ORGANELLE
REVIEW | OPEN ACCESS

Jing *et al.* Organelle (2025) 3:7
https://doi.org/10.61747/0ifp.202503007

Advances in Brain Organoid Modeling and Its Applications in Brain Disorders

Yue Jing, Feifei Li, Chao Xia, Fei Xie,* and Xuemei Ma*
College of Chemistry and Life Science, Beijing University of Technology, Beijing, China 100124
*Corresponding to: Fei Xie. No.100, Pingleyuan, Chaoyang, Beijing100124, China. E-mail: xiefei990815@bjut.edu.cn. Xuemei Ma. No.100, Pingleyuan, Chaoyang, Beijing100124, China. E-mail: xmma@bjut.edu.cn.

Received: 24 August 2025; Revised: 9 September 2025; Accepted: 1 October 2025

Journal homepage: https://www.organelles.org

ABSTRACT

Brain organoids, as 3D cell culture models simulating human brain development and structure, have made significant progress in neuroscience research in recent years. This review systematically summarizes the current research status of brain organoids, including their construction methods-specifically patient-derived brain tumor tissues and iPSC induced brain organoids. It further discusses the applications of brain organoids (alone or in combination with microfluidic technology) in disease model construction, mechanism research, and drug screening. Additionally, the technical limitations and challenges inherent to this model system are discussed. Finally, the future development directions of brain organoids are prospected, with the aim of providing a comprehensive reference for related research fields.

Key words: Brain organoids; Brain disorders; iPSC; Brain organoids-on-a-Chip; Microfluidics

List of abbreviations:

iPSC	Induce pluripotent stem cells
ESC	Embryonic stem cells
EB	Embryoid bodies
PDTO	Patient-derived tumor organoids
GBM	Glioblastoma multiforme
GBO	Glioblastoma organoids
GSCs	Glioma stem cells
PDX	Patient-derived xenograft
PDOX	Patient-derived orthotopic xenografts
IDH	Isocitrate dehydrogenase
TMZ	Temozolomide
OPC	Oligodendrocyte precursor cell
NPC	Neural Progenitor Cell-like cells
IPTO	Individualized patient tumor organoids
EMT	Epithelial-mesenchymal transition
NDDs	Neurodevelopmental disorders
TCGA	The Cancer Genome Atlas
MDS	Miller-Dieker syndrome
FXS	Fragile X syndrome
RTT	Rett syndrome
ASD	Autism spectrum disorder
SNRs	Single neural rosettes
AD	Alzheimer's disease
Aβ	β-Amyloid
p-Tau	Phosphorylated tau
NFTs	Neurofibrillary tangles
BBB	Blood-brain barrier
ALS	Amyotrophic Lateral Sclerosis
PDC	Parkinsonism dementia complex
PD	Parkinson's disease
FTD	Frontotemporal dementia
GRN	Granulin precursor gene
HD	Huntington's Disease
HUVECs	Human umbilical vein endothelial cells
COC	Cyclo-Olefin Copolymer
RA	Retinoic acid
RXR	Retinoid X receptor
SHH	Sonic hedgehog homology
FGF8	Fibroblast growth factor 8
TGF-β	Transforming Growth Factor-β
BMP	Bone Morphogenetic Protein
MSNs	Medium spiny neurons

INTRODUCTION

The brain, acknowledged as the most complex organ in the human body, presents significant challenges in studying its structural functions and in development treatment for related diseases. Among neurological disorders, glioblastoma multiforme (GBM) is the most common primary brain tumor in adults, accounting for 45.2% of primary malignant tumors in the brain and central nervous system, with a median survival period of only 15 months [1,2]. Another major public health challenge, Alzheimer's disease (AD), increases sharply with age. It is estimated that by 2036, the global number of AD patients will reach 19.117 million, as current medications are unable to repair damaged neurons or reverse the progression of the disease [3]. These neurological diseases, characterized by high disability rates and low cure rates, pose a significant long-term challenge for modern medicine [4]. Traditional research methodologies relying on animal models and 2D cell cultures face limitations: the former fails to accurately reflect human brain pathology due to interspecies

The Article Processing Charge for this work was funded by the authors.

differences, while the latter cannot recapitulate the intricate cell-cell interactions within a 3D microenvironment [4,5]. Consequently, the development of in vitro models capable of simulating the complex physiological processes of human organs is critically important.

In 2009, Sato et al. [6] first demonstrated that Lgr5-expressing mouse intestinal stem cells could be cultured in vitro to form micro-tissues exhibiting crypt-villus structures, marking the initiation of organoid technology. In 2013, Lancaster et al. [7] successfully generated brain organoids in vitro from induced pluripotent stem cells (iPSCs) derived from both healthy individuals and microcephaly patients. This breakthrough provided the first iPSC-based three-dimensional model for studying developmental neurobiology, catalyzing the rapid advancement of brain organoid technology. Brain organoid construction techniques involve the directed differentiation of iPSCs or ESCs, or the direct use of patient-derived brain tumor tissues [8,9]. These techniques generate miniature organs in culture dishes that replicate key structural and functional characteristics of human brain or tumor tissue, thereby providing a revolutionary platform for investigating brain development and disease pathogenesis [10,11]. This "in vitro brain model" not only simulates physiological processes such as cortical layering and neural circuit formation but also facilitates the investigation of cellular and molecular mechanisms underlying disease pathogenesis, as well as the modeling of tumor microenvironments under controlled conditions [7]. Consequently, it is widely considered to be a critical bridge connecting fundamental research with clinical applications.

Currently, research on brain organoids has advanced in three significant directions. First, to tackle the challenge of brain tumor heterogeneity, researchers are constructing brain tumor organoids using either primary tumor cells extracted from patients or induced pluripotent stem cell iPSC-derived tumor cells. This approach offers an ex vivo model for developing personalized anticancer drugs [12]; Secondly, individualized brain organoids derived from iPSC technology have made significant advancements. By reprogramming patients' somatic cells into iPSCs followed by directed differentiation, researchers have constructed organoids containing gene mutations associated with familial AD, Huntington's disease, and other disorders, these models successfully recapitulate patient-specific phenotypes of neurological disorders in vitro [13].Thirdly, the interdisciplinary convergence of organoid and microfluidic technologies is enhancing the reproduction of human physiological structures and functions through model systems, microfluidic chips enable precise modulation of fluid shear stress, nutrient gradients, and oxygen tension, effectively addressing the issue of hypoxia-induced core necrosis commonly observed in conventional organoid cultures [14,15]. More importantly, these integrated systems can create functional in vitro blood-brain barrier (BBB) models that replicate dynamic barrier functionality, thereby offering a novel platform for investigating neuro-immune crosstalk and conducting high-throughput drug permeability screening for brain-targeted therapeutics [14].

This review highlights recent advances in brain organoid modeling related to disease mechanisms and applications while also addressing some of the challenges associated with organoids. It thereby offers a comprehensive reference for the current state of organoid technology.

1. PATIENT-DERIVED ORGANOIDS

Brain tumors, encompassing both primary and metastatic types, present a significant threat to human survival due to limited treatment options and poor prognoses [16]. Patient-derived tumor organoids (PDTOs), as emerging research tools, retain the cellular heterogeneity, genetic characteristics, and spatial organization of the original tumor, allowing them to accurately mimic tumor growth, invasion, and response to treatment [16]. The procedure for establishing PDTOs is shown in Figure 1. In summary, tumor tissues are aseptically obtained during surgery and processed within two hours to maintain their viability. Necrosis-free, dense tumor regions are meticulously dissected into 0.5-1 mm pieces and cultured in a growth factor-enriched medium

