CN-BIO

AUTUMN WEBINAR SERIES

The Rhythm of Life

Using Microfluidics To Mimic Blood Flow in Single- and Multi-Organ-on-a-Chip Models

Q&A

A full run down of questions & answers from our September 22nd webinar

Q&A participants

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Abbreviations

Two-dimensional	**2D**
Three-dimensional	**3D**
Computational Fluid Dynamics	**CFD**
Extracellular matrix	**ECM**
Gastrointestinal	**GI**
Good laboratory practices	**GLP**
Human stellate cell	**HSC**
Immunocytochemistry	**ICC**

iPSC	Immuno-pluripotent stem cell
KC	Kupffer cell
MPS	Microphysiological system
OOC	Organ-on-a-chip
PFA	Paraformaldehyde
PHH	Primary human hepatocyte
TEER	Transepithelial electrical resistance

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: When you create a disease model and apply the drug, how do you change the media? Do you add new media every time or do you add disease causing agent and then drug in the same media? Could you also tell me about volume and timings of media change?

A: From Alysha Bray, Scientist, CN Bio:

Like in any standard cell culture experiment, media is changed regularly - every 2 to 3 days depending on the experimental plan - using standard P1000 pipettes and tips in a Microbiological Biosafety Cabinet.

Whether you want to trigger a disease or add a drug, the agent/molecule is added to the culture media and refreshed at every media change.

Volumes of media vary between organ models and plates. For example, our liver-on-a-chip (powered by the PhysioMimix™ **MPS-LC12 plate**) uses an average volume of 1.6 ml per well whereas our gut-on-a-chip (powered by the PhysioMimix™ **MPS-T12 plate**) uses less media (up to 1 ml).

For experiments requiring a longer incubation time, such as low clearance compounds or time courses, the volume of media can be increased to suit longer incubation periods – for example 4 to 5 days.

Q2

Q: For your 3D-like gut model, which goblet cells are you using? HT29?

A: From Alysha Bray, Scientist, CN Bio:

Yes, we currently use HT-29-MTX-E cells along with caco-2 epithelial cells to create our 3D-like gut tissue. Goblet and epithelial cells are seeded onto a standard 24-Transwell® which is then incubated in our PhysioMimix™ **MPS-T12 plate**.

To learn more about our gut-on-a-chip model, **watch our animated video** on the PhysioMimix™ Organ-on-a-chip (OOC) System page.

Q3

Q: What is the physiologically relevant flow rate for liver? Should the liver experience any meaningful shear?

A: Dr David Hughes, CEO, CN Bio:

In a liver, hepatocytes experience very low (near zero) shear stress as they are separated from the blood by the fenestrated endothelium. However, as the endothelium is fenestrated (i.e. has holes), there will be substantial mass transport, and this will be influenced by the flow rate in the sinusoid.

Our PhysioMimix™ OOC System provides sufficient flow to ensure good mass transport with low shear stresses of a similar order of magnitude to those observed in the liver sinusoid/capillary.

To know more about the flow in our system:

Domansky et al, 2010 "Perfused multiwell plate for 3D liver tissue engineering"

Ebrahimkhani et al, 2014 "Bioreactor technologies to support Liver function *in vitro*"

Q4

Q: Can you briefly describe the process of retrieving tissues for analysis such as histology and immunocytochemistry (ICC)?

A: From Alysha Bray, Scientist, CN Bio:

Our PhysioMimix™ OOC system allows for the easy removal of any tissues for microscopic or -omics analysis.

A tool supplied with the MPS-LC12 plate can be used to easily recover 3D liver microtissues, as demonstrated in the **animated video** on the PhysioMimix™ OOC product page of our website. When working with **MPS-T12 plates**, we use forceps to remove Transwell® inserts from the plate. 3D-like gut tissue is subsequently recovered by cutting out the Transwell® membrane.

Tissues can be fixed using your preferred fixative protocol (we use a standard 4% paraformaldehyde (PFA) fixing protocol with slightly longer incubation period), prior to embedding or staining using optimised standard protocols.

