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Bridging the gap in Parkinson's disease research: from general pathophysiology to advanced 3d cell culture models

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder, having a substantial negative impact on the quality of life of patients with its wide range of motor and non-motor symptoms. PD has a complex etiology involving aging, genetic, and environmental factors. The hallmark of the pathology in PD is the accumulation of misfolded α-synuclein, a presynaptic vesicle-associated protein, in dopaminergic neurons, causing neuronal death in the substantia nigra pars compacta (SNpc) and other brain regions. This review focuses on the main pathophysiological mechanisms of PD and in vitro cell culture models. Conventional 2-dimensional (2D) systems can not properly mimic living tissue and reflect disease pathology. On the other hand, 3-dimensional (3D) cell culture models bring an innovative perspective to PD research because they offer tissue-like 3D environments that support the functioning and viability of cells. The potential use of spheroids and organoid systems, 3D bio-printing, microfluidic systems, and organ-on-chip models is discussed in comparison with traditional approaches using 2D. These methods have great potential for more realistic simulation of the dynamics of the disease, allowing the investigation of therapeutic molecules and targets. This review synthesizes current knowledge on PD mechanisms and 3D in vitro models, and explains why 3D offers advantages over 2D for studying disease and testing therapies.

Keywords Parkinson's disease, Pathophysiology of PD, 3D cell culture, Co-culture, PD research

Background

Parkinson's disease (PD) is a progressive neurodegenerative disorder that involves both motor and non-motor symptoms. While bradykinesia, rigidity, and resting tremors are key diagnostic motor features, numerous well-documented non-motor symptoms can appear over ten years before the onset of the motor symptoms, offering essential insights into the disease's development and prognosis [1]. The onset of PD generally occurs at about the age of 60, with its global prevalence rising sharply from approximately 0.3% to over 3% in individuals over 80 [2]. The global burden of the disease has been reported as 6.1 million cases of PD in 2016 and 1.02 million new

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cases of PD in 2017 [3]. According to published prevalence studies, the number of cases is estimated to reach approximately 9 million individuals by 2030 in five Western European and ten of the world's most populous countries [4]. Although aging is the predominant risk factor for PD, genetic mutations, environmental toxins, and lifestyle contribute to its complex pathogenesis.

Since the identification of the first causative gene, *SNCA*, in Parkinson's disease in 1997, genetic studies have revealed several other causative genes and risk variants explaining the underlying mechanisms of PD [5]. Neuropathologically, PD features loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and Lewy bodies—intracytoplasmic inclusions composed primarily of misfolded α-synuclein—in surviving neurons. Based on the origin of alpha-synuclein accumulation and the progression pattern of pathology, two Parkinson's disease subtypes, "body-first" and "brain-first," have been proposed, each exhibiting distinct clinical phenotypes, particularly during the prodromal phase [6]. Beyond dopaminergic loss, cholinergic, serotonergic, and noradrenergic systems are also affected, broadening the symptom spectrum [7]. Several co-pathologies are observed in brain cells, contributing to the complexity of the disease and its diverse clinical manifestations. These include Aβ plaques, tau abnormalities, TDP-43 inclusions, neuroinflammation, small-vessel changes, and mitochondrial dysfunction [8].

Modeling helps dissect PD biology at the molecular and cellular levels and supports target discovery and therapy testing. Approaches include in vivo animal models, in vitro 2D culture, and newer 3D systems. Animal models can reproduce dopaminergic neuron loss, motor deficits, and some non-motor features through genetic or toxin paradigms [9], but they do not fully reproduce human physiology or the progressive course of PD and have limited predictive value for clinical translation. Moreover, the use of animal models is costly, ethically controversial, and time-consuming [10]. Conventional 2D culture is simple and low-cost, but it lacks the tissue context; cell–matrix and cell–cell interactions are limited. 3D culture systems, including spheroids, organoids, bioprinted constructs, and microfluidic chips, more closely mimic native microenvironments and tissue organization than 2D monolayers [11]. They enable the study of protein aggregation, neuronal loss, and neuroinflammation over time. Furthermore, patient-specific treatment strategies and pathways can be studied with patient-derived cell models and potentially be used in translational research. These platforms support screening by enabling more physiologically relevant, richer, and longer-term readouts at the cellular level [12].

This review synthesizes PD pathophysiology and compares in vitro models, with emphasis on how 3D platforms differ from conventional 2D culture. We assess how well each approach recapitulates key PD processes, including α-synuclein aggregation, mitochondrial dysfunction, and neuroinflammation, and we outline strengths, limitations, and use cases. The 3D systems covered include spheroids, organoids, 3D bioprinting, microfluidics, and organ-on-chip technologies, with attention to their translational value in patient-derived systems and screening.

Pathophysiology of parkinson's disease

The hallmarks of Parkinson's disease include loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) with striatal dopamine depletion, and the presence of Lewy bodies in surviving nigral neurons (intracellular inclusions enriched in misfolded α-synuclein) [13]. The upstream triggers of α-synuclein misfolding and accumulation remain unclear; genetic variants and post-translational modifications can promote aggregation and overload cellular clearance pathways such as the ubiquitin-proteasome system and autophagy (Fig. 1). Mitochondrial dysfunction, neuroinflammation, and oxidative stress also contribute to PD pathogenesis [14].

Dopamine (DA) plays a vital role in modulating movement. It also shapes reward, behavior, cognition, attention, memory, and sleep [15]. The motor circuit includes corticostriatal projections from motor-related cortical areas to striatal medium spiny neurons. A hyperdirect pathway provides direct glutamatergic input from the motor cortex to the subthalamic nucleus. The basal ganglia output nuclei, the globus pallidus internus (GPi) and substantia nigra pars reticulata (SNr), project to the brainstem and ventrolateral thalamus. In the direct pathway, D1 receptor–expressing medium spiny neurons inhibit GPi/SNr, disinhibiting the thalamus and facilitating movement. In the indirect pathway, D2 receptor–expressing neurons inhibit the globus pallidus externus (GPe), which disinhibits the subthalamic nucleus (STN); STN then excites GPi/SNr, increasing thalamic inhibition and suppressing movement. Dopamine from the substantia nigra pars compacta (SNpc) excites D1 MSNs and inhibits D2 MSNs, balancing these pathways; dopamine loss shifts activity toward the indirect pathway and impairs movement, producing Parkinsonian symptoms [2].

Misfolded protein aggregation

Alpha-synuclein

α-synuclein (α-synuclein) is a 140 amino acid presynaptic protein that helps regulate neurotransmitter release, SNARE complex assembly, and synaptic-vesicle homeostasis [16]. In PD, an imbalance between the production and degradation of this protein leads to its aggregation and the formation of intraneuronal inclusion bodies

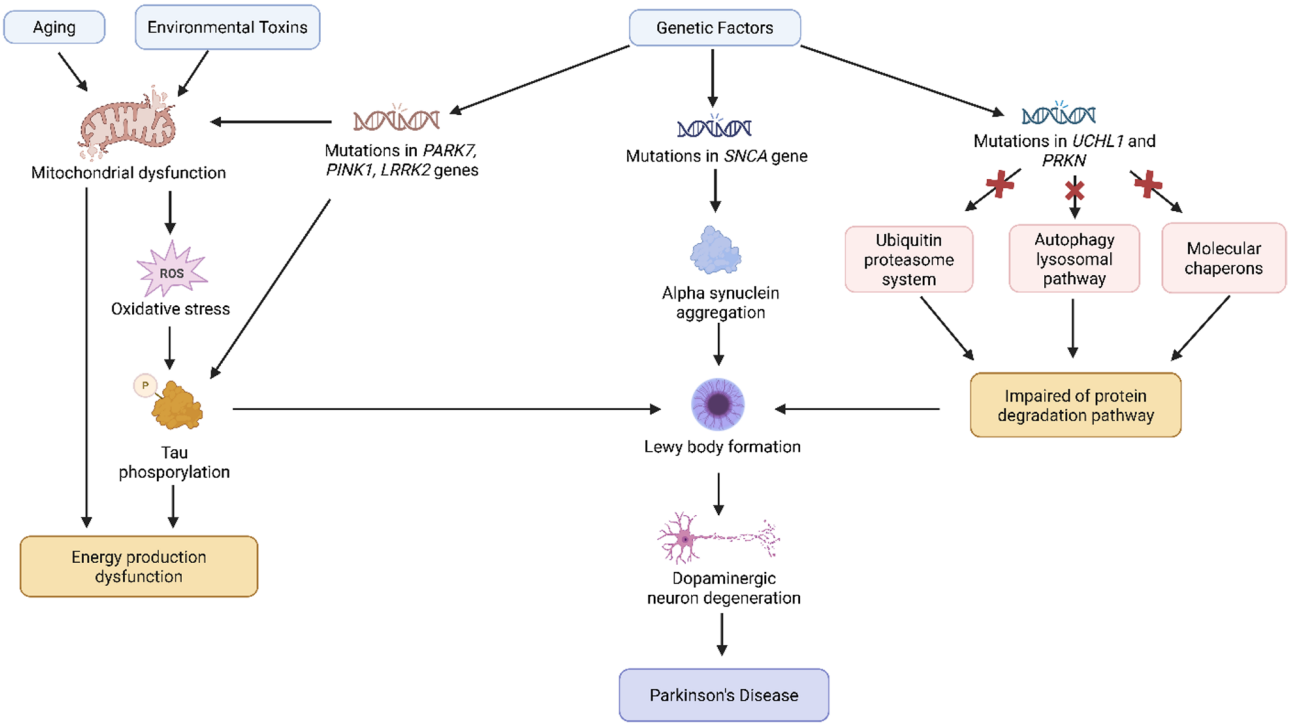


Fig. 1 Schematic of Parkinson's disease heterogeneity showing contributing factors and pathways. Aging and environmental toxins promote mitochondrial dysfunction and increase reactive oxygen species (ROS). Elevated ROS, together with mutations in PARK7, PINK1, and LRRK2, are associated with impaired energy production and tau phosphorylation. Mutations in UCHL1, SNCA, and PRKN promote toxic protein aggregation and Lewy body formation [42]. Image created using BioRender(2024)

called Lewy Bodies (LBs) (Fig. 1). LB and Lewy neurites, as defined by Frederic Lewy, consist of α-synuclein, which form filaments that trap organelles such as mitochondria and lysosomes [17]. Pathologically, α-synuclein aggregates appear in the neuron cytoplasm before accumulating as diffuse pale bodies. After this association, these accumulations act as seeds, contributing to the aggregation of diffuse α-synuclein into filaments that form Lewy Bodies [18]. This accumulation is a key pathological feature observed in the brains of individuals with PD [19]. It was discovered by Ganjam et al. that overexpression of both wild-type and mitochondria-targeted α-synuclein causes mitochondrial membrane potential loss, an increase in mitochondrial reactive species, and cell death in differentiated dopaminergic neurons [20].

