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Studying Human Neurological Disorders Using Induced Pluripotent Stem Cells: from 2D Monolayer to 3D Organoid and Blood Brain Barrier Models

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Abstract

Neurological disorders have emerged as a predominant healthcare concern in recent years due to their severe consequences on quality of life and prevalence throughout the world. Understanding the underlying mechanisms of these diseases and the interactions between different brain cell types is essential for the development of new therapeutics. Induced pluripotent stem cells (iPSCs) are invaluable tools for neurological disease modeling, as they have unlimited self-renewal and differentiation capacity. Mounting evidence shows: 1) various brain cells can be generated from iPSCs in 2-dimensional (2D) monolayer cultures; 2) further advances in 3D culture systems have led to the differentiation of iPSCs into organoids with multiple brain cell types and specific brain regions. These 3D organoids have gained widespread attention as *in vitro* tools to recapitulate complex features of the brain, and 3) Complex interactions between iPSC-derived brain cell types can recapitulate physiological and pathological conditions of blood-brain barrier (BBB). As iPSCs can be generated from diverse patient populations, researchers have effectively applied 2D, 3D and BBB models to recapitulate genetically complex neurological disorders and reveal novel insights into molecular and genetic mechanisms of neurological disorders. In this review, we describe recent progress in the generation of 2D, 3D and BBB models from iPSCs and further discuss their limitations, advantages, and future ventures. This review also covers the current status of applications of 2D, 3D and BBB models in drug screening, precision medicine, and modeling a wide range of neurological diseases (e.g., neurodegenerative diseases, neurodevelopmental disorders, brain injury, and neuropsychiatric disorders).

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Introduction

Neurological disorders, such as neurodegenerative, neurodevelopmental, and psychiatric disorders, have emerged as a predominant healthcare concern in recent years, due to their severe consequences on quality of life and prevalence throughout the world. The causes and risk factors behind these diseases, including a combination of environmental and/or genetic factors, are complex and not well understood. Many neurological disorders are chronic and incurable conditions with debilitating effects that may continue for years or even decades post-diagnosis. For instance, Alzheimer's Disease (AD) is one such prominent disease taking a major toll on the aging population and places a huge burden on the healthcare system. In its 2018 Annual Report (473), the Alzheimer's Association estimated 5.7 million Americans are living with AD. Further research into the mechanistic causes of neurological disorders is essential for the development of new therapeutic approaches for disorders like AD.

Since there are many inherent barriers to conducting research directly on human subjects and primary brain samples, animal models have played a major role in studying neurological disorder mechanisms for decades and have been at the forefront of evaluating novel therapeutic approaches (39, 41, 71, 225, 234, 242, 247, 347, 354, 414). Nevertheless, considerable questions have emerged regarding the translatability of such animal-based research to human disease treatment since there are many differences in physiology, genetics and developmental patterns between human and animal brains (143, 144, 173, 237, 273, 416, 494). For example, microcephaly is a neurodevelopmental disorder in which brain size is markedly reduced. However, genetically engineered mice expressing several human microcephaly-related gene mutations have failed to recapitulate the severely reduced brain size seen in human patients (25, 164, 237, 268, 361). Discordance between preclinical drug studies conducted in animal models and human clinical trials has also raised significant concerns. Many prospective drugs for stroke, traumatic brain injury (TBI) and AD were found to be effective in animal experiments; however, these same drugs failed in clinical trials (4, 276, 402, 440, 458). The high clinical failure rate in drug development is based at least in part on the inability to adequately model human neurological disorders in animals. The advent of human induced pluripotent stem cells (iPSCs) with their ability to differentiate into different types of neural cells provide unprecedented opportunities to decipher the mechanisms of neuronal loss occurring in neurological diseases and to develop therapeutic approaches in conditions that may better translate to humans.

In 2007, the team of Shinya Yamanaka showed that human iPSCs could be reprogrammed from a small sample of skin-derived fibroblasts in a culture dish through the combined expression of pluripotency-associated transcription factors SRY (sex determining region Y)-box 2 (SOX2), octamer-binding transcription factor 4 (OCT4), Kruppel like factor 4 (KLF4) and myc pronto-oncogene (c-Myc) (428). Since skin biopsy remains an invasive approach,

more and more studies appeared in recent years describing the successful reprogramming of iPSCs from many other human somatic cell types such as blood and urine cells (82, 367, 374, 411, 456). Although there may be some methylation profile differences between iPSCs and embryonic stem cells (ESCs) due to the reprogramming process (117, 219), iPSCs and ESCs are considered similar in regards to cell morphology, proliferation and differentiation capacity (283), without the same ethical barriers as for cells in extra-embryonic tissues (e.g. placenta) (22, 68, 217, 428, 433, 488). Thus, human iPSCs are ideally suited for obtaining large quantities of neural cells required for disease modeling, drug screening and cell-based therapy (Figure 1). Importantly, iPSCs can be derived from patients who have neurological disorders, allowing researchers to study nervous system diseases within an endogenous human system. Development of iPSC technology, therefore, offers unique possibilities to investigate the cellular consequences of genetic vs. environmental factors in a human model as well as the underlying mechanisms (21, 39, 414).

The central nervous system (CNS) is composed of two major cell types: neurons, which are the primary signaling cells, and glial cells (astrocytes, oligodendrocytes, and microglia), which support neurons in various ways. Neural stem cells (NSCs) proliferate and differentiate into neurons, astrocytes, and oligodendrocytes, and play important roles in brain development (125, 482). Microglia, the innate immune cells of the CNS, are present throughout the CNS, but they are not derived from NSCs. Nevertheless, microglia play important roles in synaptic plasticity, neurogenesis, homeostatic functions, and immune activity (1). The blood-brain barrier (BBB) forms the critical biological barrier between the peripheral circulation and the CNS and maintains the strict environment required for normal brain function through selective substance crossing. The BBB can be altered under pathological conditions often exacerbating disease phenotypes. The BBB consists of four cell populations: brain microvascular endothelial cells (BMECs), pericytes, neurons, and astrocytes (404). Importantly, these various human brain cells have been generated from iPSCs in 2-dimensional (2D) monolayer cultures (1, 68, 217, 359, 431, 459), and the BBB can be constructed in the culture dish using human iPSC-derived astrocytes, neurons, and endothelial cells (373). Recent advances in 3-dimensional (3D) culture systems have led to the generation of brain organoids from human iPSCs by mimicking routine neurological development. This article includes 10 sections: generation of different brain cells from iPSCs in 2D monolayer cultures, blood-brain barrier modeling using iPSCs, generation of 3D organoids from iPSCs, the strengths and limitations of 2D and 3D culture approaches, modeling neurodegenerative diseases, modeling traumatic brain injury, modeling neurodevelopmental disorders, modeling neuropsychiatric disorders, screening drug toxicity, translation of human iPSCs: using of iPSCs in screening drug efficacy and precision medicine for neurological disorders. The focus of this article is to review recent progress in 1) generating 2D, 3D and BBB systems from human iPSCs in culture dishes, and 2) the applications of these systems to neurodegenerative, brain injury, neurodevelopmental, and neuropsychological disease modeling, drug screening, and precision medicine. The limitations, advantages, and future ventures associated with the use of iPSCs as *in vitro* models of neurological disorders are also discussed.

Generation of Different Brain Cells from iPSCs in 2D Monolayer Cultures

Neural stem cells

During fetal mammalian neurodevelopment, and to some extent in postnatal through adulthood, NSCs or neural progenitors cells (NPCs) act as self-renewing cells that can differentiate into multiple types of brain cells (27, 232, 323, 397), although there may be some restriction on their differentiation and proliferation potential (191). The development of multiple different protocols to generate NSCs has been of great interest for the study of neurodevelopment, as well as for identifying potential therapeutics targeted at neurodegenerative diseases (52). There are ongoing clinical trials in the United States and around the world that utilize NSC transplantation for a variety of diseases, including PD and ALS (163, 399).

Following developmental cues, some of the protocols grow iPSCs as uniform flat colonies (Figure 1) before being cultured in a low-attachment dish with chemically defined medium to drive the formation of embryoid bodies (EB) to mimic early human embryogenesis. EBs can then be cultured with specific growth factors [e.g., fibroblast growth factor 2 (FGF-2), usually presence of B27 and/or N2 medium formulation] for the formation of neural rosettes. Paired box protein-6 (Pax6)-positive neural rosettes are radial arrangements of cells that mimic the developmental pattern of neuroepithelial cells in the neural tube. The rosettes can then be re-plated in a monolayer culture, which consists primarily of NSCs (139, 489). One of the limitations to using these protocols to generate NSCs was the variation among different iPSC lines and batch-to-batch variation (49, 196). Other protocols used longer EB formation periods, and specific sorting methods alongside different growth factors, to generate clonal neural rosettes more efficiently, with longer pluripotency and the possibility of easy expansion (75, 141, 326). Circumventing the EB formation, Ebert et al proposed a method that generates pre-rosette stem cells with the help of FGF-2 and epidermal growth factor (EGF). This protocol was more efficient in generating NSCs, with a more simple and economic approach (135). In general, NSCs can be characterized by cell morphology and cell-specific marker expression. iPSCs express pluripotent stem cell markers OCT4 and stage-specific embryonic antigen 4 (SSEA4). NSCs show triangle-like morphology distinct from the flat morphology of iPSCs (Figure 1) and express NSC markers SOX2 and Nestin. NSCs have strong proliferative potential and are passaged every 5–6 days to allow for population expansion. They can differentiate into cells of various neural lineages (108, 165, 189). A summary of NSC differentiation strategies can be found in Table 1. It is important, however, to point out that there are many other protocols that have been reported with small changes compared to the summarized table 1, but the principles described remain similar.

Neurons

While the exact number of neurons within the human brain is unknown, most estimates place this number around 85 billion (17, 381). Neurons receive, transmit and process information through intricate networks formed by different specialized types of neurons, many of which are affected by complex neurological diseases. Being the primary functional component of the CNS, understanding how neurons function in both healthy and normal processes is essential. Non-specific neurons can be also directly differentiated from iPSC-

derived NSCs by culturing NSCs in neural differentiation medium including brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), dibutyryl cyclic adenosine monophosphate (cAMP), and ascorbic acid (165).

There has been a push to generate specific types and subtypes of neurons. These neurons can mimic neurotransmitter outbursts, defined electrophysiological patterns and present distinct receptors and markers. Some of these include nociceptors, motor, dopaminergic (DA), GABAergic, glutamatergic, cortical, hippocampal, and serotonergic neurons (Table 2). The ability to produce different subtypes of neurons has significantly contributed to recent advances in phenotyping diseases that might affect only certain populations of neurons (118, 221, 358, 380).

There are a number of similarities between the protocols developed to generate these different types of neurons, with most of them modulating the bone morphogenetic protein (BMP)/SMAD signaling cascade and Wnt signaling in early development. Chambers et al in 2009 showed that dual SMAD inhibition via SB431542/Noggin treatment was an efficient and fast way to achieve neural induction without the need for EB formation (77). The SMAD inhibition protocol (77) provided the foundation for many subsequent neuronal differentiation protocols. There are, however, several variations to any given strategy. As pointed out by Sances et al, considering the available techniques to generate only motor neurons, there has been more than 10 different publications with slightly modified differentiation protocols used for different timeframes and subtype specifications (386). With that, Table 1 offers a representative example of the protocols to generate the various neuronal subtypes, but it is not an exhaustive list of the potential differentiation protocols.

Glial cells

Glial cells include oligodendrocytes, astrocytes, ependymal cells, Schwann cells, microglia, and satellite cells. On average, glial cells make up about 50% of the brain volume, with a total number of cells similar to the number of neurons (17). They provide support and protection for neurons and maintain homeostasis, while also helping neurotransmission. Several diseases previously thought to be affecting neurons where later discovered to be affecting these support cells, e.g. multiple sclerosis (MS), and Guillain-Barré syndrome (332). Other pathologies, like schizophrenia, have also been recently linked to glial cell dysfunctions, such as white matter abnormalities (32). Glial cells are also essential for scar formation in response to CNS injury - whether traumatic, ischemic or in different models of AD and PD (28, 58, 417). Thus, understanding the processes behind normal gliogenesis during development is important in devising better therapeutic strategies (124). Below we summarize a few of the protocols used to generate different glial cells from iPSCs (Table 1).

Oligodendrocytes

In the CNS, oligodendrocytes are responsible for myelinating neurons, a role played by Schwann cells in the peripheral nervous system. During normal development, they are the last cell type to be generated, although their progenitors tend to arise alongside the first neurons (432). Similar to other cells in the developing brain, these progenitors follow a complex spatiotemporal differentiation, where each area of the brain gives rise to different

specific types of oligodendrocytes (432). Diseases affecting mature oligodendrocytes are called demyelinating diseases and include MS and leukodystrophies; these diseases are usually associated with decreased motor function and sensation (248). Early studies with embryonic stem cell transplantations showed that oligodendrocyte progenitor cells (OPCs) transplanted into myelin-deficient adult rats were able to migrate, myelinate and later recover locomotor abilities (81, 213, 263). While there is no iPSC-based therapy yet, ESC-derived OPCs are being used in a clinical trial for spinal cord injury, and iPSC-based models of MS have been developed as a tool to understand the disease progression (477).

Using a similar approach to EB-based neuron differentiation protocols, Wang et al. created OPCs that were, in fact, capable of generating both myelinated astrocytes and oligodendrocytes (460). This was done by generating neural rosettes as described above. These were cultured for 35–40 days, then switched to a glial induction media supplemented with platelet-derived growth factor-AA (PDGF-AA), insulin growth factor-1 (IGF-1) and Neurotrophin-3 (NT3). These factors drove the formation of gliospheres, which can be replated in small cell clusters to form oligodendrocytes. The overall differentiation duration from iPSC to oligodendrocytes varies from 110–150 days, generating myelin basic protein (MBP)- and oligodendrocyte transcription factor (OLIG2)-positive cells that were shown to myelinate immunodeficient mouse brains, with the ultrastructure showing mature compacted myelin (460). A more recent protocol was also established by Douvaras and colleagues, in which neural induction was achieved via dual SMAD inhibition prior to the formation of EBs (129). This allowed for a more efficient formation of OLIG2-positive cells in the absence of any exogenous FGF2. This new protocol was also able to generate OPCs from primary progressive MS (PPMS) patients, a more severe form of the disease. A summary of oligodendrocyte differentiation strategies can be found in Table 1.

Microglia

Microglial cells in humans are derived from myeloid progenitors in the yolk sac, and in the CNS they act as the first line of immune defense (16, 161, 249). They are constantly scanning the brain environment for plaques, cell debris, and possible infectious agents, even though the BBB usually excludes most pathological factors (157). Because of their role, microglia are essential for the inflammatory responses of the CNS, and they have been shown to be dysregulated in several different diseases, including AD, PD, and MS (385).

