CN-BIO

2022 WEBINAR SERIES

A breath of fresh air

Novel approaches to human lung disease modelling for accelerated drug discovery

A full run down of questions & answers from our 29th March webinar

Acronyms

3D: 3-Dimensional

3D FTIR: Fourier-Transform Infrared Spectroscopy

ALI: Air-liquid interphase

CDER: Center for Drug Evaluation and Research

COC: Cyclic olefin copolymer

COPD: Chronic obstructive pulmonary disease

FDA: Food and Drug Administration
IHC: Immunohistochemistry

miRNA: Micro RNA

MPS: Microphysiological system

OOC: Organ-on-a-chip

PBMC: Peripheral blood mononuclear cells

PDMS: Polydimethylsiloxane

TEER: Transepithelial electrical resistance

THP-1: Human leukaemia monocytic cell line

TJ: Tight Junction

Q&A participants

Professor Wojciech Chrzanowski

Faculty of Medicine and Health, University of Sydney

Dr Emily Richardson

Lead Scientist - Assay Development, CN Bio

Another question?

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

Questions

Q1

Q: How long can the lung tissue be cultured in the PhysioMimix™ OOC system?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

In general, we culture our lung tissues for 14 days and we see excellent differentiation of tissue at that time. We have cultured tissues up to 21 days and the barrier integrity still looked good. In the future I would like to see how far we can push this model but obviously there is some question as to the usefulness of extending culture versus the time, effort, money and resources it would require.

Q2

Q: Why use the THP 1 cell line instead of primary monocytes?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

That's a really good question. We decided to start to use the human leukaemia monocytic cell line (THP-1) because they are easy to culture compared to primary monocytes. Something we really like to ensure at CN Bio is that the models we develop are applicable to every laboratory. We believe that most labs have a vial of THP-1s in their liquid nitrogen somewhere and so, this makes it a little bit more cost effective. However, we do plan to use primary cells, including primary immune cells in the future. Ultimately, we plan to have peripheral blood mononuclear cells (PBMCs) recirculating in the basolateral side of the model .

Q3

Q: Why do you think the model is reacting to the lentivirus as it's not a live virus? Are you planning on using live SARS-CoV-2 in your model?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

Another good question. So, it was a bit of a surprise, but a nice surprise, to us when we saw that our lung model was reacting in an inflammatory way to the lentivirus. In the literature, it has been proven that the Spike protein of SARS-CoV-2 in its cleaved form (S1-S2) causes pro-inflammatory responses, particularly in the microvasculature (**Buzhdygan et al., 2020**, **Perico et al., 2022,**

Raghaven et al., 2021). In future studies, we hope to look further into this damage caused in the lung MPS.

To answer the second part of the question, we would have loved to use live SARS-CoV-2. Unfortunately, we only have a BSL-2 laboratory which makes it impossible in house; however, we are collaborating with the **Biagini group at the Liverpool School of Tropical Medicine** who are using real SARS-CoV-2 with our **PhysioMimix OOC** system. We look forward to viewing results from this group soon. Stay tuned to learn more on this topic later in the year.

Q4

Q: Are you planning on using this lung model for other assays?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

Absolutely yes, we already use our models for other applications and so do our collaborators. The U.S. Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) use our models to evaluate inhaled medications. This work is ongoing, but we hope to present some of their findings in the future. We would also love to model diseases like asthma and chronic obstructive pulmonary disease (COPD) to test the efficacy of novel therapeutics. Obviously, the other area to explore is additional infection models, aside from COVID-19. Within our lab, we would like to complete further experiments on BSL-2-safe respiratory pathogens, such as influenza virus, and use our multi-organ plate to understand crosstalk between the lung and liver during these infections. We would also like to include more immune cell types into these models to increase physiological relevance and therefore the models' ability to predict responses.

Q5

Q: Could you expand more on the alveolar-like structures that you see? Do you have any idea of why they are produced with treatment and why the numbers of tight junctions are increased?

A: Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

This is a very important question and, while we are still investigating the mechanisms responsible for the formation of these structures, we strongly believe that this is associated with the treatment that we developed and designed. This treatment contains multiple classes of micro RNAs (miRNAs) and proteins which simultaneously activate

different types of cells which synergistically act to promote structure repair. Because we are targeting not only the upper airway but also the small airway, this treatment is very likely to trigger and promote the repair and regeneration of structures in the small airway. This may be the reason why we see the formation of these alveolar-like structures.