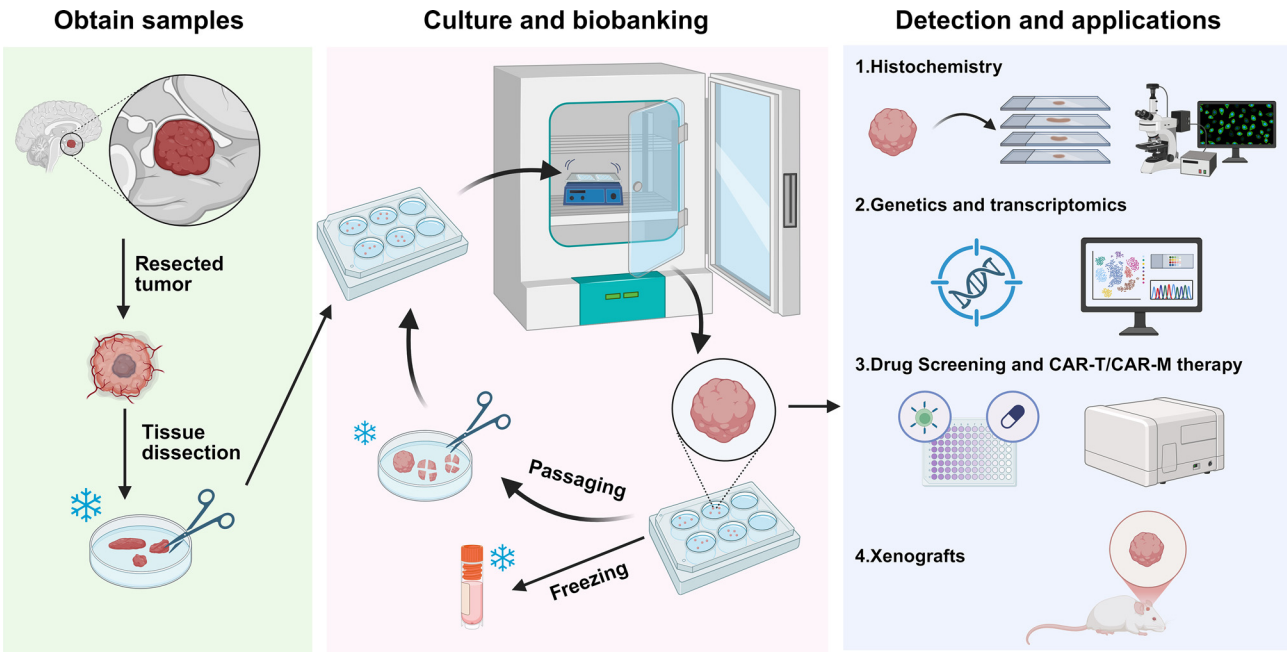

Figure 1. Schematic of Tumor Organoid Biobanking: Construction and Applications (Figure created with BioRender).

under dynamic conditions (using rotating bioreactors or shakers), the formation of organoids is evaluated after 1-2 weeks. Subculturing and biobanking begin approximately four weeks post-establishment [17,18].

High-grade gliomas have become a significant challenge in clinical treatment due to their infiltrative growth and a recurrence rate exceeding 90%, the development of high-fidelity in vitro models is essential for overcoming this treatment dilemma [19]. In 2016, Hubert et al. [20] pioneered direct culture GBM tumor organoids from patient samples, replicating the hypoxic gradient-driven spatial heterogeneity of tumor stem cells, which is a hallmark of in vivo microenvironments. This innovative approach established an effective platform for oncology drug screening. Logun et al. [21] employed glioblastoma organoids (GBOs) to replicate the responses to CAR-T therapy, demonstrating that tumor cytolysis was directly correlated with the levels of CAR-T engraftment in patient cerebrospinal fluid; and the cytokine release kinetics were consistent with in vivo profiles. Similarly, Zhai et al. [22] demonstrated that patient-derived autologous Tanζ-T28-Δ7R CAR-T cells exhibit potent anti-tumor activity and favorable safety profiles in GBM models, these findings are consistent with results validated in patient-derived glioma stem cells (GSCs), orthotopic xenograft models, and patient-derived xenograft (PDX) models. A study collected tumor tissues from 241 patients with different glioma subtypes, including isocitrate dehydrogenase1 (IDH1) mutation, EGFR amplification and other molecular subtypes, and successfully established over 160 organoids and 40 patient-derived orthotopic xenografts (PDOX) models to validate the efficacy of temozolomide (TMZ) and VAL-083, providing an excellent platform for high-throughput drug screening [23]. Beyond drug screening applications, tumor organoids have proven instrumental in revealing novel molecular features and tumorigenic mechanisms. Researchers established PDTOs from low- and high-grade IDH-mutant gliomas, proposing that IDH-mutant gliomas form when oligodendrocyte precursor cell (OPC) transform into slow-growing OPC-like malignant cells, driven by DNA hypermethylation-related epigenetic changes, and indicating that tumor progression is related to a switch from epigenetic to genetic drivers [24]. Olga Kim et al. [25] used samples from the same patient with IDH1 R132H mutant glioma to simultaneously establish low-grade glioma and high-grade glioma organoid models, dynamically modeling tumor progression. This revealed critical roles of epithelial-mesenchymal transition (EMT) and Notch signaling in malignant transformation, along with metabolic reprogramming links (such as glutamine dependency, activated fatty acid metabolism) to malignant progression, providing novel insights into aggressive mechanisms in IDH-mutant gliomas [25]. Using organoids derived from adamantinomatous craniopharyngiomas in conjunction with single-cell sequencing and spatial transcriptomics, Chen et al. [26] revealed aberrant activation of the PROS1/GAS6-AXL signaling axis between cancer-associated fibroblasts and tumor epithelial cells in the tumor microenvironment, and further demonstrated that the AXL inhibitor bemcentinib effectively blocks tumor progression, providing multi-omics evidence for the clinical translation of AXL inhibitor combined with immunotherapy.

Although PDTOs offer significant advantages in mimicking tumor heterogeneity and facilitating personalized treatment, they encounter several limitations in the clinical translation process, such as the restricted availability of samples, the success rate of culture being influenced by multiple factors, and the challenge of accurately replicating the invasiveness and metastatic capabilities of in vivo tumors [16]. iPSC-derived organoids in combination with tumor organoids are expected to overcome these limitations.

2. IPSC-DERIVED BRAIN ORGANOIDS

2.1. Construction of brain organoids

iPSCs can be generated through the reprogramming of somatic cells from patients (such as dermal fibroblasts, peripheral blood mononuclear cells, etc.) via the introduction of specific transcription factors (including Oct3/4, Sox2, Klf4, c-Myc, etc.) [27,28]. Lancaster et al. [7] pioneered the establishment of brain organoids using iPSCs derived from healthy donors and microcephaly patients. In 2014, the protocol for constructing brain organoids was published (Figure 2, Top panel) and this protocol employed unguided methods, which rely entirely on the spontaneous morphogenesis and intrinsic differentiation capabilities within iPSC aggregates then form mixed and unorganized brain regions that mimic the complexity of whole-brain development [29]. However, organoids generated with this method show greater randomness, significant batch-to-batch variation, and standardization challenges [16,30,31]. The alternative approach employs guided differentiation protocols, which utilize external patterning factors to induce region-specific organoids and precisely control developmental signaling pathways, this minimizes stochasticity while enhancing regional specificity and reproducibility, with the process typically segmented into three phases: an initial induction phase, a differentiation induction phase involving region-specific patterning, and neuron maturation phase for functional development [10,32]. Forebrain organoid construction typically involves dual SMAD inhibition during early neural induction to promote forebrain neuroectoderm formation, followed by telencephalic patterning via specific factors such as WNT pathway inhibitors and transforming growth factor-β (TGF-β) inhibitors in the culture medium [11]. Forebrain organoids can simulate processes such as neurogenesis, neuronal migration, and synapse formation in the forebrain, and have significant value in the study of neurodevelopmental disorders and neurodegenerative diseases [33]. The addition of SMAD inhibitors and WNT activators during the neuroectodermal development stage, followed by treatment with factors such as sonic hedgehog homology (SHH) and fibroblast growth factor 8 (FGF8) during the patterning stage, promotes the differentiation of midbrain dopaminergic neurons [11,34]. iPSC-derived midbrain organoids contain electrophysiologically active, mature dopaminergic neurons and generate structures similar to human substantia nigra neuromelanin granules, these organoids are valuable models for studying dopaminergic neuron development, function and related disease mechanisms [35]. Retinoic acid (RA) is a key factor in hindbrain development, and its signaling plays a central role in patterning the hindbrain's anteroposterior (A-P) axis [11]. Hindbrain organoids model cerebellum and brainstem development, providing platforms to investigate cerebellar developmental disorders such as ataxia and brainstem dysfunction [36]. In addition to the dual SMAD inhibition, which is added during the initial induction phase, the cytokines required for region-specific patterning differentiation are shown in Table 1.

2.2. Modeling Human Neurological Disorders with Brain Organoids

Brain tumors: iPSC-derived brain organoids serve as a transformative platform for brain tumor research by mimicking the tumor microenvironment, preserving spatial heterogeneity, and predicting clinical drug responses [43–45]. Researchers used gene-editing technology to introduce oncogenic mutations in brain organoids. By testing 18 single-gene mutations/amplifications and 15 distinct mutation

