CamBioScience Liver Organoids Course – Homerton College, Cambridge, 1-2 July 2019

Human primary liver cancer-derived organoid in vitro (taken from Broutier *et al*. 2017)

History of organoids

Early organ culture in vitro

In 1907 Henry Van Wilson sparked interest in the creation of organs through a series of dissociation-re-aggregation experiments from which he discovered that dissociated sponge cells could re-aggregate to form a whole organism in vitro. Following these findings, multiple labs went on to create 'mini-organs' applying the same method using different primary tissue samples, including those derived from amphibians and embryonic chicks (Weiss & Taylor. 1960). It was later discovered that embryonic and pluripotent stem cells could also be used for the creation of organs due to their differentiation potential and capacity to self-organise into different structures resembling those found in multiple tissue types (Evans. 1981).

Organoids in a 3D culture system

An 'organoid' is a mini three-dimensional biological model of an organ grown in vitro. They are self-organising structures derived from primary tissue or stem cells and once formed have the ability to represent realistic micro-anatomy of a particular organ (Prior *et al*. 2019).

An early study carried out by Bissell and colleagues, in rats, showed that when hepatocytes interact with the extracellular matrix (ECM) their function improved. They also showed that mammary gland epithelia had the capability to self-organise in 3D secretory ducts when cultured with ECM extracts (Bissell *et al*. 1987). These studies were crucial to the advent of 'organoids', they provided evidence that 3D culture systems had the potential to support the growth of more complex mini-organs, that they had the capacity for morphological rearrangement and further tissue differentiation. These initial cultures however lacked the capacity for self-renewal and were limited to short-term expansion (Prior *et al*. 2019). More recently Hans Clevers and colleagues discovered that mouse Lgr5 positive adult stem cells could form self-renewing cultures when embedded in matrigel as ECM and grown in media supplemented with R-spondin and other niche factors. The inclusion of R-spondin was a key finding in this study as it up-regulates Wnt signalling, which is necessary for the maintenance of stem cell populations (Sato *et.al.* 2009). Following their findings in 2009 Clevers and colleagues went on to generate intestinal organoids from human stem cells (Sato *et.al.* 2011).

To date several organoid systems have been generated including retinal, brain, pancreas and liver. Organoid creation, in comparison to 2D culture, is particularly challenging due to the requirement of a 3D culture system with ECM and a specialised growth media containing various niche factors. Therefore extra training in this field is incredibly useful for researchers considering using organoids to further their studies.

How can organoids benefit research?

In comparison to 2D culture, cells in 3D culture more closely resemble functional and architectural properties of tissues *in vivo*, due to their ability to generate cell-cell, or cell-ECM, interactions. Organoids also more closely mimic cells within tissues as they are surrounded by concentration gradients of signalling effector molecules, nutrients and waste products as a 3D cluster rather than a horizontal plane exposed to a uniform concentration of factors (Prior *et al.* 2019). This makes organoids more physiologically relevant than 2D culture models, especially with the establishment of more biochemical and biomechanical micro-environments that affect cell proliferation, morphogenesis and survival. This could be a benefit for research in terms of drug development and screening giving a more realistic outcome as seen *in vivo*. As well as this, organoid signalling pathways can be manipulated and genome editing can also be performed (Prior *et al.* 2019).

Liver organoids course

Introduction to CamBioScience and the liver organoids course

CamBioScience is a division of OBRIZUM (The AI-Learning Platform) based in Cambridge, UK. They have provided intensive training courses since 2015 in various life science technologies for professionals, in both academia and industry, worldwide. Due to the novel and complex nature of culturing organoids, CamBioScience set up a 'liver organoids' training course, in collaboration with CRUK, AIRC, AECC and the HUNTER HCC Expeditor Network. The course took place in Cambridge, from the 1st to the 2nd of July 2019, enabling researchers from collaborating centres across Europe to learn the pioneering techniques of liver organoid culture through a series of absorbing lectures and laboratory practical's. The course was led by Dr Michelle Ware who was an extremely enthusiastic and helpful host. Dr Ware has a background in neuroscience and was very knowledgeable on organoids, she put together a fantastic course with excellent speakers and practical sessions.

Overview of the course

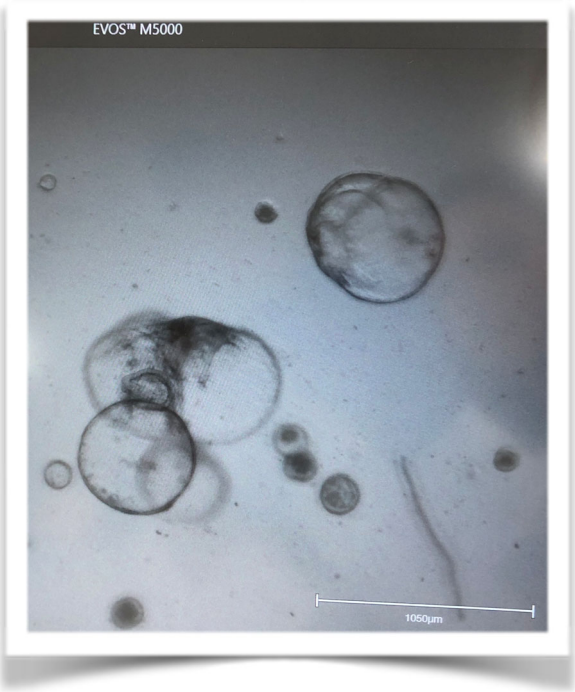
Dr Ware welcomed everyone to the course and went on to lead the opening session. This first session included a team-building exercise, allowing all participants to get to know each other's background and role within HUNTER. The first lecture was given by Dr Laura Pellegrini, a Postdoctoral Research Fellow at Cambridge University currently working on cerebral organoids. Dr Pellegrini gave an insightful introduction to organoids and how they can be used as a model system to understand development and disease. In the following session, participants were asked to give a 3-minute flash talk on their current research projects. These flash talks, not only provided an interesting snippet into everyone's work but also, introduced different ways in which organoids can be used in research. The first practical session was run jointly by Dr Lucía Cordero Espinoza (a research assistant) and Mewanthi Flaminia Kaluthantrige Don (a PhD student) from the Meritxell Huch Lab, where they work with 3D liver organoid technology.

