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Review article

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Human midbrain organoids: a powerful tool for advanced Parkinson's disease modeling and therapy exploration

Xin Cui[1,2,3,7], Xinwei Li[1,2,3,7], Huimin Zheng[1,3,7], Yun Su[1,3,7], Shuyu Zhang[1,4], Mengjie Li[1,3], Xiaoyan Hao[1,3], Shuo Zhang[1,2,3], Zhengwei Hu[1,2,3], Zongping Xia[1,3,5], Changhe Shi[1,3,6], Yuming Xu[1,3,6] ✉ & Chengyuan Mao[1,3] ✉

Parkinson's disease (PD) is a neurodegenerative disorder marked by the loss of dopaminergic neurons in the substantia nigra. Despite progress, the pathogenesis remains unclear. Human midbrain organoids (hMLOs) have emerged as a promising model for studying PD, drug screening, and potential treatments. This review discusses the development of hMLOs, their application in PD research, and current challenges in organoid construction, highlighting possible optimization strategies.

Parkinson's disease (PD) is one of the most common neurodegenerative disorders associated with movement disabilities, affecting more than 6.1 million people worldwide[1,2], with the mean age of onset at 55[3]. Clinically, as a debilitating neurological disorder, PD patients mainly present with motor symptoms such as resting tremor, bradykinesia, rigidity, postural instability, loss of coordination and shuffling or freezing gait[4–6]. Non-motor symptoms may also present including depression, anxiety, constipation, sleep disturbances, hyposmia, paresthesia, and cognitive abnormalities[7–10]. The key histopathological hallmark of PD is the gradual loss of midbrain dopaminergic (mDA) neurons and the presence of intraneuronal protein inclusions named "Lewy Bodies" (LB) in substantia nigra (SN)[3,10–12]. Composed of abnormal α-synuclein (α-syn) protein aggregations[13], LBs-associated pathology has been attributed to mitochondrial metabolism alteration and proteasomal and autophagy-lysosomal dysregulation, which ultimately bring about the death of mDA[14,15].

Up to now, there are three prevalent tools to explore the underlying mechanism of PD, including the post-mortem brain tissue from PD patients, animal models and in vitro cell models. The post-mortem brain of PD patient is an ideal source for the PD analysis which directly reflect the actual inner environment. The use of human brain tissue, however, is strictly restricted by practical constraints[16–21]. Furthermore, post-mortem brain tissue may have undergone irreversible changes during the process of death that limit its utility for the study of PD[16]. Animal models of PD can be further divided into two groups, the toxin-based model and gene-based model[3]. By introducing neurotoxins such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat, and rotenone, toxin-based models suffer from overloaded oxidative stress which provokes the speedy degeneration of mDA that mimic the sporadic PD[17,18]. On the other hand, gene-based models are built via overexpress or knockdown/knockout certain genes such as LRRK2, Parkin, DJ-1, and PINK1, to study the molecular mechanism of their involvement in PD pathology[18–20]. However, the value of animal models is limited because of their inherent differences from humans[21,22]. In vitro cell models are one of the most easily obtained approaches to investigate PD pathogenesis, with the advantage of shorter time consumption and smaller inter-batch differences, not until the advent of iPSCs technology revolutionized the modeling of human diseases including PD[23]. Combined with genome editing techniques[24], this approach offered the opportunity to give rise to disease-relevant neuronal subtypes that retain the certain genetic background of the patient. Nonetheless, current approaches for mDA neuron generation are typically based on two-dimensional (2D) conditions, however, mDA neurons yielded from iPSCs without other cell co-cultivation clearly neglect the fact that in vivo neuron growth and maturation does not work on their own[25–27]. What's more, the granular pigments named neuromelanin (NM) synthesized by DA in human SN has not been seen in the 2D-derived mDA neurons[28–31].

Thus, an appropriate in vitro model that reflects human neurobiology to advance our insight of the pathology of PD is urgently needed. The introduction of midbrain organoids allowing us to overcome some of the obstacles, given either by the use of animal models, such as species specificity of cellular pathways, ethical issues and the limited availability of post-mortem human brain tissue. This review briefly summarizes the development of midbrain organoids since their inception and focuses on their PD-related application, providing new hints for further condition optimization and clinical transformation of midbrain organoids.

[1]Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou, China. [2]Academy of Medical Sciences of Zhengzhou University, Zhengzhou, China. [3]Henan Key Laboratory of Cerebrovascular Diseases, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou, China. [4]Neuro-Intensive Care Unit, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China. [5]Clinical Systems Biology Laboratories, Zhengzhou University, Zhengzhou, China. [6]Institute of Neuroscience, Zhengzhou University, Zhengzhou, China. [7]These authors contributed equally: Xin Cui, Xinwei Li, Huimin Zheng, Yun Su. ✉e-mail: xuyuming@zzu.edu.cn; maochengyuan2015@126.com

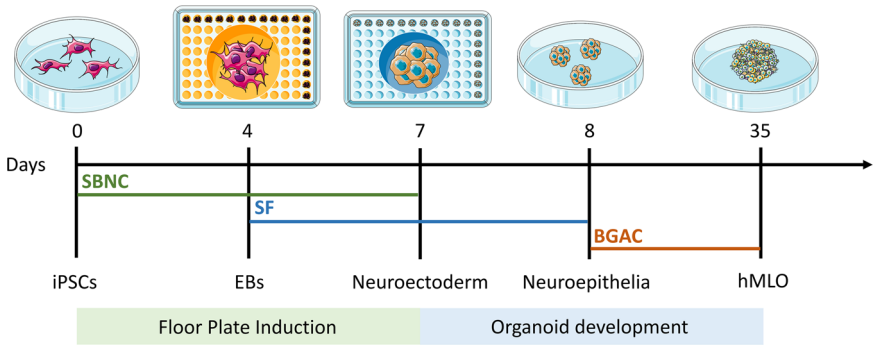

Fig. 1 | Generation of hMLOs using human iPSCs. Schematic diagrams illustrating the overall strategy to generate hMLOs. Differential interface contrast (DIC) images illustrate the typical morphology of cells at each stage. SBNC: SB431542, Noggin, and CHIR99021; SF: SHH-C25II and FGF8; BGAC: BDNF, GDNF, ascorbic acid, and db-cAMP. This image was independently created by us using Adobe Illustrator. All elements are original, and no third-party materials were used.

The midbrain organoids and Parkinson's disease

Over the last decade, three-dimensional (3D) organoid technology has become popular in stem cell research. Organoids are miniature in vitro 3D cellular clusters generated from iPSCs or isolated organ progenitors[32–36]. Due to their capacity for self-renewal and self-organization, organoids can mimic many aspects of in vivo organs. Organoids have been generated that model various parts of the brain, including the forebrain, midbrain, cerebellum, cortex, and hippocampus[37–39]. Various methods are used to generate these organoids, many of which aim to model the development of the human brain. In addition, brain organoids have been used to recapitulate human disease. In this context, the rise of human stem-cell derived brain 3D organoid cultures, which recapitulate features of the brain's composition, organization, and function[40], has led to significant advances in our understanding of neurodevelopment and in disease modeling. Although midbrain and mDA markers have been found to spontaneously arise in non-directed whole brain organoids[41], the proportions of cells expressing such markers tend to be small and highly variable, thus warranting the development of more directed differentiation protocols. Some approaches have led to the development of "neurospheres," which contain an increased proportion of DA neurons, along with excitatory, inhibitory neurons as well as glial cells[42]. Most efforts have been directed at specifically reproducing mesencephalic development in the dish, in order to generate mDA neurons in representative human "midbrain organoid" (hMLO) structures. In general, the development of hMLOs involves two stages, namely floor plate induction and organoid development. By incorporating specific components, iPSCs are differentiated from a 2D state into 3D spheres with midbrain-specific markers (Fig. 1).

