Using multi-organ culture systems to study Parkinson's disease

Perspective:

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Abstract

In recent years, it has been revealed that Parkinson's disease pathology may begin to manifest in the gastrointestinal track at a much earlier time point than in the brain. This paradigm shift has been suggested following evidence in humans that has been reproduced in animal models. Since rodent models cannot recapitulate many of the human disease features, human induced pluripotent stem cells (iPSCs) derived from Parkinson's patients have been used to generate brain organoids, greatly contributing to our understanding of the disease pathophysiology. To understand the multifaced aspects of Parkinson's disease, it may be desirable to expand the complexity of these models, to include different brain regions, vasculature, immune cells as well as additional diverse organ-specific organoids such as gut and intestine. Furthermore, the contribution of gut microbiota to disease progression cannot be underestimated. Recent biotechnological advances propose that such combinations may be feasible. Here we discuss how this need can be met and propose that additional brain diseases can benefit from this approach.

Keywords: Parkinson's disease, brain organoids, intestine organoids, organ on-chip, patient on-chip, gut-brain

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33 **Parkinson's disease**

34 The synucleinopathy Parkinson's disease (PD), is a neurodegenerative disease characterized
35 by abnormal accumulation of the alpha-synuclein (α-Syn) protein in the brain. A key
36 neuropathological hallmark of PD are neuronal inclusions positive for the protein α-synuclein
37 known as Lewy bodies and Lewy neurites. Most patients present a movement disorder that
38 can include tremor, slowness of movement, rigidity and postural instability [1]. Additional non-
39 movement symptoms include neuropsychiatric problems, altered smell sense, sleeping
40 difficulties as well as orthostatic hypotension, constipation, and urinary incontinence [2]. The
41 motor symptoms are attributed to dopaminergic cell loss within the substantia nigra (SN) pars
42 compacta, resulting in subsequent dysfunction of the basal ganglia, a cluster of deep nuclei
43 that participate in the initiation and execution of movements [3]. Genetics plays an important
44 role in Parkinson's disease, with disease–susceptibility loci including more than 90 genes,
45 including *SNCA (Synuclein Alpha), LRRK2 (Leucine Rich Repeat Kinase 2), GBA*
46 *(Glucosylceramidase Beta),* and *MAPT (Microtubule Associated Protein Tau)* [4]. However,
47 genetics is not the only contributing factor to the disease; it is likely that the interactions
48 between age, genetics, epigenetics and environmental factors can trigger the disease [5-7]. It has
49 been recently suggested that COVID-19 may enhance disease progression, yet additional
50 studies are needed to investigate this suggestion in depth [8-10]. The genetic information
51 facilitated modelling of some of the common monogenic Parkinson's mutations using genetic
52 approaches in cellular and animal systems. For example, it has been demonstrated that *GBA*
53 mutations in mouse models result in increased levels of α-Syn [11-13]. Forced expression of the
54 GBA enzyme in mouse brains ameliorated histopathological and memory aberrations [13].
55 Accumulation of the α-Syn protein in the brain occurs rather late in the disease. Interestingly,
56 about 14 years after fetal dopaminergic neurons are implanted in the striatum of PD patients,
57 these neurons exhibit Lewy pathology in the cell bodies and axons [14, 15]. These findings and
58 others resulted in the theory that PD may be a prion-related disorder, yet some of the criteria
59 fall short for the full definition [16, 17]. Postmortem brain sections of these PD patients at
60 different time points post-implantation suggest the inflammation and microglial activation in
61 the grafts are present long before the accumulation of α-Syn (Fig. 1A). These findings
62 contributed to the concept that microglia participate in the propagation and spread of α-Syn
63 pathology [16, 17]. Additionally, a number of studies suggest the adaptive immune system is
64 involved in disease progression [18]. In recent years, it has become evident that accumulated α-
65 Syn protein can be observed in the gastrointestinal (GI) tract in the early stages of the disease

