
Review

Advances in human brain organoids for Alzheimer's disease modeling

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Abstract: Alzheimer's disease (AD) is a critical neurodegenerative disorder with only a small number of effective disease-modifying therapies currently existing. Establishing a translational-relevant preclinical AD model is considered the bottleneck in AD drug development. Human brain organoids (hBOs) are suitable candidates to model AD in the context of human physiology. In recent years, hBO platforms have evolved to incorporate the robust population of microglia, astrocytes, and vascular endothelial cells. Concurrently, the adoption of innovative modeling strategies has expanded the realm of AD modeling in hBOs, extending from familial AD to sporadic AD and beyond. This review discusses recent advances in hBO designs for modeling AD, highlighting emerging strategies to model different facets of AD, and evaluates the current extent to which AD phenotypes are recapitulated in hBO systems. hBO models exhibit varying levels of recapitulation in AD phenotypes, with degrees ranging from strong, moderate, emerging but limited, weak but consistent, and weak and variable. Collectively, advances in AD modeling using hBOs have partially alleviated limitations related to cellular diversity and complexity, while expanding strategies for modeling distinct subtypes of AD. These advances have also been successful in strengthening the recapitulation of several AD phenotypes and unraveling emerging AD phenotypes in hBOs. However, early-generation AD hBO models lack cellular and physiological complexity, as well as modeling strategies for different AD subtypes. Future work is still required to enhance the robustness, credibility, and translational utility of AD hBO models in biomedical applications.

Keywords: human brain organoid; Alzheimer's disease; tissue engineering; neurodegenerative disease; disease modeling

1. Introduction

In 2021, approximately 52.6 million people worldwide were living with dementia, with Alzheimer's disease (AD) accounting for the majority of the cases [1]. AD is a neurodegenerative disorder associated with progressive cognitive decline and memory loss [2]. AD at the cellular level is historically characterized by the accumulation of extracellular amyloid plaques composed of amyloid-beta ($A\beta$) peptides and intracellular neurofibrillary tangles composed of hyperphosphorylated tau [3]. However, the pathophysiological landscape of AD is now understood to be highly complex, encompassing neuroinflammation, synaptic loss, cellular apoptosis, network impairment, and mitochondrial dysfunction [4–6]. AD is stratified into early-onset familial AD (fAD) and late-onset sporadic AD (sAD) [7]. FAD is predominantly driven by autosomal dominant mutations in *APP* or *PSEN1/2* genes in a Mendelian pattern of inheritance [8]. Conversely, the etiology of sAD remains elusive, currently viewed as an intricate interplay between genetic susceptibilities and environmental factors [9–11].

Despite decades of drug discovery and development efforts, disease-modifying therapies for AD remain limited to aducanumab and lecanemab. Those are FDA-approved monoclonal antibodies targeting $A\beta$ for microglia-mediated clearance [12,13]. The slow progress of AD drug discovery and development is largely attributed to poor translational relevance of conventional preclinical AD models, which primarily consist of animal models and human two-dimensional (2D) cell culture systems. Limited translational relevance has been attributed primarily to the physiological differences arising from the unique biological characteristics of the human, which are not fully recapitulated in animal models, resulting in species-specific differences in AD pathogenesis [14]. Rodent amyloid precursor protein (APP) differs from its human ortholog by three amino acid substitutions (R5G, T10F, and H13R) within the $A\beta$ peptide domain [15], resulting in a low propensity for spontaneous $A\beta$ aggregation and plaque formation [16]. Therefore, successful modeling of amyloid pathology in rodents necessitates the implementation of transgenic overexpression of human APP [17,18]. Rodent tau lacks the human-specific 3R/4R isoform balance, exhibiting a reduced tendency to form paired-helical filaments and neurofibrillary tangles [19]. Despite these limitations, transgenic and humanized mouse models remain valuable for investigating disease mechanisms within an intact organism and for evaluating systemic responses and therapeutic efficacy [20]. Similarly, other AD experimental systems, including xenotransplantation and organ-on-chip systems, enable the study of human neural cells within the living host and provide greater control over tissue-tissue interactions in dynamic and microenvironmental conditions, respectively [21,22]. However, these models demand greater technical complexity and may not fully resemble the three-dimensional cytoarchitecture of the developing human brain.

The advent of human brain organoids (hBOs) provides an unprecedented opportunity to recapitulate human brain development and function in vitro. hBOs are three-dimensional, self-organizing neural tissues derived from human pluripotent stem cells (hPSCs), primarily comprising neurons and, to a variable extent, glial cells [23]. Unlike animal models and 2D neuron culture, hBOs follow intrinsic developmental programs during hPSC differentiation, thereby providing better recapitulation of in vivo human brain development. This innate patterning equips hBOs with cytoarchitecture, cellular diversity, and physiological characteristics that resemble those of the developing human brain [24]. A significant breakthrough was achieved with the generation of self-organizing cerebral organoids that mimic features of the multi-regional architecture of the human brain [25].

Despite their potential, conventional hBOs possess limitations that must be addressed to faithfully model human brain development and function. Primary among these is the lack of robust and mature non-neuronal populations, specifically mature astrocytes, microglia, and vascular endothelial cells [26]. This is particularly important for AD modeling, as astrocytes and microglia are critical mediators of neuroinflammatory processes, while vascular endothelial cells form an essential component of the blood-brain barrier (BBB), whose dysfunction is increasingly recognized as a key contributor to AD pathogenesis [27,28]. Furthermore, neurons and glial cells in hBOs exhibit developmentally immature states with transcriptional and functional profiles resembling early to mid-gestational stages of human brain development [29–31]. Nevertheless, their maturation remains heterogeneous depending on the organoid generation protocol and culture duration. These limitations restrict their capacity to form the complex functional synaptic networks required for advanced disease modeling [32]. Moreover, hBOs continue to face reproducibility issues, in which variability and uncontrolled heterogeneity persist in terms of morphology, size, cell type composition, and functional output [33,34]. Therefore, rather than replacing existing preclinical models, hBOs should be considered as complementary platforms that bridge the gap between *in vitro* and *in vivo* systems.

Most existing studies modeling AD in hBOs have focused predominantly on fAD modeling, while sAD modeling remains scarce, despite approximately 95% of AD cases being sporadic [35,36]. This disparity is largely attributable to the well-defined genetic etiology of fAD [10]. Sporadic AD modeling, however, is fundamentally challenging due to its complex, multifactorial, and incompletely understood pathogenesis, which emerges predominantly in the context of ageing [37]. Recently, the field has introduced innovative strategies to circumvent these limitations, to establish more robust AD hBO models. Such models can be leveraged for broader translational applications, including, but not limited to, drug discovery, precision medicine, biomarker identification, and investigation of disease mechanisms related to AD. Together, these applications will pave the way for a better understanding of AD biology, more precise AD diagnosis and prognosis, and more effective AD therapy and treatment regimen. Ultimately, the rising trend of AD global cases can be mitigated.

Hence, this review serves to provide a focused evaluation of recent advances in hBO-based AD modeling and the current capacity of these platforms to recapitulate AD phenotypes. First, we examine advances in hBO design and modeling strategies for capturing diverse AD subtypes, with particular emphasis on the comparative advantages, disadvantages, and technical complexity of each approach. Next, we critically analyze the extent and consistency of AD phenotype recapitulation across existing hBO models, highlighting differences between modeling strategies and identifying phenotypes that remain incompletely reproduced. Finally, we discuss the persisting challenges in recapitulating AD phenotypes within hBOs and outline future directions to advance the translational frontiers of hBO-based AD modeling.

2. Improving cellular diversity and regional identity in hBOs

Recent advances in human brain organoid (hBO) technology have considerably expanded the capability of *in vitro* models to recapitulate the cellular complexity and pathological microenvironment associated with Alzheimer's disease (AD). Early hBO systems were primarily limited by insufficient cellular diversity, lack of vascularization, incomplete neuronal maturation, and the absence of resident immune populations, all of which restricted their physiological relevance for modeling multifactorial neurodegenerative processes. To overcome these limitations, emerging bioengineering and stem cell-

based approaches have focused on incorporating additional brain-resident and peripheral cell populations, enhancing regional specification, and reconstructing intercellular interactions that more closely resemble the human brain. These developments have enabled hBOs to evolve from relatively simple neuroepithelial structures into increasingly sophisticated multicellular systems capable of modeling neuroinflammation, blood-brain barrier interactions, vascular dysfunction, and glial-mediated pathology in AD.

