Webinar Q&A Report:
It's Life, but Not As We Know It: How Predictive Human Organ Models Are Transforming and Reshaping the Efficiency of Drug Discovery

Questions in this Q&A Report are answered by:
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1. Can any organ be recreated in vitro using OOC/MPS? What other organs have you developed in your system?

A. Dubourg: In an ideal world, any organ could be recreated using organ-on-a-chip (OOC), also known as Microphysiological systems (MPS). However, many factors need to be considered when developing human organ mimics *in vitro* including*:*

- the complexity of the organ itself
- the critical phenotypes and functions required to achieve human relevance
- the type of platform/technology (e.g., chip *versus* plate) and its ability to recreate those functions
- the microfluidics required to adequately perfuse the organ (e.g., gravity-driven, recirculating media, or a unidirectional flushing of media via pneumatic pumps)
- the ability to deliver a physiologically-relevant flow rate
- the support/matrix required (e.g., use of scaffold or extracellular matrix (ECM))
- the accessibility of human-relevant and 3D-validated cells

Want to learn more? Read the following blog: Flowing to the Beat of Your Heart.

At CN Bio, we are focusing our time in developing organs that are key to assessing drug efficacy and predicting drug safety to bridge the gap between preclinical and clinical drug development phases. We have currently developed, characterized, and validated our liver-on-a-chip model (presented in

this webinar) which can be used for a plethora of purposes such as modeling common liver diseases (e.g., Hepatitis B virus (HBV) and non-alcoholic steatohepatitis (NASH)) predicting drug metabolism, or evaluating drug-induced liver injury (DILI).

Another area of interest for us is the lung. Pulmonary disease rates are on the rise, whilst the inhaled route of drug administration is currently underutilized. In response, we have recently developed two lung models (bronchial and alveolar) using primary epithelial and endothelial lung cells to meet these needs.

The most common route of drug administration is the oral route, therefore we have also developed a gut model, currently using immortalized cells, that can be used in isolation to measure drug absorption, or interconnected to a liver model to investigate first pass metabolism/drug bioavailability. This is interconnection of single organ models into systems to model processes and facilitate inter-organ crosstalk is also possible for lung-liver too.

2. In your NASH model, you only show Elafibranor (ELF) and Obeticholic acid (OCA) data. Have you tested other anti-NASH compounds or new drug modalities in your platform?

G. Guenigault: OCA and ELF are two of the most advanced anti-NASH compounds in the drug development pipeline. These well-known drugs have enabled us to accurately characterize, validate and refine the NASH model that we developed, in part via collaboration with AstraZeneca. We now use ELF and/or OCA as positive controls for our in-house NASH experiments, including those we run for customers via our contract research services. Through our Contract Research Services, we have also tested additional anti-NASH compounds and new drug modalities. For example, we investigated the efficacy of an siRNA-mediated therapy against β2-spectrin (SPTBN1) and HSD17B13 inhibitors highlighting the potential of those two modalities for the treatment of NASH.

3. Can your liver model be used to study low clearance compounds?

G. Guenigault: Yes, it can. One of the big advantages of long-term culture longevity (up to 4 weeks) is that this enables the effects of drug exposure to be measured over longer periods of time. The key determinant for low clearance studies is how long cells remain viable, with good hepatic functionality and metabolic activity without a media change, or fresh media/supplements. Currently, there's a 4-day window in which to detect slowly metabolized/low clearance compounds. A recent publication from Roche characterized our liver model for low clearance studies with well-known compounds, such as Tolbutamide and Lorazepam, as well as compounds with fast clearance (e.g., Diclofenac). By integrating mathematical modeling with experimental Liver-on-a-chip studies, the *in vitro* to *in vivo* extrapolation (IVIVE) data they observed closely matched the reported clinical data for the compounds tested – highlighting the value of this approach to improve drug metabolism and efficacy predictions (Docci *et al.*, 2022).

