CN-BIO

AUTUMN WEBINAR SERIES

Testing on Humans

How to Predict Hepatotoxicity and Drug Clearance ahead of Clinical Trials using Liver-on-a-Chip

Q&A

A full run down of questions & answers from our October 13th webinar

Q&A participants

Audrey Dubourg
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MBA, PhD
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Abbreviations

Air-Liquid Interface	**ALI**	**NPC**	Non-parenchymal cell
Body-on-a-chip	**BOC**	**OOC**	Organ-on-a-chip
European Medicines Agency	**EMA**	**PBMCs**	Peripheral blood mononuclear cells
U.S. Food and Drug Administration	**FDA**	**PHH**	Primary human Hepatocyte
Human leukocyte antigen	**HLA**	**RNA-seq**	RNA sequencing
Microphysiological system	**MPS**	**SCS**	Single cell suspension
Next-generation Sequencing	**NGS**	**TEM**	Transmission electron microscopy

Another question?

Drop an email to one of our experts - **sales@cn-bio.com**

Missed the webinar?

Watch an on demand - **recording of the webinar here**

Questions

Q1

Q: How long do liver microtissues stay alive in your Liver-on-a-chip platform?

A: From Dr Tomasz Kostrzewski, Director of Biology, CN Bio:

The liver microtissues in our Liver-on-a-Chip model are viable, with a functional conserved phenotype, for over 28 days. We have published data (**Ortega-Prieto *et al*, 2018**) showing viable functional cultures up to 40 days, so the system could potentially be used for longer period of time.

Q2

Q: Is the scaffold used made of a synthetic or natural material?

A: From Dr Tomasz Kostrzewski, Director of Biology, CN Bio:

The current scaffold, used in our **MPS-LC12** consumable plate, is a polystyrene scaffold coated in collagen. Other scaffold structures can be used in our PhysioMimix™ OOC consumable plates made from a wide variety of materials (both synthetic and natural).

Q3

Q: How do we deal with chances of contamination in open-well model in your PhysioMimix™ OOC system?

A: Brian Manning, Head of US Sales, CN Bio:

The open-well set up of our **PhysioMimix™ OOC system** makes it easier to maintain a sterile environment following standard laboratory operating procedures and good aseptic techniques using a microbiological safety cabinet.

The PhysioMimix™ **MPS-LC12** consumable plates, containing the liver microtissues, have standard loose-fitting lids used for Cell culture well-plates which means they remain sterile in the incubator for the duration of the experiment when good laboratory practices are followed. The pneumatic connection within the PhysioMimix™ hardware is a fully closed system, therefore not exposed to the environment. An HEPA filter is also used in the system as an extra precautionary measure.

Q4

Q: How does this model address Immune-mediated DILI? Especially, adaptive immune-mediated toxicity issues?

A: Brian Manning, Head of US Sales, CN Bio:

Co-culture models containing primary human hepatocytes (PHH) and human non-parenchymal cells (NPCs) can be used to assess immune-mediated toxicological responses. HLA-matched PBMCs can also be added to assess adaptive immune responses.

Q5

Q: How are you engaging with EU regulators? Do you have similar collaborations to that of CDER?

A: From Dr Tomasz Kostrzewski, Director of Biology, CN Bio:

We are in regular contact with EMA regulators about our technology and they are abreast of developments in the Microphysiological System (MPS) field. However, we do not have a formal collaboration akin to the team at the FDA/CDER setup as yet.

Q6

Q: How do you account for scaling of organs, in multi-physiological systems? Do you have separate media for each cell type or are they connected together with a common media? How do you ensure that they all have their requirements met?

A: From Dr Tomasz Kostrzewski, Director of Biology, CN Bio:

We use a variety of scaling methodologies to build our multi-organ platforms and do not have a one-size fits all approach. Most commonly, we focus on a functional methodology to scale microtissues to ensure crosstalk between organs is physiologically relevant, but other methods are available.

Q7

Q: Do you have any real time measurements, or do you need to remove plates from the platform to manually measure things?

Light microscopy? Fluorescence *in situ*?

A: From Dr Tomasz Kostrzewski, Director of Biology, CN Bio:

We have used some sensing technology to monitor cells in the

platform in real time, however this is not commonly undertaken. Our system does not currently allow for live cell imaging due to the current setup of the hardware. Scaffolds containing each microtissue can be removed at any desired time point for imaging purposes (from basic brightfield to confocal immunofluorescence or TEM).