In recent years, at least five different protocols to generate microglial cells from iPSCs have been proposed (1, 128, 169, 317, 349). A common component in all of these protocols is Colony-stimulating factor 1 (CSF1) receptor ligands. CSF1 receptor signaling is essential for normal macrophages proliferation, differentiation, and survival (85, 86). Muffat et al proposed an early protocol utilizing EB formation using CSF1 and IL-34 as the main differentiation factors (317). Pandya et al later presented a similar method that also included an astrocytic co-culture layer (349). To bypass the formation of EBs, the related problems of batch-to-batch variability, and generation of contaminating cell types, Abud and colleagues proposed a different protocol in which iPSCs are directly differentiated into hematopoietic progenitors with the help of FGF-2 and BMP4 (1). These cells were further differentiated into microglial-like cells, capable of migration, secretion of cytokines and phagocytosis.

Both Douvaras et al and Haenseler et al further improved upon this method using IL-34 along with granulocyte-macrophage colony-stimulating factor (GM-CSF) instead of CSF1 (128, 169). That methodological change provided them with higher efficiencies and fewer starting iPSCs, and in the case of Douvaras et al, no need for the co-culture feeder layer. A summary of microglia differentiation strategies can be found in Table 1.

Astrocytes

Astrocytes are often referred to as the housekeeping cell of the brain based on their invaluable role in brain homeostasis, synaptic plasticity, and response to pathological states of the CNS (26, 91). Astrocytes are a heterogeneous population and have varying morphological and physiological characteristics largely dependent on regional localization with each astrocyte contacting up to a million individual synapses (170, 187, 309, 441, 490, 496). Significant differences exist between human and rodent astrocytes, which is a limiting factor in the ability to capture human clinical pathophysiology phenotypes in an animal model. Human astrocytes are up to 30-fold larger and extend up to 10 times more processes compared to their rodent counterparts (337, 338). Additionally, human astrocytes react to neuronal synapses at a much faster rate by rapidly propagating calcium waves and responding to glutamate faster than rodent astrocytes (172, 337, 425, 498). The inability to obtain viable adult human astrocytes and potential ethical concerns utilizing fetal-derived astrocytes significantly hindered early studies (313). iPSC-derived astrocytes overcome some of these barriers, and although they are not as well studied as neuronal subtypes, iPSC-derived astrocytes have demonstrated several functional characteristics.

The significance of astrocytes in the CNS has led to a number of iPSC-derived astrocyte protocols attempting to replicate the physical and functional properties of *in vivo* astrocytes (Table 1). To successfully differentiate astrocytes from stem cells, several requirements need to be met. First, a loss of pluripotency while transitioning to a neural progenitor cell type is most commonly done by modulating SMAD (77, 102, 224, 230, 231, 261). Next, a neuron-to glial switch must occur; this default switch can occur following extensive elongated periods of culture (224, 230, 261), serum addition (251, 348, 378) or modulation of the Janus kinase/signal transducers and activators of transcription (JAK/STAT), bone morphogenic protein (BMP), and NOTCH signaling pathways (46, 230, 337, 403). Regional specificity can be ascertained by modulating RA, BMPs, and sonic hedgehog (SHH) in differentiating astrocytes from stem cells (230, 261, 378). The ability to derive mature and regionally specific astrocytes will be critical for future disease modeling applications.

Characterizing iPSC-derived astrocytes has been a challenge, as scientists attempt to discern between subtypes and maturation levels. Outside of a morphological appearance of iPSC-derived astrocytes, a number of markers can be utilized (228, 231, 498). Nuclear factor I-A, a transcription factor, is commonly expressed in immature astrocytes (72, 116). Astrocyte progenitors express S100 calcium binding protein β (S100β) (114), although varying cell types of the CNS can also express these markers. Additionally, Aquaporin-4, Aldehyde dehydrogenase family 1 member L1, glutamate transporter 1 and glutamine aspartate transporter 1 have been utilized in the identification of astrocytes representing different maturation states (378). Glial fibrillary acidic protein (GFAP) has been the gold standard in

characterizing mature astrocytes; however, its expression varies dramatically in different regions of the CNS and expression of additional markers can begin to identify subtypes (62, 229, 498). A summary of astrocyte differentiation strategies can be found in Table 1.

Generation of 3D Organoids from iPSCs

Different 3D culture systems have existed for more than a decade, with the inclusion of both scaffold-based cultures, and scaffold-free cultures (e.g. spheroids) (179, 311). Here we will follow a definition for organoids as provided by Huch et al in 2017: “a 3D structure derived from either pluripotent stem cells (ESCs or iPSCs), neonatal or adult stem/progenitor cells, in which cells spontaneously self-organize into properly differentiated functional cell types, and which recapitulates at least some function of the organ” (201). In the case of cerebral organoids, this includes neurons, astrocytes, and oligodendrocytes that form a dorsal cortical organization reminiscent of the ventricular zone layer, a transient layer present at an early embryonic stage. The neurons present in cerebral organoids are also functionally active, demonstrating spontaneous calcium surges and action potentials blocked by the application of tetrodotoxin (236).

Many improvements in an organoid generation have been made in recent years by making minor modifications of the original Lancaster protocol. This method takes between 1–2 months to differentiate iPSCs into organoids, which can then be kept for up to a year; however, growth is reduced by 2 months, and cerebral organoids start shrinking after 5–6 months (237). Similar to the 2D model procedure described above, the start of this 3D cerebral organoid generation protocol begins with the formation of EBs from iPSCs on ultra-low attachment plates with medium containing decreased FGF-2. After forming an ectoderm, the EBs are then transferred to a neural induction medium composed of DMEM-F12, N2 supplement, and heparin, which generates neuroectoderm within 3–4 days. Following the formation of neuroectoderm, the tissues are embedded in Matrigel, a gelatinous mixture that can mimic an extracellular matrix (ECM). This allows for further development of the organoids, aided by differentiation media composed of Neurobasal media, and B27 supplement; RA is added at a later stage to caudalize the organoids. The last discovery made by Lancaster and colleagues was that agitating the organoids after differentiation was completed improved the diffusion of nutrients and allowed for prolonged organoid survival and growth (237). While it is difficult to pinpoint the exact neurodevelopmental age at which organoids correlate with the *in vivo* human brain, 2-month-old organoids seem to be most similar to the developing brain of the first trimester (237). A representative image from our group highlights the heterogeneity and organized structure in 2-month-old cerebral organoids, showing the presence of NSCs, astrocytes, neurons, and synapse structure (Figure 4).

The field of cerebral organoids has seen significant advances in the last 4–5 years (214), with the inclusion of more complex and specific protocols, and more recently, vascularized organoids (286, 357). One of the first advances was the discovery that, when compared to human fetal brains, cerebral organoids have significant similarities in their methylome and transcriptome (67, 274). Analysis of these epigenomic signals also showed that the organoids are strongly correlated to early-to-mid fetal cortical development, with many

genes related to the ECM being differentially expressed (274). This is possibly due to the use of Matrigel as an embedding material. Other studies using single-cell RNA-sequencing showed that 6-month-old organoids had 10 transcriptionally distinct cell population. There were, however, concerns about the reproducibility of such studies, since the authors did report a high degree of batch effects (274).

By modifying specific parts of the established protocol, several groups have been able to generate organoids with different properties. Quadrato et al. have shown that organoids with an enhanced maturity (achieved by plating fewer iPSCs in the beginning and supplementing the media with BDNF) are able to form photosensitive cells, which is in agreement with the findings of retinal-like cells in organoids (366). The enhanced organoids also had dendritic spines, the protrusions responsible for helping the electrochemical signaling between neurons, and an indication of increased neuronal maturation (366).

The two main types of neurons in the cortex are either excitatory glutamatergic or inhibitory GABAergic, and these are predominantly generated by the dorsal forebrain progenitors and ventral forebrain progenitors, respectively (174). Therefore, a recent development was achieved by fusing two different organoids, one with a dorsal and one with ventral identity (19). The formation of a dorsal-ventral axis allows for more elegant models involving neuronal cell migration, and it also opens the doors for other types of fusion-based protocols. While undoubtedly complex, the organoids presented so far are highly self-organized, with most of the possible changes coming from chemical signals. Lancaster et al changed that when they introduced microfilament-engineered cerebral organoids (enCORs) (19). These are organoids that, with the help of poly(lactide-co-glycolide) copolymer fibers, form elongated neuroepithelia, display improved cortical development, a polarized cortical plate, and even increased reproducibility. In contrast with fused organoids, these organoids contain dorsal and ventral identities in a single organoid, while also showing a complex organization of the cortical plate (235). Alongside the advancements in BBB modeling in 2D previously mentioned, 3D cultures have also seen a push towards complex models. Nzou et al recently created a spheroid model of the NVU, which included the presence of endothelial cells, pericytes, astrocytes, microglia, oligodendrocytes and neurons that generated organoids exhibiting tight junctions, adherens junctions, and transport proteins (335).

Blood Brain Barrier Modeling using iPSCs

Multiple barriers exist within the CNS, including the epithelial blood-cerebrospinal fluid barrier and the arachnoid barrier; however, iPSC-derived models have only been utilized to study the BBB. The BBB is responsible for the homeostasis between the cerebral vasculature and the brain and implements active interaction between the bloodstream and CNS (215). The BBB consists of specialized microvascular endothelial cells, pericytes, neurons, and astrocytes (404). Neurovascular unit (NVU) is a structural functional basis of the BBB. NVU enables tight regulation of blood flow through the vasculature, which has a unique structure in the brain. NVU, consisting of brain microvascular endothelial cells (BMECs), basement membrane, pericytes, astrocytes and microglial cells, couples local neuronal function to local cerebral blood flow and regulates transport of blood-borne molecules across the BBB. Individual cell components have their respective roles within the

NVU (279, 296). The regulation of molecular transport enables the brain to properly function under physiological conditions and modulate the transport of pharmaceuticals into the brain. However, a number of pathological conditions display dysfunctional BBB. Thus, the need for physiological *in vitro* BBB models is critical for both pharmaceutical screens and modeling disease states. Astrocytes, neurons, pericytes, and NSCs have all been shown to induce, regulate, and maintain these BBB properties in BMECs (23, 207, 369, 375, 421, 437, 464). Taken together, the enhanced barrier tightening, tight junction protein expression, active nutrient and efflux transporters, reduced para-cellular diffusion, reduced trans-cellular transport, and diminished leukocyte adhesion molecule in BMECs contribute to the tightly regulated movement of molecules, ions, and cells between the cerebral vasculature and the brain tissue.

The majority of BBB models have focused on the interplay between astrocytes and BMECs (207). Astrocyte co-culture has been demonstrated to enhance barrier tightening in BMECs (113). However, following neuron co-culture, BMECs can display continuous tight junctions (69, 391–393, 437). More recently, pericytes, similarly to astrocytes, can increase TEER and decrease permeability (256, 320, 321). Surprising, astrocyte and pericyte-secreted factors alone have upregulated BBB properties in endothelial progenitor populations (50, 73). However, the ability to derive multi-cellular models comprising combinations of pericytes, neurons, astrocytes, or NSCs in co-culture with BMECs have induced the greatest BBB properties compared to co-culture with any single NVU cell type (56, 69, 190, 256, 258, 259, 320). To further advance *in vitro* BBB models, especially their application in understanding neurovascular dysfunction in several CNS diseases, patient specific iPSCs can be utilized to derive each component of the multicellular BBB model. A number of approaches have been utilized to optimize the *in vitro* multicellular BBB model: transwell co-culture system, microfluidic devices, and cell aggregate model (Figure 2). The following subsections include the recent progress of generating two BBB cell components (endothelial cells and pericytes) and *in vitro* BBB models from iPSCs.

Brain microvascular endothelial cells

BMECs are specialized endothelial cells that form a physical, metabolic, and transport barrier between the blood and the brain. Compared to peripheral endothelial cells, BMECs have a much greater restriction to para-cellular diffusion of ions, molecules, and proteins. This markedly reduced diffusion is a result of the expression of tight junction proteins: occludin, claudin-5 and zonula occludens-1 (ZO-1) (150, 315, 331). The formation of the tight junctional proteins results in elevated barrier tightness, represented by an increase in trans-endothelial electrical resistance (TEER). BMECs display elevated TEER levels greater than 1000 Q x cm^2 compared to peripheral endothelial cell TEER levels that often don't do not exceed 30 Q x cm^2 (64, 104, 105, 342, 370). The markedly reduced para-cellular diffusion and restricted trans-cellular transport enable BMECs to regulate nutrient and metabolic movement from the blood to the brain. A variety of molecular transport systems including solute carriers (SLC; nutrient transporters) and efflux transporters assist in the regulation of ion and small molecule movement across BMECs. Glucose transporter 1 (GLUT1), L-type amino acid transporter 1 (LAT-1) and monocarboxylate transporter 1 (MCT-1) are responsible for the transport of glucose, large amino acids, and

monocarboxylates, respectively (42, 103, 159, 216, 334, 340, 341, 499). Efflux transporters: p-glycoprotein (P-gp), multidrug resistance-associated protein 1 (MRP-1) and breast cancer resistance protein (BCRP) are vital in human transendothelial transport (99, 101, 138, 243, 282, 307, 394, 495, 497).

Considering their clear role in modulating molecule flow around the brain, a number of models have been utilized to study BMEC. Rodent, porcine and bovine models have all shown capabilities of expressing several BBB properties including elevated TEER, tight junction protein expression, response to the co-culture NVU cell-types and active efflux transporters (182, 469). However, due to species variations, these models need to be interpreted carefully when comparing them to human conditions (113, 427, 462). Primary and transformed BMECs exhibit reduced barrier properties once removed from the brain microenvironment and can begin to de-differentiate following prolonged culture periods (65, 146, 285, 465). Therefore, iPSC-derived BMECs offer a unique opportunity to generate BMECs and model various diseases affecting components of the BBB. Lippmann et al developed the first iPSC-derived BMEC population by creating a micro brain environment with co-differentiating neural-like and endothelial cells (258). The resulting BMECs could reach barriers exceeding 2000 $\Omega \times cm^2$ when treated with RA and over 5000 $\Omega \times cm^2$ when placed in co-culture with pericytes, neurons, and astrocytes (256). Additionally, iPSC-derived BMECs expressed active efflux transporters: P-gp, MRP-1 and BCRP and a number of solute carrier (SLC) genes: LAT-1, GLUT-1, and MCT-1 (256, 258). Several iPSC-derived BMEC protocols have been developed in an attempt to further define differentiation conditions, expedite the differentiation timeline and incorporate pathological conditions (11, 119, 190, 363, 365, 373, 449, 461). A summary of BMEC differentiation strategies can be found in Table 1.

Pericytes

CNS-pericytes are essential for BBB maintenance, regulation, and development. Pericytes directly contact BMECs and contribute to several critical BMEC properties. Pericytes first contact BMECs in the developing CNS capillaries during embryogenesis. This initial contact between BMECs and pericytes enables a substantial decrease in paracellular transcytosis and inflammatory protein expression (110). Following initial barrier development, pericytes assume a regulatory and maintenance role of the BBB. Pericytes have been demonstrated to regulate both the stability and diameter of cerebral capillaries (29, 123, 171, 353) while also contributing to BMEC basement membrane proteins (123, 382, 422, 447). Importantly, pericytes have demonstrated a striking reduction in transcytosis, a type of transcellular transport of macromolecules across the interior of the BMECs (15).

