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REVIEW

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Organoid technology for brain and therapeutics research

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Summary

Brain is one of the most complex organs in human. The current brain research is mainly based on the animal models and traditional cell culture. However, the inherent species differences between humans and animals as well as the gap between organ level and cell level make it difficult to study human brain development and associated disorders through traditional technologies. Recently, the brain organoids derived from pluripotent stem cells have been reported to recapitulate many key features of human brain in vivo, for example recapitulating the zone of putative outer radial glia cells. Brain organoids offer a new platform for scientists to study brain development, neurological diseases, drug discovery and personalized medicine, regenerative medicine, and so on. Here, we discuss the progress, applications, advantages, limitations, and prospects of brain organoid technology in neurosciences and related therapeutics.

KEYWORDS

brain organoid, drug discovery, neurosciences, pluripotent stem cell, regenerative medicine

1 | INTRODUCTION

Previous studies have already demonstrated that pluripotent stem cells (PSCs) have the immense self-organizing capacity and hold the potential to develop into the whole organ.[1,2] Organoids' culture system is based on the foundation of the self-organization of PSCs. When placed within proper three-dimensional (3D) scaffold (often Matrigel) and given proper biochemical factors, PSCs can develop into tissue-specific organoids. Currently, various organoids have been established including intestine,[2] liver,[3] kidney,[4] and brain.[5] In general, organoids contain organ-specific stem or progenitor cells which can self-renew and differentiate into heterogeneous tissue-specific cells rather than a single-cell lineage. Moreover, organoids recapitulate some key structures and functions of organs. For instance, intestinal organoids built from Lgr5$^{(+)}$ stem cells recapitulate structures of distinct intestinal crypts and villus domains and these structures contain various differentiated cells of intestine in vivo.[2] Compared with traditional cell culture, organoids have better recapitulated cell-cell interactions, cell-matrix interactions and better simulate cellular functions and signaling pathways presented in tissues. In addition, with the technology of reprogramming differentiated human somatic cells into PSCs,[6] organoids cultured from patient-derived induced pluripotent stem cells (iPSCs) have enormous potential to model diseases that can hardly be recapitulated by animal models and traditional cell culture. In other words, organoids provide a new model in research and have extensive application values in biomedical area.

2 | PROGRESS IN BRAIN ORGANOID TECHNOLOGY

Brain is the most intricate and complex organ in the body. Our current understanding of brain is mainly based on the animal models, postmortem examination, and traditional cell culture. However, owing to the complexity of the human brain and inherent species differences, it is difficult to have a further understanding of brain through traditional methods. Brain organoids which recapitulate a lot of key features of the developing brain provide a new platform for scientists to study brain development and brain disorders. Based on the foundation that PSCs have enormous self-organizing capacity to develop into whole

The first two authors contributed equally to this work.

tissues and inspired by intestinal organoids culture protocol (embedding the tissues in Matrigel[2]), Lancaster et al. developed a protocol for growing 3D neural tissue termed brain organoid from human PSCs.[5] They firstly generated neuroectoderm from embryoid bodies and then embedded neuroectodermal tissues in Matrigel droplets which could provide a scaffold to promote self-organization of PSCs. After a period of stationary culture in dish, Matrigel droplets were transferred and placed within a spinning bioreactor to strengthen the exchange of nutrients and oxygen, as shown in the Figure 1.

In the method, pattern growth factors are not used, such as the SMAD inhibitors which can promote the differentiation of embryonic stem cells (ESCs) into endoderm.[7] Instead, the protocol relies on the self-organization of ESCs and provides necessary growth conditions for brain organoids development where brain tissues develop pretty quickly; 8-10 days after these Matrigel droplets transferred to the spinning bioreactor, cells within brain organoids show neural identity and in 20-30 days, these brain tissues form defined brain regions. The cultured brain organoids reach up to 4 mm in size and can survive about 10 months when maintained in the spinning bioreactor.

