Minireview

Neural Organoids, a Versatile Model for Neuroscience

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Three-dimensional cultures of human neural tissue/organ-like structures in vitro can be achieved by mimicking the developmental processes occurring in vivo. Rapid progress in the field of neural organoids has fueled the hope (and hype) for improved understanding of brain development and functions, modeling of neural diseases, discovery of new drugs, and supply of surrogate sources of transplantation. In this short review, we summarize the state-of-the-art applications of this fascinating tool in various research fields and discuss the reality of the technique hoping that the current limitations will soon be overcome by the efforts of ingenious researchers.

Keywords: central nervous system, human pluripotent stem cells, in vitro modeling, neurodevelopment, neurological disorders, organoid

INTRODUCTION

The advent of new techniques often drives new discoveries that can change our concepts in science. It is likely that three-dimensional (3D) culture of brain-like organoids is one such technique. After the first report on the 3D culture of neural cell aggregates that exhibit some aspects of brain histotarchitecture (Eiraku et al., 2008), there has been an explosion of technical improvements in culture methods, and new discoveries have been made using these techniques (Kadoshima et al., 2013; Lancaster et al., 2013; Nakano et al., 2012; Qian et al., 2016; Warmflash et al., 2014). These 3D cultures of neural cell aggregates, collectively called neural organoids (NOs), are formed by recapitulating the developmental processes and organization of the developing human brain in vitro. It is still unclear how 3D culture of human pluripotent stem cells (hPSCs) resulted in strikingly different and in vivo-like consequences compared to the two-dimensional (2D) monolayer culture (Costa et al., 2016; Duval et al., 2017). However, it is widely speculated that position-dependent cellular signaling and increased signal tone by compact cellular communications provide favorable conditions for in vivo-like developmental progress (Kapałczyńska et al., 2018). The ability of hPSCs to produce the brain in vivo or NOs in vitro is associated with their self-organization property, defined as a cell’s ability to organize specialized morphology and histotarchitecture (Brassard and Lutolf, 2019; Eiraku et al., 2008; Kadoshima et al., 2013). These processes often coincide with cell type specification, and the separation of these two events is difficult to achieve in vivo. In this respect, NOs provide a unique experimental model to segregate cell differentiation and morphogenesis processes and to explore neural development and disease processes beyond the currently available levels. NOs have been successfully used to supplement or replace animal models and to address the unique features of human nervous system development (Arm and Pašca, 2018; Di Lullo and Kriegstein, 2017; Kim et al., 2020; Koo et al., 2019). This review elaborates on the usefulness and potential
of NOs, for promoting their use in research.

**NEURAL ORGANOIDS FOR DEVELOPMENTAL STUDIES**

NOs are produced by mimicking developmental processes, and our knowledge about neural development is the most important resource for the establishment of protocols for the region-specific NO production. Typically, protocols for NO generation consist of 3-4 steps, each mimicking the continuous but conceptually segregated in vivo developmental cascades (Fig. 1). hPSCs are first induced into neuroepithelia-like cell populations (neural induction step) by applying factors known to determine neural fates. Induction of anterior and posterior neural parts occurs through different developmental processes: anterior parts are induced by the conventional ‘dual SMAD inhibition’ procedure (Chambers et al., 2009), whereas caudal neural induction requires transient neuromesodermal fate specification (Denham et al., 2015; Gouti et al., 2014; Lippmann et al., 2015). After initial neural induction, 3D neural cell clusters can be further specified into specific domains depending on the applied regional morphogen (regional specification step). Because local signals pointing to specific brain regions have been identified in vivo, many region-specific NO protocols have been successfully established by screening efficient recipes of these factors. These specified NOs can then be cultured in the appropriate conditions for neural differentiation, long-term maturation, and advanced neural development (neural differentiation/maturation step; summary in Table 1).

During the maturation period, NO exhibits many features of histogenesis including migration, neurogenesis, and laminar formation. Studies on cerebral organoids have demonstrated the formation of human-specific outer subventricular zone neurogenic niches, highlighting that hPSC-derived NOs recapitulate the human-specific features of brain development (Bershteyn et al., 2017; Kadoshima et al., 2013; Lancaster et al., 2013; Qian et al., 2016). An extended NO culture can reproduce gliogenesis and myelination, which mainly occur during the postnatal stages (Bershteyn et al., 2017; James et al., 2021; Madhavan et al., 2018; Shaker et al., 2021). However, the occurrence of these postnatal events does not directly indicate that the NO reaches mature stages resembling the postnatal brain. Transcriptome or epigenome analyses suggest that an approximately 10-month culture is required to obtain perinatal features, even though gliogenesis is evident by 2-4 months of culture (Amiri et al., 2018; Gordon et al., 2021; Yoon et al., 2019). These discrepancies might be caused by the protocols for NO production, asynchronized developmental progress in NOs, and different experimental sensitivities/methodologies. Considering that later brain development is more influenced by nearby structures and cell migration across embryonic brain regions, and that neural circuit formation with other brain regions is essential for later brain development (Tau and Peterson, 2010; Valiente