4. How easy is it to transition from 2D culture into 3D PhysioMimix™ assays?

A. Dubourg: It is fairly straightforward to transition from 2D cell culture into 3D culture using our PhysioMimix™ OOC systems. The PhysioMimix system is very easy to set-up and user-friendly. The hardware takes around 20 min to install and does not require an engineer to be on site.
Our PhysioMimix Multi-chip consumable plates feature an open-well design that will feel familiar to anyone who is used to working with standard cell culture plates. This approach lowers the adoption curve versus other organ-on-a-chip solutions, where smaller futuristic-style chips are used. There are some elements of assay set up that will be new to users, such as priming the plates' microfluidic pumps (which provide fluidic flow/perfuse cultures like the blood stream) prior to cell seeding but this is not a complex procedure.

If new users are concerned about the transition to 3D when acquiring a PhysioMimix system, we offer online and onsite training plus a range of SOPs and "how-to" videos.

In addition, we also have a catalogue of 3D-validated cells, which are primary cell lots that we have thoroughly tested to ensure that they thrive in 3D, in our PhysioMimix system, maintaining their function and phenotype for up to 4 weeks when cultured under perfusion. So, rather than spending your valuable time, resources, and budget validating cell lots to find those that are OOC compatible, you can focus on making new discoveries!

Finally, this year we launched our new NASH-in-a-box kit which contains everything a researcher needs to recreate our *in vitro* NASH model in their own laboratory, including step-by step guided software protocols.

For further reading, this blog provides a useful overview of some of the points raised above (and more).

5. Can you customize PhysioMimix models e.g., add in extra cell types such as circulating immune cells?

G. Guenigault: It is totally possible to customize the type of cells used in the PhysioMimix system. The architecture of our multi-chip plates is open, rather than closed – similar to a standard cell culture 24-well plate. This makes it easy to customize your MPS/OOC models to make them your own by adding more cell types in.

For example, we currently offer three types of pre-validated liver models:

- Monoculture of primary human hepatocytes (PHH) – used for drug metabolism studies and disease modeling (e.g., non-alcoholic fatty liver disease (NAFLD) or HBV)
- Coculture of PHH with human Kupffer cells (HKCs), used in our work with the FDA presented in this webinar, for drug-induced liver injury (DILI) and immune-mediated toxicity
- Tri-culture of PHH, HKCs and human stellate cells (HSCs) to recreate a NASH phenotype and presented in this webinar as well

If required, these models can and have been adapted by our customers (published in a poster at SOT in 2022) to include Liver sinusoidal endothelial cells (LSECs) and, as the system has recirculating media in each well, circulating immune cells (such as peripheral blood mononuclear cells – PBMCs), for studying immune-mediated DILI responses.

6. Have you tried other liver cells such as induced-Pluripotent Stem Cells (iPSCs)?

G. Guenigault: We have indeed used different types of iPSCs in our system to show proof of concept, but we have not fully validated the models. In the main, we work with primary cells whenever possible because of their human relevance.

For example, for our liver model, we have demonstrated that the iPSC-Hepatocytes (iPSC-Hep) form a nice tissue, similar to our PHH model. The tissue offers improved hepatic function and metabolic activity compared to standard 2D iPSC-Hep models but in our hands, primary human hepatocytes function more highly than iPSC-derived hepatocytes, for example in determining cytochrome p450 activity. Primary cells also remain in a differentiated state for a month in culture when perfused to replicate blood circulation. This allows us to run chronic dosing studies, to profile human drug metabolism, and to identify metabolite-driven toxicity in both healthy and diseased liver models. However, in jurisdictions in which the use of human tissues is restricted, stem cells represent the obvious alternative.