Pericytes along with vascular smooth muscle cells are typically categorized as mural cells. Vascular smooth muscle cells are typically associated with larger vascular systems such as arterioles and venules where pericytes are typically associated with smaller vascular structures such as capillaries. Mural cells express a variety of markers including platelet-derived growth factor receptor-beta, neural/glial antigen-2, smooth muscle protein 22-alpha and calponin-1 (14). Potassium voltage-gated channel subfamily J member 8 (KCNJ8) and ATP-binding cassette, sub-family C member 9 (ABCC9) have been identified as pericyte-

specific transcripts in the murine CNS vasculature but has been unconfirmed in the human CNS vasculature (44, 200). Unfortunately, an *in vitro* distinction is difficult as no specific marker will discern smooth muscle cells from pericytes. However, developmentally, CNS-derived pericytes arise from neural crest stem cells, while other peripheral mural cell types originate from a mesodermal lineage (140, 226). iPSCs have demonstrated their capability to be differentiated into CNS-mural cells through an intermediary neural crest stem cell stage (84, 299) although their ability to enhance BBB properties remains unknown. A summary of pericyte differentiation strategies can be found in Table 1.

iPSC-based blood brain barrier model

Recent discoveries of significant species differences in the abundance and function of key BBB transporters have highlighted the need for the development of human BBB models (31, 373). Canfield et al first demonstrated that neurons, astrocytes, and BMECs could be derived from the same iPSC cell line (69). Following co-culture with neurons and astrocytes, BMECs displayed significantly improved barrier tightening and tight junction localization. Several studies confirmed the reproducibility and application of iPSC-derived multicellular BBB transwell models displaying elevated barrier tightening, improved tight junction localization, efflux transporter activity, appropriate para cellular permeability, and transport activity (11, 190, 373). Hollmann and Qian demonstrated that BMECs could be differentiated under defined conditions and in a reduced timeframe while exhibiting elevated barrier properties (190, 363). iPSC-derived BBB models have been utilized to further understand the pathological barrier in Huntington ‘s disease and AHDS (253, 449). However, a limitation of these models is the utilization of the transwell-system. Direct contact between NVU cell types and BMECs is severely limited or even absent due to BMECs being seeded on top of the porous transwell, while co-culture subtypes are seeded on the bottom of the transwell or in the plate itself.

Microfluidic devices can be incorporated to further improve BBB phenotypes by concentrating BBB enhancing-secreted factors, introducing flow-mediated shear stress, and allowing for a more appropriate interaction between BMECs and NVU cell types to occur. Several studies have demonstrated that microfluidic devices can be utilized to model the BBB utilizing primary and animal sources (3, 457). Wang et al established a microfluidic device utilizing iPSC-derived BMECs and primary astrocytes that displayed elevated, stable, and near *in vivo* TEER and para-cellular permeability (461). The complete incorporation of all human iPSC-derived components and the ability to monitor BBB properties in real time may further improve the soundness and scalability of multicellular BBB microfluidic models. Finally, a cell-aggregate model that allows for direct contact between the NVU cell types has displayed tight junction expression, adherins junctions, and active P-gp efflux transporters (88, 444). Interestingly, human cortex spheroids displayed charge selectivity through the barrier and were susceptible to hypoxic conditions, indicating potential applications in drug discovery and toxicity testing (336). However, the incorporation of iPSC-derived cell types remains to be investigated in cell aggregate/spheroid models.

The Strengths and Limitations of 2D, 3D, and BBB Culture Approaches

Since iPSCs provide multiple opportunities for modeling cell types and interactions as occurs within the brain, it is important to look carefully at the strengths and weaknesses of each system prior to application and data interpretation. In the following subsections and Table 3, we will summarize major considerations of 2D, 3D, and BBB systems; techniques, applications, and data interpretation will be discussed, in addition to new directions in applying the various models.

2D Monolayer models

The iPSC-derived 2D monolayer model was first to originate and represents a classical approach to isolate specific neural cell types and perform mechanistic cellular and molecular studies. The initial ability of scientists to use what is known of the *in vivo* brain development patterning to achieve neural cell differentiation has been groundbreaking, and lead to protocols using chemically defined medium and appropriate culture environments to mimic internal and cues to achieve neural cell types of interest (260). As discussed above, specific subtypes of neural cell types, e.g., cholinergic neurons, can be generated as well (13). In contrast to more complex BBB co-culture and cerebral organoids, the various methods to generate homogenous neural cell types from iPSCs, in addition to performing stationary 2D culture, is relatively less challenging, time consuming, and expensive (185). The likelihood of reproducibility through differentiation batches is higher, and it is easier to analyze the types of cells present, and cell type-specific physiology (134). Confounding variables of multiple cell types and unknown physiological components are reduced in data interpretation, and 2D monolayer models also provide great resolution of cell morphology. Accordingly, disease modeling has limitations in various neurodegenerative conditions in achieving certain disease phenotypes. In more recent years, the primary strength of 2D monolayer models has been highlighted in personalized medicine and disease modeling. While this topic will be discussed in greater detail later in this review, the ability to obtain cells from healthy and diseased patients, reprogram them to iPSCs, and then generate the neural cell of interest, represents the next generation of medicine. Since 2D monolayer culture is more feasible to perform for the reasons discussed above, drug responsiveness and disease phenotypes unique to different individuals can be assessed.

The feasibility and relative simplicity of the 2D monolayer model relative to other models imply its greatest weakness, in the lack of structure, dynamic growth expansion, heterogeneous cell types, organ complexity, *in vivo* organ environment, vascularization, and maturity. Many skeptics question the viability of studying singularized cells apart from an intact system and believe that physiological pathways, drug responses, and phenotypic background/outcomes cannot be modeled in a manner that would resemble occurrences in the *in vivo* human (297). Additionally, recent studies have reported how the quality of 2D monolayer culture, specifically cell health, differentiation efficacy, depend greatly on cell plating density, specific patient-derived iPSCs, and the individual handling the cells and performing differentiation (134). The concept of micropatterning has become more important, which emphasizes cell orientation and positioning in combination with providing appropriate environmental factors and supporting cell types. Studies have begun to discover

the significance of area, mechanical forces, and attachment surface for optimal stem cell growth and proliferation, in order to achieve more viable cultures and cells through resemblance of the polarity and dynamics of brain development observed *in vivo* (250). Through continued attempts to overcome these limitations, 2D monolayer models remain the most widely used iPSC-derived approach based on financial and technical feasibility, and for the high throughput potential in personalized medicine/drug testing (59). Future work remains focused on gene editing to determine the significance of genetic background/mutations in various human diseases (260), in addition to applying 2D monolayer protocols to generate co-culture systems, e.g., BBB models, or 3D organoids.

3D Organoid models

Cerebral organoids contain multiple brain cell types, show distinct brain regions, and contain highly complex tissue organization and dimension. Thus, the 3D cerebral organoid model has been recognized as the next advancement from 2D monolayer models and BBB co-culture systems an *in vitro* tool to recapitulate complex features of the *in vivo* human brain. More so than comparatively static 2D monolayer models and BBB constructs, cerebral organoids are accepted as a highly dynamic model of the embryonic developing brain over time.

Compared with the widely used iPSC system cultured as a 2D monolayer and BBB cultures, cerebral organoids are more similar to developing human brains in the aspects of complexity, structure, and function furthering the study of neurodevelopment and neurological diseases (106, 364, 366). Specifically, there is enhanced opportunity to study multifaceted human diseases and gene mutations that affect many cell types, their interactions, components of the constantly changing neurodevelopmental process, and the function of neuronal circuits (151, 237, 333, 351, 364). Starting the differentiation process with singularized, detached iPSCs allows for the process of self-organization and patterning occurring during embryonic development, and gives rise to significant tissue architecture not possible when tissues are attached the plate in 2D monolayer and BBB methods (13). Additionally, Matrigel coating provides a basement membrane and growth factors to allow maturation and differentiation of heterogeneous neural cell populations over time (236). In summary, the neural environment is best mimicked *in vitro* by employing 3D cerebral organoid cultures and has made cerebral organoids the most desirable method for physiologically understanding mechanisms of disease (260), drug action, personalized medicine (136).

One of the greatest limitations in the different types of organoids thus far is their size limitation, due to the lack of vascularization. Although many studies on neurodegeneration and aging suggest a need for a longer duration of organoid culture, the emergence of a necrotic center has been observed in turn with cerebral organoid maturation. In order to minimize the amount of cell death in the center of the organoid as it gets bigger, bioreactors are often used to oxygenate the culture medium, but this is only sufficient for a short period of time (237). In relation to determining which stage of development cerebral organoids represent, and despite the fact that cerebral organoids are nearly as sophisticated as fetal brain tissues in the early second trimester and recapitulate developmental dynamics and a greater degree of maturation, it is still a challenge to define the exact real human brain-

equivalent age of cerebral organoids (229). Using brain region emergence and specific neural cell types to estimate the age of cerebral organoids has been limited by an imprecise recapitulation of specific cortical layers and orientation of brain regions in cerebral organoids compared to what is observed in the *in vivo* brain (236). These 3D models are generated from iPSCs, they have the same limitations as all other iPSC models, including differentiation efficacy across cell lines and batch to batch variability. Therefore, some limitations still exist in modeling diseases associated with aging and neurodegeneration over time (185). Finally, cost, technical difficulties to generate cerebral organoids, and time commitment, are the greatest for cerebral organoids. Despite the limitations described above, the 3D cerebral organoid field is rapidly moving forward with methods to vascularize the organoids (286), scaffolding approaches to ensure uniform growth via an external structure (235), patient-specific cells, drug testing, and disease-related gene editing (214). A summary of neural cell types in 2D modeling is shown in figure 3.

BBB model

Scientists began to create BBB models to overcome some of these limitations of the 2D model in resembling the environment surrounding neural cells, including cell composition, chemical properties, and physical selectivity. Overall, the goal is to better mimic *in vivo* physiological function of the brain and create a system in which diseases and drug action can be better assessed (69). Since BBB properties greatly differ across species, careful attention is taken to achieve human-specific molecular regulated molecular transport across endothelial cell layers and neural cell types of used in the iPSC-derived model (205).

In order to accurately set up the BBB model, as discussed above, iPSCs have been a favorable cell type, to begin with, as they can be differentiated into all of the cell types required for the co-culture BBB construct (BMECs, astrocytes, pericytes, etc.) (11). The ability to harness this advantage of iPSCs to be the single source for different co-cultured cell types has led to a BBB that is less likely to degenerate/de-differentiate over time, compared with cells derived from animals, multiple progenitor cell sources, or BBB constructs in which certain necessary cell types are missing (239). Since BMEC transporter and membrane characteristics are essential in the physiological relevance of artificial BBB models, it has been a strength to observe specific transporters, the polarized flux of substrates, and *in vivo* TEER properties and values (205). Since the BBB is often a contributing factor in the development of many neurodegenerative diseases, iPSCs from diseased individuals can also be used to generate the various cell types required for the model. The BBB offers more opportunities than a 2D monolayer and 3D culture to focus on environmental contributions to phenotype (257), and also allows environmental factors to be manipulated by the experimenter compared to the spontaneous environment arising from differentiation of a particular cell type or organoid.

Similar to what was discussed as a limitation of 2D monolayer systems, widely different differentiation processes are possible based on the starting iPSC line (257), highlighting issues in variability, phenotypic outline, and general experimental protocols. The *in vivo* BBB is defined by its ability to determine the selectivity of which substances can cross from the circulating blood into the brain. Thus, the primary weakness of the BBB is clear in its

isolation from a vascularized system containing variations in perfusion, and an environment containing capillaries and vascular beds (205). Cost and technical difficulty compared with 2D monolayer systems also limit the application of BBB models (239). Another major limitation in iPSC-BBB models is the lack of contributing cell types of the BBB including iPSC-derived pericytes and microglia. The role of pericytes in regulating several BBB properties has been well documented in various primary/animal models. iPSC-derived brain pericytes will certainly enhance future BBB models in an attempt to recapitulate the *in vivo* BBB in both physiological and pathological state. Due to the relative novelty of the BBB system, only a small number of drugs have been tested for their permeability through BMECs and their effect on neural cell types. Advancements in iPSC-derived BBB models will certainly benefit from bioengineering approaches. Advanced BBB models will both improve our understanding of the BBB under pathological conditions but will also enhance our ability to deliver neuro-pharmacological agents across the BBB. Combining approaches mentioned in the previous sub-sections including addition of contributing iPSC-derived NVU cell types, direct contact between all cell types, the addition of flow-induced shear stress, and allowing BBB properties to be monitored in real time will be critical in understanding critical in understanding neurovascular disorders of the CNS. Additionally, there is a great avenue for pharmaceutical and disease assessment using iPSC-based BBB model (257), so this remains an area that both basic scientists and commercial ventures are focused on. In addition, the long-term viability of BBB models is being assessed, in addition to methods that can more accurately assess flow conditions, and recapitulate *in vivo* TEER, transporters, and polarized cell composition (11, 239).

Overall, different iPSC-based models described above have benefits and limitations. It is also important to highlight the human relevance of studies employing iPSCs compared with an animal model, as human conditions can be modeled that would be impossible *in vivo.* We will continue to discuss how 2D, 3D, and BBB iPSC-based systems are utilized to study disorders and diseases of the CNS. Attention to specific neural cell types, stages in the differentiation process, and patient-specific studies will highlight both the strengths and limitations of iPSC-derived models. As discussed above, 2D monolayer models have been the most widely used for cell culture, but 3D cerebral organoid and BBB models are gaining momentum and being applied to model brain disorders.

Modeling Neurodegenerative Diseases

Neurodegenerative diseases, such as AD, PD, Huntington’s disease (HD) and ALS, represent a significant health concern, affecting approximately 6.8 million people in the United States (293, 472, 474, 475). These diseases rob those affected of their independence in the latter half of their lives, each disease in their own unique way. Effective treatment options for these diseases remain limited, and no cures are available. This is in part due to the fact that attempts at modeling these diseases, while numerous, are imperfect.

Rodent models of neurodegenerative diseases are valuable in that they allow researchers to study these diseases in an *in vivo* context. Yet, many of these models require the overexpression of a mutant protein in order to reveal a disease phenotype. This is likely required because these symptoms take decades to manifest in humans, and rodents simply

do not live that long. Additionally, having to wait for the rodents to reach an advanced age, relative to their own lifespan, is not conducive for research on a timely scale. However, overexpressing proteins, both wildtype and mutant, is likely to alter the protein function (160). In particular, protein aggregation is a common feature of neurodegenerative diseases, and increasing the copy number of any protein to a sufficient level could cause it to aggregate. Therefore, it is difficult to parse out what observed phenotypes are inherent to the mutant protein itself and not due to the non-physiological levels present in the models. This is especially true given that many studies do not compare the mutant models to transgenic mice overexpressing the wild-type protein at the same level. Therefore, human *in vitro* models have also been utilized, including primary samples, immortalized cell lines, and iPSCs.

