Q&A session: What's needed to realise the potential of organoids?

From standardisation of imaging techniques, to more reproducible & cost-effective organoid culture media

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Is it best to microinject treatments directly into organoids or add the treatment to the culture medium?

RO: Microinjecting treatments into organoids would be quite labour intensive and not suitable for high-throughput screening. For the majority of compounds that are able to permeate the cells, especially small molecule compounds, it doesn't really matter whether you add them basolaterally or apically. In this case, I'd suggest adding them to the media directly so you have a higher throughput assay. I'd use microinjecting only for very specific compounds that aren't able to permeate the cells e.g. an antibody-based treatment.

RS: With organoids the problem of diffusion is something that everyone needs to deal with. This leads to variability in the core and the peripheral cells. But that being said, this holds value for mechanistic and drug clearance studies. In future we might be able to overcome some of these limitations that we see today using endothelial cells.

DP: I totally agree with René. Most clinically important compounds should be able to enter the cells. There might be two clinical compounds that you need to look at injecting directly into organoids but then you're really looking at working with individual organoids rather than say 50 organoids per well.

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Do you always use passage five when developing organoids or does it depend on the cell type?

DP: Passage number five is just an empirically chosen passage number based on when we find that the culture has stabilised vs. crashing out after being passaged further. This is why we usually pick passage number five to determine the success rate for our pancreatic cancer organoids, but it can vary for different organoids and tissue types.

RO: It doesn't matter too much which passage number you take but, saying that, the organoid size can affect which passage number is best to use to ensure your subculture is successful. Passage five seems to be a reasonably good number.

Is this also the case for liver organoids?

RS: The number five is a synchronisation of your process and even in primary cells it could be passage three or five as you have to take the cells through a protocol. It's a midpoint because after that the cells, if they are primary, start to senesce.

Could organoids speed up and reduce the cost of drug development? If so, how can they do this?

RS: Animal testing is an expensive process and method, which organoids could help to reduce. You can also use organoids to look into drug metabolism. Putting these into a process itself if enough to reduce the cost, not entirely but in every process 25-20% of the cost can be reduced.

Could organoids also be beneficial in precision medicine?

RO: Yes, organoids are much more representative of the actual patient and therefore any drugs you develop using organoids will have a much higher chance of being successful in the clinic. All the failed drugs are really the majority of costs in pharmaceutical development. Therefore, by tweaking the success rate you can already gain huge margins.

DP: In our experience, organoids help to overcome lots of false positives in drug screens. When we do side-by-side comparisons of 2D vs. 3D, we see less hits in a 3D setting but then these would be considered the true hits.

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Where can you obtain organoids? Is it best to make them yourselves or can you purchase them?

DP: As part of an NIH-funded project with ATCC and other large research institutes around the world, we're building a biorepository of well-characterised organoids including sequencing and clinical data, as well as a 6-month follow-up. This is probably the easiest resource of organoids at the moment.

RO: At the HUB we're also participating in the NIH project. One of the main reasons for us wanting to join this collaboration was because of the long time scales and ethical regulations associated with collecting all the necessary patient consents. The ATCC is set up in such a way that is makes this much easier so we're contributing to this biobank to help researchers to easily access organoids, as well as adding other models to our own repository.

RS: We have all the necessary licences and informed consent for all our lines and samples, so are able to provide them directly to people via our website.

Do you have any insight into which growth factors can have a big impact on organoid cultures?

Rob Nixon (Qkine): We need to be producing growth factors in an animal-free way, for example our TGF-β1, to help with the scale-up and translation of stem cell and organoid applications from bench to clinic. As Dennis discussed earlier, R-spondin 1 is another important organoid growth factor and we need to take away those key differences and heterogeneity that we see between growth factor batches and help to standardise organoid culture media. We already have a more reliable, optimised version of R-spondin 1 in our portfolio to offer customers. I think the other big area is Wnt3a, which is a very complex signalling story, so we need to bring a higher purity, more reliable version of this key growth factor to the market.

RS: Growth factors have a huge role to play because you can see variability and differentiation starting to happen in your processes. As you often run assays over several months, it's important to have consistency in your growth factor batches and a good production process.

RO: The growth factor space is something that has been developing a lot recently particularly being able to get good growth factors. When we started working with organoids, we had to make everything ourselves and therefore it was a huge time investment for anyone wanting to work with organoids. The wider availability of organoid growth factors is enabling smaller labs to start doing organoid experiments.

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DP: The harmonisation is still very important. As we work with patient samples, there isn't a one-size-fits all approach. However, having a standardised culture media to use across a population of organoids or particular tissue type is very important.

How does organoid size affect the outcome of assays? Is it important to always use a similar size to ensure reproducibility or does the size not matter?

RO: It really depends on the question you are asking. If you take a dense tumour organoid and you test a compound on a large organoid, then the outer cells will start dying before the inner cells. However, the same might not be the case in a smaller organoid – so in this instance, yes size does matter for reproducibility. The total proliferation can also be lower if you have very large organoids compared to very large compact organoids. But if you're talking about cystic organoids then the size is a lot less important.

RS: There are a lot of studies looking into the impact of variable diffusion within organoids. You need to homogenise cultures and make sure that over a period of time you have reproducibility that you can control - you can't have complete control over the size, but there should be some threshold that you come up with.

DP: If you're doing an image-based assay and you just want to see morphological changes then size is important to a certain extent. However, if you're doing a drug screen and you want a homogeneous seeding density, then you need a homogeneous cell solution to achieve this.

Thank you to all our speakers for their contributions to this insightful Q&A session