As summarized in Figure 1, current advances in hBO design include the integration of microglia-like cells, endothelial and vascular components, region-specific assembloids, and multicellular coculture systems to generate more physiologically relevant AD models. Recent strategies, such as the incorporation of induced microglia (iMGs), vascular progenitor cells, ganglionic eminence organoids, and blood vessel organoids, have substantially improved the modeling of neuron-glia communication, immune responses, and cerebrovascular interactions in AD-related neurodegeneration. In parallel, assembloid technologies and transcription factor-mediated lineage engineering have further enhanced cellular heterogeneity and regional complexity within hBO systems. These advances will be discussed further in the following subsections.

2.1. Integration of robust microglial population into hBOs

Microglia are specialized, brain-resident macrophages that originate from yolk sac mesoderm [38]. They orchestrate neurodevelopment by balancing the depletion of the neuronal progenitor cell (NPC) pool via modulation of apoptosis and the promotion of neurogenesis by supporting NPC proliferation and neuronal maturation [39]. They are equipped to sense pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) and subsequently mount a neuroinflammatory response [40]. Depending on the context, this neuroinflammation, together with its associated cytokine profile, can be either neuroprotective or neurotoxic [41,42]. In the pathological state of AD, microglia become maladaptive, accelerating the aggregation of A β and tau species rather than facilitating their clearance. Additionally, microglia in AD mediate synapse loss through complement-dependent synaptic pruning activity, together with the secretion of proinflammatory cytokines that contribute to neuronal damage [43]. Importantly, the absence of a robust microglia population in conventional hBO models represents a significant limitation in recapitulating the cellular complexity of the human AD brain.

Conventional AD hBO models lack a robust microglial population due to inherent biological constraints during organoid development. Specifically, standard hBO protocols direct the differentiation of hPSCs down the neuroectodermal lineage, whereas microglia arise from the mesodermal lineage [44,45]. Nevertheless, a minute microglial population has been demonstrated to appear spontaneously in conventional hBOs [46]. A spontaneous emergence of microglial population in hBOs is the result of incomplete neural induction, leading to a minute population of hPSCs undergoing mesodermal differentiation. Despite its success in incorporating microglia in hBOs, this method suffers from an intensified heterogeneity issue, most likely due to the presence of unintended mesodermal cell subtypes apart from microglia [46]. Hence, a method to reproducibly incorporate the robust microglia population into hBOs is warranted.

Recently, several strategies for incorporating a robust microglial population into AD hBOs have been explored (Figure 1). These include coculturing immature hBOs, which are predominantly composed of neural progenitors, with either human-induced pluripotent stem cell (hiPSC)-derived microglia termed iMG [47] or with hiPSC-derived primitive macrophages termed hiMACs [48].

Specifically, one study cocultured 5×10^4 iMG cells at day 40 with human cerebral organoids at day 60 [47]; another study cocultured 1×10^4 and 5×10^4 hiMACs at day 40 with human cortical organoids at day 44 [48]. HiMACs that are cocultured with hiPSC-derived neurons have been reported to undergo tissue-specific maturation into microglia-like cells, expressing the microglia-specific marker, Iba1, and phagocytosis-like ability toward A β_{42} [49]. Alternatively, immature hBOs have also been cocultured with primitive macrophage progenitors (PMPs) [50] or with common myeloid progenitors (CMPs) [51]. Specifically, one study cocultured 1.2×10^4 PMPs with 3×10^4 neural progenitor cells to allow self-assembly [50], and another study dissociated 21-day-old forebrain organoids into single cells that were then cocultured with 2.5×10^4 CMPs to allow aggregate formation [51]. It is noteworthy that in vivo CMPs are the precursors of circulating macrophages and not of tissue-resident macrophages, which are derived from erythro-myeloid progenitors [49,52]. Nevertheless, CMPs that are exposed to neural tissue-specific cues in vitro undergo differentiation into microglia-like cells, expressing microglia-specific markers including CD11b, TREM2, and Iba1 (Table 1) [51,53].

Notably, in all coculturing approaches, microglia and their progenitors are incorporated into developmentally immature hBOs. Evidence suggests that integrated microglia modulate neurogenesis and neuronal maturation, although the extent of their true influence remains underexplored [54,55]. Conversely, neural cells in hBOs are actively secreting chemical cues, including IL-34, which can influence the differentiation of microglia/macrophage progenitors into mature microglia identity [56]. Successful integration of functional microglia into hBOs was validated by their ability to migrate and subsequently proliferate in hBOs, adopting a ramified morphology with extended processes. Importantly, these microglia were found to exhibit phagocytosis-like capability toward A β aggregates [48,50].

Incorporation of microglia into hBOs for AD modeling has provided unprecedented opportunities to delineate the influence of microglia on neurogenesis. hBOs with integrated microglia exhibited an increased proportion of MAP2-positive neurons [47]. This observation is possibly explained by the role of microglia in expanding NPC pools that later differentiate into neurons [39]. Moreover, these advances offer insights into how microglia affect AD pathogenesis and how the AD milieu alters microglia behavior and functions. It has been reported that microglia harboring a fAD mutation acquired a morphological shift from ramified to amoeboid phenotype, accompanied by upregulated TREM2 and downregulated P2RY12 expression, indicating a disease-associated microglia (DAM) phenotype [57]. Notably, these fAD microglia exhibited a reduced capacity to phagocytose A β . It was proposed that this phenomenon is due to autophagy dysregulation in AD microglia, thereby impairing lysosomal degradation of A β while leaving the phagocytic uptake process largely unaffected [58].

Complementary to this, microglia in AD hBOs upregulated expression of Iba1, CD44, and CD9, indicating an activated phenotype [50,51]. Moreover, microglia in AD hBOs were reported to exhibit upregulation of IL-6 and TNF- α (proinflammatory markers) and downregulation of S100A10 (anti-inflammatory marker), consistent with a proinflammatory microglial phenotype [47,51]. A β aggregates in the vicinity of microglia have been observed to acquire more circular and smoother surface morphology compared to those that are distant [48]. This is due to the proposed ability of microglia processes to form a barrier that prevents the formation of protofibrillar A β_{42} hotspots [59]. It was demonstrated that microglia in AD hBOs enhance synaptic pruning activity, resulting in synaptic loss [50,60]. Together, these coculture approaches for incorporating robust microglia populations into AD hBOs have endowed these systems with a better recapitulation of microglia-mediated pathogenesis in AD.

2.2. Induction of astrocytic lineage in hBOs

Astrocytes constitute a major glial cell population that performs diverse physiological functions in the human brain [61]. They mediate the communication between neurons and cerebral vasculature that fine-tunes the rate of blood flow to the level of neuronal activity. Apart from that, astrocytes are active players in the tripartite synapse model by releasing gliotransmitters and growth factors that modulate synaptic transmission [62]. Astrocytic end feet constitute a key component of the blood-brain barrier (BBB), interacting with endothelial cells and pericytes to support BBB functions [63]. Notably, in AD, astrocytes actively participate in immune-like responses by releasing pro- and anti-inflammatory cytokines in a context-dependent manner [64]. Moreover, astrocytes in AD acquire a reactive state by displaying hypertrophy of the astrocytic process, together with the upregulation of intermediate filaments expression. Activation of the NF- κ B pathway in reactive astrocytes contributes to the release of C3 protein. Binding of C3 protein with its receptor, C3aR, on microglia and neurons alters A β phagocytosis and neural network activity, respectively [65].

Considering the prominent roles of astrocytes in the brain environment and pathophysiology, the inclusion of a robust astrocyte population is critical for AD modeling in hBOs. Despite being derived from neuroectodermal lineage, astrogliogenesis is instigated later in the neurodevelopmental timeline, after the completion of neurogenesis [66]. Conventional hBOs have been reported to harbor a spontaneously emerging astrocyte population, but the emergence of astrocytes varies substantially depending on the protocol, with the unguided method commonly requiring an extended culture period [67,68]. The ability to accelerate astrogliogenesis reduces the need for prolonged culture periods, hence minimizing hBOs' susceptibility to time-dependent variability [69].

A recent strategy has been introduced to establish a robust astrocyte population in hBOs within just 45 culture days through an induction-based method (Figure 1) [70]. The strategy aimed to specifically induce astrocytic lineage in hBOs by transducing hPSCs with lentiviral vectors carrying the astrogenic transcription factors, *NFIB* and *SOX9* [70]. Successfully transduced hPSCs were then cocultured with non-transduced hPSCs to form chimeric embryoid bodies that differentiated into hBOs comprising robust astrocyte populations. Astrocytes' identity and functionality were confirmed by the expression of astrocytic markers, including GFAP, S100B, and CD44, as well as by glutamate uptake activity, indicating successful astrocyte development in hBOs [70]. hBOs with induced astrocyte populations were reported to exhibit elevated numbers of MAP2-positive neurons and increased formation of synaptophysin-PSD95-positive synaptic puncta. These improvements contributed to better neural network functionality compared to conventional hBOs lacking a robust astrocyte population [70], which aligns with another study that investigated the role of astrocytes on organoid neural networks [71]. Collectively, these findings suggest that the incorporation of robust astrocyte populations accelerates hBO maturation, consistent with the established roles of astrocytes in human brain development.