66 [19]. Interestingly, neuronal cell loss in the enteric nervous system of PD patients and animal
67 models has not been observed, despite the accumulation of α-Syn aggregates in the GI tract
68 [20, 21]. It has been proposed that there could be transfer of the aggregated α-Syn protein from
69 the GI tract to the brain [22].
70 Indeed, animal models have demonstrated that α-Syn protein aggregates can move from the
71 gut to the brain via the vagus nerve, particularly in aged mice [20, 23-25]. This transfer was also
72 observed in a mouse model exposed to an environmental toxin that induced the production
73 and secretion of the α-Syn protein, and that the recession of the autonomic nerve halted this
74 process [26] (see Fig. 1B). Supporting this notion, longitudinal analysis of people that have
75 undergone bilateral vagotomy suggested a decreased risk for the development of PD [27].
76 Introduced expression of the GBA enzyme using an inducible viral expression system with
77 high affinity for the peripheral nervous system in enteric neurons partially restored the GI
78 phenotype observed in mice overexpressing the α-Syn protein in Thy1-positive projection
79 neurons [25]. However, not only is the enteric nervous system relevant to the disease etiology in
80 the GI, rather both the gut endothelium [28], and gut microbiome contribute significantly [29-33].
81 The human body hosts a rich collection of microorganisms, most of them residing in the gut,
82 where they are involved in food digestion as well as providing the host different by-products
83 [34]. The brain gut-axis is bidirectional. The brain affects the intestinal activity and function [35]
84 and the gut microbiome is involved in the maintenance of the mucus in the gut epithelium,
85 and its metabolites affect the immune system and brain function [34]. Interestingly, PD patients
86 have been found to have a different composition of microbiota [32, 36, 37] and the patients exhibit
87 altered concentrations of short chain fatty acids, as well as altered plasma concentrations of
88 different cytokines, suggesting the involvement of the immune system [31, 36]. The intestinal
89 microbiota has an instructive role in PD, and is required for the motor deficits, microglia
90 activation and α-Syn pathology [30]. Supporting this notion is the finding that the introduction
91 of specific microbial metabolites is sufficient to induce the pathology [30]. Implantation of PD
92 fecal microbes into mice resulted in a more pronounced phenotype in comparison with those
93 obtained from healthy controls [30] (Fig. 1C). It has been proposed that the exposure to amyloid
94 proteins existing in gut microbes can promote the aggregation of α-Syn [38].
95 The complex interactions between PD, gut microbes, and the immune system has been
96 demonstrated in PTEN Induced Kinase 1 (*Pink1*) knockout mice [39].
97 Mutations in either *PINK1*, a ubiquitin kinase, or in Parkin RBR E3 Ubiquitin Protein Ligase
98 (*PRKN*), also known as Parkinson Disease Protein 2, *PARK2*, are associated with PD. The

99 function of the encoded proteins is related to mitophagy and they were found to contribute to
100 the understanding of the gut-brain axis in PD pathology [40, 41]. In relation to PD, knockout
101 mice for both *Pink1* and *Park2* demonstrated a pronounced inflammatory response to
102 exhaustive exercise [42]. Another study revealed there is an increase in the presentation of
103 mitochondrial antigens in immune cells in the absence of PINK1 or Parkin, suggesting that
104 autoimmune mechanisms are involved in the development of PD [40]. Furthermore,
105 autoimmune mechanisms evolve when the intestines of *Pink1* knockout mice are infected
106 with bacteria, resulting in the establishment of a group of cytotoxic mitochondria-specific T
107 cells in the periphery and in the brain [39]. These specific T cells are able to kill dopaminergic
108 neurons *in vitro.* The mice develop motor impairment, which can improve following L-
109 DOPA treatment [39]. L-DOPA (**l**-3,4-dihydroxyphenylalanine), an amino acid precursor that
110 passes the blood-brain barrier (BBB) to be taken up by the dopaminergic neurons and
111 converted into dopamine, is commonly used for treatment of PD patients. The gut microbiota
112 can also be involved in metabolism of L-DOPA, which can interfere with the disease
113 management [43]. Eradicating some bacteria, such as *Helicobacter pylori,* more commonly
114 found in PD patients, has been shown to improve patients' symptoms and enhanced the
115 effectiveness of L-DOPA treatment [37]. A systematic study identified microbial species that
116 convert L-DOPA to dopamine by tyrosine decarboxylase enzymes, then determined which
117 species can dehydroxylate dopamine to *m*-tyramine. This second activity was found to be
118 related to a single nucleotide polymorphism that induced an amino-acid substitution. To
119 facilitate L-DOPA application and reduce its processing, the investigators identified a small
120 molecule inhibitor that can inhibit the dehydroxylation of dopamine [43]. Despite all these
121 studies indicating the role of the microbiome in PD, it should be noted that mice lacking the
122 microbiome do not show any major neuronal dysfunction or PD like symptoms.
123
124 **Development of organoid models to study PD**
125 Organoids are 3D structures grown from stem cells, consisting of organ-specific cell
126 types that self-organize through cell sorting and spatially restricted lineage commitment [44-46].
127 The popularity of human derived organoids for modeling development and disease has been
128 increasing in recent years (Fig. 2A). Prerequisite for the development of organoid models for
129 human diseases was the introduction of cell reprogramming by Yamanaka [47]. This
130 technological breakthrough was used to generate iPSCs from PD patients, which were then
131 differentiated into dopaminergic neurons [48, 49]. Nevertheless, it should be noted that neurons
132 and other tissue-like structures that are derived from iPSCs usually lack maturity, and this is