Tieng et al.[43] were the first to adapt a widely-used 2D differentiation protocol[25] to 3D suspension through the use of microwells to create homogeneously sized embryonic bodies, which were then placed on an orbital shaker for 3 weeks, before being seeded and grown at air-liquid interface. Although the suspension-culture phase of their protocol was short, they proved that such an approach could efficiently generate mDA progenitor cells (~80% of all cells expressed FOXA2 and LMX1A) as well as TH-expressing cells after only 3 weeks. Following these results, 3 new protocols were published within 1 year[39,44,45], describing the generation and long-term maintenance of hMLO (up to 5 months). These papers were the first to provide in depth characterization of the model, and proof that these organoids could be maintained in long term cultures in order to favor neuronal maturation. Although each protocol presents differences in timing, specific molecules used and their concentrations, these approaches mainly rely either on the sequential[44] or simultaneous[39,43,45] use of morphogens to induce midbrain floor plate identity, as described earlier. These organoids developed features of organization similar to the midbrain floor plate, namely a ventricular zone containing OTX2+FOXA2+ cells, as well as intermediate (LMX1A+, NURR1+) and mantle layers containing progressively maturing neurons (MAP2+, TH+). Several markers of pan-mDA neuronal identity have been consistently observed in hMLO, including the dopamine transporter (SLC6A3/DAT), DOPA decarboxylase enzyme DDC, and TF PITX3[44,46]. Studies also found that NM granules can spontaneously appear in long term cultures, their structures resembling those found in adult human SN tissue. NM-containing cells were found to be enriched in transcripts expressed in A9 SN mDA neurons[44,47–49]. Exogenous DA treatment could also significantly increase the accumulation of NM, suggesting that these granules may indeed be by-products of DA metabolism[44]. There have been reports demonstrating that transplantation of hMLOs into mouse brains can establish classical substantia nigra-striatum network connections, further confirming the presence of functionally relevant SN components in organoids[50]. The hMLOs were also found to produce DA, and mDA neurons showed characteristic electrophysiological pacemaker activity which was responsive to the use of D2/D3 agonist quinpirole. Beyond mDA neurons and their progenitors, excitatory and inhibitory neurons[43,44] were found in these hMLO, as well as astrocytes and myelinating oligodendrocytes, consistent with the composition of the midbrain[44,45].

The construction of hMLOs offers an attractive alternative to 2D culture models, they recapitulate some of the complex characteristics and physiology of the human midbrain that are lacking in 2D culturation. They may also provide the missing link between in vitro 2D models and in vivo animal models. Moreover, the ability to maintain organoids for long-term culture, up to 1 year, means that they may be used for aging studies, and/or long-term drug interventions. Due to the multifactorial nature of neurodegenerative diseases such as PD, the use of single cell type models might limit target discovery and the potential for phenotypic screening. The usage of animal models can also be limited due to species differences[51]. hMLOs provide a human-based 3D culture system that may combat the limitations of other models in the drug screening process, either. In addition, since iPSC carry the same genetic signature as the patient they are derived from, the generated organoids will express any pathogenic mutations present.

Since the advent of hMLO in 2014[40], some of the major breakthroughs in the PD field are the results of hMLO usage. Human brain-based models of PD have contributed greatly to our understanding of disease mechanisms and pathogenesis, while also facilitating a means to test viable drug candidates[52–54]. PD treatment is also a promising application for hMLO[55].

Application of hMLOs in PD

In the following sections, we will delve into three key aspects of recent advancements in the application of human midbrain-like organoids: constructing PD models; predicting PD toxicity; and developing treatments for PD (Fig. 2).

PD model construction

For the past few years, organoids have widely been used in neurodegenerative disease, including Alzheimer's disease[56,57], amyotrophic lateral sclerosis[58,59], spinal muscular atrophy[60] and Huntington's disease[59]. As mentioned earlier, the conventional models for PD studying (in vitro 2D cell models, animal models and the post-mortem brain tissue) all have their unneglected drawbacks, the advent of organoids is considered a major breakthrough in stem cell research and has enabled advancements in the applications of human iPSC for disease modeling. Since then, various

