

The importance of cerebral organoid technology in medicine

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Abstract. Because of the intricate nature of the nervous systems, neurological diseases have always been one of the least studied areas of pathology and medicine. Currently, there is no cure for these kinds of diseases but only medications or therapies that relieve symptoms and minimise suffering. Thus, cerebral organoids derived from human pluripotent stem cells are produced in order to study the development and pathology of the human brain, especially the embryonic stage, and to model neurological diseases. In this dissertation, I will make a judgement on the appropriate usage of cerebral organoid in investigating neurological disease through exploring and assessing the effectiveness of the cerebral organoids modeling Zika Virus and Alzheimer's disease and examining the ethical issues arising from this practice.

Keywords: Cerebral organoid technology, Vascularisation, Zika Virus.

1. Overview of Cerebral Organoids

Cerebral organoids are artificial 3D miniature organs grown in vitro that replicate structure and function of the human brain. They are mainly used to recapitulate embryonic brain development because they cannot live for a long time. This will be further discussed in the paper. They are developed from human pluripotent stem cells (hPSCs), which can be divided into two categories, induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs). Pluripotent cells refer to cells that are not yet specialised to cells such as vascular, lung cells are more. iPSCs are derived from somatic cells that resemble the features of ESCs and ESCs originate from undifferentiated cells in the embryo (Qian et al., 2019). Cerebral organoids can be developed from two kinds of methods: unguided and guided. In the unguided method, hPSCs aggregate and form embryoid bodies (EBs). Then, they are implanted into an extracellular matrix (ECM), a jello-textured base placed on the bottom of a dish to provide an environment for cells to grow in, to which is added cell culture media that provides nutrients and other growth factors required for the organoids' development. Lastly, the dish is put into a spinning bioreactor for neural differentiation. Because of the lack of interference, the formation of the organoid relies entirely on the spontaneous growth and differentiation of the cells. As a result, the organoids obtained are heterogeneous which means they contain various types of brain cells (Qian et al., 2019). There are two different guided methods. The first one is the insertion of patterning factors to manipulate the differentiation of hPSCs into forming a desirable brain region. Thus, the organoids obtained are homogeneous. The second method uses fused organoid technologies. This minimises the heterogeneity of the cerebral organoid. Firstly, hPSCs are differentiated into specific brain cells with the use of patterning factors to form organoids. Then the organoids that represent different cells are fused together to represent distinct brain regions (Qian et al., 2019).

The principal aims of this dissertation are to explore what cerebral organoids are and their existing applications in the understanding of pathology and discovering potential treatments of neurological disorders, and investigate relevant ethical issues of the utilisation of organoids in research.

For the first aim, we will describe what these artificial "brains" are and the two different types of methods they are usually made. The method the scientists take in creating the organoids determines the overall structure and simplicity of the organoid, so it is essential to discuss the circumstances of selecting the appropriate approach. After presenting some fundamental knowledge of these in vitro brains, I will outline the most recent advancement of cerebral organoids and the existing limitations they face in recapturing an actual human brain. During this description, I evaluate the effectiveness

of these organoids with support from published journal articles in the field. Then we investigate the usage of organoids in researching the pathogenesis and potential medication and therapies for Zika Virus disease and Alzheimer's disease. I chose these two diseases because Zika Virus is a neurodevelopmental disorder and Alzheimer's disease is a neurodegenerative disorder, so by comparing these two diseases, the significance of organoids in understanding the two main stages of human brain development is emphasised. In each study, I will also review and assess the value of the existing research discoveries. In doing so, I incorporate detailed procedures of an experiment carried out by a group of scientists led by Yan-Peng Xu in determining the potential clinical application of enoxacin in treating Zika Virus.

Lastly, I explore the existing and potential ethical issues of using cerebral organoids for research. I identify the current threats and analyse scenarios where such research is unethical. Furthermore, I pinpoint the probable moral dilemmas in the future and discuss why scientists and researchers have raised concerns. I think it is necessary to understand the perspectives of the specialists in this field in deciding the conditions in which organoids can be ethically used. This will support my main argument of identifying and evaluating the extent of the usage of these miniature organs.

