CN-BIO

SUMMER WEBINAR SERIES

The future of Pre-Clinical Drug Research: An Introduction to Organ-On-Chip

Q&A

A full run down of questions & answers from our June 2nd webinar

Question subject areas

Abbreviations

BBB – Blood brain barrier

COC – Cyclic olefin copolymer

iPSC – Induced-pluripotent stem cell

MGBA – Microbiota gut brain Axis

OOC – Organ-On-Chip

Q&A participants

Miss Alysha Bray
Scientist

Dr Graham Broder
Associate Director, Bio Engineering

Dr Audrey Dubourg
Product Manager

Dr Tomasz Kostrzewski
Director of Biology

Miss Vaishnavi Manoharan
Account Manager

Another question? Drop an email to one of our experts - **sales@cn-bi.com**

Missed the webinar? Watch an on demand **recording of the webinar here**

Biology

Q1

Q: Using your system, is it possible to create a skin model where malignant T cells could be in circulation?

I work with cutaneous T cell Lymphoma and trying to establish a model where malignant T cells infiltrate and proliferates inside the skin model.

A: From Alysha Bray, Scientist

Yes you can. Most skin models utilise Transwell inserts, for which our T12 plate has been specifically designed to accommodate. T cells can recirculate in our T12 model. Whether T cells infiltrate the model is dependent on the actual model, the number of different skin cell types, how dense the tissue is as well as the T-cells to skin cells ratio.

Q2

Q: What types of breast cancers have been modelled using these OOC systems? Are there any 3D OOC systems fabricated by CN Bio?

A: From Audrey Duborg, Product Manager

Our collaborators at the University of Pittsburgh use several breast cancer cell mixtures within our Liver-on-chip model to study the effects of breast cancer metastasis on the liver. You may find these recent publications from Prof Alan Wells' team useful.

Beckwitt *et al.*, 2018 **Clark *et al.*, 2014**

We supply research labs with true 3D OOC technology, the PhysioMimix™ OOC. Our solution enables researchers to create complex 3D co-cultures for long-term cell culture (over 4 weeks).

Q3

Q: Which part of heart tissue do you have experience with? What has been done with heart cells? What aspects of cardiac function can be demonstrated?

A: From Audrey Dubourg, Product Manager and Vaish Manoharan, Account Manager

So far, we have worked with iPSC-derived cardiomyocytes to model cardiac tissues linked to our liver model to study heart-liver toxicity crosstalk.

It is in our product roadmap to develop a more complex cardiac model.

Q4

Q: What are the research initiatives at CN Bio for Skin model? What has been done or achieved so far?

A: From Vaish Manoharan, Account Manager

As mentioned previously, for skin tissues, we use Transwell inserts in our T12 consumable plate, which has been specifically designed to accommodate Transwells. Skin can be generated from numerous sources including pre-formed tissues. These can then be put into our OOC platform, the PhysioMimix™, under perfusion (or linked to other organs such as liver). This approach can be used for several applications including toxicity testing, and drug absorption.

Q5

Q: Is there any OOC system available that can be used for behavioural neuroepigenetic studies?

A: From Audrey Dubourg, Product Manager

The brain is one of the most complex organs to mimic in vitro. Recent developments in the OOC field include the creation of organoid and spheroid models, however, these models still lack vascularisation. To date, most OOC technologies have focused on modelling the blood brain barrier (BBB) which is obviously "simpler" to recreate than the entire brain. The BBB is also key to stopping infection, or for the passing of drugs to the brain. Unfortunately, current models are not sophisticated enough for modelling MGBA and neuroepigenetics. however, it is hoped that reliable complex in vitro MGBA models will develop over time.

A good 2020 review covering this topic can be found here: **Raimondi *et al.*,2020**.

Q6

Q: If we are working with Hypoxia in Brain. How is your system compatible to study neuronal micro-physiology under hypoxic stress?

A: From Audrey Dubourg, Product Manager

We have used iPSC-derived astrocytes and neurons as well as NPC (neural progenitor cells) derived from the human H1 ES line so growing neurons in our system is entirely possible. The microfluidics in our system are designed to create fluidic movement within the plate, allowing cells better access to oxygen and nutrients present in the media than in standard static cell culture.

However, the system does not enable oxygen levels to be reduced. To create a hypoxic environment, hypoxic chambers and incubators are commercially available, however whether our system would fit into one of those chambers would need to be assessed (in terms of footprint and practicality).