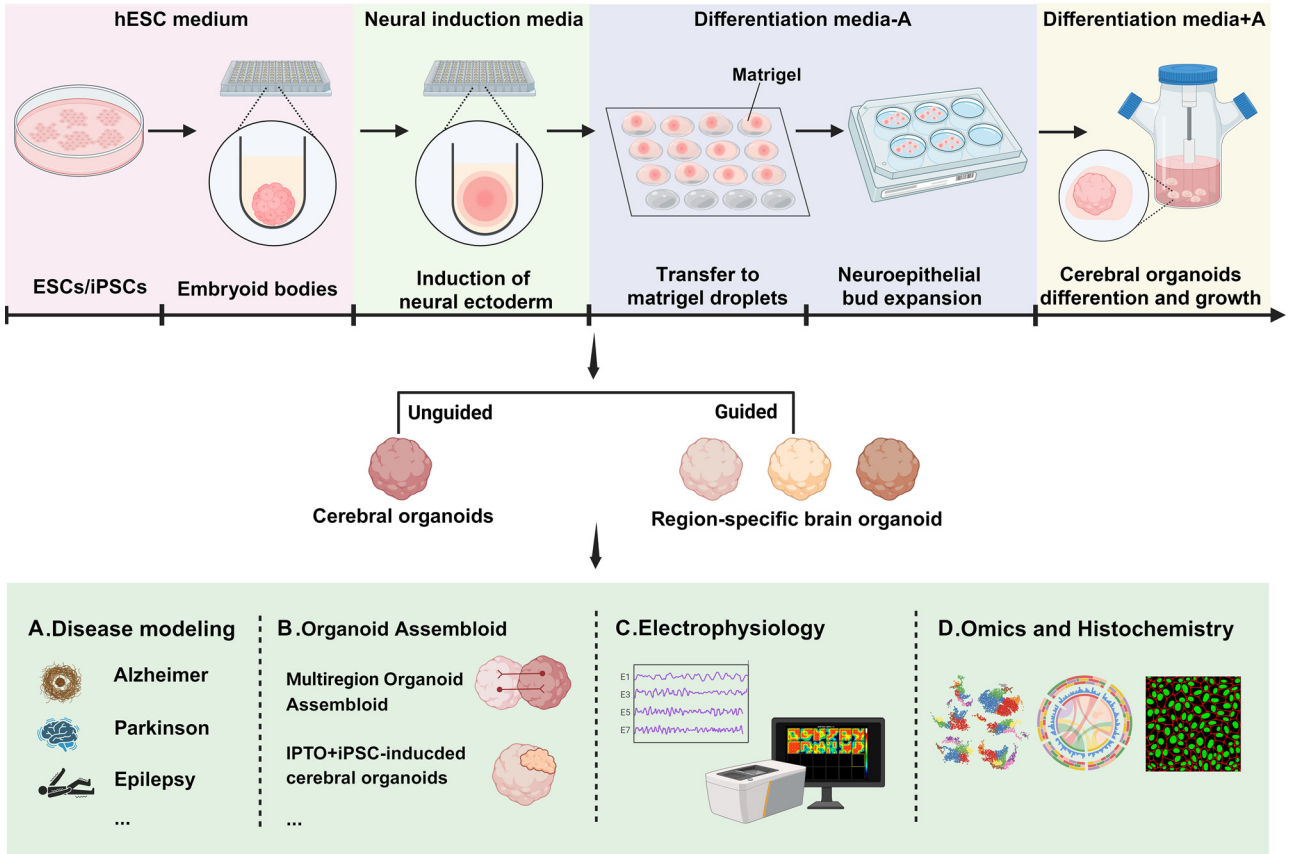

Figure 2. iPSC-Derived brain organoid development and applications (Figure created with BioRender).

combinations derived from The Cancer Genome Atlas (TCGA), involving GBM, pediatric central nervous system primitive neuroectodermal tumor, atypical teratoid/rhabdoid tumor, and medulloblastoma, they discovered that specific mutation combinations could induce the formation of GBM-like and CNS-PNET-like tumors [46]. Similarly, through knockout of key GBM tumor suppressor genes (including various combinations of PTEN, TP53, CDKN2A, CDKN2B, and NF1) integrated with multi-omics analysis, this study revealed how genotypes determine the molecular characteristics and functionality of tumors, identified genetic heterogeneity-particularly NF1 mutations-as a central driver of molecular and functional heterogeneity in GBM (notably the mesenchymal phenotype), and established the pivotal role of glycerolipid metabolism in brain tumors [47]. Peng et al. [12] developed individualized patient tumor organoids (IPTO), a novel brain tumor model created by implanting fresh patient tumor tissue blocks directly into human iPSC-derived brain organoids, this model accurately replicates patient-specific brain tumors' cellular ecosystem (including tumor heterogeneity and microenvironment/TME) and molecular pathology. IPTO features rapid 10-day generation, high success rates, and broad applicability. In clinical trials, it successfully predicted patient responses to chemotherapy (temozolomide) and targeted therapy (osimertinib), enabling precision oncology [12]. The Hi-Q platform innovatively achieves high-throughput, high-homogeneity, low-stress, and cryopreservable production of brain organoids, enabling medium-throughput screening of therapeutic agents for glioma [48].

Neurodevelopmental disorders: Neurodevelopmental disorders (NDDs) are a complex group of disorders characterized by impairments in the growth and development of the brain or central nervous system, resulting in deficits in cognitive, emotional, physical, and social functioning [49]. For a long time, research on NDDs has faced challenges due to difficulties in accessing the developing human brain, ethical constraints, and inadequate reproduction of human-specific processes in traditional models, however, significant progress has been made through brain-like organoid technology [50,51]. For example, iPSC-derived brain organoids from Miller-Dieker syndrome (MDS) patients successfully mimicked the cellular features of lissencephalic malformations, revealed a delayed mitosis phenotype in outer radial glia, and reproduced increased apoptosis and defective spindle orientation due to deletion of the LIS1/YWHAE gene in the organoids [52]. Researchers used isogenic FMR1-KO iPSCs in 2D/3D models to demonstrate for the first time that FMRP deficiency causes neural developmental imbalance, including astrocytic hyperplasia and excitatory synaptic dysfunction-a core feature of Fragile X syndrome (FXS). This method overcomes species limitations in animal models and offers a new platform for drug screening and mechanistic studies [53]. Samarasinghe et al. [54] used Rett syndrome (RTT) patient-derived iPSCs to create fused cortical-ganglionic eminence brain organoids, these organoids modeled key pathological features of human neural networks, including excitation/inhibition imbalance and epileptiform high-frequency oscillations. The study identified impaired inhibitory neuron function as the core disease mechanism and showed that Pifithrin-α partially reversed these defects, demonstrating the utility of organoid platforms for studying neurological diseases and drug development [54]. In autism spectrum disorder (ASD), Li et al. [55] developed the CHOOSE system (CRISPR-human organoids-single-cell

Table 1. Cytokines required for region-specific pattern differentiation in brain organoids.

	Inducing factors	Function	Ref.
Forebrain			
Cortex	WNT inhibitor + TGF-β inhibitor	Neuroepithelial extension, maintain forebrain properties	[10,37]
Hippocampus	WNT activator + BMP activator/SHH inhibitor	Activate dorsal nerve precursors, Blocking ventral lateralization signals	[38]
Striatum	BMP inhibitor + TGF-β inhibitor + WNT inhibitor + activin A + RXR	Lateral ganglion bulge induction, strengthen the ventralization signal,induce specialization of striatum interneurons and MSNs	[39]
Hypothalamus	BMP inhibitor + TGF-β inhibitor + WNT activator/inhibitior + SHH + FGF	Ventral forebrain specialization, hypothalamic nuclei regionalization	[40]
Midbrain	BMP inhibitor + TGF-β inhibitor + WNT activator + SHH + FGF8	Neural precursor proliferation and establishment of the midbrain-hindbrain boundary, Ventral lateralization and dopaminergic neuron fate determination	[10,40]
Hindbrain			
Brainstem	BMP inhibitor + TGF-β inhibitor + RA + bFGF + EGF + Insulin + Transferrin + Progesterone	Induction of the midbrain, portions of the peripheral brainstem and neural crest regions behind it for neuronal stem/progenitor cell expansion	[41]
Cerebellum	TGF-β inhibitor + BMP inhibitor + Insulin + Wnt activator + FGF8b + SDF1a	Designation of the midbrain-hindbrain boundary and inhibition of midbrain fate, Support for precursor cell expansion, forms organized laminar layering, and develops functional neurons	[36]
Spinal Cord	TGF-β inhibitor + WNT activator + FGF + BMP4 + RA	Induction of dorsal spinal cord and sensory interneurons	[42]
	TGF-β inhibitor + WNT activator + FGF + RA + SHH	Induction of ventral spinal cord and motor neuron production	

RNA sequencing) to study 36 high-risk ASD genes, revealing their roles in cell fate determination, and they verified in patient-derived organoids that ARID1B mutations in the BAF complex lead to differentiation defects in oligodendrocyte and interneuron precursor cells. Brain organoids derived from schizophrenia patients with NRXN1 deletions exhibit aberrant developmental trajectories in early neural progenitor cells (NPC) and are significantly enriched for risk genes associated with schizophrenia and ASD [56]. A study demonstrates that low-intensity ultrasound promotes neural progenitor cell proliferation, neuronal maturation, and synaptogenesis in human brain organoids by activating the YAP signaling pathway, it enhances structural and functional connectivity of transplanted organoids in injured brains and rescues neurodevelopmental defects in microcephaly models, offering a non-invasive treatment for neurodevelopmental disorders and cortical injuries [57]. Another study generates human telencephalic brain organoids from stem cell-derived single neural rosettes (SNRs), which can stably produce structured tissues containing dorsal (cortical) and ventral (striatal) neural progenitor cells, excitatory and inhibitory neurons, as well as astrocytes, oligodendrocytes [58,59]. Using SNR-derived organoids, we demonstrated that SHANK3 deficiency leads to abnormal intrinsic neuronal excitability, excitatory synaptic deficits, and significant downregulation of clustered protocadherins, providing novel molecular insights into the pathological mechanisms of ASD [60].