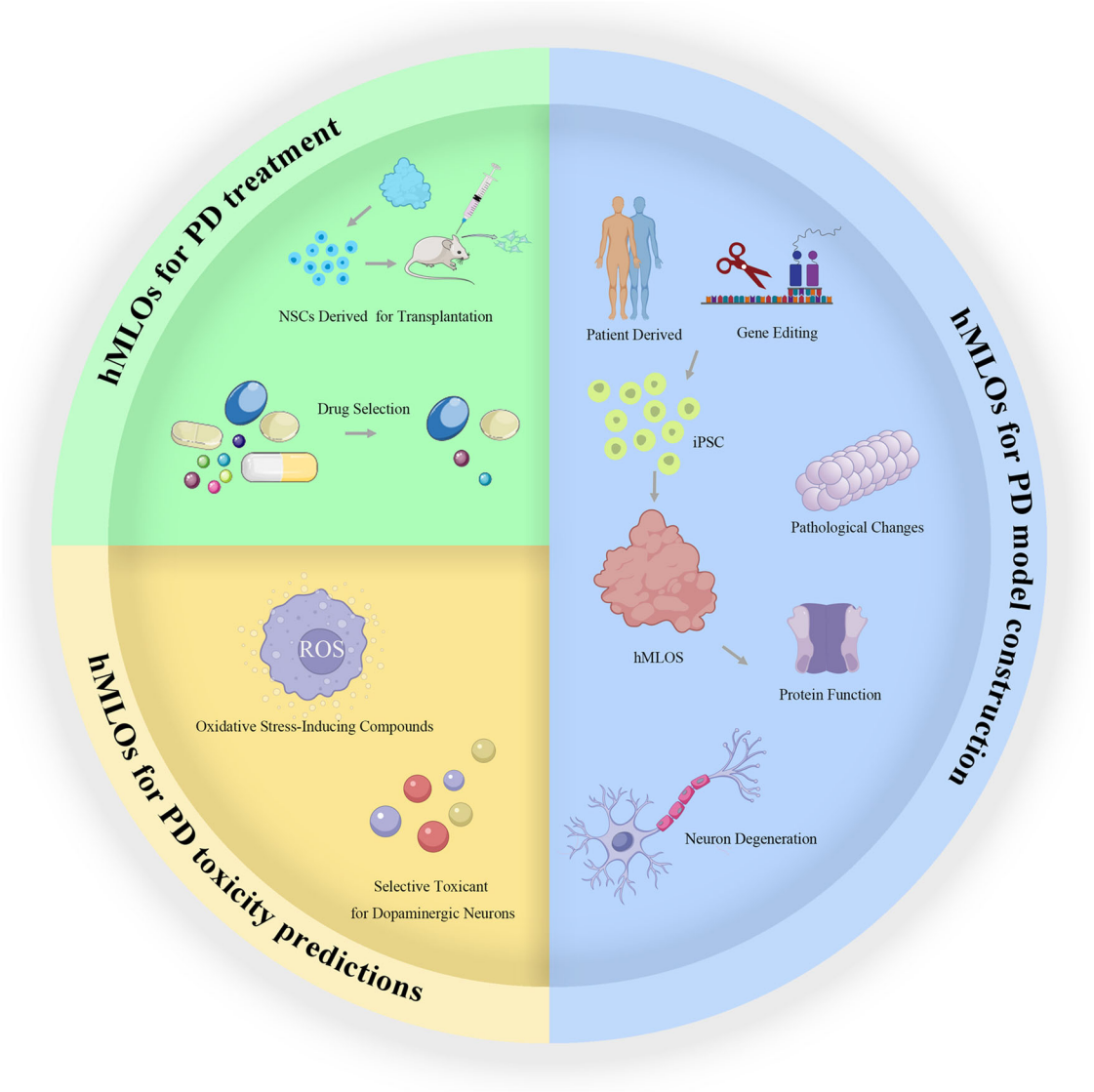

Fig. 2 | Applications of hMLOs in Parkinson's disease research. Three main directions of hMLOs in PD research: PD model construction, PD toxicity predictions and PD treatment. PD model construction involves deriving iPSCs from PD patients or using gene-edited iPSCs to generate midbrain organoids, which are then used to investigate PD-related pathological changes, protein dysfunction, and neuronal degeneration. PD toxicity predictions explore the effects of oxidative stress-inducing compounds and selective toxins that target dopaminergic neurons. PD treatment research includes the extraction of neural stem cells (NSCs) from midbrain organoids for transplantation in mouse models and the selection of drugs that may provide therapeutic benefits. This image was independently created by us using Adobe Illustrator. All elements are original, and no third-party materials were used.

methods have been developed to generate models aiming to mimic human midbrain contents.

With the prevalence of hMLOs, they are increasingly used in PD modeling. Similar to the construction of traditional PD animal models, there are 2 popular strategies in PD-related hMLOs induction, which are genetic-based and toxin-based, respectively. The development of hMLOs provides a more advanced model for the in-depth study of PD.

Construct hMLOs carrying monogenic PD disorders. With the advancement of genetic techniques and population studies, over 20 monogenic forms of PD have been described and over 100 loci have been identified as risk factors for PD[61–64], however, most of their pathogenesis are poorly understood. Recent advances in 3D organoid technology offer promise in advancing the understanding of PD on a platform more physiologically[44,65,66]. The combination of specific genetic mutation with hMLO construction shed light on the further exploration on PD pathogenesis. We have summarized the recent research using hMLOs in the study of PD genetic mutations and their pathogenetic mechanisms in Table 1.

SNCA

SNCA, the first gene associated with familial Parkinson's disease, encodes the protein α-syn[67]. Missense mutations in the SNCA gene cause a rare, autosomal dominant inherited form of Parkinson's disease[68–72]. Moreover, copy number variations (CNVs) in the SNCA gene were also identified in patients with Parkinson's disease. Indeed, the clinical phenotype of SNCA duplications resembles typical late-onset sporadic Parkinson's disease whereas SNCA triplications lead to a more widespread neurodegeneration with early-onset parkinsonism and dementia[72–77]. This implies that there is a direct relationship between SNCA gene dosage and disease severity[73,78]. Beyond these rare genetically determined forms, the abnormal accumulation of α-syn in the brain is a classical pathological hallmark for a group of related neurodegenerative disorders, collectively referred to as synucleinopathies. To date, the majority of studies examining α-syn pathophysiology

Table 1 | Genetic mutations and phenotypic outcomes in human midbrain-like organoids for Parkinson's disease research

Gene	Mutation	Phenotype	Reference
SNCA	SNCA triplication	Detergent-resistant, β-sheet-rich α-syn aggregates and spherically symmetrical Lewy body-like inclusions with eosinophilic cores form	Jo et al.[80]
	SNCA triplication	Elevated α-synuclein levels, age-dependent increases in α-synuclein aggregation, and selective loss of dopaminergic neurons	Mohamed et al.[81]
	SNCA triplication	Accumulation of pathological αSyn, morphological resemblance to diverse stages of LB formation, and loss of DA neurons	Becerra-Calixto et al.[82]
	SNCA triplication	α-synuclein aggregation, dopaminergic neuron loss, and astrosenescence with nuclear and chromatin alterations	Muwanigwa et al.[83]
LRRK2	G2019S LRRK2 mutation	Increased α-synuclein aggregation, aberrant clearance, and gene expression profiles mimicking LRRK2-associated sporadic PD	Kim et al.[31]
	G2019S LRRK2 mutation	Decrease in number and complexity of mDANs, and increased FOXA2 expression	Smits et al.[97]
	G2019S LRRK2 mutation	Untimely and incomplete differentiation with reduced cellular variability	Zagare et al.[99]
PINK1	PINK1 knockout	CCCP/NH4Cl treatment reveals a PINK1- and Tom40-positive 720-kDa HMW complex in WT but not in PINK1 KO hMLOs	Eldeeb et al.[109]
	PINK1 knockout	Reduced growth rate and impaired DA neuronal differentiation	Brown et al.[110]
GBA1	L444P GBA1 mutation	Significant reduction in GCase activity and TH+ neurons, increased insoluble α-synuclein, and phosphorylated α-synuclein	Baden et al.[115]
	N370S GBA1 mutation	Reduced GCase activity, impaired autophagy, mitochondrial dysfunction, altered lipid metabolism, fewer complex dopaminergic neurons, etc	Rosety et al.[116]
DNAJC6	DNAJC6 knockout	Pathological α-synuclein aggregation, increased neuronal firing, mitochondrial and lysosomal dysfunctions, and neurodevelopmental defects	Wulansari et al.[121]
PARKIN	PARKING knockout	Increased oxidative stress and enhanced dopaminergic neuron death upon differentiation	Ahfeldt et al.[123]
ATP13A2	ATP12A2 knockout	Elevated oxidative stress levels	Ahfeldt et al.[123]
DJ-1	DJ1 knockout	Elevated oxidative stress levels	Ahfeldt et al.[123]
	DJ1 knockout	AGE accumulation, increased α-synuclein phosphorylation, and protein aggregation	Morrone Parfitt et al.[124]

have relied on 2D cell culture systems and rodent models[78,79]. With the advent of human induced pluripotent stem cells (iPSCs) and 3D brain organoids, it is now possible to use patient-derived models to more faithfully reconstitute human brain-region specific features of the disease in vitro.