133 an issue that has not been completely solved yet [50]. Some approaches to solve this caveat has
134 been co-culturing of different cell types, for example co-culturing of human neuronal
135 progenitors with rodent astrocytes resulted in mutually synergistic maturation [51]. Generation
136 of isogenic lines in which a particular mutation was corrected, or a new mutation was
137 introduced into a control line, markedly reduced the variability due to different genetic
138 backgrounds [52, 53]. Most of these studies investigated a common mutation in *LRRK2* [48, 49, 53].
139 Genetic engineering of human cells was dependent upon the introduction of efficient genome
140 editing tools as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases
141 (TALENs), and more recently clustered regulatory interspaced short palindromic repeat
142 (CRISPR)/Cas-based RNA-guided DNA endonucleases [54]. An additional technological
143 breakthrough was the ability to generate different types of human organoids from iPSCs
144 derived from patients, human embryonic stem cells, or organ-restricted stem cells [44]. Early
145 success has been noted with the establishment of organoids from endodermal-derived organs
146 such as the esophagus, gut, stomach, liver, pancreas and lung [55-57]. Mesodermal-derived
147 organs include kidney, heart, cartilage, bone, reproductive organs and muscle. Successful
148 renal and endometrium organoids have also been generated [55, 58, 59]. Ectoderm-derived organs
149 include two main tissues; the surface ectoderm that will develop into skin and associated
150 glands and hair, and the neural ectoderm that will develop into the brain, the spinal cord and
151 the neural crest [60]. Most relevant to current research on PD are brain organoids. Pioneering
152 research from the lab of the late Yoshiki Sasai demonstrated that stem cells can recapitulate
153 several features of organogenesis, including cell differentiation, spatial patterning and
154 morphogenesis, and successfully generated organoids resembling different brain regions and
155 retina [45]. Subsequent research from Lancaster and Knoblich demonstrated that it is possible to
156 obtain a mixed regional identity using a relatively simple media [46, 61]. This field has increased
157 dramatically over the last few years with multiple protocols [62]. Scientists can now generate
158 connections between different brain regions by fusing structures known as "assembloids",
159 thus mimicking a higher organization level, which may prove critical in modeling diseases [63].
160 There have also been some advances in regards to generating functional networks [64]. Multiple
161 studies have characterized the cell repertoire and diversity in organoids obtained from
162 different protocols using single-cell (sc) analyses [65]. Overall, the general notion in the field is
163 that although brain organoids are not identical to the developing human brain, these are
164 useful models. Comparisons of sc-RNA-seq data from multiple brain organoids to data
165 derived from the developing human brain indicate that the developmental trajectories and cell
166 types in the organoids resemble those observed in the human embryonic brain [66]. A different

