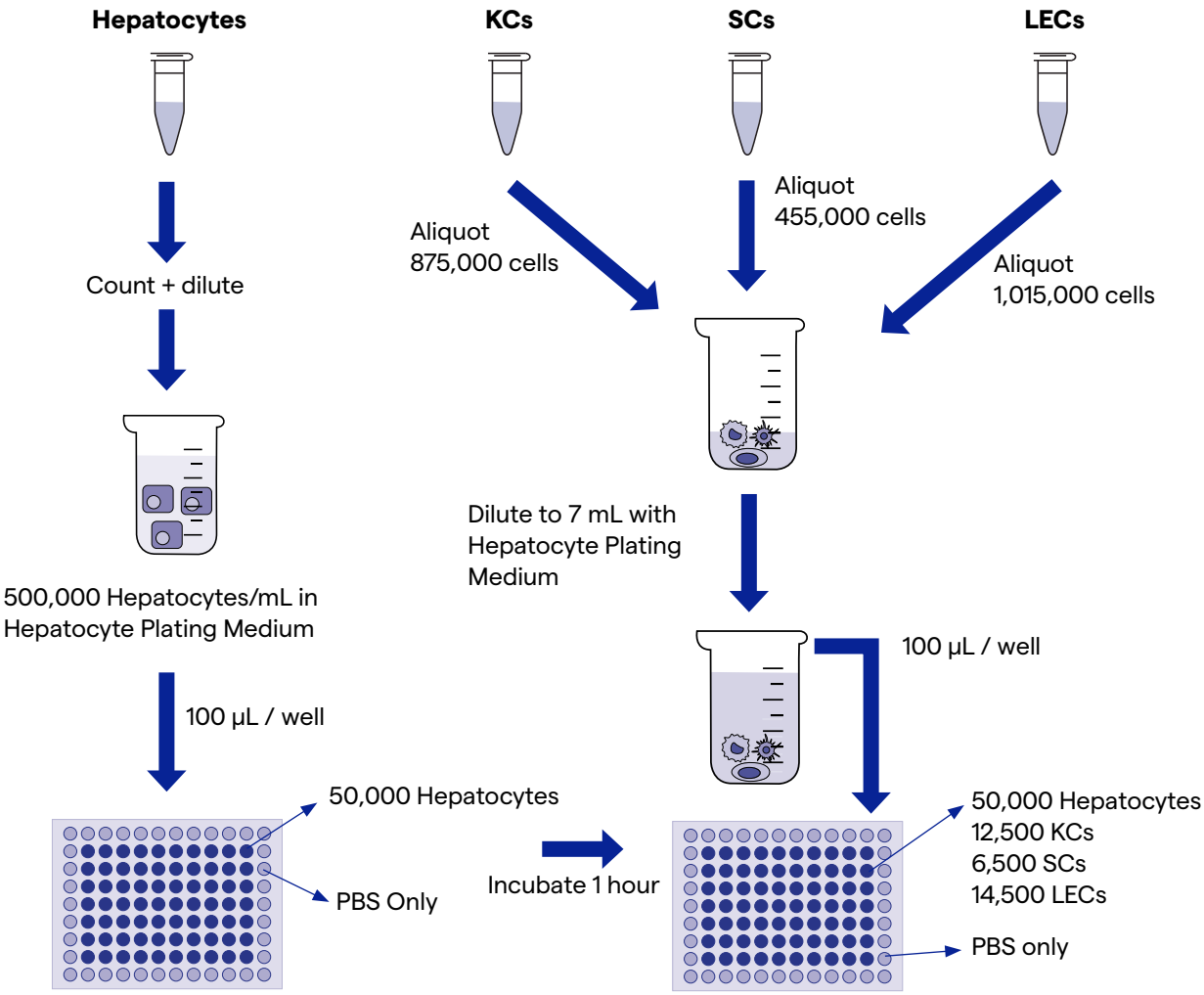

Figure 1.
Visualized protocol for plating hepatocytes and NPCs in the correct ratio in a 96-well, high-throughput format. Note, this example uses 70 co-culture wells. If using a different number, adjust the total volumes accordingly but ensure the ratios of hepatocytes to each NPC are kept the same.