Up to now, there are four comprehensive studies about building SNCA mutant hMLOs using different cell resourses[80–83]. Mohamed et al.[81] use three-dimensional midbrain organoids differentiated from iPSCs derived from patients carrying a triplication of the SNCA gene and from CRISPR/Cas9 corrected isogenic control iPSCs. These human midbrain organoids recapitulate key features of α-synuclein pathology observed in the brains of patients with synucleinopathies. The SNCA triplication human midbrain organoids express elevated levels of α-synuclein and exhibit an age-dependent increase in α-synuclein aggregation, hinting that hMLOs carrying SNCA gene multiplication can reliably model key pathological features of PD and provide a powerful system to study the pathogenesis of synucleinopathies. Jo et al.[80] further generated and characterized hMLOs from GBA1−/− and SNCA overexpressing isogenic embryonic stem cells by gene-editing technic to investigate genotype-to-phenotype relationships in PD, with the particular aim of recapitulating α-syn-and Lewy body-related pathologies and the process of neurodegeneration in the hMLO model. They identified for the first time that the loss of glucocerebrosidase, coupled with wild-type α-syn overexpression, results in a substantial accumulation of detergent-resistant, β-sheet-rich α-syn aggregates and Lewy body-like inclusions in hMLOs. Becerra-Calixto et al.[82] showed that fPD-hMLOs from patients with SNCA triplication spontaneously accumulated pathological α-syn and exhibited significant neurodegeneration. Similarly, Muwanigwa et al.[83] found that 3xSNCA hMLOs from PD patients displayed elevated levels of α-syn, progressive loss of dopaminergic neurons, and a senescent-like phenotype in astrocytes.

LRRK2

Missense mutations in the leucine-rich repeat kinase 2 (LRRK2) gene locus are the most common known causes of late-onset familial and sporadic PD[84,85]. Previous studies have suggested that the LRRK2 G2019S gene mutation is associated with α-synuclein accumulation, mitochondrial dysfunction, and impaired dopamine signaling in the human brain, eventually resulting in the progressive loss of dopamine neurons[86–89]. However, a particularly difficult challenge in understanding the role of LRRK2 in PD research has been the generation of models that accurately recapitulate the LRRK2 mutant-associated disease state. For example, animals that harbor genetic mutations mimicking the familial forms of parkinsonism, including LRRK2 mutations, fail to show clear evidence of progressive midbrain dopamine neuron loss or Lewy body formation[90–93]. Another approach that has been taken to model PD is the use of patient-derived iPSCs directed to differentiate into dopamine neurons. These models also show variable dopamine neuron toxicity, but other features of PD pathology, such as Lewy body aggregates, are not as prominent as in the human brain[94], and such culture systems are generally immature[95].

The first two in-depth reports of PD modeling in hMLO focused on the effects of the LRRK2 G2019S mutation, which has been associated with both sporadic and familial forms of the disease due to its variable penetrance[96], and which constitutes the most common genetic risk factor for PD. To do so, the researchers relied on Crispr-Cas9 gene editing to either introduce the mutation in a control iPSC line[31], or to combine this with a correction in a mutant patient line[97]. Smits et al.[97] found that while the number of mDA progenitors (FOXA2+TH− cells) was significantly increased after 1 month of differentiation in LRRK2 vs. control hMLO, an apparent impairment of differentiation led to a reduction in the number and complexity of mDA neurons (FOXA2+TH+) after longer periods of culture (day 70). Interestingly, the increase in the number of progenitors was significantly higher in LRRK2 PD hMLO compared to those from controls with the knock-in mutation. This result thus highlights the importance of the genetic

background in the penetrance of the LRRK2 G2019S variant[96]. In line with these findings, Kim et al.[31] observed that while LRRK2 G2019S hMLOs were no different in size compared to controls, mDA neurite length and expression of mDA identity markers were decreased (such as TH, DAT, NURR1, PITX3, EN1) by day 60. The LRKK2 hMLO also contained higher levels of phosphorylated α-syn in endosomal compartments, and higher expression levels of markers of mitophagy and autophagy. The authors also identified TXNIP[98] as an important mediator of LRRK2-G2019S pathological mechanisms, and proved that knocking-down its expression reversed the accumulation of phosphorylated α-syn. Additionally, a study by Zagare et al.[99] explored the LRRK2 p.Gly2019Ser mutation within midbrain organoids, revealing through single-cell RNA sequencing that this mutation induces early neurodevelopmental alterations. Specifically, it affects differentiation patterns and reduces cellular variability, suggesting critical early-stage disruptions in Parkinson's disease progression.

PINK1

Autosomal recessively inherited mutations in the PINK1 gene typically cause early onset PD[100,101]. PINK1 has been implicated in the regulation of mitophagy, mitochondrial function and oxidative stress[102–108], however, the underlying mechanisms of PINK1-mediated PD are not fully understood. Pink1 knockout mice do not display reductions in DA neurons in the substantia nigra10. In contrast, PINK1 deficiency in zebrafish results in both reduced numbers of DA neurons in larval and adult zebrafish as well as impaired mitochondrial function and morphology[107].

Recent studies employing hMLOs have shed light on the functional roles of the PINK1 gene in Parkinson's disease. In the research conducted by Eldeeb et al.[109], iPSC-derived DA neurons and hMLOs were utilized to investigate the endogenous high-molecular-weight (HMW) PINK1 complex in physiologically relevant models. Their findings revealed that treatment with CCCP and ammonium chloride induced a PINK1- and Tom40-positive 720-kDa HMW complex in wild-type DA neurons and hMLOs, but not in PINK1 knockout models, underscoring the essential role of PINK1 in the assembly of this complex, which is crucial for mitochondrial functionality. Another study by Brown et al.[110] explored the impact of PINK1 deficiency on dopaminergic neurogenesis using isogenic midbrain-specific organoids derived from small molecule neural progenitor cells. This study found that PINK1-deficient organoids exhibited a significantly reduced growth rate and impaired differentiation of DA neurons, highlighting the gene's importance in neurodevelopmental processes related to PD. Together, these studies emphasize the utility of hMLOs in modeling PD at a cellular level, particularly in understanding how genetic variations such as those in PINK1 affect disease progression and neuronal functionality.

GBA1

Mutations in the GBA1 gene are widely recognized as one of the most significant genetic risk factors for PD, particularly associated with the early onset and more severe progression of the disease[111,112]. The GBA1 gene encodes the lysosomal enzyme GCase, which is crucial for the degradation of glycolipids. Deficiencies or dysfunctions in GCase due to GBA1 mutations result in the accumulation of glycolipid substrates, contributing to lysosomal dysfunction and α-synuclein aggregation, which are hallmark features of PD pathology[113,114].

Research utilizing iPSC-derived midbrain organoids has significantly advanced understanding of GBA1 mutations in Parkinson's disease. Baden et al.[115] developed organoids to study mitochondrial dysfunctions associated with GBA1 mutations, demonstrating reduced GCase activity and increased insoluble α-synuclein, which impacts dopamine neuron health. Meanwhile, Rosety et al.[116] focused on the neurodevelopmental effects of the N370S GBA mutation, revealing impaired neuronal differentiation and increased oxidative stress, alongside metabolic alterations predisposing cells to neurodegeneration. Collectively, these studies underscore the critical insights provided by midbrain organoids into the complex mechanisms of GBA1 mutations within disease-relevant models.