The autophagy-lysosome pathway (ALP) and ubiquitin-proteasome system (UPS) are two complementary proteolytic mechanisms responsible for intercellular protein quality control. While the UPS primarily degrades short-lived and misfolded proteins through ubiquitination by E3 Ub ligases, the ALP clears long-lived proteins, protein aggregates, and damaged organelles [21]. Failure in protein quality control mechanisms is associated with several neurodegenerative diseases, including Alzheimer's Disease (AD), Huntington's Disease, and Parkinson's disease (PD).

Mutations in *SNCA* (e.g., A30P, A53T) or overexpression of wild-type α-synuclein impair lysosomal function, leading to elevated intralysosomal pH, reduced lysosome numbers, and altered lysosome/ER calcium signaling, which is essential for autophagy activation and α-synuclein clearance [22]. It has been reported that overexpression of α-synuclein causes early catalytic dysfunction of the 26 S proteasome, leading to dysfunction of the ubiquitin-proteasome system. In dopaminergic neurons, this dysfunction corresponds to the selective accumulation of α-synuclein that is phosphorylated on serine 129, a pathogenic modification linked to neurotoxicity [23].

Misfolded α-synuclein oligomers and fibrils cause neuroinflammation by disrupting cell membranes, contributing to oxidative stress, mitochondrial dysfunction, and neuronal death [24]. A single intracerebroventricular injection of α-synuclein oligomers in mice reduced tyrosine hydroxylase and dopamine levels while also causing motor and non-motor impairments within 45 days [25]. Such toxicity might be linked to the structural properties of oligomers: an exposed hydrophobic N-terminal region that strongly binds to lipid bilayers and a β-sheet-rich oligomeric core that disrupts membrane integrity [26].

On the other hand, the A53T mutation linked to the familial form of PD is known to cause severe non-motor

symptoms such as anxiety, gastrointestinal problems, anosmia, and motor symptoms [27]. In the transgenic mouse model overexpressing A53T-human-α-synuclein, dopaminergic neuron degeneration and mitochondrial inclusions have been reported [28]. Mutant A53T-α-synuclein forms oligomers more rapidly than WT-α-synuclein, causing ROS production and impaired mitochondrial trafficking [29]. Kilpeläinen and colleagues showed that the double A53T and A30P mutation results in behavioral deficits in seven-month-old mice. After 12 months, mice showed dopaminergic deficiency and reduced extracellular dopaminergic markers in the SN and striatum, accompanied by elevated levels of α-synuclein oligomers [30].

Accumulating data suggest that α-synuclein can be released from neurons and taken up by neighboring cells, thereby spreading pathology among interconnected populations of neurons. This process occurs, at least in part, through calcium-dependent secretion in extracellular vesicles, and conditioned media containing secreted α-synuclein have been shown to induce neurodegeneration in recipient cells [31]. The transmissible α-synuclein pathology concept is further supported by long-term follow-up of fetal mesencephalic grafts in PD patients. In these patients, a significant proportion of grafted neurons have lost dopaminergic markers such as tyrosine hydroxylase and dopamine transporter, yet many exhibit α-synuclein immunoreactivity and even LB-like inclusions decades after transplantation, supporting α-synuclein spreads between cells, establishes pathology in grafted neurons, and drives disease progression [32].

Tau

The *MAPT* gene encodes the Tau protein, which regulates microtubule stability and axonal transport. Dysfunction in Tau is associated with several tauopathies, such as Alzheimer's Disease and Progressive Supranuclear Palsy. Post-mortem brain analyses of PD patients have revealed that grafted tissue contains hyperphosphorylated Tau aggregates and neurofibrillary tangles in the cortex and striatum. Additionally, 16 years after transplantation, co-localization of Tau (phospho-Tau Ser202 and Thr205) and α-synuclein has been reported [33]. Moreover, after MPTP treatment, phosphorylated α-synuclein (p-α-synuclein), α-synuclein, phosphorylated-Tau, and Tau levels were increased in the SNpc and hippocampus of mouse models. Furthermore, p-α-synuclein and p-Tau were co-localized in the hippocampus, SN, and dentate gyrus. As a result of Tau phosphorylation, microtubule levels are decreased, and neuronal degeneration is induced via microtubule depolymerization and axonal myelin sheath disruption [34]. Studies have demonstrated that mutations in *LRRK2* (PARK8) and subsequent dysregulation of the LRRK2 protein are among the most contributory factors in familial PD, promoting Tau neurotoxicity through disorganization of actin and mitochondrial dynamics.

α-synuclein has been shown to promote Tau fibrillization, and their co-incubation synergistically accelerates the aggregation of both proteins [35]. This co-interaction has also been detected in Alzheimer's disease patients with LB pathology and in transgenic mouse models combining AD and Lewy body dementia. Aβ, tau, and α-synuclein act synergistically to influence each other's accumulation and aggregation [36]. In vitro studies have demonstrated that the microtubule-binding domain of tau and the negatively charged C-terminus of α-synuclein promote co-aggregation and fibrillation [37]. Similarly, such collaborative interactions between tau and α-synuclein in PD may increase the progression.

Failure of protein degradation mechanisms

The two main mechanisms regulating protein homeostasis in mammalian cells are the ubiquitin–proteasome system (UPS) and lysosomal proteolysis (LP). The UPS is an ATP-dependent intracellular protein degradation pathway that cooperates with molecular chaperones; hence, altered ATP availability or chaperone activity can contribute to UPS dysfunction [38]. The UPS regulates protein quality by tagging misfolded or damaged proteins with ubiquitin (via E1/E2/E3 enzymes) and degrading them into peptides in the 26 S proteasome [39]. McKinnon et al. showed that overexpression of A53T-α-synuclein resulted in catalytic proteasome impairment and UPS dysfunction, leading to dopaminergic cell death and behavioral impairments in rats. Furthermore, UPS failure is accompanied by selective accumulation of α-synuclein that is phosphorylated at serine 129 (pS129) and may represent an early event in synucleinopathy that contributes to dopaminergic neurodegeneration [23]. Treatment with proteasome inhibitors induces early apoptosis, the formation of cytoplasmic inclusions, and elevated α-synuclein expression in cell culture models. In animal models, it also results in dopaminergic cell degeneration, inflammation in the substantia nigra pars compacta (SNpc), and neurodegeneration across various brain regions [34, 40].

Among genes linked to lysosomal function, *ATP13A2 (*PARK9*)* encodes a lysosomal P-type ATPase involved in cation transport, lysosomal homeostasis, and autophagy. Mutations in this gene disrupt the proteolytic processing of lysosomal enzymes and impair autophagosome clearance, thereby weakening cellular protein degradation. In post-mortem PD brains, *ATP13A2* protein levels are reduced in dopaminergic SNpc neurons, highlighting its contribution to PD pathology [41, 42].

Molecular chaperones are proteins that maintain proteostasis in living cells by interacting with and stabilizing

client proteins. Chaperone-mediated autophagy (CMA) is a lysosomal pathway that degrades proteins with the help of molecular chaperones. Heat shock proteins (HSPs) are a major chaperone family, and key HSPs, including HSP26, HSP40, HSP60, HSP70, HSP90, and HSP100, are associated with PD [43]. HSPs can recognize regions in monomeric α-synuclein and bind weakly to it, preventing aggregation [44]. Studies showed that chaperons could prevent α-synuclein aggregation by recognizing the N-terminus [45]. For instance, HSP70 can induce solubilization and refolding of aggregated proteins and inhibit the formation of aggregates. Furthermore, Moloney and colleagues showed that overexpression of HSP70 reduced α-synuclein–induced early dystrophic neurite formation in the striatum of an AAV-α-synuclein rat model of PD [46]. Another chaperon, HSP90, prevents aggregation of mutant A53T α-synuclein by forming a stable complex with A53T oligomers along the aggregation pathway [47].

Mitochondrial impairment and oxidative stress

Mitochondria are responsible for energy production, calcium buffering, and homeostasis in eukaryotic cells. In PD, mitochondrial dysfunction and α-synuclein accumulation are the core pathological mechanisms, where intracellular α-synuclein aggregates induce mitochondrial oxidative stress in substantia nigra pars compacta (SNpc) dopaminergic neurons, attributed to activity-dependent calcium entry and activation of NADPH oxidase activity [48].

A key line of evidence emerged in 1983, when several drug users developed acute Parkinsonian motor symptoms after exposure to MPTP [49]. MPTP crosses the blood–brain barrier, where it is converted by monoamine oxidase B in astrocytes to MPP^+. After entering neurons via the dopamine transporter, MPP^+ accumulates in mitochondria and inhibits complex I of the electron transport chain. The resulting energy failure, membrane depolarization, and oxidative stress cause dopaminergic neuron degeneration and motor deficits such as reduced rearing [50, 51].

Cells rely on mitochondrial quality control mechanisms, particularly mitophagy, to counteract such damage. Selective removal of damaged mitochondria via mitophagy helps maintain mitochondrial homeostasis and is mediated by PINK1 (PTEN-induced kinase 1) and parkin (an E3 ubiquitin ligase) [52]. Parkin acts downstream of PINK1 and is recruited to depolarized mitochondria to promote mitophagy [53]. Mutations in *PINK1* and *parkin* are among the first genetic abnormalities linked to autosomal-recessive early-onset PD [54]. Parkin knockout flies show shortened lifespans and motor deficits due to muscle apoptosis from mitochondrial failure, while gram-negative intestinal infection in *PINK1* knockout mice causes dopaminergic neuron loss and levodopa-responsive locomotor dysfunction [55, 56].

Genetic mutations

Genetic factors are estimated to account for ~ 25% of PD risk [57]. Since 1997, 24 genes have been identified concerning monogenic PD. Genes with an autosomal dominant type of inheritance include *SNCA*, *LRRK2*, *HTRA2*, and *VPS35*, whereas genes with an autosomal recessive type of inheritance include *PRKN* (parkin), *PINK1*, *PARK7* (DJ-1), and *ATP13A2*; these are classified by inheritance pattern [58, 59].

The *SNCA* (PARK1) gene, first linked to PD in 1997, encodes α-synuclein, the first gene implicated in hereditary forms of PD. Pathogenic SNCA variants are associated with Lewy body pathology, degeneration of dopaminergic neurons, and persistent motor deficits [49, 60]. Other genes reported in hereditary PD include *LRRK2* (PARK8), *UCHL1*, *PARK7* (DJ-1), *ATP13A2*, *PINK1* (PARK6), PARK3, PARK9, PARK11(loci), *PRKN* (parkin), *GBA*, and *VPS35* (PARK17) [61]. A CRISPR/Cas9-generated D620N variant in the *VPS35* gene causes reduced mitochondrial membrane potential and mitochondrial dysfunction, impairing *PINK1*/parkin-mediated mitophagy [62].