The brain organoids recapitulated many key features of human brain in vivo and developed various distinct brain regions including retina, dorsal cortex, ventral forebrain, midbrain-hindbrain boundary, choroid plexus, and hippocampus. The outer subventricular zone (OSVZ) which is completely absent from mice brain[8] was also recapitulated in brain organoids. Consistently, brain organoids derived from mice ESCs did not have OSVZ. The outer radial glia cell zone was also identified within brain organoids, which exhibited typical behavioral and morphological features. What worth mentioning is that a study used single-cell RNA sequencing to analyze gene expression programs of cells within human brain organoids and fetal neocortex. They found that genetic features of human cortex were accurately recapitulated in brain organoids.[9]

After that, brain organoid technology has been constantly adjusted. Pasxca et al. developed specific brain subregions through the introduction of dual-SMAD inhibition and SFEB (serum-free culture of embryoid bodies) method.[10] The protocol did not use bioreactors and Matrigel, and the obtained brain organoids contained various neuron expressing markers of both superficial and deep layer cortex.

Although the above-mentioned culture methods of brain organoids promote the study of human brain development and disorders, there are still many deficiencies that need to be overcome such as low homogeneity and high cost. Encouragingly, a more reproducible, simple-to-use, cost-effective brain-region-specific organoid platform is established by Qian et al.[11] With the introduction of miniaturized spinning bioreactor and the use of induced factors, pure forebrain-specific organoids, as well as midbrain-specific and hypothalamic-specific organoids are generated from human iPSCs. These brain-region-specific organoids recapitulate several more key features of human cortical development with higher homogeneity compared with previous methods. Furthermore, the improved protocol reduces the use of medium volume and allows for mass production of brain organoids, providing an accessible and affordable technology to model human organogenesis and human disorders and to boost brain drug testing and screening.

3 | APPLICATIONS AND ADVANTAGES OF BRAIN ORGANOID TECHNOLOGY

Compared with traditional methods, brain organoid technology largely overcomes the species differences between humans and

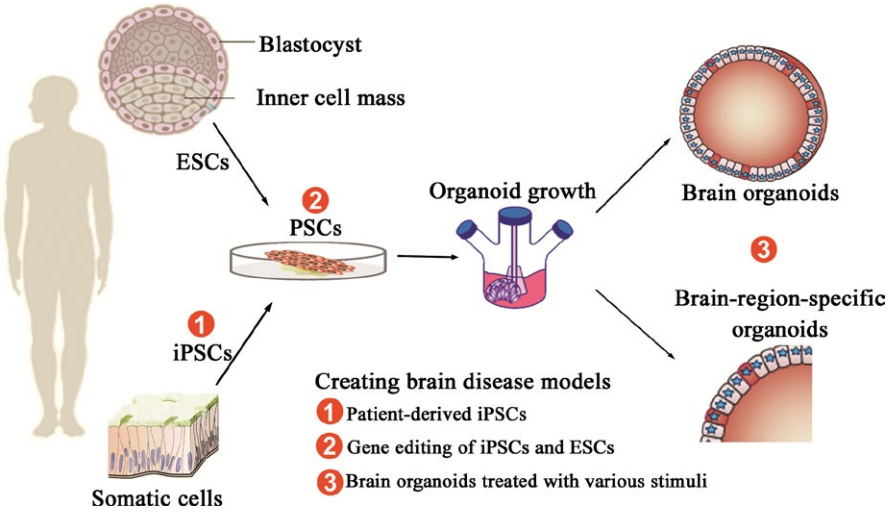

FIGURE 1 The generation of brain organoids and brain disease models. Pluripotent stem cells (PSCs) include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). ESCs can be generated from blastocyst, and iPSCs can be reprogrammed from somatic cells. Brain organoids are induced and differentiated from PSCs successively through different developmental stages, and eventually maintained in the spinning bioreactor. PSCs can develop into brain organoids by virtue of self-organization and differentiate into brain-region-specific organoids with the use of inducing factors. As for diseased brain organoids, three kinds of methods are used to create certain brain disease models: (1) using patient-derived iPSCs that are reprogrammed from patient-derived somatic cells, such as skin cell and blood cell; (2) applying gene editing to modify disease-linked alleles of PSCs, including ESCs and iPSC; (3) treating brain organoids with various stimuli, such as oxygen-glucose deprivation for an in vitro ischemic stroke model

other animals, bridges the gap between cell level and organ level, and shows great advantages. Brain organoid technology as a novel technology has wide applications in biology research, as shown in Figure 2.