Q5

Q: Has your Transwell®-based product also been used with 3D cell culture?

A: From Alysha Bray, Scientist, CN Bio:

As demonstrated by our gut model, the flow within our **MPS-T12 plate**, in which Transwells® are used, enables the creation of 3D tissues. We are also developing models using spheroids directly plated onto Transwell® inserts within Matrigel™, however this project is still in the early stages of development.

Q6

Q: If you added endothelial cells to the liver spheroid on the scaffold, could you develop vascularized liver organoids with the flow?

A: From Alysha Bray, Scientist, CN Bio:

It is important to note that the microtissues in our PhysioMimix™ OOC platform are not 'organoids' in the traditional sense; but totally perfused 3D microtissues resulting in the long-term maintenance of cell health, function and viability (**see our latest video on our Liver-on-a-chip model**) in comparison to the traditional organoid which commonly sees cell death in the centre, in a few days, due to the lack of nutrients.

A vascularisation of the liver tissue may occur when adding endothelial cells; however, we have yet to establish this internally. Some of our collaborators are currently working on developing their own co-culture model using endothelial cells along with parenchymal and non-parenchymal cells which should help shed some light on this question.

Q7

Q: How do you benchmark your results against clinical data?

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

To benchmark, we compare our organ-on-a-chip data against clinical data for reference compounds. **Tsamandouras et al, 2017** is a great example. In this collaborative study with Prof. Linda Griffith's lab at Massachusetts Institute of Technology (M.I.T.), various well-known drugs (e.g.: lidocaine, ibuprofen), were investigated to gage the level of translatability between the advanced 3D *in vitro* model and human. This study also researched the effects of donor-donor variability to establish if similar trends were observed *in vitro* as *in vivo*.

Q8

Q: Don't you want a less permeable gut (i.e. to prevent inflammation/infection)?

A: From Alysha Bray, Scientist, CN Bio:

Developing *in vitro* gut models can be challenging as the gut barrier should be permeable yet also maintain its tight junction formation.

To accurately mimic the human gut, we aimed to keep the permeability as high as possible in our model to allow for the passage of nutrients and oxygen through gut tissue, while still maintaining the barrier formation as seen by the tight junction expression.

If we had aimed for a tighter barrier, it would impact the passage/diffusion of nutrients and other compounds through the gut barrier, as is observed in standard *in vitro* 2D Caco-2 tissues, and thus compromise data accuracy and human-relevance.

Q9

Q: Are different types of collagen or other matrix proteins used as substrates for 2D vs 3D?

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

Our PhysioMimix™ OOC platform allows for the use of various cell formats (e.g.: primary cells, immortalised, iPSCs or even commercial inserts) which are all supported by the same kind of matrix whether they are in 2D or 3D. For example, the scaffolds used in our liver-on-a-chip model are coated with Type I collagen, which is the standard matrix for the *in vitro* cell culture of liver cells such as primary human hepatocytes.

Q10

Q: How was inflammation created using your gut model? How was inflammation verified and monitored?

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

The presented gut model was not designed for inflammatory studies but for DMPK studies, as no immune cells were included. We have previously worked with gut model co-cultures in our platforms that include dendritic cells (**Chen et al, 2018**) which allow inflammatory cross-talk studies to be performed. Here, inflammation in the gut was induced using TLR agonists and

cytokine profiles in the cell culture media were monitored using Luminex analysis.

Q11

Q: What additional organ models are you interested in developing, and how will you integrate these models into your PhysioMimix™ OOC platform?

A: From Alysha Bray, Scientist, CN Bio:

We are interested in developing many different organ models including, but not limited to, skin, heart, kidney, as well as further developing our lung model.

Initially, these organ models are developed using our current single-organ **MPS-T12 plates**, which allows for any organ model to be developed in a Transwell® format. Where it is clear that a different bioengineering approach is required to improve the translatability of data, this leads to the development of bespoke plates like our Liver-on-a-chip **MPS-LC12 plate**.