In addition to these well-characterized familial genes, other loci such as the Tau-encoding gene *MAPT* are also found to be associated with PD pathology. Tau can interact with α-synuclein, induce aggregation, and colocalize with α-synuclein in Lewy bodies. Several studies report an association between PD and *MAPT* haplotypes; for example, PD patients more often carry the H1/H1 genotype than controls. Tunold et al. reported that the apolipoprotein E ε4 allele and the MAPT H1 haplotype are related to the progression of dementia in PD [63].

Genes associated with PD can be grouped into two categories. Familial PD genes (e.g., *SNCA*, *LRRK2*, *VPS35*, *PRKN*, *PINK1*, *PARK7*, *ATP13A2)* harbor mutations sufficient to cause disease in an inherited manner. Genetic risk factors (e.g., *GBA*, *TMEM175*, *COMT*, *GRIN2A*) do not directly cause PD but increase susceptibility or modify severity in combination with other genetic or environmental influences. While causal mutations define hereditary PD, risk variants contribute to the disorder's more complex, multifactorial forms [43].

SNCA (PARK1)

SNCA is the first identified gene related to the autosomal-dominant form of PD (Table 1). It was discovered in an Italian family that developed the autosomal-dominant form of PD [64]. A missense mutation in *SNCA*, causing the p.A53T amino-acid substitution, was identified, and the same variant was found in unrelated Greek families [65]. Other studies identified missense mutations, such

Table 1 Genetic mutations in parkinson's disease

Gene	Chromosome	Type of Inheritance	Mutation Type	Clinical Phenotype	Pathogenic Mechanisms	References
SNCA (PARK1/4)	4q22.1	Autosomal dominant	Missense mutation (*A53T*, *A30P*, *E46K*, *H50Q*, *G51D*), duplications, triplications	Early- and late-onset PD, variable severity; dementia in some cases	α-synuclein aggregation, LB formation, mitochondrial dysfunction, impaired synaptic function, and elevated ER stress	[5, 102, 103]
PRKN (PARK2)	6q26	Autosomal recessive	Exon deletions/duplications, nonsense, frameshift, missense	Early-onset PD, slow progression, dystonia	Impairs the ubiquitin-proteasome system, resulting in a deficit in mitochondrial respiration	[76–78]
PINK1 (PARK6)	1p35.12	Autosomal recessive	Missense, nonsense mutations	Early-onset PD, dystonia	Impairment in mitochondrial homeostasis and mitophagy, decreased levels of calcium levels in mitochondria	[54, 80, 81]
PARK7 (PARK7)	1p36.23	Autosomal recessive	Missense, nonsense, deletions	Early-onset PD	Elevated levels of mitochondrial oxidative stress and autophagy, and decreased calcium uptake by mitochondria	[5, 54, 88–105]
LRRK2 (PARK8)	12p11.2-q13.1	Autosomal dominant	Missense (*G2019S*, *Y1699C*, *R1441C/G/H*, *I2020T*) mutations, rare deletions	Late-onset PD is often clinically similar to sporadic PD	α-synuclein aggregation, altered kinase activity, mitochondrial and lysosomal impairment	[88, 106–108]
ATP13A2 (PARK 9)	1p36.13	Autosomal recessive	Missense, nonsense, deletions	Kufor-Rakeb syndrome (early-onset Parkinsonism, pyramidal signs, dementia	α-synuclein accumulation, lysosomal dysfunction, increased ROS, and autophagy impairment	[42, 109, 110]
VPS35 (PARK17)	16q11.2	Autosomal dominant	Missense mutation (*D620N*, others rare)	Late-onset PD is clinically similar to sporadic PD	Defective retromer complex, impaired endosomal trafficking, mitochondrial dynamics, and α-synuclein pathology	[111, 112]

as p.A30P, p.E46K, and p.G51D. In addition to point mutations, copy-number multiplications of the *SNCA* locus, including duplication and triplication, have been reported. Both lead to autosomal-dominant PD, with triplication generally associated with earlier onset and more severe phenotypes [66]. Heterozygous duplication often results in a phenotype similar to idiopathic PD, whereas heterozygous triplication is associated with diffuse Lewy body disease, consistent with a gene-dosage effect that correlates with increased α-synuclein expression [67] Recent hiPSC-derived midbrain organoid models carrying *SNCA* triplication recapitulate this phenotype, showing time-dependent increases in α-synuclein aggregation (oligomeric and phosphorylated forms) in neurons and glia and selective loss of dopaminergic neurons [68]. Studies indicate that the A53T mutation impairs nuclear function by disrupting Ran (Ras-related nuclear protein)–dependent import of essential nuclear proteins through the nuclear pore complex. Altered Ran expression increases A53T-related nuclear envelope defects and disrupts nuclear architecture [69]. Regensburger and colleagues reported that the A53T mutation decreases neurite growth, synapse development, and cell death in vitro. Moreover, A53T α-synuclein impairs dendritic spine density in mouse models [70]. Administration of preformed α-synuclein fibrils into mice overexpressing A30P-α-synuclein caused microglial activation, aggregation of p-α-synuclein, and decreased tyrosine hydroxylase (TH) levels; these effects were more severe than in wild-type mice [71].

A recent cryo-EM study of N-terminally acetylated E46K α-synuclein fibrils showed that E46K disrupts native electrostatic contacts, yielding a looser fibril architecture that is more sensitive to proteolysis yet more prone to fragmentation, with enhanced seeding compared with WT fibrils; this may reflect less-compact fibril surfaces that more readily recruit monomeric α-synuclein [72].

VPS35 (PARK17)

The retromer complex supports lysosomal function by regulating endosomal trafficking, thereby influencing the autophagy–lysosome pathway. The *VPS35* gene encodes VPS35, a core retromer subunit that controls endosomal cargo sorting and recycling. Retromer abnormalities have been reported in several neurodegenerative diseases, including PD and Alzheimer's disease [73]. In neurons, VPS35 contributes to the trafficking of membrane proteins to dendrites. A missense mutation in the *VPS35* gene was identified in autosomal-dominant, late-onset PD in Swiss and Australian families (Table 1) [57]. Overexpression of *VPS35* leads to neurodegeneration and contributes to Parkinson's disease-related cellular stress and disrupted neurite formation. The D620N mutation in *VPS35* induces substantia nigra (SN) dopaminergic neuron degeneration and axonal impairment [74].

Parkin (PARK2) and PINK1 (PARK6)

The *PRKN* gene encodes parkin, a 465-amino-acid E3 ubiquitin ligase that regulates protein clearance pathways, mitochondrial maintenance, cell metabolism, Ca^{2+} homeostasis, and cell death [75]. Parkin ubiquitinates substrates, promoting degradation of misfolded proteins. Across populations with early-onset Parkinson's disease, diverse *PRKN* (PARK2) mutations, including deletions and point mutations, disrupt ubiquitin-ligase activity and lead to loss of parkin function; such mutations are the most common cause of autosomal-recessive early-onset PD (Table 1) [76–78]. Parkin knockout mice show motor and non-motor deficits, striatal mitochondrial-respiration defects, and markers of oxidative damage [79].

The *PINK1* (PARK6) gene was linked to early-onset recessive PD in 2001, in a large Italian pedigree. Individuals with *PINK1* mutations show clinical features that are often similar to sporadic PD [80, 81]. PINK1 has been reported to interact with α-synuclein and to promote clearance of excess α-synuclein, helping protect mitochondria and prevent cell death [82]. Loss of *PINK1* leads to enlarged, dysfunctional mitochondria in the striatum and subsequent mitochondrial-respiration failure in the cerebral cortex of mouse models [83].

In mice with *PINK1* exon 4–5 deletions, striatal dopamine levels decrease and Ca^{2+} storage is impaired, whereas exon 2–3 deletions cause serotonergic neurodegeneration and olfactory deficits [84, 85].

PINK1 and parkin act together in mitochondrial quality control by regulating mitophagy. Upon mitochondrial damage, PINK1 accumulates on the outer mitochondrial membrane and recruits parkin, which ubiquitinates outer membrane proteins to trigger selective autophagic removal of damaged mitochondria [53]. Mutations in *PINK1* and/or *PRKN* impair this pathway and underlie autosomal-recessive early-onset PD. Clinically, *PRKN*- or *PINK1*-associated PD often shows a predominantly motor phenotype with good levodopa responsiveness compared with other cases [86].

In *PINK1* knockout mice, α-synuclein overexpression using an adeno-associated viral vector in the substantia nigra causes dopaminergic neuron loss and increased α-synuclein phosphorylation, indicating that *PINK1* loss heightens sensitivity to α-synuclein toxicity [60].

DJ-1 (PARK7)

DJ-1, encoded by the *PARK7* gene, is expressed primarily in microglia, astrocytes, and neurons. It is involved in several cellular processes, including mitochondrial function, chaperone activity, protease activity, and responses to oxidative stress [87]. DJ-1 protects cells from hydrogen peroxide–induced injury via oxidation-dependent activation. *PARK7*-knockout cells are more sensitive to neurotoxin exposure and show loss of the oxidized form of DJ-1, highlighting DJ-1's role in defense against reactive oxygen species (ROS) [88]. Mutation or ablation of *DJ-1* in dopaminergic cell lines results in mitochondrial inner membrane potential loss, a decrease in cellular ATP levels, and increased inner-membrane permeability. These findings suggest that DJ-1 has an important role in dopaminergic neuron maintenance and mitochondrial ATP-synthesis pathways [89]. *PARK7*-related early-onset PD typically exhibits an autosomal-recessive type of inheritance; oxidized DJ-1 is also observed in idiopathic PD brains, and DJ-1 mutations are associated with rigidity, tremor, dyskinesia, and non-motor symptoms including anxiety and cognitive impairment [90]. DJ-1 also shows chaperone-like activity in addition to antioxidant properties. It may modulate α-synuclein aggregation and reduce its toxicity through HSP70 upregulation; conversely, DJ-1 downregulation reduces antioxidant capacity and impairs HSP70 upregulation [91]. In postmortem PD brain tissue, activated microglia are present in the substantia nigra [92]. DJ-1 impairment increases microglial activation and enhances microglia-mediated neurotoxicity. DJ-1 interacts with p65 in microglia to limit microglial activation and protect against dopaminergic neuron death caused by neuroinflammation [93]. In summary, *PARK7* deficiency leads to activation of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) and downregulation of anti-inflammatory pathways, resulting in an elevated microglial inflammatory response [94].

