GROW YOUR OWN BRAIN

Jenny Straiton explores the miniature world of organoids and discusses how these small models are making big changes in the world of neurological research.

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Initially, scientists learned about disorders when disaster happened – Paul Broca made his mark when he linked posterior left frontal lobe damage to speech production; losing his hippocampus made Henry Molaison, better known as H.M., the world's most famous amnesiac; and being run through with a railroad spike turned Phineas Gage into a psychological phenomenon.

Later, neuroscientists relied on *in vitro* cell cultures or animal models to see what was happening. Although less devastating for the subjects, this route still has many limitations. A rat's brain is significantly different to a human brain, particularly regarding the cortex, and a single type of cell in a dish can in no way represent the magnitude or the complexity of the human brain as a whole.

The limitations shown in the traditional methods of neurological research raised an important question, as summarized by Sergiu Pasca of Stanford University (CA, USA): “Can we capture in a dish more of these elaborate processes that are underlying brain development and brain function?”

The answer? Yes; scientists have developed ways to take stem cells and grow cerebral organoids (Figure 1), or ‘mini-brains’, which, as they grow, model the development of the human brain.

Starting as induced pluripotent stem cells (iPSCs), organoids are lab-grown (Figure 2), miniature versions of the body's organs. The 3D anatomy and variety of tissue and cell types make them the most realistic models of human anatomy to date. Researchers have developed organoids for all major organs, from the heart to the pancreas, but one of those gaining serious attention is that of the brain.

First developed by Lancaster *et al.* in 2013 [1], these cerebral organoids were shown to be realistic models of the human brain, modeling distinct regions and cellular organization patterns. They can grow to 3–4mm and can survive up to 10 months. Staining experiments demonstrated the presence of a distinct forebrain at 16 days of development and further sub-regionalization to a ventral and dorsal cortex at later stages of growth. Hippocampal cells can be observed, although they lack the localization seen *in vivo* and are found throughout the organoid.

“I think that these brain organoids hold incredible potential for modeling human neurological disease in completely new ways,” commented Paola Arlotta of Harvard's Department of Stem Cell and Regenerative Biology (MA, USA), who has been described as a pioneer in brain organoids [2].

“Human brain organoids generated from cells of patients carry extraordinary potential to serve as transformative models to understand and develop treatments for prominent human neuropathologies, like neuropsychiatric disease.”

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BACK TO THE START; REWINDING NEURAL DEVELOPMENT

The development of psychiatric diseases is poorly understood. Often starting in the womb, it is difficult to capture the nature of abnormal brain development and watch as these changes occur.

“What if we could somehow go back, so to speak?” questions Arlotta. “What if we could take a sample of blood from a child with autism, make his or her own stem cells and turn those into a model of their brain? Could we then begin to watch in some small part how the brain had formed?” [2].

A recent study from the SALK institute (CA, USA)

did just that, and demonstrated that neurons derived from skin cells of individuals with autism spectrum disorder (ASD) show different patterns of development and growth when compared with cells of neurotypical individuals [3].

When re-differentiating skin cell-derived iPSCs into neurons, researchers noticed that the neurons derived from people with ASD grew at a faster rate and had longer, more complex branches. The team then used a subset of the neurons to develop cerebral organoids and found that those from ASD cells were larger and had an increased thickness of the cortical plate.

"The current diagnostic methods are mostly subjective and occur after the emergence of behavioral abnormalities in young children," commented lead author Simon Schafer. *"We hope these studies will serve as a framework for developing novel approaches for diagnosis during an early period of child development – long before behavioral symptoms manifest – to have the maximum impact on treatment and intervention."*

These results supported those of a smaller 2015 study where the organoids grown from ASD patient's stem cells showed increased expression of genes that control neuronal growth and development [4]. Overexpression of the transcription factor FOXG1 in particular was found to lead to the overproduction of inhibitory GABAergic neurons, creating an imbalance in the excitatory/inhibitory neuron ratio. ASD-derived organoids were also found to have an accelerated cell cycle.