![Fig. 1. The strategy to generate NOs.](image-url)
Table 1. Summary of the region-specific NO generation procedures

| NO type                  | Neural induction | Regional specification | Neural differentiation/maturation | Reference         |
|--------------------------|------------------|------------------------|-----------------------------------|-------------------|
| Cerebral organoid        | - (N2 medium)    | -                      | -                                 | Lancaster et al., 2013 |
| Cortical organoid        | DKK-1 (Wnt inhibitor) LeftyA (TGFβ inhibitor) BMPRIA-Fc | -                      | -                                 | Eiraku et al., 2008 |
| Cortical organoid        | IWP1e (Wnt inhibitor) SB431542 (TGFβ inhibitor) | 40% O₂, FBS 10%          | Matrigel (2% in medium)           | Kadoshima et al., 2013 |
| Forebrain organoid       | Dorsomorphin (BMP inhibitor) A83-01 (TGFβ inhibitor) | WNT3A, CHIR99021 (GSK3 inhibitor) SB431542 (TGFβ inhibitor) | BDNF, GDNF (neurotrophic factors) TGFβ Ascorbic acid (vitamin C) cAMP | Qian et al., 2016 |
| Ventral forebrain organoid | Dorsomorphin (BMP inhibitor) SB431542 (TGFβ inhibitor) | FGF2, EGF               | BDNF, NT3 (neurotrophic factors) | Paşca et al., 2015 |
| Subpallium spheroid (ventral forebrain) | Dorsomorphin (BMP inhibitor) SB431542 (TGFβ inhibitor) | FGF2, EGF, IWP2 (Wnt inhibitor) SAG (SHH agonist) | BDNF, NT3 (neurotrophic factors) | Birey et al., 2017 |
| Choroid plexus organoid  | - (Wnt inhibitor) | CHIR99021 (GSK3 inhibitor) BMP4 | Matrigel (2% in medium)           | Pellegrini et al., 2020 |
| Hippocampus organoid     | IWP1e (Wnt inhibitor) SB431542 (TGFβ inhibitor) | CHIR99021 (GSK3 inhibitor) BMP4 | 40% O₂                             | Sakaguchi et al., 2015 |
| Optic cup organoid       | IWP1e (Wnt inhibitor) | CHIR99021 (GSK3 inhibitor) SAG (SHH agonist) | -                                 | Nakano et al., 2012 |
| Thalamus organoid        | SB431542 (TGFβ inhibitor) LDN193189 (BMP Inhibitor) Thioglycerol | BMP7                      | BDNF (neurotrophic factors) Ascorbic acid (vitamin C) | Xiang et al., 2019 |
| Hypothalamus organoid    | SB431542 (TGFβ inhibitor) LDN193189 (BMP Inhibitor) | PD325901 (MEK-ERK inhibitor) WNT3A | FGF2, CNTF | Qian et al., 2016 |
| Midbrain organoid        | SB431542 (TGFβ inhibitor) Noggin (BMP inhibitor) CHIR99021 (GSK3 inhibitor) | FGF8, SHH | BDNF, GDNF (neurotrophic factors) Ascorbic acid (vitamin C) cAMP | Jo et al., 2016 |
| Midbrain organoid        | SB431542 (TGFβ inhibitor) LDN193189 (BMP Inhibitor) | LDN193189 (BMP Inhibitor) SHH, purmorphamine (SHH agonist) FGF8 | BDNF, GDNF (neurotrophic factors) TGFβ Ascorbic acid (vitamin C) cAMP | Qian et al., 2016 |
| Midbrain organoid        | Dorsomorphin (BMP inhibitor) A83-01 (TGFβ inhibitor) IWP2 (Wnt inhibitor) CHIR99021 (GSK3 inhibitor) | FGF8, SAG (SHH agonist) | BDNF, GDNF (neurotrophic factors) Ascorbic acid (vitamin C) cAMP | Kwak et al., 2020 |
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and Marin, 2010), regionally isolated NOs cannot replicate this interaction-based neural development. Further, non-neuronal lineage cell populations, such as blood vessels and microglia (Adams and Eichmann, 2010; Lenz and Nelson, 2018), also play important roles in brain development, which cannot be generated by simple NO induction. Accordingly, many approaches to include multiple components in NO have successfully demonstrated that some aspects of these insufficiencies can be overcome by complex technologies such as fusion, assembly, connection, and polarization (Fig. 2).

Cumulative results have demonstrated that NOs faithfully replicate many aspects of the developmental progress of the human brain. Thus, NOs provide a great opportunity to glimpse human brain development in vitro with many destructive technologies. However, in vitro NO growth is not completely the same as brain development in vivo, and observations from the NO model cannot provide direct evidence that the same events occur during human development. In this respect, to identify novel developmental processes of the human brain, human specimen studies need to be combined with NO-based observations, increasing the need for access to the embryonic human brain using innovative imaging methods or embryonic human brain banks (Kretzschmar, 2009; Lee et al., 2016; Ueda et al., 2020).