With respect to the second question, which asks why we see an increased number of tight junctions (TJs). The answer is very similar. Our treatment is targeted at improving barrier function. Barrier function improvement is represented by the formation of a uniform structure, uniform TJs and improving permeability, or inhibiting permeability through this epithelial barrier in our lungs. The treatment again is responsible for the formation and the regeneration of the tissue by formation of well-developed and very functional TJs.

Q6

Q: Did you use primary or immortalized cell lines in your models and if you used both did you see any differences?

A: Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

We used both immortalized cell lines and primary cells, however, immortalized cell lines were only used to optimize conditions, and to help us understand how the PhysioMimix OOC system works, because these cells have certain limitations. We use primary cell models for safety and efficacy assessments because primary cells allow us to create models that represent human physiology. They can differentiate properly, they can form cilia, they can secrete mucous, and they form functional lung mimicking structures. The additional advantage of using primary cells is that we can take cells directly from patients to perform personalized screening, or personalized testing of formulation preparations for specific patients.

Q7

Q: The 3D FTIR data looks very exciting, could you expand more on this and how it can be used in the future?

A: Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

Thank you this is also a very interesting question. Whether used in material science, or biomaterials, 3D-Fourier Transform Infrared (FTIR) spectroscopy gives you very precise information about a structure. We use 3D-FTIR, or more broadly vibrational spectroscopy, in our research all the time to provide a fingerprint, or molecular fingerprint of structures.

Here, we used high-throughput and high-content FTIR to look for changes in the molecular composition of our lung models that indicate lung injury. In the maps I presented, the colours and the position of specific peaks look different in injured lungs. Subsequently, if lung tissues recover from injury, the colours and the peaks will be shifted, and their intensities will also be different.

This approach enables us to perform a fast and precise assessment of a treatment's functionality, or its efficiency, or efficacy. We also use this approach to look at the injury itself. For example, if we induce injury, do we induce it uniformly across the whole model? And, post-injury, how well does the treatment improve recovery from the specific insult that we applied?

Q8

Q: Can 3D printing assist the development of the models?

A: Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

Very good question. 3D printing and so-called organ-on-a-chip (OOC) are two mega trends. By combining them, we synergize their capabilities. Indeed, we can print directly, or assist the formation of our models by 3D bioprinting them directly onto Transwells®. This is a huge benefit of the Transwell-based system because we can do extruding printing or bioprinting on these models. So, if in the development of your model, you need to use a matrix to assist the formation of certain structures, it's the ideal setup.

We are very fortunate to have a very strong collaboration in this space with a Canadian company called **Aspect Biosystems**. We use a unique 3D printer that they have, which is based on the microfluidic printhead with a relatively long needle for printing. This allows us to go to the bottom of the plate to print one model and then put a secondary model into the Transwells®. This combination gives us additional opportunities and additional capabilities to develop certain models. It is not always necessary and, as I presented, formation of the lungs may not always require 3D bioprinting.

Q9

Q: Why is perfusion important in establishing and testing the models?

A: Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

Very important question. Perfusion serves two main functions. Firstly, perfusion provides mechanical cues. We live because of mechanical forces and mechanical stresses - our heartbeat, our movements, our muscle tension - provide critical and fundamental biomechanical cues to ourselves, which trigger many regenerative processes. Especially in our lungs. When we breathe, there is a significant expansion of the lungs and significant changes in the mechanics of these structures. By introducing the shear stresses of the microcirculation into our models, it adds value as it mimics something that is physiologically present.

But there is a second critically important aspect which is cell to cell communication. Perfusion moves all the molecules which are secreted by cells - cytokines, proteins, vesicles - between cells to recreate similar conditions to the human body.

So, to summarise, there are these two key aspects - mechanical cues that provide a certain level of stimuli to the cells, plus nutrient exchange and informational crosstalk between cells. We could clearly see a huge difference between the models which were established in static conditions versus the models which were established in dynamic conditions with microcirculation.

Q10

Q: How can these models replace and reduce animals in research?

A: Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

The key element here is that some animal models do not represent human physiology or human disease. There is a lot of progress in the area of humanized mice etc., but there are limitations. We know that the correlation is sometimes close to zero. These *in vitro* models mimic certain parts of the human physiology, so they allow us to test things more physiologically, or more importantly pathophysiologically. The formation of the human specific-, or

human mimicking, structures within these models is isolated from the whole system - there's no enzymatic regulation, there's often only one humanized organ and therefore limited interaction with other organs.