Due to the cell types that are affected by neurodegenerative diseases, access to primary samples is limited to post mortem tissue. These samples, therefore, can only provide insight on the end-stage of the disease. Additionally, evaluating whether a particular therapy is effective is not possible with these tissues. Therefore, before the advent of iPSCs, researchers routinely used immortalized cell lines, fetal derived NPCs, and ESC-based systems to investigate disease pathways and the benefits of therapeutics. However, these models also required the overexpression of mutant proteins. Therefore, human iPSCs have distinct advantages as they can be derived from specific patient populations and retain the specific genetic background. This is particularly important for studying diseases that primarily lack a clear genetic influence. Neurodegenerative diseases are primarily considered in terms of which neuron sub-type is affected during the disease progression, i.e. motor neurons (MNs) in ALS and medium spiny neurons (MSNs) in HD. Therefore, the majority of studies utilizing iPSCs to model these diseases have focused on generating the affected neurons in 2D monolayer models, and as such, are the focus of this review. A section was also included to discuss more recent approaches in 3D cerebral organoids. As multiple cell types are clearly affected in neurodegenerative disease, including disorders inclusive of multiple cell types comprising the BBB, we have also included some discussion of the involvement and of these cell types and potential for BBB modeling in the context of the specific disease.

2D models to study neurodegenerative diseases

Alzheimer's Disease

AD affects millions of people worldwide, making it the most common cause of dementia as well as the most common neurodegenerative disorder (436, 484). It initially affects short-term memory, but progresses into widespread cognitive impairment, leaving those affected without the ability to independently perform daily tasks on their own (436, 484). While AD can be inherited through mutations in the amyloid precursor protein *(APP)* gene, presenilin 1 *(PSEN1)* gene or presenilin 2 *(PSEN2)* gene, over 95% of all AD cases are sporadic (436). On a cellular level, AD is characterized by extracellular amyloid-p (Ap) plaques and intracellular neurofibrillary tangles that are made of hyperphosphorylated tau (436, 484). While it is unknown whether these pathological hallmarks play a causal role in the observed cell loss, it is known that they occur in the regions that show severe atrophy, including the

hippocampus, entorhinal cortex, temporal lobe, parietal lobe, frontal cortex, and cingulate gyrus (436).

iPSCs have been utilized extensively to model AD, primarily by use of 2D monolayer models, and there is a remarkable amount of overlap in their findings (Table 4). However, these studies have been largely limited to characterizing the lines generated and determining whether they recapitulate the key pathological hallmarks of AD. In this regard, while the majority of studies have shown signs of elevated Aβ production and tau hyperphosphorylation, only one study was able to specifically detect Aβ aggregates (223). As such, future work with AD iPSC-derived neurons should be focused on identifying novel disease mechanisms so that effective therapeutics can be developed.

Microvascular damage could play a role in the progression of AD (54) as studies have indicated that basement membrane dynamics are altered in AD and may contribute to BBB leakiness (33, 127, 384). Although some studies have demonstrated that no BBB effect has been detected in AD (149, 212, 246), others have shown a compromise in BBB integrity in AD patients (6, 418, 452). Soluble Ap reduces P-gp expression, a key efflux transporter in BMECs in animal models (176, 350). Observations in animal studies and in iPSC-derived neurons indicate that the recent advent of iPSC-derived BBB models could be vital in understanding the underlying microvasculature impairment in AD patients. An in-depth discussion of AD iPSC disease modeling has been reviewed elsewhere (436, 484).

Parkinson's Disease

PD is the second most common neurodegenerative disease and is caused by the loss of DA neurons within the substantia nigra pars compacta. The loss of these neurons within the midbrain causes a variety of motor issues including tremors, rigidity, and bradykinesia which progressively worsen over time. Although PD is primarily sporadic, about 10% of cases are caused by a genetic mutation. As such, using iPSCs reprogrammed from PD patients allows us to model both sporadic and familial forms of the disease within an endogenous system.

A summary of PD studies utilizing iPSC-derived DA neurons in 2D monolayer culture and their findings can be found in Table 4. The majority of these studies did not observe a reduction in cell viability or differentiation ability without exogenous stressors. However, a few groups were able to detect cell viability or neuronal differentiation defects (343, 387, 406). Moreover, a number of studies showed α-synuclein accumulation, suggesting that iPSC-derived DA neurons from PD patients can recapitulate at least one hallmark phenotype of the disease. There is a great deal of overlap between the studies published thus far (Table 5). The main phenotypes found in PD patient iPSC-derived DA neurons are mitochondrial dysfunction, oxidative stress, decreased neurite outgrowth, alpha-synuclein accumulation, and increased susceptibility to external stressors. However, only a few studies have addressed a possible mechanism by which these phenotypes are manifesting (194, 270, 396). Furthermore, correcting PD mutations using zinc-finger nucleases (ZFN) can reverse the observed phenotype, implying a causal link from the mutations to the observed pathologies (371, 388). Given that the field has shown that iPSC-derived DA neurons can be used to model both sporadic and familial PD, future work should focus on further elucidating the

mechanisms underlying PD pathology and identifying drug targets. Further analysis of PD disease modeling using iPSCs can be found in the review written by Cobb and colleagues (92).

Huntington's Disease

HD is a neurodegenerative disorder that is caused by a mutation in a single gene and is autosomal dominantly inherited. In HD there is a repeat expansion of the amino acid sequence for glutamine (CAG) within exon 1 of the *Huntingtin* (Htt) gene; CAG repeats of approximately 40 or more causes HD, with repeats of more than 60 causing juvenileonset HD (70, 438). This expansion, while ubiquitously expressed, primarily causes the death of MSNs in the striatum, but cells in the cerebral cortex, hippocampus, and hypothalamus are also affected (94). The dysfunctions in these brain regions lead to both motor deficits as well as cognitive and psychiatric phenotypes.

iPSC modeling of HD has been reviewed extensively by Tousley and Kegel-Gleason (438). Given that HD has a clear developmental phenotype, many labs choose to study both undifferentiated iPSCs and NSCs in 2D monolayer culture to better understand the early deficits of the disease (438). However, for the purpose of this review, we have chosen to highlight only the papers that generated functional neurons for study, detailed in Table 6. In this pursuit, it has been well documented that HD iPSC-derived neuron cultures do not exhibit a cell death phenotype until BDNF has been removed from culture media (Table 6). Additionally, while the mutated, higher molecular weight protein can be detected by western blot, aggregated Htt has either not been detected (181, 479) or not specifically addressed (Table 6). These data indicate that despite HD being a monogenetic disease, current iPSC techniques do not recapitulate two of the characteristic disease phenotypes without exogenous stressors. Nevertheless, some key phenotypes have been identified in iPSC-derived MSNs. For instance, it has been found that ATP levels are decreased in HD MSNs (87, 167). This may be important for disease pathogenesis as recent work has indicated that ATP is intimately involved in maintaining protein solubility (352). In accordance, efforts to increase the expression of the adenosine receptor $A_{2A}R$ reduce DNA damage after H_2O_2 treatment (87). In addition, conferring with the developmental deficits seen in studies utilizing undifferentiated iPSCs/NSCs, the HD iPSC Consortium has found alterations in the expression of genes relating to neuronal development and function that indicate a reduction in the maturity of HD iPSC-derived MSNs (180).

A number of studies have indicated that HD can impair cell types of the NVU and have detrimental effects on the BBB (130, 198, 255). Impairment of *Htt* has been directly linked to alterations of the microvasculature. For example, changes in cerebral blood volume, density, and BBB permeability have been found in both rodent models and HD patient-isolated tissue (130, 147, 193, 198, 255). Drouin et al observed decreased tight junction protein expression, increased transcytosis and increased permeability of the BBB in an HD mouse model (130). Utilizing HD patient-isolated tissue the authors observed a similar decrease in occludin and claudin-5 expression correlated to an increase in permeability in HD samples compared to control. However, these studies could not discern the role of each cell type in the NVU and the corresponding leakiness of the BBB. It, therefore, remained

unclear whether BMECs contribute to the observed HD pathologies or if it is secondary to neurodegeneration. Lim et al generated HD iPSC-derived BMECs, which interestingly displayed increased angiogenic properties and dysfunctional BBB properties, including decreased TEER and increased trans-cytosis (253). Targeted intervention of altered angiogenic properties partially rescued some of the deficits observed in HD-derived BMECs. From a therapeutic aspect, the ability to address neurodegenerative diseases with effective treatments relies heavily on the status of new techniques to employ the iPSC-derived human BBB model.

Amyotrophic Lateral Sclerosis

ALS, or Lou Gehrig's Disease, is caused by the loss of the upper and lower MNs of the brain and spinal cord. The loss of these neurons leads to progressive paralysis and death, typically within 3–5 years of symptom onset. ALS is a less common neurodegenerative disease, with at most 3.6 new diagnoses per 100,000 people a year, although the exact incidences vary (166). Yet, ALS is particularly challenging to model due to its heterogeneity. While the vast majority of all cases are sporadic, like AD and PD, approximately 10% can be traced through families and caused by a genetic mutation (502). However, mutations in over two dozen different genes have been identified as causative for ALS, and more continue to be identified (502). This has made generating mouse models of every gene unrealistic and made iPSCs an especially valuable tool.

A summary of common ALS disease phenotypes in iPSC-derived MN cultures in a 2D monolayer format by genetic mutation can be found in Table 7. The majority of the literature has utilized iPSCs with mutations in one of the four most common genes - superoxide dismutase 1 *(SOD1),* chromosome 9 open reading frame 72 *(C9orf72),* transactive response DNA binding protein 43 kDa *(TDP-43),* and fused in sarcoma *(FUS)* (502), and only a small number of studies modeling sporadic ALS have been published (Table 7). Although familial cases account for a small percentage of ALS cases similar disease phenotypes exist between familial and sporadic subsets. While some studies were able to detect a reduction in basal viability of the MN cultures (35, 38, 208), the majority showed no reduction in cell viability or differentiation ability without the addition of exogenous stressors (Table 7). Alternatively, almost all of these studies showed evidence of proteinopathy, suggesting that ALS iPSC-derived MN cultures were able to recapitulate some key disease phenotypes. Interestingly, genetic correction of the mutations using CRISPR 9 Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 has been shown to reverse the observed disease phenotypes including hyperexcitability, ER stress, morphological alterations, and increased DNA damage (35, 325, 400, 453), confirming that the observed alterations are due to the mutation itself rather than normal population variation. A major limitation in the current iPSC-derived MN studies detailed here is the limited overlap in the general lines of inquiry. Endoplasmic reticulum (ER) stress, mitochondrial abnormalities, and alterations in excitability are the three most common phenotypes found in ALS iPSC-derived MNs (Table 7). However, the exact mitochondrial phenotype found varies from study to study, making it challenging to validate which alteration is the most relevant (9, 35, 109, 325). Additionally, findings on excitability changes are contradictory, as some studies reported hyperexcitability (121, 453) while others report hypoexcitability (324, 325, 390), and another study found no alterations

in excitability (400). As MNs with the hexanucleotide repeat expansion in C9orf72 have been found to be hyperexcitable (121, 453), hypoexcitable (390), and regularly excitable (400), it is unclear which accurately represents the true disease state. However, as recent work has suggested that ALS MNs shift excitability phenotypes during maturation *in vitro* (121), this could be indicative of the variability in maturation in culture between labs, lines, and differentiation protocols.

ALS astrocytes have also been studied extensively to determine their role in the disease progression, which has been reviewed nicely by others (241, 481). It has been shown that ALS astrocytes cause MN death in both co-culture experiments and when MNs are treated with conditioned medium from astrocyte cultures (111, 122, 277, 288). Interestingly, further highlighting the important role astrocytes play in ALS, expressing mutant SOD1 in astrocytes alone has been shown to be sufficient to induce MN death (319). Additionally, Qosa et. al. demonstrated that mutant SOD1 astrocytes upregulated P-gp expression and increased reactive oxygen species (ROS) in endothelial cells. The roles of matrix metalloproteinases, inflammatory cytokines, and oxidative stress have all been indicated in ALS progression and certainly require further studies (7, 34, 45, 95, 115, 142, 376).

Evidence has also suggested that entire NVU comprising of BMECs, pericytes, astrocytes, neurons and the extracellular matrix is compromised in ALS (178, 448). As early as 1984, ALS patients demonstrated altered blood-cerebrospinal fluid barrier phenotypes with elevated cerebral spinal fluid IgG and albumin levels (10, 245). Garbuzova-Davis et. al observed leaky barriers in SOD1 mice in addition to degenerated endothelial cells and astrocytes, mitochondrial dysfunction and dissociation of astrocyte end feet and endothelial cell interactions (153–155). Additionally, animal ALS models have several alterations in the endothelium: reduced expression of platelet-endothelium cell adhesion molecule, altered tight junction proteins, reduced basement membrane components, altered efflux transporter activity (306, 312, 328). In human studies, familial and sporadic ALS patients were found to have reduced expression of tight junction proteins: ZO-1 and occluding (183). Similarly to previous studies, ALS patients exhibited astrocyte end-feet dissociated from the endothelium (312). BBB models utilizing iPSCs will enable a more thorough evaluation of multiple ALS disease subsets as well as dissecting the role of each BBB cell type in disease progression. The capabilities of an iPSC-derived BBB model can meaningfully improve ALS disease modeling applications and may unveil sites of therapeutic intervention. iPSC modeling for ALS has been further discussed in a review by Guo and colleagues (166).

Aging cell culture models

Most neurodegenerative disorders first appear in adulthood. However, iPSC-derived neurons resemble fetal brain cells (51, 289), thereby potentially limiting how accurately they can model the neurons of an aged individual. Many different methods of increasing the age of iPSC-derived cell types have been developed. One such method is to express progerin in iPSC-derived cells (308); progerin is the mutant form of lamin A that causes the premature aging disease Hutchinson-Gilford progeria syndrome. The addition of progerin to the culture conditions has been shown to increase many marks of aged cells, including abnormalities in nuclear morphology, DNA damage, shortened telomeres, and senescence-associated p-

galactosidase (SA-β-gal) expression, among others (308). However, it is challenging to determine whether the observed phenotypes in these “aged” cultures are due to the age of cells or the exogenous application of progerin. Another method to induce aging in iPSC cultures is to treat them with a telomerase inhibitor which results in shorter telomeres (450). Like progerin expression, telomerase inhibition has been shown to increase evidence of DNA damage and mitochondrial ROS production in iPSCs (450). Although effective, telomerase inhibition still relies on exogenous treatments to obtain an aged phenotype. Finally, it has been suggested that using high passage iPSCs (i.e. iPSCs cultured for one year) induces many aging marks, including changes in nuclear lamina structure and disruption of nucleocytoplasmic shuttling (355). This method, while a much less artificial aging method compared to the other two strategies discussed here, has its limitations. Namely, this method requires waiting a year to age iPSCs before they can be used for further experiments. Additionally, long-term culturing of iPSCs increases the possibility for chromosomal abnormalities, which again causes a potential confounding variable in the resulting data (355).