### NEURAL ORGANOIDS FOR DISEASE MODELING AND DRUG SCREENING

Modeling human brain diseases using NO technology is one of the major goals that researchers pursue. Considering that NOs are produced via a developmental program for organogenesis, modeling developmental defects were immediately addressed upon the advent of the NO technique. Observation of known developmental defects with specific genetic mutations in NO cultures is considered per se as efficient validation method of the NO culture system. Therefore, modeling of developmental defects and establishment of NO culture methods have emerged inseparably in most early studies (Birey et al., 2017; Lancaster et al., 2013; Li et al., 2017; Qian et al., 2016). Further, as early developmental defects are closely associated with morphological defects, histological analyses are the major tools used to evaluate them. Therefore, defects in early morphogenesis, neurogenesis, and cell migration can be addressed by the morphology, size, and cellular composition of NOs. For instance, early neureulation-related cellular specification is closely associated with tube morphogenesis, and the NO model has been successfully used for modeling tube morphogenesis (Karzbrun et al., 2021; Lee et al., 2020). Similarly, microcephalic phenotypes in human genetic mutations are not faithfully replicated in mouse models, whereas NO-based assays can successfully replicate these phenotypes (Lancaster et al., 2013; Qian et al., 2016; Zhang et al., 2019).

Considering that many rare genetic mutations that cause developmental brain disorders have been identified by state-of-the-art whole-genome screening, combinations of gene editing and NO techniques have great potential to address these rare genetic diseases. A list of the successful studies of these types of developmental disease modeling is presented in Table 2.

| NO type | Neural induction | Regional specification | Neural differentiation/maturation | Reference |
|---------|------------------|------------------------|----------------------------------|-----------|
| Cerebellum organoid | SB431542 (TGFβ inhibitor) | FGF2 | BDNF, GDNF, NT-3 (neurotrophic factors) | Muguruma et al., 2015 |
| Brainstem organoid | Dorsomorphin (BMP inhibitor) | SB431542 (TGFβ inhibitor) | BDNF, GDNF, NT-3 (neurotrophic factors) | Eura et al., 2020 |
| Spinal cord organoid | CHIR99021 (GSK3 inhibitor) | SB431542 (TGFβ inhibitor) | BDNF, GDNF, NT-3 (neurotrophic factors) | Ogura et al., 2018 |
| Spinal cord organoid | CHIR99021 (GSK3 inhibitor) | SB431542 (TGFβ inhibitor) | BDNF, GDNF, NT-3 (neurotrophic factors) | Lee et al., 2020 |

BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; CNF, olfactory neurotrophic factor; CAMP, cyclic adenosine monophosphate; EGF, epidermal growth factor; FBS, fetal bovine serum; FGF, fibroblast growth factor; GDNF, glial cell-derived neurotrophic factor; GSK3, glycogen synthase kinase-3; NT3, neurotrophin-3; SAG, smoothened agonist; SD1, stomal cell-derived factor 1; SHH, sonic hedgehog; TGFβ, transforming growth factor-beta.
Defects in later developmental programs often do not exhibit overt morphological changes; therefore, more precise analyses with physiological tools are required. Methods for detecting the physiological responses of NOs have been widely used only recently (Osaki and Ikeuchi, 2021; Trujillo et al., 2019; Zafeiriou et al., 2020), and the physiological defects in these models are only beginning to be reported (Andersen et al., 2020; Birey et al., 2017; Mariani et al., 2015; Samarasinghe et al., 2021; Ye et al., 2017). For instance, epileptic episodes can be recognized by altered neuronal burst activity and local field potentials. Accordingly, using these physiological tools, Rett’s syndrome and Schizophrenia were successfully modeled using NOs derived from patient iPS cells (Samarasinghe et al., 2021; Ye et al., 2017). The use of NOs for psychiatric diseases is more challenging because these pathologies are known to be associated with neural circuit formation, synaptic plasticity, and other sophisticated abnormalities without gross signs of structural changes (Das et al., 2020; McCleague et al., 2017; Van Spronsen and Hoogenraad, 2010). Many brain regions are involved in the pathogenesis of psychiatric diseases, and region-specific NOs may have limited potential to exhibit these circuit-dependent pathologies. Furthermore, psychiatric diseases are often modeled in experimental animals exhibiting similar behavioral defects. However, the current NO models have limited complexity compared to multiregional neural circuits in vivo and have no readout systems comparable to animal behavior. Therefore, these limitations impede the use of NO in modeling these types of diseases.

NOs have also been successfully used to model neurodegenerative diseases. NOs produced from iPSCs of patients with Alzheimer’s disease (AD)-related mutations exhibit AD-like symptoms, such as amyloid-beta deposits (Jack et al., 2010; Murphy and LeVine, 2010). The NO-based Parkinson’s disease model has also been reported to exhibit Lewy body-like inclusions and alpha-synuclein aggregations (Jo et al., 2021). It is unclear why these late-onset symptoms in aged human patients were observed in the NOs, which replicate a much younger aged brain. It is plausible that the current ‘standard’ cultures of NO may be pro-degenerative owing to non-humanized media and the lack of sufficient signals/factors for healthy growth, which is presumably supplied from the blood or other parts of the body in vivo. Supporting this, NOs have been reported to exhibit increased transcriptome signatures associated with oxidative stress compared to stage-matched embryonic brains (Bhaduri et al., 2020). Alter-

