CN-BIO

AUTUMN WEBINAR SERIES

A Microphysiological Model of Metastatic Progression

Q&A

A full run down of questions & answers from our November 3rd webinar

Q&A participants

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Abbreviations

2D - Two-dimensional

3D - Three-dimensional

EdU - 5-ethynyl-2'-deoxyridine

MPS - Microphysiological system

OOC - Organ-On-A-Chip

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: Thank you for a nice presentation. I am wondering if you investigated the role of cyclic dosing on your quiescent cells and chemotherapeutic resistant cells?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

In the experiments described, tumour cells were exposed to two consecutive rounds of chemotherapy over a 72-hour period. No break was given between the two doses.

Q2

Q: What investigative method did you use to track quiescent cells which re-entered the proliferation status again?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

We tracked quiescence by harvesting scaffolds prior to treatment (day seven), three days after treatment had ceased (this was also immediately prior to stimulation; day 13) and two days after cells re-entered the cell cycle (day 15). During each time point we tracked active proliferation through incorporation of 5-ethynyl-2'-deoxyridine (EdU). Depending on the experiment, tumour cells were exposed to EdU for 48 up to 96 hours.

Q3

Q: Very interesting! Were these studies performed under normoxic conditions (for liver)? Do you have any information on the impact of fluctuations in oxygenation on dormancy?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

All studies presented were performed under normoxic liver conditions. At this time, we have not fluctuated oxygenation levels in our experimental set-up.

Q4

Q: Are metastatic tissues less stiff than primary cancer tissues?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

A comparative study across all tumour types and associated metastatic lesions has not been conducted. However, with respect to colorectal cancer, tissue stiffness has been found to be higher in the liver metastases compared to the primary tumour.

Q5

Q: Are there any differences between metastatic tissue fibroblasts and primary site fibroblasts?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

The source of cancer-associated fibroblasts found in metastatic sites is still a controversial question. There are those who propose that metastatic tumour cells co-operate and rebuild their specialized cancer-associated fibroblasts compartment, while others suggest the interesting possibility that tumour cells may bring their fibroblasts from the primary site along with them.

Q6

Q: What microenvironmental gradients did you investigate while studying bidirectional crosstalk between parenchymal cells?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

Within the Legacy LiverChip®, we have investigated the impact of co-culturing donor matched non-parenchymal cells with parenchymal cultures (hepatocyte alone) on soluble signalling crosstalk (77 different cytokines, chemokines and growth factors were assessed). We also looked at these differences in the standard high impact polystyrene and the hydrogel scaffolds.

Q7

Q: When you were sourcing the right liver-on-a-chip model for your studies, what key features did you look for?

A: From Dr Amanda Clark, Research Assistant Professor, University

of Pittsburgh

When researching a liver-on-a-chip model which could meet our research needs, we looked at the following key features as being essential for the development our metastatic model:

1. **Cellular composition:** support cultures comprising the full complement of resident hepatic cells (both parenchymal and non-parenchymal cells) to accurately recapitulate liver functioning as well as cell-cell and signalling interactions
2. **Microfluidics:** controllable and tuneable fluid flow for physiologically relevant shear forces, oxygenation and extended culture
3. **Scaffolding or matrices:** enable the creation of 3D, multicellular tissue and promote self-organization
4. **Readouts:** The ability to examine an array of biological and pharmacological information (e.g. genomic, proteomic, phenotypic, metabolic, therapeutic efficacy indices etc.)

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

Physiologically relevant shear forces, oxygenation and the *in vitro* recapitulation of liver functions are critical factors to consider when building a liver-on-a-chip model. The ability to fine tune microfluidics to replicate organ-specific blood flow is also necessary for more *in vivo*-like 3D liver microtissue creation. For more information about the impact of flow on *in vitro* liver model recreation, watch our on-demand webinar: **The Rhythm of Life**.

Q8

Q: What system requirements are critical when developing a 3D liver model for studying metastatic diseases?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

In addition to the features described in Question 7, a metastatic disease model also requires:

- **Cellular composition:** a full complement of parenchymal, stromal and immune cells is crucial as metastatic tumour behaviour and therapeutic sensitivity are strongly influenced by the bidirectional relationship that exists between them and all the cellular components of the metastatic microenvironment.