2. Advances and Limitations of Cerebral Organoids

In recent years, methodologies of differentiating brain organoids have been refined to improve its resemblance to the human brain. Cerebral organoids are a big family of various kinds of brain organoids and the cerebral cortex organoid is the most researched.

2.1 Vascularisation

Vascularisation has always been a problem of organoids because they cannot survive for a prolonged period of time relying only on the ECM. A necrotic core, a cluster of dead cells, often forms in the center of an organoid because essential nutrients and oxygen cannot be diffused into the inner core. However, with the presence of a vascular network, these materials can be easily transported around the entire organoid and the organoid can survive longer to model later stages of human brain development. Besides, vascularisation is required for proper neurogenesis in the organoids, which is also critical when studying growth and differentiation of brain cells. Although scientists have been able to utilise this technology to model neurological diseases in prior to growing the vascular system in organoids, the realisation of vascularisation has vastly improved the usefulness of cerebral organoids in disease modeling and treatment research.

2.2 Electrophysiological Activity

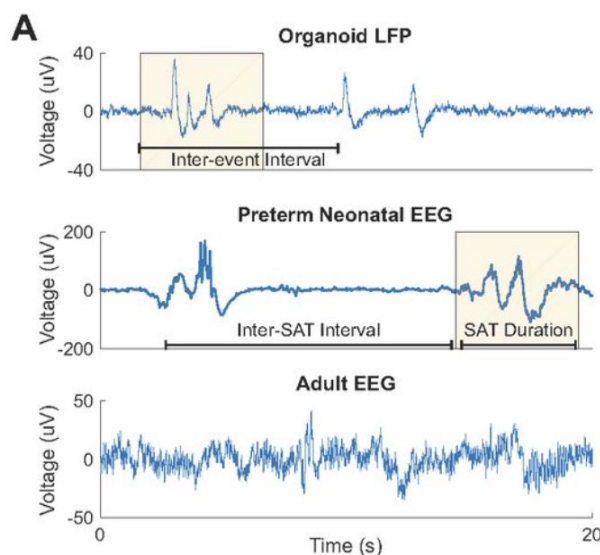


Figure 1. High content validity

A Nature news article published in 2018 reported that neuroscientist Alysson Muotri and his colleagues compared the electroencephalogram (EEG) activity of cortical organoid and a mature human brain. They found out that the organoids are unable to recapitulate “synchronized networks that fire with predictable rhythms” which is shown in the human mature brain, but instead the EEG of organoids shows great similarities with the EEG of 25 to 39 weeks old infants. (<https://www.nature.com/articles/d41586-018-07402-0>) Another research led by Cleber A. Trujillo reaffirms Muotri’s results discovering that there were comparable patterns between cortical organoid beyond 28 weeks EEG and human preterm neonate EEG at 35 weeks of gestational age (<https://www.biorxiv.org/content/10.1101/358622v2.full>). This shows high content validity as both groups used EEG activity to compare the electrophysiological activity, therefore it can accurately display the correlation between the two sets of data.

However, although both parties had the same results, the projection made by the researchers working on such research varies. Hongjun Song, a developmental neuroscientist at the University of Pennsylvania in Philadelphia, expressed his approving attitude and claimed that these findings suggest cortical organoids can be very useful in studying brain developmental disorders. On the other hand, Sampsa Vanhatalo, a neurophysiologist at the University of Helsinki who provided the infant’s EEG database for Muotri’s study, expressed his uncertainty and claimed that these similarities cannot be that representative to the extent that these waves are illustrating the actual brain waves of an infant’s brain. Trujillo from the latter study also commented that we could not state the cortical organoids have “functional equivalence” as a full neonatal cortex at this point but we can suggest that the organoids have potential in modeling human electrophysiology in the future.

2.3 Blood Brain Barrier (BBB) Organoid

Apart from this discovery, organoids that model the blood brain barrier (BBB) are also successfully grown. It is essential to model the BBB as many infections or malfunctions of the central nervous system (CNS) are closely related to it, such as Alzheimer’s disease and Parkinson’s disease. The blood brain barrier is a layer of endothelial cells that separate the capillaries in the brain and the other brain cells. It regulates the transportation of critical nutrients in the CNS. This layer also acts as a barrier to block any toxins or pathogens in the flowing capillaries from entering the brain tissues and prevents further infection of the nervous system. However, because of this function, BBB also blocks many drugs from entering the brain, hindering treatments for neurological and psychological disorders. As a result, BBB organoids are useful tools in examining potential methods for drugs to be transported through the barrier.