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**Fig. 2. Complex NO models.** Fusion is defined as a hybrid among region-specific NOs. This approach is considered a way to investigate the interaction of different regional NOs, and can be easily obtained by positioning two or more NOs close to each other. It is suitable for exploring cellular migration or innervation of nerve fibers. Assembly is defined as a connection or co-culture of NO and non-neural tissue/cells. Upon the developmental program, tissues derived from mesoderm/endoderm that cannot be simultaneously induced from neural induction protocol are generated separately and then mixed with NO. For examples, the assembloid with microglia is provided as a model to observe neuro-immune responses within NOs. Connection technology provides a system capable of building a sophisticated neural network between NOs. To establish axonal connectivity, microdevices are required to allow directional axon outgrowths. Polarized NOs represent enhanced patterning features by the external stimulation. Polarization can be achieved by latest technologies such as inducible focal gene expression, microfluidic gradients, or micropatterning.
| NO type                     | Disease                                                                 | Causes or risk factors                                                                 | Map associated disease phenotype in NOs                                                                 |
|----------------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Cerebral organoid          | Neurodevelopmental diseases (morphological defects)                     | CDK5RAP2 mutation                                                                     | Neuronal progenitor apoptosis and increased neuronal progenitor proliferation                             |
| Forebrain organoid         | Zika virus-induced microcephaly                                         | Zika virus infection                                                                   | Overall smaller NOs                                                                                   |
| Cerebral organoid          | Neurodevelopmental diseases (physiological defects)                     | Cerebral organoid Deletions of chromosome 17 (17p13.3)                                 | Mitotic defect in outer radial glial cells, increased apoptosis of neuroepithelial stem cells, and defective neuronal migration of cortical neurons |
| Telencephalic organoid     | Autism spectrum disorder                                                | Idiopathic ASD patient-derived hiPSC                                                  | Antiepileptic drug                                                                                   |
| Spinal cord organoid       | Neural tube defect                                                      | CACNA1C mutation                                                                       | Fused organoid (dorsal forebrain and ventral forebrain)                                               |

| Disease NO type            | Causes or risk factors                                                                 | Map associated disease phenotype in NOs                                                                 |
|----------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Neurodevelopmental diseases (morphological defects) | CDK5RAP2 mutation                                                                     | Neuronal progenitor apoptosis and increased neuronal progenitor proliferation                             |
| Neurodevelopmental diseases (physiological defects) | Cerebral organoid Deletions of chromosome 17 (17p13.3)                                 | Mitotic defect in outer radial glial cells, increased apoptosis of neuroepithelial stem cells, and defective neuronal migration of cortical neurons |
| Telencephalic organoid     | Idiopathic ASD patient-derived hiPSC                                                  | Antiepileptic drug                                                                                   |
| Spinal cord organoid       | CACNA1C mutation                                                                       | Fused organoid (dorsal forebrain and ventral forebrain)                                               |

| Disease NO type            | Causes or risk factors                                                                 | Map associated disease phenotype in NOs                                                                 |
|----------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Neurodevelopmental diseases (morphological defects) | CDK5RAP2 mutation                                                                     | Neuronal progenitor apoptosis and increased neuronal progenitor proliferation                             |
| Neurodevelopmental diseases (physiological defects) | Cerebral organoid Deletions of chromosome 17 (17p13.3)                                 | Mitotic defect in outer radial glial cells, increased apoptosis of neuroepithelial stem cells, and defective neuronal migration of cortical neurons |
| Telencephalic organoid     | Idiopathic ASD patient-derived hiPSC                                                  | Antiepileptic drug                                                                                   |
| Spinal cord organoid       | CACNA1C mutation                                                                       | Fused organoid (dorsal forebrain and ventral forebrain)                                               |
### Table 2. Continued

| Disease | NO type | Causes of risk factors | Major associated disease phenotype in NOs | Potential therapeutic approaches | Reference |
|---------|---------|------------------------|------------------------------------------|----------------------------------|-----------|
| Rett syndrome | Fused organoid (cerebral cortex and ganglionic eminence) | MECP2 mutation | Hyperexcitability and hypersynchrony defects in the balance of excitatory and inhibitory synapses | Pifithrin-a (TP53 target inhibitor) | Ye et al., 2017 |
| Schizophrenia | Forebrain organoid | DISC1 mutation | Abnormal neural oscillation and delayed cell-cycle progression of radial glia cells | Ye et al., 2017 |
| Neurodegenerative disease | | | | | |
| Alzheimer's disease | Cortical organoid | APP duplication - PSEN1 mutation | Increased Aβ aggregation and hyperphosphorylation of tau protein | Compound E (γ-secretase inhibitor), BACE-1 (β-secretase inhibitor) | Raja et al., 2016 |
| Alzheimer's disease | Cortical organoid | APOE ε4 | Increased Aβ and tau protein localization in extracellular space and hyperphosphorylation of tau in neurons | 6 FDA-approved candidate drugs | Zhao et al., 2020 |
| Alzheimer's disease | Cerebral organoid | Sporadic AD patient-derived hiPSC APOE ε4 | Increased Aβ plaques in extracellular space and hyperphosphorylation of tau in neurons | 6 FDA-approved candidate drugs | Park et al., 2021 |
| Parkinson's disease | Midbrain organoid | LRRK2 mutation MPTP-induced neurotoxicity | Reduced dopaminergic differentiation and decreased neurite length | GSK2578215A (LRRK2 kinase inhibitor), TXNIP knockdown | Kim et al., 2019 |

*NO = Neurospheres Organoids*
natively, considering that the survival and growth of neurons are dependent on proper connectivity in vivo, the absence of appropriate neural connectivity in the NO model may be responsible for its pro-degenerative nature. Improvement of culture techniques and precise control of normal-like NO maturation are thus required for better modeling of neurodegenerative diseases.

