

Deep Dive series

The future of organoid intelligence

21 June 2023



Question and answer compilation

1 Organoid practicalities: lifespan, size restrictions and differentiation

1.1 What are the requirements to keep the organoids in microelectrode arrays (MEAs) alive, and what is their typical lifespan? What are the challenges that could be tackled in this area?



Answered
by Lena Smirnova

The challenge here is to provide nutrition to the center of the organoids. We are therefore developing microfluidic devices to advance these systems further and make them more complex. We keep our organoids in cultures for 6 months or longer. However, without shaking (if smaller than 500 μm) or perfusion, they will develop a necrotic core. So, perfusion is the key to keep them big, complex, and alive.



Answered
by Brett Kagan

Lifespan is an open question as Professor Alysson Muotri described in his talk. Most people look at a lifespan for organoids of weeks to a few months, but there are also researchers aiming for 12 months-plus of lifespan now, as well as extending this in the future. Requirements to keep organoids alive in relatively simple terms are to:

- Control temperature
- Provide nutrients
- Remove waste.

This can be done in a number of ways, but there are likely benefits to automating it and coupling it to perfusion through an organoid.

1.2 What is the maximum size of brain organoids currently, and what factors would determine that? What are the main differences you notice during the differentiation process comparing 2D cultures and brain organoids (time to obtain mature differentiate cells, efficiency of differentiation, neural cell functionality)?



Answered
by Lena Smirnova

The model we developed is small on purpose - we keep our model under 500 μm to avoid a necrotic core. This is because as a toxicology lab, the main focus of our research was toxicity testing of chemicals and drugs, so we needed to have a healthy system inside and outside. But other groups, such as Prof Muotri's lab, use much bigger organoids. There are certain limitations with the size - we want to make our tissues healthy throughout the entire organoid. Therefore, many researchers are trying to make a vascularization of those organoids to make them healthy inside as well. The problem with larger organoids, which is well reported and confirmed, is that the core will be dying. How big we need them is a good question and what we're trying to understand. We're testing our small system to understand what the roof is for our research; can we induce synaptic plasticity for example? Increasing size and complexity will allow us to move forward if we get stuck on the way with the smaller organoids.



Answered
by Alysson Muotri

Different groups have different protocols - we can grow organoids up to 0.5 cm, so they're very big, you can see them with the naked eye. At one point the center becomes necrotic because nutrients are coming by diffusion, they are not vascularized. This is a serious problem in the field that many labs are trying to solve.



Answered
by Lena Smirnova

In terms of differentiation, the density of the cell cultures is very different, and we immediately see, even with a simple spheroid of one cell type, a difference in how they respond to toxicants, or how long we can keep them in culture. For example, we have a model of dopaminergic neurons that we can keep in a monolayer culture for a maximum 10 days, but if you put them in 3D they're happy sitting there for weeks or months. The lifespan is one aspect, complexity is another, especially when we differentiate the different neuron types. In the monolayer you mainly get neurons and some astrocytes. It's very hard to differentiate oligodendrocytes in the monolayer and even harder to have myelination in the monolayer. 3D cultures allow you to do that. In addition, what Prof Muotri showed in his talk is the layering of the cortex architecturally - so the structure of the brain organoids you can only do in 3D, you cannot do this in the monolayer.

1.3 How will the culture media requirements be met for moving forwards with OI? Cell cultures will require the constant replenishment of DMEM, FBS etc. along with waste removal from cultures?



Answered
by Lena Smirnova

Our media is xeno-free, so no FBS. But yes, our idea is the perfusion system to create the large and complex organoid, which can stay alive and active. Currently we are keeping them in cultures due to gyratory shaking, and we change the medium every two days.

2 Applications of results from organoid-based studies

2.1 How similar are the electrophysiological properties of organoids with the developing human brain, i.e., how can you practically correlate MEA readings to in-vivo data?



Answered
by Lena Smirnova

This question was answered by Prof Muotri in his talk, but we are also working with clinicians to compare the human EEG from different patients with brain organoid recordings to see whether certain 'model' patterns can be developed and compared between human the EEG and MEA recording for the organoids. This work is still ongoing.

2.2 What is your view on neuromorphic chips? Do they do a good job in somehow mimicking the brain? Is there potential in such technology?



Answered
by Brett Kagan

This is an interesting technology with some potential applications. However, I would say that so far it has had limited success in strictly mimicking the brain, except in some very simple ways. This isn't necessarily bad, as long as we understand that neuromorphic chips are a model, not the brain itself, and there are several very big differences in both structure and function. Of course, just because

there are differences between neuromorphic and biological brains, doesn't say anything about the potential of this technology. I think it comes down to the right tool for the right job. I suspect there are key applications that neuromorphic chips are ideally suited for, just as there are key applications where biology will be ideal.

2.3 An advantage of the organoid is the 3D structure. How do you obtain a 3D read-out of synaptic membrane potentials, as would be required to understand phenomena like synaptic plasticity in learning and memory?



Answered
by Lena Smirnova

This is exactly what we are working to establish, with different types of systems developed to record in 3D from inside and from the outside (shell, mesh electrodes, shank electrodes, nanowires etc.). We are also looking into the network dynamics with high density MEAs, to see how the synaptic activity can be modulated, whether we can establish a model of long-term potentiation (LTP) in 3D, and show LTP without performing patch clamping.

