Developed the model, built the assay, now a focus on THROUGHPUT! The Liver-48, designed for industry adoption

POSTER: 218

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1. Intro

CN Bio's PhysioMimix® OOC Systems turn pressure changes into recirculating fluidic flow, to mimic the bloodstream. By perfusing media into highly tailored microenvironments, functional 3D microtissues are generated from validated primary human cells, creating microphysiological systems (MPS).

Developed the model

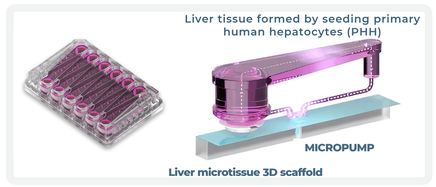


Fig 1. A: Liver-12 plate, B: slice through well & flow path

Our Multi-chip Liver-12 plate has many applications, is FDA-characterized [1] and has supported a successful regulatory submission[2]. This demonstrates the potential of MPS, however, industry adoption has been incremental, with throughput cited as a limiting factor.

Built the assay

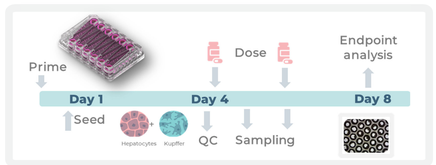


Fig 2. PhysioMimix DILI assay workflow

The challenge therefore was to retain an SBS-standard footprint and maintain Liver MPS functionality whilst increasing throughput. Here, we describe the development of a Multi-chip Liver-48 plate, with 48 chips per plate and 144 per 3 plate docking station. Fig.3 below shows an example plate plan of how two compounds "P" and "T" could be tested with 7 concentrations and controls all in one plate.

Now to focus on throughput...

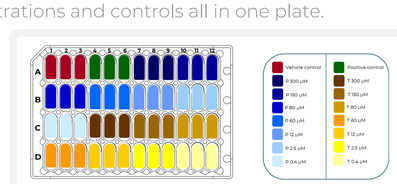


Fig 3. Example plate plan, (compounds "P" & "T")

2. Methods

Initially mathematical and computational fluid dynamics models informed the design for items such as flow rates, oxygen concentrations in media and mechanics of the consumable.

Computational Fluid Dynamics

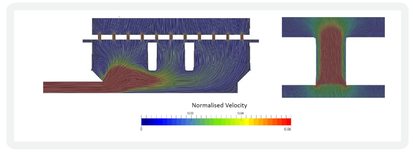


Fig 4. Example CFD: flow through the scaffold pores

Techniques such as finite element analysis were used to ensure forces across each well/chip in the consumable were even to minimize overall variability.

Finite element analysis

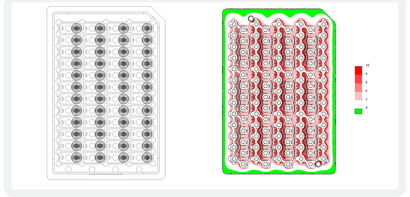


Fig 5. FEA simulation: force distribution

Plate design was focused on delivering reproducibility by avoiding well/chip variation. This was confirmed through metrology and techniques such as Micro-CT.

Manufacture and assembly

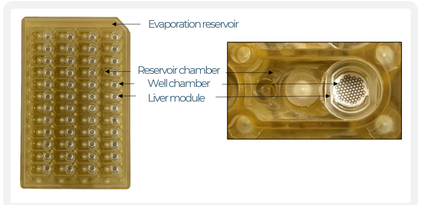


Fig 6. Liver-48 consumable, assembled with components

Micro-CT

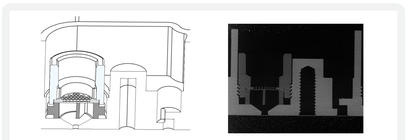


Fig 7. CAD section (left) and Micro-CT slice (right) of assembled components within Liver-48

3. Results

A series of engineering tests were performed on the plate to optimize its function and verify the design. Design variation was minimized, with flow variation (CVs) across the Liver-48 plate typically less than 10%.

Flow rate variation

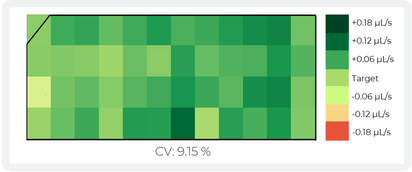


Fig 8. Plate map with flow rates compared across the 48 individual wells

The Liver-48 plate was tested for its ability to produce viable and functional microtissues in an equivalent manner to the Liver-12 plate. The same donor combination of hepatocytes and Kupffer cells were seeded into each plate and cultured for 8 days. Liver microtissues with robust "doughnut" structures formed within pores of both plates, representing the liver sinusoidal structure. Albumin and urea quality control (QC) metrics were analyzed as endpoints, data showed an increase in albumin with time and stability of urea over 8 days

Liver-12 model comparison

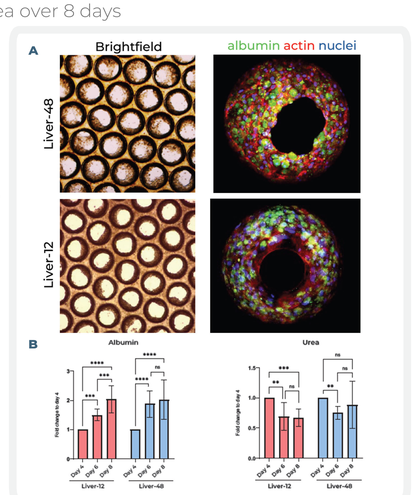
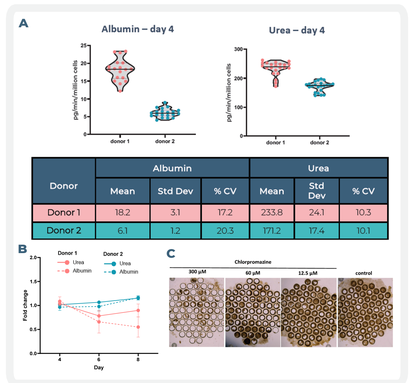


Fig 9. A: Brightfield and confocal images of microtissues within scaffold pores, B: Albumin and Urea QC data from each plate over time

To test the Liver-48 plates utility in assessing hepatotoxicity, two different hepatocyte donors were seeded into the plate and cocultured with the same Kupffer cell donor for 8 days. Donors were chosen based on different characteristics/lifestyles. QC metrics, albumin and urea, at day 4 appear different between the two donors, despite a similar coefficient of variation CV. On day 4, tissues were dosed with 3 concentrations of Chlorpromazine. Albumin and urea levels decreased over time in only one of the donors, highlighting differences in donor sensitivity. Microscopy images indicate a dose response.

Donor sensitivity



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

Fig 10. A: D4 QC metrics, B: Albumin and urea post dosing, C: Brightfield images

4. Discussion

By miniaturizing each Liver chip, 48 tissues can now be cultured per plate, enabling a total of 144 chips per PhysioMimix Single-organ HT System. By increasing the throughput, more drug candidates, replicates and controls can be tested over a broader range of concentrations, conditions, or cell donors. The format delivers a fast, robust and cost-effective understanding of human drug safety profiles earlier in the pipeline.

PhysioMimix® OOC System

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References

1) Rubiano, Andrés, et al. (2021) "*Clinical and translational science* 14.3: 1049-1061.

2) https://cn-bio.com/physiomimix-data-supports-inipharms-ini-822-for-metabolic-liver-disease-treatment/