To counteract the limitations in these aging strategies while still achieving the desired aged cell type, many labs have directly reprogrammed somatic cells into the cell type of interest and completely by-passing the iPSC state, summarized in Table 8. It has been shown that these cells retain the aging marks of the somatic cell, in this case, fibroblasts (301, 430), thereby potentially providing a more accurate model of a symptomatic patient’s neurons. To date, neurons of many different cell types have been made, including MNs, DA neurons, and MSNs, which are applicable for modeling ALS, PD, and HD respectively. While these studies have primarily focused on optimizing the method by which the desired cell type is produced, a few studies have shown that directly converted patient fibroblasts to the affected cell type resulted in phenotypes that more closely mimicked the disease state (262, 266, 451). Notably, directly reprogrammed cells from HD patient fibroblasts formed Htt aggregates (266, 451) and exhibited a reduction in viability without exogenous stressors (451). These are hallmark pathologies of HD that are not observed in iPSC-derived MSN cultures, thus supporting the use of directly reprogrammed MSNs to model symptomatic HD. Additionally, directly converted MNs from ALS patient fibroblasts have also been shown to recapitulate characteristic pathologies, including reduced viability and protein mislocalization (262). Given this success in better recapitulating the disease phenotype, directly converted cells will likely be helpful in future studies to better understand the disease mechanism as well as be used for drug screening.

Despite the benefits of directly reprogrammed cells exhibit, there some major limitations to their application as disease models. One of these limitations is that these cultures introduce a lot of error into the study, as each well represents a separate reprogramming event that is unlikely to be identical across the entire plate. Additionally, since the majority of these studies converted fibroblasts into terminally differentiated neurons, the converted cells are generally incapable of expansion in culture in order to maximize the sample size. Similarly, fibroblasts are not indefinitely proliferative in culture, which limits researchers’ ability to generate a large number of cells for conversion. Therefore, many groups have attempted to directly generate NPCs from fibroblasts so that the reprogramming occurs only once, reducing introduced error, and the resulting cells would be proliferative (192, 303, 310).

These induced NPCs (iNPCs) have been shown to be capable of differentiating further into specific neuronal subtypes that exhibit many disease relevant phenotypes (192, 303, 310). However, it is not yet clear whether the neurons derived from iNPCs more closely mimic the disease state compared to iPSC-derived neurons.

3D models to study neurodegenerative diseases

One common limitation that all studies discussed so far share is that the models have all been 2D, thus inherently restricting how well they can model a 3D human. Therefore, much effort has been put into developing various 3D models of neurodegenerative disorders, summarized in Table 9, each with their own strengths and weaknesses.

3D models have most extensively been utilized to model AD (Table 9), the simplest of which merely involves differentiating NSCs into neurons and glia while they are embedded into Matrigel (89). This model is clearly not as structured as other 3D models of AD; however, when NSCs overexpressing mutant AD proteins are grown this way, extracellular deposits of Aβ can be detected (89). This is a key phenotype of AD brain tissues that has not been directly observed in 2D iPSC models of AD, indicating that even simple 3D model can improve on disease modeling. If this phenotype can be recreated with AD patient iPSC-derived NSCs, this could be a powerful model of AD that may be technically easier than other organoid models.

Various organoid models have also been generated in order to model AD and PD (Table 9). Cortical organoids have primarily been utilized in AD models, and midbrain organoids have been described for PD modeling. However, in both cases, the majority of the work done with these organoids has been largely descriptive in an attempt to prove that these models can be generated and that they are structured similarly to the specific brain region of interest. Indeed, all of the studies describing midbrain organoids have only been proof of concept reports rather than true disease modeling papers (210, 314, 364, 434). Cortical organoids for AD modeling studies have gone slightly farther in that these studies determined whether the organoids recapitulated any AD phenotypes (240, 368, 483). In this pursuit, evidence of Aβ accumulation and tau hyperphosphorylation has been found (240, 368, 483). Additionally, one study was able to show that γ-secretase inhibition, which has been used extensively in AD models, was less efficacious in the organoids compared to 2D models, indicating the importance in considering the 3D nature of the brain when performing future drug screening experiments (240).

3D models of HD and ALS have not yet been widely used (Table 9); a single report of 3D models have been published for each disease. For HD, the neurodevelopmental phenotypes have been modeled well in cortical organoids generated from HD iPSC lines (93). However, as it is unlikely that these organoids contain MSNs, this model will not be able to be utilized for studying deficits of the MSNs specifically. ALS 3D modeling has so far taken a different approach than organoids. Osaki and colleagues describe the development of MN spheroids that are then plated onto endothelial cells in a microfluidic device (346). These spheroids express various MN markers more highly than monolayer cultures, indicating an improvement in the maturation of these neurons (346). Additionally, the presence of endothelial cells in the microfluidic device mimics the vasculature that would be present in

an *in vivo* system, which has been largely missing from other organoid models. It would, therefore, be very interesting to see if other organoids can be grown with endothelial cells in a similar manner to MN spheroids and how this would improve upon the model.

Modeling Traumatic Brain Injury

TBI is defined as an insult to the brain from an external mechanical force, unrelated to degeneration and congenital conditions (300). Multiple causes exist, including car accidents, firearms, explosions, athletic injuries, and falls, and the consequences of TBI can be relatively acute, chronically debilitating physically and psychologically, or fatal. TBI can be the result of an open head injury, close head injury, deceleration, metabolic, hypoxic, oncogenic, infectious, or stroke-related (360, 467). The effects on the brain may be undetectable and are physically manifested in loss of consciousness, headache, nausea, altered sensory output, drowsiness, and difficulty sleeping. In more severe cases, penetration of brain tissue, tissue tears, bruising, bleeding, and altered brain morphology occurs (36, 284, 316). Moreover, brain injury extends to multiple cell types within the brain, including neurons, astrocytes, oligodendrocytes, and microglia, and leads to complex effects on synaptic plasticity, metabolism, and cognitive function (383).

More has been discovered regarding the prevalence of TBI in recent years, with the goal of better diagnoses, biomarkers, and treatment options. Accordingly, TBI modeling has begun to be studied in iPSC-derived neurons. In one study investigating a simulation of closed-head TBI, cell culture plates containing iPSC-derived neurons were lined with silicone membranes able to stretch. When neurons were exposed to repeated strain, it was observed that increasing strain augmented neural injury, with phenotypes such as cell death, reduction in neurite length, and altered neuronal morphology (410). In a 3D cerebral organoid model, Zander and colleagues utilized explosives to simulate blast pressures capable of producing TBI. At two different blast pressures, there was no difference in cell viability, but an increase in reactive oxygen species was observed. Additionally, intracellular calcium and sodium were increased following the two blast pressures (491). Therefore, these studies indicate that subtle effects from TBI may be identified using iPSC model systems. Additionally, generating homogenous populations of different cell types in 2D cultures (e.g., astrocytes, neurons, and oligodendrocytes), and heterogeneous populations of cells in BBB culture and cerebral organoid approaches have potential in being used to dissect the complex effect of TBI on multiple neural cell types, mentioned above.

Modeling Neurodevelopmental Disorders

Brain development begins within the first month of gestation and is believed to extend through adolescence (199, 435). In addition to the complex cellular and molecular patterning events influencing the complex processes within neurodevelopment, environmental exposures are critical in determining the fate of the brain (162). The extensive process includes structural formation within the embryo, fetal development of brain regions, and the prolonged period of neurogenesis (57). The events during neurogenesis are strong determinants of the neural cell survival, neuronal migration, and network connections formed within the brain and its constituent cell types (222). A summary of iPSC-derived

models to study neurodevelopmental disorders, such as viral exposure, autism, and Allan-Herndon-Dudley syndrome, is shown in Table 10. It should be noted that other developmental disorders (e.g. Fragile × Syndrome, Down Syndrome, and Angelman's Syndrome) have been extensively modeled using iPSC technology and are discussed elsewhere (2, 12, 55, 145, 202, 265). So far, to our knowledge, only 2D monolayer and 3D cerebral organoid cultures have been used to model the below disease conditions.

Developmental viral conditions

Viral infection extending to the developing brain, usually through maternal transmission during prenatal development, has a devastating effect on the formation of the brain, in addition to neurocognitive outcomes (100, 412). Cytomegalovirus (CMV) is a herpes virus which may not harm healthy individuals but can be detrimental to infants as fetal infection can cause deformities and mental disability (446). CMV only infects human cells, so there are no appropriate animal models to study the neurological effects of CMV infection. As such, the majority of studies examining the CMV infection has been performed on human fibroblasts. These cells are valuable for understanding various mechanisms of viral infection and spread, they are insufficient to evaluate the neural consequences of CMV infection. As such, iPSCs, NSCs, and human neural cell lines are valuable model systems. CMV infection has been successfully modeled by exposing healthy iPSCs, NSCs, and neurons at various stages of the differentiation process to CMV. Interestingly, iPSCs were not permissible to CMV infection when experimentally treated; however, neural progenitor cells were susceptible to CMV, which significantly impacted the differentiation to more mature neurons. Bigley and colleagues also found that CMV reduced NSC differentiation into neurons and astrocytes and further showed that a viral kinase inhibitor to restore some of the differentiation capabilities (37). CMV infection has also been shown to impair neural calcium release (107). A similar study found that iPSC-derived NSCs underwent apoptosis as a result of cytomegalovirus infection (322). Thus, these data may offer insight into the cellular and molecular mechanisms of neurological deficits resulting from infection during the vulnerable developmental period.

Along these lines, Zika virus has similar effects on the developing brain and has made headlines in the recent years. Specifically, the virus is transmitted by mosquitos, and when pregnant women contract Zika, the effects on the fetus lead to reduced brain size and altered morphology, ophthalmic problems, and neurodegeneration extending to multiple neural cell types (407, 445). In iPSC-derived cortical neurons, MNs, and astrocytes, African and Asian viral strains produced infection, with the Asian Zika strain showing greater ability to replicate in host cells (238). In contrast to iPSC-derived 2D model to study Zika, iPSC-derived 3D cerebral organoids have been more recently employed as a model to look at the transient, dynamic nature of the developing brain. Exposing cerebral organoids to African, American, and Asian viral strains was sufficient to infect cerebral organoids. At the level of NSCs, premature differentiation and reduced proliferation were reported following infection by African and American Zika virus, specifically affecting proliferating ventricular zone apical progenitor cells. These adverse effects on neural progenitor cells attenuated neurogenesis and led to cortical degeneration (151). A second cerebral organoid study generated forebrain-specific organoids and observed increased apoptosis, reduced

proliferation, and subsequently diminishing cell volume following infection over time with African and Asian viral strains. Accordingly, ventricular size was increased within cerebral organoids, and both the ventricular zone and neuronal layer were reduced in size (364). The iPSC-derived cerebral organoid model affords the opportunity to assess infection as it progresses over time to identify the time-, spatial-, and cell type-specific effects.

With an increasing number of studies focusing on Zika virus, specific genes have been implicated in the progression of viral infection. In iPSC-derived cerebral organoids, AXL receptor tyrosine kinase gene has been studied for its role in propagation of Zika virus infection through mediating viral entry into the cell (298). Initial RNA-sequencing of astrocytes, radial glia, neurons, endothelial cells, and microglia, showed high expression of the AXL receptor in human cortical primary cell cultures, suggesting cell-specific susceptibility to Zika viral entry. The researchers then utilized iPSC-derived cerebral organoids to asses AXL receptor expression and saw enrichment in regions resembling the ventricular zone. Closer examination revealed that these cell types showed markers of the choroid plexus and radial glia, with reduced AXL receptor expression in neurons (333). Despite cell-specific profiling of the candidate AXL-receptor, a more recent finding refuted its prominent role in Zika virus infection. Wells et al used CRISPR/Cas9 to knockout AXL in iPSCs, and then observed the effect of Zika virus infection in iPSC-derived neural progenitor cells and iPSC-derived cerebral organoids that had been genetically edited. Knockout of the AXL receptor did not protect the neural progenitor cells from Zika infection or Zika-induced apoptosis, and that cells expressing a radial glia phenotype within cerebral organoids were also infected. AXL receptor knockout also did not protect from microcephaly, and the authors concluded that Zika virus must have an alternative mechanism for cell entry and infection (240). Collectively, the multiple models for Zika virus have shown how iPSC-derived 2D and cerebral organoid models can complement each other. In the field related to Zika virus, and in other fields, there is utility in assessing different cell types individually and then translating that knowledge into a more heterogeneous model system in which the different cell types can be studied throughout development and in synchrony.

Autism

Autism spectrum disorder originates as a neurodevelopmental condition and includes a vast range of disability in social, behavioral, and functional activity. From a very young age, individuals with autism can exhibit attention deficits, challenges with social interactions, impaired or loss of speech, repetitive or inappropriate behavior, and inappropriate reactions to novel circumstances. In a subset of patients, copy number variation 16p11.2 has been linked to autism (454). To better study the phenotypic outcomes of these patients with this copy number variant, patient-specific iPSCs were differentiated into cortical neurons. These neurons showed morphological abnormalities such as neuronal hypertrophy, altered soma size, and differences in dendrite length and branching relative to neurons derived from iPSCs of healthy individuals. Individuals with the 16p11.2 deletion showed increased neuronal action potential firing, while the opposite was true for neurons from individuals with the 16p11.2 upregulation. Both upregulation and downregulation of 16p11.2 resulted in enhanced synaptic strength (118). Changes in synapse morphology and activity were also

observed in iPSC-derived neurons from individuals with Rett Syndrome, a condition within the autism spectrum caused by a mutation targeting methyl-CpG-binding protein-2 (MECP2) gene on the X chromosome. These patient iPSC-derived neurons showed a reduction in glutamatergic synapses and altered neuronal morphology, reduced calcium release, and a lower frequency of postsynaptic currents (287). Another iPSC-based study related to autism generated iPSC-derived placodal neurons from individuals with microdeletions in the SH3 region of the multiple ankyrin repeat domains 3 (SHANK3) locus (211). SHANK3 is a protein found within the post-synaptic density. Neurons from autistic patients showed a reduction in synaptic markers. Additionally, time course analysis in iPSC-derived NSCs and early post-mitotic neurons showed that autistic patients exhibited reduced SHANK3 throughout the differentiation process and may be responsible for the altered neuronal morphology in autistic patients. SHANK3 rescue through lentiviral transduction in placodal neurons from autistic patients was sufficient to attenuate the neural dysmorphology (211). A better understanding of gene expression and alterations in synaptic events throughout the differentiation process will be valuable to defining developmental outcomes and treatment approaches.

Allan-Herndon-Dudley syndrome

Allan-Herndon-Dudley syndrome (AHDS) was one of the early X-linked mental retardation syndromes mapped onto the X chromosome. Mutation analysis identified the monocarboxylate transporter 8 gene (MCT8) within each family diagnosed with AHDS. Inactivating mutations of MCT8 alters thyroid hormone levels and ultimately severe neuro-psychomotor impairments (133, 148). Several animal models expressing MCT8 knockout failed to represent the human pathological conditions (133, 439). Additional MCT8-deficient fibroblasts and human cell lines have been utilized to model AHDS, however, they lack key characteristics of affected neurons or endothelial cell populations. The ability to derive neurons and BMECs from iPSCs initially reprogrammed from AHDS patients diminishes the shortcoming of the inadequate models. Vatine et. al generated iPSCs from patients with MCT8 mutations along with associated and corrected controls utilizing CRISPR/Cas 9 technology (449). iPSC-derived neurons deficient in MCT8 transporter displayed depressed thyroid hormone uptake, but still displayed normal triiodothyronine neural maturation. Interestingly iPSC-derived BMECs were deficient in transporting the thyroid hormone into the brain and was ultimately responsible for the decreased concentrations of triiodothyronine in MCT8-deficient brains, unveiling mechanistic insight and potential therapeutic approaches (449). Thus, this adverse transport phenotype of AHDS could potentially be more accurately modeled by employing a BBB model in the future.

