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Analysis of the Research Progress in Constructing Brain Organoids with Stem Cells

Ziyu Guo

Xiamen University, Xiamen, China
3113908440@qq.com

Abstract: Organoids, three - dimensional cell aggregates, are formed through the differentiation of stem or progenitor cells in controlled lab settings. They can replicate aspects of real organs, revolutionizing life science research. Brain organoids are created via two main techniques. Non - guided differentiation depends on the natural, spontaneous transformation of stem cells to yield diverse cell types. Guided differentiation, on the other hand, uses specific cues to form organoids mimicking distinct brain regions or nuclei. However, accurately representing brain nuclei features remains a major hurdle. Brain organoids have a rich cellular composition, including neurons and glial cells, similar to fetal cortical tissue, which allows them to model brain functions. Over time, they've evolved from single - donor models to more complex ones derived from multiple donors. This evolution is crucial for studying differences in drug responses among individuals and advancing personalized medicine. Using induced pluripotent stem cells (iPSCs) to generate neural stem cells and brain organoids circumvents ethical issues related to embryonic stem cells. It also enables the creation of patient - specific models, facilitating precise research into neural development mechanisms and neurological disease pathogenesis. This article comprehensively reviews the progress in building brain organoids from stem cells, providing key insights for future brain research.

Keywords: Brain Organoids, Stem Cells, Construction Methods, Cell Type Diversity, Alzheimer's Disease

1. Introduction

In recent years, organoids have emerged as a revolutionary tool in life science research, attracting significant interest across various fields. These small, three-dimensional structures, composed of multiple cell types, are created by cultivating stem cells in controlled laboratory conditions. Organoids possess the remarkable ability to replicate certain aspects of the structure and function of human tissues and organs in vitro, making them valuable models for studying biological processes and disease mechanisms [1]. The idea of organoids first took shape in the early 20th century, when scientists sought to culture tissues outside the body in an effort to better understand their development and physiological roles. However, due to the limited technological capabilities at the time, progress was slow, and it was not until recent decades—thanks to significant advances in stem cell technology, cellular biology, and biomaterials—that major breakthroughs in organoid research began to emerge. A critical element in the formation of organoids is the use of stem cells. These cells are characterized by their unique abilities to self-renew and differentiate into various specialized cell types, which make

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them indispensable for organoid construction. The primary types of stem cells employed in organoid development are embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [2]. ESCs, derived from early-stage embryos, have a remarkable capacity to differentiate into a wide array of cell types, thus offering a rich reservoir for constructing organoids that resemble different organs. In contrast, iPSCs are generated by reprogramming adult somatic cells through advanced gene editing technologies. Despite being derived from differentiated cells, iPSCs exhibit similar pluripotency to ESCs, without the ethical concerns associated with the use of embryonic cells. Additionally, iPSCs offer the exciting potential for creating patient-specific organoids, which is particularly beneficial for personalized medicine and advancing disease modeling.

Brain organoids, a pivotal area within organoid research, are typically generated from human pluripotent stem cells (hPSCs). These intricate, three-dimensional structures spontaneously organize into formations that closely resemble the fetal human brain. They are composed of diverse cell types, including progenitor cells, neurons, and glial cells, which work together to mimic the complex cellular composition of the brain [3]. The emergence of brain organoids has unlocked unprecedented opportunities for neuroscience research. For the first time, scientists can replicate brain development in vitro, investigate the mechanisms underlying neurological diseases, and explore novel drug candidates for therapeutic purposes.

One of the most pressing and challenging neurological conditions is Alzheimer's disease (AD), a widely prevalent neurodegenerative disorder that severely impacts cognitive functions and quality of life. AD not only places a substantial emotional and financial burden on both patients and their families, but it also remains a major scientific and medical challenge due to the complexity of its pathogenesis. Although substantial progress has been made in understanding AD, current animal and cell-based models still fall short of fully capturing the multifaceted nature of the disease. This gap in research tools has hindered the development of more effective treatments. In this context, brain organoids present an innovative and promising model that holds the potential to bridge these gaps, offering a more accurate simulation of human brain pathology in AD research.

