CN-BIO

SUMMER WEBINAR SERIES

Go with the flow

Application of Microfluidic 3D Liver Chip Models for Genotoxicity Testing

Q&A

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

Abbreviations

GLP - Good laboratory practices

3D - Three dimensional

OOC - Organ-on-chip

MPS - Microphysiological system

ADME - Absorption, Distribution, Metabolism and Elimination

NGS - Next generation sequencing

HPLB - Human peripheral blood lymphocytes

Q&A participants

Dr Renato Cardoso

Scientific Director of Genetic Toxicology

Charles River Laboratories (Montreal - Canada)

Dr Thalita Zanoni

Senior Research Scientist

Charles River Laboratories (Montreal - Canada)

Dr Audrey Dubourg

Product Manager

CN Bio

Another question?

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

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Questions

Q1

Q: How does setup differ between liver sandwich, open liver chip and closed liver chip?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

The liver sandwich model is a prototype that we created to check whether the metabolic activity of hepatocytes would increase due to interactions with other cell types. Because this liver sandwich model does not incorporate flow, which can lead to a loss of metabolic activity, we subsequently decided to develop and test liver models using different organ-on-chip technologies that incorporate microfluidics. Both the open and closed liver-on-a-chip models we tested work similarly in terms of flow, the only major difference is that the open liver-on-a-chip model enables the culture of more cells, which is better suited to our needs.

Q2

Q: How do you monitor the cellular layer intactness of 2D or 3D models?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

The T12 plate allows us to visualize cell morphology under the microscope which enables us to assess the cell health of the hepatocytes. When building the liver-on-a-chip model, endothelial cells and hepatocytes were seeded separately within a 24-hour window to ensure optimum cell adhesion and health. In addition, other parameters, such as albumin, urea, CYP activity and cell viability, were also evaluated to ensure that the cellular layers in the models were intact and fully functional.

Q3

Q: Apart from albumin and urea what other parameters have you investigated?

A: From Dr Thalita Zanoni, Senior Research Scientist, and Dr Renato Cardoso, Scientific Director of Genetic Toxicology, Charles River Laboratories:

We have tested the metabolic activity of the culture by measuring the expression of several CYP enzymes. Those results are still very preliminary but show great promise. We plan to test more CYP enzymes to generate a CYP induction metabolic profile and look at the morphology of cultures using H&E staining.

We are, however, more interested in amplifying pro-mutagen dosing to test how effective the liver-on-a-chip system is, as well as looking into specific genotoxicity applications to assess if cultures are able to metabolise pro-mutagen in the system.

Q4

Q: Have you used immune cells in the liver-on-a-chip platform?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Our current goal is to keep our liver-on-a-chip model as simple as possible whilst meeting our genetic toxicology testing needs. Therefore, we do not plan to include any cells into our model that are currently not required for genetic toxicology testing. However, we are confident that adding immune cells to the liver-on-a-chip model could easily be done and would be a great additional feature for immunological testing.

Q5

Q: Why did you use a TK6 cell overlay?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

TK6 cells are human-derived lymphoblastoid cells which are p53 competent. Since they are proliferative cells, they are routinely used for genetic toxicology experiments and represent a good alternative for the *in vitro* micronucleus assay. The inclusion of TK6 cells into our co-culture system was important because it allowed us to perform two distinct genetic toxicology assays within the one model. The two assay tests performed in this co-culture system were the micronucleus assay (performed in TK6 cells) and the comet assay (performed in the liver tissue within the liver-on-a-chip model).

Q6

Q: How long was the exposure period for these samples?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

We followed the dosing period used in standard *in vivo* genotoxicity studies. We dosed cultures at 0, 24 and 45 hours and took cultures down at 48 hours. Collecting cells at the 48-hour endpoint is critical for assessing the true extent of DNA damage caused by pro-mutagen exposure since it lowers the chances of cell repair post-dosing.

Q7

Q: Do you typically compare and validate *in vitro* results with mouse assays or human clinical data?

A: From Dr Renato Cardoso, Scientific Director of Genetic Toxicology, Charles River Laboratories:

As there is still a lot of groundwork to be done validating these models, we do not compare *in vitro* results to human clinical data only. Currently we compare and validate our *in vitro* results to *in vivo* animal models and the literature. For now, clinical data could be used to verify negative results and to eliminate any potential false positives derived using the *in vitro* models.

Q8

Q: Why did you choose the specific media for hepatocytes? Is that media suitable for endothelial cells too?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Whilst developing our co-culture model, we performed a series of test to assess the survival and health of endothelial cells in hepatocyte-specific media. From those data, we concluded that endothelial cells survive and settle well in the chosen hepatocyte media.

Q9

Q: Do you see any constrains in microfluidic devices? What do you think should be improved to create good microfluidic liver models?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

When kicking-off this project, we tested several microfluidic systems to assess which would be best for our research needs. We opted for the open-well liver-on-a-chip platform because the system is very easy to use compared to the other technologies tested. We did not meet any major constrains when building our model. By comparing the model with and without flow, we concluded that flow improved the detection of false-positive controls.

Q10

Q: How do you recommend balancing the use of cells like HepaRG compared to primary hepatocytes? Do you feel HepaRG cells are sufficiently predictive?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

According to the latest literature, HepaRG can be a useful cell line to predict metabolization in the liver, however, we hypothesized that primary hepatocytes would provide a more predictive tool.

Although we did obtain good results with HepaRG cells, we obtained better datasets using primary hepatocytes. In the open liver-on-a-chip model, we also observed a greater detection of positive controls and results that were in line with those reported in genotoxicity publications.