GROWING YOUR CONNECTIONS: DO NEURONS THAT FIRE TOGETHER REALLY WIRE TOGETHER?

Neuropsychologist Donald Hebb first coined the phrase 'cells that fire together wire together' in respect to synaptic plasticity and the associative learning process. Though not to be taken literally – neurons firing together results in seizures – the abstract concept is valid; firing of cell A is needed to initiate the firing of cell B and with repeated or persistent activity, the efficiency of cell A in causing the firing of cell B is increased.

This form of synaptic strengthening is believed to be the underlying cause of neural plasticity, that being the alteration of neural networks throughout life, as well as the learning process and beginning of structuring the brain.

Modeling the development of this synaptic activity in organoids may help in understanding how neural connections develop before birth and potentially into early stages of life. As the brain develops, electrical activity lacks the synchronized activity as shown in mature human brains and in the brain of a premature infant activity is disorganized and chaotic.

In work presented at the 2018 SfN conference, researchers from the University of California San Diego (CA, USA) found that, at 6 months of growth, cerebral organoids spontaneously produce human-like brain waves that show great resemblance to the disorganized patterns of the premature infant (Figure 3) [5]. This similarity to the early, still developing brain suggests that neuroscientists will

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now also be able to model the early stages of developmental epilepsy, something previously restricted due to the difficulty of *in utero* research.

However, not all agree with the translatability of the information that can be gained from such models. Arnold Kriegstein, also of the University of California, holds a more negative view, pointing out that *"proving that they're the same will be difficult because researchers know so little about how babies' brains are wired. The organoids could be missing key components that drive EEG patterns in real brains."*

This limitation was acknowledged by study leader Alysson Muotri. *"The organoids aren't close to being real human brains. They don't contain all the cell types found in the cortex, and they don't connect to other brain regions."*

However, his group are conducting more work with the organoids, growing them for longer in order to see whether they will continue to develop and form the synchronous activity associated with the mature brain. They also plan to test the cortical function of these organoids by forming connections with organoids modeling other parts of the body.

DRUG DEVELOPMENT AND GROWTH

With the addition of the blood–brain barrier (BBB) [6], researchers from the Wake Forest Institute for Regenerative Medicine (NC, USA) have developed a 3D cerebral organoid that, as explained by senior author Anthony Atala, *"is the first engineered tissue equivalent to closely resemble normal human brain anatomy, containing all six major cell types found in normal organs including neurons and immune cells."* Researchers have also developed ways to vascularize the organoids [7]. Taking a standard, stem cell-derived cerebral organoid and surrounding it with their newly developed endothelial cells, researchers grew an organoid that contained capillaries that penetrated its inner layers (Figure 4).

Combining this vascularization with the addition of the BBB, scientists have the ability to mimic strokes and may be able to use this to create more effective methods to reduce the secondary side effects of stroke. ▶

Figure 1. A cross-section of the cerebral organoid [1].

Figure 2. The process of making organoids from stem cells [1].

Figure 3. A slice through a brain organoid, shows mature cortical neurons of the outer edge of the structure. Credit: Muotri Lab/UC San Diego, CA, USA [5].

► These more realistic models have the potential to assist in the development of novel drugs and treatments, creating more translatable models to be used in preclinical testing. *“The shortage of effective therapies and low success rate of investigational drugs are due in part because we do not have a human-like tissue model for testing,”* commented Atala. *“The development of tissue-engineered 3D brain tissue equivalents such as these can help advance the science toward better treatments and improve patients’ lives.”*

When discussing the potential for organoids in drug development, Mindy Goldsborough, Chief Science and Technology Officer at ATCC (VA, USA) commented, *“There are several complexities of the nervous system that present challenges for developing optimal in vitro models; among these is that the in vivo nervous system is generally composed of multiple cell types working in a coordinated effort whereas in vitro model systems are often single cell types. Another challenge is that cells within the nervous system tend to be terminally differentiated, and to mimic that differentiation process in vitro can require specialized cell culture conditions that can take several weeks.”*

Because organoid models can overcome these challenges, they are likely to play an important role in improving the success rate of

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investigational drugs, especially as the range of available disease models diversifies. *“This will enable researchers to have a wide breadth of models to use for drug screening applications”* added Goldsborough.