This article provides an in-depth examination of recent advancements in the generation of brain organoids from stem cells. It outlines the various techniques used to create these organoids, the diversity of cell types they contain, and the potential applications of brain organoids for disease modeling, with a particular focus on Alzheimer's disease (AD). Special attention is given to the processes involved in generating brain organoids for both familial Alzheimer's disease (FAD) and sporadic Alzheimer's disease (SAD), highlighting the unique challenges associated with each form of the disease. Furthermore, the article explores the obstacles currently faced in the field of AD brain organoid research and discusses possible strategies for overcoming these challenges.

The value of this review lies in its comprehensive synthesis of the latest developments in brain organoid research, providing a systematic framework for future studies. It offers a structured overview of current methodologies and explores potential avenues for further research, serving as a vital resource for researchers in the field. Additionally, the article emphasizes the potential of brain organoids to drive progress in understanding AD pathogenesis and developing more targeted and effective treatments. Ultimately, it is hoped that the insights gained from this research will pave the way for significant advancements in combating Alzheimer's disease and improving patient outcomes.

2. Key Aspects of Brain Organoids

2.1. Overview of Brain Organoids

Brain organoids represent a distinctive type of organoid derived from human pluripotent stem cells (hPSCs). These organoids self-assemble into a complex, three-dimensional structure that mirrors various aspects of the fetal human brain. They consist of a variety of cell types, such as progenitor

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cells, neurons, and glial cells, which collectively form a tissue that resembles the human brain both in terms of cellular composition and organization. Unlike traditional two-dimensional cell cultures, brain organoids provide a more accurate representation of brain development, closely simulating the tissue architecture and developmental pathways of the human brain. This makes them invaluable models for investigating the developmental processes and functional mechanisms of the brain. In recent years, advances in several cutting-edge technologies have significantly propelled the field of brain organoid research. These include patient-derived human induced pluripotent stem cell (hiPSC) technologies, genetic modification techniques, genome editing tools, as well as high-throughput methods for single-cell transcriptomics and epigenetic analysis. These technological innovations have not only improved the precision and efficiency of brain organoid construction but also enhanced our ability to study human brain development and pathology. As a result, these advancements have reshaped the research landscape, offering new opportunities to explore neurological diseases and the evolutionary aspects of the human brain [4].

2.2. Construction Methods of Brain Organoids

Brain organoids can be generated using two main strategies: guided and unguided methods.

The unguided approach leverages the inherent capacity of human pluripotent stem cells (hPSCs) to undergo spontaneous morphogenesis. In this method, aggregates of hPSCs are cultured in conditions that allow them to self-organize and differentiate without external guidance. The cells naturally progress through a series of differentiation steps, leading to the formation of structures that resemble brain tissues. As the hPSCs differentiate, they give rise to a heterogeneous mixture of cell types, which together form organoids that reflect various regions of the developing brain. This approach is advantageous in its ability to mimic the complexity of brain development, although the resulting organoids can exhibit variability in terms of their size, organization, and tissue composition.

In contrast to the unguided construction approach, the guided construction method incorporates the introduction of specific external signaling factors that influence the differentiation process of human pluripotent stem cells (hPSCs) into particular cell types. This method involves the application of a variety of small molecules and growth factors, which effectively direct hPSCs to develop into spheroids that mirror specific tissue types. These external patterning factors are crucial during the initial stages of differentiation, as they help determine the lineage commitment of progenitor cells. Once the desired cell types begin to emerge, the patterning factors are gradually removed, allowing the cells to continue maturing into their final forms. This approach ensures a more controlled and predictable differentiation process. A distinctive feature of the guided method is its ability to generate organoids that replicate specific regions of the brain. By manipulating the timing and composition of the applied patterning factors, researchers can create organoids that mimic distinct areas of the brain, such as the cortex, hippocampus, or basal ganglia. These region-specific organoids can then be fused together to create a single, integrated “combinatory body,” which simulates the complex interactions that occur between different brain regions. This fusion enables the study of how various parts of the brain communicate and work together, providing valuable insights into brain connectivity and the functional relationships between distinct regions [4].