Q7

Q: You mentioned a few organs which are modelled on the OOC, do you think it could also eventually be used to model brain disorders (e.g. Alzheimer's)?

A: From Audrey Dubourg, Product Manager

Recreating an in vitro model of the entire brain represents an enormous challenge since all the different cell populations and the surface dimension of the brain need to be considered. Current models are rudimentary by comparison, requiring more advanced work on cell-culture techniques and microfluidic technologies before they become anywhere near representative. The more we continue to research and develop the complexity of 3D models, however, the closer we will get to modelling brain-like tissues in vitro and eventually brain disorders such as Alzheimer's.

Q8

Q: Can your system be adapted to mimic the blood brain barrier (BBB)?

A: From Audrey Dubourg, Product Manager

Yes it is possible to mimic the BBB in our system. We have

successfully developed brain tissues using iPSC-derived astrocytes and neurons as well as NPC derived from human H1 ES line (**Edington *et al.*, 2018**).

Q9

Q: Do you have any products supporting bone related diseases?

A: Audrey Dubourg, Product Manager

No, we currently do not have any products supporting bone-related diseases. Bone-on-chip models are still very much in their early stage of development with only a handful of publications from academic institutes.

Q10

Q: We are interested in gut on a chip specifically small intestine. Will your company provide protocols and help in developing the gut on the chip in our lab including culture media advice, etc.?

A: From Audrey Dubourg, product Manager

Yes it is possible. We are currently working with immortalised cells but are moving towards iPSCs and primary cells to develop complex co-culture models. We are always happy to assist and guide our customers and collaborators as and where required.

Q11

Q: Does FDA support drug testing by microfluidic chips instead of in vivo test?

A: From Audrey Dubourg, Product Manager

In vivo testing is required to move a drug along the various preclinical and clinical phases before approval by any regulatory agencies. The FDA, however, is currently assessing OOC and microfluidic technologies.

To learn more, please join us for our webinar on the 15th of July where the FDA will present on “Establishing Strategies To Evaluate Microphysiological Systems for Drug Development”.

You can register here

Q12

Q: Would a positive result in terms of efficacy for a compound be needing further clinical trials in humans?

A: From Audrey Dubourg, Product Manager

Although OOC technologies can bring more confidence in the preclinical stages of the drug development process by reducing the gap between animal models and human, human clinical trials are still required as current OOC models are still very simple compared to the complexity of the human physiology. It is hoped that the more progress we make in the OOC field, the closer we will get to decreasing our dependence on expensive animal testing and human clinical trials.

Q13

Q: Can OOC results be submitted to regulatory authorities for IND filing?

A: From Vaish Manoharan, Account Manager

Yes, they can. All regulators have said they would encourage IND submissions with OOC data. OOC data can be used in both efficacy and toxicology data packages in IND submissions.

Q14

Q: You mentioned that regulators are evaluating the OOC for drug applications- could you tell us any more about that, please? Any examples you could share?

A: From Vaish Manoharan, Account Manager

We have an ongoing collaboration with the FDA to fully assess OOC systems such as our PhysioMimix platform as a preclinical tool for drug development.

If you'd like to know more about this, we have an upcoming webinar from our collaborators at the FDA who will be discussing their evaluation strategies of MPS systems. I would really recommend you listen in to this webinar.

You can register here

We also have a paper which will be published shortly detailing the collaboration and data with the FDA.

Q15

Q: Can the system be used technically for any cell lines e.g. cancer cells, non-mammalian, etc?

A: From Audrey Dubourg, Product Manager

Yes, our system is flexible and can be adapted for any cell type. Commercial inserts can also be used in our system; and we also have validated certain cell lines within our system which we can recommend upon request.

Q16

Q: How does the system model organ-organ interaction?

A: From Tom Kostrzewski, Director of Biology

Our PhysioMimix™ OOC system can be used for single organ or multi-organ studies. Multi-organ studies are performed with consumable plates that contain multiple fluidically linked chambers. Cell culture medium in the well is pumped between these chambers in a manner that mimics blood flow in the body. Using this approach, we can begin to model organ-organ interaction looking at how secreted markers from one organ will affect a second organ.

Q17

Q: Could you give some examples of parameters can be measured in this system?