2.3. Integration of vascular endothelial cells into hBOs

The roles of endothelial cells in the brain extend beyond forming vasculature for the transport of nutrients and oxygen to the brain parenchyma. They also include active participation in the clearance system of toxic proteins like A β , tau, and α -Syn [72]. Endothelial cells are the main component of the BBB that limits and selects for the substances that can penetrate the brain parenchyma by the

expression of tight junction components, including claudins, occludins, and junctional adhesion molecules (JAMs) [73]. These components are crucial in protecting the brain parenchyma from neurotoxin and pathogen exposure [74]. In AD, elevated levels of A β are a risk factor for cerebral amyloid angiopathy, a pathological condition that compromises the integrity of the BBB [75].

BBB dysfunction increases the vulnerability of brain cells to neurotoxins and pathogens, which have been implicated in sporadic AD pathogenesis. Recapitulating BBB dysfunction in sporadic AD models is not feasible in conventional hBOs due to the mesodermal origin of endothelial cells [76]. Different strategies have been adopted to integrate vascular endothelial cells into hBOs (Figure 1). These include coculturing immature hBOs with vascular progenitor cells [50] or the endothelial cell line, VeraVecs [51]. Specifically, one study cocultured 7×10^3 vascular progenitor cells with 3×10^4 neural progenitor cells to allow self-assembly [50], while another study dissociated 21-day-old forebrain organoids into single cells that were then cocultured with VeraVecs at a ratio of 1:1, forming cell aggregates at a cell density of 1.5×10^6 [51]. These methods enabled vascular endothelial cells to mature alongside other brain cells in hBOs, forming vessel-like structures [50,51]. Nevertheless, the functionality of this vessel-like structure requires further characterization and validation. Successful differentiation of vascular progenitors (VPs) into endothelial cells was validated with the expression of the endothelial marker, CD31 [77]. Apart from endothelial cellular fate, these VPs are competent to differentiate into PDGFR β -positive pericyte-like cells in hBOs [50]. This highlights an advantage of using VPs over a terminally differentiated endothelial cell line, which cannot possibly yield a pericyte population (Table 1). hBOs with a robust endothelial cell population have been reported to exhibit an increased astrocyte population compared to conventional hBOs [51]. This highlights the role of endothelial cells in promoting astrocyte development [78]. Therefore, endothelial cell incorporation not only facilitates the formation of vessel-like structures but also promotes astrocyte development that is delayed in conventional hBOs.

A more sophisticated method to integrate vasculature into brain organoids has been established through the fabrication of assembloids composed of cortical organoids (COs) and blood vessel organoids (BVOs) [79]. Independent generation of BVOs yielded organoids containing multiple mesoderm-derived cell types, including endothelial cells, pericytes, and macrophages. These cells constitute a vascular network structure with the formation of a lumen and basement membrane, composed of collagen type IV. BVOs have been shown to undergo an angiogenesis-like process, by the formation of an angiogenic front [80]. Coculturing COs with BVOs resulted in fused cortical-blood vessel organoids (fCBOs). Notably, BBB-like features were observed in fCBOs, as evidenced by the expression of tight junction proteins (ZO1 and claudin-5) and adherens junction proteins (VE-cadherin and β -catenin). These fCBOs also exhibited higher expression levels of glial markers for astrocytes (GFAP) and microglia (IBA1). SARS-CoV-2-associated sAD fCBO models exhibited a condensed and fragmented endothelial cell phenotype, implying damaged endothelial lining. Collectively, advances in endothelial cell integration into hBOs involve strategies including coculturing brain organoids with endothelial cell lines, vascular progenitor cells, or BVOs. Among the currently available approaches, BVO-based vascularization appears to provide the closest recapitulation of human brain vasculature. This can be substantiated by the formation of BBB-like structures in hBOs that are not detected in the models using endothelial cell lines or vascular progenitor cells. Nevertheless, the extent to which BBB-like structures in fCBOs recapitulate the human BBB in terms of functions remains poorly characterized and warrants further investigation.

2.4. Regionalized organoids and multi-regional assembloids

Unguided methods generate self-patterned brain organoids with mosaic forebrain and hindbrain identities [25]. The drawback of this method is the uncontrolled variability and heterogeneity with respect to cell type composition and region formation [81]. Guided methods generate patterned brain organoids that are commonly more regionalized with the benefit of enhanced reproducibility [82]. Two common regionalized brain organoids that are commonly utilized to model AD are forebrain organoids [83,84] and cortical organoids [48,51,85,86]. It has been established that the earliest cholinergic neuron degeneration in the AD human brain emerges in the basal forebrain region, specifically the nucleus basalis of Meynert (NbM), which acts as the predictor for entorhinal cortex tau pathology [87]. Hence, regionalized forebrain organoids have the potential to model the early anatomical origins of AD in the human brain. However, it remains unclear whether current forebrain organoid protocols faithfully recapitulate NbM development. Cortical organoids, on the other hand, are relevant to modeling AD phenotypes in the cortical region of the brain: it has been well-documented that advanced stages of AD involve the spread of tau pathology throughout regions of the cortex [88,89]. Cortical organoids have been demonstrated to reproduce several AD-like phenotypes, including amyloidopathy, tauopathy, synaptic dysfunction, and cellular stress. However, reproducing the chronic progression toward late-stage AD remains challenging due to relative cellular immaturity and limited ageing phenotypes [90].

To investigate the inter-regional interaction in the AD pathogenesis, recent approaches have shifted from utilizing self-patterned brain organoids toward the use of assembloids, which offer enhanced reproducibility in terms of regional identity formation [91]. This involves integrating different modules of regionalized hBOs into a single system to study specific interactions that cannot be modeled in regionalized organoids per se (Figure 1) [82]. One study has reported the application of assembloids to model enhanced neural network maturity, comprising excitatory and inhibitory neurons and their implication in AD. This was achieved by the fusion of two regionalized organoids, namely cortical organoids (COs) and ganglionic eminence organoids (GEOs) [92]. COs harbor predominantly excitatory glutamatergic neurons; meanwhile, GEOs serve as a source of GABAergic interneurons in the brain [91]. During human brain development *in vivo*, these inhibitory interneurons migrate tangentially to the developing cortical region to form a mature neural network. The fusion of COs and GEOs enables the migration of GABAergic interneuron progenitors from GEOs into the COs region.

By comparing CO-GEO assembloids carrying either the APOE3 or APOE4 allele, it was found that APOE4 CO-GEO assembloids exhibited higher coordination and synchronicity of network activity compared to APOE3 assembloids, indicating altered neural network activity associated with AD [92]. This finding contrasts with observations from conventional regionalized hBO models of AD that reported reduced coordination and synchronicity of neural network functionality [50,85]. This discrepancy may be influenced by the presence of a more abundant GABAergic interneuron population in assembloid models that is limited in regionalized AD hBO models. A different scenario has been reported in self-patterned cerebral organoids in which similar increases in neural network activity were observed [93]. Due to the diverse regional identity in self-patterned cerebral organoids, it is possible for ganglion eminence-like structures to develop spontaneously, contributing to a robust GABAergic interneuron population [68]. Nevertheless, enhanced neural network activity is not consistently recapitulated across self-patterned cerebral organoid models, likely owing to substantial variability in regional identity formation [94,95].

