Q&A Webinar Organ-on-a-chip March 9, 2022

Questions related to the Intestinal Explant Barrier Chip

Have you ever tested the difference in the transport results when two or more different pieces gut tissues are tested? How many replicates are needed to obtain reliable results?

We have tested donor-to-donor variation with regards to transport of drugs and nutrients, and we do observe interdonor variations reflecting patient-to-patient variation. Intraexperimental variation is rather low, but always slightly higher compared to single-cell model like Caco-2 due to biological variation. We therefore always advice to include n=4/test condition to ascertain statistical power.

I was wondering how do you keep the intestinal explant in place and how do you make sure that there is no "leakage" from the sides?

This is exactly where technology meets biology ! Together with engineers, TNO has designed the molds for static InTESTine and microfluidic chips for Intestinal Explant Barrier Chip (IEBC), in which the tissue segments are mechanically fixed. In each incubation we include markers to not only guarantee tissue functionality (paracellular transport and transcellular transport, LDH, ATP), but also proper mounting of the tissue and barrier integrity (e.g. FITC-Dextran 4kD: FD4).

For how long is the intestine explants viable in the intestine chip?

In most of the experiments we have performed we only included time points up to 24h, without depriving the tissue quality and functionality. Currently we are performing experiments up to 72h of incubation, after which the intestinal tissue still is functioning comparable to earlier time points.

Where is the outlet in your explant barrier chip system?

The apical (luminal) and basolateral (blood side) media are recirculating in the design of the IEBC, so the outlet is directly after the intestinal tissue segment.

How long can you keep the intestine in this system? Especially when looking at inflammatory responses/more longer term responses, have you compared this to the in vivo situation?

You are now comparing to caco-2 cells, have you tried intestinal organoids?

In the InTESTine system (static) we can study intestinal processes for a period of maximal 6h, in the IEBC we can determine intestinal processes for at least 24h (preliminary data show that we can increase to 72h of incubation). This indeed opens opportunities to study intestinal processes that require more time to start, e.g. immunological responses or ROS production, in line with clinical data (for example we have shown the anti-inflammatory properties of sulfasalazine in the combined microbiome-intestine platform).

Intestinal organoids are also available, which can be cultured for at least a week as a barrier model, mainly to be applied to study interindividual and interpopulation variations in drugs absorption and metabolism. For immunological responses we have not tested the opportunities yet.

How do you get rid of the mucosal microbiota when stimulating the inTESTine with LGG or Klebsiella without disturbing the mucus layer?
The intestinal tissue that is being used in the model is flushed and washed with ice-cold storage buffer to get rid of access of mucus and luminal content. Upon including intestinal tissue segments in either in InTESTine or IEBC, access of mucus is removed before applying the dose solution (e.g including pathogenic or probiotic bacteria) in the apical compartment. Endogenous microbiota from the patient (human material) or animal (pig, dog, rat, chicken) that remain in the firm mucus layer in close contact with epithelial tissue is left undisturbed.

Can you provide some information on microchip fabrication? Is PDMS used to fabricate the chips? If yes, PDMS could absorb drugs, did this affect your results? Are you looking for more developed material to fabricate microchip?
When TNO started its work on organ-on-a-chip models we were amazed by the fact that PDMS is that often used exactly because of the reasons you mentioned in your question. As our ultimate goal is to have models used in compound testing we decided right from the beginning to not use PDMS in our model systems. We make use of in-house developed 3D printable material (BI-OC) that is inert and biocompatible with cells and tissues, and has similar binding profiles to standard cell culture plastics.

Questions related to the liver-on-a-chip

Did you test a time-response and saw a maximal collagen deposition/production?
We did indeed do a time response. Some background: we prefer to correct collagen deposition for total cellular protein. Of course the more cells you have, the more collagen can be produced. Earlier time points (days 1 – 2 after start stimulation) give a limited degree of collagen deposition, both when you correct for total protein or not, despite high levels of collagen mRNA transcription. At day 4 after start of stimulation with TGF-β we typically reach a plateau. In absolute values collagen deposition will still increase somewhat after day 4, but not when you correct for total protein. After day 8 the stellate cells start to detach, so that is a clear end point.

What was the method for total collagen measurement? A hydroxyproline assay?
That is correct, we use a hydroxyproline-based assay to measure the total collagen levels. This assay was developed in-house at TNO but is commercially available via QuickZyme Biosciences.

What are the validation parameters for the 3d model? Gene expression markers, albumin by hepatocytes, cytochrome genes?
We can use a large set of markers to examine functionality of the different cell types or to monitor disease induction. Among them are indeed measurement of human albumin levels in the medium and functional assays for CYP enzymes for hepatocyte functionality. Concerning the last one, we typically monitor CYP3A4 activity. Another marker would be urea levels in the medium as a read-out for protein metabolism. These are great ways to monitor hepatocyte functionality during the study. We also determine expression of markers of hepatocyte functionality by immunostaining (mostly IF). Markers we use there are A1AT, HNF4α and MRP2. But of course, immunostainings are end point measurements, so not suitable for monitoring purposes.

Why don't you combine the steatotic medium and the fibrotic medium from day 4?
The fibrotic medium indeed contains the same levels of fructose and fatty acids as the steatotic medium, but it also contains additional components to stimulate fibrogenesis and to mimic the NASH stage.

I might have missed this, but what is the fibrotic medium? TGF-b is included?
The fibrotic medium for the 3D model contains among others immunomodulatory components, such as a low dose of LPS, but also high fatty acid and fructose levels. In contrast to medium for the 2D stellate cell model, the fibrotic medium for the 3D model does not contain recombinant TGF-β.

Is your liver model also applicable or adjustable to other liver diseases than NASH?
When we started to work on our 3D liver model we chose to focus on NASH and liver fibrosis, as that is our primary area of interest of my research team. But indeed we are working on expanding the use of the model for other liver diseases. One important example is the coupling of our gut and liver platforms for use in ADME / drug metabolism studies. This would be an ideal setting to introduce diabetic or hyperlipidemic conditions and to determine possible effects of (subclinical) liver disease on drug metabolism.