Challenges and future perspectives for 3D cerebral organoids as a model for complex brain disorders

NEUROSCIENCE RESEARCH NOTES

OPEN ACCESS | EDITORIAL ISSN: 2576-828X

Challenges and future perspectives for 3D cerebral organoids as a model for complex brain disorders

Pike-See Cheah [1,2,*], John O. Mason [3] and King-Hwa Ling [2,4,5]

[1] Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2] Genetics and Regenerative Medicine Research Centre, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3] Centre for Discovery Brain Sciences, The University of Edinburgh, Edinburgh EH8 9XD, United Kingdom.
[4] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[5] Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.
* Correspondence: cheahpikesee@upm.edu.my; Tel.: +603-8947 2355

Published: 12 January 2019
https://doi.org/10.31117/neuroscirn.v2i1.28

Keywords: cerebral organoids; 3D culture; microfluidic platform; precision medicine;

1.0 INTRODUCTION

The human brain is made up of billions of neurons and glial cells which are interconnected and organized into specific patterns of neural circuitry, and hence is arguably the most sophisticated organ in human, both structurally and functionally. Studying the underlying mechanisms responsible for neurological or neurodegenerative disorders and the developmental basis of complex brain diseases such as autism, schizophrenia, bipolar disorder, Alzheimer's and Parkinson's disease has proven challenging due to practical and ethical limitations on experiments with human material and the limitations of existing biological/animal models. Recently, cerebral organoids have been proposed as a promising and revolutionary model for understanding complex brain disorders and preclinical drug screening.

Cerebral organoids are *in vitro*-derived structures that resemble broad regional identities of the brain that recapitulate key features during brain development [1]. Various methods for neural culture have been published [2]. From embryoid body-derived neural rosette culture [3] to serum free floating culture of embryoid body-like aggregates approaches [4,5], the Lancaster protocol [6] is probably one of the most widely used methods to culture cerebral organoids. To more accurately mimic the complexity of the human brain, human embryonic stem cell lines (hESCs) and induced pluripotent stem cells (iPSCs) have been used to establish miniature replicas of human brain. These cells can self-organize into complex structures, which model both proper 3D organization and the normal development of multiple brain regions. This model also recapitulates normal