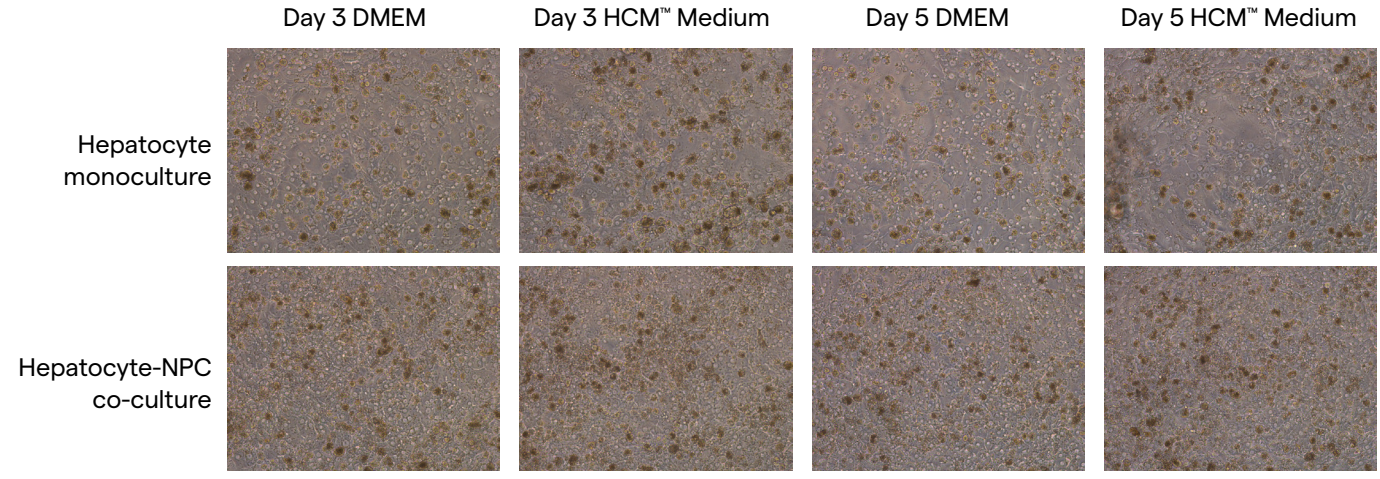

Figure 2.
Representative hepatocyte donor (Donor 2) cultured with and without NPCs, in DMEM medium versus HCM™ Medium over the course of a five–day culture. Images at 20x magnification.

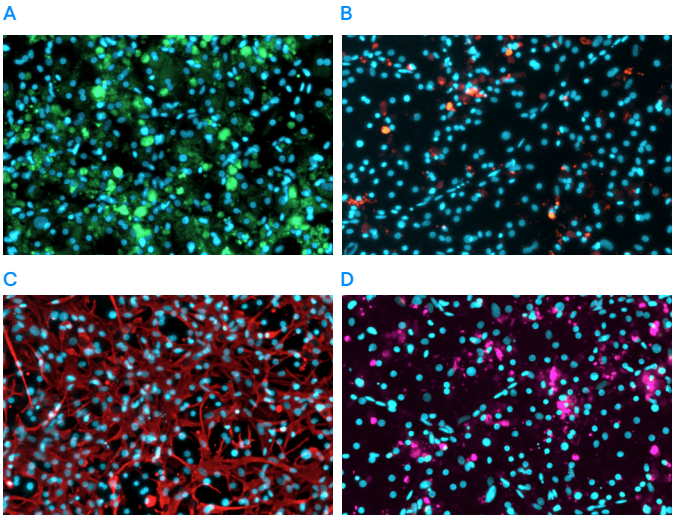

Figure 3.
Fluorescent images of ICC-stained hepatocytes (A), KCs (B) SCs (C), and LECs (D) after five days of co-culture in HCM™ Medium. Images were captured from Donor 1 at 10x magnification.