3.1 | Brain development

Owing to the ethical and legal issues, various animal models and postmortem examinations are main research approaches for current human brain study, providing a rough understanding of vertebrate and mammalian brain development. However, due to the structure differences, such as the inner fiber layer and the OSVZ, which play important roles in the development of human brain but absent from mice brain,[8] comprehensive and deep studies of human brain through animal models cannot be carried out successfully.

Except for animal models and postmortem examination, neural stem cells (NSCs) derived from PSCs or iPSCs also have been conducted to study the nervous system. In 2001, the first 2D neural tube-like structures called neural rosettes derived from ESCs were established by Zhang et al.[12] The neural rosettes had apical-basal polarity and epithelial characteristics which were similar to the features of the embryonic neural tube. Furthermore, the neural rosettes recapitulated the function of radial glial stem cells through generating intermediate progenitor types and even could form progenitor zones which were similar to the ventricular zone and subventricular zone in vivo.[13,14] In a certain degree, the neural rosettes had a better recapitulation of some aspects of brain development than the homogeneous NSCs derived from PSCs. Nevertheless, the neural rosettes still cannot recapitulate the overall development of human brain because of the limitation of 2D property.

Taken together, brain organoids cultured by 3D method have been the most promising model to study human brain development so far. Brain organoid technology and traditional cell method allow scientists to conduct a deeper study of human brain. For example, brain cortex contains huge number of neurons and the size of brain cortex causes the differences in cognitive abilities between humans and other primates.[15,16] However, the mechanism of substantial expansion of the neurons in brain cortex is still unclear. Otani et al. performed both brain organoids and traditional cell culture to study the variation in brain cortex size between humans and other animals. The study found that a major determinant of brain cortex sizes in primates is a species-specific program controlling the output of cortical progenitor cells. In other words, cortical size is largely determined by the difference in the cortical progenitor cell outputs.[17] In the study, brain organoid technology played an important role and had huge advantages over traditional cell culture. There is no doubt that brain organoid technology as a novel tool provides a new platform for scientists to better understand human brain and helps to shape its development, unveil human brain mysteries.

3.2 | Neurological diseases

Neurological diseases such as Parkinson's disease, autism spectrum disorder (ASD), Alzheimer's disease, and bipolar disorder have a devastating impact on human lives and cause a heavy financial burden on their families and society.[18] The pathogenesis and mechanism of these diseases are always complex and the symptoms are always diverse