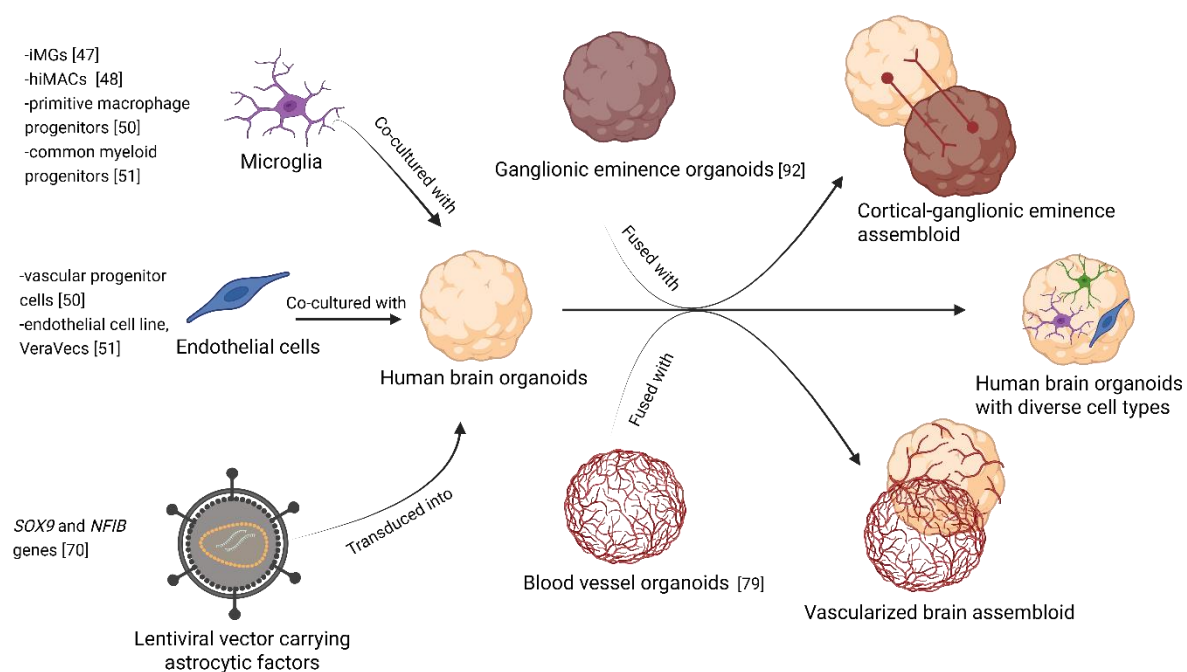


Figure 1. Overview of advances in human brain organoid design for Alzheimer's disease modeling.

Table 1. Comparative overview of emerging strategies to enhance human brain organoid complexity.

Advances	Methods/subtypes	Age of hBOs (days) (reference)	Advantages	Disadvantages	Technical complexity
Incorporation of microglia	Human iPSC-derived microglia (iMGs)	120 [47]	Control over microglia identity and maturation	In vitro maturation of iMG in isolation may not fully recapitulate the maturation cues provided by neuron–microglia interactions within the developing hBO	High
	Human iPSC-derived primitive macrophages (HiMACs)	50 [48]	Enable maturation within the hBO microenvironment, allowing exposure to endogenous neural cues	Limited experimental control over the final maturation state of HiMAC in hBO	Moderate
	Primitive macrophage progenitor (PMPs)	65 [50]	Allows PMPs to undergo differentiation within the developing hBO microenvironment, potentially exposing them to endogenous developmental cues	Variable differentiation efficiency may result in limited control over the final microglial population	Moderate
	Common myeloid progenitor (CMPs)	70 [51]	Allows myeloid progenitors to migrate into the mature hBO and subsequently mature into microglia-like cells	Introduction into a more mature neuronal environment may limit exposure to developmental cues present during progenitor-stage neural development	High
Induction of astrocytes	Transduction with <i>NFIB</i> and <i>SOX9</i>	45 [70]	Accelerates astrocyte generation within the developing hBO, enabling astrocytes to interact with neural cells during organoid development	Genetic manipulation of iPSCs may alter the endogenous trajectory and relative abundance of astrocytes within the developing hBO	High
Incorporation of vasculature	Vascular progenitor cells	65 [50]	Developmentally relevant; enables vascular–neural interactions	Limited control over vascular development	Moderate
	Endothelial cell line	70 [51]	Simple; reproducible; controllable endothelial incorporation	Non-brain-specific endothelial cells; limited representation of the neurovascular unit	Moderate
	Fused cortical blood vessel organoid (fbBO)	180 [79]	Multicellular vascular compartment; better mimics the neurovascular unit	Requires the independent generation and subsequent fusion of two distinct organoid systems; highly variable	High

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Advances	Methods/subtypes	Age of hBOs (days) (reference)	Advantages	Disadvantages	Technical complexity
Regionalized hBO	Forebrain organoid	120 [83]	Covers a broader range of forebrain cell populations and regional identities; can capture interactions between cortical and other forebrain regions	Greater regional heterogeneity can reduce anatomical specificity and experimental consistency	Moderate
	Cortical organoid	50 [48]	High cortical specificity; relevant to cortical AD pathology	Limited regional representation	Moderate
		70 [51]			
		84 [85]			
Multi-regional assembloid	CO-GEO assembloids	180 [86]	Recapitulates cortical–interneuron interactions and interneuron migration; greater cellular and circuit complexity	Requires the generation and fusion of distinct regional organoids	High
		180 [92]			

3. Emerging strategies for modeling AD in hBOs

Human brain organoids have increasingly been adapted to model the heterogeneous mechanisms underlying Alzheimer's disease (AD), moving beyond single-pathway approaches toward more diversified disease-modeling strategies. Since AD encompasses both inherited and non-inherited forms, current hBO-based models have been designed to reflect different pathogenic contexts, including familial AD driven by highly penetrant mutations, sporadic AD associated with genetic susceptibility and environmental risk factors, and chemically induced models that reproduce selected pathological features such as amyloid accumulation, tau aggregation, mitochondrial dysfunction, or blood-brain barrier disruption. These approaches allow researchers to examine how distinct upstream triggers may converge on shared downstream mechanisms, including synaptic dysfunction, neuroinflammation, oxidative stress, altered proteostasis, and neuronal vulnerability.

As illustrated in Figure 2, existing AD modeling strategies in hBOs can be broadly categorized into familial AD models, mechanism-specific AD models, sporadic AD models, and hybrid models that combine genetic predisposition with environmental or pathological stressors. Familial models commonly rely on patient-derived iPSCs or genome editing to introduce mutations in AD-associated genes such as *APP*, *PSEN1*, and *PSEN2*, whereas sporadic models incorporate risk factors such as APOE4 genotype, blood-brain barrier dysfunction, viral infection, neurotoxin exposure, and impaired protein clearance. Mechanism-specific models, in contrast, focus on individual disease-related processes such as mitochondrial dysfunction, tau aggregation, amyloidosis, or per- and polyfluoroalkyl substance (PFAS)-induced neurotoxicity. A more detailed discussion of these strategies is provided in the following subsections.

3.1. Modeling familial AD (fAD) in hBOs

The emergence of gene-editing techniques has markedly strengthened fAD modeling in hBOs by enabling the generation of isogenic hBOs derived from a common parental hPSC line (Figure 2) [85,94,96]. Hence, organoid-to-organoid genetic variability can be minimized. Conventionally, fAD modeling in hBO systems relied on patient-derived hiPSCs carrying fAD mutations [97]. Although fAD mutations are genetically defined, uncharacterized genetic variability between fAD patients may underlie the phenotypic inconsistencies and irreproducibility in fAD hBO models (Table 2) [83]. A similar concern arises when comparing fAD hBOs with non-isogenic controls that might lead to erroneous conclusions of the experimental findings [84,98]. In this context, gene editing technology offers the opportunity to autonomously introduce specific familial or novel mutations into resulting fAD hBO models. This approach enables systematic investigations of the molecular mechanisms that drive fAD pathogenesis associated with specific, site-directed mutations [85,94]. Alternatively, using this approach, the difference in fAD pathogenesis implicated by the gene-specific mutations in *APP* and *PSEN1/2* has been explored [96].

Conventional fAD hBO models partially recapitulate tauopathy by exhibiting increased phosphorylated-tau (p-tau) levels but lack neurofibrillary tangles (NFTs) or paired helical filaments (PHFs) [98,99]. This observation may be explained by the fact that the progression from pre-NFTs to mature NFTs naturally requires an extended period of time, probably exceeding the culture period of hBOs [100]. To overcome the limitation of the absence of higher-level tau aggregates in fAD hBO models, the field has developed models that employ adeno-associated virus (AAV)-mediated mutant

tau gene (P301L) transfer to accelerate tauopathy manifestation in fAD hBO models (Figure 2) [83]. Using this approach, the sequential cascade of tau aggregation from monomeric p-tau to oligomeric species, filamentous assemblies, and, ultimately, to NFTs has been successfully recapitulated. These findings suggest that viral-mediated transfer of mutant *MAPT* represents an effective strategy to overcome the attenuated tauopathy recapitulation in conventional fAD hBO models [83]. However, because tau pathology in this context is artificially induced, the kinetics of tau aggregation may no longer recapitulate endogenous tau aggregation processes, potentially influencing downstream pathogenic mechanisms. As a result, the translational relevance of these fAD hBO models warrants careful evaluation, underscoring the need for additional validity studies.

