CN-BIO

2021 WEBINAR SERIES

Pathologically Scarred by Fibrosis

How to Model and Quantify Human NASH in a Microphysiological System

Q&A

A full run down of questions & answers from our April 27th webinar

Abbreviations

2D: Two dimensional

3D: Three dimensional

ASO: Antisense oligonucleotide

DEGs: Differentially expressed genes

HSC: Human Stellate cell

iPSC: Induced-pluripotent stem cell

LPS: Lipopolysaccharide

MPS: Microphysiological system

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

NPC: Non-parenchymal cell

PHH: Primary human hepatocytes

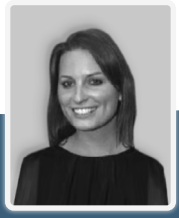
PNPLA3: Patatin-like phospholipase domain containing 3

QC: Quality control

siRNA: Small interfering RNA

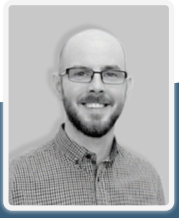
SNPs: Single nucleotide polymorphisms

Q&A participants



Dr Samantha Peel

Principal Scientist
AstraZeneca



Dr Gareth Guinegault

Senior 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: What are the advantages of the CN Bio microphysiological system model over spheroid models of fibrosis?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

Spheroids are a very good system to study fibrosis, better than 2D immortalised line model. However, as good as they are, spheroids models are still limited in terms of scale and longevity.

Compared to spheroids, our NAFLD/NASH microphysiological system (MPS) model enables many more endpoints to be measured per replicate, thanks to the large numbers of cells used to create healthy and diseased 3D liver microtissues (over 600,000 cells per well) and much larger volume of media (1 mL) from which to sample. For example, scaffolds containing the 3D liver microtissues can be cut in half; one half can be used for microscopy while the other is used for -omics analysis e.g.; RNAseq for transcriptomics. The large sampling volume allows for the analysis of various soluble biomarkers, from cell health (LDH, albumin, ALT/AST) to cell function (CYP activity), inflammation (IL-6, TNF-α, etc.) and fibrosis (TIMP1, Fibronectin, etc.) (**Kostrzewski et al, 2019**).

Large scaling isn't the only benefit of our model over standard spheroid models. Use of media flow in the **PhysioMimix™ system** enables healthy (and diseased) liver tissue to remain functionally and metabolically active for over 4 weeks, much longer than is possible when culturing under static conditions. A recent publication from the FDA demonstrates the benefits to assay performance of culturing using flow versus gold standard spheroid and liver sandwich approaches (**Rubiano et al, 2020**).

Q2

Q: What are CN Bio's future plans with this technology? For example, are there plans to increase the scale from 12 wells?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

Up to 6 plates can be run at the same time using 1 PhysioMimix Controller, i.e. a total of 72 conditions. Compared "like for like" to technologies who use microfluidic flow (pneumatics that permit

organ-specific tuning) instead of gravity-driven flow, we provide a higher throughput. Increasing the throughput for our 3D liver model is part of our roadmap, however, currently, our efforts have been focussed on developing our **new Multi-organ platform** and new organ models, such as **gut-liver** and **lung**.

Q3

Q: Where do you source the human primary cells from? Have you used different donors? Do you see much variation in the level of fibrosis induction between donors?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

When developing *in vitro* models, each cell lot/donor will react differently to the environment/conditions used. Some may do really well in 2D, under static conditions (e.g.: monolayer, liver sandwich or spheroid) but perform poorly under flow in a microphysiological system (MPS), or vice-versa. To develop the best model for your research, it is therefore important to source and test a variety of donors. We source our cells from a wide range of large cell suppliers such as Lonza, ThermoFisher and BioIVT, thoroughly testing each cell lot/donor in our PhysioMimix system to identify the best donors for our disease models. To save time and effort, customers can access CN Bio validated cell lots (i.e. those that have been internally tested and proven to generate healthy, functional 3D liver tissues) through our Cell Supply Service. If you'd like to know more, please contact us at **enquiries@cn-bio.com**.

Although using the same donor each time to generate data with consistency is ideal, it is impossible to guarantee as stocks of donors will run out at some point. As such, it is also important to observe donor-donor variability to gain a better understanding of variations in metabolism between patients. Therefore, when starting a project, we tend to use the same donor for a specific set of experiments to ensure consistency and build confidence in the data. Then, we will use a different donor, or more, to assess donor-donor variability; this will greatly depend on the application and the question asked in the project.