167 study using both their own data combined with published data doubts the fidelity of the
168 model and claims that despite presenting a broad cell classes, brain organoids do not
169 recapitulate distinct cellular subtype identities or the appropriate progenitor maturation [67].
170 Midbrain organoids containing dopaminergic neurons are of special interest for
171 understanding PD. Two studies aimed at the generation of midbrain organoids started with
172 3D aggregates of neuroepithelial stem cells treated with activators of the WNT and Hedgehog
173 pathways, embedding them in Matrigel droplets, followed by inducing the differentiation into
174 human midbrain organoids [68, 69]. In these midbrain organoids they observed a large
175 population of TH-, LMX1A-, and FOXA2-positive neurons, which were also positive for
176 other ventral midbrain identity markers. They further demonstrated the presence of both A9
177 and A10 subtypes of midbrain dopaminergic neurons (GIRK2 and TH; CALBINDIN and
178 TH, respectively). The presence of astrocytes and oligodendrocytes was also verified, and
179 myelination was observed. Synaptic connectivity and electric activity were also demonstrated
180 [68, 69]. A more recent study has optimized the protocol for midbrain organoid generation which
181 could enable efficient drug testing [70].
182 The relevance of this model is underscored by studies demonstrating that midbrain-
183 specific organoids derived from PD patients carrying the LRRK2-G2019S mutation can
184 recapitulate disease-relevant phenotypes [71, 72]. In one study, isogenic 3D midbrain organoids
185 with or without the PD *LRRK2-G2019S* mutation were generated and shown to recapitulate
186 pathological hallmarks [71]. These mutant organoids exhibited increased susceptibility to
187 induced neurotoxic damage, resulting in increased apoptosis. Phosphorylated α-Syn was
188 localized in endosomes, and there was an increase in mitophagy. Reduction in the expression
189 of a specific thiol-oxidoreductase, TXNIP, can significantly decrease aggregated α-Syn [71].
190 Another study focused on the cause for the decreased number and complexity of midbrain
191 dopaminergic neurons in *LRRK2-G2019S* mutant organoids compared to controls [72]. The
192 floor plate marker FOXA2, required for midbrain dopaminergic neuron generation, is
193 increased in PD patient-derived midbrain organoids, suggesting a neurodevelopmental defect
194 in midbrain dopaminergic neurons expressing LRRK2-G2019S [72].
195
196 **Future possibilities**
197 Despite being a movement disorder, it is clear that PD etiology is not restricted to the
198 brain. PD is a multisystem condition that involves several organs and systems. Evidence of
199 early involvement of the intestine and its' interaction with the microbiome are accumulating.

200 In parallel the understanding of the involvement of the immune system both innate and
201 adaptive components are revealed. The cross-talk between the gut and the brain unfolds
202 through transfer of substances/proteins/metabolites via the enteric nervous system and the
203 vagus nerve to the brain, and with a possible contribution of vasculature system. This view
204 dictates a more complex approach to PD modeling. Is it possible to combine all of these
205 systems for *in vitro* studies? (see Fig. 2B,C for possible schemes).
206 Intestinal organoids have already been used in personalized medicine approaches,
207 such as predicting the efficacy of Cystic Fibrosis drug treatments [73, 74]. Bioengineering
208 approaches has enabled incorporating human intestinal organoids into small micro-
209 engineered Chips [75]. Both intestinal and epithelial organoids can be initiated from single
210 adult stem cells obtained from patient epithelial biopsies, thus skipping the long
211 reprogramming process required when starting from a somatic cell. However, the
212 introduction of the microbiome to this culture is not a trivial task. One possibility may be to
213 introduce specific bacterial-derived metabolites to the media, which would first require in-
214 depth analysis and screening of multiple bioactive metabolites. Bacterial metabolites could be
215 sensed either by G-protein-coupled receptors that are often expressed by intestinal epithelial
216 cells, specific subsets of immune cells, or by tissues and/or cell types that are central to the
217 host metabolism, such as pancreatic islet cells, adipocytes and enteroendocrine cells of the
218 gut [76]. A large proportion of the endogenous microbiota is anaerobic, and are localized on the
219 apical cell surface, facing the lumen. However in culture, micro-organisms added to the
220 media will only be able to interact with the basal and not the apical side of the cells, and be in
221 an environment with relatively high oxygen concentration. To overcome these challenges,
222 one approach has been to reverse the polarity, creating an "inside out" organization of the
223 organoids [77]. Another approach has been to introduce the microorganisms into the lumen by
224 microinjection [78]. This study managed to achieve a complex and stable microbiome by
225 implementing innovations in bioengineering, using advanced organoid culture,
226 microfabricated culturing devices, computer vision and semiautomated injection devices [78].
227 Organs-on-chip are microfluidic cell cultures that were generated as possible alternatives for
228 the use of animal models with the promise that they can recapitulate the structure, function,
229 physiology, and pathology of living human organs *in vitro* and possibly mimic inter-organs
230 interactions [79-83]. Most of the organoid systems cannot be precisely controlled and they have a
231 limited capacity to provide for instructive cues that are required for organogenesis [82]. The
232 initial intestinal organoids are limited as they are lacking endothelium-lined blood vessels
233 and immune cells and are not exposed to fluid flow and other mechanical constraints. These