3.2. Modeling sporadic AD (sAD) in hBOs

Modeling sAD requires prior understanding of the risk factors that initiate sAD progression. The etiology of sAD is highly complex, encompassing genetic and non-genetic factors that make it difficult to identify a single initiating trigger [37]. Most of them remain hypothetical and are therefore regarded as risk factors rather than bona fide causal triggers [9,101]. The APOE4 allele is considered to be the strongest genetic risk factor for sAD [102,103]. Hence, the inclusion of APOE4 has become a mainstream modeling strategy for sAD in hBOs (Figure 2). To establish APOE4-related sAD hBO models, organoids are fabricated using either AD patient-derived iPSCs harboring the APOE4 genotype [92,99] or healthy individual-derived APOE3 iPSCs that are subsequently edited to the APOE4 genotype [70]. These models have been shown to recapitulate amyloidopathy by the manifestation of increased A β levels and A β aggregation [70,92,99]. Tauopathy, reflected by an increased p-tau level, was observed in sAD hBO models [70,99], whereas such changes were not reported by another hBO model [92]. It could be speculated that the unsuccessful recapitulation of tauopathy is due to the absence of multi-regional interactions in their regionalized cortical organoids [92]. This raises a critical question of whether multi-regional interactions are indeed indispensable for robust tauopathy manifestation in APOE4-related sAD hBO models.

Reduced synaptic density was successfully recapitulated in one hBO model [99] but not in another [70]. A similar trend was observed for impaired neuronal network functionality, which was successfully recapitulated in the APOE4-related AD hBO models [92] but not in the gene-edited APOE4 model [70]. It is important to highlight that this study established the APOE4 genotype in hBOs by CRISPR-Cas9 gene editing of APOE3 hiPSCs [70] instead of deriving them from AD patients, as done in other hBO models [92,99]. These findings raise the argument that APOE4 alone might be insufficient to drive synaptopathy in sAD (Table 2). This is because hiPSCs derived from sAD patients might not only harbor the APOE4 allele but also other uncharacterized genetic risk factors for sAD. On the contrary, the establishment of the APOE4 allele by gene editing of APOE3 hiPSCs will miss out on those uncharacterized genetic risk factors that are only present in sAD patient-derived hiPSCs. Collectively, these findings highlight the multifactorial nature of sAD pathogenesis, suggesting that models based on a single risk factor may only partially recapitulate AD phenotypes.

On top of that, BBB dysfunction has been strongly linked to sAD progression [104]. BBB dysfunction can be modeled in hBOs by treatment with human serum (Figure 2) [95,105]. In these studies, BBB dysfunction-related sAD hBO models were reported to recapitulate multiple AD phenotypes, including amyloidopathy through increased A β aggregate formation, tauopathy via elevated p-tau level, synaptopathy reflected by reduced SYN1 synaptic puncta, and cellular loss

indicated by increased expression of the apoptotic marker, cleaved caspase-3. One study reported altered neural network functionality, reflected by a decreased number of spikes, bursts, mean firing rate, and asynchronous calcium oscillation [95]. Together, these studies demonstrate that the exposure of brain parenchyma to human serum represents a useful surrogate model of BBB dysfunction. This might be due to the diversity of human serum components that instigate AD pathogenesis through convergent pathways in accordance with the multifactorial nature of sAD.

Pathogen infection has been posited to be a risk factor for sAD [106]. This hypothesis can be modeled by infecting hBOs with Herpes Simplex Virus 1 (HSV-1) [107–109] or SARS-CoV-2 (Figure 2) [79]. HSV-1-infection-related sAD hBO models were shown to recapitulate amyloidopathy through increased extracellular A β aggregations [108,109]. Tauopathy was recapitulated through increased total tau levels, elevated p-tau levels, and enhanced expression of 4R tau isoforms [107]. Neuroinflammation was recapitulated by increased mRNA expression of proinflammatory cytokines (TNF- α and IL-6) and decreased mRNA expression of anti-inflammatory cytokines (IL-10 and IL-4), while cellular loss was recapitulated by impaired neurogenesis resulting in smaller organoid sizes [108]. SARS-Cov-2 infection-related sAD hBO models were reported to recapitulate amyloidopathy through increased A β 42 levels, A β aggregation, and the formation of amyloid fibril-like structures. Tauopathy was recapitulated by increased p-tau levels; meanwhile, neuroinflammation was recapitulated by increased mRNA expression and secretion of proinflammatory cytokines, including TNF- α , IL-1 β , and IL-6. In addition, cellular loss was recapitulated by increased apoptosis, evidenced by TUNEL-stained cells [79]. It is noteworthy that synaptopathy has not been consistently reported in current viral infection-related sAD hBO models. This hints that synaptopathy might not be robustly captured in the current viral infection-related sAD hBO models, which reflect temporal limitations of phenotype assessment or poor assay sensitivity. These observations align with the infection hypothesis, which proposes pathogen-induced neuroinflammation as a trigger for downstream AD phenotypes [110].

Environmental neurotoxins have been hypothesized as a risk factor for sAD [111,112]. In recent years, this hypothesis has been modeled in hBOs by treatment with PFAS (Figure 2) [113]. PFAS are neurotoxins that interfere with glutamatergic and dopaminergic neurotransmission [114]. These neurotoxin exposure-related sAD hBO models were reported to recapitulate amyloidopathy through increased A β aggregate formation, tauopathy via increased p-tau levels, synaptopathy by decreased synaptic puncta, and cellular loss through increased apoptotic marker, cleaved caspase-3. The detection of decreased synaptic puncta perfectly aligns with the proposed mechanism of PFAS neurotoxicity. Hence, this suggests that in neurotoxin exposure-related sAD, synaptopathy might be the initiating event, followed by downstream amyloidopathy and tauopathy.

AD has been proposed to spread through a prion-like mechanism [115,116]. Supporting this hypothesis, infusing blood containing A β oligomers into normal mice has been shown to accelerate amyloid pathology [117]. A similar phenomenon has been observed in humans, particularly recipients of cadaveric growth hormone preparations containing A β seeds [118]. Modeling of this phenomenon was implemented by treating hBOs with brain extract from sAD patients (Figure 2) [50]. Amyloidopathy was recapitulated by increased A β aggregates and plaque-like structure formation. Tauopathy was observed by increased p-tau levels and NFT-like structure formation. Synaptopathy was exhibited by decreased synaptic puncta. Neuroinflammation was confirmed by an increase in mRNA expression of proinflammatory genes (*IL-6* and *CCL2*). Cellular loss was evidenced by the increased apoptotic marker, cleaved caspase-3. Altered neural network functionality was indicated by the reduction of the number of spikes, bursts, and mean firing rate. The relatively broad range of AD

phenotypes detected in this model can be attributable to the presence of multiple pathogenic factors in the sAD patient brain extract, in particular A β and tau seeds, but might include other uncharacterized seed proteins implicated in AD pathogenesis.

Collectively, these studies highlight that no single sAD model is capable of recapitulating the full spectrum of AD pathology. Models based on APOE4, BBB dysfunction, pathogen infection, environmental neurotoxins, and patient-derived brain extracts capture distinct aspects of disease pathogenesis. This reflects the multifactorial nature of sAD and suggests that future modeling strategies may require the integration of multiple risk factors to achieve more comprehensive disease recapitulation.

3.3. Modeling hybrid fAD-sAD

Although fAD and sAD are generally considered to arise from distinct etiologies, hybrid models combining familial mutations with environmental risk factors warrant investigation. Such models may provide novel insights into how sAD-associated risk factors influence ongoing fAD progression and vice versa (Table 2). One study reported a hybrid AD model in hBOs by combining *PSEN1/2* familial mutations with HSV-1 infection (Figure 2) [119]. The study found no significant difference in A β aggregation in hybrid hBO models compared to fAD-only hBO models, suggesting that HSV-1 infection may not substantially exacerbate amyloidopathy in fAD. Transcriptomic and proteomic analyses were conducted, and no exacerbation of the AD-related phenotypes between hybrid and sAD-only hBO models in terms of pathways implicated in innate immune response and neurodegeneration was found. These observations suggest that modeling hybrid fAD-sAD in hBOs through *PSEN1/2* mutation-HSV-1 infection does not yield any exacerbation of the ongoing AD pathogenesis implicated independently by *PSEN1/2* mutations or HSV-1 infection. However, this study lacked a comprehensive functional evaluation of AD phenotypes, particularly tauopathy, synaptopathy, and neuronal loss as determined by immunostaining, western blot, ELISA, and other functional assays. Future studies that aim to model hybrid AD models in hBOs might employ different combinations of AD risk factors, for example, *PSEN1/2* mutations and serum exposure or *APP* mutations and neurotoxin exposure. These alternative combinations may reveal interactions between fAD and sAD risk factors that are not captured by current hybrid models.

