Validation of a high-throughput MPS for 3D culturing of primary hepatocytes

Isavella Georgiades[1], Catriona Hamilton[1], Daniel Carney[1], Dharaminder Singh[1], Joanna Michalowska[1], Omid Siddiqui[1], Emily Richardson[1], Tomasz Kostrzewski[1]

1. CN Bio Innovations, 332 Cambridge Science Park, Cambridge, UK

Introduction

Advances in microphysiological system (MPS) technology are enabling more physiologically relevant *in vitro* models that bridge the gap between conventional cell culture and *in vivo* studies. The CN Bio PhysioMimix® Core System recreates tissue-specific microenvironments through pneumatic-driven perfusion, delivering media flow across 3D microtissues to support long-term cell viability and function.

To meet growing demands for higher throughput in drug discovery and development, we have designed the Liver-48 plate, an expansion from our widely adopted Liver-12 plate (**Figure 1**). This innovation increases experimental capacity four-fold while maintaining the biological fidelity and fluidic control that underpin the PhysioMimix® platform.

Figure 1: Comparison of PhysioMimix® Multi-chip Liver plates. Left: Liver-48 plate, a high-throughput format. Right: Liver-12 plate.

In this study, we present the engineering characterization and biological assessment of the Liver-48 plate, validating its performance from microfluidic flow dynamics through to hepatocellular function. By integrating experimental flow measurements and comparing biological outputs to the well-established Liver-12 plate, we demonstrate that scaling to higher throughput does not compromise physiological relevance.

Materials & Methods

The scaffold pore size and environmental conditions of Liver-12 were optimised in a module format for tissue formation and replicated in Liver-48 (**Figure 2)**. To achieve the same velocity and shear stress per pore; the flow rate was adjusted from 1µl/s in Liver-12 to 0.24µl/s in Liver-48.

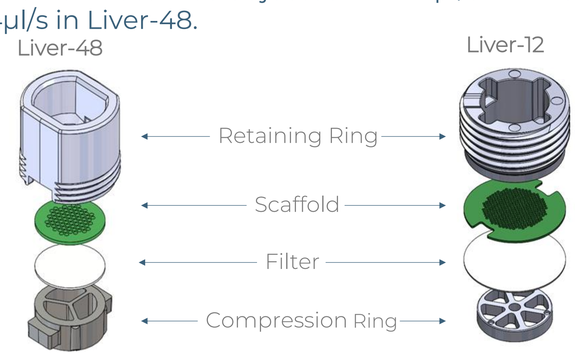


Figure 2: Comparison of liver modules Left: Liver-48, Right: Liver-12. Not to scale.

Flow rate measurements were taken for each well using a custom adapter, alongside Sensirion SLI-2000 liquid flow sensors and Sensirion data capture software. At lower flow rates, quantisation error and low relative accuracy dominate. Data conditioning was thus applied in post-processing to correct for this using an in-house Python script (**Figure 3**).

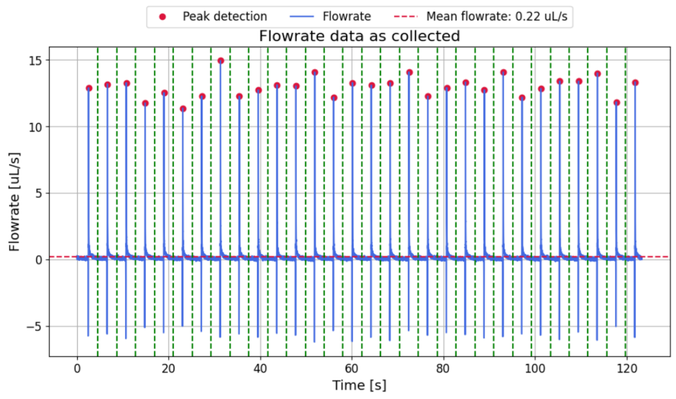


Figure 3: In-house Python script which segments pulses using peak detection algorithm to calculate mean flow rate per well