Donor variability can often be observed in our model; however, we generally see a trend between donors. For example, when inducing severe fibrosis (by adding cues such as TGF-□), although the severity of fibrosis varies between donors, an increase in fibrosis is always observed.

Q4

Q: Is it possible to image the tissues live?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

The current set-up of our PhysioMimix system does not allow for live microscopy due to the pneumatics that are built in to the bottom of our **consumable plates** to drive microfluidic flow inside the plates. However, we are currently working on a solution that will enable live microscopy in our system.

Q5

Q: You show modulation of fibrosis via small molecule inhibition, have you tried any form of genetic modulation of the microtissues via CRISPR (for example) or via siRNA/ antisense?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

We have used and adapted various genetic modulation methods in our system. We have looked at key single nucleotide polymorphisms (SNPs), for example, knocked-out patatin-like phospholipase domain containing 3 (PNPLA3) in Human Stellate cells (HSCs). In this study we confirmed that mutated SNPs on PNPLA3 gene in HSC enhanced the overall NASH disease state (**Kostrzewski et al, 2019**).

We have also successfully used small interfering RNA (siRNA) and antisense oligonucleotides (ASOs), both of which were shown to be effective in our model.

Q6

Q: What combination of these added components gives the most NASH-like phenotype?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

When comparing cues/components, we look at expression profile using transcriptomics/RNA seq and compare the data to that of NASH patients to define the overlap. Although all cues are very good and show great NASH-like phenotype, TGF-β drives the NASH phenotype a little bit further than the other components. -

Depending on what specifically researchers want to do with the model, adding different cues to subtly change the profile is possible. For example, if an inflammatory response is needed, lipopolysaccharide (LPS) is a very component to add. Which cue to add at what dose greatly depends on the question being asked.

Q7

Q: For the image analysis, do you treat each of the circular structures as an individual data point, or is it intensity across the entire sample?

A: Dr Samantha Peel, Principal Scientist, AstraZeneca:

To obtain an intensity value for the whole tissue in each sample, we capture 8 filter views for each microtissue/scaffold. Each one of these filter views contains several of the circular structures (microchannels within the collagen-coated scaffold in which cells are seeded in a 3D set-up within the **PhysioMimix MPS-LC12 plate**).

We then calculate the fluorescence intensity of each microchannel and average that across the filter view. We can get a separate value for each filter view and average those 8 filter views to get an intensity value that is representative of the microtissue as a whole.

Q8

Q: Have you tried normalising the mean intensity of the fibrosis markers to anything (such as phalloidin or Hoescht)?

A: Dr Samantha Peel, Principal Scientist, AstraZeneca:

For the standard assay described here, we have not normalised the data but this is definitely something to consider. We track the area of the microtissue, which is really important if, for example, a compound totally destroyed the tissue due to a non-specific toxicity effect. This would affect the level of collagen deposition but not necessarily have anything to do with the pathway of interest. If this has happened, we can therefore flag the compound and change the total area of the tissue that is captured for analysis. We have images of tissues so we can QC them and observe whether the tissues look unusual. We can then look for common toxicity phenotypes that may be appearing. These toxicity phenotypes could potentially be incorporated into the image analysis to be automatically detected.

Q9

Q: Is this confocal image analysis available as a readout for studies performed as a service at CNBio?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

Confocal imaging analysis is available for all our models and is frequently used for our NASH service. The method established and demonstrated in this webinar has been modified to be used by CN Bio without Astra Zeneca's equipment but it does give similar end results.

Q10

Q: How are NAFLD genes defined on the 'Modeling NASH - RNAseq' slide? Are inflammatory cytokines not included among them?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

The NAFLD genes were defined using published meta-analysis o f multiple data sets comparing obese and NAFLD patients and identifying differentially expressed genes (DEGs) that were consistently altered. These include genes from multiple pathways so may include inflammatory cytokines.

Q11

Q: Has the model been created with iPS-derived hepatocytes instead of PHH?

A: From Dr Gareth Guinegault, Senior Scientist, CN Bio:

Our primary focus has always been to use primary cells to be as close to the human liver's physiology as possible. However, there is a growing interest in developing iPS-derived hepatocytes models, especially for researchers who cannot easily access primary cells. We have successfully developed a monoculture liver model with iPS-derived hepatocytes, however this model has yet to be used to replicate a disease state or be used in a co-culture with other non-parenchymal cells (NPCs)

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