Neurodegenerative diseases: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline. Core pathological features include amyloid-beta plaques, neurofibrillary tangles (NFTs), neuronal loss, and brain atrophy, which collectively disrupt neural networks and exacerbate impairment [61]. A study describes a rapid and reproducible method to recapitulate tau pathology within three weeks, by exposing human iPSC-derived neurons to human tau oligomers and co-culturing them with iPSC-derived astrocytes to form 3D assembloids [62]. Another study overcomes the limitation of slow astrocyte differentiation in conventional brain organoids by constructing chimeric organoids-induced by mixing iPSCs expressing NFIB/SOX9 and APOE3/APOE4-with functional astrocytes detected as early as 45 days [63]. Chen et al. [64] modeled Sporadic AD BBB breakdown using serum-exposed hiPSC brain organoids, demonstrating β-Amyloid (Aβ) aggregation, phosphorylated tau (p-tau), synaptic loss, and neural impairment, and that serum exposure exacerbated pathology by upregulating BACE and GSK3α/β expression. Researchers have developed a novel human-derived AD brain organoid model by knocking in three familial APP mutations into iPSCs harboring the APOE3 Christchurch variant and differentiating them into forebrain organoids, this comprehensively demonstrates that the rare APOE3ch mutation confers robust protection against Aβ deposition, tau pathology, gliosis, as well as neuronal apoptosis and necroptosis [65]. Zhao et al. [66] also confirmed that isogenic conversion of APOE4 to APOE3 reversed most AD-related phenotypes in AD brain organoids. Using iPSC-derived brain organoids from PSEN1 M139V fAD patients, Wang et al. [67] discovered that G3BP2 inhibits Tau aggregation by masking its microtubule-binding region, this identifies G3BP2 as a natural neuronal defense against tau pathology and a potential early therapeutic target for tauopathies. Hong et al. [68] generated amyotrophic lateral sclerosis (ALS)-parkinsonism dementia complex (PDC) brain organoids from patient-derived iPSCs, establishing a microglia-containing brain organoid platform, they demonstrated that IFN-γ supplementation restores M2 microglia levels and enhances β-amyloid uptake in ALS-PDC-affected organoids. The study of these organoids will help to gain insight into the pathogenesis of AD and identify new therapeutic targets.

Midbrain organoids for parkinson's disease (PD) can recapitulate pathological processes such as progressive degeneration of dopaminergic neurons and α-synuclein aggregation observed in PD patients [69]. A study constructed 3D organoids by mixing iPSC-differentiated astrocytes with human fibroblasts reprogrammed using FUW-Ascl1, Pitx3, Nurr1, and Lmx1a. Mature dopaminergic neuronal markers were observed at 20 days, with increased neuromelanin-containing cells at 30 days, more faithfully recapitulating the aged human midbrain milieu [70]. Another research successfully generated 3D midbrain-like organoids using human embryonic stem cells (hESCs), in

which TH^+ neurons exhibited biochemical and electrophysiological properties of mature functional dopaminergic neurons, these neurons produced neuromelanin-like granules and formed functional neuronal networks, demonstrating the organoids' potential for studying synaptic connectivity and neuronal function [35]. Transplantation of hiPSC-derived midbrain organoids into the striatum of PD mouse models enables effective survival and differentiation into functional dopaminergic neurons. These neurons establish bidirectional neural connections within the host brain, including axonal projections to the substantia nigra and cortex, and significantly ameliorate motor dysfunction, demonstrating robust neural integration and functional recovery [71]. The ventral midbrain-striatum-cortex assembloids successfully models the long-distance functional projections of dopaminergic neurons from the ventral midbrain to the striatum and cortex in vivo, serving as a novel in vitro dopaminergic system model, particularly applicable to cell therapy for PD and research on neural circuit mechanisms underlying addictive disorders [72]. DNAJC6-mutant hESCs, differentiated into midbrain organoids and dopaminergic neurons, revealed early ventral patterning defects, DNAJC6 deficiency impaired clathrin-mediated endocytosis, disrupting the WNT-LMX1A feedback loop and causing sustained suppression of midbrain developmental genes, thereby recapitulating the full DNAJC6-linked PD phenotype [73]. An et al. [74] developed a novel mesoporous Au nanodot-patterned 3D concave electrode for drug evaluation in PD patient-derived midbrain organoids.

ALS and frontotemporal dementia (FTD) share core pathologies of neuron death, glial activation, and aberrant TDP-43 aggregation, with ∼20% of patients having C9ORF72 mutations (30-50% in familial cases) and ∼15% exhibiting ALS-FTD syndrome [75]. A novel biohybrid motor neuron spheroid, incorporating reduced graphene oxide, human umbilical vein endothelial cells (HUVECs), and iPSCs from ALS patients, mitigates core necrosis and enhances neurogenesis in 3D neural models, thereby improving electrophysiological outcomes, when integrated with muscle bundles, this spheroid forms a neuromuscular junction model for simulating ALS pathology and conducting drug screening [76]. Furthermore, the researchers developed a mature brain organoid model using human iPSCs and were able to spontaneously replicate TDP-43 protein dysfunction in vitro in the absence of granulin precursor gene (GRN) dysfunction. This included TDP-43 mislocalization, hyperphosphorylation, loss of function, and aberrant splicing of STMN2, effectively addressing the long-standing challenge of simulating TDP-43 protein lesions in vitro [77]. Furthermore, a study demonstrates at the 3D organoid level that the C9ORF72 mutation induces mitochondrial dysfunction by promoting the accumulation of astrocyte-specific mitochondrial DNA mutations [78].

Huntington's disease (HD), an autosomal dominant neurodegenerative disorder caused by abnormal CAG trinucleotide repeat expansion in the HTT gene, manifests core pathology through selective degeneration of striatal and cortical neurons, which clinically presents as motor impairments (including choreiform movements), cognitive decline, and neuropsychiatric abnormalities [79]. Currently, no cure exists for this disease. Research indicates that healthy cells, through direct contact, can rescue key HD phenotypes-including developmental defects, transcriptional abnormalities, maturation trajectories, synaptic density, and communication pathways-in human telencephalic organoids via non-cell autonomous mechanisms, which is predominantly observed in ventral lineages whereas HD cells exert minimal influence on healthy counterparts, these findings strongly support therapeutic strategies that employ cell replacement or gene silencing [80]. Another study successfully modeled neuronal projection defects using assembloids composed of striatal-like organoids and midbrain substantia nigra-like organoids, which exhibit a higher proportion of medium spiny neurons (MSNs) and dopaminergic neurons, thereby enhancing their similarity to the human brain in vitro [81]. Using gene-edited iPSCs to generate brain organoids with different HTT genotypes (biallelic 70Q, monoallelic mutations, poly-Q-free controls), mutant HTT was shown to cause neurodevelopmental toxicity before neurodegeneration in human organoids, this revealed the CHCHD2-mISR axis as a critical pathogenic pathway [82]; inhibiting its effector ADAM10 rescued early phenotypes in vitro, improving neuronal differentiation [79]. Furthermore, cortico-striatal assembloid models have revealed that the ARF1/Golgi pathway-whose dysregulation disrupts Golgi-mediated protein trafficking and neuronal maturation-constitutes a potential therapeutic target for neurodevelopmental impairments in HD [83].

3. BRAIN ORGANOIDS-ON-A-CHIP WITH MICROFLUIDIC INTEGRATION

The complex functions of the human brain critically rely on its precise 3D architecture and dynamic microenvironment, wherein an efficient vascular network and functional BBB are essential for neuronal survival, synaptic connectivity, and overall neural circuit operation [84]. Conventional 2D cultures and non-vascularized 3D brain organoids, which lack functional vascularization and a BBB, develop necrotic cores due to inadequate nutrient/oxygen diffusion and metabolic waste accumulation, thereby fundamentally restricting their capacity to model neurovascular interactions, drug penetration dynamics, and neurodegenerative disease mechanisms [85]. Organ-on-a-Chip technology, particularly Brain Organoid-on-a-Chip integrated with microfluidic systems, provides a revolutionary platform to address key challenges-such as vascular absence, necrotic core formation, and functional immaturity-while advancing vascularization research in brain organoids [15,86]. Saberi et al. [87] engineered a microporous PDMS chip with dual culture chambers separated by an 8-μm membrane-a configuration that permits axonal penetration to establish functional neural networks while preserving tissue compartmentalization-and thereby recapitulated epilepsy's core pathology of transregionally propagating abnormal discharges in a 3D in vitro model (Figure 3A). Based on the current research advancements in microfluidic technology, simple 2D and 3D model chips have been developed to simulate the BBB and human microenvironment, which typically incorporate organoids, neurons, human HUVECs, and glial cells to mimic key physiological processes in blood circulation such as shear stress and nutrient exchange (Figure 3B-D) [88–91]. A microfluidic chip that employs microcontact printing to create Geltrex adhesive islands within its central channel facilitates the formation of tubular structures from hPSCs, which achieves 3D spatial patterning of the human neural tube along both rostral-caudal and dorsal-ventral axes in vitro for the first time, recapitulates key embryonic nervous system developmental processes [92]. Consequently, it provides an in vitro platform that closely simulates the in vivo microenvironment (Figure 3E). Quintard et al. [93] designed a four-chamber organ-on-a-chip that incorporates two circuits, a 'surrogate blood circuit' whose channel dimensions were scaled to human physiology, and an 'excretory circuit' with the specific organ combination selected to enable research evaluating compound absorption, distribution, metabolism, and excretion profiles and personalized drug screening (Figure 3G). The PDMS material exhibits certain limitations in hydrophobic compound compatibility and small