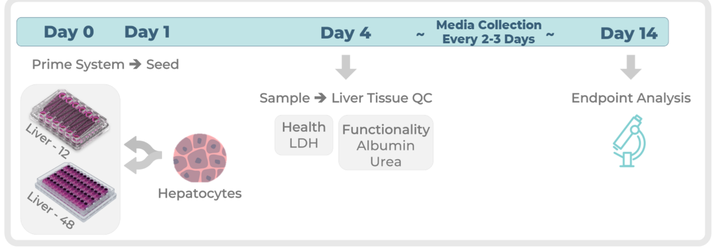


Figure 4: Standard PhysioMimix Human Monoculture Assay.

Two different primary human hepatocytes (PHHs) donors, sourced from Lonza (Donor 1) and LifeNet Health (Donor 2), were seeded into PhysioMimix Liver-12 and Liver-48 plates and maintained under perfused conditions for 14 days (**Figure 4**). Tissue health and function were monitored by LDH release and albumin production, with media collected every 2–3 days. Endpoint imaging and analysis were performed on day 14.

Results

The flow rate test showed values from 0.090 µL/s to 0.134 µL/s across the plate (**Figure 5**) with a CV of 8.3%. Well D1 measured at our lower threshold of 0.090 µL/s. We proceeded with biological validation to further understand cell functionality at the edge of the set flow rate thresholds.

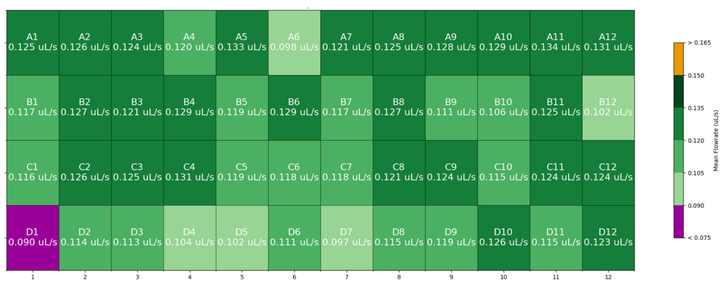
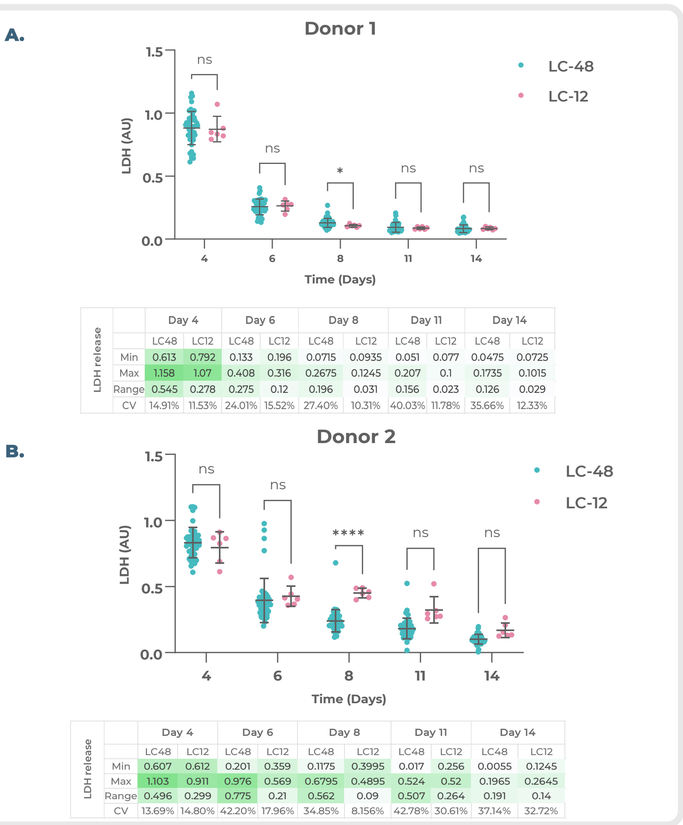