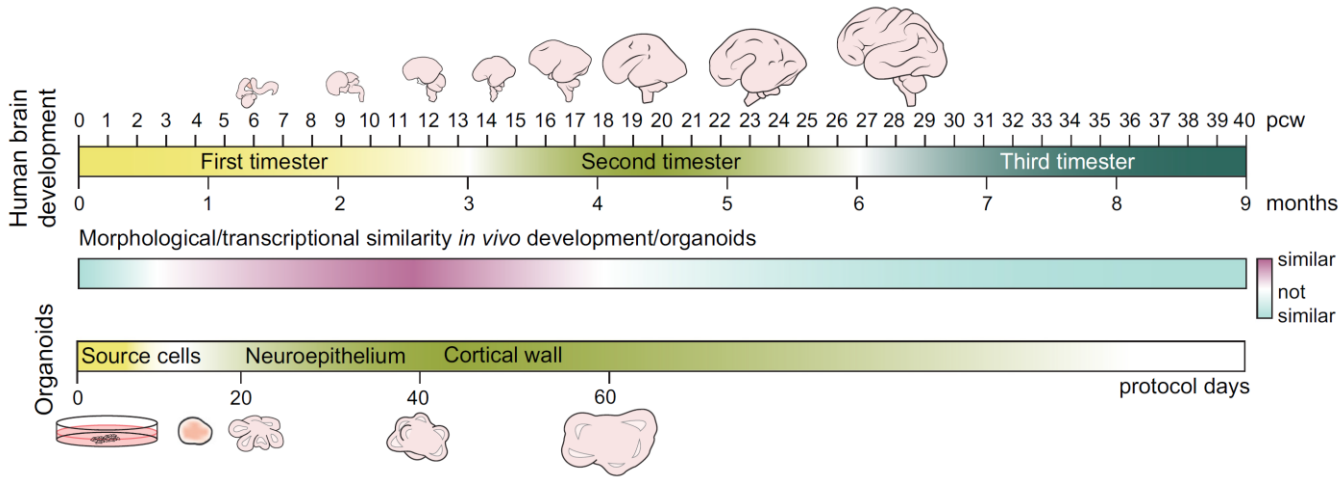

Figure 1. Timeline of human brain and cerebral organoid development and their similarity throughout development (this figure is reproduced without any modifications from Kelava and Lancaster, 2016 [7] under the open access terms and creative common license CC BY 4.0,).

brain development on a gene expression, cellular and biological level, exhibiting almost all the major functional and neuroanatomical structures found in a developing fetal brain (Figure 1) [7].

2.0 CHALLENGES FACING CEREBRAL ORGANOIDS AS A MODEL

Cerebral organoids have become increasingly popular as models to study various neurological, neurodegenerative and neuropsychiatric disorders. Despite their superiority in mimicking key aspects of physiological brain development and function, various challenges remain unresolved.

- ***Random positioning of the developing brain structures***

  Cerebral organoids develop in the absence of extra-embryonic tissues that provide essential cues to set up the antero-posterior axis of the embryonic brain. As a result, cerebral organoids lack normal [8] embryonic axis formation, an important mechanism that instructs the ordered formation of specific brain regions in the correct orientation.

- ***Cerebral organoids are not vascularized***

  The lack of vascularization imposes constraints on the growth and development of organoids. Cerebral organoids often contain a necrotic core of cells that fail to survive in the absence of a blood supply. The absence of vascularization also currently precludes the use of organoids to study brain disorders that specifically involve the circulation, such as stroke. At rest, the richly vascularized adult brain receives approximately 15-20% of cardiac output. The absence of a vascular network in current cerebral organoids limits their application in the study of angiogenesis and cerebrovascular diseases. Current approaches to vascularize organoids including transplantation into mouse brain [9] and co-culture of neural cells with endothelial cells [8]. With recent development of engineered 3D vascular and neuronal networks [10], scientists can grow cerebral organoids directly on a microfluidic chip platform with combination of the relevant signaling molecules to enable proper patterning [11]. Vascularized organoids may be helpful for studying tumors as the vasculature is needed for growth of cancerous cells and facilitates metastasis.

- ***Organoids lack a blood-brain barrier (BBB)***
  Although some aspects of a normal vascular network could be mimicked in a microfluidic platform, this could probably not be used to study the BBB. Attempts to reproduce the BBB in organoids include the co-culture of a single layer of brain microvascular endothelial cells together with pericytes and astrocytes [8]. The presence of a realistic BBB in cerebral organoids would make the model valuable for studying drug delivery and safety. Despite the success in mimicking BBB in various mixed cultures, the difficulty in modulating the tightness of BBB junctions and trans endothelial electrical resistance may stand in the way of making the system fully functional [12].

- ***Difficult to orchestrate the development, support and function of glia architecture***
  Glial architectural is essential to support the microenvironment of the CNS. The glial network includes microglia (resident innate immune cells derived from the mesoderm), astrocytes and oligodendrocytes (also known as neuroglia and derived from the ectoderm). They play crucial roles in the development and function of the brain and have been linked to various neurodevelopmental and neurodegenerative disorders [13,14]. The colonization timepoints of microglia, astrocytes and oligodendrocytes at different brain regions during neurodevelopment vary. The random positioning of different brain regions in cerebral organoids makes it difficult to recapitulate timely glial cell colonization and normal neuron-glia interaction in cerebral organoids. Despite these limitations, it was recently reported that microglia can also be innately derived from mesodermal cells within early stage cerebral organoids. The glia exhibited typical morphology, molecular profile, behavior and function of microglia *in vivo* [15].