On the other hand, the unguided construction method relies on the innate ability of hPSCs to self-organize and differentiate into neural tissues without the aid of external patterning factors. This approach involves carefully crafting the culture conditions, including the formulation of specialized culture media and the addition of specific additives. These factors encourage the hPSCs to adopt neural characteristics and initiate the process of brain tissue formation in vitro. In this method, hPSCs are aggregated to form embryoid bodies (EBs), which are then embedded in an extracellular matrix to provide structural support. These EBs are cultured in rotating bioreactors, a setup that promotes tissue expansion and facilitates the neural differentiation process [5]. The significant advantage of the

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unguided approach is the degree of self-organization it provides to the hPSCs. As the cells differentiate, they spontaneously form a variety of brain cell types, including those from the forebrain, midbrain, hindbrain, retina, choroid plexus, and mesoderm. This process mirrors the natural development of the human brain, producing organoids that exhibit a diverse and heterogeneous cellular composition, similar to that seen in early brain development [6].

In addition to chemical patterning factors, the guided method can be further enhanced by the use of synthetic biomaterials, which provide additional structural guidance for the developing brain organoids. These materials can help direct the spatial organization of the cells, ensuring the formation of more complex and orderly tissue architectures. One such technique, known as microfilament-engineering, involves the use of polymer microfilament scaffolds around which embryoid bodies (EBs) are cultured. The scaffold serves to support the growth of the organoid, encouraging the expansion of the ventricular structure and aiding in the consistent formation of the neuroepithelium. This method not only enhances the physical organization of the organoid but also improves the reproducibility of the results, making it a promising tool for developing standardized brain models for research purposes [7].

2.3. Diversity of Cell Types in Brain Organoids

Brain organoids serve as complex in vitro models that replicate the cellular diversity and structural organization of the human brain. These organoids are capable of generating a wide array of cell types, which can vary depending on the specific research objectives and the culture methods employed. As shown in Table 1, different protocols can yield a variety of brain cells, each with distinct characteristics and functions. The creation of brain organoids involves generating several types of neural and glial cells, including radial glial cells (RG), neurons, interneurons, astrocytes, and oligodendrocyte precursor cells (OPCs). These cell types closely mirror the cellular composition found in the human cerebral cortex, making brain organoids valuable tools for studying brain development, pathology, and disease mechanisms [3].

The process of generating brain organoids can be adapted to produce specific cellular populations, depending on the experimental needs. By manipulating various factors, including the choice of stem cell type, exogenous signaling molecules, and the use of bioreactors and extracellular matrices, researchers can direct the differentiation of pluripotent stem cells into desired brain regions. For instance, when using embryonic stem cells (ESCs), the organoid system can be influenced by external signaling cues such as the smoothened agonist (SAG) to induce the formation of specific structures like the adenohypophysis. Similarly, the incorporation of cell-type-specific molecules can guide the differentiation process to generate particular cell populations within the organoid [8].

In addition to ESCs, another valuable source for constructing brain organoids is induced pluripotent stem cells (iPSCs). These cells can be directed towards specific brain regions, such as the forebrain, through the application of various signaling molecules. Examples include Dorsomorphin, a BMP inhibitor, and A83, which inhibits both BMP and TGFβ pathways. Other molecules like SB-431542, WNT3A, CHIR99021, BDNF, and GDNF play key roles in promoting the formation of forebrain structures by modulating critical signaling pathways involved in neural differentiation and regional specification [9].