2.4 In your workshop report you say: 'Once we understand the brain's structural and functional connections (the connectome), drug and toxicological tests on the organoid would provide valuable insight into understanding the impact of chemicals and drugs on functionality and how this may affect structure and circuitry.'

Does this mean there needs to be complete understanding of the brain's connectome for organoids to be used in drug and chemical screenings? If so, when do you expect the brain's structure and function to be understood?



Answered
by Brett Kagan

Great question, but fortunately not. For instance, researchers currently use animals in some cases to test or screen drugs, yet we don't have a complete understanding of animal brains and are still able to draw conclusions about the impact of drugs of toxins etc. However, the benefit of more controllable systems such as envisioned in OI is that we may be able to more completely explore how the system works and responds in ways that are difficult when using animals or people, and thereby end up with a better understanding through these methods.

2.5 **Have you ever tried to do something with the currents you measure with your MEA set-ups? Can you use them somehow, for example, to light up a lamp? Would this be possible in the near future with the existing technologies?**



Answered
by Brett Kagan

The currents from neural systems are very small. So, we've not used them as a power source, and I would not foresee it in the future as there are likely better sources to generate power.

3 Ongoing and future research

3.1 **What research can we expect to read about in the next year or so? Is the interest in AI always going to overshadow possible improvements such as OI?**



Answered
by Brett Kagan

All of the presenters on this webinar have groups developing new research. If you're interested, I'd say follow them on social media and you'll see some cool things coming out as there is a lot going on!

3.2 **What approaches could we consider for creating large organoids with separate sub-regions to study interactions between model brain areas? Has there been progress in creating effective circulatory systems in vitro?**



Answered
by Lena Smirnova

This research is ongoing by several groups. For example, Professor Sergiu Pasca's lab is developing assembloids of different types of neurons or regions and letting them 'communicate'. Professor Kenneth Kosik's lab also recently showed circulatory systems, and there's also similar work being done by Professor Alysson Muotri's lab (speaking at this webinar).

3.3 Who are the teams, institutes and universities participating in the development of CL1? How far along is this specific project?



Answered
by Brett Kagan

CL1 stands for Cortical Labs 1 and is the first tool being built by Cortical Labs. You can look at <https://www.corticallabs.com/> for some more information on this. I'll add we're hoping to be releasing the alpha version by the end of the year. So, if you follow closely, we'll be providing more updates as developments occur.

3.4 Will JHU set up OI doctoral programs in the future?



Answered
by Lena Smirnova

Great idea! We should keep that in mind as we move forward with the field, thank you!

3.5 Do you foresee applying an MPS-sensors-AI platform to modeling the gut-brain axis, in the near future?



Answered
by Lena Smirnova

We have been discussing this with one of our collaborators and are actively looking into this at the moment.

3.6 In what other organs/organoids/tissues has OI being studied or have the potential to be studied?



Answered
by Thomas Hartung

The human brain is a fantastic computer, it is hardly outperformed by real computers. That's because it's not working by being fed zeros and ones. It's being fed millions of different signals which are also more or less analogous signals. I think the brain can only shine if you give similar inputs. That's why combining it with sensory organs like retinal organoids is a big way forward. We have co-authors on our paper who are planning on doing exactly this with us. I'm also aware of some of Prof Muotri's work, which is doing this already - with these types of combinations.

I also mentioned nociceptors in my presentation. I think this would possibly be the simplest test path, and it could be an important model for chronic pain to have nociceptors combined with the brain organoid. We can also dream of completely different architecture. We don't need to have one brain organoid which is bigger and bigger. We could combine a cortical brain organoid with a brain stem organoid. David is already working on some models of combining units of brain

organoids. These are things that are all in the making. The limit is the sky here. It can also be hyper-physiological, we are not bound to any boundaries, we don't have to have it in a skull. We can even give it new types of input we'd not even thought about in biology.

4 Ethical implications of work with brain organoids

4.1 In light of the fact that autistic adults embrace their neurodivergence and are opposed to therapies that are designed to 'cure their autism', how are you considering the ethical implications of potentially eradicating a brain type that is considered by many to be a part of natural human diversity? In other words, what about those (me included) who do not want to be neurotypical?



Answered
by Karen Rommelfanger

This is such an important point. One of the first steps is for researchers to be aware of is that tissue donors might have reservations about research oriented at 'curing' autism. Secondly, researchers should be able to communicate about their intentions/goals for research in their consent forms in such a way that potential research participants can decide NOT to participate if the research does not align with their values. Donors also need to be aware if their tissue is being collected under 'broad consent' mechanisms where their tissue can be used for research beyond the original intent (so not initially being used to study 'cures' for autism that may eventually be re-used for that purpose in future research studies) or more circumscribed use.

4.2 Would the clinical trials (or preparation for the trials) be completely animal-free or would animal models still have to be used at some point?



Answered
by Lena Smirnova

We see a bright future, especially for drug efficacy testing. The safety testing might take more time to move away completely from animals, and in certain areas animals will be still required, but less and less as micro-physiological systems (MPS) advancement progresses.