7. Can this system replicate the liver endothelial fenestrae?

A. Dubourg: As mentioned in Q5, we routinely use three types of liver cells: hepatocytes, Kupffer and stellate cells in our liver models but one of the advantages of the PhysioMimix approach is that customers can adapt OOC models to meet their differing needs. We have yet to incorporate endothelial cells into our liver model, however, a customer has incorporated LSECs into their DILI model. We would expect to recreate, or mimic, most of the microarchitecture and some part (if not all) of the liver fenestrae as we can recreate the liver bile canaliculus in our current models, but this will need to be confirmed, well-characterized and validated.

8. What are the advantages of PhysioMimix systems compared to competitors?

A. Dubourg: The advantages of our PhysioMimix system compared to other OOC platforms greatly depends on the type of OOC/MPS technology we are comparing to and the customer's needs. For the purposes of simplicity, if we compare the PhysioMimix to chip-based technology with physiologically-relevant microfluidics, like ours, rather than gravity-driven flow solutions that tend to be lower content/higher throughput, the PhysioMimix offers:

- Fast/easy set-up of the system itself (see Q4).
- Although not high throughput, PhysioMimix enables users to run up to six multi-chip plates (72 samples) simultaneously. This means 24 conditions can be run in triplicate per PhysioMimix (4

conditions per 12-well Liver plate), minimizing sample to sample variability/generating more robust data. The multi-chip plate-based approach has the added benefit of being scalable. To increase throughput, we simply need to incorporate more chips into the plate rather than redesigning the whole system.

- High content data output is also a key advantage of our system. This is achieved because we operate at a larger scale than the "micro" chip-based systems. Up to 1 mL of media can be sampled, enabling large-scale biomarker testing including clinical biomarkers (such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) for the liver). The tissue generated in the system is also big enough to allow for microscopy and -omics to be run in parallel on the same tissue.
- Open system. Customers find the open nature of our OOC system is an advantage. For example, when inducing/studying the mechanism of disease or performing longitudinal studies, it is easier to access the cultures to change experimental conditions, or manipulate the models, or remove samples using an open system versus a closed system.
- Recapitulating the immune system. An advantage of our system is the possibility to add in circulating immune cells to assess immune responses. Most chip-based OOC technologies have a flushing-type of microfluidics in which the liquid is flushed from one side of the chip to the other and unable to recirculate through, or around, the cellular tissue. This more limiting for those wishing to investigate immune-mediated responses.

Most chip-based technologies use polymethylsiloxane (PDMS) to create their chip and microfluidics. PDMS is a relatively cheap and easy to manipulate material which allows for the creation of very thin membrane. It is commonly used in bioengineering to develop cell culture supports. However, drugs bind highly and non-specifically to PDMS, which is problematic when assessing a compound's efficacy. When developing our PhysioMimix Multi-chip plates, we avoided using PDMS, choosing to use cyclic olefin copolymer (COC), instead because of its low non-specific binding properties. We can therefore accurately predict a drug's efficacy without having to worry about any effect of the plate's material on the test compound (Tsamandouras *et al.*, 2017).

9. Does the CN BIO system support high-throughput screening of drugs/small molecules?

A. Dubourg: I'll split the question into two parts. Firstly, there is generally an inverse relationship between high throughput assays and assay complexity. High throughput *in vitro* screens are generally performed using simplistic but convenient 2D immortalized cell-based assays. They usually provide one output measurement from which yes/no decisions can be made at scale. For evaluating DILI, the trade-off is that they offer around 30% predictability. MPS/OOC is the polar opposite!

The point is explored in more detail in this blog but OOC/MPS are more suited to late-stage preclinical drug evaluation. Here, fewer drugs are screened in complex human-relevant preclinical assays that use primary cells. These assays deliver greater sensitivity (a recent study from Emulate suggests in the 80% range for DILI) and ours generates high-content data that can be used to provide deeper insights,

such as mechanism of action. PhysioMimix systems offers medium throughput. As mentioned in Q8, the system can run up to 6 multi-chip plates simultaneously, therefore enabling the users to screen up to 72 samples/run. However, we are currently investigating how to increase throughput (without compromising on the aforementioned benefits) so that researchers can benefit from the insights that PhysioMimix delivers earlier in their drug discovery workflows.