CAN YOU GROW A CONSCIENCE?

As with many scientific advances, cerebral organoids do not come without their critics.

In a 2018 neuroethics workshop [8], Walter Koroshetz, Director of the NIH’s National Institute of Neurological Disorders and Stroke (MD, USA), presented the crux of the issue: *“The question here is, as those cells come together to form information processing units, when do they get to the point where they’re as good as what we do now in a mouse? When does it go beyond that, to information processing you*

only see in a human? And what type of information processing would be to a point where you would say, ‘I don’t think we should go there?’”

However, the question of ethics may also be argued on the side for cerebral organoids, as proposed by Arlotta. *“As a society, we must consider the ethical structure of this work. As a scientist, I must consider also the responsibility I have to make a difference, now that we can, for patients affected with some of the most devastating diseases of our time. Is it ethical or unethical not to take this opportunity to understand disease to alleviate suffering? I think the choice there is very clear”* [2].

The likely unanswerable philosophical question of mind versus body is undoubtedly going to be a major factor in this ethical issue; is the mind a distinct conscious being or is it just the physical body alone? Biology has yet to provide a definite answer to this question of consciousness and is even further away from developing a way to measure it. *‘Cogito ergo sum’* may be so, but how can you determine thinking?

Overall, the ethical debate around cerebral organoids creates an almost paradoxical argument; more realistic models are needed to gain more information about human disorders, although more ►

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► realistic models may have consciousness and therefore it is immoral to use them. With no marker that can draw a clear line between right and wrong in this situation, the advancement of this technology may also be its downfall.

GROWING UP: WHAT'S IN THE FUTURE FOR ORGANOID?

Despite the ethical controversy, improvements in the cerebral organoid models are happening thick and fast.

As well as observing neurogenesis, organoids also show promise for modeling neurodegeneration; mimicking diseases such as Alzheimer's, Parkinson's and multiple sclerosis could lead to greater understanding of the pathways and progression of such diseases. Outside of understanding disease development, organoids are also assisting the advancement of personalized and regenerative medicine. Testing drug efficacy on the organoid grown from a patient's biopsied tissue allows for greater precision in treatment and reduces the risk currently experienced with the trial and error method, something that has already proven successful in cystic fibrosis treatment [9].

In regenerative medicine, the future could be in the use of 'spare parts' to replace what has been lost. In something akin to the fictitious work of Mary Shelley, researchers have found that, when inserted into mouse models, the human organoids were shown to integrate with the mouse brain, forming new connections and sharing the blood supply [10]. Transplanting tissue as an organoid rather than individual neural progenitor cells has been shown to have improved engraftment and more robust vascularization [11]. This new method of transplantation has been suggested as an alternative strategy for promoting neural repair in cases of traumatic brain injury and neurodegeneration.

However, the sheer complexity of the human brain means that it is unlikely scientists will ever be able to construct a full replica.

"If getting to a full understanding of the brain is a mile, we have walked at least six inches," commented Jeff Lichtman (Harvard University; MA, USA). *"You look at the actual structure of the brain or even an organoid and it's just extraordinarily complicated. It's much more complicated than anything humans have ever built. This is a little humbling"* [2].

Organoids allow for the observation of otherwise inaccessible brain development. However, they show variability and are only in the early stages of research. More control is needed over their growth and the ability to guide anatomical organization and reduce variability in order to produce more reliable and translational research models.

It must be pointed out that the labeling of these organoids as 'mini-brains' may in fact be a misnomer, nomenclature that is potentially harmful to the field. Although showing unprecedented abilities, it is important to remember that, in reality, they are just a ball of cells.

That being said, each additional discovery and advancement creates greater potential for the cerebral organoid. The initial development of these models opened a new door in the field of neuroscience and provided a starting point for further research into the inner workings of the brain.

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Figure 4. A vascularized organoid – shows that blood vessels have penetrated both the outer, more organized layers as well as the inner core of the organoid [7].

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