

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

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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

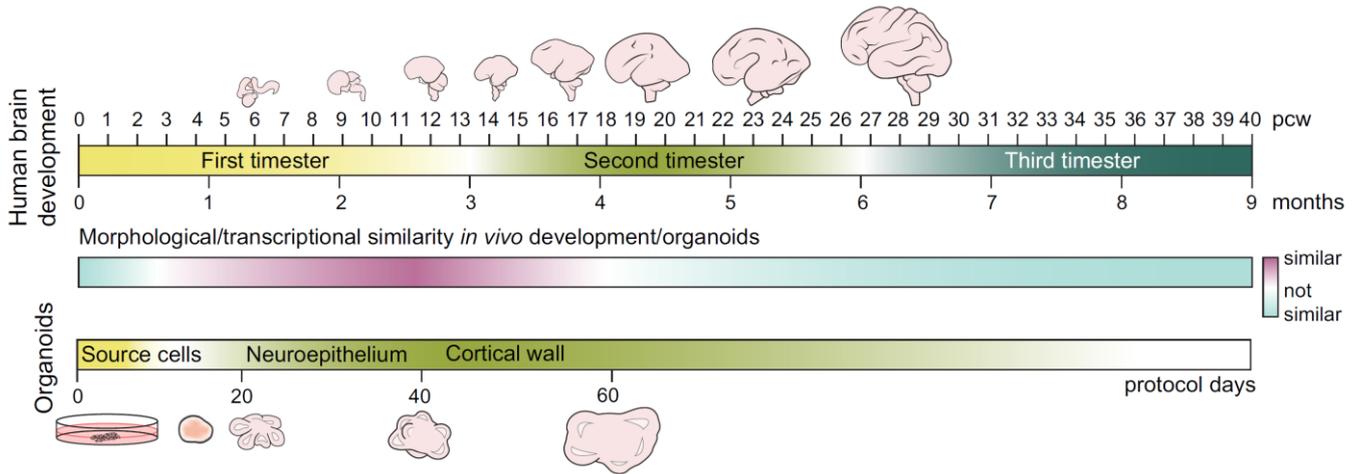


Figure 1. Timeline of human brain and cerebral organoid development and their similarity throughout development (this figure is reproduced without any modifications from Kelava and Lancaster, 2016 [7] under the open access terms and creative common license [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/),).

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 architecture 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 ORGANIDS

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].

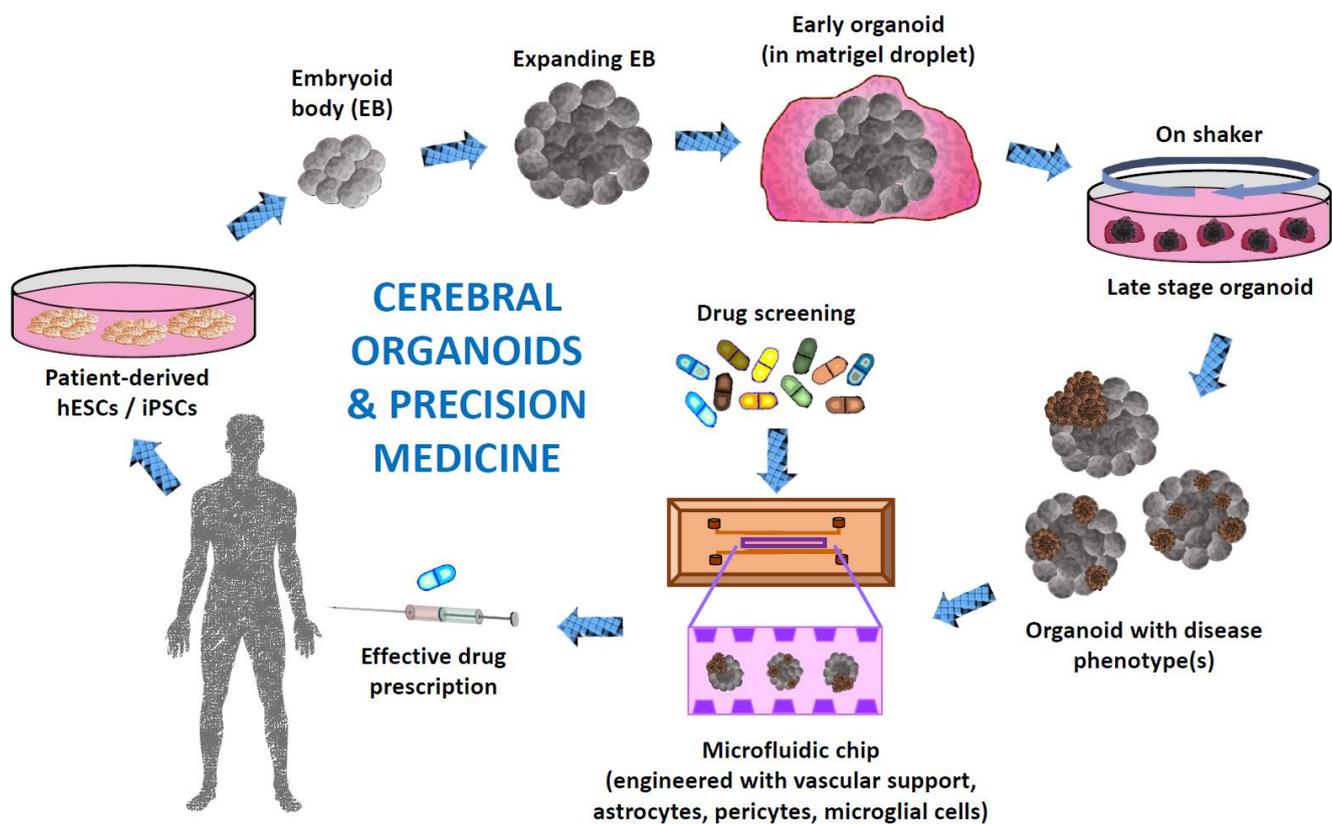


Figure 2. Schematic representation of 3D culture technology in generating cerebral organoids from patient-derived hES/iPS cells for precision medicine. The cerebral organoids can be generated in large scale and cultured in microfluidic chip, further subject for drug screening, eventually leading to personalized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

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