A: From Audrey Dubourg, Product Manager

Thanks to the large sampling capabilities of our PhysioMimix™ OOC platform , various parameters such as cell health (LDH, Albumin), metabolic activity (CYP), cytotoxicity to -omics analysis and microscopy can be performed for one sample.

Q18

Q: How reliable/consistent are these systems over the entire 28-day use period? Hepatocytes typically decline in function over time, and so how well validated are these systems in terms of the function of drug clearance (CYPs, UGT, transporters, both influx and efflux etc)

A: From Tom Kostrzewski, Director of Biology and Audrey Dubourg, Product Manager

We have published data for 28 days showing the stability of the system for a variety of applications including drug metabolism, toxicology and disease modelling. Hepatocyte performance in the system is significantly improved compared to other standard approaches such as collagen-sandwich cultures and spheroids. CYP activity can be detected throughout the culture period as can the expression of transporters.

To know more about our liver model and its various applications, you can listen to our next webinar on NASH which will be held on the 16th of June by Dr Michele Vacca, one of our collaborators at the University of Cambridge.

Q19

Q: Does your system allow organoid culture?

A: From Audrey Dubourg, Product Manager

Yes, using **our T12 plate** it is possible to use organoids in our system. We are currently developing several collaborative projects with industrial and academic partners to further develop organoid models.

Q20

Q: Are there any publications on this system using organoid monolayers?

A: From Alysha Bray, Scientist

It is clear that utilising monolayer organoids in our system is beneficial, but we're only at a preliminary stage to show any data or publish.

Q21

Q: You mentioned the system can be used to study human specific metabolic profile of large and small molecules, is this system also used to study drug transporters?

A: From Tom Kostrzewski, Director of Biology

We have used the system to look at drug metabolism, drug transport and drug intracellular accumulation. The publication by **Vivares *et al.*, 2015** shows the expression of key transporters in our system over time.

Q22

Q: Can you please elaborate the influence of immune cells? How these will be assessed?

A: From Audrey Dubourg, Product Manager

The addition/incorporation of immune cells in OOC systems, such as ours, has been exponentially increasing over the last couple of years. The reasons behind the need to include immune cells into OOC system/models can differ from one project to another but are mainly due to:

Immune mechanisms play a crucial role in major diseases, such as cancer, and are the primary cause of other common conditions, such as autoimmune diseases.

Molecularly targeted therapies, such as engineered T cells, stem cells or nanoparticle, can cause unwanted immune responses, which can sometimes be catastrophic.

Selective activation or blockade of the immune system has shown to be a powerful therapeutic approach; however, this approach can lead to adverse effects such as cytokine-release syndrome.

Lack of translatability between animal models and the human immune response which limit the study of the human immune response responses and mechanisms

Therefore, the development of reliable in vitro models of the human immune system are needed to fully assess the immune system play in both pathogenesis and therapy. Immune cells can be added into the system at any given time to be recirculated within the 3D tissue. Immune cells-tissue interactions can be assessed by various methods such as biomarkers, cytotoxicity assays, microscopy or -omics analysis.

Q23

Q: Could this system be used in other species - say feline or canine?

A: From Vaish Manoharan, Account Manager

Yes, absolutely, both can be studied on the platform which is not limited to human. We have successfully worked with rat cells also.

Q24

Q: You mentioned gut, skin lung etc.. are you referring to the tissues or just the cells - eg.. skin is made up of different cell types... does your system recreate the complexity of the skin tissue?

A: From Audrey Dubourg, Product Manager

When talking about an organ model e.g. gut model, skin model or lung, we are talking about a gut-like tissue generated by seeding several gut cell types together in a co-culture.

We have successfully worked with commercial inserts, such as EpiSkin™. But it is also possible to build a model using several cell types to recreate the complexity of an organ.

Q25

Q: For the organ crosstalk studies with the TL6 (multi-organ gut liver plate), is the media a customized media or commercialized media?

A: From Audrey Dubourg, Product Manager

We use standard media, such as DMEM or WEM, with an adapted concentration of supplements. The type of media and supplements will depend on the cell type used in the experimental protocol.

BioEngineering

Q26

Q: What's the main process used to manufacture the OOC in plastics? Are there others materials?

A: From Graham Broder, Associate Director of BioEngineering

The component parts of OOC plates are manufactured by injection moulding. Most of the plate including almost all of the

fluid contacting area is made from Cyclic olefin copolymer (COC), specifically chosen for its demonstrated low binding with small molecules, drugs and biomolecules. Following component part manufacture, plates are assembled and inspected prior to packing and sterilization.