In terms of disease modeling, there have been many significant attempts to upgrade NO culture systems suitable for high-throughput screening (HTS). At least two important features have been addressed and improved to achieve this goal. First, the variability of NOs in different batches and within batches should be precisely controlled. Notably, the NO induction response is highly dependent on batches. It is unclear what causes these differences, but it is predictable that the quality and/or condition of hPSCs may greatly affect the consequences of treatment. Further, the format of cell culture may affect the homogeneity of cellular responses regardless of batches. For instance, because cultured hPSCs in 3D appear to exhibit more variable responses to induction reagents depending on their relative position in the cluster, there have been attempts to induce hPSCs into neural-lineage cells in 2D (Lee et al., 2020; Renner et al., 2020). The resultant ‘primed’ neural-fate cells can then be dissociated and reaggregated into 3D spheroids with a precisely controlled number of cells. These 3D spheroids can also form a brain histoarchitecture as seen in 3D-initiated NOs. These approaches have obvious benefits in terms of quantifiability because better control of the initial cell number and conditions strongly contributes to the reduction of variations. Considering that this approach may sacrifice some aspects of the histoarchitectures in conventional NOs, it is especially suitable for producing NOs for brain regions where the histoarchitecture is less obvious or ignorable. Second, culture formation should become large-scale and suitable for automation. To achieve this goal, several culture platforms have been proposed using micro- or macro-fluidic designs. These include micro-spin culture systems and microcavity arrays (Brandenberg et al., 2020; Cho et al., 2021; Qian et al., 2016). With the combination of robotics, large-scale cultures and HTS can be achieved, and NOs can be used for drug screening or toxicology testing at the industrial front.

**UNIQUE OPPORTUNITIES OFFERED BY THE NO MODEL**

The NO model provides unique opportunities that cannot be addressed by other experimental models. These irreplaceable features are mainly derived from the maximal flexibility of the in vitro system (Fatehullah et al., 2016; Kim et al., 2020). Human-based studies are mostly observational due to difficulties in accessing manipulation or intervention; thus, they provide only correlational information obtained by non-experimental research methods. On the contrary, genetic modification, testing for unidentified drugs, or surgery to change pre-existing condition of biological systems are possible with animal models (Barré-Sinoussi and Montagutelli, 2015). Although they are valuable tools to identify the importance of genes at the organismic level and to implicate some aspects of human
diseases, they are limited by the constraints of biological systems. For instance, in vivo development of the brain is under the constraints of non-neural tissues surrounding it, and it is quite complicated to consider neural development separately from these non-neural components. In this respect, the in vitro growth of NOs from single cells enables maximal flexibility in the modulation of environmental and genetic factors. Thus, NO models provide important experimental approaches for addressing questions that cannot be accessible otherwise. Some examples of ingeniously used NO models are as follows.

**Analysis of morphogenesis with physical factors**

Morphogenesis is controlled by multiple biochemical and physical factors such as cellular polarity, cell adhesion, and viscoelasticity (Von Dassow and Davidson, 2011; Zallen, 2007). Genetic mutation or treatment of small molecules that regulate biochemical pathways have been successfully used to elucidate the role of these multiple factors. However, these changes in in vivo models often affect both morphogenesis and cell differentiation, and the separation of two biological processes is complicated. Accordingly, the mechanism of morphogenesis has been less addressed. Furthermore, physical factors that may be important for controlling morphogenesis are difficult to evaluate in vivo and remain largely ignored. The advent of 3D culture of NOs opened up new possibilities for tackling this problem. For instance, the culture of NOs caged in a physically constrained microchamber resulted in cerebral gyrus-like folding (Karzbrun et al., 2018). The underlying mechanism appeared to be related to the surface-to-core differences in cell density and stiffness, and theoretical simulations supported these notions. Neural tube morphogenesis was also replicated in the NO model, either in the absence of extracellular matrix (ECM) or with the support of ECM/non-neural cells (Karzbrun et al., 2021; Lee et al., 2020). Thus, the physical factors underlying tissue morphogenesis will be more addressed based on the experimental alteration of physical factors and simulations using NO models.

**Evolutionary studies**

Owing to ethical and technical constraints on human research and the absence of an appropriate model, human evolution research has been almost impossible. Advances in biotechnology, including whole-genome sequencing, comparative genomic analysis, and the NO models, provide new opportunities to investigate human evolutionary studies (Mora-Bermúdez et al., 2016). At least two strategies have been reported for NOs as a model to explore human evolution, and comparison of NOs from human and other non-human primates has revealed that the emergence of human-specific cortical lamina features can be reproduced in vitro (Kanton et al., 2019; Pollen et al., 2019). Because invasive biological examinations are feasible with NO cultures, single-cell transcriptome analysis further provides potential biological cascades determining the evolution of human-specific features. For instance, experimental and comparative genomic research has reported the evaluation of the hominoid-type NOs regulated by evolutionary regulator gene, ZEB2 (Benito-Kwiecinski et al., 2021). The NOs derived from human PSC-manipulated ZEB2 was similar to the hominoids, showing less size expansion and atypical neural activity, compared to control human NOs. With more complete gene editing to achieve better homology with hominoid genotypes, these approaches can provide rich information on human brain evolution.