Other related genes

DNAJC6 encodes auxilin, which acts as a co-chaperone to recruit HSC70 to clathrin-coated vesicles for disassembly[117]. Homozygous loss-of-function mutations of DNAJC6 have been identified in familial juvenile parkinsonism[118–120]. However, the pathogenic mechanism for PD caused by DNAJC6 mutations remains unclear. Wulansari et al.[121] built hMLOs carrying DNAJC6 mutation with key PD pathologic features, i.e., mDA neuron degeneration, pathologic α-synuclein aggregation, increase of intrinsic neuronal firing frequency, and mitochondrial and lysosomal dysfunctions. In addition, neurodevelopmental defects were also manifested in hMLOs carrying the mutations. Transcriptomic analyses followed by experimental validation revealed that defects in DNAJC6-mediated endocytosis impair the WNT-LMX1A signal during the mDA neuron development. Furthermore, reduced LMX1A expression during development caused the generation of vulnerable mDA neurons with the pathologic manifestations. These results suggest that the human model of DNAJC6-PD recapitulates disease phenotypes and reveals mechanisms underlying disease pathology, providing a platform for assessing therapeutic interventions.

Mutations in the E3 ubiquitin ligase (PARKIN), the protein deglycase (DJ-1) and the presumptive cation-transporting ATPase 13A2 (ATP13A2) are inherited in an autosomal recessive fashion and cause completely penetrant early-onset PD in homozygous or compound heterozygous carriers[122]. It is unclear how dysregulation of these genes results in the relatively selective death of nigral dopaminergic neurons. To address this question, Ahfeldt et al.[123] used hMLO to study the roles of 3 severe PD-associated mutations mentioned above (in PARKIN/PRKN, DJ1/PARK7, and ATP13A2/PARK9) through genomic editing of a healthy control iPSC line. Increased levels of oxidative stress are found in all PD lines. Increased death of DNs upon differentiation was found only in the PARKIN knockout line. Using quantitative proteomics, they observed dysregulation of mitochondrial and lysosomal function in all of the lines, as well as common and distinct molecular defects caused by the different PD genes. Dysregulation of PD-relevant pathways indicate shared and distinct molecular signatures among the isogenic PD cell lines. Identified shared or specific dysregulated candidate genes may inform efforts to find therapeutic targets and to stratify PD pathology and patients on a molecular level. Besides, Parfitt et al.[124] focused on the role of DJ1 in an iPSC-derived midbrain organoid model deficient for DJ1 activity, which illustrated how disruption in lysosomal proteolysis led by astrocytes contributes to the accumulation of advanced glycation end products (AGEs) and increased α-syn phosphorylation. This study emphasized the importance of astrocytes in maintaining proteostasis within the neuronal environment, particularly highlighting their role in reversing proteolysis deficits in DJ1 knockout midbrain neurons. In another study[125], midbrain organoids were used as a tool to help researchers identify a unique subtype of neurons, characterized by the expression of the Parkinson's disease risk gene RIT2.

Construct hMLOs using PD causative exogenous stimuli

In general, the hMLO models of PD are mainly induced by iPSCs carrying PD-related mutations, with downstream experiments principally study the pathogenic molecular mechanism of mutation-related PD. However, similar to the establishment of animal model of PD, the induction of PD hMLOs can also use neurotoxins. Compared with gene-based methods, toxin-based method has its irreplaceable advantage: the mechanisms of these neurotoxic drugs in PD pathogenesis can be comprehensively studied. So far, two successful in-depth cases have been reported, use MPTP and 6-OHDA as exogenous neurotoxic stimuli, respectively[46,126].

To replicate the neuropathology of PD, many previous studies had used animal models with a variety of dopaminergic toxins[127]. Among them, MPTP is known to be the most reliable and frequently used toxin due to its ability to stably induce clinical symptoms that are indistinguishable from PD[128,129]. After crossing the blood-brain barrier, MPTP is converted first into 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP) by monoamine oxidase B (MAO-B) and then into 1-methyl-4-phenylpyridinium (MPP+) in astrocytes[129]. Though astrocytes play a key role in the mode of action of

MPTP[129,130], MPTP, a representative and reliable dopaminergic neurotoxin, has not been properly used in previous iPSC-based in vitro modeling studies of PD. Kwak et al.[46] generated a special type of hMLO named DAC3.0 MOs which contain a large number of glial cells, such as astrocyte. MPTP-treated DAC3.0 Mos exhibited massive cell death, and the number of apoptotic cells increased in a dose-dependent manner, indicating that the cell death induced in DAC3.0 MOs is mediated solely by MPTP treatment. Notably, MPTP-mediated cell death is observed largely in TH-positive mDA neurons but rarely in other cellular components (GABAergic neurons, astrocytes, and oligodendrocytes etc.) Taking the information together, their data indicates that DAC3.0 MOs produced by their modified protocol contain functional glial cells that facilitate the action of MPTP, a representative dopaminergic neurotoxin, which could be used for the in vitro modeling of PD.

Another similar research is done by Monzel et al.[126]. They developed a neurotoxin-induced PD organoid model by neurotoxic compound 6-OHDA treatment. Cell quantification by flow cytometry revealed that exposure to 6-OHDA caused a concentration-dependent reduction in the amount of living DANs. 6-OHDA treatment leads to a decrease in the amount of TH+ cells and to neurite fragmentation, in other words, it led to a decrease in the complexity of DANs and increase of fragmented neurites. Results showed that it is a valuable tool for advanced in vitro PD modeling.

Using hMLOs for appropriate PD toxicity predictions

Approximately 90% of cases are of sporadic and idiopathic origin[131,132], and many studies hypothesize that genetic and environmental factors, including exposure to toxicants, may play a role in the long-term etiology of PD[133–135]. However, Parkinsonism in humans can also have more direct causes, where specific compounds can selectively and acutely ablate dopaminergic neurons. For example, MPTP, a toxic byproduct of drug synthesis, caused PD-like symptoms in illicit drug users that had unintentionally exposed themselves to the compound[136,137]. Following the discovery of MPTP's effects, similar specific toxicities were identified for other compounds, including the common pesticides rotenone and paraquat[138,139]. Furthermore, many therapeutic drugs are known to cause drug-induced Parkinsonism (DIP) in patients (the second most common form of Parkinsonism in aged patients after PD)[139–141]. While DIP is often reversible as it is mostly caused by the blockage of the dopamine receptor at the post-synapse[141], some drugs, including the antipsychotic haloperidol, may also have additional neurotoxic effects and thus directly damage dopaminergic neurons[142–144]. Human data on the molecular details of these side effects are scarce due to limited access to tissue samples. Toxicity testing is a crucial step in the development and approval of chemical compounds for human contact and consumption. However, existing model systems often fall short in their prediction of human toxicity in vivo because they may not sufficiently recapitulate human physiology. The complexity of 3D human organ-like cell culture systems ("organoids") can generate potentially more relevant models of human physiology and disease, including toxicity predictions. As previously described in section "Construct hMLOs using PD causative exogenous stimuli", neurotoxic drugs, including MPTP and 6-OHDA, can be used for the construction of hMLOs with PD phenotypes. Compared with the mainstream gene-based models, toxin-based models can be further used as an ideal platform for the further study of PD pathogenesis caused by these neurotoxins. Followed by advanced technics such as microscopy-based phenotyping in a high-content fashion, neurotoxic effect on dopaminergic neurons can be assessed[123].