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Table 1: Cell Types of Brain Organoids and Their Origins

Starting Material	Induction Condition	Cell Types and Corresponding Brain Regions
ESC	Sonic Hedgehog agonist (SAG) and other cell type-specific molecules	Gonadotropic pituitary gland
iPSC	Dorsomorphin (BMP inhibitor), A83 (BMP and TGFβ inhibitor), SB-431542, WNT3A, CHIR99021 (GSK3β inhibitor), BDNF, GDNF	Prosencephalon
iPSC or ESC	Endogenous or CycA (an antagonist of the SHH signaling pathway), cultured in Matrigel and a rotating bioreactor	Dorsal cortex
iPSC or ESC	Induced by IWP2 (a WNT inhibitor) and SAG, supplemented with Matrigel and using a rotating bioreactor	Ventral cortex
ESC	Induced by WNT inhibitor, SB-431542, GSK3 inhibitor, and BMP4	Hippocampus and choroid plexus
iPSC	Inductively cultured with LDN-193189, SB-431542, WNT3A, SHH, SAG, FGF2, and CNTF	Hypothalamus
iPSC	Dorsomorphin, SB-431542, EGF, FGF2, BDNF, and NT3	Cerebral cortex

2.4. Existing Models of Brain Organoids

A range of brain organoid disease models has been established, each offering unique insights into the pathophysiology of various neurological disorders. These models are valuable tools for investigating a wide array of brain diseases, as summarized in Table 2. One of the most prominent applications of brain organoids is in the study of congenital brain malformations, which may arise from genetic mutations or infections. For example, brain organoids have proven instrumental in replicating the development of cortical structures and in simulating conditions such as primary microcephaly, particularly those linked to mutations in the ASPM gene. In these organoid models, researchers can observe a reduction in neural progenitor cell (NPC) numbers during cortical development, offering crucial insights into the mechanisms underlying microcephaly and other developmental disorders [10,11]. Moreover, brain organoids serve as robust systems for investigating the roles of specific proteins and signaling pathways involved in disease progression. For instance, the DISC1 gene plays a critical role in neurogenesis by regulating the attachment of the Ndel1/Nde1 complex to the centromere during cell division. Disruptions in this process can lead to developmental and neuropsychiatric disorders [12]. The Zika virus, through its encoded protein NS2A, interferes with neurogenesis in the developing mammalian cortex by causing the degradation of key adherens junction proteins. This disruption has significant implications for brain development, as adherens junctions are crucial for maintaining cellular architecture and communication. Additionally, brain organoids derived from individuals diagnosed with Autism Spectrum Disorder (ASD) demonstrate a notable shift in the balance between excitatory and inhibitory neurons. This imbalance, which is believed to contribute to the neurodevelopmental abnormalities seen in ASD, has been linked to the overexpression of FOXG1, a transcription factor that plays a vital role in the development of the forebrain [13]. Furthermore, brain organoids are valuable models for investigating neurodegenerative diseases, particularly Alzheimer's disease. These models have been shown to replicate the hallmark pathological features of Alzheimer's, such as the accumulation of amyloid-β plaques and tau tangles, which are central to the disease's progression in human brains [14]. Human neural cultures derived from extracellular matrix (ECM)-based neurospheres, specifically from Alzheimer's disease patients, can accurately replicate AD-associated pathological phenomena. These include the aggregation of amyloid-β, the hyperphosphorylation of tau protein, and the presence of endosomal abnormalities, all

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of which are integral to the disease's progression. Despite these promising advancements, the current state of brain organoid development is still in its early stages. While these organoid models offer a valuable approximation of in vivo brain conditions, they are still relatively simple and often fail to fully capture the complexity of human brain pathology. As a result, there remains a significant gap between the simulated models and the true biological environment of the brain, necessitating further improvements in organoid technology to enhance their accuracy and relevance for disease modeling.