Q12

Q: Will other organs be added into your multi-organ plate, in the future?

A: From Alysha Bray, Scientist, CN Bio:

The Transwell®-Liver (MPS-TL6) plate, in which our gut-liver model is currently being developed, can be adapted to suit a variety of two-organ models. The plate's design allows for the culture of a variety of organ types in a standard Transwell® format alongside the culture of 3D liver microtissue in our liver-on-a-chip to facilitate interaction and cross-talk studies between lung-liver, brain-liver, skin-liver and more. Which organ we will add into the multi-organ platform, once the gut-liver has been fully validated, will greatly depend on what our collaborators need to achieve their research goals. This multi-organ plate is currently in the late phase of development and will be commercially available in 2021. If you'd like to know more, please contact our Commercial team at **sales@cn-bio.com**.

Q13

Q: What about the gut microbiota? Is this system compatible with such an application?

A: From Alysha Bray, Scientist, CN Bio:

Our gut-on-a-chip can potentially be used to study gut microbiota, as material can be applied to the gut culture and local or systemic effects studied. To date, we have limited experience in this area, however a number of our collaborators are working on developing **gut microbiota models in an aerobic environment**.

Q14

Q: What are the differences in the scaffold requirements for different tissues? Can the liver tissue scaffold work for a muscle or skin tissue organoid?

A: From Alysha Bray, Scientist, CN Bio:

Scaffold requirements are very organ-dependent and will vary according to each organ microarchitecture. Although our MPS-LC12 scaffold has been optimised to primarily work with primary liver cells and immortalised liver cell lines, it could be adapted for use with other organs either with a different scaffold or ECM coating.

Q15

Q: How many Hepatocytes are seeded in one experiment?

A: From Alysha Bray, Scientist, CN Bio:

Our PhysioMimix™ OOC platform enables you to run up to 4 conditions in triplicate per plate, with a well-defined and optimised seeding number of 600,000 Primary Human Hepatocytes (PHH) per well either in mono- or co-culture with non-parenchymal cells. This number of cells is critical for the generation of truly high content data. Various endpoint analysis, such as -omics and microscopy, and clinical endpoints can be performed at the same time on one sample.

Q16

Q: What are the cells used in your Liver-on-a-chip model?

A: From Alysha Bray, Scientist, CN Bio:

The 3D liver microtissues created in our liver-on-a-chip model are generated either from the monoculture of PHH; or a more complex tri-culture of PHH with Kupffer cells (KCs) and Human Stellate

Cells (HSCs). Both models are used for various applications such as **DMPK**, **ADME** and **Toxicology** as well as disease modelling (e.g.: **steatosis**, **NASH**, or **Hepatitis B**).

Q17

Q: What do you mean by human relevant properties of the gut barrier (TEER value)?

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

The permeability of barriers can be assessed in a number of ways, measuring the transepithelial electrical resistance (TEER) is a common one.

The human gut is estimated to have a low TEER value of approximately 50 $\Omega.cm^2$, whereas *in vitro* cultures typically have values in the thousand and, therefore, do not truly reflect the human gut physiology. Our gut model has significantly lower TEER values of a few hundreds $\Omega.cm^2$, still higher than the human gut but much closer and therefore more physiologically relevant.

Q18

Q: How do you maintain a sterile environment?

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

As for any cell culture-based experiment, good aseptic techniques should be maintained along with the use of a microbiological safety cabinet to conserve a sterile environment over time when experiments are being set up and at any time whilst plates are de-lidded and out of the incubator. To understand more about how our PhysioMimix™ OOC system is set-up, **watch our animated video**.

Q19

Q: Flow scaling seems to be key to the successful development of any 3D *in vitro* models, but what other biological parameters do you think should be considered when recreating a 3D *in vitro* organ model?

A: Dr David Hughes, CEO, CN Bio:

This will greatly depend on the organ you are trying to model. The biological model should capture the organ's key features for the intended application.