234 deficits may be overcome in gut chip models [79, 84]. It is possible to generate one layer of
235 intestinal epithelial cells on top of a lower layer of microvascular endothelial cells to
236 fabricate a three-dimensional villi structure [84]. Furthermore, it is possible to co-culture these
237 villi structures with living microbes [85, 86]. It should be noted that organs-on-chip and
238 organoids represent two different but complementary approaches and the possibility to
239 integrate these two approaches in a synergistic way is extremely exciting [82]. More recently,
240 the organoid and organ chip approaches have been combined to develop a microfluidic
241 primary human intestine chip model [87]. A possible caveat is that most microfabricated
242 devices rely on the silicone-based polymer polydimethylsiloxane (PDMS), which could
243 absorb small and hydrophobic molecules.
244 Introduction of the enteric nervous system (ENS) into these complex models may prove to be
245 a challenge [88, 89]. The ENS develops both from the vagal and the sacral neural crest which
246 can be differentiated from human pluripotent stem cells (ES). Following their production, the
247 cells were co-cultured in the presence of smooth muscle cells, and were able to form
248 interconnections [90]. These cells were also transplanted into immunodeficient mice and were
249 found to repopulate the host colon. A tissue-engineering approach was used to develop
250 human intestinal organoids incorporating ENS [91]. To incorporate vagal neural crest cells and
251 ENS precursors into the developing intestinal organoids, mid/hindgut spheroids and neural
252 crest cells were mechanically co-aggregated. The crest cells were derived from human stem
253 cells by low-speed centrifugation and then the aggregates were transferred to three-
254 dimensional growth conditions for twenty-eight days. To achieve a complete maturation and
255 add vascularization, these cultures were transplanted in mice. This approach yielded a tissue
256 that was highly organized, integrated into the intestinal smooth muscle and drove NO-
257 dependent relaxation. The authors noted that although CHAT-positive neurons were detected
258 *in vitro,* they were not detected following transplantation, possibly reflecting the fetal nature
259 of the transplanted organoids. Another study improved the maturity of the organoids by using
260 an early *in vivo* co-implantation of the stem cell derived enteric neural crest cells with the
261 intestinal organoids [92]. Thus, so far there has not been an *in vitro* solution for combing the
262 ENS with the intestine as there was a need to implant different components to mice.
263 Advances have also been made in introducing the immune system into organoid cultures [93].
264 In one study, human intestinal stem cell-derived enteroid monolayers were co-cultured with
265 human monocyte-derived macrophages [94]. The addition of macrophages changed the
266 physiology of enteroid monolayers by enhancing their barrier function and maturity. Another
267 study added human polymorphonuclear leukocytes (PMNs), a cell population comprised

268 mainly of neutrophils, to human intestinal organoids, and then introduced either commensal
269 or pathogenic bacteria [95]. While the commensal bacteria did not harm the development of the
270 intestinal organoids, the pathogenic bacteria induced the loss of epithelial integrity and the
271 production of interleukin 8 (IL-8). IL-8 induced rapid recruitment of neutrophils [95]. Another
272 study used coculturing of intestinal organoids with fetal TNF-α producing CD4+ T cells,
273 demonstrating that when T cells are introduced in low numbers they promote epithelium
274 development, while in high numbers they mediate inflammation [96]. In a different study,
275 human intestinal organoids were cocultured with human T lymphocytes, inducing the *in vitro*
276 maturation of the organoids [97]. IL-2 was identified as the major factor that induced
277 maturation [97]. A different approach involves transplanting the human organoids under the
278 kidney capsule of immunocompromised mice in order to recruit missing cell types [98].
279 For PD studies, the main focus has been on midbrain-specific organoids, yet the
280 ability to increase the complexity and the representation of additional cell types as well as
281 defining the conditions that support the growth of other brain regions may be advantageous.
282 Microglia are one of the target cell types to be included into PD models, and is of great
283 importance for studying the involvement of innate immunity in the disease onset and
284 progression [99]. Activation of microglia are thought to promote the disease or alternatively be
285 involved in some neuroprotective aspects [100, 101]. Here we will discuss two potential methods
286 for including microglia (reviewed in [99, 102]). The ability to generate functional microglia is not
287 trivial and requires careful characterization of the cells [99, 102]. The first method is to develop
288 brain organoids that contain endogenous microglia. Minimal modifications to the original
289 Lancaster cerebral organoid protocol [61] resulted in efficient development of microglia from
290 mesodermal progenitors [103]. These cells resemble adult microglia by gene expression and
291 likely reached maturation through the interaction with the other cell types in the culture.
292 However, the organoids represent a relatively early stage of development in a limited portion
293 of the brain [103]. Another protocol used microglia-like cells that were derived from hiPSCs
294 using a simplified protocol with stage-wise growth factor induction [104]. The second method is
295 to exogenously add iPSC-derived microglia to the brain organoids. For generation of iPSC-
296 derived microglia, several protocols have been developed, and there are commercially
297 available cells as well [99]. In one study, the microglia-like cells were co-cultured with brain
298 organoids [105]. Adaptive immune system cells are also to be considered in the context of a
299 complex modeling entity. CD4+ T cells have a role in the intestine but T cells that recognize
300 self-antigen that are CNS derived can invade the brain during PD progression [106, 107]. These