Modeling Neuropsychiatric Disorders

The spectrum of neuropsychological illness is very broad and is becoming more prominent in contributing to disability, difficult diagnoses, and the need for a personalized approach to treatment options. Neuropsychiatric disorders are especially challenging for basic scientists as animal models are very limited in their behavioral capabilities and their inability to report and communicate (83, 330, 463). So far, a component of heritability has been reported in humans for some psychological conditions, and there have been multiple genome wide

associations purposed to elucidate underlying genes, environmental factors, and any clues of the underlying cellular and molecular pathways (379, 420). The use of patient-specific iPSCs and the ability to perform differentiation protocols to recapitulate vulnerable cell types in specific disorders is invaluable in addressing some of these previous limitations. A summary of iPSC-derived models to study neuropsychiatric disorders is shown in Table 11. These models have been applied in 2D monolayer and 3D organoid culture approaches.

Schizophrenia

Schizophrenia is a severe, chronic, psychological condition with effects extending to emotion, perception, and behavior. There are three classes of schizophrenia: 1) positive, with symptoms being hallucinations, delusions, and disordered thought and movement; 2) negative, with symptoms of reduced emotions related to pleasure, diminished speaking ability, and difficulty in performing activities; 3) cognitive, where patients exhibit poor understanding of information and subsequent decision making, trouble with focus and attention, and a reduction in utilizing learned information (177, 429). Schizophrenia is a heritable disease and is believed to be polygenic. Additionally, there are other potential causes of schizophrenia, such as pre- and postnatal neurodevelopmental problems, and exposure to viruses (158). At a cellular and molecular level, reduced brain volume, dendritic spine density, and an abnormal distribution of neural cell within the prefrontal cortex have been reported, with DA and glutamatergic neurons being most impacted (53).

With such complex genetic and environmental components to schizophrenia, in addition to little understanding of the disease relative to other brain conditions, studies in the field have begun to dissect similarities and differences across affected individuals. Brennand et al obtained iPSCs from a patient with child-onset schizophrenia and other patients with a family history of schizophrenia (51). iPSCs from all patients differentiated to glutamatergic and DA neurons but showed deficits in the connection between neurons and neurite outgrowth. Loxapine, an anti-psychotic drug used in humans, improved neuronal network connectivity (53). In order to look more closely at this dysregulation in synapse formation and neuronal morphology, iPSCs from keratinocytes of three individuals with paranoid schizophrenia receiving clozapine treatment were differentiated into DA and glutamatergic neurons (377). Mitochondrial dysfunction was implicated to play a role in the disruption of neural networks. At different points in the differentiation process, starting from the keratinocytes derived from the affected individuals, basal mitochondrial respiration was reduced. In both patient-derived keratinocytes and iPSCs, mitochondrial complex I showed greater dopamine-induced inhibition. Furthermore, mitochondrial membrane potential was reduced in keratinocytes, iPSCs, DA NSCs, and glutamatergic NSCs, accompanied by abnormal distribution of mitochondria within the cells (377). Mitochondrial dysfunction has been implicated in various neurodegenerative conditions, but this was the first time such an in-depth resolution was achieved by using iPSCs to observe the manner in which schizophrenia alters the dynamics of neural circuitry.

Bipolar disorder

Bipolar disorder is a type of depression in which episodes of mania occur with a varying degree of frequency on a patient-specific basis. The depressive aspect is manifested as

chronic periods of sadness, hopelessness, anxiety, and other symptoms. The mania within the depression may cause abnormal elation, irritability, and uncontrollable behavior (206).

As is the case with Schizophrenia and other psychological disorders, the disease is believed to be heritable, polygenic, and/or environmental, posing challenges in considering differences among individuals. In focusing on genes underlying network connections between neurons and within the brain in bipolar disorder, Chen et al collected iPSCs from bipolar disorder patients and performed a microarray to look at transcript variants in both iPSCs and differentiated neurons relative to healthy controls (78). Pathway analysis revealed that iPSCs from bipolar patients showed alterations in calcium signaling-related genes involved in differentiation, neuroplasticity, and response to stress. Bipolar disorder neurons showed increased gene enrichment in genes related to neuronal morphology, differentiation, synapse formation and organization, neurotransmitter action, and growth factor release (80). To further comprehend this polygenic aspect of bipolar disorder, a second study obtained iPSCs from family members with and without bipolar disorder (278). A close look at the neuronal differentiation process showed that iPSCs from bipolar disorder patients sequentially formed neural rosettes, but only a subset of cell lines from bipolar patients was able to be differentiated to NSCs. Furthermore, NSCs from bipolar disease patients showed reduced proliferation and a smaller yield of fully differentiated neurons (278). The development of well-defined iPSCs to neuron differentiation protocols allows focusing on specific points during the neurodevelopment process and leads to fine control of experimental design and dysfunction at various stages that underlie bipolar disease.

Screening Drug Toxicity

As described above, iPSC-derived neurons have enabled mechanistic insights into human cells that are otherwise difficult to obtain. For many years, drug exposures and drug actions have been studied in animal models, allowing scientists to elucidate cellular, molecular, and behavioral phenotypes. In some cases of drug exposures on the human brain, e.g., developmental anesthetic exposure (21, 48, 295), almost nothing is known of the human relevance of animal studies. Neurocognitive studies in animals are also limitations in translating phenotypes to what is observed in human populations. Additionally, it has been challenging to dissect the specific effects of drugs only affecting specific types of neurons and their subsequent output altering behavior (156, 275). As addiction becomes more and more of a worldwide epidemic, in addition to exponential growth in pharmacological development, iPSCs have been utilized to generate neurons and other neural cell types to shed light on potential drug toxicity and the underlying mechanisms. Here are two representative research fields regarding applications of human iPSC models in testing the neurotoxic effect of additive drugs and anesthetic agents. A summary of iPSC-derived models to study drug toxicity is shown in Table 12, which has mostly been modeled in 2D monolayer models.

Additive drugs

Certain genetic backgrounds have been reported to increase susceptibility to addictive disorders. Once gene polymorphisms have been identified that render individuals more

susceptible to conditions, iPSCs can be obtained from these individuals, as they maintain their genetic signature prior to reprogramming and throughout the differentiation process. Knowing that the nicotinic receptor alpha 5 has been linked with risk of addiction, iPSCs were obtained from individuals containing either the major or minor single nucleotide polymorphism and were differentiated into DA or glutamatergic neurons (344). Interestingly, the minor allelic polymorphism resulted in greater neuronal excitability in response to current injections. While both populations of DA neurons showed spontaneous action potentials, DA neurons with the minor allele variation were more responsive to nicotine exposure (344). Similar to the DA neuron response, glutamatergic neurons from individuals with the minor allelic variant showed a greater response to nicotine (344). This elegant study highlights the value of the iPSC system to model human disease. A study employing this same concept collected iPSCs from opioid-dependent individuals and control subjects, both containing different polymorphisms in the dopamine receptor transporter, and generated DA neurons (409). Neurons from opioid-dependent individuals showed increased dopamine release, and administration of valproic acid, a treatment for addiction and other neuropsychological disorders, increased expression of DA genes (409). These two studies show great advantages in dissecting the effects of drugs on specific cell subtypes, and methods for differentiation. They also provide direct findings applicable to the human population and highlight a high-throughput approach to study patient-specific outcomes.

Anesthetic drugs

The field of anesthetic-induced developmental neurotoxicity has posed a different, unique epidemiological paradox relative to addiction: despite years of evidence that anesthetics are harmful to the developing brain of rodents and nonhuman primates studied in the lab (267, 423, 482, 492, 501), and recently in human ESC-derived neurons (20, 442, 443), there is no direct evidence that anesthetics result in harm to the brain during childhood.

To cross this barrier from basic science to the clinic, iPSCs have begun to be utilized to test for any detrimental effect of anesthetics on human neurons. First in this field to use this approach was Ito et al, who tested the effect of ketamine on iPSC-derived DA neurons (204). High doses of ketamine resulted in adverse neuronal morphology, with decreased network formation and reduction in neuronal processes. High doses also increased apoptosis, reactive oxygen species, and NADH/NAD^+ production, and reduced ATP production, via ATP synthase, mitochondrial membrane potential, and neurotransmitter reuptake (204). This iPSC-based approach provided much greater cellular and molecular insight than previous animal studies, in addition to looking at the effect of anesthetics on a particular cell type without the confounding effects of the entire brain. Additionally, similar mitochondrial-related findings to what was observed in iPSCs have been reported using human ESC-derived neurons (442, 443), further promoting the use of iPSCs due to consistent conclusions and a more ethical approach. A second study on developmental anesthetic exposure utilized iPSCs to generate NSCs and exposed the NSCs to different doses of propofol. Toxicity was observed following high doses of propofol, with increased apoptosis and reduced cell viability. There was no effect of propofol on neural stem cell proliferation (269). Combined and independently, these two studies represent the strength of iPSCs to look at the effect of anesthetics at different time points in the developmental neurogenesis

process. Additionally, the opportunity exists to test different drug doses, different durations, and frequencies, which is impossible in humans *in vivo* and limited in animal models.

Translation of Human iPSCs: Using of iPSCs in Screening Drug Efficacy and Precision Medicine for Neurological Disorders

Precision medicine has also been termed “personalized” and “individualized” medicine. Over recent decades, there has been a persistent movement toward precision medical treatment tailored for the individual patient based on genetic information derived from a patient’s own biological samples. The major aims of precision medicine are 1) prescribing the right drug at the right dosage and time for each patient, thereby improving drug efficiency, minimizing drug-induced adverse events, and improving the overall cost-effectiveness of health care, and 2) forming earlier diagnosis so that patients can receive preventive therapeutics before a disease causes irreversible damage (168). Several recent studies have taken a step toward using iPSC-derived neurons to model disease-specific neuronal features that may in the future facilitate personalized treatments for neurological disorders. The following subsections will highlight a few examples of how iPSCs have been used in precision medicine-related studies. iPSC-derived 2D monolayer models remain the most widely used so far, but techniques in 3D and BBB models continue to evolve and be put to use.

Screening drug efficacy on neurological disorder treatment

Since certain types of neurons have been found to be differentially affected in leading to neuropsychological dysfunction, obtaining pure populations of specific neuronal subtypes has been invaluable in studying human neurons inaccessible *in vivo.* Serotonin has been recognized for its contribution to mood, emotion regulation, sleep, and hunger, while dysfunction in the serotonergic system leads to adverse psychological disorders, such as depression, bipolar disorder, anxiety, and eating disorders. Here, we discuss example studies on the individualized response to treatment options (Table 13).

The need to model these pressing conditions lead to a protocol to obtain serotonergic neurons through iPSC differentiation by activation of the WNT/sonic hedgehog signaling pathway (271). Following generation of rostral hindbrain NSCs, serotonergic neural progenitor cells originated, and ultimately led to a population of serotonergic neurons. These neurons tested positive for CNS-specific serotonergic markers, such as serotonin, tryptophan hydroxylase 2, and GATA binding protein 3. Electrophysiologically, serotonergic neurons showed a characteristic action potential profile, serotonin release following stimulation, and responded to tramadol and escitalopram oxalate, drugs used to treat anxiety and depression in humans (271). Development of these differentiation protocols is central to personalized medicine and drug testing. A large-scale drug screen was used to test the effect of thousands of potential drugs on treating Zika virus infection (478). iPSCs were used to generate neural progenitor cells and astrocytes, and activated caspase 3 was measured following Zika virus exposure. In addition to the FDA approved drug Niclosamide, inhibitors of cyclin-dependent kinase activity were effective at preventing the Zika virus from replicating (478). As exemplified by this study, iPSCs provide a great opportunity to further inform clinical trials

to ensure that drugs will be safe and effective in humans *in vivo.* Although proving the concept of the BBB is perhaps in its infancy relative to 2D monolayer and 3D cerebral organoid cultures, it has already begun to be used to test drug action. For example, two anti-brain tumor drugs were assessed in an iPSC-derived BBB model, and it was found that the drugs were better able to permeate into the brain than what has been observed *in vivo* (195). This highlights the potential of BBB models to also inform drug action and targeting, in addition to assessing disease phenotypes specific to the individual.

Another application combining drug testing and personalized medicine is that patient specific iPSCs can be differentiated, and their individual response to drugs can be observed. In this regard, one study utilized iPSC-derived DA neurons from PD patients carrying mutated acid p-glucocerebrosidase 1 which is responsible for Gaucher disease and also implicated in PD (294). These patient-specific DA neurons displayed dysregulation of calcium homeostasis and increased stress vulnerability involving elevated cytosolic calcium; furthermore, correction of the acid p-glucocerebrosidase mutations rescued the pathological disease phenotypes (168, 395). Another exciting demonstration for the application of iPSC model in precision medicine research is the comparison of iPSC-derived hippocampal neurons derived from healthy and bipolar disorder individuals, as well as between bipolar disorder patients who were clinically responsive or nonresponsive to lithium. The bipolar disorder patient-specific iPSC-derived neurons were hyperactive compared with control neurons. This hyper-excitability phenotype was reversed by lithium treatment only in neurons derived from bipolar disorder patients who responded to lithium treatment in the clinic (302). These data suggest that patient specific iPSC-based model is a valuable tool in precision medicine research of studying the mechanisms of neurological disorders, screening drug efficiency, and developing new therapies.

Gene editing

Genomic editing in iPSCs has led to major breakthroughs in the realm of personalized medicine, understanding unique mutations, and also providing a human model without the uncertainty of translating an animal model to human disease (188). We discuss a few example studies, summarized in Table 14. Multiple methods of altering gene expression and/or the genome itself in vitro are possible, including, but not limited to, RNA interference (RNAi) strategies such as small interfering RNA (siRNA) or short hairpin RNA (shRNA), transcription activator-like effector nucleases (TALEN), ZFN, and CRISPR/Cas9 (280, 419).

RNAi, in general, is a relatively simple method of temporarily knocking down the expression of genes by affecting their mRNA transcripts (476). While this does not take into account the genetic background of a patient (5), it has been helpful in identifying the unknown significance of genetic mutations and correlating phenotypes seen in human disease to a specific gene(s). In a patient presenting developmental and speech delay, and language disability, sequencing detected rearrangements in the glycosyltransferase like domain containing 1 gene (GTDC1). In this patient, iPSC-derived neural progenitors had reduced ability to proliferate and alterations in neurogenesis. GTDC1 was then knocked down via shRNA in human embryonic stem cells and in zebrafish, and confirmed to be involved in adverse effects on the CNS (5). In this case, iPSCs were used for initial

identification of phenotypes underlying the uncharacterized human condition, and the work done in iPSCs could also be validated in other model systems.