These *in vitro* models enable us to test multiple formulations, screen the formulation,

test the safety and efficacy in a much faster and much more representative way than some of the animal models. Obviously, they will not push away the use of animals completely, but they will reduce the number of animals which we

need to test our formulations in. The reason for this is because, after testing in human physiology mimicking models, we will have a very high degree of confidence in our formulations, our drugs, new compounds, new molecules.

I personally think there is a chance that these advanced *in vitro* OOC models will completely remove the need for some animal models at certain points in drug development and maybe they will become a prerequisite before human clinical trials.

OOC models have a very strong presence at the moment. They are likely to provide key answers to questions in terms of drug safety and efficacy. I would even say, I advocate to use them in many cases instead of animals, as they may provide more informative

data in terms of their impacts on human health. Critically, they could be essential in terms of optimizing the conditions of the treatment - whether single dose or repeated doses.

Q11

Q: There have been a number of instances of pneumothorax in SARS-CoV-2 high dependency cases. Would there be clues on the nature of biophysical, biomechanical characterization?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

I think it would be difficult to study pneumothorax in this case as we are not looking at the whole organ. However, pneumothorax is generally caused by destruction of the lung, which then causes air escape from the lung into the chest wall. In the lung microphysiological system (MPS), we could detect this by looking for destruction of the tissue integrity, particularly by using techniques like immunohistochemistry (IHC) where we can see the cross section of the tissue - like the example I showed with poly(I:C) in the talk. This would be particularly powerful in the alveolar model, where we can

determine whether the alveolar-sac-like structures get destroyed. There are other ways to detect this sort of destruction in the lung MPS, using trans-epithelial electrical resistance (TEER) to measure barrier integrity, for example. When we use high concentrations of lentivirus or other more destructive methods, we can absolutely detect it using both IHC and TEER methods in our lab.

Q12

Q: Have you characterized single cell transcriptomics of the lung/endothelial cell model? If so, do you see large differences across gene expression just by adding/subtracting the flow system?

A: Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

Unfortunately, we have not characterized single cell transcriptomics. Since such experiments would be very valuable and can bring pivotal information about the model functionality, disease, and recovery, we would like to conduct them, and to do it we would be interested in developing collaboration with experts in this area.

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

No, we have not undertaken single cell transcriptomics in the lung models – but it's something we would like to do in the future to get a full picture of the model and how it compares to the human lung. As I showed in the presentation, we have performed qPCR analysis to demonstrate the presence of cell markers of key cell types (AT I / II markers in the alveolar model. Goblet, Club cell and ciliated epithelium markers in the bronchial model). This analysis showed an upregulation of these cell markers in the tissues cultured by the **PhysioMimix** system. Large differences in gene expression is also something we have seen and can quantify using microscopy. When we add further complexity to the model (e.g., via the incorporation of endothelial cells), we observe further upregulation of these cell markers, which is indicative of enhanced cell-cell communication and a more physiologically relevant phenotype.

Q13

Q: Have you successfully observed migration of immune cells from blood to airway side?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

This is something we are looking forward to doing in the future. At

the moment, the size of the Transwell® pores we use are 0.4 µm and therefore THP-1 cells cannot migrate through. Last year, we looked to purchase membranes with larger pores, however there were significant supply issues. Once available, we will purchase larger pore-sized membranes to determine immune cell migration into the apical side of the Transwell®. We are particularly interested to do these experiments with mixed population PBMCs. This should give us some interesting data on immune infiltration, particularly into the alveoli, with infection.

Q14

Q: What materials are MPS-T12 plates made of? Can plate materials absorb small molecule drugs?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

PhysioMimix consumable plates are made of COC (cyclic olefin copolymer) which is an amorphous thermoplastic. COC offers great thermic properties and represents the most inert plastic currently available for cell culture. The latter means that COC plates are less adsorbent than commonly used polydimethylsiloxane (PDMS) plates or chips. Experiments using COC plates are therefore less prone to drug loss from non-specific binding to the plate surface than those made of PDMS and, by default, more suited to detailed compound evaluation studies.

If you'd like to know more, please read: **van Midwoud et al**, Comparison of biocompatibility and adsorption properties of different plastics for advanced microfluidic cell and tissue culture models, 2012.

