CN-BIO

2022 WEBINAR SERIES

The Dash for NASH

How to Succeed in NASH Therapeutic Development

Q&A

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

Acronyms

ATP - Adenosine triphosphate

CYP - Cytochrome P450

KCs - Human Kupffer cells

IFA - Immunofluorescence assays

LSECs - Liver sinusoidal endothelial cells

NAFLD - Non-Alcoholic Fatty Liver Disease

NASH - Non-Alcoholic Steatohepatitis

OOC - Organ-on-a-chip

PDMS - Polydimethylsiloxane

PHHs - Primary human hepatocytes

HSCs - Primary human stellate cells

3D - There-dimensional

TGF-β - Tumor growth factor-beta

Q&A participants

Dr Audrey Dubourg

Product Manager, CN Bio.

Dr Ovidiu Novac

Senior Scientist, CN Bio.

Raul Silva

Scientist, CN Bio.

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: Does CN Bio plan on launching more disease model kits?

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

So, we are currently discussing the development of additional kits. Kits enable us to package up a healthy, or disease model so that customers (irrespective of experience) can get started quickly and easily. We have various projects to develop new **disease models** and applications for our **single- and multi-organ models**. Part of the process includes investigating how we enable **PhysioMimix™** users to adopt these in the most simple and effective manner, so stay tuned and watch out for new kits in the future!

Q2

Q: Have you tried to test progression of Non-Alcoholic Steatohepatitis (NASH) into cancer or to combine your existing NASH model with cancer cells?

A: Dr Ovidiu Novac, Senior Scientist, CN Bio.

This is a very good question. Fibrosis is key to the disease progression into severe NASH, cirrhosis and, if left untreated, liver cancer. Developing *in vitro* models that mimic the more advanced clinical phenotypes of the disease is therefore crucial, and it is something that we have recently started to investigate. For example, by adding cues such as tumor growth factor-beta (TGF-β), our 3D *in vitro* model can already progress to an advanced and more severe NASH stage with a quantifiable fibrosis phenotype (**Kostrzewski *et al*, 2021**), but I think we can go even further to reach a critical point where we generate, or trigger, hepatocellular cancer.

If you want to know more about how modeling fibrosis in our 3D *in vitro* liver MPS model can help understand disease progression and generate advanced NASH, watch our webinar: **Pathologically Scarred by Fibrosis**.

Q3

Q: Can you please describe how the 3D structure of the liver model is generated?

A: Raul Silva, Scientist, CN Bio.

The **PhysioMimix Multi-chip Liver plate** contains 12 wells, each containing an individual, enclosed recirculating perfusion system and a bespoke collagen-coated scaffold containing hundreds of microchannels in which primary liver cells (hepatocytes and non-parenchymal cells) are seeded to develop a liver microtissue. Microfluidics inside the plate are connected to and controlled by the **PhysioMimix OOC system**. They ensure that the scaffolds are continuously perfused by cell culture media. Put it simply, the microfluidics in the system mimic human blood flow to the liver and provide nutrients, oxygen, and biomechanical stimuli to all the cells within the three-dimensional (3D) structure to generate a **physiologically-relevant 3D liver microtissue**.

Q4

Q: Are you able to quantify the liver fat droplets?

A: Dr Ovidiu Novac, Senior Scientist, CN Bio.

Yes, we can. We use an automated imaging assay to quantify the number and the size of the liver fat droplets, so that we can further categorize them as macro- or micro-vesicles.

Q5

Q: Can you create a healthy liver model by incorporating more liver cell types?

A: Raul Silva, Scientist, CN Bio.

The short answer is yes but this really depends on what you are trying to achieve. When developing *in vitro* models, we have to assess which cell type is required for end application as it is currently impossible to fully recapitulate a human liver in a dish. For example, our standard healthy liver model, mainly used for **drug metabolism studies**, is comprised of a monoculture of primary human hepatocytes (PHHs). However, for studies requiring inflammation or other hepatic features, we will add non-parenchymal cells (NPCs) such as Kupffer or Stellate cells, or recirculating immune cells. A typical example of one of our coculture liver model, aside from NASH, is our PHH/Kupffer cells

(KCs) model used to investigate **drug-induced liver injury (DILI)**, in which KCs are added to add an inflammation element as they act as non-circulating hepatic macrophages.

If a more complex model is needed, for example a tri-culture with PHH/KCs and human stellate cells (HSCs), the nature of the stellate cells has to be accounted for. HSCs remain challenging to use *in vitro,* for studies other than **hepatic metabolic diseases**, due to their fibrotic profile. Once extracted from a donor, HSCs remain somewhat activated and can express some level of fibrosis even without any cues which may bias any investigation. Their pro-fibrotic phenotype can be further exacerbated when cultured on a plastic surface prior to their incorporation into a liver co-culture model. This is why we currently only include HSCs in our NASH assay, however, we are looking at ways to prevent their activation upon seeding to use them for more diverse applications.