Oxidative stress is a crucial causative factor for PD progression[145], which is caused by an imbalance between the generation and detoxification of reactive oxygen and nitrogen species (ROS/RNS). This imbalance plays an important role in brain aging and age-related neurodegenerative diseases. In the context of PD, the sensitivity of dopaminergic neurons in the substantia nigra pars compacta to oxidative stress is considered a key factor of PD pathogenesis. David et al.[146] studied the effect of different oxidative stress-inducing compounds (6-OHDA, MPTP or MPP+) on the population of dopaminergic neurons in an iPSC-derived hMLO model. Treatment with 6-OHDA, MPTP or MPP+ at 4 weeks of differentiation disrupted the dopaminergic neuronal phenotype in hMLOs. 6-OHDA increased ROS production and decreased mitochondrial function most efficiently. It further induced the greatest changes in gene expression and metabolites related to oxidative stress and mitochondrial dysfunction. Co-culturing hMLOs with an endothelial barrier using a transwell system allowed the assessment of differential penetration capacities of the tested compounds and the damage they caused in the dopaminergic neurons within the hMLOs. In conclusion, treatment with compounds known to induce PD-like phenotypes in vivo caused molecular deficits and loss of dopaminergic neurons in the hMLOs model. This approach therefore recapitulates common animal models of neurodegenerative processes in PD at similarly high doses.

So far, the inherent biological heterogeneity and cumbersome generation and analysis of organoids has rendered efficient, unbiased, high throughput evaluation of toxic effects in these systems challenging. Recent advances in both standardization and quantitative fluorescence imaging enabled researchers to dissect the toxicities of compound exposure to separate cellular subpopulations within human organoids at the single-cell level in a framework that is compatible with high throughput approaches[147,148]. Renner et al.[147] screened a library of 84 compounds in standardized human automated midbrain organoids (AMOs) generated from two independent cell lines correctly recognized known nigrostriatal toxicants. This approach further identified the flame retardant 3,3',5,5'-tetrabromobisphenol A (TBBPA) as a selective toxicant for dopaminergic neurons in the context of human midbrain-like tissues for the first time. Further, 3D AMOs demonstrate higher sensitivity in than in 2D cultures to the known neurotoxic effects of the pesticide lindane, which proved the feasibility of quantitatively assessing cell-type-specific toxicity in human organoids in vitro.

Using hMLOs for appropriate PD treatment. At present, the mainstream treatment of PD is drug therapy such as levodopa and surgeries like deep brain stimulation[149–151]. These treatment options were designed to relieve the symptoms of PD or mitigate side effects of antiparkinsonian drugs rather than intervene in the underlying disease process. To achieve meaningful progress in halting or delaying the progression of cell loss in PD, a greater understanding of the disease process is needed. As an optimized in vitro model, the appearance of hMLOs may provide a new perspective for PD treatment[152]. We have compiled in Table 2 the recent advancements in using hMLOs for the therapeutic applications targeting PD.

PD treatment using neural stem cells derived from hMLOs

Recent studies have demonstrated that stem cell-derived mDA neurons or mDA progenitors could indeed functionally integrate into striatonigral circuits[153], and provide some symptomatic relief in a non-human primate model of PD without forming tumors[154]. The extent of recovery in the neurologic score and spontaneous movement of PD monkeys grafted with iPSC-derived cells was similar or lower than those produced by L-DOPA drug administration[154]. The outcome of PD clinical trials cannot be predicted with the information currently available, but studies to improve cell therapeutic efficacy and safety should not be abandoned but continued until a complete success in the clinical transition is reached. Therefore, as organoids have been shown to efficiently integrate into rodent neural circuits after transplantation[155], using dopamine-producing hMLOs may prove to be a useful development for therapeutic purposes. In this regard, the following issues in donor cell preparation need to be further addressed for a successful clinical translation of PD cell therapy, which are, the high expression of midbrain factors, the existence of astrocyte differentiation, and the stability and reproducibility of hMLOs.

Successful clinical translation of stem cell-based therapy largely relies on the scalable and reproducible preparation of donor cells with potent therapeutic capacities. Kim et al.[55] use hMLOs to prepare cells for PD therapy. In this study, neural stem cells (NSCs) were isolated from midbrain organoids (Og-NSCs), which expanded stably and differentiated into mDA

Table 2 | Applications of human midbrain-like organoids in toxicity predictions and treatment approaches for Parkinson's disease

Application	Reference	Key Findings
Toxicity predictions	David et al.[42]	6-OHDA, MPTP, and MPP+ significantly disrupted the dopaminergic neuronal phenotype, with 6-OHDA most effectively increasing ROS production and reducing mitochondrial function, revealing differential penetration capabilities of the compounds and their specific damage to neurons.
	Renner et al.[147]	Standardized human automated midbrain organoids (AMOs) effectively identified known nigrostriatal toxicants and revealed 3,3',5,5'-tetrabromobisphenol A (TBBPA) as a selective toxicant for dopaminergic neurons. The method demonstrated higher sensitivity in 3D AMOs compared to 2D cultures for detecting neurotoxic effects, such as those from the pesticide lindane, with high reproducibility in dose-response experiments.
Treatment	Kim et al.[55]	Midbrain organoids from human pluripotent stem cells produced stable neural stem cells that differentiated into functional, midbrain-type dopamine neurons with minimal variability and enhanced resistance to stress. Transplanted cells showed significant therapeutic effects in Parkinson's disease models, supporting their potential for clinical applications.
	Zheng et al.[159]	The hMOs derived from induced pluripotent stem cells, transplanted at Day 15, demonstrated high survival and maturation rates of dopaminergic neurons in a Parkinson's disease mouse model. These neurons effectively restored motor functions and formed bidirectional connections without tumor formation, showcasing hMOs as a promising and safe cell therapy for Parkinson's disease.
Drug Selection	Kim et al.[31]	Isogenic 3D midbrain organoids with a Parkinson's disease-associated LRRK2 G2019S mutation successfully mimicked the pathological features of sporadic Parkinson's disease. Protein analysis revealed the critical role of TXNIP in LRRK2-related disease mechanisms, validating the utility of 3D organoids in Parkinson's disease research and therapeutic development.
	Jarazo et al.[160]	In PD patient-derived midbrain organoids, CRISPR/Cas9 correction of PINK1 mutations enhanced neuronal function and differentiation. Treatment with 2-hydroxypropyl-β-cyclodextrin improved autophagy and neuroprotection in these organoids, highlighting its therapeutic potential for Parkinson's disease.
	Kim et al.[161]	The optogenetics-assisted system OASIS effectively induced α-synuclein aggregates in PD iPSC-derived midbrain organoids, identifying BAG956 as effective in reversing PD phenotypes by promoting autophagic clearance. OASIS provides a viable animal-free model for drug development.
	Shin et al.[162]	A biohybrid robot-on-a-chip featuring a human motor system with brain organoids, motor neuron spheroids, and muscle bundles effectively evaluates drug impacts on Parkinson's disease. This model demonstrated increased muscle movement in response to levodopa, validating its potential as a standard model for drug testing in neurodegenerative diseases.