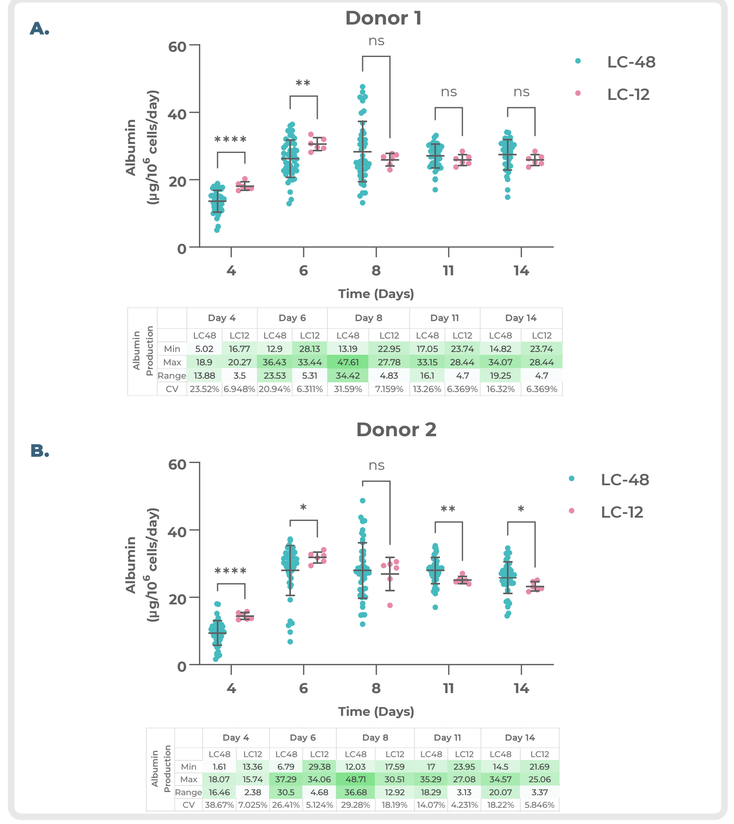
Figure 5: Mean flowrate per well across a Liver-48 plate

In biological validation tests, LDH release showed minimal differences between Liver-48 and Liver-12, with both plates displaying the expected decline over 14 days, reflecting stable hepatocyte health (**Figure 6**). Albumin production was robust throughout, with Liver-48 showing comparable or enhanced levels relative to Liver-12 (**Figure 7**). Donor-related differences in LDH and albumin levels were evident, but plate throughput had no effect. These results confirm that scaling from Liver-12 to the high-throughput Liver-48 format preserves tissue viability and liver-specific functionality.



LDH release		Day 4		Day 6		Day 8		Day 11		Day 14	
		LC48	LC12	LC48	LC12	LC48	LC12	LC48	LC12	LC48	LC12
Donor 1	Min	0.613	0.792	0.133	0.196	0.0715	0.0935	0.051	0.077	0.0475	0.0725
	Max	1.158	1.07	0.408	0.316	0.2675	0.1245	0.207	0.1	0.1735	0.1015
	Range	0.545	0.278	0.275	0.12	0.196	0.031	0.156	0.023	0.126	0.029
	CV	14.91%	11.53%	24.01%	15.52%	27.40%	10.31%	40.03%	11.78%	35.66%	12.33%
Donor 2	Min	0.607	0.612	0.201	0.359	0.1175	0.3995	0.017	0.256	0.0055	0.1245
	Max	1.103	0.911	0.976	0.569	0.6795	0.4895	0.524	0.52	0.1965	0.2645
	Range	0.496	0.299	0.775	0.21	0.562	0.09	0.507	0.264	0.191	0.14
	CV	13.69%	14.80%	42.20%	17.96%	34.85%	8.156%	42.78%	30.61%	37.14%	32.72%

***Figure 6: Liver-12 and Liver-48 microtissues show minimal LDH release differences over 14 Days in two donors. A** Donor 1 (Lonza) and **B** Donor 2 (LifeNet Health).*