Q8

Q: Can you expand on your experience with the liver-lung model? Is that a healthy model? Was the lung model an ALI Transwell® culture inserted into the organ-on-a- chip system?

A: From Dr Tomasz Kostrzewski, Director of Biology, CN Bio:

Yes, the lung model used was an ALI culture of bronchial epithelium cultured on a Transwell®. The lung tissue was a healthy model exposed to lung toxicants. Investigations to determine the subsequent effects of toxicants on the liver were then studied; however, this model could easily be adapted for disease modelling purposes. The lung-liver co-cultures in this model were shown to be stable for up to 10 days.

Q9

Q: What is in the boxed system shown in the title page? Microfluidics, incubator, imaging?

A: From Dr Audrey Dubourg, Product Manager, CN Bio:

Our PhysioMimix™ OOC system comes with:

- A Controller that sites outside the incubator and controls the microfluidics in the system
- A 3-way Docking Station which sits in the incubator and allows for the use of 3 MPS Driver/plate.
- 3 x MPS drivers in which the MPS consumable plates are inserted onto the Docking Station to run the microfluidics in the consumable plates. The MPS driver recognises both our **MPS-LC12 plate** (used for our liver-on-a-chip model) and our **MPS-T12 plate** (used for our gut-on-a-chip model and other barrier models)
- Note one Controller can control two Docking Stations to increase capacity to 6 MPS Driver/plates if required

The Docking Station fits on the shelf of a standard incubator and the scaffolds and other inserts in our consumable plates can be used to run any standard microscopic analysis using the microscope of choice.

To learn more about how the system works, **watch our latest video**.

Q10

Q: How would you highlight the advantage of your system over other MPS available on the market?

A: Brian Manning, Head of US Sales, CN Bio and Dr Audrey Dubourg, Product Manager, CN Bio:

The OOC market offers a wide range of technologies, from very simple gravity-driven plates to complex true microfluidics systems such as our PhysioMimix™ OOC platform which can be used for longer term culturing (up to 1 month). Use of these technologies is not mutually exclusive. We developed our system with large scale sampling capability to enable deep investigations into efficacy, causality or mechanism of action to be made, whereas more simple approaches can be used in a complimentary way to satisfy higher throughout yes/no decision making upstream. Despite the complex biology that it mimics, the PhysioMimix™ OOC is very simple to install and to integrate into existing workflows. For example, Scientists benefit from the familiarity and comfort of working with open-well plates rather than having to learn new techniques.

With that in mind, our PhysioMimix™ OOC MPS system:

- Allows for multiplexing of assays allowing temporal data to be collected as well as assay endpoints. This includes enhanced use of supernatants (via Luminex and other platforms), real-time imaging technologies, as well as analytes for endpoints such as ATP, RNA etc.
- Can employ 'omics-based' technology particularly NGS (next gen sequencing), SCS (single cell sequencing), proteomics, lipidomics etc. These technologies are evolving at a rapid rate, requiring a fraction of the input material that would have been needed a few years ago.
- Enables the user to experiment under physiologically relevant flow-based conditions that more accurately mimic an *in-vivo* environment versus static cultures. In particular, better media and flow conditions improve tissue viability, longevity and organotypic performance. To know more about how flow impacts the cell culture condition, check our first webinar in our Autumn series **The Rhythm of Life: Using Microfluidics to Mimic Blood Flow in Single- and Multi-Organ-on-a-Chip Models**.
- Provides human-relevant 3D disease models for diseases such as **NASH**, **NAFLD**, Fibrosis, T1/2 Diabetes, Immuno-oncology, **HBV** and Lung Fibrosis.
- Encompasses both single organ-on-a-chip (OOC) and body-on-a-chip (BOC) multi-organ platform approaches allowing more complex investigational toxicity studies to be performed and questions to be answered.

Q11

Q: How do you perform secreted and staining readouts at the same time?

A: From Dr Tomasz Kostrzewski, Director of Biology, CN Bio:

The open-well set up of our system allows for regular sampling of up to 1 ml of media which can be analysed to identify secreted and circulating biomarkers. The relatively large scaling of our set up also allows for isolation of the 3D liver microtissues from which cellular phenotype readouts can be reported.

To learn more about our Liver-on-a-Chip model used for DMPK and Safety Toxicity Screening, **watch our latest animated video**.

Q12

Q: Can this system be used to culture skin tissues to identify the toxicity of topical transdermal drugs?