TALEN gene editing, on the other hand, acts directly on the desired DNA sequence, inducing cleavage and therefore cause a complete knockdown (43). Wen et al reprogrammed iPSCs from schizophrenic patients with the Disrupted in schizophrenia 1 (DISC1) frameshift mutation and differentiated them into forebrain neural progenitor cells. By correcting the DISC1 mutation by use of a donor vector TALEN gene editing, DISC1 protein rescue was achieved, and reduced the adverse synaptic phenotypes observed in the iPSC-derived neurons from patients with the frameshift mutation (466). ZFN was one of the first gene editing tools to be introduced, and it acts similar to TALEN, through non homologous end joining repair mechanisms, although its efficiency is not as high (152). As mentioned before, ZFN gene editing was used to reverse mutations in the leucine rich repeat kinase 2 (LRRK2) gene. Mutations in this gene are associated to mitochondrial dysfunction in PD patients, and increased vulnerability to oxidative stress in iPSC-derived NSCs from PD patients. Sanders et al and Reinhardt et al showed that repairing LRRK2 G2019S mutation in iPSCs improved mitochondrial viability, and opened new mechanisms behind PD, respectively (371, 388).

To better address this type of study on rare human diseases, a gene editing platform utilizing CRISPR/Cas9 was combined with the differentiation strategy of generating neurons from iPSCs. Specifically, iPSCs were transfected as single cells with a CRISPR/Cas9 system targeting Glutamate Ionotropic Receptor NMDA Type Subunit 2B, a gene implicated in rare neurodevelopmental disorders. The single iPSCs could then be differentiated to neural progenitor cells, midbrain cells, and neuronal cells within cerebral organoids, with achievement of a stable, persistent knockout throughout the entire process (30). With a greater push for personalized medicine, these rapid pipelines for addressing questions via genetic manipulations are key and facilitate the understanding of disease phenotypes at both a cell-specific and organismal basis.

Conclusion

Brain diseases are among the most serious health problems facing our society and cause human suffering and enormous economic costs. Different diseases might target different types of brains cells and the different development stages of tissues. Unlimited human iPSC-derived 2D cultured brain cells, 3D brain organoids, and BBB provide researchers with invaluable human models that can be applied in the following brains disease research areas: 1) studying the progression from early progenitor cells to neurons and other brain cells, 2) allowing experimental analysis of disease pathogenesis linking them to molecular phenotypes, 3) dissecting the contribution of different types of brain cells to brain diseases, and 4) investigating the effect of individual disease cause or risk factors on the different types of cells. Compared with the simple 2D model, 3D cerebral organoid development allows the iPSCs to differentiate into realistic layers similar to those of real developing brains (237), representing a more clinically relevant model for brain disease research. Many studies have provided convincing evidence supporting the use of iPSC-derived 2D, 3D, and BBB models in human brain disease modeling, drug screening, and novel therapeutic development.

Besides the great potential of these iPSC models, recent work also highlights the following major limitations of iPSC models that must be overcome or be taken into consideration. 1) Using current protocols, generation of patient-specific and matched healthy individual-derived iPSCs and iPSC-derived brain cells are expensive and time consuming, which leads to small sample sizes for most reported studies. 2) One of the major caveats with this *in vitro* stem cell study lies in the relevance of the *in vitro* model compared to a true *in vivo* system. 3) The current *in vitro* platforms used to configure cells to replicate the BBB (e.g., transwell model) is not perusable capillaries seen in brains (205). 3D organoids lack vascularization, which results in the impaired passage of nutrient and oxygen deep within organoids, therefore, leading to necrosis at the center of organoids (Figure 4) and preventing prolonged growth. 5) There is an absence of immune cells such as microglia in 3D organoids, which restricts the use of organoids in modeling inflammation responses to infection. 6) Most neurodegenerative disorders first appear in adulthood. However, iPSC-derived neurons resemble fetal brain cells (51, 289). Although cerebral organoids were nearly as sophisticated as fetal brain tissue in the early second trimester (237), it is still a challenge to define the exact real human brain-equivalent age of cerebral organoids.

These limitations reflect some of the current concerns for employing iPSC-based models for studying brain diseases. However, these should be viewed as features that need to be clarified and considered for improvement rather than as shortcomings of the innovative research tool. Great strides have been made toward potentially overcoming some limitations of iPSC models. For instance, it has been shown that skin fibroblasts could be directly converted into expandable NSCs by timely restricted expression of four genes OCT4, SOX2, KLF4, and cMyc. These NSCs were able to differentiate into neurons (304). Recently, Pham et al developed a protocol for vascularization of brain organoids. On day 34 after the initiation of differentiation, brain organoids were reembedded in Matrigel with human iPSC-derived endothelial cells. On day 54, vascularized organoids were transplanted into mice. The human CD31-positive blood vessels were detected within the center of the organoids 2 weeks after the transplantation (357). In summary, since the first discovery in 2007 that human skin fibroblasts can be reprogrammed to iPSCs, many studies have continued to apply this iPSC technology and successfully recapitulating a variety of brain diseases *in vitro.* iPSC-based disease modeling is a newly developing field. Rapid developments in iPSC technology and other advances in cellular, molecular and developmental neurobiology, and global collaboration among researchers in academia and industry will be the future driving forces to accelerate development of more clinically relevant human iPSC models and new treatments for people with brain diseases. It is predicted that over the next 5 to 10 years, researchers will resolve many fundamental questions about the technology, including generating more mature neurons from iPSCs (408). Perhaps the best advice at present is to take multiple levels of study using a combination of iPSC-derived 2D monolayer, 3D organoid, BBB, and various animal models, to provide a reliable understanding of the pathological phenotypes and underlying mechanisms of human brain diseases.

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Didactic Synopsis

Major teaching points

- Induced pluripotent stem cells (iPSCs) are able to differentiate into different types of neural cells (e.g., neurons, astrocytes, and oligodendrocytes) by being cultured in chemically defined induction media in 2-dimensional (2D) monolayer cultures.
- iPSC-derived 3-dimensional (3D) cortical organoids are composed of multiple neural cell types and exhibit defined brain regions. Cortical organoids have received widespread attention as *in vitro* tools to recapitulate function, architecture, and geometric features of human brain tissues and offer an unprecedented opportunity to study complex human diseases that affect multiple cell types, their interactions, and the function of neuronal circuits.
- iPSC-derived astrocytes, endothelial cells, neurons, and pericytes have been utilized to construct the blood-brain barrier (BBB) in culture dishes. Human iPSC-derived BBB replicates key features of the BBB seen *in vivo* and enables new mechanistic investigations of BBB functions in neurological diseases and drug screening.
- 2D cell models, BBB models, and 3D organoids have different advantages and limitations for studying neurological diseases.
- Human iPSC disease modeling has key advantages compared to animal models such as providing a complicated genetic signature of patients and unlimited cell resource. Human iPSC-derived 2D,3D, and BBB systems have been used for modeling various neurological disorders to study the pathological phenotypes and mechanisms. Specifically, patient-specific iPSC-derived neural cells provide a promising human model for precision medicine research in dissecting genetic contribution to disease development, testing the efficiency and toxicity of drugs, and developing new therapeutics for neurological disorders.
- iPSC-derived 2D cell models 3D organoids and BBB models, have been used for *in vitro* modeling of neurodegenerative diseases [e.g., Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and traumatic brain injury (TBI), Huntington's disease, neurodevelopmental disorders (e.g., cytomegalovirus (CMV) infection, Zika virus infection, autism, Rett syndrome, and Allan-Herndon-Dudley syndrome (AHDS), and neuropsychiatric disorders (e.g., schizophrenia and bipolar disorder)]. The findings have provided novel insights into molecular and genetic mechanisms of brain diseases.
- Techniques in iPSCs, gene editing, and patient-specific cells are at the forefront of approaches towards personalized medicine initiatives. Gene editing allows for disease modeling and obtaining cells from patients

suffering from conditions allows for the study of response to therapeutics and phenotypic analysis.

- Limitations of iPSC-based models for neurological disorders (e.g., immature features of differentiated neural cells and lack of vascularization) should be taken into consideration in future studies. Developments in iPSC technology and other rapid advances in cellular, molecular and developmental neurobiology will be the future driving forces to accelerate development of more clinically relevant human iPSC models and new treatments for people with neurological disorders.

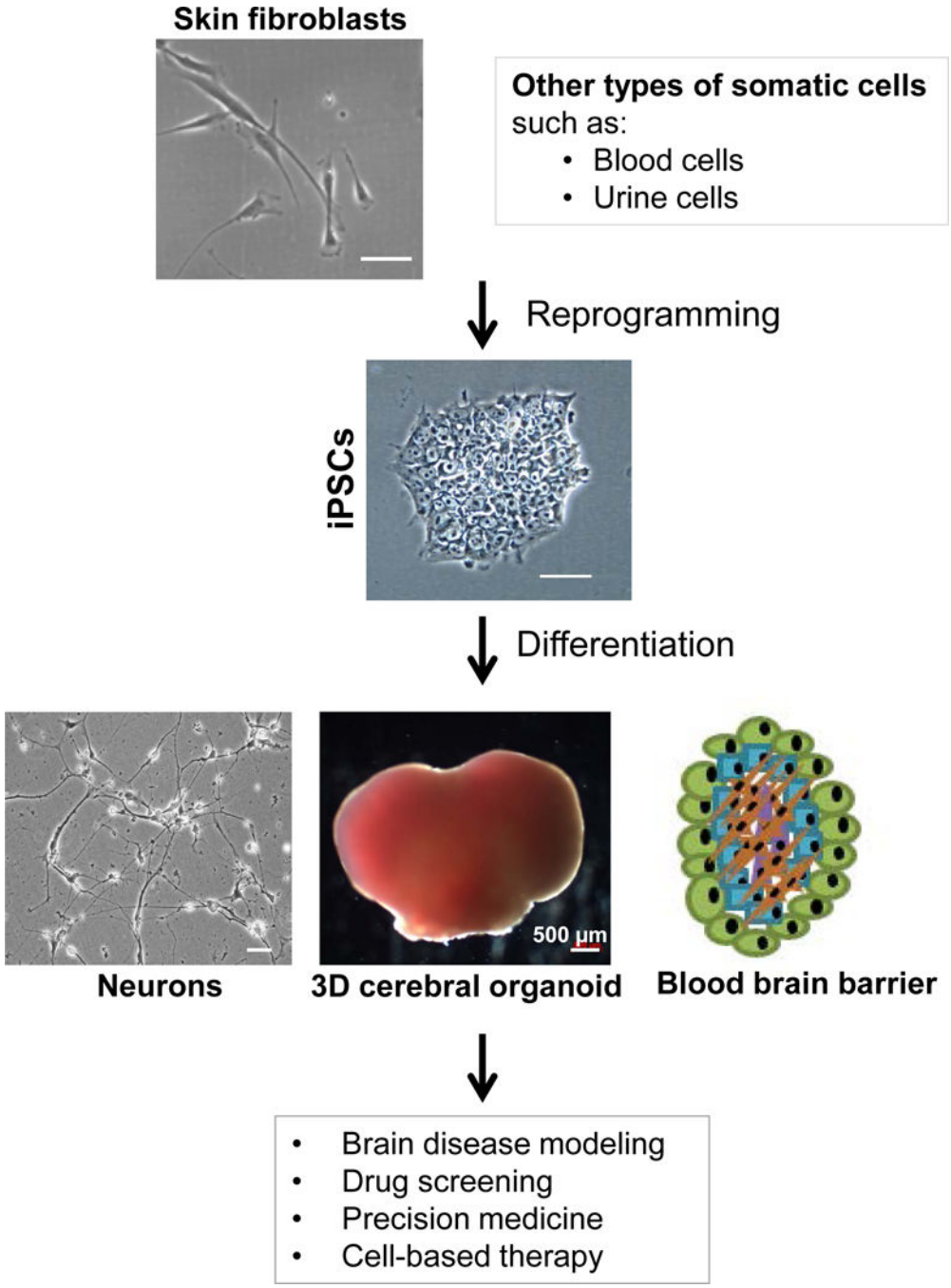

Figure 1.

Schematic representation of induced pluripotent stem cell (iPSC) generation and application. Many somatic cells (e.g., skin fibroblasts, blood cells, and urine cells) can reprogram into iPSCs, and differentiated into neuronal cell types of interest in both a 2-dimensional (2D) monolayer culture and 3-dimensional (3D) brain organoids, which can be used for modeling human brain disease, elucidating underlying molecular and genetic mechanisms, high-throughput drug screening, precision medicine, and tissue regeneration. Scale bar cale bars without labels: 20 μm.

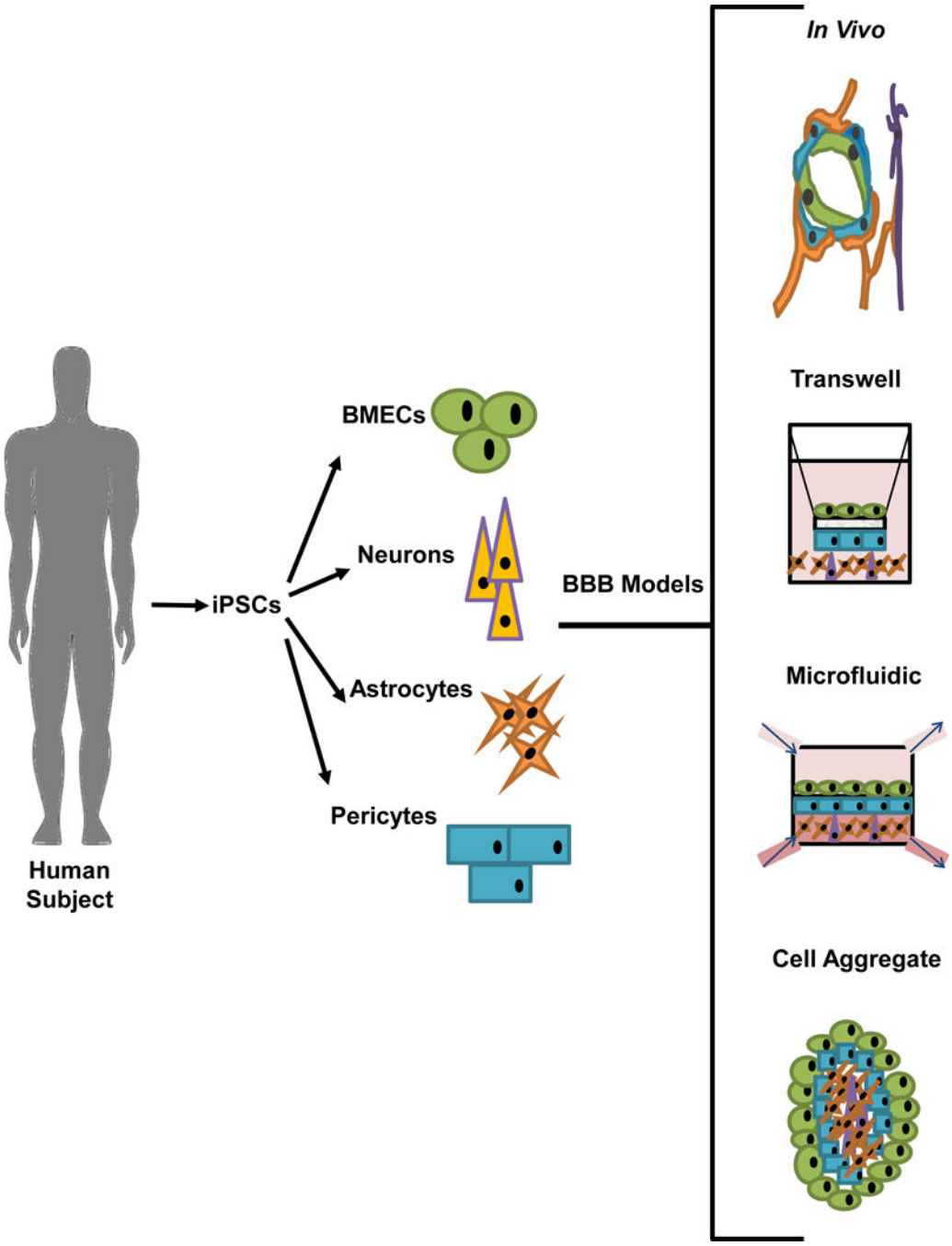

Figure 2.