**Complex organoids/assembloids**

Since the cellular composition in NOs can be customized as desired in vitro, various genetically/environmentally distinguishable cell populations can be mixed to explore the specific contribution of specific cell populations during brain development or pathogenesis (Shi et al., 2020; Song et al., 2019; Wörsdörfer et al., 2019). Although mosaic animal models are available through genetic manipulation or cell transplantation approaches, the technological limitations of the utility are larger. On the contrary, the NO model provides greater freedom for experimental design. For instance, Rett syndrome was addressed using this strategy of mosaic fused organoids (Samarasinghe et al., 2021). hPSCs from Rett syndrome patients and control individuals were separated and used for producing cerebral organoids and ganglion eminence organoids, which provided inhibitory neurons to neural circuits in the cortical organoid. By reciprocal assembly of NOs from these different sources, it was discovered that epileptic symptoms in NOs from patients with Rett syndrome were strongly associated with ganglion eminence. In combination with cell-type-specific gene modulation, using cell populations from different sources and mosaic fused organoid/assembloid approaches will provide fruitful opportunities to tackle otherwise impossible questions.

**OUTLOOK**

NOs cannot replicate the brain accurately, and there are many limitations to be addressed. Some of these limitations might be overcome in the future, whereas others are too fundamental problems to solve them. By improving culture conditions and utilizing bioengineering techniques, NOs can be generated more similar to the human brain. Although most information on NO production has been borrowed from developmental studies, knowledge derived from NO-based studies will soon provide valuable insights into human brain development. Considering ethical principles, such as the risk-benefit ratio, it is very difficult to obtain normal human brain specimens. In this respect, even for disease modeling, the potential ability of NOs to replicate the normal human brain is valuable.

The flexibility of the experimental modulation of NO development may open up the so-called field of synthetic embryology. In combination with gene editing, synthetic embryology may serve as a tool for evolutionary biology, as synthetic biology utilizes the fundamental evolutionary concepts. This also means that synthetic neural networks can be produced based on artificial design, similar to the design and production of electrical circuits. These issues have never been addressed using either animal models or human studies; thus, such synthetic approaches will provide answers (and questions) about how neural circuits work and how they produce sophisticated...
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Brain functions. Finally, the importance of an industrial infrastructure for NOs cannot be emphasized. Considering the value of NOs as a disease model for basic research and drug development, enhancing the accessibility of NOs to the equivalent level of experimental animals will be key to maximizing the impact of NO techniques: for example, a biobank of organoids will facilitate NO-based research and development.

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AUTHOR CONTRIBUTIONS
W.S. conceived the project. W.S. and J.H.L. wrote the manuscript.

CONFLICT OF INTEREST
The authors have no potential conflicts of interest to disclose.

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REFERENCES

Adams, R.H. and Eichmann, A. (2010). Axon guidance molecules in vascular patterning. Cold Spring Harb. Perspect. Biol. 2, a001875.

Amin, N.D. and Paçá, S.P. (2018). Building models of brain disorders with three-dimensional organoids. Neuron 100, 389-405.

Amiri, A., Coppola, G., Scuderi, S., Wu, F., Roychowdhury, T., Liu, F., Pochareddy, S., Shin, Y., Safi, A., Song, L., et al. (2018). Transcriptome and epigenome landscape of human cortical development modeled in organoids. Science 362, eaat6720.

Andersen, J., Revah, O., Miura, Y., Thom, N., Amin, N.D., Kelley, K.W., Singh, M., Chen, X., Thete, M.V., Walczak, E.M., et al. (2020). Generation of functional human 3D cortico-motor assembloids. Cell 183, 1913-1929.

Bagley, J.A., Reumann, D., Bian, S., Lévi-Strauss, J., and Knoblich, J.A. (2017). Fused cerebral organoid model interactions between brain regions. Nat. Methods 14, 743-751.

Barré-Sinoussi, F. and Montagutelli, X. (2015). Animal models are essential to biological research: issues and perspectives. Future Sci. OA 1, FSO63.

Benito-Kwiecinski, S., Giandomenico, S.L., Sutcliffe, M., Riis, E.S., Fatehullah, A., Tan, S.H., and Barker, N. (2016). Organoids as an in vitro model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 15, 142-148.

Birey, F., Andersen, J., Makinson, C.D., Islam, S., Wei, W., Huber, N., Fan, H.C., Metzler, K.R.C., Panagiotakos, G., Thorn, N., et al. (2017). Assembly of functionally integrated human forebrain spheroids. Nature 545, 54-59.

Brandenberg, N., Hoehnel, S., Kuttl, F., Homicsko, K., Ceroni, C., Ringel, T., Gjorevski, N., Schwank, G., Coukos, G., Turcatti, G., et al. (2020). High-throughput automated organoid culture via stem-cell aggregation in microcavity arrays. Nat. Biomed. Eng. 4, 863-874.