neurons. Unprecedentedly high proportion of cells expressed midbrain-specific factors, with relatively low cell line and batch-to-batch variations. Single cell transcriptome analysis followed by in vitro assays indicated that the majority of cells in the Og-NSC cultures are ventral midbrain (VM)-patterned with low levels of cellular senescence and mitochondrial stress, compared to those derived from 2D-culture environments. Notably, compared to traditional hMLO that aims to mimic the whole midbrain area, the VM- patterned hMLOs are prone to produce more A9 DA neurons, which is essential in PD development molecular profile[55,156–158]. Moreover, in contrast to current methods yielding mDA neurons without astrocyte differentiation, mDA neurons that differentiated from Og-NSCs were interspersed with astrocytes as in the physiologic brain environment. Thus, the Og-NSC-derived mDA neurons exhibited improved synaptic maturity, functionality, resistance to toxic insults, and faithful expressions of the midbrain-specific factors. They further transplant Og-NSCs into 6-OHDA-lesioned hemi-parkinsonian rats to testify their therapeutic effect, the results showed reproducible behavioral restoration and mDA neuron engraftment in PD animals transplanted with Og-NSCs. Though this experiment broadens our horizon in PD treatment using hMLO-derived NSCs, only the H9 cell line is used for PD treatment, for a wide range of clinical utility of the Og-NSC method, more conclusive results should be verified in a variety of hESC/iPSC lines. Zheng et al.[159] explored the potential of hMLOs derived from iPSC for treating PD. Their study found that hMLOs, particularly at Day 15 of differentiation, are effective in replenishing dopaminergic neurons when transplanted into PD mouse models. Post-transplantation, these cells demonstrated survival, maturation, and integration into the host brain, effectively restoring motor functions and establishing bidirectional connections with natural brain targets, without tumor formation.

Using hMLOs for PD drug selection

The road to discover novel therapeutics for neurological disorders including PD has been severely hampered by the lack of access to relevant testing platforms. One of the reasons responsible for this low success rate is that conventional 2D cell culture models are not accurate enough predictors of how drugs will work in humans. 3D brain organoids differentiated from iPSCs to resemble specific parts of the human brain, which include architecture composition and physiology, can provide an alternative system that may lead to breakthroughs in key areas of drug testing and toxicological evaluation. Having reliable and scalable iPSC-derived brain organoid models that can much more accurately predict human drug responses will significantly increase success rate in developing treatments for brain-related disorders. In this background, organoids constitute a relevant platform to identify novel therapeutic compounds and to assess their efficacy on specific phenotypes. For example, Kim et al.[31] showed that α-synuclein accumulation could be reduced in LRRK2 G2019S hMLO through treatment with a LRRK2 kinase activity inhibitor (GSK2578215A), but also by knocking down the expression of TXNIP, which their study had identified as a central mediator of G2019S pathology. Jarazo et al.[160] also found that treatment with the HP-ß-CD compound improved mDA neuronal differentiation in PINK1 and PRKN-mutated hMLO, likely through increased mitophagy. Besides, Kim et al.[161] developed an optogenetics-assisted system, OASIS, which rapidly induces α-synuclein aggregates in PD iPSC-derived midbrain neurons and organoids. This method identified BAG956, a compound that effectively reverses PD phenotypes by enhancing autophagic clearance of pathological α-syn aggregates. Recently, Shin et al.[162] introduced a novel biohybrid robot-on-a-chip incorporating a brain organoid, motor neuron spheroids, and muscle bundle for evaluating drug effects on neurodegenerative diseases like PD. This system uniquely integrates a patient-derived midbrain organoid to measure muscle movement, demonstrating significant improvement in muscle bundle movement in response to levodopa. This model overcomes previous limitations by successfully mimicking human motor system functions, providing a promising tool for PD drug evaluation.

Up to now, the two main approaches for drug discovery are target-based screenings, where the modulation of the activity of a previously identified druggable target is used as readout, and phenotypic screenings, in which the rescue of a phenotype in a disease model (cells, tissue, animal etc.) serves as readout[163,164]. In the past four decades, the majority of investment for drug discovery was biased towards target-based screenings[164]. These highly standardized large-scale assays can be performed in classical cell lines

or even with isolated proteins in vitro. However, despite increasing investment, drug discovery has a high failure rate especially when it comes to neurodegenerative diseases (NDD)[165]. In fact, there are still no disease modifying drugs approved for the major NDD, Alzheimer's disease (AD) and PD[165]. In the last 20 years only 5 first-in-class small molecule drugs for NDD were approved, of which none was discovered in target-based screenings but in phenotypic screenings[166]. Multifactorial diseases like PD are only poorly represented in a screening focusing on a single target molecule, as the "one-drug-one-target" approach does not take into account the complexity of such diseases[167]. Patient-derived hMLO models become an increasingly powerful tool to model human diseases for precision medicine approaches. The identification of robust cellular disease phenotypes in these models paved the way towards high throughput screenings (HTS) including the implementation of laboratory advanced automation. Recently, researchers describe an integrated, complex automated platform for HTS in translational research setting[168]. The comprehensive integrated automated platform can be used for designed to maintain and expand different cell types (adherent and suspension cells), differentiate iPSC-based hMLOs and to perform high throughput and HTS. This allows for produce sufficient cells for HTS/HCS purposes and can be adapted to meet special requirements of other cell types enabling novel precision medicine approaches across diseases. However, due to the needs of high-throughput, high content and small variations between batches during HST, there is a long way to go for adequate combination of these two prevalence techniques[169–171].

Discussion

Parkinson's disease, by its progressive and irreversible nature, has been challenging for clinicians to diagnose and prognosticate. Reliable markers of early disease have yet to be identified and brain tissue biopsies rarely provide enough benefits to outweigh the risks of the procedure[16]. Besides, there are still no disease-modifying drugs available to prevent or reverse these neurodegenerative phenomena. One of the existing barriers to potential therapy development is the remarkably unclear understood nature of disease development[142]. Similarly, scalability and ethical barriers limit the high-throughput drug-testing potential of the established animal models. The emergence of iPSCs technology brought light to mimicking the age-dependent neurodegenerative diseases such as PD, AD, and amyotrophic lateral sclerosis (ALS). Researchers have been exploring the potential of iPSC-derived cells for disease modeling, drug screening, and cell replacement therapy in these disorders. The advent of organoids has elevated the application of iPSC-based technology to new heights. It presents a novel model for PD simulation, disease progression prediction, drug screening, and therapeutic interventions[55].