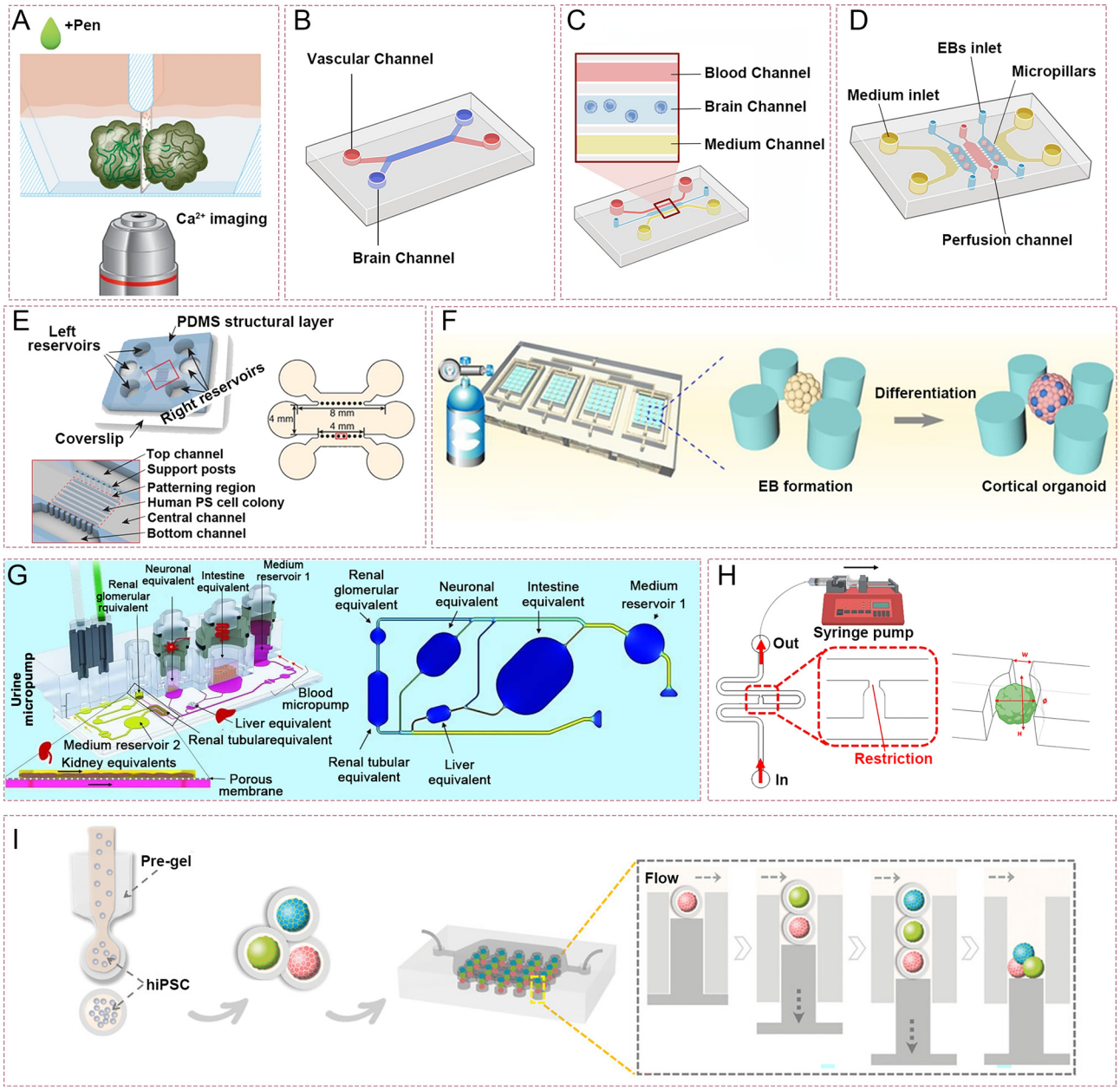

Figure 3. Brain Organoids-on-a-Chip with Microfluidic Integration.

A. Device that simulates the propagation of epileptiform discharges between brain organoids [87]. B. Schematic diagram of a dual-channel microengineered brain organoids-on-a-chip modeling the nigrostriatal pathway [88]. C. 2D BBB chip consisting of three channels [89,90]. D. Schematic of a perfusable brain organoids-on-a-chip featuring dual culture medium channels separated by micropost arrays, with permeable channels enabling nutrient/oxygen exchange [91]. E. Dorsal–ventral patterned microfluidic forebrain-like structures with spatially segregated dorsal and ventral regions and layered apicobasal cellular organizations that mimic development of the human forebrain pallium and subpallium, respectively [92]. F. Chips with micropillars to generate EBs and brain of consistent size and morphology [95]. G. Physiologically inspired model of the four-organ-chip [93]. H. Perfusable organoid vascularization chip [14]. I. Finalization of formed brain assembloids using electrospray technology and microfluidics [96].

molecule absorption. In contrast, chips manufactured with cyclic olefin copolymer (COC) demonstrate long-term stability, suitability for large-scale production, excellent optical properties, and low chemical absorption [94]. Quintard et al. [14] developed a COC-based organ-on-a-chip that incorporated 10 parallel microchannels, each featuring a U-shaped cup-like trap structure for precise organoid positioning, through which functional vascularization and perfusion of multiple organoid types were successfully achieved using advanced hydrogel encapsulation and endothelialization protocols (Figure 3H). Additionally, chips fabricated from other materials exist, such as a photocurable resin chip composed of functional acrylic ester-based monomers, photoinitiators, and crosslinkers, which comprises a top layer with channels for cell injection/fluid flow and a bottom layer containing four independent chambers embedded with micropillar arrays; these chambers enable precise control over embryoid body dimensions to prevent organoid fusion while the four independent chambers

supports parallel drug screening [95] (Figure 3F). Zhu et al. [96] encapsulated hiPSCs in sodium alginate-carboxymethyl cellulose core-shell microcapsules using microfluidic electrospray, which functioned as 3D culture scaffolds to protect cells from mechanical stress, enable nutrient exchange, and promote self-organization into uniform embryoid bodies (EBs), which were subsequently differentiated into region-specific brain organoids (Figure 3I). Additionally, Szebényi et al. [97] pioneered a human brain organoid slice model that resolves hypoxia in conventional organoids. By sectioning 50-day-old cerebral organoids into 300-μm slices for air-liquid interface culture, they extended viability to 240 days and recapitulated early C9ORF72-linked ALS/FTD pathology.

In summary, microfluidic systems not only provide a more physiologically relevant culture environment for brain organoids but also establish the physical foundation for subsequent on-chip induction and integration of vascular networks. Simultaneously, they hold immense potential for applications including screening blood-brain barrier permeability of drugs, assessing neurotoxicity, investigating neurovascular coupling mechanisms, and studying the pathogenesis of brain disorders.

4. CURRENT LIMITATIONS AND FUTURE PERSPECTIVES

Brain organoids, which are self-organizing 3D tissues derived from human pluripotent stem cells, can replicate the structure and function of the human brain. The methods for generating these organoids have been continuously optimized, resulting in the successful development of vascularized organoids [14,30]. When combined with immunostaining and single-cell sequencing technologies, these organoids effectively model the dynamic spatiotemporal processes of early neural development [13]. Additionally, they provide valuable models for a variety of brain diseases, including GBM, AD and PD, thereby offering a novel preclinical platform for investigating pathogenesis and conducting drug screening [13]. However, the current advancements in organoid technology continue to face several challenges and limitations.