Q11

Q: Have you tried doing DILI tests using the chip and if so, how do you determine IC_{50}?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

To date, we have not yet used our liver-on-a-chip models to assess DILI. However, since cells can be detached easily and with high viability, calculating an IC_{50} using the liver-on-a-chip model should not be difficult.

Q12

Q: Is live cell imaging possible in microfluidic systems?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Yes. The cover from plate is detachable.

Q13

Q: How do you stain the cells in microfluidic systems?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Depending on the microfluidic systems, cells could be stained within the liver-on-a-chip platform directly or cells are transferred into a standard 24-well plate to be stained.

From Dr Audrey Dubourg, Product Manager, CN Bio:

In our PhysioMimix™ OOC platform, cells are seeded on removable inserts that can easily be taken out of the plate for staining, immunostaining or to extract genetic material. Immunostaining of cells in the multi-layer co-culture can be performed by following an adapted version of any standard SOP.

Q14

Q: Have you tested the difference between the HepaRGs and primary hepatocytes in the other models (e.g. Spheroids)?

A: From Dr Thalita Zanoni, Senior Research Scientist, and Dr Renato Cardoso, Scientific Director of Genetic Toxicology, Charles River Laboratories:

We have not. The principle of the test would be the same so we decided to move on to the other models to develop and test.

Spheroids are great and deliver valuable datasets, depending on the applications you use them for. For the tests we use, spheroids are too convoluted to get optimum data. We decided to move away from spheroids, not because they do not work but because the open liver-on-a-chip model was better suited to the purposes of our research.

Q15

Q: Is % Hedgehog a significant index for the Comet Assay?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

The % Hedgehog obtained in our results showed acceptable values within the normal excepted range.

Q16

Q: Since there is a current focus on decreasing animal use in science, I would like to know if your department offers other 3D model assays that could serve as an alternative to animal testing?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Yes, within our department this is one of our current research interests.

We have recently developed 3D skin reconstruct assays and now offer a GLP format 3D skin comet assay. In addition, we will soon be offering the micronucleus assay in reconstructed epidermal equivalents.

Q17

Q: I really like the idea of creating one assay that is capable of evaluating 2 different genotoxic endpoints. Do you see the possibility of integrating the liver chip with other cells/tissues?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

The main advantage of this liver-chip platform is that the system can be used to co-culture a wide variety of cells and tissues which expands the range of applications it can be used for.

Q18

Q: Could this liver chip be applied to other purposes other than genotoxicity testing?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Yes, absolutely. The liver chip could be applied for various types of *in vitro* testing.

From Dr Audrey Dubourg, Product Manager, CN Bio:

As Thalita mentioned, our PhysioMimix™ OOC Microphysiological (MPS) platform is flexible, enabling scientists to adapt it to meet their research needs. Our customers and collaborators use the platform for various applications such as disease modelling, ADME, Pharmacology, immuno-oncology and many more.

Q19

Q: What effect would this liver chip have on drug development efforts in the pharmaceutical sector?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

We are currently still in the validation phase of this liver-on-a-chip model; therefore, this will depend on the upcoming results we will obtain. But we are optimistic.

If this approach works, we believe that these liver-on-a-chip models could be useful tools to predict metabolism more efficiently in pre-clinical studies and therefore decrease animal usage going forward.

Q20

Q: Will this liver-chip model be available commercially?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Our main goal is to develop fully validated liver-on-a-chip models that are commercially accessible to our customers. Our current objective, however, is to continue with the validation studies to prove the efficacy of this model before developing the offering. This model should be available commercially soon. In the meantime, due to several requests from different companies, we can offer customised models.

Q21

Q: What first attracted you to the CN Bio system? How do you view CN Bio technology compared with other Organ-on-chip technologies?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Having tested several microfluidic systems in house, we liked the fact that CN Bio system comes in an open plate format. Furthermore, the cost per culture was also attractive and the system seemed simple to use.

Q22

Q: How challenging was it to start off your 3D liver models?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

It looks much harder than it is. Our scientist had experience with other 3D models so it was straight forward for us to get started.

Q23

Q: If you were to develop this 3D liver model further to be more *in vivo*-like, what would you consider adding or modifying?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

We do acknowledge that incorporating other cells into the system (ie. Stellate, Kupffer cells etc) could result in a more *in vivo*-like liver-on-a-chip model. However, it also all depends on what you are trying to achieve.

Right now, if we prove that metabolization is efficient within our developed co-culture model and that it allows us to recognize mutagens and pro-mutagens, there would be no reason to further elaborate. Furthermore, we have performed some preliminary testing using next-generation sequencing (NGS) but this is not a priority to investigate right now.

Q24

Q: Would you consider adding any non-parenchymal cells in your model?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

Compared to the number of hepatocytes, the number of non-parenchymal cells in a liver is relatively low. Nevertheless, incorporating some of these cells for other specific evaluation would be a good idea.

Q25

Q: Do you plan on using other immune cells than TK6?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

TK6 cells are not immune cells but are derived from a human B lymphoblastoid cell line. The main benefits of TK6 cells are that they are p53 proficient and karyotypically stable. One of the advantages of using TK6 cells for micronucleus assay is that they are proliferative; therefore, eliminating donor to donor variability seen while using human peripheral blood lymphocytes (HPBL). They also reduce the percentage of non-relevant positive results compared to p53-mutated cell lines.

Q26

Q: What is the next step for your 3D liver model?

A: From Dr Thalita Zanoni, Senior Research Scientist, Charles River Laboratories:

We aim to further expand the number of pro-mutagens and mutagens tested in the system. We also intend to amplify the testing of CYP450 enzymes which are key players in drug metabolism and prodrug activation.

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