Another frequently requested cell type is liver sinusoidal epithelial cells (LSECs) which we have yet to incorporate into our model. We are currently working on incorporating LSECs in our healthy and disease models to enhance their physiological relevance.

Q6

Q: Do you use PDMS in your Liver plates?

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

We do not use Polydimethylsiloxane (PDMS) in our Liver plates. This polymer is quite widely used but it has limitations. PDMS is a very cheap, easy to use cell culture material that can be turned into a very thin membrane. Because of this, it is frequently used - even within some Organ-on-a-chip (OOC) technologies. However, PDMS has very high non-specific binding properties, which poses a challenge for applications where drugs or other chemicals are being tested. When developing our **PhysioMimix™ OOC systems** and their **Multi-chip consumable plates**, we decided to move away from PDMS and to use Cyclic Olefin Copolymer (COC) instead. For cell culture applications, COC offers one of the lowest non-specific binding properties (**van Midwoud *et al*, 2012**). This ensures that we can confidently assess therapeutic compounds and report their pharmacokinetic and pharmacodynamic properties.

Q7

Q: Can NAFLD or steatosis only (rather than NASH) be recreated with your kit?

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

NAFLD, or steatosis only, can indeed be recreated in our model by using PHHs only. The **NASH-in-a-box kit** comes with PHHs, KCs and HSCs, but if someone wants to just recreate steatosis or Non-Alcoholic Fatty Liver Disease (NAFLD), they simply need to follow the supplied protocol, using PHHs only.

Q8

Q: Do you supply a healthy liver model as control in your kit?

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

We do not supply a healthy liver model in our kit for two reasons. Number one is that there is little interest in assessing the effect of an anti-NASH compound in a healthy tri-culture as only NASH-diagnosed patients are ever likely to take the treatment. The second reason is because the HSCs are always activated, even if thawed and seeded directly with the other cell types, there will always be a certain level of fibrosis, even in a healthy liver model. Therefore, there may be some bias in the assessment. However, if a healthy control is necessary for a study, it is entirely possible to coculture the three cell types, using the standard maintenance media used for triculture. Normalizing fibrosis levels to the control will be key to an accurate assessment of the drug's effects.

Q9

Q: Are you able to have HSCs activated just with the NASH media in the system and without TGF-β addition?

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

Stellate cells always have some level of activation, even when simply thawed and seeded. However, in this protocol, we increase their level of activation, and thus their pro-fibrotic phenotype, by first seeding them in a cell culture flask for a week prior to culturing with the other liver cell types. It is totally possible to observe fibrosis without using tumor growth factor-beta (TGF-β), as described in this webinar. Our **NAFLD/NASH models** offer the flexibility to develop early-stage steatosis through to advanced and severe fibrosis, depending on the cell types and cues added to the milieu.

Q10

Q: Are all the cells from a same donor?

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

In an ideal world, this would be the case. Unfortunately, it is still challenging to access/provide all three cell types from the same donor. This is not something we can offer at the moment. However, we do thoroughly test and validate each cell type (using the PhysioMimix OOC) prior to supplying them in the kit. PHHs are first validated alone to verify 3D tissue formation and adequate tissue function (albumin, urea, CYP3A4, etc.). Once performance in monoculture has been established, PHHs are subsequently tested with KCs and HSCs under NASH conditions to ensure good cell-cell interaction and disease phenotype generation in the **PhysioMimix Multi-chip Liver plate**, using our bespoke NASH media.

Q11

Q: How do you maintain shear stress?

A: Raul Silva, Scientist, CN Bio.

Shear stress is driven by the microfluidics integrated in the **PhysioMimix Multi-chip Liver plate**. The base of the plate contains micropumps which continuously drive the circular flow of media inside each well. Flow rates can be adjusted via the controller's touchscreen to either increase or reduce shear stress to the microtissues.

Q12

Q: Which cell lines were used to create this model?

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

To ensure that we recreate an *in vitro* model that is as human relevant as possible, cell lines (also known as immortalized cells), are not used in this model. Instead, we use primary cells to generate physiologically relevant liver tissues and ensure an accurate recapitulation of the disease phenotype. We source our primary cells form various vendors and offer them for purchase individually as **PhysioMimix 3D validated cells**, or within our **PhysioMimix NASH-in-a-box kit**, which also contains everything required to recreate our industry-proven NASH assay in your own laboratory.

Q13

Q: Can one harvest the cells for downstream analysis?

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

Yes, it is entirely possible to access liver tissue cells at the end of the experiment. Being rather big, the scaffold on which cells are seeded can be cut in half. One half of the recovered microtissue can be used for -omics or adenosine triphosphate (ATP) content quantification, and the other half can be fixed for immunofluorescence assays (IFA) using confocal microscopy. By combining, for example, gene expression (RNA sequencing) and protein expression (IFA), this enables an accurate investigation of the disease and an anti-NASH compounds' mechanistic effect on the liver tissue.

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

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