Firstly, the lack of internationally standardized protocols for organoid construction, quality assessment, and drug evaluation significantly limits data comparability and reproducibility across research platforms, while mass production of organoids has been achieved via electrospray technology and the Hi-Q platform, their inadequate structural and functional maturity impedes accurate simulation of the highly intricate architecture of native organs (particularly the brain) and the characteristics of adult tissues [48,96]. Furthermore, this field is limited by excessively high production costs and technical complexities, which involve resource-intensive processes such as the induction of pluripotent stem cells, the optimization of 3D culture systems, and high-throughput analysis [98]. These factors hinder the potential for large-scale applications. While vascularized organoids and microfluidic systems have significantly mitigated issues related to vascular deficiency-induced necrosis through dynamic perfusion and engineered designs that preliminarily simulate multi-organ interactions, challenges concerning the deep integration of functional vascular networks, neuro-immune co-simulation, and scalable production remain unresolved [99]. The potential development of consciousness in brain organoids, driven by advances in organoid technology, underscores the imperative for ethical norms and guidelines to mitigate associated risks [99]. To address these challenges, we propose leveraging multi-omics integration to imultaneously acquire transcriptomic, proteomic, metabolomic, and electrophysiological data at single-cell and spatial resolutions [100]. Furthermore, by incorporating machine learning techniques, we can identify key factors influencing cellular development, which will enable the optimization of culture conditions, enhance overall organoid maturity, and improve consistency across experimental batches [100,101].

In summary, this review discusses the construction of cerebral organoids and their applications in disease modeling. It also highlights the ongoing challenges in organoid technology, including drug evaluation, functional simulation, cost efficiency, and vascularization. Future advancements, particularly breakthroughs in vascularization, immunomodulation, and systematization, expected to enhance the utility of organoids. At the same time, the establishment of standardized protocols and ethical frameworks is a crucial prerequisite for translating organoid technology from laboratories to clinical practice.

AUTHOR CONTRIBUTIONS

Conceptualization, F.X., and X.M.; writing-original draft preparation, Y.J. and F.X.; writing-review and editing, F.L. C.X., and X.M.; visualization, Y.J., F.L. and C.X.; funding acquisition, X.M. All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was supported by the Military Logistics Key Open Research Projects (BHJ17L018).

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Not applicable.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Hu D, Xia M, Wu L, et al. Challenges and advances for glioma therapy based on inorganic nanoparticles[J]. *Mater Today Bio*. 2023, *20*: 100673. doi:10.1016/j.mtbio.2023.100673
2. Kanderi T, Munakomi S, Gupta V. Glioblastoma Multiforme[J]. 2025.
3. Xu L, Wang Z, Li M, et al. Global incidence trends and projections of Alzheimer disease and other dementias: an age-period-cohort analysis 2021[J]. *J Glob Health*. 2025, *15*: 4156. doi:10.7189/jogh.15.04156
4. Rubio A D, Hamilton L, Bausch M, et al. A Comprehensive Review on Utilizing Human Brain Organoids to Study Neuroinflammation in Neurological Disorders[J]. *J Neuroimmune Pharmacol*. 2025, *20*(1): 23. doi:10.1007/s11481-025-10181-x
5. Bai L, L. Reis R, Chen X, et al. Organoid research: Advanced models, precision medicine, and translational medicine[J]. *Organoid Research*. 2025, *1*(1): 25060009. doi:10.36922/OR025060009
6. Sato T, Vries R G, Snippert H J, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche[J]. *Nature*. 2009, *459*(7244): 262–265. doi:10.1038/nature07935

7. Lancaster M A, Renner M, Martin C A, et al. Cerebral organoids model human brain development and microcephaly[J]. *Nature*. 2013, *501*(7467): 373–379. doi:10.1038/nature12517
8. Yang H, Wang Y, Wang P, et al. Tumor organoids for cancer research and personalized medicine[J]. *Cancer Biol Med*. 2021, *19*(3): 319–332. doi:10.20892/j.issn.2095-3941.2021.0335
9. Azar J, Bahmad H F, Daher D, et al. The Use of Stem Cell-Derived Organoids in Disease Modeling: An Update[J]. *Int J Mol Sci*. 2021, *22*(14). doi:10.3390/ijms22147667
10. Maisumu G, Willerth S, Nestor M, et al. Brain organoids: building higher-order complexity and neural circuitry models[J]. *Trends Biotechnol*. 2025. doi:10.1016/j.tibtech.2025.02.009
11. Chiaradia I, Lancaster M A. Brain organoids for the study of human neurobiology at the interface of in vitro and in vivo[J]. *Nat Neurosci*. 2020, *23*(12): 1496–1508. doi:10.1038/s41593-020-00730-3
12. Peng T, Ma X, Hua W, et al. Individualized patient tumor organoids faithfully preserve human brain tumor ecosystems and predict patient response to therapy[J]. *Cell Stem Cell*. 2025, *32*(4): 652–669. doi:10.1016/j.stem.2025.01.002
13. Sun N, Meng X, Liu Y, et al. Applications of brain organoids in neurodevelopment and neurological diseases[J]. *J Biomed Sci*. 2021, *28*(1): 30. doi:10.1186/s12929-021-00728-4
14. Quintard C, Tubbs E, Jonsson G, et al. A microfluidic platform integrating functional vascularized organoids-on-chip[J]. *Nat Commun*. 2024, *15*(1): 1452. doi:10.1038/s41467-024-45710-4
15. Li Z, Yu D, Zhou C, et al. Engineering vascularised organoid-on-a-chip: strategies, advances and future perspectives[J]. *Biomater Transl*. 2024, *5*(1): 21–32. doi:10.12336/biomatertransl.2024.01.003
16. Xu H, Jiao D, Liu A, et al. Tumor organoids: applications in cancer modeling and potentials in precision medicine[J]. *J Hematol Oncol*. 2022, *15*(1): 58. doi:10.1186/s13045-022-01278-4
17. Jacob F, Ming G L, Song H. Generation and biobanking of patient-derived glioblastoma organoids and their application in CAR T cell testing[J]. *Nat Protoc*. 2020, *15*(12): 4000–4033. doi:10.1038/s41596-020-0402-9
18. Abdullah K G, Bird C E, Buehler J D, et al. Establishment of patient-derived organoid models of lower-grade glioma[J]. *Neuro Oncol*. 2022, *24*(4): 612–623. doi:10.1093/neuonc/noab273
19. Xu X, Li L, Luo L, et al. Opportunities and challenges of glioma organoids[J]. *Cell Commun Signal*. 2021, *19*(1): 102. doi:10.1186/s12964-021-00777-0
20. Hubert C G, Rivera M, Spangler L C, et al. A Three-Dimensional Organoid Culture System Derived from Human Glioblastomas Recapitulates the Hypoxic Gradients and Cancer Stem Cell Heterogeneity of Tumors Found In Vivo[J]. *Cancer Res*. 2016, *76*(8): 2465–2477. doi:10.1158/0008-5472.CAN-15-2402
21. Logun M, Wang X, Sun Y, et al. Patient-derived glioblastoma organoids as real-time avatars for assessing responses to clinical CAR-T cell therapy[J]. *Cell Stem Cell*. 2025, *32*(2): 181–190. doi:10.1016/j.stem.2024.11.010
22. Zhai Y, Li G, Pan C, et al. The development and potent antitumor efficacy of CD44/CD133 dual-targeting IL7Rα-armored CAR-T cells against glioblastoma [J]. *Cancer Lett*. 2025, *614*: 217541. doi:10.1016/j.canlet.2025.217541
23. Golebiewska A, Hau A C, Oudin A, et al. Patient-derived organoids and orthotopic xenografts of primary and recurrent gliomas represent relevant patient avatars for precision oncology[J]. *Acta Neuropathol*. 2020, *140*(6): 919–949. doi:10.1007/s00401-020-02226-7
24. Wu J, Gonzalez C L, Battaglia S, et al. Evolving cell states and oncogenic drivers during the progression of IDH-mutant gliomas[J]. *Nat Cancer*. 2025, *6*(1): 145–157. doi:10.1038/s43018-024-00865-3
25. Kim O, Sergi Z, Yu G, et al. A patient-derived cell model for malignant transformation in IDH-mutant glioma[J]. *Acta Neuropathol Commun*. 2024, *12*(1): 148. doi:10.1186/s40478-024-01860-6
26. Chen Y, Liu X, Ainiwan Y, et al. Axl as a potential therapeutic target for adamantinomatous craniopharyngiomas: Based on single nucleus RNA-seq and spatial transcriptome profiling[J]. *Cancer Lett*. 2024, *592*: 216905. doi:10.1016/j.canlet.2024.216905
27. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors[J]. *Cell*. 2006, *126*(4): 663–676. doi:10.1016/j.cell.2006.07.024
28. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors[J]. *Cell*. 2007, *131*(5): 861–872. doi:10.1016/j.cell.2007.11.019
29. Lancaster M A, Knoblich J A. Generation of cerebral organoids from human pluripotent stem cells[J]. *Nat Protoc*. 2014, *9*(10): 2329–2340. doi:10.1038/nprot.2014.158
30. Qian X, Song H, Ming G L. Brain organoids: advances, applications and challenges[J]. *Development*. 2019, *146*(8). doi:10.1242/dev.166074
31. Li Y, Zeng P M, Wu J, et al. Advances and Applications of Brain Organoids[J]. *Neurosci Bull*. 2023, *39*(11): 1703–1716. doi:10.1007/s12264-023-01065-2
32. Birtele M, Lancaster M, Quadrato G. Modelling human brain development and disease with organoids[J]. *Nat Rev Mol Cell Biol*. 2025, *26*(5): 389–412. doi:10.1038/s41580-024-00804-1
33. Adlakha Y K. Human 3D brain organoids: steering the demolecularization of brain and neurological diseases[J]. *Cell Death Discov*. 2023, *9*(1): 221. doi:10.1038/s41420-023-01523-w
34. Chambers S M, Fasano C A, Papapetrou E P, et al. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling[J]. *Nat Biotechnol*. 2009, *27*(3): 275–280. doi:10.1038/nbt.1529
35. Jo J, Xiao Y, Sun A X, et al. Midbrain-like Organoids from Human Pluripotent Stem Cells Contain Functional Dopaminergic and Neuromelanin-Producing Neurons[J]. *Cell Stem Cell*. 2016, *19*(2): 248–257. doi:10.1016/j.stem.2016.07.005
36. Atamian A, Birtele M, Hosseini N, et al. Human cerebellar organoids with functional Purkinje cells[J]. *Cell Stem Cell*. 2024, *31*(1): 39–51. doi:10.1016/j.stem.2023.11.013
37. Velasco S, Kedaigle A J, Simmons S K, et al. Individual brain organoids reproducibly form cell diversity of the human cerebral cortex[J]. *Nature*. 2019, *570*(7762): 523–527. doi:10.1038/s41586-019-1289-x
38. Pomeshchik Y, Klementieva O, Gil J, et al. Human iPSC-Derived Hippocampal Spheroids: An Innovative Tool for Stratifying Alzheimer Disease Patient-Specific Cellular Phenotypes and Developing Therapies[J]. *Stem Cell Reports*. 2020, *15*(1): 256–273. doi:10.1016/j.stemcr.2020.06.001
39. Ozgun A, Lomboni D J, Aylsworth A, et al. Unraveling the assembloid: Real-time monitoring of dopaminergic neurites in an inter-organoid pathway connecting midbrain and striatal regions[J]. *Mater Today Bio*. 2024, *25*: 100992. doi:10.1016/j.mtbio.2024.100992
40. Qian X, Nguyen H N, Song M M, et al. Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure[J]. *Cell*. 2016, *165*(5): 1238–1254. doi:10.1016/j.cell.2016.04.032
41. Lui K N, Li Z, Lai F P, et al. Organoid models of breathing disorders reveal patterning defect of hindbrain neurons caused by PHOX2B-PARMs[J]. *Stem Cell Reports*. 2023, *18*(7): 1500–1515. doi:10.1016/j.stemcr.2023.05.020
42. Ogura T, Sakaguchi H, Miyamoto S, et al. Three-dimensional induction of dorsal, intermediate and ventral spinal cord tissues from human pluripotent stem cells[J]. *Development*. 2018, *145*(16). doi:10.1242/dev.162214
43. Wang X, Sun Y, Zhang D Y, et al. Glioblastoma modeling with 3D organoids: progress and challenges[J]. *Oxf Open Neurosci*. 2023, *2*: d8. doi:10.1093/oons/kvad008
44. Correia C D, Calado S M, Matos A, et al. Advancing Glioblastoma Research with Innovative Brain Organoid-Based Models[J]. *Cells*. 2025, *14*(4). doi:10.3390/cells14040292
45. Ogawa J, Pao G M, Shokhirev M N, et al. Glioblastoma Model Using Human Cerebral Organoids[J]. *Cell Rep*. 2018, *23*(4): 1220–1229. doi:10.1016/j.celrep.2018.03.105
46. Bian S, Repic M, Guo Z, et al. Genetically engineered cerebral organoids model brain tumor formation[J]. *Nat Methods*. 2018, *15*(8): 631–639. doi:10.1038/s41592-018-0070-7
47. Wang C, Sun M, Shao C, et al. A multidimensional atlas of human glioblastoma-like organoids reveals highly coordinated molecular networks and effective drugs[J]. *NPJ Precis Oncol*. 2024, *8*(1): 19. doi:10.1038/s41698-024-00500-5
48. Ramani A, Pasquini G, Gerkau N J, et al. Reliability of high-quantity human brain organoids for modeling microcephaly, glioma invasion and drug screening[J]. *Nat Commun*. 2024, *15*(1): 10703. doi:10.1038/s41467-024-55226-6