A: From Dr Tomasz Kostrzewski, Director of Biology, CN Bio:

The PhysioMimix™ OOC system can be used to incorporate Transwell-based skin models using our **MPS-T12** consumable plate. These skin inserts can be studied in isolation in a perfused system or in our multi-organ set up by connecting them to liver cultures.

Q13

Q: How many endpoints readouts can you perform on each sample?

A: Brian Manning, Head of US Sales, CN Bio:

As it is possible to sample a large volume of media (up to 1 ml per sample), many different endpoints can be measured in parallel. For example, a Luminex panel may contain 20 or more analyte readouts. Clinical biomarkers can be analysed which are traditionally difficult to detect in *in vitro* cultures

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Q14

Q: What is different about your solution that enables clinical measurements such as ALT/AST to be measured as these are typically hard to detect in other systems?

A: Brian Manning, Head of US Sales, CN Bio:

The sensitivity of clinical measurements, such as Serum transaminase assay, depends greatly on the amount of material available for the assay. Other systems are highly limited in measuring clinical markers due to the small amount of cells/ supernatant available for the assay, whereas our Liver-on-a-Chip model contains 6 x 10^5 PHH in large volume of media; therefore, allowing for the reliable quantification of clinical markers that are notoriously challenging to measure *in vitro*.

Q15

Q: What are the advantages of your approach versus other 3D models such as spheroids?

A: Brian Manning, Head of US Sales, CN Bio:

Spheroids are limited in some assays as they only contain 1,000 to 3,000 cells. Spheroids are also typically cultured in static cell culture conditions rather than microphysiological flow conditions which usually leads to cell death in the centre of the spheroids, in a few days, due to lack of nutrients. Our PhysioMimix™ OOC system allows for the culture of large-scale 3D liver microtissues with a maintained viability, phenotype and metabolic activity for over 30 days enabling for long-term experimental assessment of toxicity and DMPK.

Q16

Q: How long does it take to induce DILI or any disease states and for how long can you test thereafter using your system?

A: Brian Manning, Head of US Sales, CN Bio:

It usually takes 4 days to induce fatty liver diseases such as NAFLD or NASH in our Liver-on-a-Chip model, whereas, inducing acute or chronic DILI takes a bit longer, usually around 14 days. Diseased 3D Liver microtissues can be cultured and treated with drug/compound of choice for 28 days or more.

To know more about our fatty liver diseases and DILI model, download our poster booklet: **Microphysiological System for Studying Fatty Liver Diseases and its Impact on Drug-Induced Liver Injury**.

Q17

Q: You have spoken a lot about drug safety and investigational toxicology, can you also run genotoxicity assays using this Liver-on-a-chip model?

A: Brian Manning, Head of US Sales, CN Bio:

Yes. We have developed sophisticated genotoxicity models in partnership with Charles River Laboratories. To know more about how to perform genotoxicity assays in our PhysioMimix™ OOC system, watch the third webinar of our Summer 2020 Series:

Go With the Flow – Application of Microfluidic 3D Liver Chip Models To Genotoxicity Testing.

Q18

Q: What is your standard Investigational tox service offering? What is the timeframe for a couple of compounds at 2 or 3 different concentrations? Where can I learn more about your services?

A: Brian Manning, Head of US Sales, CN Bio:

Our standard Investigational tox service offering includes various endpoints:

- Cell viability and health (ATP, Albumin, LDH, ALT)
- Metabolic activity (CYP activity)
- Imaging (from brightfield to immunostaining of target proteins)
- Additional mechanistic markers, such as RNA-seq, can also be added as required

The timeframe to deliver a full report will greatly depend on the complexity of the study as well as on the number of compounds/concentrations, time points and endpoints requested in the study.

For example, a standard experimental plan using 2 compounds with 3 concentrations, in a single dosing set up, will take 2 weeks to run and up to a month to a month and a half to have a final report with all the endpoints analysis done.

To learn more or to further discuss, contact us at

sales@cn-bio.com, or visit our website

https://cn-bio.com/drug-metabolism-and-safety-toxicity-testing-services/

Our autumn webinar series continues

3rd Nov

A microphysiological model of metastatic progression

Dr Amanda Clark
University of Pittsburgh

Register here

24th Nov

Engineering Mucosal Barriers

From Organoids to Organs-on-Chips

Prof Linda Griffith
Massachusetts Institute of Technology (MIT)

Register here

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