LRRK2 (PARK8)

Leucine-rich repeat kinase 2 (LRRK2), encoded by the *LRRK2* gene (PARK8 locus), regulates mitochondrial function and synaptic transmission and has both kinase and GTPase activities. Pathogenic variants in *LRRK2* cause late-onset autosomal-dominant PD, and common variants at PARK8 have been identified as risk factors for sporadic PD [23]. Stereotactic delivery of viral vectors encoding human G2019S LRRK2 into the substantia nigra or striatum of adult rats induces robust dopaminergic neurodegeneration; this effect depends on both kinase and GTPase activities and can be prevented by kinase inhibition or by GTPase-reducing mutations [95]. In PD brain tissue, LRRK2 is enriched in regions with α-synuclein phosphorylation and accumulation and co-localizes with α-synuclein in Lewy bodies. LRRK2 can interact with α-synuclein and promote its aggregation and fibrillization; conversely, *LRRK2* ablation increases small inclusion bodies in vitro [96]. Overexpression of G2019S LRRK2 in the mouse forebrain produces behavioral impairment and α-synuclein pathology via kinase-dependent mechanisms [97].

ATP13A2 (PARK9)

ATP13A2, encoded by the *ATP13A2* gene, is a lysosomal P-type ATPase that plays a crucial role in transporting inorganic cations across membranes. It also regulates the functions of various organelles, including lysosomes, the endoplasmic reticulum, and mitochondria [98]. Mutations in *ATP13A2* have been associated with the autosomal recessive form of early-onset Parkinson's disease [42]. Specifically, ATP13A2 promotes α-synuclein release via exosomes, whereas its impairment reduces exosomal release [99].

Furthermore, ablation of *ATP13A2* leads to loss of lysosomal membrane integrity and impaired autophagy, which is linked to α-synuclein aggregation and multimerization, while overexpression of *ATP13A2* enhances ubiquitin–proteasome pathway activity and suppresses α-synuclein multimerization [100]. Additionally, *ATP13A2* mutations reduce ATP13A2 protein expression, disrupting metal-ion homeostasis [101].

Neuroinflammation

In the CNS, microglia are the primary cell type responsible for inflammatory mechanisms. Upon activation, microglia release a broad spectrum of pro- and anti-inflammatory mediators, including TNF-α, IL-1β, IL-6, TGF-β, reactive oxygen species (ROS), nitric oxide, and pro-apoptotic factors; many of these have been detected in the substantia nigra pars compacta (SNpc), striatum, and cerebrospinal fluid of PD patients [113]. Grozdanov and colleagues showed that microglia are sensitive to pathological α-synuclein, which activates immune signaling and is taken up by microglia [114]. Neuroimaging studies using PET have revealed widespread microglial activation in the pons, basal ganglia, and frontal regions in PD patients compared with controls [115]. Microglia can undergo pro-inflammatory and anti-inflammatory phenotypes; the former is characterized by elevated secretion of cytokines such as TNF-α, IL-1β, IL-6, IL-12, nitric oxide, and ROS [116]. Postmortem PD brain tissue shows elevated IL-1β, TLR4 activation, and increased microglial and astrocytic markers, consistent with innate immune activation (Fig. 2) [92, 117]. Experimental studies support that chronic expression of these cytokines contributes to nigral dopaminergic degeneration and motor deficits [118]. During disease progression, apoptotic neurons release pathological molecules (aggregated α-synuclein, ATP, and MMP-3). These activate microglia and amplify inflammation, leading to further neurodegeneration [119].

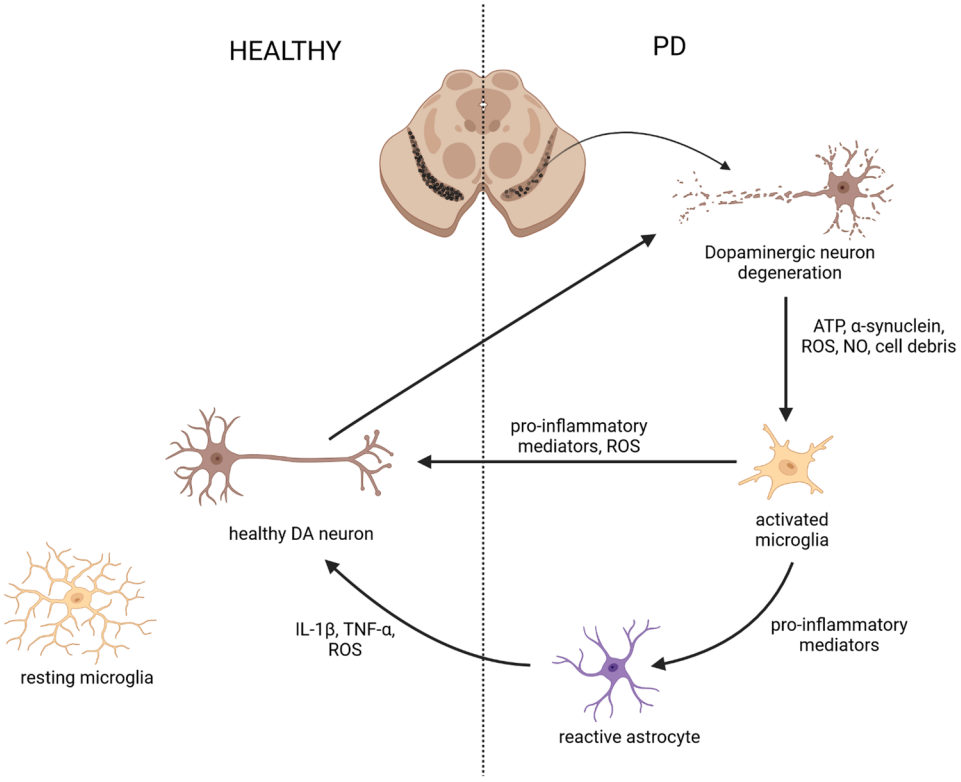


Fig. 2 Schematic of microglia-mediated neuroinflammation during PD progression. Microglia, activated in a pro-inflammatory phenotype in PD, release mediators that activate astrocytes, resulting in increased generation of proinflammatory factors such as nitric oxide and reactive oxygen species (ROS), leading to dopaminergic neuron degeneration [116]. Image created using BioRender (2024)

Consistent with this, immunohistochemical studies of postmortem PD brains have detected microglial activation and increased expression of human leukocyte antigen (HLA) class II (MHC class II) molecules in affected regions. Sulzer et al. showed that α-synuclein peptides presented by HLA molecules trigger both $CD4^+$ and $CD8^+$ T-cell responses, with nearly 40% of PD patients exhibiting reactivity, highlighting a genetic link between HLA alleles and adaptive immune activation in PD [120]. Concurrently, infiltration of $CD8^+$ T lymphocytes into the SNpc has been detected before dopaminergic neuron loss, indicating that cytotoxic T cells can contribute to neuronal death even prior to overt Lewy body (LB) formation [121, 122]. A recent study demonstrated that immune-triggered lysosomal dysfunction can accelerate PD pathology. Human iPSC-derived dopaminergic neurons form LB-like inclusions when exposed to α-synuclein preformed fibrils, but only when combined with a pro-inflammatory stimulus (either interferon-γ or IL-1β) or when co-cultured with activated microglia-like cells. By impairing neuronal lysosomal function, interferon-γ facilitates the development of membrane-bound LB-like inclusions, reflecting dysfunction of the autophagy–lysosome pathway [123]. These findings suggest that activation of the innate and adaptive immune responses exacerbates the PD molecular pathology [124].

Modeling approaches in parkinson's disease research and advances in 3D in vitro modeling

Experimental models of PD have been widely used to understand and clarify disease mechanisms, evaluate the contribution of genetic and environmental risk factors, and examine potential therapeutic targets and molecules. Cellular pathologies such as protein aggregation, mitophagy, and apoptosis can be stimulated in cellular PD models. Although these models enable study of cell-specific pathways, they cannot replicate the PD microenvironment or overall disease progression [9, 10].

Experimental animal modeling of PD is mainly based on neurotoxin administration or genetic manipulation; however, neither approach fully recapitulates the complete disease pathology. Neurotoxin models are commonly used to investigate therapies targeting motor symptoms, but they do not mimic the progressive loss of dopaminergic neurons or key hallmarks of PD pathogenesis (for example, Lewy body inclusions). MPTP has been used in mice and non-human primates; however, it causes acute degeneration and deficits that can recover within days after injection, limiting long-term screening. Moreover, MPTP only partly recapitulates the spectrum of behavioral impairments seen in human PD, and MPTP-induced phenotypes are influenced by age, strain, and sex [125–130]. By contrast, exposure to herbicides and pesticides often has high mortality and lacks mechanistic specificity. There is substantial variation in the percentage of animals that develop dopaminergic inclusions and in their severity [131–135]. Transgenic animal models help link genetic mutations to disease mechanisms, yet they often show divergent pathophysiology and behaviors. For instance, transgenic models for point mutations of α-synuclein (A30P, A53T, and E46K) lack dopaminergic cell loss in SNpc [136, 137] contrast, models of *LRRK2* point mutations (G2019S and R1441C) mimic dopaminergic degeneration but lack α-synuclein aggregates and Lewy bodies (LBs) [138, 139].

In conventional 2D cell cultures, cells are grown as a single layer on a flat surface, resulting in limited cell-cell interactions. In 2D systems, cell-cell interaction can be maintained with direct or indirect co-culture. Two or more cell types can be mixed and cultivated for direct co-culture. For instance, neurons develop longer and more numerous neurites than in monoculture when co-cultured with murine astrocytes and microglia [140]. Alternatively, systems using cell-culture inserts (along with other indirect methods such as conditioned medium transfer and feeder-cell layers on coverslips) provide a more dynamic environment by enabling intercellular communication via paracrine factors, exosomes, and cytokines. In these setups, different cell types are cultured on opposite sides of a semipermeable membrane, allowing interaction via soluble molecules without physical contact [141]. Commercially, permeable collagen membranes, plastic porous transwell membranes, and disposable inserts such as Koken® and Falcon® are available. For instance, co-culture of Nurr1-overexpressing neural stem cells and microglia in a Transwell system can mimic the microglial milieu to investigate the effects of pro-inflammatory mediators (e.g., TNF-α, nitric oxide, IL-1) released by activated microglia and to assess Nurr1's role in PD pathogenesis. Nurr1 exhibits anti-inflammatory effects and promotes differentiation of neural stem cells into dopaminergic neurons [142]. Although insert-based co-cultures can partially recapitulate intercellular communication, they lack 3D cytoarchitecture and direct cell contact and are influenced by gravity and the mechanical properties of the membrane [143].

Despite the efforts to improve 2D culture models to better mimic the in vivo microenvironment, their inherent limitations persist. The lack of extracellular matrix (ECM) components and 3D spatial organization significantly affects cellular differentiation, proliferation, and overall function, while the flattened architecture in 2D cultures alters proliferation, morphology, and cellular dynamics [144]. For example, it has been demonstrated that Rainbow trout liver cell spheroids exhibit tissue-like organization characterized by cell-cell junctions, interdigitations, and endocytic and exocytic activity at the cell-cell interfaces. Moreover, genes essential for lipid

metabolism are upregulated in 3D cultures compared with 2D monolayers. Ultrastructural studies further confirm that 3D cultured cells display characteristics of metabolically active cells, such as a prominent Golgi apparatus, abundant mitochondria, and extensive endoplasmic reticulum networks [145]. Although in vivo and in vitro models have provided key information, they generally fail to fully capture human-specific disease mechanisms. By contrast, midbrain dopaminergic neurons and organoids derived from human iPSCs offer a powerful platform for disease modeling and translational drug screening in synucleinopathies and have enabled the development of an optogenetics-based α-synuclein aggregation–induction system [146].