Q15

Q: From your TEER data in endothelial+epithelial models, it seems there is a synergic effect of both layers, i.e., the total resistance is higher than the sum of both. Any thoughts on why this is happening?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

The total resistance in the coculture is due to two things, the synergistic effect of two cell layers, plus the positive effect that endothelial cells have on the differentiation and survival of the epithelial cells (and vice versa). When we add endothelial cells, we see increased differentiation of the epithelial cells into physiologically relevant phenotypes (shown in the qPCR data – particularly alveolar

model). We also observe (although have not quantified) that the bronchial epithelium also becomes thicker when endothelial cells are present – perhaps also due to the positive differentiation into an even better pseudostratified epithelium. Together, both the dual cell layer and increased differentiation of the epithelium causes this increased TEER we see in the coculture.

Q16

Q: Why there is such a big difference in the structure of alveolar model once basolateral perfusion is added? Sheer force should not be involved due to the membrane - is this an effect of better oxygenation?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

This is a great question, and certainly something we are intrigued by ourselves! We believe that shear forces affect the cells through the Transwell membrane, after all the membrane is only 10 µm thick and porous. Fluidic flow modelling in our **PhysioMimix Barrier (MPS-T12) plates** also supports the suggestion that shear forces are present on the bottom of the Transwell. However, I also agree with your point that much of the extended differentiation and survival we see in the MPS is probably due to better oxygenation and flow of nutrients from the media. Again, going back to the previous question, I think including the endothelium on the basolateral side furthers these phenotypes as crosstalk between the cells can occur.

Q17

Q: The air sacs you describe in the alveolar model are 3D, are the alveolar cells embedded in a 3D matrix from beginning or do they assemble these sacs spontaneously at Air-Liquid Interphase (ALI)?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

The easy answer is no, we do not use 3D matrix/scaffolds in these models. Cells are plated directly onto Transwell® membranes and left to differentiate at ALI. The air sac-like structure formation is not solely due to ALI culture, although this certainly aids cell differentiation to an extent. We do not see any air sac-like formation in tissues cultured in static conditions, so therefore we attribute the formation of these structures to the sheer stress/additional oxygenation/nutrient availability effects that result from fluidic media flow (as controlled by the **PhysioMimix OOC System**).

Professor Wojciech Chrzanowski, Faculty of Medicine and Health,

University of Sydney.

Because we do not use any scaffold which encourages such assembly, cells initially form layers and then spontaneously form air sacs in some areas. While these sacs are assembled spontaneously, they are almost exclusively formed in the models that were treated with specific formulations.

Q18

Q: Can you examine O2 utilization in the flow layer?

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio & Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

Yes, it would be possible to embed an oxygen probe/sensor within the MPS and monitor the oxygen level. As the **PhysioMimix OOC system** has an open well, accessible format, this would be relatively simple to do. It is something we would like to include in future iterations of this model/**PhysioMimix Barrier (MPS-T12) plates**, where we could also include other internal probes, for example to measure barrier integrity (TEER). Doing so would allow us to learn more about the models in a highly quantitative, live and unobtrusive manner.

Q19

Q: What are you planning to do next with the models?

A: Professor Wojciech Chrzanowski, Faculty of Medicine and Health, University of Sydney.

Our next steps are: (1) the validation against animal models, (2) refining the quality control standard/process for the models, (3) testing 'conventional' drugs and correlating the results with clinical outcomes and literature, (4) testing toxicity of air pollutants (inhalation injury) on the models, and (4) development of SOPs for safety and efficacy assessment with the models. We also intend to develop models of different diseases; however, our current focus remains COPD. We have also some technical projects in the pipeline which aim to automate some of the processes and tests.

A: Dr Emily Richardson, Lead Scientist – Assay Development, CN Bio.

Now that we have validated our lung models for COVID-19 research, our internal focus has switched to the development of multi-organ lung-liver coculture models using PhysioMimix Dual-organ (**MPS-**

TL6) plates. Thus far this has been very successful, so we hope to share these results in the near future. We are also validating our lung models for use in drug evaluation studies, such as inhaled medication absorption and permeabilization through lung tissues. Later in the year, we plan to develop additional lung disease models that will enable researchers to better understand the mechanism of disease and test the efficacy of developmental drugs - as shown so brilliantly by Wojciech in his presentation. Together, we believe this range of single- and multi-organ lung assays will enable researchers to study a diverse spectrum of topics including basic lung biology, pathogen infection and preclinical drug safety/ efficacy testing.