3.4. Modeling AD-specific mechanisms

Mechanism-specific AD models are valuable for elucidating molecular pathways implicated in AD pathogenesis (Table 2). Familial- and sporadic-based AD models are useful for evaluating the extent of AD phenotype recapitulation. However, they are often less suited for dissecting the contribution of individual molecular mechanisms to AD progression. Recently, mechanism-specific AD modeling in hBOs has been explored. One study reported the modeling of mitochondrial dysfunction-related AD by fabricating hBOs with loss-of-function mutations in *PITRM1*, a crucial gene in mitochondrial homeostasis and function (Figure 2) [120]. In this model, AD phenotypes were recapitulated, including amyloidopathy by increased A β levels and formation of amyloid-like aggregates, tauopathy by increased p-tau levels, neuroinflammation by increased levels of secreted proinflammatory cytokine (TNF- α), synaptopathy by pathway analysis of dysregulated synaptogenesis and long-term potentiation, and cellular loss by increased apoptotic marker, cleaved caspase-3. These findings underscore the feasibility of modeling AD in hBOs through mitochondrial dysfunction, which

recapitulates several key AD phenotypes and is consistent with the established role of mitochondrial dysfunction as an ageing-related driver of sporadic AD [121].

Tauopathy-related AD can be modeled in hBOs harboring the Tau P301S mutation, a risk mutation implicated in familial frontotemporal dementia (Figure 2) [51]. Tauopathy was moderately recapitulated by the increase in total tau and p-tau levels, despite no tau tangle formation being reported. This model recapitulated neuroinflammation by the upregulation in the mRNA expression of the proinflammatory marker (TNF- α) and downregulation in the gene expression of the anti-inflammatory marker (S100A10). It remains unclear whether this model recapitulates amyloidopathy, synaptopathy, or other AD phenotypes, as these outcomes were not evaluated. Overall, the extent of AD phenotype recapitulation in this model remains difficult to determine because only a limited set of phenotypes was assessed. Future studies using this modeling strategy in hBOs are recommended to optimize the protocol and evaluate AD phenotype recapitulation in a more comprehensive manner.

Trisomy 21 in Down syndrome (DS) individuals introduces an extra copy of the *APP* gene, resulting in its overexpression [122]. Investigating the effect of the overexpressed *APP* gene is feasible by developing hBOs from hPSCs derived from Down syndrome individuals (Figure 2) [86,123]. Both models recapitulated amyloidopathy by increasing A β levels and A β aggregates and tauopathy via increased p-tau levels. However, only one study has reported recapitulating neuroinflammation by the formation of the inflammasome and oxidative stress by increased production of thioredoxin-interacting protein (TXNIP) [123]. Taken together, these observations imply that trisomy 21-related AD hBO models were able to recapitulate AD phenotypes, with amyloidopathy being the most prominent one. This suggests that amyloidopathy may represent an early pathogenic event in DS-associated AD, mirroring the amyloid-first cascade proposed in familial AD. Nevertheless, none of these current trisomy 21-related AD hBO models are reported to recapitulate the end-stage pathology of cellular loss and altered neural network functionality. This situation is possibly explained by the mechanism of resilience and resistance to AD pathogenesis reported in some individuals with Down syndrome [124,125]. Collectively, these findings suggest that while amyloidopathy emerges relatively earlier in DS-related organoid models, downstream AD phenotypes may require longer culture periods to fully develop.

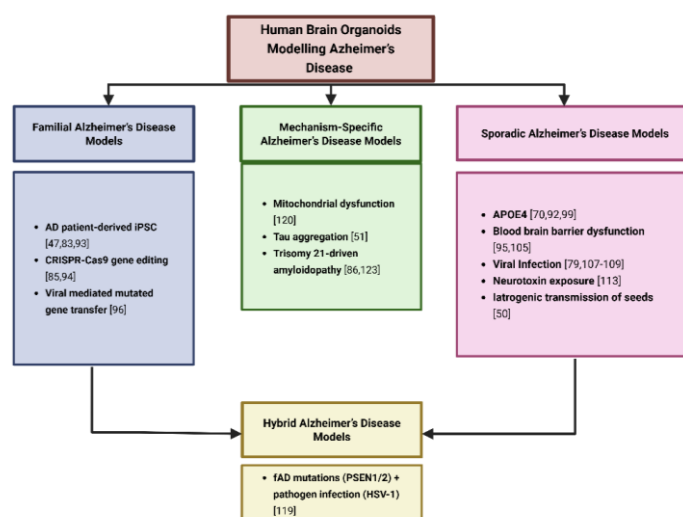


Figure 2. Overview of the current modeling strategies for Alzheimer's disease in hBOs.

Table 2. Comparative overview of strategies for modeling Alzheimer's disease using human brain organoids.

Advances	Methods/subtypes	Age of hBOs (days) (reference)	Advantages	Disadvantages
Familial AD	AD-patient-derived	120 [47]	Preserves patient-specific genetic backgrounds, enabling personalized AD modeling and precision medicine	Patient-to-patient genetic variability may lead to inconsistent AD phenotype recapitulation due to uncharacterized genetic factors that confer susceptibility or protection
	iPSC	45 [83]		
		90 [84]		
		120 [93]		
		84 [98]		
	Viral-mediated mutated gene transfer	70 [96]	Enables the effects of specific fAD mutations to be isolated, minimizing confounding from uncharacterized genetic backgrounds present in patient-derived models	May not recapitulate the physiological expression level of the mutated gene, potentially affecting AD phenotype and model fidelity
Sporadic AD	CRISPR-Cas9 gene editing	45 [85] 180 [94]	Enables precise and controllable editing of fAD-associated genes, facilitating the dissection of mutation-specific effects and functional characterization of novel variants	Off-target editing may introduce unintended genetic alterations, compromising model fidelity and reproducibility
	APOE4	45 [70] 180 [92] 84 [99]	Models a major genetic risk factor for sAD and enables investigation of APOE4-associated AD phenotypes	APOE4 effects may be insufficient to induce robust sAD pathology without additional major risk factors, including aging
	Blood–brain barrier dysfunction	90–110 [95] 142 [105]	Enables neurovascular interactions to investigate BBB breakdown-associated AD phenotypes	Establishing functional vasculature and BBB remains a major challenge
	Viral infection	68 [79] 57 [107] 87 [108] 120 [109]	Provides a controllable environmental insult for investigating infection-associated mechanisms that may promote AD-related phenotypes	Viral-induced pathology may reflect acute infection-related injury rather than chronic and progressive AD pathogenesis
	Neurotoxin exposure	70 [113]	Provides a controllable approach to delineate the neurotoxicity aspect of AD	Chemical-induced neurotoxicity may not faithfully reproduce the multifactorial nature of sAD
	Iatrogenic transmission of seeds	28 [50]	Enables investigation of cell-to-cell propagation of seeds and their role in driving AD pathogenesis	Exogenous seeds may not fully recapitulate the endogenous initiation, maturation, and chronic progression of protein aggregation in AD

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Advances	Methods/subtypes	Age of hBOs (days) (reference)	Advantages	Disadvantages
Mechanism-specific AD	Mitochondrial dysfunction	60 [120]	Direct modeling of mitochondrial and metabolic dysfunction implicated in neuronal vulnerability and AD progression	Mitochondrial impairment is not AD-specific and may produce general cellular stress that complicates the interpretation of AD-specific phenotypes
	Tau aggregation	75 [51]	Directly targets a key AD pathology and enables mechanistic investigation of tau phosphorylation, aggregation, and propagation	Induced tau pathology may require artificial stimulation and may not reproduce the full temporal progression or molecular complexity of human tauopathy
	Trisomy-21-driven amyloidopathy	84 [86] 150 [123]	Provides a genetically predisposed human model with <i>APP</i> gene dosage elevation, recapitulating early amyloidogenic processes associated with Down syndrome-associated AD	AD pathology arises from a broad chromosomal imbalance, making it difficult to attribute phenotypes specifically to <i>APP</i> or AD mechanisms
Hybrid AD	FAD mutation-viral infection	93 [119]	Combines genetic susceptibility with an environmental insult, enabling investigation of interactions between fAD mutations and sAD pathological triggers	Multiple interacting variables complicate causal interpretation and may reduce reproducibility compared with single-factor models

4. Recapitulation degree of AD phenotypes in hBOs modeling AD

The goals of disease modeling are to achieve a high-fidelity recapitulation of pathological phenotypes representative of human disease and to advance our understanding of underlying disease mechanisms. With the fast-progressing field of AD modeling in hBOs, the recapitulation of AD phenotypes has expanded to include not only classical hallmarks of AD but also several emerging AD phenotypes. This is essential for the application of hBOs as an AD drug testing platform, since it is increasingly recognized that classical hallmarks of AD per se are insufficient to explain the cognitive impairment in AD patients [5]. Instead, clinical symptoms of AD are the result of multiple interconnected pathologies that emerge with distinct temporal and spatial dynamics throughout disease progression. Therefore, a robust AD hBO model must be able to recapitulate a broader spectrum of AD phenotypes beyond the classical hallmarks. In current AD hBO models, a broader AD phenotype recapitulation has indeed been reported, although the degree of recapitulation for each pathology varies. To assess the degree of recapitulation for each AD phenotype, we classify them into five categories: strong, moderate, emerging but limited, weak but consistent, and weak and variable. The classification was established based on two parameters: the depth of recapitulation details that resemble human AD pathology and the reproducibility of findings across studies (Table 3). A list of AD phenotypes and their degree of recapitulation in current AD hBO models is given in Table 4.