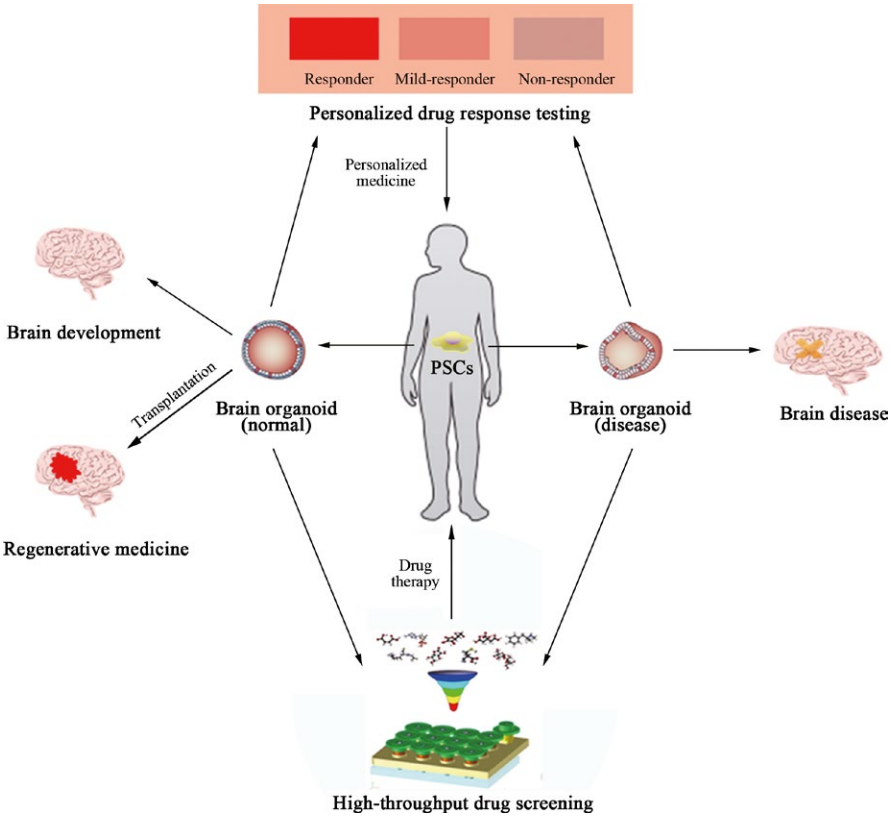

FIGURE 2 The applications of brain organoids. Pluripotent stem cell (PSC)-derived brain organoids can be applied to study brain development and transplant into brain injury site to repair injured tissues as a regenerative medicine therapy. Disease models of brain organoids can be used to study brain diseases. Both normal and diseased brain organoids have a great potential application in personalized medicine through personalized drug response testing and contribute to drug discovery and therapy through high-throughput screening to test drug efficacy and toxicity with the introduction of miniaturized spinning bioreactor

and complex, which make neurological diseases hard to receive early diagnosis and effective treatments. Our current understanding of neurological diseases is almost relied on the animal models and postmortem examination. Considering that neurological diseases are always involved in multigene mutation and the inherent species differences, it is difficult for animal models to well mimic neurological diseases. In addition, neurological diseases are always related to lesions of multiple cell types and affect more than one region of the brain; thus, it is almost impossible for traditional cell culture technology to model neurological diseases.

Brain organoids provide an ideal platform for studying neurological diseases in virtue of their various cell types, multiple regions of brain, and the characteristic of recapitulating many key features of human cortical development. What is more, brain organoids have been already applied to study neurological diseases such as the microcephaly and ASD and achieved many inspiring results.[5,11,19,20]

Mutation of the gene CDK5RAP2 can lead to the microcephaly.[21,22] However, when using the mouse with gene CDK5RAP2 mutation to model the microcephaly, several studies found that the size of the brain between the normal and the mutant had no significant difference.[23-25] Yet, the size of human brain with gene CDK5RAP2 mutation is severely reduced. There is an urgent need to find a proper model to study microcephaly. Interestingly, brain organoids cultured from a microcephaly patient skin fibroblasts derived iPSCs with Cdk5rap2 mutations shared several similarities with human microcephaly.[5] The patient-derived brain organoids were smaller than the normal organoids and only had occasional neuroepithelial regions. Furthermore, the number of the radial glial stem cells in the patient-derived brain organoids was less and the number of neurons was more compared to the normal group. These results illustrated the premature differentiation of neurons and accounted for the phenotype of patient tissue.

It is beyond doubt that using brain organoids to model and study neurological disease still has a long way to go, but the 3D brain organoid technology in combination with gene-editing techniques open a new door to study complex brain disorders. More recently, Mariani et al.[20] used brain organoids derived from patients with ASD to study autism spectrum disorder. They found that ASD-derived organoids had an accelerated cell cycle, produced more GABAergic inhibitory neurons and the overexpression of the transcription factor FOXG1 contributed to the overproduction of GABAergic neurons which may be the initiation factor of ASD. These studies mentioned above build a framework of studying neurological disease and demonstrate that brain organoids as a new technology have a great prospect and bright future in studying neurological disease.