Unlike 2D cultures, 3D cultures let cells grow in three dimensions and interact with the extracellular matrix (ECM), better mimicking living tissue (Fig. 3). Cell-cell and cell-ECM interactions in 3D models allow cell proliferation, differentiation, and morphology in an in vivo-like environment [147]. For these reasons, 3D cell cultures better recapitulate disease microenvironments than 2D cultures and help bridge the gap between in vitro and in vivo models.

Spheroids and organoids

Because of self-assembly, cells in suspension readily form aggregates. In this process, single cells adhere to one another to produce multicellular spheroids; cadherins and extracellular-matrix (ECM) components facilitate aggregation, creating tissue-like microenvironments [149]. Spheroids, generally smaller than 1 mm, can be generated by pellet culture, liquid-overlay, or hanging-drop methods (Fig. 4). Traditional approaches use low-adhesion surfaces for spontaneous self-assembly or hanging drops in which cell suspensions are cultured on inverted plates; these methods are simple and widely used, but often yield heterogeneous spheroid sizes [150]. While these methods are simple and widely used, they usually result in heterogeneous spheroid sizes. In order to overcome this limitation, microwell-based platforms like AggreWell™ have been developed. In these systems, individual cells are spatially confined within microwells, which directs them into uniform aggregates and

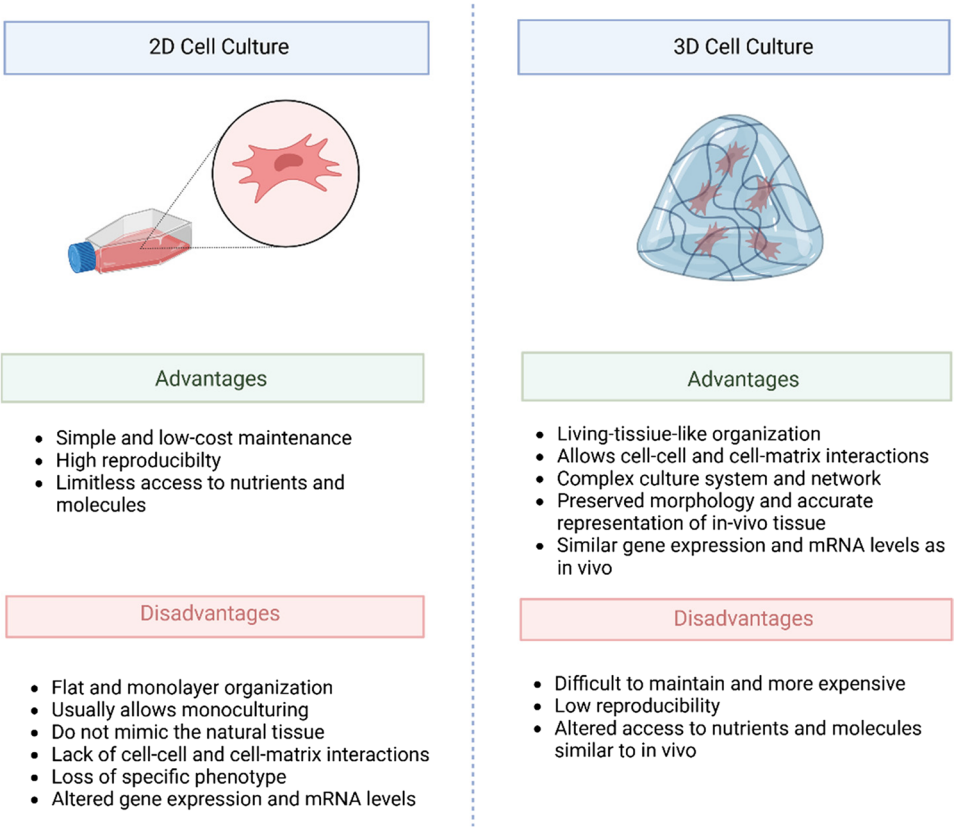


Fig. 3 Comparison of 2D and 3D cell culture systems. This figure compares 2D and 3D cell culture systems, indicating the advantages and disadvantages of both systems. Two-dimensional (2D) cultures are low-cost and easy to use, but lack the native microenvironment and do not capture physiological complexity well. In contrast, 3D cell culture allows cells to grow in a three-dimensional, tissue-like environment. With this approach, cell-cell and cell-matrix interactions can be modeled to reflect physiological processes more accurately. However, 3D systems can be harder to standardize and are more costly [148]. Image created using BioRender(2024)

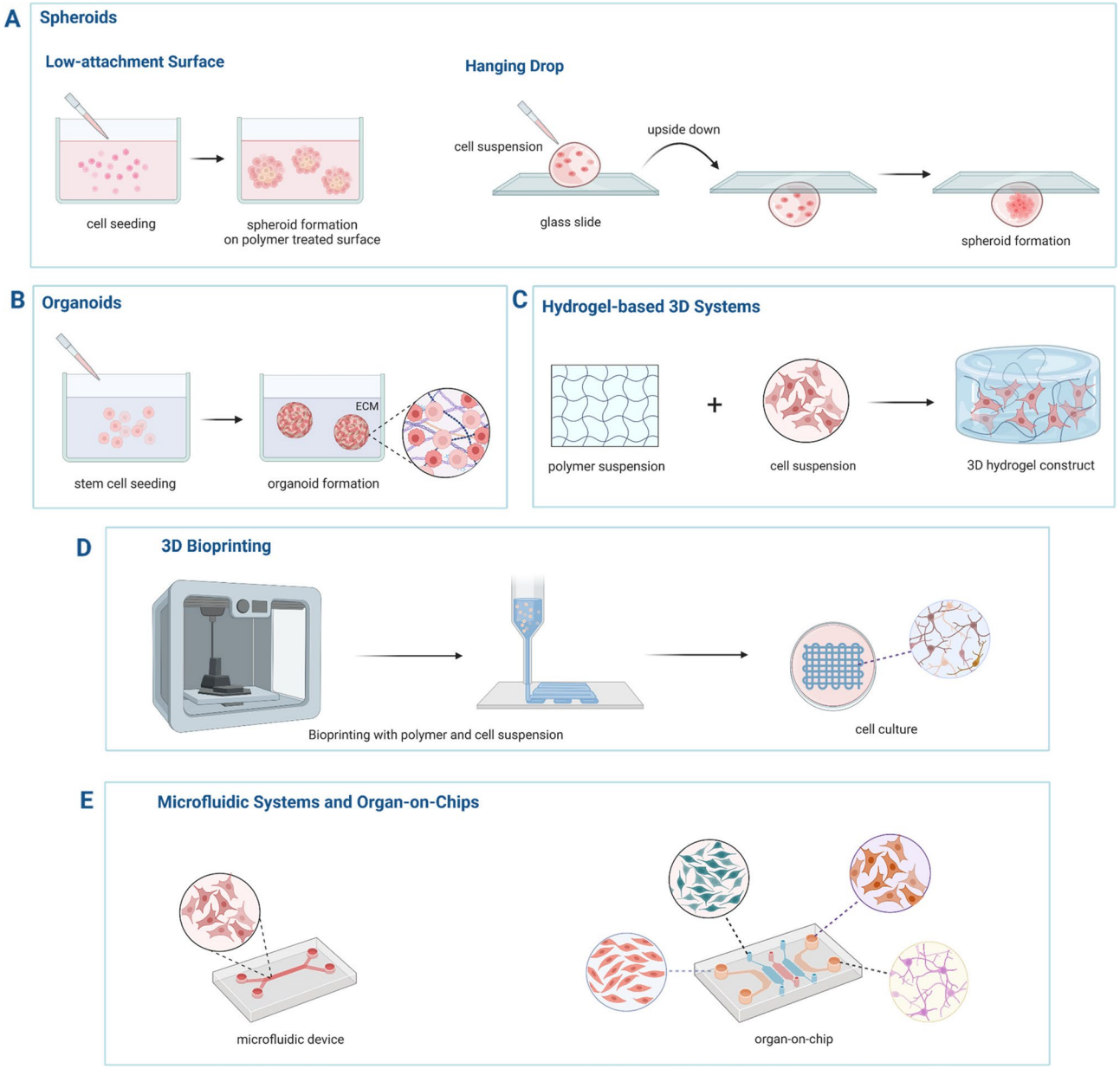


Fig. 4 Overview of 3D culture techniques. **A** Spheroids: formed on low-adhesion surfaces (cells self-aggregate) or by the hanging-drop method (cells seeded on inverted lids/plates). **B** Organoids: generated by culturing stem/progenitor cells in extracellular matrix (ECM)–containing gels. **C** Hydrogel-based 3D culture: cells embedded in natural or synthetic hydrogel matrices. **D** 3D bioprinting: deposition of cell-laden bioinks (biopolymers) to build tissue-like constructs. **E** Microfluidic devices and organ-on-chip systems: model tissue microenvironments and organ-level functions in a controlled, standardized manner [159]. Image created using BioRender (2024)

significantly reduces size variability. The high density of microwells (hundreds of wells per square centimeter of culture surface) enables large-scale production. As a result, many uniform spheroids can be produced in parallel [151]. Spheroids can be used in neurodegenerative disease modeling as their advantage is to examine cell-cell and cell-ECM interactions. It has been shown that SH-SY5Y spheroids overexpressing A53T α-synuclein can be generated and used to study PD pathology [152]. Additionally, Strong and colleagues used iPSC-derived neurons to generate ventral tegmental area–like (VTA-like) spheroids containing A53T-expressing dopaminergic neurons to investigate neural activity [153].