Amyloidopathy can be conceptualized as a continuum of aggregation states, but some frameworks group them into early (soluble A β) and later (aggregated A β) stages [126]. Early-stage amyloidopathy is characterized by an increase in the A β species production, often evidenced by elevated A β 42 levels and an increased A β 42/A β 40 ratio prior to overt fibrillar deposition. In current hBO models, these early amyloid-related changes are reproduced in numerous studies (Table 4). The later stage entails the aggregation of A β from soluble species into oligomers, protofibrils, and fibrils, culminating in plaque deposition, including neuritic plaques in the advanced disease stage. However, current AD hBO models do not yet consistently capture the full temporal sequence of the amyloid aggregation pathway. Instead, later-stage amyloid pathology in many studies is inferred from pan-A β immunostaining, which often does not discriminate among oligomeric, protofibrillar, and diffuse plaque-like A β assemblies. An amyloid fibril-like structure is detected in AD hBO models by Thioflavin-S and Congo Red staining. However, key defining features of the neuritic plaque, particularly the presence of dystrophic neurites, have not yet been consistently demonstrated in current AD hBO models [127].

Early tauopathy entails hyperphosphorylation of the tau protein [128]. This pathology has been consistently recapitulated in current hBO models by numerous studies (Table 4). The later stage of tauopathy involved progressive aggregation of misfolded p-tau from soluble oligomers to paired helical filaments and ultimately neurofibrillary tangles. The recapitulation of this sequential cascade is emerging in hBO models; however, it currently relies largely on artificial induction through mutant *MAPT* gene expression rather than spontaneous pathogenesis. Therefore, further validation using independent modeling approaches is required to achieve a more robust recapitulation of tauopathy in AD hBO models.

Neuroinflammation is increasingly recognized as a central pathological component in AD [129]. However, the precise cellular, temporal, and spatial profiles of neuroinflammation in AD patients remain incompletely characterized. This is because neuroinflammation is not merely a molecular event but also includes intricate alterations at the cellular and tissue levels, including changes in glial states, cell-cell interactions, and tissue organization. Currently, the recapitulation of neuroinflammation in hBO models is categorized as moderate, considering that extensive characterization revolves around molecular alterations, with relatively limited evaluation of cellular and tissue-level inflammatory responses (Table 4). Future AD hBO models should therefore integrate robust evaluation of neuroinflammation at the cellular and tissue level to enhance biological relevance and translational utility.

Synaptopathy recapitulation in current AD hBO models remains relatively limited, particularly with respect to robust functional readout (Table 4). This is partly because synaptopathy is frequently inferred from reduced synaptic marker density by immunostaining, whereas direct functional assessment remains less common. In AD patients, the extent of synaptopathy is not constrained to synapse loss but also includes functionality impairment of long-term potentiation [130]. To date, only one study has inferred synaptic plasticity-related alterations in AD hBOs from transcriptomic pathway analysis, but direct experimental evidence of LTP-like plasticity remains scarce [120]. In the future, synaptogenesis and LTP impairment in hBO models should be validated by functional phenotypic assays such as electrophysiology, multi-electrode array recordings, and calcium imaging, alongside structural synapse quantification.

Cellular loss in AD takes place through various mechanisms and is not limited to apoptosis [131]. Recapitulation of cellular loss in current hBO models is generally considered moderate and largely centered around apoptotic mechanisms by the activation of caspase 3, DNA fragmentation, and upregulation of apoptotic pathways identified through transcriptomic analyses (Table 4). To date, only

two studies have reported the distinct mechanism of cellular loss due to impaired neurogenesis during development in AD hBO models [48,85]. Thus, recapitulating other mechanisms of cellular loss, such as necroptosis and parthanatos, will be critical for improving the biological relevance of hBO models.

Altered neural network functionality recapitulation is considered weak in current hBO models. This is due to the limitation of current hBOs to constitute mature neural circuits that constitute specific brain functions. Moreover, current neural network functionality assessment in hBO models is confined to neuronal activity or localized neural networks rather than interconnected neural circuits. Additionally, the findings between different studies are varied, with some studies reporting enhanced synchronicity of neural network activity and the rest reporting impaired synchronicity (Table 4). It is noteworthy that AD hBO models that reported enhanced synchronicity are predominantly self-patterned organoids or assembloids [92,93]. In contrast, AD hBO models that reported impaired synchronicity have been reported across a mixture of patterned and self-patterned hBOs [50,85,94,95]. This suggests that multi-regional composition in self-patterned organoids and assembloids might, to some extent, contribute to the enhancement of network synchronicity observed in those AD hBO models. Thus, the establishment of mature and more comprehensive neural networks is proposed to be a main focus of future works that aim to evaluate altered neural network activity in AD hBO models.

Recapitulation of mitochondrial dysfunction and oxidative stress is an emerging area in current AD hBO models. However, these recapitulations are substantiated by limited evidence derived from transcriptomic pathway analyses and an increased level of thioredoxin-interacting protein (TXNIP) only (Table 4). Future studies are warranted to validate these findings by capturing diverse aspects of mitochondrial dysfunction and oxidative stress at the molecular and cellular levels.

Table 3. Description of the classification system used to categorize the degree of recapitulation.

Degree of recapitulation	Resemblance to human AD pathology	Reproducibility across study
Strong	The extent of recapitulation in hBO models almost or completely matches human AD pathology	Reproducible across numerous studies
Moderate	The extent of recapitulation in hBO models matches several aspects of human AD pathology	Reproducible across numerous studies
Emerging but limited	Novel aspect of AD recapitulated in hBO models	Reproducible across a limited number of studies
Weak but consistent	The extent of recapitulation in hBO models matches minor aspects of human AD pathology	Reproducible across a limited number of studies
Weak and variable	The extent of recapitulation in hBO models matches minor aspects of human AD pathology	Irreproducible across a limited number of studies

Table 4. Degree of AD phenotype recapitulation in current hBOs modeling AD.

AD phenotypes	Recapitulation details	Degree of recapitulation	Level of evidence	References
Early stage of amyloidopathy	Increased total A β peptide level in supernatant	Strong	Biochemical	[70,83,84,86,95,96,113,120,123]
	Increased total and insoluble A β levels in organoid lysates			[95,98]
	Increased A β 42 and A β 43			[85,93]
	Increased A β 42/A β 40 ratio			[85,86,92,93,99,120]
Later stage of amyloidopathy	Increased extracellular A β aggregate	Moderate	Histological	[47,50,83–86,92,95,96,98,105,113,119,123]
	Formation of intracellular A β aggregates			[50]
	Formation of extracellular amyloid plaques			[50,83,96,120]
Early stage of tauopathy	Increased p-tau levels	Strong	Biochemical	[50,51,70,83,84,86,94–96,98,99,105,107,113,120,123]
	Increased total tau levels			[51,107]
	Increase in expression of 4R tau			[107]
	Detection of insoluble p-tau			[83,86]
Later stage of tauopathy	Increased aggregated/oligomeric tau	Emerging but limited	Histological	[83]
	Formation of tau filaments			[83]
	Formation of tau tangles			[50,83,96]
Neuroinflammation	Exhibited a neuroinflammation profile by transcriptomic analysis	Moderate	Transcriptomic, biochemical, and cellular	[47]
	Increased mRNA expression of IL-6, TNF- α , and CCL2 (proinflammatory markers)			[50,51]
	Decreased mRNA expression of S100A10 (anti-inflammatory marker)			[51]
	Increased secreted level of TNF- α and IL-1 β			[120]
	Inflammasome formation by increased ASC-speck released			[123]
	Activation of microglia by the increase in TREM2, Iba1, CD44 and CD9 expression and decrease in P2RY12 expression			[47,50,51]