3.3 | Drug discovery and personalized medicine

Biological activity, pharmacological action, and medicinal value need to be assessed in the drug discovery, where animal and cell models are introduced to accomplish associated researches. However, the animal models have the limitation of species specificity and the heterogeneous human population also cannot be reflected by the use of inbred strains.[26] Traditional cell culture forms relatively pure cell linage which cannot reflect the cell-cell interaction and the cell-matrix interaction. To a certain degree, these factors are responsible for the fact that it takes more than 10 years and costs more than 2 billion dollars to create a new drug.[27,28] The lack of sufficiently mature model of neurological diseases causes the current dilemma that drug discovery for treating neurological diseases is at a near standstill.[29]

Brain organoids not only provide a new platform for modeling diseases but also for drug discovery. Brain organoids infected by the Zika virus (ZIKV) showed many features of microcephaly. For example, the overall size of infected brain organoids reduced and the neuronal layer thickness within infected brain organoids became thin.[11] Further experiments found that ZIKV targeted on neural progenitor cells (NPCs) and led to the increased cell death and suppressed proliferation of infected NPCs.[11,19] The ZIKV study with aid of brain organoid technology not only revealed the relationship between ZIKV outbreak and the increased congenital microcephaly in Brazil but also held great potential for drug testing including potential ZIKV antiviral drugs. The improved protocol and miniaturized spinning bioreactors reduce the production cost and allow for enlarging the production of brain organoids which is important to drug discovery.[11]

Besides, neuroprotective drugs have always been the research focus for the treatment of brain disorders. Although most of neuroprotective agents have been demonstrated neuroprotection in rodent animal models and cell models, they have been shut down in clinical trials.[30] Brain organoid technology provides a new thought for drug screening through establishing disease models to replace relevant regular experimental models or even nonhuman primate models. For example, brain organoids can be used to establish ischemic model to replace or supplement animal middle cerebral artery occlusion model and cell oxygen-glucose deprivation model to narrow species difference and increase the success rate of clinical translation.

Organoids also have potential applications in testing efficacy and toxicity of drugs and compounds.[31-33] More recently, brain organoids derived from human PSCs were performed to predicting neural toxicity. The brain organoids were treated with 34 toxic and 26 nontoxic chemicals and able to accurately predict 90% of neurotoxic compounds with the use of RNA sequencing and machine learning.[34]

Moreover, organoids can be used to test individualized patient's response to different drugs possible as organoids can be cultured from patient-derived somatic cells. Recently, rectal organoids derived from patient with cystic fibrosis were performed to test individual responses to cystic fibrosis transmembrane conductance regulator-modulating drugs. The study found that drug responses in rectal organoids were positively correlated with published outcome data from clinical trials.[35] Up to now, there has been no report about such application of brain organoids, but the above study offers a kind of thought for brain organoids to assess drug response at the level of the individualized patient with brain disorders and then take personalized therapy.

3.4 | Regenerative medicine

Regenerative medicine as an important part of modern medicine aims at replacing or regenerating cells, tissues, or organs to restore

or establish normal function.[36] Stem cells have the ability of self-renewing and differentiating into various cell lineages, and stem cell-based strategies hold promise in curing traumatic brain injury[37-39] and stroke.[40-42] Our group has done much work in identifying therapeutic targets and screening effective neuroprotective drugs against stroke.[43-45] Recently, we have demonstrated that nicotinamide phosphoribosyltransferase-NAD cascade which is particularly important for the proliferation, self-renewal, and differentiation of neural stem cells can promote regenerative neurogenesis after ischemic stroke.[46-48] In addition to promoting tissue regeneration by stimulating endogenous stem cells, progress in stem cell transplantation also enhances the development of regenerative medicine.[49,50]