- ***Cellular heterogeneity and technical variability affect the consistency of cerebral organoids***
  Different methods of culturing cerebral organoids and technical variability influence the cellular diversity and morphology of organoids, potentially affecting phenotypes of interest. Seeding different numbers of cells, timing of embedding, variations in genetic background and use of incompletely-defined materials (such as Matrigel) significantly affect the efficiency of embryoid body formation, their size, cellular diversity and gene expression thus making batch-to-batch experimental variations hard to control [16,17].

- ***Technical challenges in long-term culture***
  Putting all the challenges above together, modeling neurodegenerative diseases that require long-term cultivation (from 9 months to years) of cerebral organoids is a very challenging task. As depicted in Figure 1, the transcriptome signatures of cerebral organoids are comparable to those of human brain during early development but not after second trimester. To model diseases in the adult or aging brain, long-term cerebral organoid culture is necessary. However, cerebral organoids are limited in their growth potential, due to the limited supporting scaffold and inaccessibility of cells within the organoids to the relevant nutrients leading to hypoxic or suboptimal growth conditions. To overcome this challenge, 3D printing of biomaterial scaffolds that couple with an artificial vascular network as well as microfluidic platforms may eventually enable much longer-term cerebral organoid cultivation.

3.0 FUTURE PERSPECTIVE OF PRECISION MEDICINE USING CEREBRAL ORGANOIDS

Despite the unresolved challenges, the limitations of 2D and genetically engineered mouse models highlight the tremendous potential of cerebral organoids in cancer therapy, neurodevelopmental diseases, neurodegenerative diseases and personalized medicine. Development of *in vitro* human cerebral organoids has offered new alternative avenues for disease modeling directly in human brain cells. Encouragingly, organoid technology together with advanced CRISPR-Cas9 gene-editing approach have successfully initiated tumorigenesis in cerebral organoids [18,19].