Compared with spheroids, which often consist of a relatively homogeneous population of a single cell type, organoids exhibit much greater cellular diversity. While organoids typically contain multiple differentiated cell types arranged in tissue-like architectures, spheroids are simplified aggregates with limited structural organization. This difference in cellular composition underpins

the broader functional potential of organoids [154, 155]. Organoids are self-organizing 3D structures, usually generated from stem cells, that recapitulate key biological and mechanical features of organs. Organoids can be derived from stem cells or tissue-derived cells, such as cancer cells from patients [156]. Cells can be mixed with scaffold material before seeding to initiate organoid formation, or aggregates in the form of embryoid bodies can be embedded in hydrogels (Fig. 4). However, spontaneous self-organization produces size variation between 0.5 mm and 4 mm, and in larger organoids, oxygen and nutrients do not reach the core. Besides traditional approaches, microwell-based platforms provide a new strategy to improve the reproducibility and scalability of organoid formation. By spatially confining cells, microwells create controlled microenvironments that promote uniform development. This system also enables high-throughput production and can direct stem cell differentiation, offering a standardized framework for studying lineage commitment and disease modeling [157]. Midbrain organoids derived from PD patients carrying *LRRK2* mutations show fewer dopaminergic neurons and reduced structural complexity relative to controls, suggesting neurodevelopmental impairment. Kim and colleagues generated isogenic 3D midbrain organoids from iPSCs engineered to carry *LRRK2* G2019S using CRISPR/Cas9; these organoids showed reduced expression of mature neuronal markers and shorter neurites compared with controls. They also observed that MPTP exposure caused abnormal localization and accumulation of pS129 α-synuclein, which was reduced by LRRK2 kinase inhibition. Thus, organoids support applications in personalized medicine, drug screening, and mechanism-specific disease modeling [158].

Hydrogel-based 3D culture systems

Hydrogels are cross-linked polymer networks with high water content that serve as extracellular matrix (ECM) mimics (Fig. 4). Synthetic and natural hydrogels are tunable in mechanical and chemical properties to match a given tissue and support cell function [160]. Synthetic hydrogels such as polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), and polyacrylamide (PAAm) lack native ECM cues; they are therefore often combined with ECM proteins, including laminin, collagen, and fibronectin, to create ECM-mimetic matrices with defined mechanics. Natural hydrogels include protein-based materials (e.g., collagen; Matrigel basement-membrane extract) and polysaccharide/biopolymer-based materials (e.g., hyaluronic acid, alginate, chitosan, silk fibroin), which provide bioactive signals found in native ECM [159]. In practice, synthetic systems offer precise mechanical control but limited bioactivity, whereas natural systems provide biological cues but greater batch variability; hybrid formulations aim to combine these advantages.

Alginate is a hydrogel-forming polysaccharide derived from brown algae, composed of two uronic acids (guluronic and mannuronic) and chemically akin to hyaluronic acid as a linear, anionic polymer. Alginate hydrogels support cell viability, electrophysiological function, and differentiation of embedded cells. Studies have shown that 3D alginate hydrogel scaffolds enhance neural maturation of hiPSC-derived dopaminergic (DA) neurons; moreover, DA neurons derived from PD patients carrying triplication of the *SNCA* gene show higher mitochondrial ROS and elevated mitochondrial membrane potential than controls, indicating PD-relevant pathology (Table 2) [161]. Matrigel, a mixture of basement extracellular matrix (ECM) proteins derived from Engelbreth-Holm-Swarm murine sarcoma cells, is widely regarded as the gold standard for mimicking the ECM, as it is enriched with natural ECM proteins such as laminin, collagen, proteoglycans, and entactin. Matrigel provides an in vivo–like environment that supports cell viability, differentiation, and formation of complex structures [11]. It supports dopaminergic and neuronal differentiation and the growth of human adipose-derived mesenchymal stem cells (hADSCs) compared with induction factor–supplemented tissue-culture plates, as confirmed by increased TH, Nurr1, DAT, and MAP2 levels, supporting its utility for PD modeling (Fig. 5) [158]. Li and colleagues observed increased pS129 α-synuclein, Lewy-body-like inclusions, and ubiquitin aggregation in differentiated SH-SY5Y cells embedded in Matrigel after rotenone or MPP^+ treatment, indicating that 3D systems more closely recapitulate in vivo physiology relative to 2D cultures (Table 2; Fig. 5) [162].

Gelatin methacrylate, a chemically modified form of gelatin with methacrylic anhydride, is a cost-effective hydrogel that supports cell survival and attachment since it is derived from gelatin, a denatured form of collagen. After methacrylation, GelMA becomes photopolymerizable in the presence of a photoinitiator. By tuning biophysical properties (e.g., pore size and stiffness), GelMA hydrogels support retinal ganglion cell axon extension, and Schwann cells extend processes and interact with dorsal root ganglion axons. In this 3D setup, myelination can be observed, making GelMA a useful scaffold for studying myelination, demyelination, and remyelination in neurodegenerative disease [164].

3D Bioprinting techniques

The three-dimensional (3D) bioprinting process deposits bioinks containing cells, spheroids, or organoids layer by layer under computer-controlled guidance following a predefined design (Fig. 4). 3D bioprinting enables reproducible control over geometry (shape, size, pore

Table 2 Methods used in 3D modeling of parkinson's disease

Methods	Cell type used	Model	Main Results	PD pathology observed	References
Midbrain organoids in Matrigel	iPSC	*LRRK2-G2019S* mutation	The results demonstrated that the *LRRK2* mutation caused fewer DA neurons and complexity in organoids compared to the controls.	Structural and behavioral impairment in DA neurons	[158, 172]
Differentiated cells in Matrigel	SH-SY5Y	MPP + and Rotenone treatment	3D cultures showed increased pS129 α-synuclein, Lewy-body-like inclusions, β-sheet deposition, and ubiquitin aggregates; 3D resembled in vivo physiology better than 2D.	pS129-α-synuclein pathology	[162]
Alginate-based hydrogels	hiPSC	*3xSNCA* mutation	Higher mROS and mitochondrial membrane potential were observed in hiPSC-derived mDA neurons carrying the 3x*SNCA* mutation compared to the controls.	Elevated mitochondrial ROS and mitochondrial membrane potential	[161]
Extrusion-based bioprinting with self-assembling tetrapeptide scaffolds	Human and mouse primary cell-derived DA neurons	6-OHDA treatment	In the DA neuron-encapsulated 3D peptide-based bio-printed model, they detected functional connectivity, neurite outgrowth, and spontaneous action potentials. With the 6-OHDA treatment, dose-dependent and selective DA neuron loss and ATP level decrease have been shown	Decreased number of TH + neurons and ATP release	[163]
Microfluidic system	Primary cortical neurons and astrocytes	Incubation with Lewy bodies from human PD brains	This study shows that human α-synuclein-containing LB fractions induce astrogliosis, and cell-to-cell transmission of α-synuclein occurs from astrocyte to neuron and neuron to astrocyte. While astrocytes uptake α-synuclein more efficiently than neurons, human α-synuclein induces endogenous α-synuclein expression in neurons than in astrocytes. Furthermore, spreading α-synuclein from astrocytes to neurons can induce apoptosis of neurons.	α-synuclein transmission, decreased neurite length, and apoptosis	[170]
Microfluidic system	Human H4 neuroglioma cells and N9 microglial cells	Co-culturing neurons with activated microglia	To investigate microglia-neuron interactions, H4 neurons were co-cultured with LPS-induced ROS-producing activated microglia. In H4 cells, they detected elevated ROS	Increased reactive oxygen species	[173]
Organ-on-chip	Substantia nigra, globus pallidus, and striatal neurons derived from the brain tissues of rat embryos	Basal ganglia circuit	Cell connectivity, axonal networks, and electrical activity were similar to those in vivo in the microfluidic device.	Neuronal connectivity and functional behavior of the basal ganglia can be simulated in future studies	[174]

architecture) and over mechanical properties (elastic/viscoelastic behavior), which supports cell viability and communication. Bioprinting combines biomaterials, bioactive factors, and cells in a tunable construct, and the bioink provides both mechanical support (e.g., elasticity/viscoelasticity) and biochemical cues (supporting cell viability, proliferation, differentiation, and signaling). Bioinks and printing techniques are selected to match the desired ECM-like and culture characteristics [165, 166]. An ideal bioink is printable, biocompatible, and viscoelastic, while maintaining cell growth and survival. Hydrogels are mainly used since they can mimic the ECM, and synthetic polymers are used to create a more mechanically tunable environment. To utilize hydrogels, which are soft and flexible bioinks, the printed structure must be crosslinked using light, enzymes, pH changes, chemical crosslinkers, or by combining them with rigid polymers [167].

Several printing techniques are available: inkjet, laser-assisted, microextrusion, and stereolithography. The choice depends on the target tissue, bioink composition, and design. Inkjet bioprinting ejects bioink droplets through a small nozzle onto a substrate. It is fast, economical, and often maintains high cell viability; however, it requires low viscosity bioinks and low cell concentrations to avoid nozzle clogging and is less suited to complex tissues. Retinal ganglion and glial cells have been successfully printed by inkjet while maintaining cell viability and morphology [168]. Extrusion bioprinting continuously extrudes bioink through a nozzle following the designed pattern. Unlike inkjet, it handles high-viscosity bioinks and high cell densities, but shear/pressure can reduce viability. With extrusion-based printing, composite bioinks can both mimic ECM and maintain 3D structure. For example, alginate provides rigidity, whereas gelatin supplies ECM-like adhesion; a sodium alginate–gelatin bioink supports neural stem cells and SH-SY5Y cells with good viability, spatial organization, cell–cell organization, and reproducibility [169]. In optic-based bioprinting techniques, a laser or projected light is emitted by the printer and then struck into the cell-enriched bio-ink, carrying out the polymerization layer by layer.