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AD phenotypes	Recapitulation details	Degree of recapitulation	Level of evidence	References
Neuroinflammation	Reactive astrocyte states by the increase in GFAP expression			[50,51]
Synaptopathy	Decreased synaptic puncta	Weak but consistent	Transcriptomic	[50,94,99,105,113]
	Dysregulated synaptogenesis and long-term potentiation pathway by transcriptomic analysis		and biochemical	[120]
Cellular loss	Reduced MAP2 and TUJ1-expressing neuron number and decreased cell viability	Moderate	Transcriptomic, biochemical, and cellular	[48,85]
	Upregulation of apoptotic related genes by transcriptomic analysis			[47,119]
	Elevated cleaved caspase 3 level			[50,93,95,96,99,105,120]
	Elevated TUNEL signal intensity			[113]
Altered neural network functionality	Reduced number of spikes, mean firing rate, number of bursts, and synchronicity index	Weak and variable	Functional	[50,85,94,95]
	Enhanced synchronicity and increased spike rate			[92,93]
	Asynchronous oscillation and reduced amplitude of calcium transient			[95]
	Asynchronous oscillation but enhanced amplitude of calcium transient			[93]
Mitochondrial dysfunction and oxidative stress	Increased production and secretion of TXNIP	Emerging but limited	Transcriptomic	[123]
	Differentially expressed genes related to oxidative stress by transcriptomic analysis		and biochemical	[119]

5. Challenges and future directions for modeling AD in hBOs

5.1. *Achieving adult-like neuronal maturation and physiological complexity in hBOs*

Advances in modeling AD using hBO models have expanded the field by ameliorating previous issues of insufficient cellular diversity in hBOs and limited modeling strategies for AD subtypes. Nevertheless, issues of neuronal maturity of hBOs remain incompletely resolved. Admittedly, integration of diverse cell types, including microglia, astrocytes, and vascular endothelial cells, has partially contributed to the enhancement of neuronal maturity. These are manifested by the expression of post-mitotic neuronal markers (e.g., NeuN) and the emergence of spontaneous electrophysiological activity, which nonetheless remains developmentally immature relative to adult brain networks. A neural network in the adult brain consists of long-range projection neurons that span different regions of the brain, exhibiting neural network regulation by excitatory and inhibitory neurons and undergoing myelination by oligodendrocytes [132,133]. This review proposes future works to explore the intrinsic mechanisms that regulate neuronal and glial maturation, including developmental programs and signaling pathways, as well as extrinsic cues, including extracellular matrix composition and stiffness, that are capable of accelerating hBO maturation toward adult-like phenotypes. Potential strategies include genetic manipulation, treatment with biologics or small molecules, and optimization of hBO culture conditions.

Beyond neuronal immaturity, the tissue-like nature of hBOs represents another fundamental limitation for faithfully modeling AD pathophysiology. As hBOs are typically maintained as isolated neural tissues, they do not fully recapitulate systemic interactions that influence brain homeostasis and disease progression, including peripheral immune signaling, endocrine regulation, metabolic cues, and gut–brain communication. These interactions may be particularly relevant to AD, as peripheral inflammation, metabolic dysfunction, and altered gut–brain signaling have been increasingly implicated in AD pathogenesis [134–136]. Therefore, future hBO-based AD models should consider integration with relevant peripheral systems to better capture the complex bidirectional interactions between the brain and the periphery. Tissue engineering approaches such as organoid-on-a-chip remain largely at proof-of-concept or early translational stages and are not yet widely adopted due to technical complexity, scalability issues, and cost [137]. This implies that further optimization is required before these technologies can be widely adopted for AD modeling using hBOs.

5.2. *Minimizing variability and heterogeneity in hBOs*

Variability and unintended heterogeneity that arise during hBO development persist as major challenges despite ongoing efforts made to minimize them. This variability is partly attributable to the sensitivity of developing hBOs to morphogen gradients [138,139] and physical cues from the stiffness of the extracellular matrix and shear stress elicited during spinning or agitated culture systems [140]. Addressing these challenges requires a deeper mechanistic understanding of how morphogen gradients and biophysical cues govern hBO development. It is therefore recommended for future works to comprehensively unravel these intrinsic and extrinsic cues and establish a more comprehensive, standardized protocol to generate hBOs with higher reproducibility. Assembloids are the current alternative to generate multi-regional brain organoids with better control of regional identity. However, current AD assembloid models present limited inter-regional interaction [92]. It would be beneficial

for future works to model AD in assembloids in a more comprehensive manner by fusing multiple distinct regionalized organoids to recapitulate long-range and multi-regional interactions observed in the human brain.

5.3. Establishing robust *sAD* phenotypes in hBOs

Although several studies have attempted to model sporadic AD in hBOs, the number and degree of AD pathological features recapitulated remain limited. This is most likely due to the employment of a single risk factor in current models, whereas accumulating evidence indicates that sporadic AD arises from the interplay of multiple risk factors, with ageing being one of the major contributing factors [141,142]. Importantly, ageing is also accompanied by alterations in the tissue microenvironment, including chronic low-grade inflammation, metabolic dysfunction, extracellular matrix remodeling, and vascular dysfunction, which may contribute to AD pathogenesis [143]. Therefore, future works that aim to model sporadic AD in hBOs are recommended to include multiple risk factors for sporadic AD, with ageing-related processes representing a central but not exclusive priority. Although current hBOs have yet to faithfully recapitulate ageing status intrinsically, certain aspects of ageing can be modeled by means of extrinsic perturbation [144,145]. Combinatorial genetic perturbations and treatments with drugs that induce multiple hallmarks of ageing are worth exploring in the future to revolutionize sporadic AD modeling in hBOs.

5.4. Defining criteria for robust AD modeling in hBOs

To date, there is no consensus on the defining criteria of what really constitutes a robust AD model in hBOs. Accordingly, different studies that reported AD modeling in hBOs often evaluated various aspects of AD phenotypes. AD phenotypes are multifaceted, encompassing classical hallmarks such as amyloid plaques and neurofibrillary tangles, as well as associated features, including, but not limited to, neuroinflammation, synaptopathy, and altered neuronal network activity. Attributing the recapitulation of classical AD hallmarks as the defining criterion for a robust AD hBO model is insufficient, as these hallmarks alone do not fully account for the heterogeneity and severity of cognitive impairment reported in AD patients. These point to the direction that broader AD-associated pathological features should be recapitulated for a system to be considered a bona fide AD model. Therefore, defining a consensus set of criteria that specifies which combination of AD-related pathological features should be recapitulated will represent an important future milestone to enhance the credibility and applicability of AD hBO models.

Additionally, the integration of multi-omics technologies would immensely benefit AD modeling in hBOs. Omics platforms, including transcriptomics and proteomics, facilitate detailed analysis of cellular states and enable inference of functional alterations. This is instrumental for benchmarking AD hBO models against human AD brain datasets to achieve higher levels of biological correlation. However, omics analysis should be used as a complementary tool rather than as a replacement for standardized phenotypic assays when evaluating AD phenotype recapitulation in hBO models.

6. Conclusion

Recent advances in AD modeling using hBOs have been driven by progress in two main areas: improving the biological complexity of hBOs and expanding modeling strategies for diverse forms of AD. Integration of robust non-neuronal cell populations, including astrocytes and microglia, into hBOs has better equipped them as AD models that recapitulate neuroinflammation. Nevertheless, the constitution of a functional BBB remains one of the major unresolved challenges in this field. Hence, the exploration to establish a validated functional BBB in hBOs should continue in the future to enable robust recapitulation of BBB dysfunction. In terms of modeling strategies for different facets of AD, progress has shown that the induction of hBOs with sAD non-genetic risk factors results in the recapitulation of limited AD phenotypes. However, the recapitulation degree of AD phenotypes varies with different risk factors. Therefore, future sporadic AD models will likely require the incorporation of multiple interacting genetic, environmental, and ageing-related risk factors to more faithfully reproduce the multifactorial nature of sAD pathogenesis. Although current hBO models consistently reproduce early amyloid and tau pathology, the recapitulation of higher-order phenotypes, including synaptopathy, complex neuroinflammatory responses, and altered neural network activity, remains incomplete. Equally important is the establishment of standardized criteria for defining and benchmarking AD phenotypes across hBO models. Looking ahead, the next generation of hBO models should move beyond reproducing individual AD hallmarks toward integrated models that recapitulate the interactions between amyloidopathy, tauopathy, neuroinflammation, BBB dysfunction, synaptopathy, and neuronal network alterations. Such models, combined with improved maturation and ageing strategies and standardized phenotypic benchmarking, could provide a more comprehensive representation of AD pathogenesis than current models that predominantly capture isolated pathological features. These advances will be particularly important for determining whether hBOs can reproduce disease mechanisms sufficiently to identify therapeutic targets and predict treatment responses. Thus, the future value of hBOs in AD research will depend not simply on how many AD phenotypes they can reproduce but on whether they can faithfully reproduce the interconnected pathological processes that drive AD progression, thereby strengthening their utility as mechanistic and preclinical models of AD.

Use of Generative-AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article. They took full responsibility for the content of the article.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Wan Mohd Naim Wan Mohd Ruslan: Writing original draft, visualization, review and editing; Siti Zaleha Raduan: Review; Maizatul Fazilah Abdul Razak: Conceptualization, writing original draft, review, editing, project administration, supervision, and funding acquisition. All authors have agreed to the final submitted version.

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