Table 2: Different Disease Models for the Application of Brain Organoids

Disease Type	Construction Methods of Brain Organoid Models	Simulating Disease Mechanism and Manifestations	Limitations of the model
Congenital brain malformations (e.g., abnormal spindle-like microcephaly)	In vitro organoid culture	Summarizes cortical development and reveals microcephaly caused by a reduction in NPCs.	very simple and immature, and the simulation results deviate from the in vivo situation.
Diseases related to the functions of proteins or pathways (e.g.,	Specific detection of a protein or pathway	DISC1 regulates neurogenesis by modulating centromere attachment of	The same as above.
DISC1 and Zika virus)		Ndel1/Nde1 during mitosis; NS2A disrupts neurogenesis in the mammalian cortex by degrading adherens junction proteins.	
Autism Spectrum Disorder (ASD)	Forebrain organoids constructed from cells of ASD individuals	Imbalance in excitatory and inhibitory neurons, linked to FOXG1 overexpression.	The same as above.
Alzheimer's disease	1. Aftin-5 induces Aβ42 peptide production. 2. iPSCs from familial Alzheimer's disease (FAD) patients. 3. Conversion of APOE3 to APOE4 in iPSCs from sporadic Alzheimer's disease (SAD) patients.	Exhibits AD features: Aβ peptides, hyperphosphorylated tau protein, NFT, synaptic dysfunction, neuronal degeneration, neuroinflammation. FAD brain organoids show amyloid and tau protein pathologies, changes in glutamatergic neurons, increased lactate dehydrogenase activity, inflammatory gene expression, and molecular alterations. SAD brain organoids exhibit Aβ aggregation and disrupted Aβ42/Aβ40 balance.	The same as above.

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3. Brain Organoids in Alzheimer's Disease Research

3.1. Induced Pluripotent Stem Cells (iPSCs) for Constructing Neural Stem Cells and Brain Organoids to Study Alzheimer's Disease

The current animal and cell-based models used to study Alzheimer's disease (AD) fall short of replicating the full complexity of the disease, limiting their effectiveness in understanding its molecular mechanisms. However, recent advancements in three-dimensional (3D) brain organoid models, particularly those derived from human stem cells, offer a promising alternative for studying AD and advancing drug discovery. These 3D organoids mimic the architecture and cellular composition of the human brain, providing a more accurate platform for investigating the molecular and cellular dynamics that drive AD pathology.

A defining characteristic of Alzheimer's disease is the accumulation of misfolded proteins, including amyloid-β (Aβ) peptides and hyperphosphorylated tau proteins, which progressively build up in specific regions of the brain. This accumulation is thought to trigger a cascade of molecular events, including the formation of neurofibrillary tangles (NFTs), impaired synaptic function, neuronal loss, and neuroinflammation. These pathological changes ultimately lead to significant cognitive decline and memory impairment, hallmark symptoms of AD. To model these processes, three primary approaches have been developed using brain organoids: 1) The first approach involves using Aftin-5, a compound that induces the production of Aβ42 peptides in brain organoids, thereby simulating the characteristics of sporadic Alzheimer's disease (SAD). 2) The second strategy involves creating brain organoids from induced pluripotent stem cells (iPSCs) derived from patients with familial Alzheimer's disease (FAD), providing a model to study the inherited forms of AD. 3) The third approach focuses on converting the APOE3 allele into APOE4 in iPSCs derived from SAD patients. This method generates differentiated brain organoids that exhibit features specific to SAD, enabling the study of the impact of the APOE4 gene variant, which is strongly associated with increased risk for AD.

These brain organoids, when used to model AD, display several key features of the disease, including the presence of Aβ peptides, tau tangles, neurofibrillary tangles (NFTs), synaptic dysfunction, neuronal degeneration, and neuroinflammation. These models hold great potential for advancing our understanding of AD and for evaluating the efficacy of potential therapeutic interventions [15].