Otherwise, PhysioMimix systems are agnostic to drug type, meaning that yes, it is compatible with small molecules and also new drug modalities. In a recent poster, we assessed the drug-liver injury (DILI) potential of two antisense oligonucleotides (ASOs) using our liver-on-a-chip model. Collaborators have also used our liver model to investigate the uptake and distribution of ASOs within liver tissue. It is therefore possible to assess the safety and efficacy of ASOs and other new modalities/small molecules in our system in its current set-up even if not for true high-throughput screening.

10. Are your plates made of glass? Are they disposable or reusable?

A. Dubourg: They are neither made of glass, nor reusable. As I mentioned in Q8, our multi-chip consumable plates are made of COC, the most inert material currently available for cell culture.

As the plates are made of cell culture plastic, they are all single-use and disposable following local H&S guidelines. Our multi-chip plates are gamma-sterilized to ensure sterility before use and can be used for over a month (depending on the plate and organ) if long-term experiments are required.

11. It is not clear to what extent one can study the 3D arrangement of cells in this system. Can you please shed some light on this?

G. Guenigault: If we focus on the model presented in this webinar, our liver model is created using our 12-well PhysioMimix multi-chip liver plate. Each of the 12-wells contains a collagen-coated engineered scaffold containing what we call microchannels (holes or pores). When liver cells are seeded into the plate, we ensure that they seed within those microchannels to generate the liver tissue. These bespoke scaffolds a) promote the 3D formation of a liver microtissue and b) ensure that the maximum surface area is exposed to flowing media to ensure all cells in the structure remain perfused with access to oxygen and nutrients.

Each scaffold is deep enough to ensure that the cells form a tissue not just across the scaffold, but they penetrate the depth of the scaffold (its microchannels) too. When the liver plate is connected to the PhysioMimix OOC system, a circular and unidirectional flow pushes the cells within the scaffold and its microchannels. Some liver cells attach to the scaffold walls (within the microchannels), while others will attach to their neighboring cells, thus creating a network that forms a liver tissue. These tissues have been shown to be polarized and feature key liver structures, such as bile canaliculi and their microvilli, to recapitulate the liver microarchitecture.

12. What 3D models exist for bone cells?

A. Dubourg: To date, there is no fully characterized and validated 3D bone model available commercially. Recreating the bone is complex and not all cell types are commercially available to reproduce a bone model consistently. A few academic groups have been working on this and have published in the last decade. We have not tackled this challenge yet as this is still very niche but if you'd like to know more, this recent review may be of use: Cells sources for human *in vitro* bone models.

13. What about centrally active compounds? At what stage are we on brain-on-a-chip applications?

A. Dubourg: The brain is one of the most complex organs to recreate *in vitro*. Recent developments in the 3D cell culture field include the creation of organoid and spheroid models, however, these models still lack vascularization and are static (no fluidic flow to recreate the physiologically-relevant blood flow) limiting their translatability. Many OOC developers are trying to recreate the blood brain barrier (BBB) to study the ADME properties of compounds, including centrally active compounds, however, this is not currently being pursued by CN Bio for the reasons stated in Q16. It is still challenging to develop a functional and physiologically-relevant brain-on-a-chip to assess the effects of compounds. Academic groups have focused on developing certain regions of the brain, but those models still require extensive work before they are sufficiently well characterized and validated for industry use.

To know more about the current states of development for Brain-on-a-chip, please read: Brain-on-a-chip: Recent advances in design and techniques for microfluidic models of the brain in health and disease.