301 immune cells invade the brain via a dysfunctional BBB which has been reported in PD
302 patients [108-110]. The BBB is a highly polarized interface between the brain and the
303 vasculature, composed of tightly connected endothelial cells that are strongly associated with
304 astrocytic endfeet processes and pericytes. There have been many advances in the *in vitro*
305 modeling of this neurovascular unit in recent years [111]. Organ-on-chip technology has been
306 used to generate a human BBB derived from iPSCs [112, 113]. The unit was composed of brain
307 microvascular endothelial-like cells, astrocytes, and neurons maintaining a "brain-side" and a
308 "blood-side". An alternative configuration used human hippocampal neural stem cells,
309 cortical microvascular endothelial cells, astrocytes, and pericytes of cortical origin in a
310 microfluidic BBB-vasculature-brain chip [114]. The study identified a previously unknown
311 metabolic coupling between the BBB and neurons [114]. Further studies introduced hypoxia
312 during the development of the BBB *in vitro,* resulting in improved functionality of the barrier
313 [115]. A recent study used the Organs-on-Chips technology to engineer a human Brain-Chip
314 representative of the substantia nigra area of the brain containing dopaminergic neurons,
315 astrocytes, microglia, pericytes, and microvascular brain endothelial cells, cultured under
316 fluid flow. They were capable of reproducing several key aspects of Parkinson's disease,
317 including accumulation of phosphorylated αSyn (pSer129-αSyn), mitochondrial impairment,
318 neuroinflammation, and compromised barrier function [116]. However, the BBB is not the only
319 entry point for immune cells into the brain, as CD4+ T cells can enter the brain via the
320 choroid plexus (CP) [117, 118]. The CP is formed in each of the four ventricles of the brain,
321 consisting of epithelium cells connected by tight junctions, blood vessels and other cell types.
322 The CP forms a barrier between the blood and the cerebrospinal fluid (CSF). The CP
323 produces CSF and also secretes many other factors and proteins that have the potential to
324 affect the proliferation of adult stem cells [119]. During the aging process, the normal functions
325 and morphology of the choroid plexus are compromised. These changes are further
326 intensified in neurodegenerative diseases such as Alzheimer's disease [120]. Choroid plexus-like
327 structures have been noticed in cerebral organoids in the Lancaster's protocol [121]. One
328 protocol that was designed towards generation of this dorsal-medial structure from stem cells
329 was developed in Yoshiki Sasai's lab is based on regulating WNT and BMP (bone
330 morphogenetic protein) signaling, and is applicable for either mouse or human cells [122, 123].
331 Recently, Lancaster modified the original protocol to enhance the formation of the choroid
332 plexus and demonstrated formation of a barrier and CSF [124].
333 Will it ever be possible to combine the gut and the brain with all the auxiliary cell
334 types and organs that are relevant for the disease onset and progression in one cultured

335 system? Such an achievement requires further understanding of the biological components
336 and advances in bioengineering. Efforts in this direction are seen in recent years with the
337 emergence of "patient-on-chip" model systems [82, 125, 126]. Initial reports describe mimicking
338 single organs, each requires its own specialized conditions, that are than combined in
339 innovative manners to form a more complex model. One approach establishes the initial
340 growth of each component individually before combining the different organs in a Lego-like
341 system [80]. More recently, human organ-chip models of the gut, liver and kidneys were
342 fluidically coupled by vascular endothelium lined channels [127]. These channels are separated
343 by a porous extracellular-matrix-coated membrane, lined with human organ-specific
344 parenchymal cells. The fluid path has an integrated arteriovenous reservoir that mimicked the
345 systemic circulation. The presence of the vascular endothelium-covered channels enables the
346 use of a blood substitute, whereas the parenchymal-covered channels of each organ uses a
347 different organ-specific optimized media. The system was designed to conduct quantitative
348 measurements of pharmacokinetic responses to drugs [127]. An alternative design involves an
349 automated culture that includes liquid-handling robotics, custom software with an integrated
350 mobile microscope, perfusion, medium addition, fluidic linking, sample collection and *in situ*
351 microscopy imaging of eight organ chips inside a standard tissue-culture incubator [128]. The
352 organs, including intestine, liver, kidney, heart, lung, skin, BBB and brain, are cultured for
353 three weeks with their fluids intermittently coupled using a blood substitute. The system is
354 modular and the configurations can be changed. This system has been used to model drug
355 kinetics and metabolism in different organs. Another multiorgan-on-a-chip platform known
356 as MINERVA (MIcrobiota-Gut-BraiN EngineeRed platform to eVAluate intestinal
357 microflora impact on brain functionality) has been designed to model neurodegenerative
358 diseases such as PD and Alzheimer's disease [129]. Overall, we envision that investigating
359 human brain diseases should be viewed in the context of a similar multi-organ configuration,
360 and could be studied either individually or in various creative combinations.
361