3.2. Developing Brain Organoids for Familial Alzheimer's Disease (FAD)

Pluripotent stem cells obtained from patients with familial Alzheimer's disease (FAD), specifically those with APP duplication or PSEN1 mutations, are utilized to generate FAD-specific brain organoids [16]. For this purpose, two induced pluripotent stem cell (iPSC) lines, APPDp1-1 and APPDp2-3, which harbor the amyloid precursor protein (APP) mutation, were chosen. Additionally, a control iPSC line, AG09173, was also included in the study. These iPSCs were cultured on irradiated mouse embryonic fibroblasts (MEFs) in a medium consisting of DMEM/F12 supplemented with 20% KnockOut Serum Replacement (KSR), non-essential amino acids (NEAA-1X), GlutaMAX (1X), and beta-fibroblast growth factor (FGF2), along with 2-mercaptoethanol. Following the culturing process, three-dimensional neural organoids were generated using established differentiation protocols. After the organoids were formed, they underwent tissue processing and were subjected to immunological analysis using primary and secondary antibodies. Laser scanning confocal microscopy was employed to visualize the tissue sections [17]. To quantify the extent of neurodegenerative features, measurements were taken for the particle count and size of β-amyloid (Aβ), EEA1, and transferrin immunoreactivity, as well as the signal intensity of phosphorylated tau

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(pTau). Enzyme-linked immunosorbent assays (ELISA) and transferrin assays were performed to further analyze the organoids. Additionally, β-secretase (BACE-1) and γ-secretase inhibitors were applied to the organoids to assess their effect on disease progression, with subsequent immunohistochemical processing conducted to evaluate the results.

These brain organoids demonstrate the characteristic pathological features of amyloid-beta (Aβ) and tau proteins, which are central to the development of Alzheimer's disease. The organoid model reveals a distinct developmental timeline: the accumulation of phosphorylated tau (P-tau) occurs after the formation of Aβ aggregates, mirroring the sequence of events seen in the pathology of human familial Alzheimer's disease (FAD). This temporal progression of amyloid and tau pathology is a key aspect of disease development in these organoids. In contrast, many mouse models used to study Alzheimer's disease typically require the introduction of multiple transgenes to exhibit strong amyloid phenotypes. Additionally, these models rarely develop tau protein pathology or neuronal degeneration, which makes the organoid system particularly valuable for studying the disease in a more human-relevant context.

Furthermore, researchers have developed a different type of FAD brain organoid by utilizing stem cells from patients with a missense mutation (A246E) in the PSEN1 gene, which is known to be associated with early-onset Alzheimer's disease [14]. These patient-derived organoids showed hallmark signs of Alzheimer's disease, including Aβ aggregation, an increase in P-tau levels, and evidence of cell apoptosis. Importantly, the formation of protein aggregates—both Aβ and P-tau—observed in these organoids aligns with previous findings in Alzheimer's research, validating the model's relevance. Additionally, the extent of cell apoptosis and the development of neurofibrillary tangles (NFTs) within these organoids were found to be positively correlated with the accumulation of these protein aggregates, further supporting the model's accuracy in replicating the key features of FAD.

The FAD brain organoids also display several additional distinctive features:

1) After 17 days of culture, a reduction in the number of glutamatergic neurons is observed in the FAD brain organoids, but by 28 days of culture, the number of these neurons shows an increase.

2) The activity of lactate dehydrogenase in the FAD brain organoids is notably elevated, which can contribute to direct toxicity in neural cells.

3) In these organoids, there is a significant upregulation in the expression of inflammatory factors such as interleukin-6 and tumor necrosis factor-α. This increase in inflammatory gene expression is believed to induce toxicity in astrocytes, which, in turn, triggers neuronal apoptosis.