14. What types of brain cells can we use for incubation with compounds?

A. Dubourg: Cells are probably the most critical parameter to consider, validate and quality control when developing an OOC model. Regardless of the model you are developing, various technical factors should be considered to validate any cells for OOC work:

- What type of cells (primary *vs* iPSC *vs* immortalized)
- Their viability (is it over 80%)
- The number of cells per vial
- How well do they perform in coculture with other cell types
- Do they form 3D tissues with the correct pathophysiology
- The number of vials accessible to ensure reproducibility

As well as biological factors:

- Are they functional (secreting any functional soluble biomarkers)

- Do they behave the way the target organ behaves?
- Any clinically-relevant endpoint I can use to translate to the human?
- How relevant is the model?

As mentioned in Q13, the brain is the most complex organ to recreate *in vitro* due to its complexity. However, progress has also been held back by a lack of commercially available primary human brain cells. Accessing primary brain cells is currently difficult as most brain cells come from donors *post-mortem*. They may have some brain damage or disease (e.g., Alzheimer's) making the cells unsuitable for a healthy *in vitro* brain model. Accessing a reliable supply of healthy cells from living donors is obviously not feasible. Therefore, currently the best way to recreate a brain in an OOC/MPS platform is by using iPS-derived brain cells which offers improved human-relevance over immortalized cell lines but with limitations.

Most commercially accessible BBB MPS models use iPS-derived astrocytes, neurons, and microglia. Some can also add some vascularization by using brain microvascular endothelial cells. The model will greatly depend on the end application. For example, when developing our 7- and 10-Organ models, as part of the DARPA race with MIT, we used neural progenitor cells and mixture of iPS-derived astrocytes and neurons to recreate the BBB. We have only used those simple models as proof of concept for complex interconnected organ studies and ADME assessment. We would therefore need to do more work to characterize and validate any such model before supplying it to our customers.

15. Any application of the PhysioMimix to neurology?

A. Dubourg: As mentioned in Q14, we developed a proof-of-concept brain model using iPS-derived neurons and connected it to other organs as part of the part of the MIT consortium in the DARPA race to develop multi-organ-on-a-chip models. This model was used along with 6 or 9 other organs in 7- and 10-organ-on-a-chip models to assess the interorgan crosstalk and determine the potential of such technologies for drug efficacy testing (Edington *et al.*, 2018). Since this time, the model has not been further developed so currently, we do not have a PhysioMimix validated model for neurology applications.

16. Do you have a model for the human eye?

A. Dubourg: Developing an eye model is not something we foresee doing in the near future. A few academic groups have developed their own in-house eye models but access to cells and model characterization limits the widespread implementation of those models. Often they are developed on in-house prototype OOC platforms which are almost impossible to implement in pharma laboratories.

At CN Bio, our R&D efforts are focused on perfecting the key organ models required by drug discovery and development, such as gut, lung, liver. For example, most gut-on-a-chip models currently offer models using immortalized gut cell lines which have many limitations and are not human-relevant

enough. Current supply of primary gut cells is not mainstream and sourcing those cells can be challenging. Moreover, those cells can be very difficult to handle *in vitro* and require expertise.

17. The hepatocytes develop bile canaliculus. Can you identify compounds specifically secreted through bile?

G. Guenigault: As presented in our webinar, we have demonstrated that the hepatocytes in our model do form bile canaliculi and bile secreted compounds have been detected. This is not an area we have internally explored thoroughly. It would require more optimization, characterization, and validation to confirm whether compounds secreted through bile can reliably be identified. Although, the model is not fully characterized for this specific application, some of our collaborators use the liver model because of its ability to recreate bile canaliculi in order assess the role of the bile canaliculus in drug safety.

18. If you can include immune cells, is there a possible use for CAR-T therapy?

We have yet to test CAR-T therapies in our system, however we have worked with circulating T cells to assess their toxic effects on the liver, when in coculture with an immunotherapy, through collaboration with a customer. It should therefore be possible to develop a protocol to investigate CAR-T therapy

Contact Details

If you have any other questions for Dr. Audrey Dubourg, Dr. Gareth Guenigault, or the CN Bio team about this webinar or about their predictive human model products or services, please go to:

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