49. Gao S, Shan C, Zhang R, et al. Genetic advances in neurodevelopmental disorders[J]. *Med Rev (2021)*. 2025, *5*(2): 139–151. doi:10.1515/mr-2024-0040
50. Pallanti S, Salerno L. Neurodevelopmental Disorders (NDDs): Beyond the Clinical Definition and Translational Approach[J]. *Children (Basel)*. 2023, *10*(1). doi:10.3390/children10010099
51. Dionne O, Sabatié S, Laurent B. Deciphering the physiopathology of neurodevelopmental disorders using brain organoids[J]. *Brain*. 2025, *148*(1): 12–26. doi:10.1093/brain/awae281
52. Bershteyn M, Nowakowski T J, Pollen A A, et al. Human iPSC-Derived Cerebral Organoids Model Cellular Features of Lissencephaly and Reveal Prolonged Mitosis of Outer Radial Glia[J]. *Cell Stem Cell* 2017, *20*(4): 435–449. doi:10.1016/j.stem.2016.12.007
53. Brighi C, Salaris F, Soloperto A, et al. Novel fragile X syndrome 2D and 3D brain models based on human isogenic FMRP-KO iPSCs[J]. *Cell Death Dis*. 2021, *12*(5): 498. doi:10.1038/s41419-021-03776-8
54. Samarasinghe R A, Miranda O A, Buth J E, et al. Identification of neural oscillations and epileptiform changes in human brain organoids[J]. *Nat Neurosci*. 2021, *24*(10): 1488–1500. doi:10.1038/s41593-021-00906-5
55. Li C, Fleck J S, Martins-Costa C, et al. Single-cell brain organoid screening identifies developmental defects in autism[J]. *Nature*. 2023, *621*(7978): 373–380. doi:10.1038/s41586-023-06473-y
56. Sebastian R, Jin K, Pavon N, et al. Schizophrenia-associated NRXN1 deletions induce developmental-timing- and cell-type-specific vulnerabilities in human brain organoids[J]. *Nat Commun*. 2023, *14*(1): 3770. doi:10.1038/s41467-023-39420-6
57. Li X H, Guo D, Chen L Q, et al. Low-intensity ultrasound ameliorates brain organoid integration and rescues microcephaly deficits[J]. *Brain*. 2024, *147*(11): 3817–3833. doi:10.1093/brain/awae150
58. Ullah H, Huang Q, Chiola S, et al. Generating and characterizing human telencephalic brain organoids from stem cell-derived single neural rosettes[J]. *Nat Protoc*. 2025. doi:10.1038/s41596-025-01197-x
59. Miura Y, Li M Y, Birey F, et al. Generation of human striatal organoids and cortico-striatal assembloids from human pluripotent stem cells[J]. *Nat Biotechnol*. 2020, *38*(12): 1421–1430. doi:10.1038/s41587-020-00763-w
60. Wang Y, Chiola S, Yang G, et al. Modeling human telencephalic development and autism-associated SHANK3 deficiency using organoids generated from single neural rosettes[J]. *Nat Commun*. 2022, *13*(1): 5688. doi:10.1038/s41467-022-33364-z
61. Guo T, Zhang D, Zeng Y, et al. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease[J]. *Mol Neurodegener*. 2020, *15*(1): 40.
62. Rickner H D, Jiang L, Hong R, et al. Single cell transcriptomic profiling of a neuron-astrocyte assembloid tauopathy model[J]. *Nat Commun*. 2022, *13*(1): 6275. doi:10.1038/s41467-022-34005-1
63. Huang S, Zhang Z, Cao J, et al. Chimeric cerebral organoids reveal the essentials of neuronal and astrocytic APOE4 for Alzheimer's tau pathology[J]. *Signal Transduct Target Ther*. 2022, *7*(1): 176. doi:10.1038/s41392-022-01006-x
64. Chen X, Sun G, Tian E, et al. Modeling Sporadic Alzheimer's Disease in Human Brain Organoids under Serum Exposure[J]. *Adv Sci (Weinh)*. 2021, *8*(18): e2101462.
65. Liu H, Mei F, Ye R, et al. APOE3ch alleviates Aβ and tau pathology and neurodegeneration in the human APP(NL-G-F) cerebral organoid model of Alzheimer's disease[J]. *Cell Res*. 2024, *34*(6): 451–454. doi:10.1038/s41422-024-00957-w
66. Zhao J, Fu Y, Yamazaki Y, et al. APOE4 exacerbates synapse loss and neurodegeneration in Alzheimer's disease patient iPSC-derived cerebral organoids[J]. *Nat Commun*. 2020, *11*(1): 5540. doi:10.1038/s41467-020-19264-0
67. Wang C, Terrigno M, Li J, et al. Increased G3BP2-Tau interaction in tauopathies is a natural defense against Tau aggregation[J]. *Neuron*. 2023, *111*(17): 2660–2674. doi:10.1016/j.neuron.2023.05.033
68. Hong Y, Dong X, Chang L, et al. Microglia-containing cerebral organoids derived from induced pluripotent stem cells for the study of neurological diseases[J]. *iScience*. 2023, *26*(3): 106267. doi:10.1016/j.isci.2023.106267
69. Morris H R, Spillantini M G, Sue C M, et al. The pathogenesis of Parkinson's disease[J]. *Lancet*. 2024, *403*(10423): 293–304. doi:10.1016/S0140-6736(23)01478-2
70. Kim H, Kang S, Cho B, et al. Parkinson's Disease Modeling Using Directly Converted 3D Induced Dopaminergic Neuron Organoids and Assembloids[J]. *Adv Sci (Weinh)*. 2025, *12*(14): e2412548.
71. Zheng X, Han D, Liu W, et al. Human iPSC-derived midbrain organoids functionally integrate into striatum circuits and restore motor function in a mouse model of Parkinson's disease[J]. *Theranostics*. 2023, *13*(8): 2673–2692. doi:10.7150/thno.80271
72. Reumann D, Krauditsch C, Novatchkova M, et al. In vitro modeling of the human dopaminergic system using spatially arranged ventral midbrain-striatum-cortex assembloids[J]. *Nat Methods*. 2023, *20*(12): 2034–2047. doi:10.1038/s41592-023-02080-x
73. Wulansari N, Darsono W, Woo H J, et al. Neurodevelopmental defects and neurodegenerative phenotypes in human brain organoids carrying Parkinson's disease-linked DNAJC6 mutations[J]. *Sci Adv*. 2021, *7*(8). doi:10.1126/sciadv.abb1540
74. An J, Shin M, Beak G, et al. Drug Evaluation of Parkinson's Disease Patient-Derived Midbrain Organoids Using Mesoporous Au Nanodot-Patterned 3D Concave Electrode[J]. *ACS Sens*. 2024, *9*(7): 3573–3580. doi:10.1021/acssensors.4c00476
75. Shao W, Todd T W, Wu Y, et al. Two FTD-ALS genes converge on the endosomal pathway to induce TDP-43 pathology and degeneration[J]. *Science*. 2022, *378*(6615): 94–99. doi:10.1126/science.abq7860
76. Shin M, Ha T, Lee S, et al. Biohybrid motor neuron spheroid composed of graphene/HUVEC/neural cell for 3D biosensing system to evaluate drug of amyotrophic lateral sclerosis[J]. *Nano Converg*. 2025, *12*(1): 29. doi:10.1186/s40580-025-00495-0
77. de Majo M, Koontz M, Marsan E, et al. Granulin loss of function in human mature brain organoids implicates astrocytes in TDP-43 pathology[J]. *Stem Cell Reports*. 2023, *18*(3): 706–719. doi:10.1016/j.stemcr.2023.01.012
78. Nie Y, Szebényi K, Wenger L, et al. Origin and cell type specificity of mitochondrial DNA mutations in C9ORF72 ALS-FTLD human brain organoids[J]. *Sci Adv*. 2025, *11*(10): r690. doi:10.1126/sciadv.adr0690
79. Conforti P, Besusso D, Bocchi V D, et al. Faulty neuronal determination and cell polarization are reverted by modulating HD early phenotypes[J]. *Proc Natl Acad Sci U S A*. 2018, *115*(4): E762–E771.
80. Galimberti M, Nucera M R, Bocchi V D, et al. Huntington's disease cellular phenotypes are rescued non-cell autonomously by healthy cells in mosaic telencephalic organoids[J]. *Nat Commun*. 2024, *15*(1): 6534. doi:10.1038/s41467-024-50877-x
81. Wu S, Hong Y, Chu C, et al. Construction of human 3D striato-nigral assembloids to recapitulate medium spiny neuronal projection defects in Huntington's disease[J]. *Proc Natl Acad Sci U S A*. 2024, *121*(22): e1978791175. doi:10.1073/pnas.2316176121
82. Lisowski P, Lickfett S, Rybak-Wolf A, et al. Mutant huntingtin impairs neurodevelopment in human brain organoids through CHCHD2-mediated neurometabolic failure[J]. *Nat Commun*. 2024, *15*(1): 7027. doi:10.1038/s41467-024-51216-w
83. Liu Y, Chen X, Ma Y, et al. Endogenous mutant Huntingtin alters the corticogenesis via lowering Golgi recruiting ARF1 in cortical organoid[J]. *Mol Psychiatry*. 2024, *29*(10): 3024–3039. doi:10.1038/s41380-024-02562-0
84. Kawakita S, Mandal K, Mou L, et al. Organ-On-A-Chip Models of the Blood-Brain Barrier: Recent Advances and Future Prospects[J]. *Small*. 2022, *18*(39): e2201401. doi:10.1002/smll.202201401
85. Papamichail L, Koch L S, Veerman D, et al. Organoids-on-a-chip: microfluidic technology enables culture of organoids with enhanced tissue function and potential for disease modeling[J]. *Front Bioeng Biotechnol*. 2025, *13*: 1515340. doi:10.3389/fbioe.2025.1515340
86. Amirifar L, Shamloo A, Nasiri R, et al. Brain-on-a-chip: Recent advances in design and techniques for microfluidic models of the brain in health and disease[J]. *Biomaterials*. 2022, *285*: 121531. doi:10.1016/j.biomaterials.2022.121531
87. Saberi A, Aldenkamp A P, Kurniawan N A, et al. In-vitro engineered human cerebral tissues mimic pathological circuit disturbances in 3D[J]. *Commun Biol*. 2022, *5*(1): 254. doi:10.1038/s42003-022-03203-4
88. Pediaditakis I, Kodella K R, Manatakis D V, et al. Modeling alpha-synuclein pathology in a human brain-chip to assess blood-brain barrier disruption[J]. *Nat Commun*. 2021, *12*(1): 5907. doi:10.1038/s41467-021-26066-5
89. Peng B, Tong Z, Tong W Y, et al. In Situ Surface Modification of Microfluidic Blood-Brain-Barriers for Improved Screening of Small Molecules and Nanoparticles[J]. *ACS Appl Mater Interfaces*. 2020, *12*(51): 56753–56766. doi:10.1021/acsami.0c17102