4) The expression levels of matrix metalloproteinases (MMP2 and MMP3) are reduced, while the expression of syndecan-3 is increased. The extracellular matrix (ECM) is essential for supporting nerve cells in the human central nervous system (CNS), providing key biochemical signals that regulate cell generation, migration, differentiation, and synaptic plasticity. Among the ECM enzymes, MMP2 plays a critical role in neurogenesis, basement membrane remodeling, and axonal regeneration. MMP3 is involved in synaptic remodeling and facilitating the degradation of Aβ protein. Syndecan-3, a member of the heparan sulfate proteoglycan family, influences cell proliferation, axon growth, and the inflammatory response. The alterations in these molecules suggest that during Alzheimer's disease (AD) progression, changes in ECM components may be linked to neuroinflammation, apoptosis, and synaptic degeneration.

5) Additionally, the brain organoids derived from familial Alzheimer's disease (FAD) patients exhibit Aβ and P-tau protein aggregates, which are consistent with findings in previous studies. The degree of neuronal apoptosis and the presence of neurofibrillary tangles (NFTs) in these organoids are directly correlated with the accumulation of protein aggregates. Researchers have successfully cultured these FAD brain organoids from the stem cells of patients with PSEN1 mutations, and these

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organoids display Aβ aggregation, elevated P-tau levels, and apoptosis, further confirming their relevance to Alzheimer's disease pathology.

3.3. Development of Brain Organoids for Studying Sporadic Alzheimer's Disease (SAD)

Brain organoids designed to investigate sporadic Alzheimer's disease (SAD) are created by stimulating brain organoids derived from healthy human stem cells with Aftin-5, a compound known to induce Aβ42 production, which in turn enhances the generation and secretion of soluble extracellular amyloid peptides [18]. To optimize and refine the construction protocol, several improvements were made to the existing method. Initially, the Sendai virus reprogramming technique was employed to reprogram fibroblasts into induced pluripotent stem cells (iPSCs). On day 0, BJ cells were infected with the Sendai virus, and after 48 hours, these cells were transferred onto inactivated mouse embryonic fibroblasts (MEFs). The stem cell culture medium, consisting of DMEM/F12 from Life Technologies, was changed daily and supplemented with 20% KnockOut Serum Replacement (KOSR), glutamine, non-essential amino acids, and basic fibroblast growth factor (bFGF) at a concentration of 10 ng/ml from Stem Cell Technologies. After 20 days of infection, selected clones of the reprogrammed cells were expanded on the MEF feeder layer before being adapted to grow in feeder-free conditions. Following the adaptation process, the tissue was treated with Aftin-5 to induce amyloid pathology, which was subsequently followed by immunohistochemical analysis, gene expression profiling, and Aβ quantification to assess the effects of the treatment.

In parallel, CRISPR/Cas9 technology was employed to introduce a mutation in the APOE gene, specifically converting the APOE3 allele to the APOE4 variant, which is strongly associated with an increased risk of developing Alzheimer's disease [19]. For this genetic manipulation, a single-guide RNA (sgRNA) plasmid targeting the APOE gene was designed, with the sgRNA sequence (5'-CCTCGCCGCGGTACTGCACC - 3') located within 10 nucleotides of the target site. An oligonucleotide pair was synthesized, annealed, and inserted into the pSpCas9-2A-GFP plasmid for precise gene editing. This plasmid was then introduced into the iPSCs using electroporation, and the successfully modified cells were screened for the APOE4 mutation. From these genetically modified iPSCs, excitatory neurons were differentiated, and further differentiation was induced to generate astrocytes and microglia-like cells, all of which were used to form brain organoids. Following the formation of these organoids, immunoblot analysis was conducted to evaluate the levels of Aβ and cholesterol, key biomarkers of Alzheimer's disease pathology, further validating the model's relevance to studying SAD.

The findings from the study suggest that the APOE4 mutation stands as the most significant single genetic risk factor for sporadic Alzheimer's disease (SAD). Specifically, organoids carrying the APOE4 mutation exhibit a notable reduction in the APOE protein levels when compared to normal organoids, despite both organoid types containing similar total cell counts. After six months of culture, APOE4 organoids demonstrate considerably elevated levels of Aβ and P-tau, with clear and significant differences when compared to APOE3 organoids.