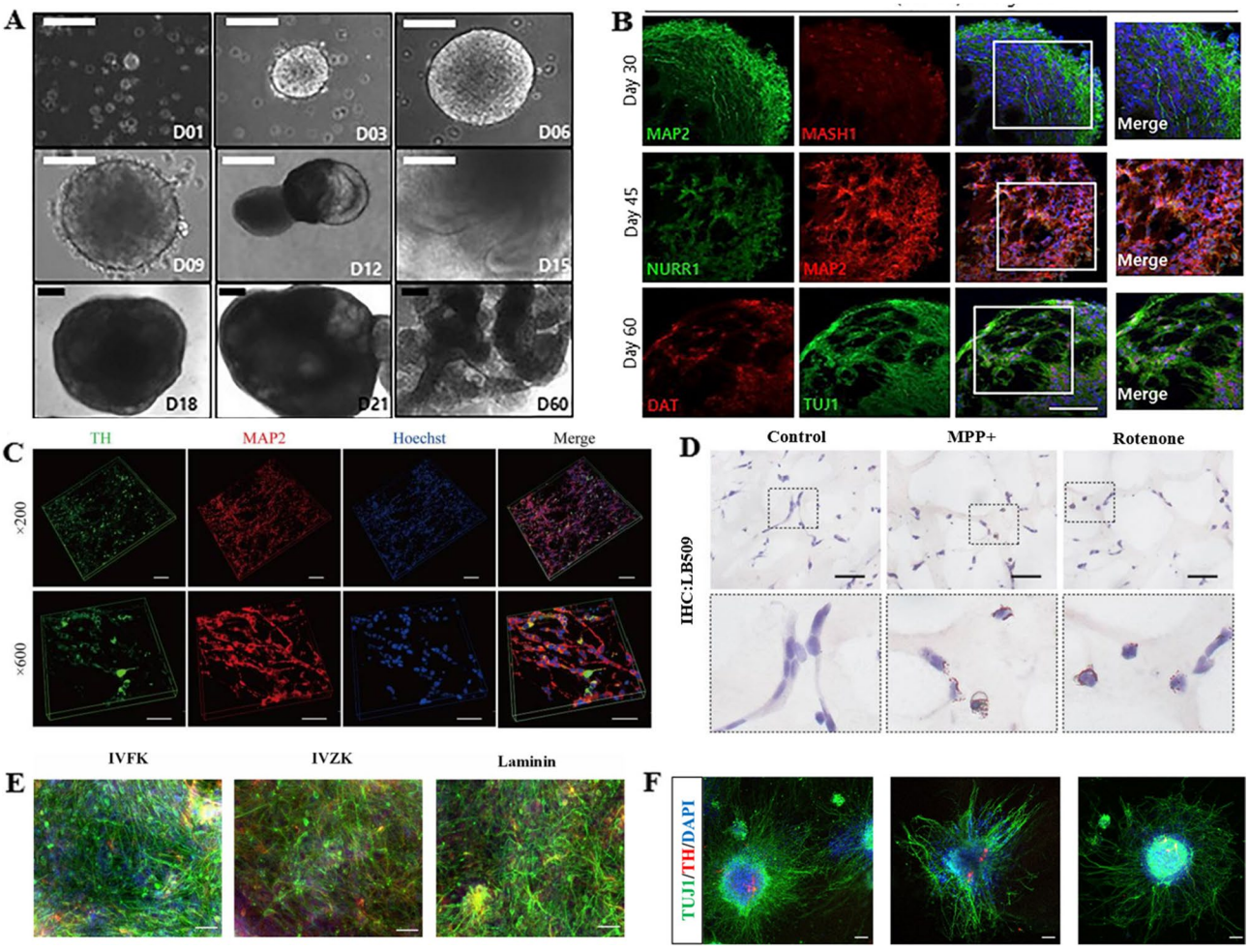


Fig. 5 Differentiated cell culture models of PD using Matrigel. **A** Brightfield images depicting the stages of 3D organoid generation over 2 months, with a scale bar of 200 µm. **B** immunofluorescence images of an in vitro Parkinson's disease model using Matrigel-embedded midbrain organoids. Images show 3D organoid formation over time, and immunofluorescence images indicate that midbrain organoids express adult dopaminergic markers. Scale bars, 100 µm [158]. **C** Three-dimensional and (**D**) immunohistochemical staining section images of SH-SY5Y cells within thin-layer 3D constructs. Immunofluorescence images show that encapsulated SH-SY5Y cells express dopaminergic markers after the differentiation period, and immunohistochemical images show Lewy Body formation after neurotoxin treatment, indicating the efficiency of disease modeling in a 3D environment—scale bars: 50 µm [162]. Figure (**E**) shows confocal images of hES cells encapsulated in self-assembling peptide scaffolds IVFK (left) and IVZK (middle) and cultured on laminin-coated plates (right), showing mature neuronal markers. TUJ1 (green), DAPI (blue), and TH (red) (**F**) immunofluorescence images of stained neurospheres with neuron markers (TUJ1 and TH) in bio-printed structures (left and middle) and laminin-coated plates showing successful migration of vmDA and vm non-DA neurons. Scale bars, 100 µm [163]

This method ensures fast manufacturing with high cell viability [10].

GelMA–Geltrex bioprinted structures, crosslinked by UV exposure, support long-term cell viability and proliferation, promote formation of complex structures, and support maturation of neural progenitor cells; dedifferentiation of murine astrocytes was also reported in vitro [170]. Combining GelMA with fibronectin and Matrigel enhanced neuron-like features in LUHMES cultures, including improved neurite morphology [171]. Abdelrahman and colleagues reported a 3D bioprinted peptide-based model using IVZK and IVFK tetrapeptides embedding primary cell-derived dopaminergic (DA) neurons. Cultured cells showed increased ATP levels (indicative of neuronal viability) and enhanced neurite branching compared with poly-D-lysine (PDL)–coated plates. Spontaneous action potentials were recorded with microelectrode arrays (MEAs) for more than one month without signal degradation, consistent with sustained neuronal functionality. The authors also noted expression of genes involved in neuronal development, maturation, and differentiation in both 2D and 3D models. After treatment with 6-OHDA (a neurotoxin widely used in PD models in vivo and in vitro), a dose-dependent reduction in ATP and a decrease in TH^+ DA neurons were observed (Table 2; Fig. 5 [163].

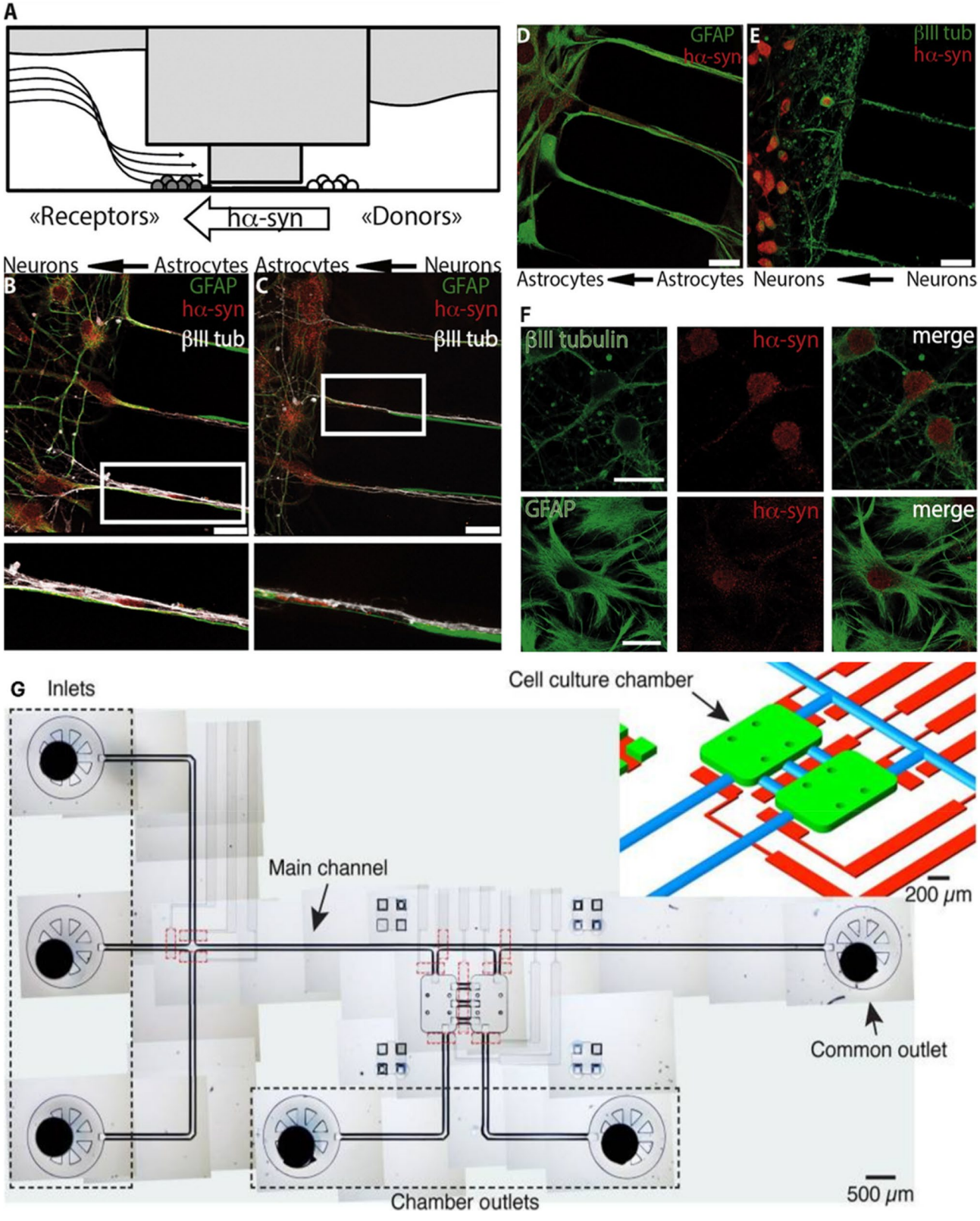


Fig. 6 (See legend on next page.)

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Fig. 6 Microfluidic systems for PD modeling. **A** Schematic of cell-to-cell transfer of human α-synuclein (hα-synuclein) between chambers. hα-synuclein is placed in a "donor" chamber and is transported intracellularly to recipient cells through microchannels, against the bulk fluid flow (direction indicated by the arrow). **B–E** Rat cortical neurons and astrocytes were seeded in the microfluidic chamber. Human Lewy Body fractions were incubated in one chamber, and hα-synuclein (red) was transported to another chamber in the direction of the arrows (labeled in green with anti-βIII tubulin) and in astrocytes (labeled in green with anti-GFAP antibodies). Different combinations of cells were assayed; Figure B shows the transport of hα-synuclein from astrocytes to neurons, Figure **C** shows the transportation from neurons to astrocytes, and Figure **D** shows the transportation from astrocytes to astrocytes. Figure E shows the transportation from neuron to neuron. Scale bar: 20 µm. **F** Immunofluorescence staining images indicate neurons internalize hα-synuclein after 24 h of incubation. Scale bar: 20 µm [170]. Image (**G**) shows a microfluidic platform with three inlets, a primary channel connected to an outlet, and two cell culture chambers, each with its own outlet. Individual cell culture chambers can be isolated by activating valves at the entry and exit points and the central channel valve. In the top-right corner, a schematic highlights the microfluidic design, including the cell culture chambers (green), pneumatic channels (red), and round cross-section channels (blue) [173]

Microfluidic systems and organ-on-chips

A microfluidic device consists of microchannels through which fluids flow, creating a tissue-like, biomimetic microenvironment. Such systems allow precise control of pH, temperature, nutrients, and oxygen, and they are reproducible, flexibly designed, and optically transparent (Fig. 4). Common device materials include polydimethylsiloxane (PDMS), polyetherimide (PEI), poly(methyl methacrylate) (PMMA), and polycarbonate (PC) because of their biocompatibility and transparency [175]. Microfluidic platforms are used to study neuronal network formation and neurite outgrowth and to direct the connection pattern [176]. Microfluidic platforms are used to study neuronal network formation and neurite outgrowth and to direct connection patterns [177]. Neurite extension is a key readout in neurodegeneration studies. These systems support the fabrication of microchannels with varied geometries and conditions and enable in vitro monitoring of growth dynamics [178]. Cells can be cultured in 2D or 3D within microfluidic devices; 3D culture better simulates the in vivo microenvironment. ECM components (e.g., collagen, fibrin, Matrigel) or synthetic polymers (e.g., PLGA, PLA, PEG, PCL) can be introduced into the device. For example, Hesari et al. designed a two-layer device with a PLGA-nanofiber–coated bottom layer to assess hiPSC differentiation; neuronal gene expression was significantly higher than on 2D tissue-culture plates or scaffold-only controls [175]. Microfluidic systems also enable co-culture to study intercellular interactions. Yang et al. co-cultured neurons and oligodendrocytes in a compartmentalized device to test whether electrical stimulation affects myelin sheath formation: neurons were seeded first to extend axons, then oligodendrocyte progenitors were added to the axonal compartment. After stimulation, myelinated segments increased significantly, and myelin sheaths were confirmed by staining [179]. Cavaliere et al. designed a microfluidic system to investigate the transportation, uptake, and release dynamics of α-synuclein and cultured primary cortical neurons and astrocytes with Lewy body fractions obtained from PD patient brains. Both cell types internalized α-synuclein aggregates; astrocytes showed greater uptake and transfer to neurons, which induced neuronal death. Human α-synuclein also promoted astrogliosis and increased endogenous α-synuclein expression in neurons (Table 2; Fig. 6) [170]. Fernandes et al. modeled neuroinflammation by activating N9 microglia with LPS to induce IL-6, TNF, IL-1β, and ROS production, then co-cultured them with H4 neuroglioma cells to study microglia-neuron interactions. H4 cells showed higher ROS than controls co-cultured with unstimulated N9 cells [173].