As organoids contain stem or progenitor cells and have the potential to develop into whole organs, organoids can provide a source of autologous cell and tissues for transplantation. Proof-for-principle animal experiments have already demonstrated that organoids had the potential for replacement therapy.[3,51] In a study, murine colon organoids were transplanted into superficially damaged mouse colon.[51] The transplanted cells quickly integrated into the mouse colon and covered the area that lacked epithelium. Four weeks after transplantation, the transplanted cells formed a single-layered epithelium which contained self-renewing crypts with normal morphology and function. In another study, liver organoids derived from single $Lg5^+$ liver cells were transplanted into fumaryl acetoacetate hydrolase mutant mice, a model for Tyrosinemia type I liver disease.[3] The transplantation significantly increased survival rate compared to the group without transplantation. Although there has not relative report about brain organoids in replacement therapy currently, brain organoids contain NPCs and various types of neurons and hold great promise for cell replacement therapy in human neurodegenerative diseases.[52] Recently, midbrain organoids containing long-lived dopaminergic neurons have been cultured. Compared with traditional cell culture, the midbrain organoids can rapidly generate a higher amount of dopaminergic neurons and neural precursor cells with higher levels of survival and longer neurite outgrowth.[53] The dopaminergic neurons tissues not only provide a tool for drug screening and toxicology test but also hold the promise for cell replacement therapy in Parkinson's disease, whose pathogenesis has a close relationship with dopaminergic neurons.

Brain organoids as a rich source of NPC can serve as a novel therapeutic strategy to promote tissue and nerve regeneration in brain injury diseases.

4 | LIMITATION OF CURRENT BRAIN ORGANOID TECHNOLOGY

Organoids as a new technology have distinct advantages over previous methods. However, it is important to keep in mind that organoids still have many drawbacks. Most organoids only represent partial components of organs. The cell types and structures within the organoids are often out of control. Thus, current brain organoids cannot well simulate relative complex neurodevelopmental conditions such as schizophrenia.

4.1 | Heterogeneity

Compared with the traditional cell culture, 3D organoids culture upon the spontaneous nature of cell self-organization exhibits more complex and typical tissue structures. However, brain organoids cultured by 3D method are highly heterogeneous. The appearance of each organoid is different from each other, and brain regions within each organoid are various and the positions of the regions are random.[54,55] The heterogeneity makes it difficult to do controlled experiments with feasible parallelity and consistency. The lack of exogenous induced factors and the spontaneous self-organization of stem cell cause the heterogeneity of organoids to a great extent. Better control of the growth and differentiation of stem cells in vitro contributes to improve the homogeneity of organoids. Treating organoids with signaling molecules such as recombinant WNT3A protein and SMAD inhibitor SB-431542 in different durations, Qian et al. generated brain-specific organoids with enhanced reproducibility and reduced heterogeneity in organoid shape and size.[11] Optimizing experimental facilities of organoids system is another feasible way to promote the homogeneity of organoids. Applying miniaturized spinning bioreactor to organoids' system, Qian et al. enhanced the reproducibility, increased the throughput, and reduced the cost of organoids system which permits comparisons of a large number of conditions in parallel for protocol optimization. Although brain-region-specific organoids with lower heterogeneity and enhanced reproducibility are crucial for controlled experiments and screening approaches, the formed brain-region-specific organoids reduce the complexity of cell types as well as the corresponding structure when compared to the whole-brain organoids. It is difficult for brain-region-specific organoids to model neurological disease that involves in cells originated from different brain regions. Much work still needs to be done to improve the homogeneity of whole-brain organoids.