In another set of experiments, brain organoids representing SAD were successfully generated from human pluripotent stem cells derived from Alzheimer's patients carrying mutations in the APOE gene [20]. These organoids not only show heightened concentrations of Aβ and P-tau proteins but also reveal notable signs of neuronal apoptosis, synaptic loss, and an increase in the formation of stress granules. These findings underscore a critical link between the APOE4 allele and the amplification of P-tau levels within brain organoids formed from induced pluripotent stem cells (iPSCs), highlighting the potential of this model in further understanding the molecular mechanisms underlying SAD.

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4. Conclusion

Brain organoids, as an emerging and innovative research tool, have opened up new possibilities for studying complex brain functions and diseases. The progress made in the development of brain organoids derived from stem cells has been remarkable, offering new insights into the intricate processes of brain development and disease. The methods used to construct these organoids can be broadly classified into two categories: unguided and guided approaches. The unguided approach relies on the natural, spontaneous differentiation of stem cells, allowing them to form heterogeneous tissues. On the other hand, the guided method involves the use of specific external signaling factors that direct the differentiation of stem cells, creating organoids that resemble particular brain regions. Furthermore, the fusion of spheroids offers a novel way to simulate the interaction between different brain regions, which forms the basis for developing diverse and more accurate brain organoid models.

The cellular diversity observed in brain organoids is striking. Depending on the initial stem cell type and the conditions under which they are cultured, these organoids can generate various cell types that mirror those found in different regions of the brain. This ability to produce multiple types of neurons and glial cells allows organoids to replicate the physiological properties and functionality of the human brain to a certain extent. When it comes to disease modeling, brain organoids have proven to be invaluable tools in studying a wide array of neurological disorders. These models have been instrumental in investigating congenital brain malformations, neurodegenerative diseases, and conditions related to dysfunctional protein pathways, such as Alzheimer's disease. Through the use of organoids, researchers have made significant strides in uncovering the underlying mechanisms of these diseases, providing potential avenues for therapeutic development.

The use of brain organoid models derived from induced pluripotent stem cells (iPSCs) represents a significant advancement in Alzheimer's disease (AD) research, offering unique benefits in understanding the disease. Specifically, familial Alzheimer's disease (FAD) brain organoids provide a powerful platform to investigate key pathological features, such as the accumulation of amyloid-β and tau proteins. These organoids also demonstrate a pathology progression that closely mirrors the timeline observed in human FAD. Notably, changes in glutamatergic neuron numbers and increased lactate dehydrogenase activity are also evident, offering valuable insights into the mechanisms underlying FAD. Similarly, brain organoids used to study sporadic Alzheimer's disease (SAD), developed through techniques like Aftin-5 induction or APOE gene editing, can replicate critical pathological alterations seen in SAD, including amyloid-β aggregation and an imbalance between Aβ42 and Aβ40 peptides.

Despite the significant promise shown by these brain organoid models, several challenges persist in current research. Existing organoid models remain relatively simplistic and underdeveloped, with considerable differences between the simulated conditions in the organoids and those in living organisms. These discrepancies hinder both the depth of disease mechanism exploration and the accuracy of drug screening efforts. To address these issues, future research should prioritize refining the construction techniques to improve the complexity and maturity of organoids, aiming to make them more representative of the true structural and functional properties of the human brain. Furthermore, the incorporation of cutting-edge technologies, such as single-cell multi-omics and gene editing, will allow for a more comprehensive investigation of the molecular processes driving brain organoid development and disease pathogenesis. In addition, further studies on the disparities between organoid models and in-vivo environments will be crucial. Enhancing our ability to translate brain organoid research into practical clinical applications will be essential for advancing neuroscience research and the development of precision medicine strategies.

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