The hands-on workshop took all participants through the process of creating mouse liver organoids. Each step was demonstrated and useful hints and tips were given along the way. Before the participants started the practical importance of preparation when creating organoids was

emphasised. There are several steps that must occur before starting the protocol to generate an organoid, including creating a specialised liver expansion and isolation medium and pre-warming culture plates. As all equipment and reagents were prepared in advance for the course, the first key step was to isolate the bile duct cells. Working in pairs, mouse liver was washed in sterile PBS and the gall bladder, diaphragm and any residual fat carefully removed. The tissue was then thoroughly minced using fine scissors, washed again with PBS and centrifuged to form a pellet. Next, the supernatant was discarded and digestion solution (collagenase + dispase II in sterile wash medium) added and placed in a water bath at 37°C. As the digestion solution was crucial for thoroughly breaking down the tissue it was important to remove all traces of PBS to avoid diluting the collagenase. After 10 minutes the tissue was disrupted to separate any residual red blood cells and fat and centrifuged again. The supernatant was removed and more digestion solution added however, this time the tissue was placed on a shaker at 37°C for an hour and a half. The last two steps were repeated and an aliquot was taken to check for the presence of clean ducts under a microscope. Once the ductal structures were observed the material was pelleted again, supernatant removed and cells washed with ice-cold wash medium to get rid of any remaining digestion solution. Ducts were then identified, collected under a bright-field microscope and centrifuged one last time. Geltrex (3D media containing ECM extracts) was thawed on ice before starting the session and was used to re-suspend the pellet. The material was then seeded by adding a droplet of Geltrex to the centre of each well in the pre-warmed culture plates. This step had to be performed quickly as Geltrex solidifies as it reaches room temperature. Finally, the plates were incubated for 10 minutes or until the Geltrex had completely solidified and then the droplets were overlaid with specialised liver isolation medium. The liver isolation medium was composed of the basal F12 medium supplemented with a variety of components including R-spondin and Wnt3 conditioned medium as well as Noggin.

Following the lab practical, networking interactions were encouraged and day 1 was brought to a close with a delightful formal dinner bringing together all of the course participants once again.

On Tuesday morning, the day began with our final practical session on passaging and overall maintenance of organoid cultures. Before the practical, we took a look at our practice organoid cultures from the day before (see image below). We were very successful in our first attempt at culturing mouse liver organoids, as we could see the organoid structure forming nicely. This was a very exciting and rewarding part of the course.



As when culturing organoids we were advised that preparation is also key for maintaining and passaging. Multi-well plates must be pre-warmed overnight or preferably 1-2 days in advance as well as thawing BME/Geltrex on ice the day before. As we were only practising passaging organoids we used our cultures created in the previous session. Firstly, the Geltrex dome containing the organoids was disrupted by scraping and pipetting up and down and then transferred into cold basal medium. The solution was then centrifuged and supernatant aspirated to remove any remaining Geltrex. We next used a narrow pasteur pipette to fully separate and break up the organoids. After a final wash, the pellet was resuspended in Geltrex, split if desired and quickly seeded in a fresh pre-warmed plate. After a 10 minute incubation the Geltrex ‘domes’ were overlaid with liver expansion media.

Following on from the laboratory practical everyone participated in a ‘Q&A Clinic’. This gave all participants a chance to ask questions about what they had learned during the practical sessions and lectures, as well as to query any topics that hadn’t been covered in the course so far.

The afternoon session included research talks on cholangiocyte organoids and their role for disease modelling (Prof Ludovic Vallier), along with senescence and control of the microenvironment (Matthew Hoare). Dr Meritxell Huch, our anticipated closing speaker, was unfortunately unable to attend on the day. Dr Lucía Cordero Espinoza (Dr Huch’s research assistant) kindly stepped in and gave a fascinating talk on the use of 3D organoids to model liver regeneration and disease.

The liver organoids course ended with a few closing remarks and a certificate ceremony to congratulate everyone for taking part on the course. There was also a chance for participants to give feedback and to continue networking with a drinks reception afterwards.

Course feedback

Having spoken to other colleagues who attended the course with me, the consensus was that the course was very well organised, informative and extremely stimulating; it was full of opportunities for discussion and networking interactions.

I learned a lot from the course and found the practical sessions to be the most useful. However, another colleague and I would have benefited even more from learning about tumouroids and how they differ from organoids in terms of culturing and maintenance as organoids and tumouroid culture will be a part of our role within HUNTER. It would have also been useful to have gone over how to create the liver expansion media as it is quite complex, containing a variety of components including conditioned medium which we will have to generate.

Overall, I thoroughly enjoyed my experience on the CamBioScience ‘liver organoids’ course and would highly recommend it to other researchers requiring intensive training in science technology.

References

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