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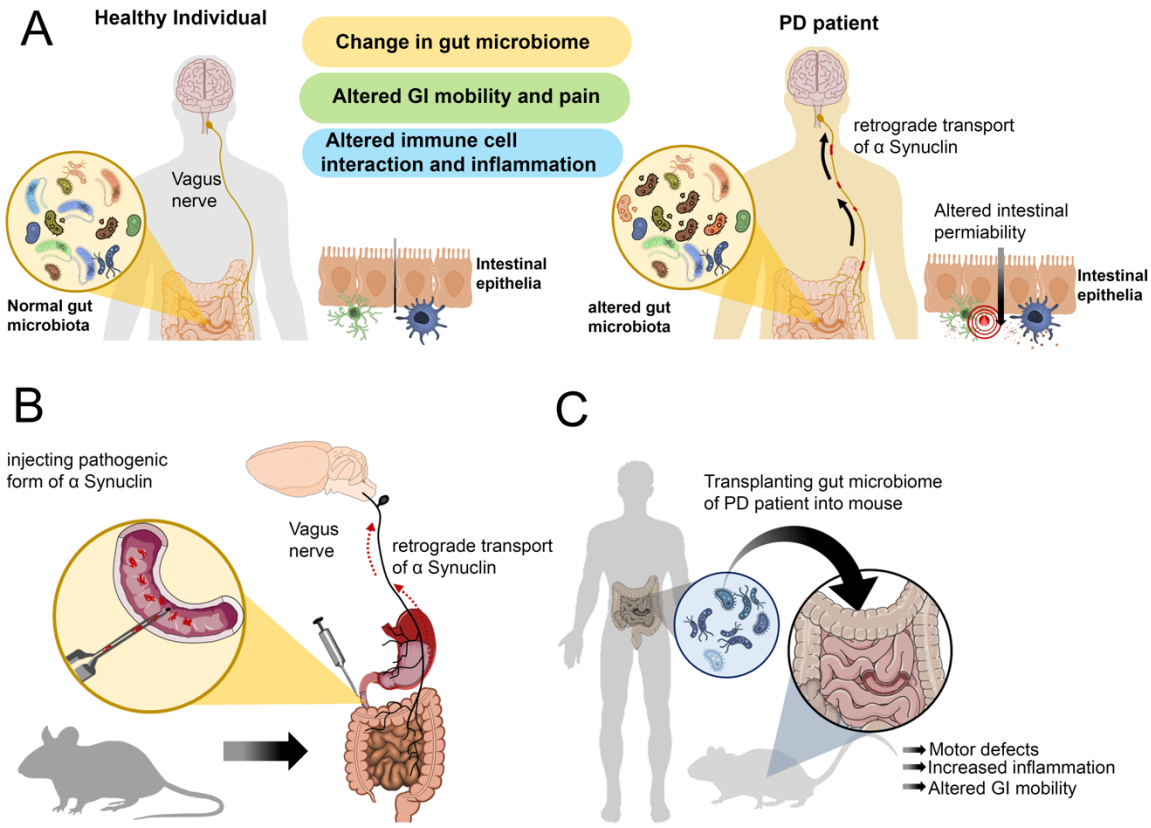

Figure 1: (A) Comparative changes in the gut-brain axis of a PD patient compared to a healthy individual. (B) α-Synuclein can be transported in a retrograde fashion from the gut to the brain, recapitulating PD pathology in the mouse. (B) Transplantation of gut microbiome from PD patient into mice leads to several PD phenotypes, including motor deficets and chronic inflamation.