Despite significant progress in modeling neurodegenerative diseases such as PD with brain organoids derived from stem cells, challenges remain in fully replicating the regional specificity and functionality of the human brain. A notable challenge is the inadequacy of vascularization, which affects not just the traditional role of vessels in supplying nutrients and oxygen, but also key aspects of disease modeling[172]. Vascularization is crucial for supporting the survival of cells at the center of organoids, preventing apoptosis due to insufficient nutrients and oxygen, and allowing the organoids to grow to a larger size, which is necessary for long-term studies of PD progression and intervention measures. Babu et al.[173] provided a detailed overview of the relevant research progress. Moreover, the integrity of the blood-brain barrier (BBB) is compromised in PD[174], and its effective simulation is essential for understanding its pathological role in PD, such as changes during neuroinflammation and neurodegenerative processes[175], and for assessing disease impact and treatment responses. Therefore, a realistic reproduction of the BBB in organoid models is critically important for in-depth study of these processes. Lastly, simulating the mechanism of drug delivery through the BBB is crucial for PD treatment strategies. The vascularization of organoid models provides a new method for studying how to improve the efficiency of drug delivery and assess the penetration capabilities of new drugs, especially considering the unique permeability characteristics of the BBB in PD patients[176]. To address the issue of vascularization in brain organoids, scientists have adopted a variety of strategies. These strategies include the use of co-culturing techniques with cells, the introduction of specific growth factors, and the development of new bioengineering methods aimed at enhancing the capability and functionality of vascular formation in organoid models[155,177,178]. Dao et al.[179] successfully developed the world's first human mini-brain that includes a fully functional BBB. These assembloids effectively recapitulated the complex anatomy and BBB breakdown seen in patients with cerebral cavernous malformations (CCMs), providing insights into disease mechanisms and potential therapeutic targets. Addressing these challenges not only enhances the biological relevance and research efficiency of organoid models but also advances the simulation and treatment research of complex neurological diseases like PD. Another issue should be taken into account is the lack of immune cell recruitment[95,180]. As immune cells participate in many neurodegenerative diseases, the role of immune cells should never be ignored. Future protocols should aim to that allow the growth of immune cells, especially microglia, in existing organoids[181]. As they regulate synapse formation and assist in the generation and maintenance of neuronal circuits in the human brain[182]. Due to lack of immune cells and vasculature, the ability of brain organoids to replicate the human brain remains less. Therefore, the recruitment of immune cells and vascular networks within organoids will expand the applicability of these systems to study more complex phenomena such as defined cell-cell interactions, circuit functionality, and later developmental events such as myelination and plasticity[183]. Recent study has reported a method for integrating microglia into iPSC-derived midbrain organoids, this midbrain-microglia assembloids shed light on the new model for neuroinflammation in Parkinson's disease[184]. Furthermore, although the advantages of 3D organoids over traditional 2D culture are obvious as mentioned above, the 2D approach has its own gains. The heterogeneity of 2D cultured cells between different batches was relatively low, with high reproducibility. Besides, 2D environment is quite convenient to use regarding the culture condition requirements, cell growth potential, flexible intervention, and possibility of close microscopic observation[185]. Moreover, 3D culture is not superior to 2D culture in all condition. Given the higher interstitial edema and tissue necrosis, which are still common in conventional induction methods, it has been reported that 3D culturation may not perform as well as that in 2D conditions[186].

In the long run, the application of hMLOs should be further explored in vary aspects, including the construction of muti-area brain organoids, the direct transplantation of organoids and the application of personalized medication. (1) The construction of area-fused brain organoids. The human central nervous system (CNS) develops from several distinct vesicles into multiple intertwined regions. During this process, a range of migratory streams arise where progenitors generated in one place migrate and integrate into other areas[187–190], and complex networks emerge, neurons branching and projecting across multiple regions[191]. Yet convoluted, the mechanism underpinning the formation of the human CNS is a highly ordered process that needs to be understood. Many diseases are the result of barriers of projection, migration or neuronal interaction between different brain regions. Although some organizing centers were observed in brain organoids[192], which are critical for regional brain patterning, most of the spatial identities in organoids appeared in an uncontrolled manner, thereby limiting the study of complex interregional interactions. Application of patterning factors and/or chemicals allows us to pattern brain organoids into different brain regions[193–196], which provides researchers "LEGO blocks" to establish fused organoid approaches. The development of fused organoids, also called assembloids[197], opens a new avenue to investigate interregional dynamics in the embryonic brain. Fusing two brain organoids

pre-patterned into different regional identities enables the study of inter-regional interactions. Organoids made by the fusion approach can help to deconstruct organogenesis by reconstructing the brain piece by piece. The development of PD is the result of abnormal projection from substantia nigra into the striatum, the application of fusion organoids can help to fully understand the pathogenesis of PD in closed neural circuits. (2) The direct transplantation of hMLOs. In a recent study, an in vivo model of vascularized human brain organoids was developed by transplanting organoids into the superficial cortex of mice[155]. Although widespread axonal extension outside the graft area was observed, region-specific long projections were not reported. Previously, reported methods produced cerebral organoids containing multiple lumens or neural tubes[37,198], which makes them difficult to use for transplantation therapy. In addition, large organoids might cause more damage to recipients than small organoids if they are transplanted in deep areas of the brain. It is possible that small brain organoids can alleviate these safety concerns and are more amenable to injection into deep brain regions. Liu et al. optimized a culturing protocol capable of efficiently generating small human cerebral organoids[199]. After transplantation into the mouse medial prefrontal cortex, the grafted human cerebral organoids survived and extended long projections to basal brain regions. Importantly, the grafted human cerebral organoids functionally integrated into pre-existing neural circuits by forming bidirectional synaptic connections with the mouse host neurons. Besides, mouse received the grafted organoids present with potentiation of the startle fear response. This study showed that subcortical projections can be established by microtransplantation and may provide crucial insights into the therapeutic potential of hMLOs for PD treatment. Recent studies also report homotopic transplantation of 2D-drived neuron into PD rat brain combined with AAV-mediate GNDF injection, the grafted mDA in SN showed a better connection with host striatum than the traditional ectopic method[200]. Together with a study showing that displaced VM-progenitors with α-synuclein triplication into the brain of PD rats can aggravate the disease phenotype[201], these studies hints that direct homotopic transplantation of hMLOs may be a promising way, which can be used to explore the pathogenicity of PD by transplanting mutation-specific organoids into normal rodent models or the therapeutic effect of by grafting healthy organoids into PD-phenotypic ones. (3) In recent years, other emerging technologies such as snRNA-seq[202,203], spatial transcriptomics[203], 3D bioprinting[204], preformed fibrils[205], and fetal brain in vitro self-organization into organoids[206] have been incorporated into research on brain-like organs, offering promising avenues for further exploration in understanding PD. These innovative techniques open up new dimensions for studying PD and hold significant potential for advancement in this field.

This paper reviews the development of hMLOs since their first appearance and systematically describes their applications in PD model construction, toxicity prediction and treatment so far. Until now, there are still some defects in the induction methods, such as lack of vascularization and immunity. Meanwhile, the application of hMLOs also needs to be further explored. The construction of complex organoids with multiple brain regions and the direct transplantation of small organoids may be a promising development direction in the future. With the continuous in-depth exploration of hMLOs, researchers have made thrilling steps in their methodology and application. For the foreseeable future, hMLOs may play an increasingly important role in the mechanism research and treatment of PD.

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Author contributions

Xin Cui, Xinwei Li, Huimin Zheng and Yun Su drafted and revised of the manuscript for content, including medical writing for the content. Changhe Shi, Shuyu Zhang, Mengjie Li, Xiaoyan Hao, Shuo Zhang, Zhengwei Hu, Zongping Xia and Changhe Shi assembled and edited the figures and tables. Chengyuan Mao and Yuming Xu conceived the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Yuming Xu or Chengyuan Mao.

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