90. Aazmi A, Zhou H, Lv W, et al. Vascularizing the brain in vitro[J]. *iScience*. 2022, *25*(4): 104110. doi:10.1016/j.isci.2022.104110
91. Wang Y, Wang L, Guo Y, et al. Engineering stem cell-derived 3D brain organoids in a perfusable organ-on-a-chip system[J]. *RSC Adv*. 2018, *8*(3): 1677–1685. doi:10.1039/C7RA11714K
92. Xue X, Kim Y S, Ponce-Arias A I, et al. A patterned human neural tube model using microfluidic gradients[J]. *Nature*. 2024, *628*(8007): 391–399. doi:10.1038/s41586-024-07204-7
93. Ramme A P, Koenig L, Hasenberg T, et al. Autologous induced pluripotent stem cell-derived four-organ-chip[J]. *Future Sci OA*. 2019, *5*(8): O413. doi:10.2144/fsoa-2019-0065
94. Rodrigues R G, Condelipes P, Rosa R R, et al. Scalable Processing of Cyclic Olefin Copolymer (COC) Microfluidic Biochips[J]. *Micromachines (Basel)*. 2023, *14*(10)
95. Zhi Y, Zhu Y, Wang J, et al. Cortical Organoid-on-a-Chip with Physiological Hypoxia for Investigating Tanshinone IIA-Induced Neural Differentiation[J]. Research (Wash D C). 2023, 6: 273.
96. Zhu Y, Zhang X, Sun L, et al. Engineering Human Brain Assembloids by Microfluidics[J]. *Adv Mater*. 2023, *35*(14): e2210083. doi:10.1002/adma.202210083
97. Szebényi K, Wenger L, Sun Y, et al. Human ALS/FTD brain organoid slice cultures display distinct early astrocyte and targetable neuronal pathology[J]. *Nat Neurosci*. 2021, *24*(11): 1542–1554. doi:10.1038/s41593-021-00923-4
98. Xu C, Alameri A, Leong W, et al. Multiscale engineering of brain organoids for disease modeling[J]. *Adv Drug Deliv Rev*. 2024, *210*: 115344. doi:10.1016/j.addr.2024.115344
99. Yu B, Zhou D, Wang F, et al. Organoids for tissue repair and regeneration[J]. *Mater Today Bio*. 2025, *33*: 102013. doi:10.1016/j.mtbio.2025.102013
100. Bai L, Jing Y, Reis R L, et al. Organoid research: Theory, technology, and therapeutics[J]. *Organoid Research*. 2025, *1*(1): 25040007. doi:10.36922/OR025040007
101. Liu H, Triffitt J T, Xia Z, et al. Artificial intelligence-enabled studies on organoid and organoid extracellular vesicles[J]. *Biomater Transl*. 2024, *5*(2): 93–94. doi:10.12336/biomatertransl.2024.02.001