4.2 | Immaturity

Currently, the size of brain organoid can reach up to 4 mm in diameter and can survive for long time when maintained in a spinning bioreactor.[5] However, the cells in the core of brain organoids begin to apoptosis after about 100 days of initial culture and do not differentiate into any new cell types partly due to the lack of blood vessels by which adequate nutrients and gas exchange may transport.[5,11] Thus, the lack of inadequate nutrient and oxygen largely limits the growth of brain organoids and affects the maturation. Another factor affects the maturation of organoids is the poor understanding of the chemical and physical cues during brain development. Although brain organoids recapitulate lots of features of brain development in vivo, many brain structures and cell types are still not recapitulated. For example, current brain organoids method can generate remarkable cortical progenitor zone, but the preplate which plays important roles in neuronal migration during cortical development has not been recapitulated.[5,11,20,56] In sum, in the context of current technical level, brain organoids can only recapitulate the development of the first trimester human fetal brain, which is not complete and comprehensive.[5,11,57]

5 | PROSPECTS OF BRAIN ORGANOIDS

It is not surprising that brain organoid technology has great advantage over traditional methods, but the shortcomings of brain organoid technology are obvious as mentioned above. In the future, tissue engineering as a good starting point can be applied to brain organoids culture and further improve brain organoid technology. For example, it may contribute to designing the niche of brain organoids and realizing the vascularization of organoids.

The niche of stem cell includes various signaling pathway, extracellular matrix (ECM), and physiochemical condition such as oxygen and osmolality. In vivo, the behaviors of stem cell are strictly regulated by the microenvironments. Compared to in vivo niche, in vitro niche is relative simple and lack of various patterning factors that cannot offer body axes for stem cell to organize. What is more, some supplements used to culture brain organoids are from animals. For example, the fetal bovine serum is used in the first step to make embryoid bodies and induce initial germ layer differentiation from human pluripotent stem cells, and albumin from bovine serum is also used as a carrier protein in the dilution for growth factor. The supplements generated from animals have an unclear impact on the development of brain organoids and may lead to the high heterogeneity of brain organoids in a certain degree. In addition, current organoid technology relies heavily on animal-derived ECM[2,5] such as Matrigel which generated from Engelbreth-Holm Swarm mouse sarcoma cells. However, the compositions of the ECM are complex and not well defined,[58,59] which may amplify the heterogeneity of organoids. Recently, Gjorevski et al.[60] designed a fully defined synthetic hydrogel and used it to culture intestinal organoids which increased the reproducibility of organoids. The study provides a feasible method for culturing organoids in a well-defined microenvironment. It is hopeful that tissue engineering will provide a niche that much closer to the physiological environment to better direct the development of brain organoids and enhance the homogeneity of organoids in the future.

The lack of vascularization is another serious challenge to organoid technology. Vascular networks allow adequate nutrition and oxygen supply for organoids; thus, integrating vascular networks into organoids is very important for organoids to further grow and differentiate. Bioengineers have already designed various approaches for generating microvascularized constructs.[61] For instance, by co-culturing mesenchymal stem cells from human bone marrow and umbilical cord-derived endothelial cells in a polymeric scaffold, the endothelial cells formed mature vascular networks in vivo 4-7 days after implantation.[62] In addition, bioprinting also offers the possibility to realize the vascularization of organoids. Using bioprinting technology, Kolesky et al. generated thick, vascularized human tissues which could be perfused on chip for long time periods (>6 weeks).[63] What is even more exciting is that vascularized and functional human liver organoids were generated from human iPSCs by transplanting liver buds into immunodeficient mice.[64] It both realizes the vascularization of liver organoids and solves the issue of nutrient supplement. The strategies mentioned above not only offer some ideas for vascularizing brain organoids but also can be integrated into brain organoids to realize the vascularization of brain organoids under appropriate modification.

6 | CONCLUSIONS

Brain organoids recapitulate many key features of normal brain in vivo that can hardly be achieved by traditional cell and animal research methods. So far, brain organoids have already been used to study brain development, model neurological diseases and test drugs. However, owing to the lack of vascularization and exogenous induced factors, brain organoids are still not ideal and cannot comprehensively recapitulate brain development as well as model brain disorders. Therefore, much work needs to be done to improve the reproducibility and maturity of brain organoids. Nevertheless, it holds out the prospects that tissue engineering can be applied to organoid technology to design organoid niches and realize organoid vascularization. By that time, brain organoids with high reproducibility can better recapitulate the complexity of human brain and model brain diseases comprehensively, which will open a new chapter in brain science research and therapeutics.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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