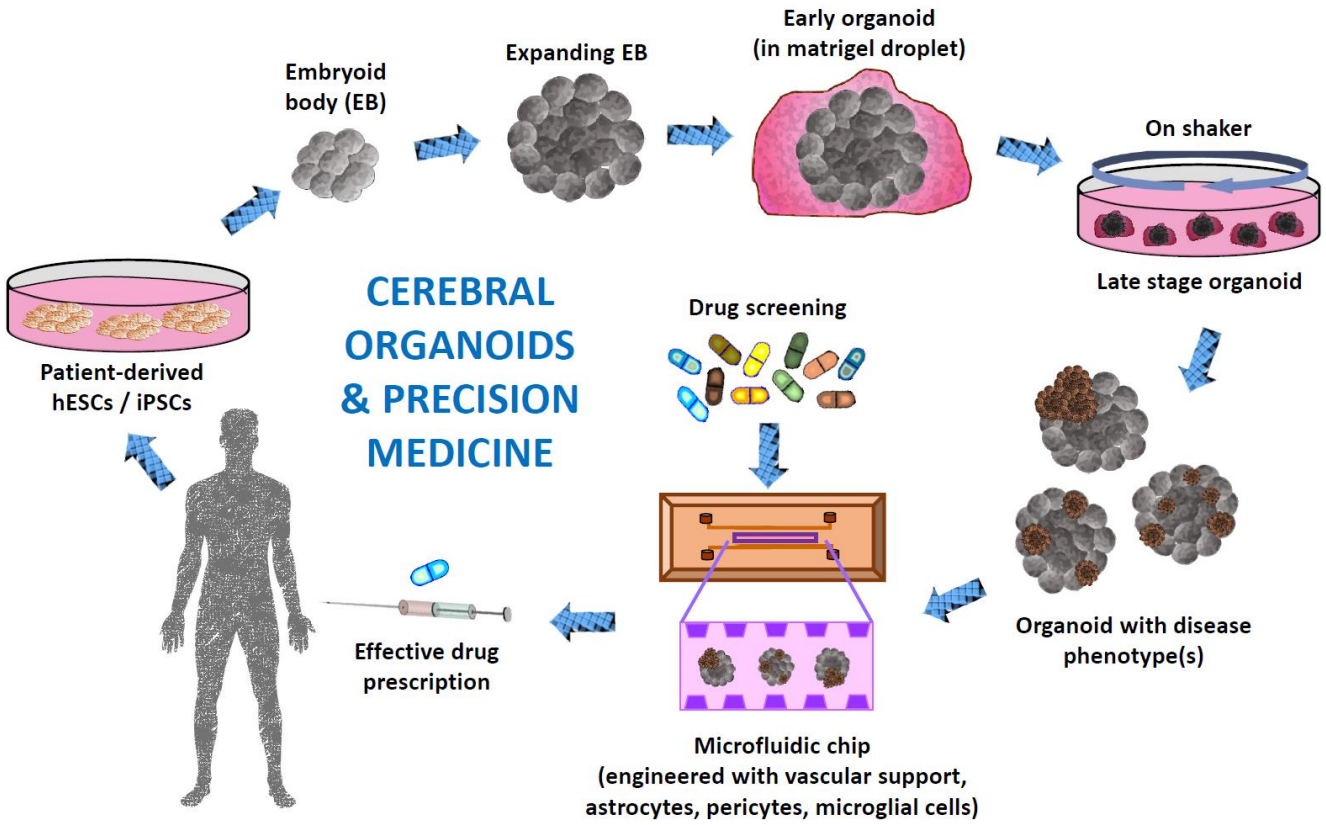

Figure 2. Schematic representation of 3D culture technology in generating cerebral organoids from patient-derived hES/iPS cells for precision medicine. The cerebral organoids can be generated in large scale and cultured in microfluidic chip, further subject for drug screening, eventually leading to personalized therapeutic approaches.

These models exhibit key features of cancer, such as cancer phenotypes, oncogenic-pathway-specific transcriptome profiles, and the potential for *in vivo* expansion and invasion.

Now, researchers are on the move to develop patient-specific organoids from a person's own stem cells, paving ways towards precision medicine [6]. In patient-derived organoids, patient's own immune cells can be incorporated to facilitate the study of immunotherapies or immune responses related to host-pathogen interactions. Such studies are difficult when patient's immune cells recognize generic organoids as foreign. In contrary, patient's immune cells will recognize the organoids of the same origin as self without the risk of rejection. This will lead to a huge prospect to model and study immune responses during host-pathogen interactions and susceptibility [20], and cancer development [21].

Conventionally, most patients with cancer receive similar "one-size-fits-all" treatment. It has recently become clear that certain treatments work well for some patients but do not show promising results in others. Individualized cancer treatments are progressively improving, and the trend has shifted towards "one dose, one patient" treatment. Personalized medicine means that clinicians and researchers need to obtain cells from a patient, grow brain organoids on a high throughout scale and test the effectiveness of a large set of drugs, finding the most appropriate for the patient (Figure 2). Personalized organoids which are directly derived from the patient, carry similar characteristics to the original tumors.

Together with drug-testing, it may result in more accurate prediction of drug response in patients and ultimately, represent an effective clinical usage of the organoid method [22].