Brassard, J.A. and Lutolf, M.P. (2019). Engineering stem cell self-organization to build better organoids. Cell Stem Cell 24, 860-876.

Cederquist, G.Y., Asciolla, J.J., Tchieu, J., Walsh, R.M., Cornachia, D., Resh, M.D., and Studer, L. (2019). Specification of positional identity in forebrain organoids. Nat. Biotechnol. 37, 436-444.

Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M., and Studer, L. (2009). Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat. Biotechnol. 27, 275-280.

Chao, A.N., Jin, Y., An, Y., Kim, J., Choi, Y., Lee, J.S., Kim, J., Choi, W.Y., Koo, D.J., Wu, S., et al. (2021). Microfluidic device with brain extracellular matrix promotes structural and functional maturation of human brain organoids. Nat. Commun. 12, 4730.

Costa, E.C., Moreira, A.F., de Melo-Diogo, D., Gaspar, V.M., Carvalho, M.P., and Correia, U.J. (2016). 3D tumor spheroids: an overview on the tools and techniques used for their analysis. Biotechnol. Adv. 34, 1427-1441.

Das, S., Ramakrishna, S., and Kim, K.S. (2020). Critical roles of deubiquitinating enzymes in the nervous system and neurodegenerative disorders. Mol. Cells 43, 203-214.

Denham, M., Hasegawa, K., Menheniott, T., Rollo, B., Zhang, D., Hough, S., Alshwaf, A., Febbraro, F., Ighianyan, S., Leung, J., et al. (2015). Multipotent caudal neural progenitors derived from human pluripotent stem cells that give rise to lineages of the central and peripheral nervous system. Stem Cells 33, 1759-1770.

Dí Lullo, E. and Kriegstein, A.R. (2017). The use of brain organoids to investigate neural development and disease. Nat. Rev. Neurosci. 18, 573-584.

Duval, K., Grover, H., Han, L.H., Mou, Y., Pegoraro, A.F., Fredberg, J., and Chen, Z. (2017). Modeling physiological events in 2D vs. 3D cell culture. Physiology (Bethesda) 32, 266-277.

Eiraku, M., Watanabe, K., Matsu-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma, K., and Sasai, Y. (2008). Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell 3, 519-532.

Eura, N., Matsui, T.K., Luginbühl, J., Matsubayashi, M., Nanaura, H., Shiota, T., Kinugawa, K., Iuchi, N., Kiriyama, T., Zheng, C., et al. (2020). Brainstem organoids from human pluripotent stem cells. Front. Neurosci. 14, 538.

Fatemi, A., Tschirridis, A., Wymeersch, F.J., Huang, Y., Kleinjung, J., Wilson, V., and Briscoe, J. (2014). In vitro generation of neuromesodermal progenitors reveals distinct roles for Wnt signalling in the specification of spinal cord and paraxial mesoderm identity. PLoS Biol. 12, e1001937.

Hor, J.H., Soh, E.S.Y., Tan, L.L.Y., Lim, V.J.W., Santos, M.M., Winanto, Ho, B.X., Fan, Y., Soh, B.S., and Ng, S.Y. (2018). Cell cycle inhibitors protect motor neurons in an organoid model of Spinal Muscular Atrophy. Cell Death Dis. 9, 1100.

Jack, C.R., Jr., Knopman, D.S., Jagust, W.J., Shaw, L.M., Aisen, P.S., Weiner, M.W., Petersen, R.C., and Trojanowski, J.Q. (2010). Hypothetical model of dynamic biomarkers of the Alzheimer’s pathological cascade. Lancet Neurology 9, 535-545.
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Peischard, S., Zeuschner, D., TsyTsyura, Y., Disse, P., et al. (2020). A fully automated high-throughput workflow for 3D-based chemical screening in human midbrain organoids. Elife 9, e52904.

Rifes, P., Iaksonov, M., Rathore, G.S., Aldrin-Kirk, P., Møller, O.K., Barzaghi, G., Lee, J., Egerod, K.L., Rausch, D.M., Parmar, M., et al. (2020). Modeling neural tube development by differentiation of human embryonic stem cells in a microfluidic WNT gradient. Nat. Biotechnol. 38, 1265-1273.

Sakaguchi, H., Kadoshima, T., Soen, M., Nari, N., Ishida, Y., Ohgushi, M., Takahashi, J., Eiraku, M., and Sasai, Y. (2015). Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. Nat. Commun. 6, 8896.

Samarasinghe, R.A., Miranda, O.A., Buth, J.E., Mitchell, S., Ferando, I., Watanabe, M., Allison, T.F., Kurdian, A., Fotion, N.N., Gandal, M.J., et al. (2021). Identification of neural oscillations and epileptiform changes in human brain organoids. Nat. Neurosci. 24, 1488-1500.

Seo, K., Cho, S., Lee, J.H., Kim, J.H., Lee, B., Jang, H., Kim, Y., Cho, H.M., Lee, S., Park, Y., et al. (2021). Symmetry breaking of hPSCs in micropattern generates a polarized spiral cord-like organoid (pSCO) with dorsoventral organization. BioRxiv, https://doi.org/10.1101/2021.09.18.460734

Shaker, M.R., Pietrogrande, G., Martin, S., Lee, J.H., Sun, W., and Wolvetang, E.J. (2021). Rapid and efficient generation of myelinating human oligodendrocytes in organoids. Front. Cell. Neurosci. 15, 631548.