Q20

Q: When measuring absorption, how do you account for the difference in total *in vivo* surface area of the gastrointestinal (GI) tract in relation to your *in vitro* model?

A: Dr David Hughes, CEO, CN Bio:

The difference in total GI surface area between our model and the human gut is accounted for by calculating the apparent permeability and scale it from *in vitro* to *in vivo* following the standard method applied in the literature.

Q21

Q: How do you accomplish flow dampening in chips for physiological relevance?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

We use a proprietary design of dampener in our PhysioMimix™ OOC system where consistent flow velocity and shear are desired. The dampening system itself consists of a semi-closed chamber between pump and tissue, a filter or flow restriction controls egress and forces a small build-up of fluidic pressure. A flexible membrane maintains this pressure between pump strokes and absorbs excess pressure during pump strokes. In this way, the extreme stop-start energy of a pulsatile flow is evened out with time.

Q22

Q: What platforms do you use for Computational Fluid Dynamics (CFD) simulation? Are they developed in-house or commercially available e.g. COMSOL? What company do you outsource the CFD modelling to?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

CFD simulations of our PhysioMimix™ OOC platform and MPS plates are generated in collaboration with a 3rd party service provider. The CFD platform used is called OpenFOAM which is an open-sourced modelling software. The results have led to a much more detailed level of understanding of our current PhysioMimix™ product portfolio and will prove invaluable in the design of all future bespoke MPS plates and organ models.

Q23

Q: Thank you for the great presentation! I wanted to ask also, what flow rates can your micropumps run? Are they peristaltic?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

You're very welcome.

Our pumps are optimised to work in the low µL s^{-1} range as this net flow rate sits well within the requirements of our overall system design: total volumes, re-oxygenation rates and mass of tissue being perfused. We could go to significantly lower or higher net flow rates with our design, but there currently is no biological need for this. The design of pump we use is a micro diaphragm pump (using valves to control direction of liquid displaced): a docked plate aligns with the pneumatic lines in the system and connects with the PhysioMimix™ controller. The on-plate pumps & valves are then actuated by the under-plate pneumatics. This means there are fewer moving parts for system reliability, and docking/undocking is a single, smooth action.

To know more about how our PhysioMimix™ OOC system works, **watch our animated video**.

Q24

Q: In the multi-organ system, can the flow be pulsed in one chamber and more continuous in the other chamber?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

Yes, the nature of flow can depend on the inclusion of a (soft) dampener or, instead, all harder surfaces. We have a number of features which provide controlled fluid movement between separate chambers, so each chamber is fluidically connected but it is not all open tubes, there are valves and micro-dams involved too.

Q25

Q: How about the material and structure of 3D scaffolds? Are these specific for different cell types?

A: From Dharaminder Singh, Principal Bioengineer, CN Bio:

It is important that we create an ideal microenvironment optimised for culturing each organ model/tissue type. This includes thoughts

surrounding the design of the scaffold, any coatings used, the materials, flow through the scaffold and many more factors. The structure of our **MPS-LC12 plate** scaffold has been carefully designed with these factors in mind. The material that we use for our liver scaffolds is collagen coated polystyrene, which is similar to the material used for tissue culture plates but optimised for liver cell culture.

Q26

Q: Is there a physical exchange of liquids and/or nutrients between the organs in the two-organ model?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

Yes, if we allow it (which we commonly do). It is the same media circulating between chambers/between tissues. Therefore, the liver tissue will be exposed to drugs absorbed by the gut tissue, likewise the gut tissue will be exposed to metabolites released by the liver tissue, reproducing an *in vivo*-like crosstalk between these two organs.

Q27

Q: How do you manage the media in the flow for different organs?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

We currently use common media which suits each tissue type within the duration of an experiment. We are continuously developing media and honing protocols alongside our physical products to expand plate utility and applications.