Organ-on-a-chip devices are microfluidic platforms that mimic the physiology of functional units of organs, and in some designs, multi-organ physiology at a small scale. Like other microfluidic systems, they allow precise control of chemical, mechanical, and physical parameters and enable studies of cell-cell and cell-ECM interactions, drug responses, and applications in personalized medicine [180]. Differentiation of iPSCs into dopaminergic (DA) neurons on organ-on-chip platforms can yield more mature and homogeneous populations than in 2D culture, in part because the automated system supplies fresh medium and removes metabolic waste [181] Because these devices contain compartmentalized and interconnected nodes, they support disease modeling with co-cultures to study cell-cell interactions, neuronal networks, and tissue-level physiology. For instance, Kamudzandu et al. reported that a brain-on-chip model reproduced the basal ganglia circuitry model to study normal and diseased functions. Neurons from the substantia nigra (SN), globus pallidus, and striatum were harvested from animal brains and cultured in separate compartments; axonal outgrowth, functional connectivity, and electrical activity were then measured. The recorded activity closely matched expectations from in vivo basal ganglia circuitry, and each neuronal population directed neurite extension as anticipated (Table 2; Fig. 7) [174].

Conclusions and future prospects

Parkinson's disease remains one of the most challenging neurodegenerative disorders to model due to its multifactorial causes, progressive nature, and the complex interaction of genetic and environmental factors. In vitro

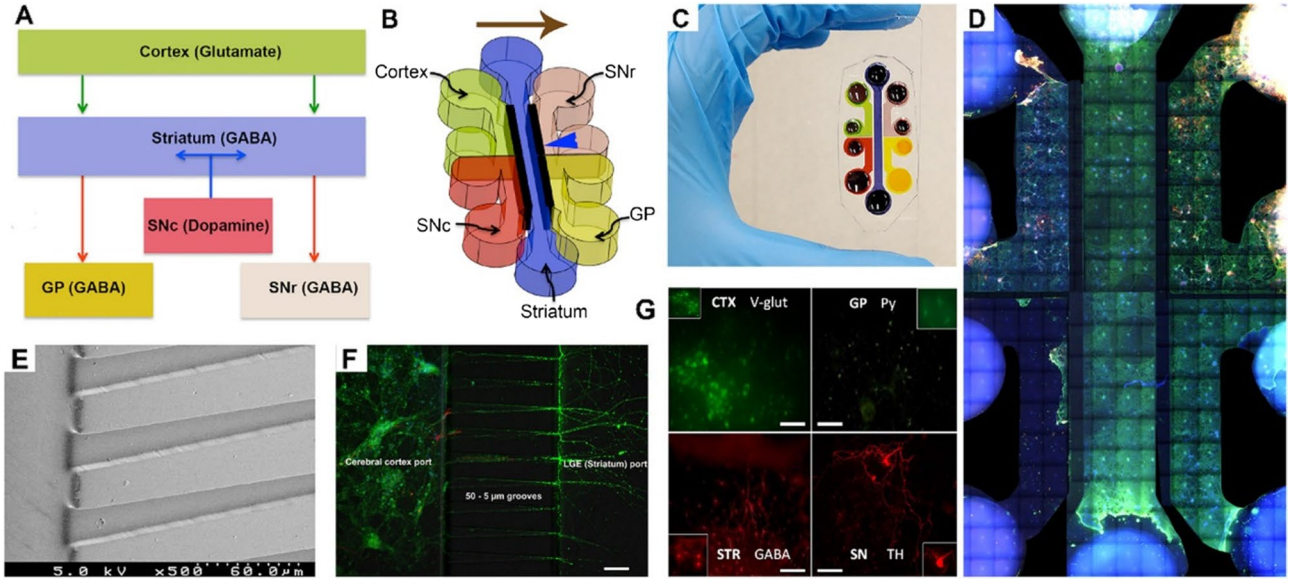


Fig. 7 Basal ganglia (BG) neuronal circuitry in an in vitro model. **A** shows a schematic representation of the BG circuit where nuclei are connected via neurotransmitter-regulated axons (arrows). Red arrows indicate GABAergic inhibition, green arrows show glutamatergic excitation, and blue arrows represent dopaminergic transmission. Figure (**B**) is the five-port microfluidic device mimicking the BG circuit; tapered micro-channels guide axon orientation (blue arrowhead). The brown arrow shows taper direction, while black lines mark micro-channel positions. **C** A PDMS microfluidic mold created by soft lithography, with colored dyes indicating fluid separation and neuronal types matching those in (**A**) and (**B**). **D** A fully seeded device with DAPI (blue), GFAP (red), and β-III-tubulin (green) staining. **E** An SEM image of the tapered micro-channels. **F** A fluorescent image of neurons (green) and astrocytes (red) across 50–5 µm micro-channels; neurons extend from the cortical to the striatal port, replicating in vivo behavior. **G** Antibody staining of neuronal subtypes in cortical, striatal, GP, and SN regions using V-GLUT2, GABA, PY, and TH antibodies, respectively [174]

systems can bridge the gap between preclinical and clinical research by enabling cell-specific interrogation, reproducibility, and scalability, while reducing animal use, cost, and ethical concerns [182–184]. Additionally, in vitro disease models can reveal cell-type-specific disturbances, uncover pathways related to neuronal vulnerability, and serve as effective platforms for testing therapeutic interventions. For example, single-cell studies of iPSC-derived dopaminergic neuron models revealed intrinsic cellular heterogeneity. Disease-associated genes, such as SNCA, MAPT, and UCHL1, were enriched in vulnerable subtypes, whereas resistant subtypes exhibited enhanced oxidative phosphorylation and stress responses, supporting the use of iPSC models to understand the molecular basis of vulnerability [185].

Current in vitro platforms have several limitations, which include: (i) inconsistencies in standardization and reproducibility across procedures and biological materials; (ii) immaturity and lack of late-onset phenotypes, such as network formation and myelination; (iii) variability in cell phenotypes, where cellular stress pathway activation disrupts cell-type specification and spatial organization; (iv) lack of a microvasculature and limited nutrient diffusion, leading to necrotic cores and elevated stress markers; and (v) constraints in scale and throughput that hinder drug screening and thorough validation [186–191].

2D and 3D models are complementary, not hierarchical. Two-dimensional cultures are still highly valuable for mechanistic work. They enable rapid studies, high-throughput drug screening, and genetic manipulation because they are simple and scalable. In contrast, 3D systems include spheroids, organoids, bioprinting, and microfluidic platforms. They are used to model complex tissue architecture, cell-cell and cell-matrix interactions, and microenvironmental effects [192, 193]. Both systems have their own disadvantages: 2D cultures may oversimplify, while 3D cultures, though more physiologically relevant in some respects, may introduce variability, hypoxia, or batch effects that complicate interpretation. Therefore, the choice of model should be based upon the specific biological question being addressed, with 2D and 3D approaches often best used in combination to validate findings across different levels of complexity [194–196].

Future research should implement these strategies to enhance physiological relevance and reproducibility, thus accelerating the translation of disease-modifying therapies and personalized approaches for Parkinson's disease. Key priorities should include: (i) incorporating vascularization and immune system components into organoid systems via co-culture with endothelial or immune cells or through bioengineered scaffolds; (ii) applying bioprinting and microfluidic technologies to create standardized and reproducible platforms with controlled spatial organization and nutrient exchange;

(iii) integrating CRISPR-based genome editing and single-cell omics to systematically dissect the genetic and molecular pathways underlying neuronal vulnerability; and (iv) combining 3D culture platforms with computational systems to establish predictive frameworks for disease progression and therapeutic response [196–201].

Abbreviations

2D	2-Dimensional
3D	3-Dimensional
6-OHDA	6-Hydroxydopamine
AD	Alzheimer's Disease
ALP	Autophagy-Lysosome pathway
α-syn	Alpha-Synuclein
BG	Basal Ganglia
CMA	Chaperone-mediated autophagy
CNS	Central nervous system
DA	Dopamine
ECM	Extracellular Matrix
ER	Endoplasmic reticulum
ETC	Electron transport chain
GelMA	Gelatin methacrylate
GPe	Globus pallidus externus
GPi	Globus pallidus internus
HLA	Human Leukocyte Antigen
HSPs	Heat shock proteins
iPSC	Induced pluripotent stem cell
LB	Lewy bodies
LDD	Dementia with Lewy Bodies
LP	Lysosomal Proteolysis
LRRK2	Leucine-Rich Repeat Kinase 2
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PAm	Polyacrylamide
PC	Polycarbonate
PD	Parkinson's Disease
PDD	Dementia with pPD
PDMS	Polydimethylsiloxane
PEI	Polyetherimide
PEG	Polyethylene glycol
PET	Positron emission tomography
PMMA	Poly(methyl methacrylate)
p-Tau	Phosphorylated tau
PVA	Poly(vinyl alcohol)
Ran	Ras-related nuclear protein
ROS	Reactive oxygen species
SN	Substantia nigra
SNpc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
STN	Subthalamic nucleus
TH	Tyrosine hydroxylase
UPS	Ubiquitin-Proteasome System
VPS35	Vacuolar Protein Sorting 35

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Authors' contributions
B.K. designed and wrote the manuscript. B.R. contributed to the manuscript writing and tables. E.S. provided ideas and suggestions for revision. G.A. and O.O.C., S.E., and Y.G.O. provided revisions. M.A.J. contributed to editing the manuscript. All authors have read and approved the final manuscript.

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