Albumin Production		Day 4		Day 6		Day 8		Day 11		Day 14	
		LC48	LC12	LC48	LC12	LC48	LC12	LC48	LC12	LC48	LC12
Donor 1	Min	5.02	16.77	12.9	28.13	13.19	22.95	17.05	23.74	14.82	23.74
	Max	18.9	20.27	36.43	33.44	47.61	27.78	33.15	28.44	34.07	28.44
	Range	13.88	3.5	23.53	5.31	34.42	4.83	16.1	4.7	19.25	4.7
	CV	23.52%	6.948%	20.94%	6.311%	31.59%	7.159%	13.26%	6.369%	16.32%	6.369%
Donor 2	Min	1.61	13.36	6.79	29.38	12.03	17.59	17	23.95	14.5	21.69
	Max	18.07	15.74	37.29	34.06	48.71	30.51	35.29	27.08	34.57	25.06
	Range	16.46	2.38	30.5	4.68	36.68	12.92	18.29	3.13	20.07	3.37
	CV	38.67%	7.025%	26.41%	5.124%	29.28%	18.19%	14.07%	4.231%	18.22%	5.846%

***Figure 7: Liver-12 and Liver-48 plates show stable albumin production over 14 Days in two donors A** Donor 1 (Lonza) and **B** Donor 2 (LifeNet Health).*

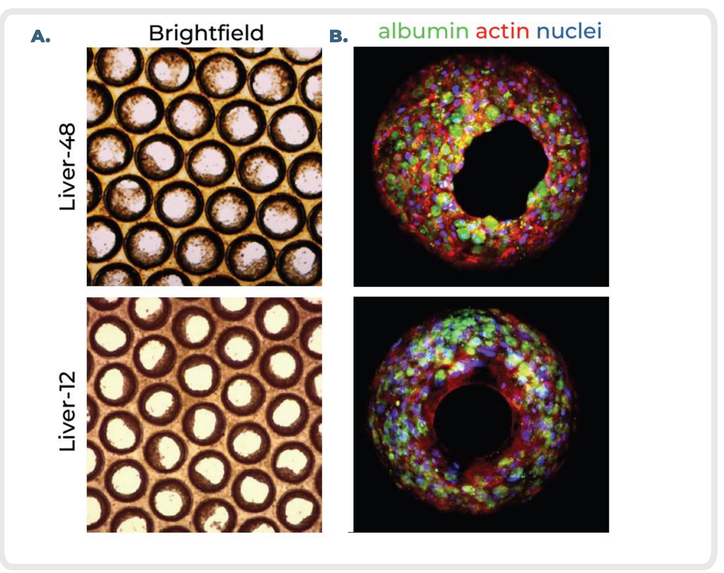


***Figure 8: A.** Brightfield and **B.** Confocal images of microtissues within scaffold pores at Day 8.*

Microscopy images (**Figure 8**) demonstrate uniform tissue formation in Liver-48 scaffolds, comparable to Liver-12. Confocal staining confirms hepatocyte functionality through albumin expression and structural integrity via actin organization, supporting the formation of healthy liver microtissues.

Conclusion

Flow rate thresholds were initially set based on Liver-12 expertise, but biological data revealed that tissues could form at the edge of these limits (e.g. well D1), indicating that current criteria may be too restrictive.

Batch analysis has established that the output flow rate is roughly half the input. Whilst this does not appear to limit biological functionality, further work is being completed to make the output flow more efficient.

Despite these limitations, combined flow and biological validation demonstrate that scaling from Liver-12 to the higher-throughput Liver-48 maintains hepatocyte viability and function. By day 14, albumin variability across wells was <20% and LDH values consistently met internal pass criteria, confirming reproducibility and robustness. The Liver-48 plate expands the PhysioMimix® platform four-fold while preserving biological fidelity and fluidic control. Liver-48 delivers robust, scalable, and reliable human-relevant data for high-throughput drug discovery.

visit **cn-bio.com**

Download this poster