Q27

Q: How is Nanotechnology applicable for OOC Technology?

A: From Graham Broder, Associate Director of BioEngineering

To date CN Bio has not explored the combined use of nanotech with our OOC platforms, but we can envision areas where the technologies are likely compatible. Nanotechnology covers a very broad spread of science & engineering, one obvious area of interest might be the study of interaction between nanoparticles and tissues, including the efficacy of novel nanotech drug delivery systems.

Q28

Q: Why don't you use PDMS for LC12 and T12? Is the biocompatible polymer used assist the cell growth?

A: From Vaish Manoharan, Account Manager

PDMS is known to be highly binding to chemicals and drugs, when assessing a drug's PK/PD, there is a need for the most inert, low-binding components in the system. We use COC which is currently known as the most inert material available. COC does not impact on cell growth.

If you'd like to know more about adsorption and efficacy in models using PDMS vs COC, please go to the following links:

van Midwoud et al, Comparison if Biocompatibility and Adsorption properties of different plastics for advanced microfluidic cell and tissue culture models , 2012

McAleer et al, Multi-organ system for the evaluation of efficacy and off-target toxicity of anticancer therapeutics, 2019

Q29

Q: Can you elaborate more on the biosensor used in OOC?

A: From Audrey Dubourg, Product Manager

We are currently looking at implementing a range of biosensors for our PhysioMimix™ system.

PhysioMimix™ OOC

Q30

Q: What is the sample volume (per well) in your system?

A: Vaish Manoharan, Account Manager

Our system allows for the sampling of up to 1mL per well, which enables researchers to perform a diverse range of endpoint analysis. Each plate has 12 independent wells.

Q31

Q: What are the dimensions of the CN Bio system? Do the plates incorporate different cell types within a tissue type? Eg. melanocytes and keratinocytes in the skin model.

A: Audrey Dubourg, Product Manager

Our system has a small footprint, and is designed to fit in a standard incubator. **You can find a more detailed specification here**.

Different cell types can be incorporated in the consumable plates. For example, using **our LC12 plate**, we recreated the liver 3D microarchitecture using primary hepatocytes, kupffer and stellate cells.

Q32

Q: Are there facilities for imaging in the PhysioMimix systems? Can you do live cell imaging with the CN Bio platform?

A: Audrey Dubourg, Product Manager

Our system currently does not allow for live cell imaging due to the current set up of the hardware; however, it is possible to perform various imaging techniques (from basic brightfield to confocal immunofluorescence or TEM) on the microtissue when taking down the experiment.

Q33

Q: Thanks for a great talk. Have you quantified cost and time savings in drug development using CN Bio product OOC? If not a full-blown study, any preliminary data?

A: Audrey Dubourg, Product Manager

This has not been fully quantified, however previous publications using known and well-established drugs in our system **Tsamandouras et al 2016**, **Sarkar et al 2017**, have shown that OOC technologies such as ours provide a reliable way to generate robust pre-clinical data so you can enter clinical trials with more confidence.

To know more about the value in terms of time and cost reduction, listen in to our fourth webinar on 15th of July which will be led by our collaborators at the FDA.

You can register here

NASH (nonalcoholic steatohepatitis)

Q34

Q: We have a single compound that we would like to study in a NASH model. Do you do assays on a contract basis or does one need to purchase the entire apparatus?

A: Audrey Dubourg, Product Manager

We offer an in-house NASH Fee-For-Service on a contract basis to test compounds in our NAFLD/NASH model. Please do not hesitate to contact us at **sales@cn-bio.com** for an informal discussion about your specific requirements.

Our summer webinar series continues

16th JUNE

The scars of fat

Register here

The transability of 3D NASH microtissues to model human/murine NASH

Dr Michele Vacca
University of Cambridge

2nd JULY

Go with the flow

Register here

The transability of 3D NASH microtissues to model human/murine NASH

Dr Renato Cardoso
Dr Thalita Zanoni
Charles River Laboratories

15th JULY

A regulators viewpoint

Register here

Establishing strategies to evaluate microphysiological systems for drug development

Dr Alexandre Ribeiro
FDA

CN-BIO

332 Cambridge Science Park
Milton Road, Cambridge, CB4 0WN

+44 (0) 1223 737941 | **sales@cn-bio.com**

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