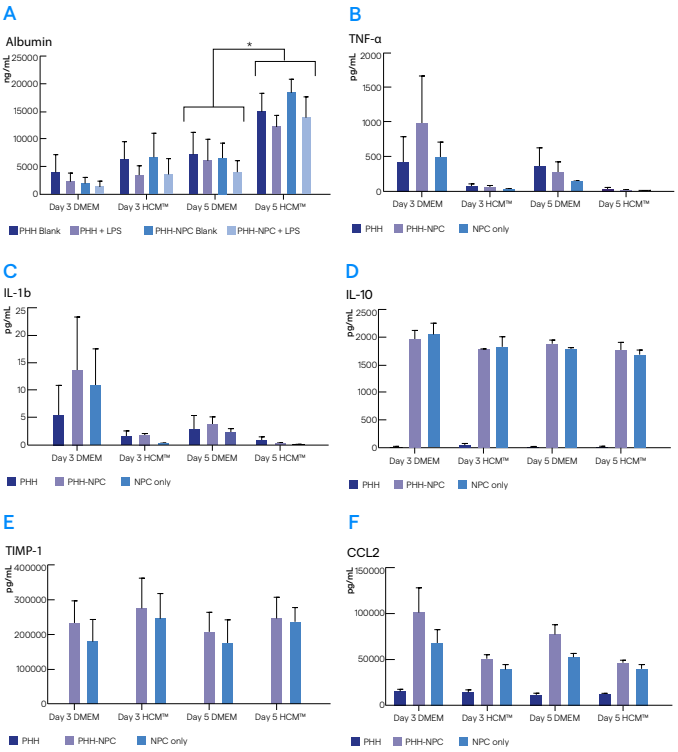

Figure 4.
ELISA assay results for hepatocyte albumin release (A) and TNF-α (B), IL-1β (C), IL-10 (D), TIMP-1 (E), and CCL2 (F) release by NPCs. All cytokine/chemokine data was taken from LPS-stimulated cultures (B-F). Data for each graph is shown as the mean of n=3 donors ± SEM. Statistical differences between DMEM and HCM™ Medium grouped by day (e.g., Day 3 DMEM vs. Day 3 HCM™ Medium) in all panels were analyzed by 2-way ANOVA with Šídák's multiple comparison test. All differences between groups were not significant except for Day 5 albumin levels in panel (A). Lack of differences in cytokine/chemokine data (B-F) indicates that NPCs function as expected in co-culture when stimulated with LPS. PHH = Primary Human Hepatocyte monoculture. PHH-NPC = Hepatocyte-NPC Co-Culture. *p<0.05.

Conclusions

This study demonstrates the development of a high-throughput, physiologically relevant hepatocyte and NPC co-culture that maintains proper functionality for up to 5 days in culture. Overall, the co-culture model performs better in HCM™ Medium (as evidenced by significantly higher albumin release by day 5; Figure 4a), while NPC populations remain robust and function as expected.

This co-culture is well suited for a wide range of high-throughput screening applications in drug discovery and toxicology where understanding inflammatory responses and crosstalk between hepatocytes and NPCs is critical.

References:

1. Hepatocyte and Non-Parenchymal Cell (NPC) 2D High-Throughput Co-Culture Instructions for Use. Link.
2. **Ly, N.,** *et al.* (2023). Hepatocytes and Non-parenchymal Cells: Development and Optimization of a Comprehensive Co-culture. Link.
3. **Ly, N.,** *et al.* (2024). Primary Human Hepatocyte 3D Spheroids Co-cultured with Non-Parenchymal Cells. Link.

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