3. Disease Modeling: Zika Virus

Cerebral organoids can mimic the embryonic development of the fetal brain and are not ethically restricted, so this makes them a good tool to investigate neurodevelopmental diseases in humans. Zika Virus (ZIKV) is carried by Aedes mosquitoes and can infect everyone. Normal people often only experience mild symptoms but the virus can cause severe effects to the fetus in a pregnant woman’s womb. This infection doesn’t cause much harm to the mother but the infant will have microcephaly and cognitive dysfunction. Currently, there is not any treatment or cure for this disease, and organoids have been utilised to investigate the pathology of ZIKV. Brain organoids are mainly used for 3 major purposes in the study of this disease: Connect and pinpoint the biological effects or changes linked to the virus and testing antiviral drugs with organoids and study the mechanism of the causes of the ZIKV.

Scientists have connected microcephaly, abnormal size of the brain, and spinal cord injury of newborn babies with ZIKV. ZIKV infects human neural progenitor cells (hNPCs) and results in neural progenitor apoptosis which is the death of hNPCs (Xu, YP., Qiu, Y., Zhang, B. et al., 2019). This is a great discovery because this reflects that ZIKV has the potential to spread apoptosis to other surrounding brain regions, resulting in considerable infection and destruction of the central nervous

system. It is also discovered that the virus reduces the thickness of the ventricular zone layer, which is also an indicator of microcephaly (Xu, YP., Qiu, Y., Zhang, B. et al., 2019). Xu's research offers a great example of how helpful organoids can be in identifying symptoms of ZIKV.

Another advantage of organoids is the testing of a range of drugs and chemicals since we cannot conduct experimental trials on humans. Brain region specific organoids are useful in recapitulating the organisation of the human fetal brain and scientists have utilised it in assessing drugs.

Yan-Peng Xu led a research study that compares the effect of enoxacin on two different brain organoids, "a hESC-derived cortical organoid of D20 that replicate the ventricular zone (VZ) and subventricular zones (SVZ)" (Xu, YP., Qiu, Y., Zhang, B. et al., 2019, p. 268), where a large number of hNPCs are present, and "a D38 organoid that recapture mature neuronal layers" (Xu, YP., Qiu, Y., Zhang, B. et al., 2019, p. 268) containing multiple cell types including hNPCs. Enoxacin, an antibiotic that acts as a RNAi enhancer (RNAi plays a role in innate antiviral immune response) is able to stop microcephaly in the organoids. The scientists discovered that ZIKV targets SOX2+ hNPCs in the VZ and SVZ and results in a reduction in thickness of the ventricular layer, further leading to a shrinking organoid. These obtained characteristics clearly illustrate the feature of microcephaly which is previously discussed in the identification of ZIKV disease. Next, enoxacin is added to the D20 organoid and the result shows that enoxacin prohibits the replication of ZIKV infected cells and retains normal cell growth. On the other hand, ZIKV messes up the structure of VZ and SVZ and leads to extensive cell death in the D38 organoid. Similarly, hNPCs survive and the inner structure of VZ and SVZ is retained with the supplement of enoxacin. These outcomes indicate that enoxacin is capable of restricting ZIKV infection in hNPCs in the simple organoid and the more complex organoid that consist of other brain cells without causing much damage to the original structure.