Shi, Y., Sun, L., Wang, M., Liu, J., Zhong, S., Li, R., Li, P., Fang, A., Chen, R., et al. (2020). Vascularized human cortical organoids (vOrganoids) model cortical development in vivo. PLoS Biol. 18, e3000705.

Song, L., Yuan, X., Jones, Z., Vied, C., Miao, Y., Marzano, M., Hua, T., Sang, Q.X.A., Guan, J., Ma, T., et al. (2019). Functionalization of brain region-specific spheroids with isogenic microglia-like cells in organoids. Sci. Rep. 9, 15663.

Song, K., Yuan, X., Jones, Z., Vied, C., Miao, Y., Marzano, M., Hua, T., Sang, Q.X.A., Guan, J., Ma, T., et al. (2019). Functionalization of brain region-specific spheroids with isogenic microglia-like cells. Sci. Rep. 9, 11055.

Tau, G.Z. and Peterson, B.S. (2010). Normal development of brain circuits. Neuropsychopharmacology 35, 147-168.

Trujillo, C.A., Gao, R., Negraes, P.D., Gu, J., Buchanan, J., Preissl, S., Wang, A., Wu, W., Haddad, G.G., Chaim, I.A., et al. (2019). Complex oscillatory waves emerging from cortical organoids model early human brain network development. Cell Stem Cell 25, 558-569.e7.

Ueda, H.R., Ertürk, A., Chung, K., Gradinaru, V., Chédotal, A., Tomancak, P., and Keller, P.J. (2020). Tissue clearing and its applications in neuroscience. Nat. Rev. Neurosci. 21, 61-71.

Valiente, M. and Marin, O. (2010). Neuronal migration mechanisms in development and disease. Curr. Opin. Neurobiol. 20, 68-78.

Van Spronsen, M. and Hoogenraad, C.C. (2010). Synapse pathology in psychiatric and neurologic disease. Curr. Neurol. Neurosci. Rep. 10, 207-214.

Von Dassow, M. and Davidson, L.A. (2011). Physics and the canalization of morphogenesis: a grand challenge in organismal biology. Phys. Biol. 8, 045002.

Warmflash, A., Sorre, B., Etoc, F., Siggia, E.D., and Brivanlou, A.H. (2014). A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. Nat. Methods 11, 847-854.

Wörsdörfer, P., Dalda, N., Kern, A., Krüger, S., Wagner, N., Kwok, C.K., Henke, E., and Ergün, S. (2019). Generation of complex human organoid models including vascular networks by incorporation of mesodermal progenitor cells. Sci. Rep. 9, 15663.

Wulansari, N., Darsono, W.H.W., Woo, H.J., Chang, M.Y., Kim, J., Bae, E.J., Sun, W., Lee, J.H., Cho, I.J., Shin, H., et al. (2021). Neurodevelopmental defects and neurodegenerative phenotypes in human brain organoids carrying Parkinson’s disease-linked DNAJC6 mutations. Sci. Adv. 7, eabd1540.

Xiang, Y., Tanaka, Y., Cakir, B., Patterson, B., Kim, K.Y., Sun, P., Kang, Y.J., Zhong, M., Liu, X., Patra, P., et al. (2019). hESC-derived thalamic organoids form reciprocal projections when fused with cortical organoids. Cell Stem Cell 24, 487-497.e7.

Ye, F., Kang, E., Yu, C., Qian, X., Jacob, F., Yu, C., Mao, M., Poon, R.Y., Kim, J., Song, H., et al. (2017). DISC1 regulates neurogenesis via modulating kinetochore attachment of Ndel1/Nde1 during mitosis. Neuron 96, 1041-1054.e5.

Yoon, S.J., Elahi, L.S., Pašća, A.M., Marton, R.M., Gordon, A., Revah, O., Miura, Y., Walczak, E.M., Holdgate, G.M., Fan, H.C., et al. (2019). Reliability of human cortical organoid generation. Nat. Methods 16, 75-78.

Zafeiriou, M.P., Bao, G., Hudson, J., Halder, R., Blenkle, A., Schreiber, M.K., Fischer, A., Schild, D., and Zimmermann, W.H. (2020). Developmental GABA polarity switch and neuronal plasticity in Bioengineered Neuronal Organoids. Nat. Commun. 11, 3791.

Zallen, J.A. (2007). Planar polarity and tissue morphogenesis. Cell 129, 1051-1063.

Zhang, W., Yang, S.L., Yang, M., Herrlinger, S., Shao, Q., Collar, J.L., Fierro, E., Shi, Y., Liu, A., Lu, H., et al. (2019). Modeling microcephaly with cerebral organoids reveals a WDR62–CP170–KIF2A pathway promoting cilium disassembly in neural progenitors. Nat. Commun. 10, 2612.

Zhao, J., Fu, Y., Yamazaki, Y., Ren, Y., Davis, M.D., Liu, C.C., Lu, W., Wang, X., Chen, K., Cherukuri, Y., et al. (2020). APOE4 exacerbates synapse loss and neurodegeneration in Alzheimer’s disease patient iPSC-derived cerebral organoids. Nat. Commun. 11, 5540.