Q28

Q: I wanted to know about oxygen zonation in liver. Do you recreate oxygen zonation as observed in liver acinus?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

We do to a degree - modelling and direct measurement agree and have shown a significant difference in pO_2 between media upstream and downstream of our liver tissue. This demonstrates

increasing oxygen depletion as the fluid passes through the lumen of liver channels, mimicking that in a sinusoid. We are actively investigating different ways to use our systems which will provide increased utility in this regard, for instance using flow to decrease the gradient and keep pO_2 high for studies focussed solely on zone 1 tissue, or vice versa for zone 3. At present, we generate a balanced model with aspects of zones 1,2 and 3.

Q29

Q: You mentioned continuous flow for your liver model and pulsatile flow for your barrier models, how different are these flows in each OOC plate and how critical is the type of flow to an organ model?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

The flow profiles can be quite different but this also depends on where you are measuring it, as the flow in two different regions of the same well can be quite different depending on the geometry of the vessel and solution properties.

What is essential is getting the local environment that the tissue experiences to be optimal. Physical properties such as shear and flow velocity act close the tissue surface. Diffusion and concentration gradients (pO_2, nutrients, waste), however, form in a region between the surface and a few 100s micros out, whilst bulk mixing and bulk flow tend to be further from the surface. It is difficult to control all these properties at the same time, so you have to be selective, focus on what's most important, and continuously test and retest to ensure the system is delivering human-relevant data.

Q30

Q: What were the biggest challenges you faced when developing your multi-organ-on-a-chip plate?

A: From Dharaminder Singh, Principal Bioengineer, CN Bio:

Understanding the specifics behind what an organ/tissue model needs can be complex, both from a microfluidics and biological perspective. This complexity is dramatically increased when multiple organ models are combined. Creating a system with controllable and consistent flow within individual organs and flow between the different organs is challenging and should not be

underestimated, however with careful and considered design and testing these can be overcome.

Q31

Q: Apart from flow, which other features do you consider as critical/key to successfully developing organ-on-chip technologies?

A: From Dr Graham Broder, Associate Director - Bio Engineering, CN Bio:

Assuming "flow" also covers nutrient (including pO_2) transport, other areas to focus on include the physical and chemical properties of the tissue scaffold (biocompatibility, geometry, surface hardness, wettability…) and the chemical/biochemical makeup of the media. These are the things each tissue experiences *in vivo* and are therefore important for the experiment. However, to realise a product there are many further aspects to consider, some of the most important include usability (easy/fun to use, access to the tissue/media for sampling, preventing infection/contamination…), throughput (how many replicates can be simultaneously run), manufacturability (reproducibility, quality control, sterilisation methods, packaging, storage-life…) and there are regulatory aspects too as any product needs to be proven safe and effective. So overall, it is a very multidisciplinary task.

Q32

Q: Do you have a platform for multi-organ experiments between liver and other organs?

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

The Transwell®-Liver (MPS-TL6) plate, in which our gut-liver model is currently being developed, can be adapted to suit a variety of two-organ models. The plate's design allows for the culture of a variety of organ types in standard 0.33 cm^2 Transwell® format alongside the culture of 3D liver microtissue, thereby facilitating interaction and cross-talk studies between lung-liver, brain-liver, skin-liver and more. This multi-organ plate is currently in the late phase of development and will be commercially available in 2021. If you'd like to know more, please contact our Commercial team at **sales@cn-bio.com**.

Our autumn webinar series continues

13th Oct

Testing on Humans

How To Predict Hepatotoxicity and Drug Clearance Ahead of Clinical Trials Using Liver-on-a-Chip

Dr Tomasz Kostrzewski
Director of Biology, CN Bio

Register here

3rd Nov

A microphysiological model of metastatic progression

Dr Amanda Clark
University of Pittsburgh

Register here

24th Nov

Physiomimetics

Integration of Organs-on-Chips with Systems Biology to Humanize Drug Development

Prof Linda Griffith
Massachusetts Institute of Technology (MIT)

Registration coming soon

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