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Di Lullo E, Kriegstein AR. The use of brain organoids to investigate neural development and disease. *Nat Rev Neurosci*. 2017;18(10):573-584. https://doi.org/10.1038/nrn.2017.107
2. Kelava I, Lancaster MA. Stem Cell Models of Human Brain Development. *Cell Stem Cell*. 2016;18(6):736-748. https://doi.org/10.1016/j.stem.2016.05.022
3. Zhang SC, Wernig M, Duncan ID, Brüstle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol*. 2001;19(12):1129-1133. https://doi.org/10.1038/nbt1201-1129
4. Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, et al. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell*. 2008;3(5):519-532. https://doi.org/10.1016/j.stem.2008.09.002
5. Watanabe K, Kamiya D, Nishiyama A, Katayama T, Nozaki S, Kawasaki H, et al. Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat Neurosci*. 2005;8(3):288-296. https://doi.org/10.1038/nn1402
6. Lancaster MA, Renner M, Martin C-A, Wenzel D, Bicknell LS, Hurles ME, et al. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501(7467):373-379. https://doi.org/10.1038/nature12517
7. Kelava I, Lancaster MA. Dishing out mini-brains: Current progress and future prospects in brain organoid research. *Dev Biol*. 2016;420(2):199-209. https://doi.org/10.1016/j.ydbio.2016.06.037
8. Bergmann S, Lawler SE, Qu Y, Fadzen CM, Wolfe JM, Regan MS, et al. Blood-brain-barrier organoids for investigating the permeability of CNS therapeutics. *Nat Protoc*. 2018;13(12):2827-2843. https://doi.org/10.1038/s41596-018-0066-x
9. Mansour AA, Gonçalves JT, Bloyd CW, Li H, Fernandes S, Quang D, et al. An in vivo model of functional and vascularized human brain organoids. *Nat Biotechnol*. 2018;36(5):432-441. https://doi.org/10.1038/nbt.4127
10. Osaki T, Sivathanu V, Kamm RD. Engineered 3D vascular and neuronal networks in a microfluidic platform. *Sci Rep*. 2018;8(1):5168. https://doi.org/10.1038/s41598-018-23512-1
11. Karzbrun E, Kshirsagar A, Cohen SR, Hanna JH, Reiner O. Human Brain Organoids on a Chip Reveal the Physics of Folding. *Nat Phys*. 2018;14(5):515-522. https://doi.org/10.1038/s41567-018-0046-7
12. Lauschke K, Frederiksen L, Hall VJ. Paving the Way Toward Complex Blood-Brain Barrier Models Using Pluripotent Stem Cells. *Stem Cells Dev*. 2017;26(12):857-874. https://doi.org/10.1089/scd.2017.0003
13. Bilimoria PM, Stevens B. Microglia function during brain development: New insights from animal models. *Brain Res*. 2014;1617:7-17. https://doi.org/10.1016/j.brainres.2014.11.032
14. Hickman S, Izzy S, Sen P, Morsett L, Khoury El J. Microglia in neurodegeneration. *Nat Neurosci*. 2018;21(10):1359-1369. https://doi.org/10.1038/s41593-018-0242-x
15. Ormel PR, de Sá RV, van Bodegraven EJ, Karst H, Harschnitz O, Sneeboer MAM, et al. Microglia innately develop within cerebral organoids. *Nat Commun*. 2018;9(1):4167. https://doi.org/10.1038/s41467-018-06684-2
16. Quadrato G, Nguyen T, Macosko EZ, Sherwood JL, Yang SM, Berger DR, et al. Cell diversity and network dynamics in photosensitive human brain organoids. *Nature*. 2017;545(7652):48-53. https://doi.org/10.1038/nature22047
17. Yakoub AM, Sadek M. Development and Characterization of Human Cerebral Organoids: An Optimized Protocol. *Cell Transplant*. 2018;27(3):393-406. https://doi.org/10.1177/0963689717752946
18. Ogawa J, Pao GM, Shokhirev MN, Verma IM. Glioblastoma Model Using Human Cerebral Organoids. *Cell Rep*. 2018;23(4):1220-1229. https://doi.org/10.1016/j.celrep.2018.03.105

19. Bian S, Repic M, Guo Z, Kavirayani A, Burkard T, Bagley JA, et al. Genetically engineered cerebral organoids model brain tumor formation. *Nat Methods*. 2018;15(8):631-639. https://doi.org/10.1038/s41592-018-0070-7
20. Iakobachvili N, Peters PJ. Humans in a Dish: The Potential of Organoids in Modeling Immunity and Infectious Diseases. *Front Microbiol*. 2017;8:2402. https://doi.org/10.3389/fmicb.2017.02402
21. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, et al. Organoid Modeling of the Tumor Immune Microenvironment. *Cell*. 2018;175(7):1972-1988.e16. https://doi.org/10.1016/j.cell.2018.11.021
22. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell*. 2015;161(4):933-945. https://doi.org/10.1016/j.cell.2015.03.053