- **Prolonged culturing:** for investigations into dormancy and recurrence, it is essential that models remain viable and functional for weeks out to months. This is predominantly achieved through active fluidic flow, which provides a continuous source of oxygen, nutrients and shear stress similar to the liver's *in vivo* environment.
- **Real-time manipulation:** the ability to seed in tumour cells after the hepatic tissue has been established. Many systems directly co-culture metastatic tumour cells and resident tissue cells; however, this is not reflective of the human situation. Additionally, the ability to therapeutically treat, stimulate and introduce molecular probes into the system at any given time is essential when investigating such a dynamic disease.
- **Specimen collection and assays:**
  - Imaging: must be able to stain and image the tissue at high and low magnifications to discern tumour burden, cell-cell interactions and therapeutic efficacy.
  - Effluent analyses: sufficient effluent samplings to assess soluble signals, isolate exosomes, determine viability and measure functionality.
  - Protein and genomic analyses: harvest tissue to obtain RNA or protein in order to identify differentially regulated genes or protein expression.

Q9

Q: Where can I find information on the Legacy LiverChip® system you mentioned?

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

The Legacy LiverChip® system has now been superseded by our **PhysioMimix™ OOC Lab-benchtop Ready Microphysiological System** which features the same functionalities as the Legacy LiverChip® but is more user-friendly, easy to adopt and flexible in terms of cell type and format.

For more information about our PhysioMimix™ OOC system, our **animated video** and our short on-demand webinar: "**An Introduction to Organ-on-a-Chip – The Future of Pre-Clinical Drug Research**" provide a great overview. Do not hesitate to contact us to discuss how the human-relevant insights achievable though organ-on-a-chip solutions can benefit your current workflows.

Q10

Q: How long can this model be cultured for when studying metastatic diseases? Have you run long-term studies?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

We have extended the culture period out to 29 days during which the tissue remained functional and viable. In the long-term studies, we retreated emergent tumour cells with a second round of chemotherapy (either the same concentration of doxorubicin or a different therapy, such as cisplatin, at different concentrations). We found that the secondary treatment with a different chemotherapy was more effective than the original, which is reflective of the response observed in patients.

Q11

Q: How easy is it to work with exosomes in this liver-on-a-chip model?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

Working with exosomes in the Legacy LiverChip® model is comparable to 2D studies. Moreover, in our hands, we are able to isolate sufficient exosomes for additional downstream experiments from a single well of the liver-on-a-chip model.

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

We have developed our system with an open-well consumable plate to allow for large scale sampling (volumes and tissues). The idea was to keep the same set-up as standard 2D cell culture in a more complex microfluidic plate which enables the culture of long-term 3D liver tissue with physiologically relevant conserved phenotype and functions.

Note. Our Legacy system has been superseded by our PhysioMimix™ OOC system which is very easy to install and use. To know more about our PhysioMimix™ OOC system, watch our **animated video**.

Q12

Q: When working with hydrogel, did you use the same MPS system as the standard scaffold used in your previous data?

A: From Dr Amanda Clark, Research Assistant Professor, University of Pittsburgh

Yes, the same Legacy LiverChip® model was utilized for these studies. We exchanged the standard high impact polystyrene scaffold for the hydrogel. All components remained the same except for the scaffolding material. We collaborated with **Prof. Linda Griffith's** laboratory at MIT to generate the hydrogels.

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

Thanks to the open-well set-up of our consumable MPS plates, scaffolds and other support can easily be integrated into our platform. The consumable plates supplied with the PhysioMimix™ OOC system have the same specifications and features as the Legacy LiverChip® consumable plates, however, **PhysioMimix™ Consumable plates** are supplied fully built, sterilised and ready-to-use.

Other episodes in our Autumn webinar series

22nd Sept

The Rhythm of Life

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

Dr Graham Broder & Alysha Bray

Associate Director - Bio Engineering & Scientist, CN Bio

Watch on-demand here

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

Watch on-demand here

Don't miss the final webinar in this series

24th Nov

Engineering Mucosal Barriers

From Organoids to Organs-on-Chips

Prof Linda Griffith

Massachusetts Institute of Technology (MIT)

Register here

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