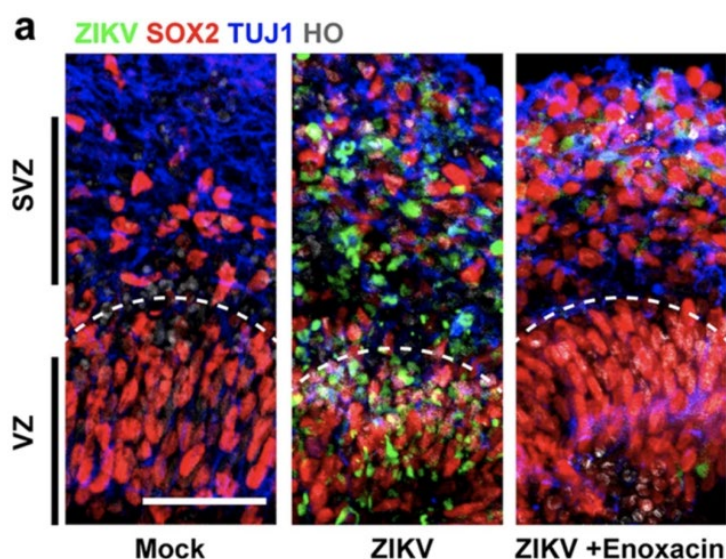


Figure 2. Organoids influence

The Researchers had not previously considered enoxacin as an antiviral drug to treat ZIKV, so the cortical organoid is a big contributor to this development. This further shows that the contributions of this technology/advancement is advantageous to medicine because we can achieve many beneficial understanding of how ZIKV attacks our immune system which are fundamental knowledge in treating this disorder.

The first diagram shows 3 different groups, mock which is the control group showing the normal organoid with the addition of enoxacin, ZIKV which is the infected group and ZIKV + Enoxacin which shows enoxacin treatment on organoids infected with ZIKV. This shows that enoxacin successfully reduces ZIKV infected cells and restores growth of SOX2 cells in the VZ and SVZ.

In the Figure 3, it shows that ZIKV greatly reduces the thickness of the VZ and with the treatment of enoxacin, the thickness of VZ returns back to the original thickness shown in the mock group.

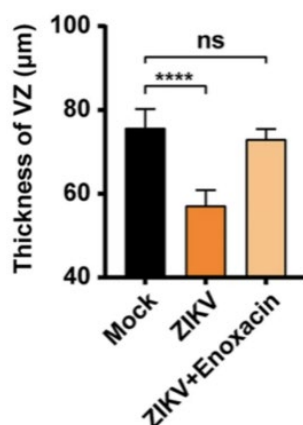


Figure 3. Influence of enoxacin

Furthermore, studies carried out show that 25-hydroxycholesterol (25HC) and Duramycin both are successful in minimising the ZIKV mRNA levels, which is useful in preventing the spread of infectious cells and reducing virus reproduction. This result is beneficial as it demonstrates that these chemicals can inhibit the virus from entering the cell and can serve as effective drugs for cortical tissue to defend against ZIKV. The research of enoxacin, 25-hydroxycholesterol (25HC) and Duramycin are all great examples of the importance of these 3D miniature organs in studying antiviral treatment. (Momoko Watanabe et al., 2017) As the structure of BBB can be recapitulated in organoids, scientists are able to observe the permeability of these drugs apart from its effectiveness on reducing the ZIKV virus in determining whether they can be potential treatments. With this understanding, it reflects that cerebral organoids are useful in treatment research because it showcases the general movement of substances across the layer of cells despite the fact that it lacks essential brain cells and tissues.

Another notable advantage of organoids is their ability to present the infection process of the brain cells which allow scientists to better study the mechanism of infection. A recent publication reveals that neural progenitor cells are more susceptible to ZIKV than other neuronal and somatic cells (Xu, YP., Qiu, Y., Zhang, B. et al., 2019). This discovery is further supported by research that claims ZIKV infects “hNPCs with high efficiency” (Tang et al., 2016, p. 588) and spreads the infectious virus to other parts of the brain. In addition, work published in Proceedings of the National Academy of Sciences of the United States of America states that ZIKV is “highly infective and cytotoxic” to hNPCs (Muffat et al., 2018, p. 7118). The identification of hNPCs as vulnerable cells reduces the scope of medication and therapy research and scientists can focus on hNPCs in treating ZIKV infection. The consensus reached by these three studies demonstrates that organoids modeling ZIKV is reliable in producing consistent results, which is essential in evaluating the effectiveness of these miniature organs. Additionally, it also reflects that organoids are proving to be effective ZIKV models that offer valuable insight into the target cells of ZIKV infection.