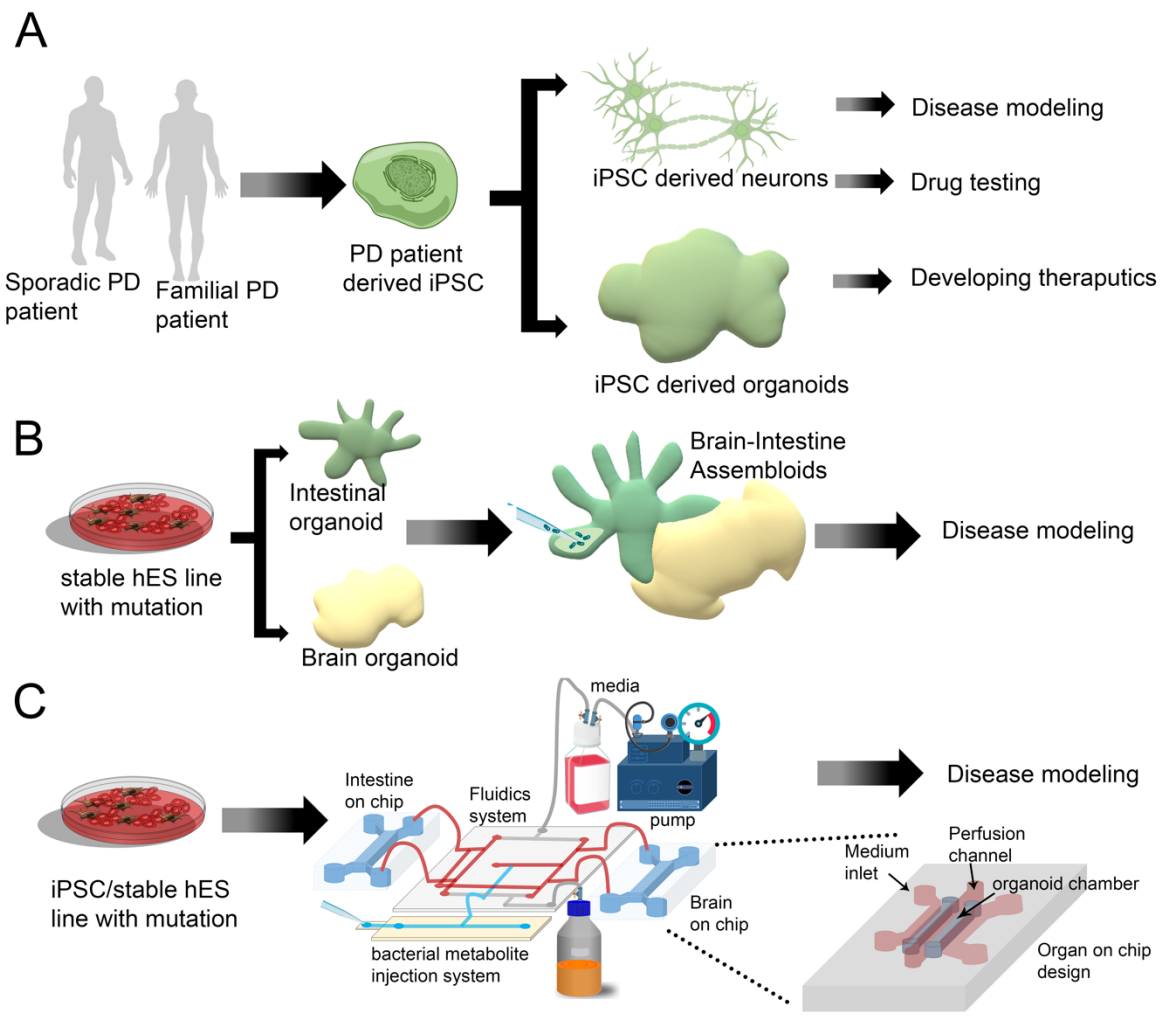

Figure 2: Present and future *in vitro* models for PD. (A) Existing approaches to model PD using iPSC-derived neurons/ brain organoids. (B) Future prospect can combine several organoids (gut & brain organoids) to make an assembloid system where microbiota derived metabolites could be injected and thus it can mimic the gut brain axis in PD. (C) Further usage of the Organ on CHIP system (the magnified view depicts the layout of Organ on chip system) could mimic the gut brain axis where the sophisticated fluidics system can establish a connection between two on Chip organs. In one CHIP it is possible to create intestine and brain in the other. The microfuidic system would establish the connection between the two and there would be an injection site for bacterial metabolites as depicted in the image. These approaches will improve the PD modeling and can be used to study disease progression or it can help in bulk drug screening.