Since we have a very limited understanding of the immunity of hNPCs, this method enables us to gain more understanding of innate immunity of hNPCs to investigate ZIKV pathogenesis and develop potential drugs. ZIKV infects hNPCs and results in neural progenitor apoptosis which is the death of hNPCs. This will further lead to the death of the uninfected neurons in the neuronal layer and ventricular zone as the infection continues to spread and causes the reduction of the volume of hNPCs and neuronal layers. This is the cause of microcephaly. With this understanding, we can conclude that cortical organoids are significant for disease modeling and research because it allows a more comprehensive understanding of the cause and effect of the disease. Without the organoids, we are limited to the study of viral infection at the cellular level, which results in an incomplete picture of infection pathology. Organoids promise a more comprehensive picture by replicating whole brain regions that are compromised by infection, and they are indispensable for drug and therapy discovery

as organoids resemble a human's brain better compared to animal's brain because of interspecies variations. Therefore, we can conclude that cerebral organoids are useful in modeling ZIKV but the usefulness is restrained when experimental results are dependent on the presence of essential cells such as glia cells and other limitations.

4. Disease Modeling: Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disease that is characterised by dementia and cognitive impairment. It can be genetically inherited or developed in the later stage of life. Recently, it is found out that people who carry the APOE4 gene variant have a greater risk of developing AD compared to the other APOE "polymorphic alleles (APOE2, APOE3)" (Zhao et al., 2020, p. 2). With that understanding, cerebral organoids are utilised to affirm this discovery and also to study the cause of this phenomenon.

In this research, AD patient-derived cells that contain APOE3 and APOE4 and normal unaffected stem cells that contain APOE3 and APOE4 are collected to compare the effect of APOE4 to both groups as AD can be familial AD and sporadic AD. The results report some main findings of AD organoids and the following is two of them, 1. APOE4 gene aggravates apoptosis and synaptic loss in the brain organoids. 2. By converting APOE4 to APOE3, the soluble A β levels (A β is a main component of A β plaques that are present in AD patients' brains) (Zhao et al., 2020). This research provides evidence that APOE4 gene variant increases the risk of getting AD. From this, it shows the significance of organoids in tracking a risk gene, allowing scientists to further study potential methods to change APOE4 in reducing vulnerability of humans getting AD. It has already been found out that by using CRISPR/Cas9 (a method of gene editing), APOE4 can successfully be converted to APOE3 in organoids, which can potentially be an effective therapy in treating AD. In addition, with the use of organoids, scientists also reaffirm that the inhibition of β and γ -secretase can greatly reduce the production of A β (It is previously tested in human clinical trials led by Stefan Viktor Vormfelde and Michael F, Egan.) (Vormfelde et al., 2020) (Egan et al., 2019). This result also indicates the benefits of organoids in studying the mechanism. With this knowledge, scientists can explore ways to inhibit these two enzymes which can potentially be a valid therapy for AD.

From these experimental data, it evidently shows that more developments have been discovered in researching ZIKV compared to AD. The success discovered in AD from organoids are mainly on connecting internal biological change resulting from AD and genetic features that increases humans' vulnerability to AD, and there hasn't been much innovation in potential drug and therapy research. Although genetic engineering may be used to tackle AD, it cannot be done in the near future. This is a reasonable identification as cerebral organoids are better at resembling the early development of the human brain and technologies that artificially mature organoids need to be further refined. Thus, we can draw the conclusion that at present, cerebral organoids are useful in studying AD, but are not very effective in recognising prospective treatments.

5. Conclusion

Cerebral organoid technology is relatively new to the field of medicine, with its first establishment around 8 years ago. Presently, there have been many advancements made in the structure of cerebral organoids such as vascularisation and blood brain barrier that refine its ability to replicate the human brain, and this further leads to approving discoveries in neurological diseases and disorders such as ZIKV and AD. These developments illustrate that this technology is useful in understanding some areas of the brain and it has started our investigation in drugs and therapies. Despite this, there are still many brain cells that haven't been grown successfully in vitro and scientists only use cortical organoids and haven't been able to differentiate cells to form organoids representing other parts of the brain to model the disease. These are all limitations that the current model fails to replicate. Therefore, we can conclude that cerebral organoid technology is currently not sufficiently useful.

Nevertheless, its development has been rapid in the last 8 years. Also, we expect this technology to develop and the limitations to be solved as this is an area of great interest. Therefore, it promises to be a useful and productive tool in the future.

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