Modeling the blood-brain barrier (BBB). iPSCs can be differentiated into several cell types of the BBB, including brain microvascular endothelial cells (BMECs), neurons, astrocytes, and pericytes. In vivo, the BBB is comprised of BMECs that form the walls of the blood vessels and are supported by pericytes, astrocytes, neurons, and additional cell types. Current approaches utilized to model the BBB include transwell models, microfluidic and tissue engineering approaches and potentially cell aggregates.

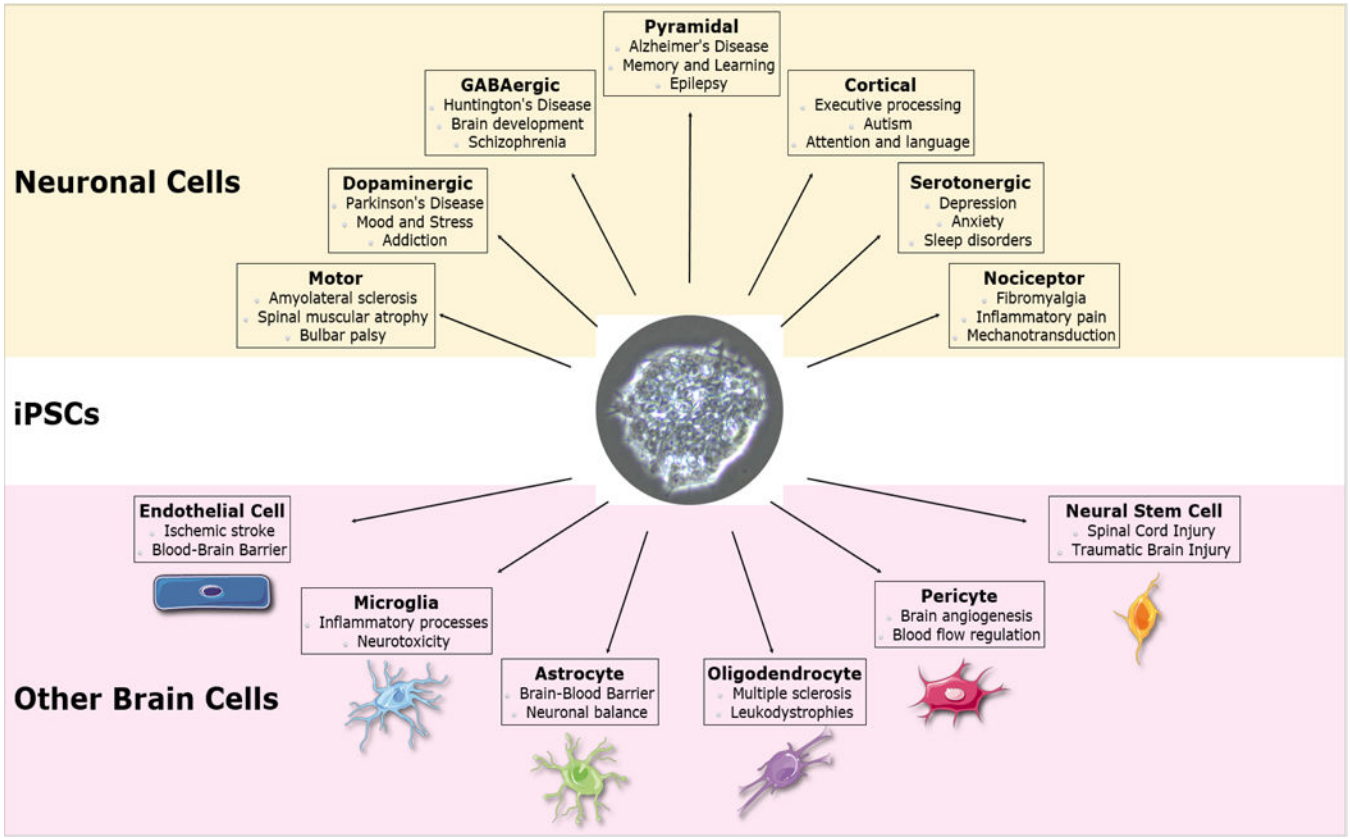

Figure 3.

The summarized reported various brain cells generated from iPSCs in 2-dimensional monolayer cultures and their application in the brain physiology and disease modeling.

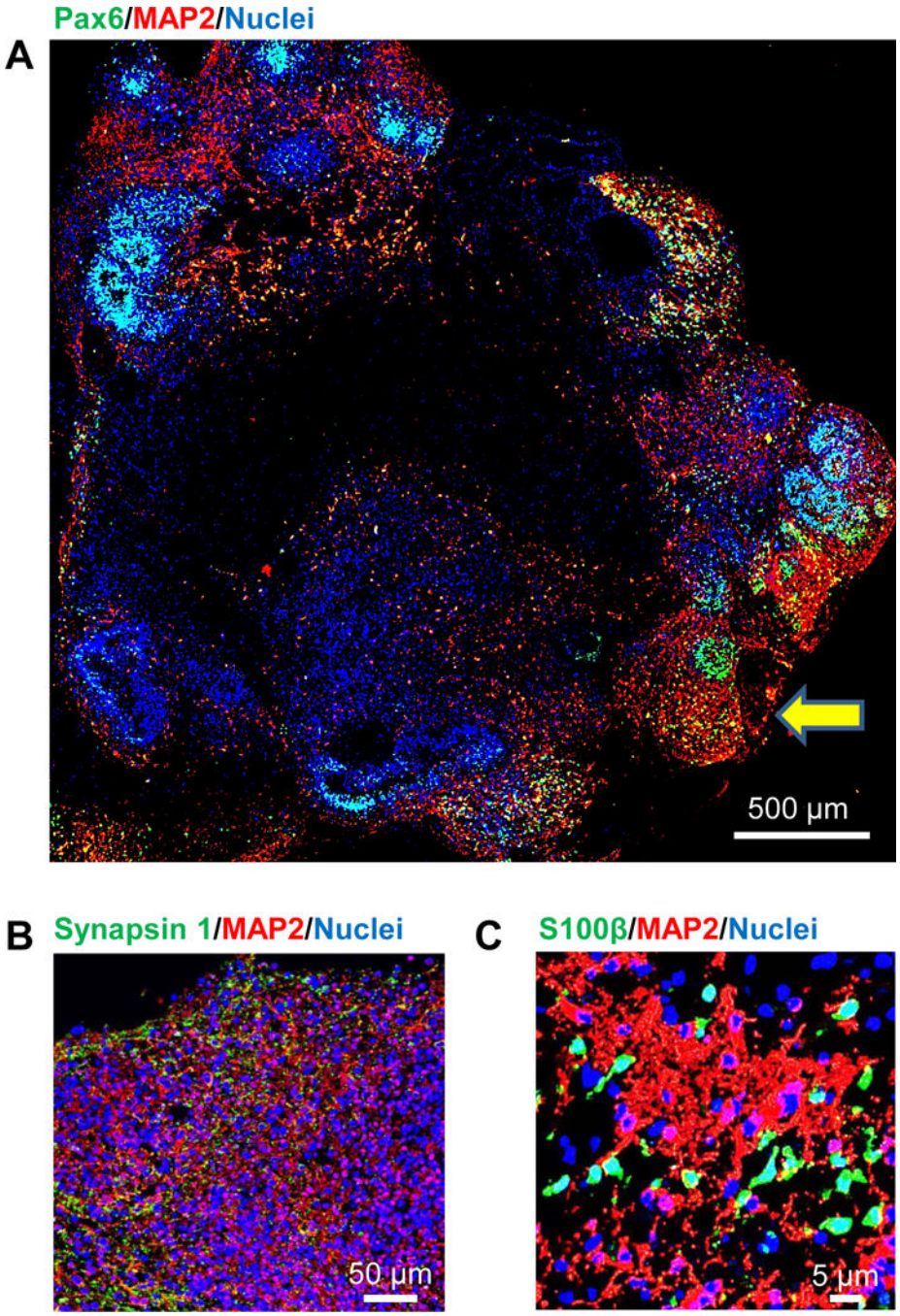

Figure 4.

Tissue sections of 8-week old cerebral organoid generated from human iPSCs. (A) In this whole section of cerebral organoid, red signals represent microtubule associated protein 2 (MAP2)-positive neurons, green are pax6-positive neuroepithelial progenitor cells, and nuclei are blue. Scale bar: 500 μm. In the area indicated by the yellow arrow, neural stem cells (green) are located in apical side and neurons located in the basal side, suggesting that neurons are differentiated from neural stem cells and migrate from the basal toward the apical side. (B) The red signal represents MAP2-positive neurons, the green signal

represents Synapsin1-positive synapses between neurons and the blue marks cell nuclei. Scale bar: 50 μm. (C) S100β-positive astrocytes are shown in green interspersed between MAP2-positive neurons in red. Scale bar: 5 μm. Blue indicates cell nuclei.

Table 1

Current protocols to generate non-neuronal iPSC-derived neural cells

Cell Type	Sequence of major induction factors	Characterization	Reference
Neural stem cells	1. RA and N2 2. EGF, bFGF, and B27	• Expression of Nestin and Sox2 • Able to differentiate into neurons and astrocytes	Yuan et al, 2013 (489)
	1. FGF-2 and EGF	• Expression of Pax6, Nestin, Sox2 and Sox1 • Able to form neural rosettes and differentiate into different neural lineages	Ebert et al, 2013 (135)
	1. N2, B27 and DMH1 2. NEAA, bFGF and EGF	• High expression of Nestin and Pax6 • Ability to differentiate into Tuj1 positive neurons	Wren et al 2015 (471)
	1. DMH1 and SB431542I 2. N2, SHH and bFGF	• Expression of Sox1, Nestin and Pax6 • Able to differentiate into DA neurons	Sugai et al 2016 (424)
Astrocytes	1. bFGF and B27 2. CNTF and BMP	• GFAP and S100β positive cells • Able to transport glutamate and enhance synapse formation of neurons	Shaltouki et al, 2013 (390)
	1. Dual SMAD inhibition 2. EGF + FGF2 + LIF 3. CNTF + RA + PM	• Expression of GFAP and S100β • Uptake of L-glutamate • Promoted synaptogenesis • Propagate calcium waves following stimulation or application of ATP	Serio et al, 2013 (403)
	1. bFGF + PM 2. CNTF + BMP	• Expression of GFAP and S100β • Uptake of L-glutamate • Enhanced synapse formation in neurons	Shaltouki et al, 2015 (405)
	1. Dual SMAD inhibition 2. CNTF + FBS 3. RA + SHH	• Expression of S100β, GFAP, CX43, Aldolase-C, and EAAT1 • Exhibited basal level Na+-dependent glutamate transport • Propagate calcium waves following adjacent astrocytes • Expressed BDNF and GDNF and enhanced survival and neurite outgrowth in motor neurons	Roybon et al, 2013 (378)
	1. Dual SMAD inhibition 2. EGF + FGF2 + LIF + FBS 3. CHIR99021 + SHH	• Expression of Vimentin, S100β, GFAP, AQP4, and EAAT2 • Uptake of L-glutamate • High activity of pyruvate carboxylase	Palm et al, 2015 (348)
Oligodendrocytes	1. bFGF and PM 2. PDGF-AA, NT3, and IGF	• OLIG2 and NKX2.2 positive cells • Able to myelinate brains of immunodeficient mice	Wang et al, 2014 (459)
	1. Dual SMAD inhibition 2. PDGF, IGF-1 and cAMP	• OLIG2 positive cells	Douvaras et al, 2014 (129)
Microglia	1. BMP4 2. bFGF, SCF, and VEGF 3. IL-3, thrombopoietin, M-CSF, and Flt3l	• Expression of known microglia markers • Highly motile processes, constantly scanning the microenvironment • Similar gene expression to human microglia (via RNA-seq)	Douvaras et al, 2017 (128)
	1. VEGF, FGF-2 2. CSF1, IL-34 and TGFβ-1	• Active cytokine secretion • Migration and phagocytosis of CNS substrates	Abud et al, 2017 (1)
Brain microvascular endothelial cells	1. Unconditioned Medium 2. bFGF + PDS 3. RA	• TEER (Ω x cm2) ~ 250 (monoculture); 3,000 (RA); 5,400 (co-culture with pericytes, neurons, and astrocytes) • Expression of tight junction proteins (occludin, claudin-5, ZO-1) • Active efflux transporters (P-gp, BCRP, MRP-1) • Nutrient transporters (TFR, LAT-1, Glut-1, MCT-1)	Lippmann et al 2012,2014 (256, 258)
	1. E6 Medium 2. bFGF + PDS 3. RA	• TEER (Ω x cm2) ~ 4700 (RA); 6,640 (co-culture with pericytes and astrocytes) • Expression of tight junction proteins (occludin and claudin-5) • Active efflux transporters (P-gp and MRP-1)	Hollmann et al, 2017 (190)

Cell Type	Sequence of major induction factors	Characterization	Reference
	1. KOEB 2. bFGF + PDS	• TEER (Ω x cm2) ~ 500 (monoculture); 1,100 (co-culture with astrocyte conditioned medium) • Expression of tight junction proteins • Expression of efflux transporters (P-gp and MRP-1) • Nutrient transporters (TFR, LAT-1, Glut-1, MCT-1)	Ribecco-Lutkiewicz et al, 2018 (373)
Pericytes	1. BMP4, VEGF 2. Activin A and GSK3 *beta* kinase inhibitor	• Expression of PDGFRβ, NG2, and CD146 • Able to form vascular plexus when cocultures with endothelial cells	Orlova et al, 2013 (345)
	1. bFGF +SB431542 + Noggin 2. PDGF-BB + TGFβ1	• Expression of TAGLN, ACTA2, CNN1, and PDGFRβ • Contractile abilities following angiotensin II stimulation	Cheung et al, 2014 (84)
	1. bFGF + EGF 2. FBS + TGFβ1	• Expression of SMA, Calponin 1, SM22α, SM-MHC	Wang et al, 2012 (455)

Abbreviations: bFGF: basic fibroblast growth factor, PDS: platelet-poor plasma-derived serum, BMP4: bone morphogenetic protein 4, Flt3l: FMS-like tyrosine kinase 3 ligand, CNTF: ciliary neurotrophic factor, PM: purmorphamine, PGDF: platelet-Derived Growth Factor, NT3: Neurotrophin-3, VEGF: vascular endothelial growth factor, EGF: Epidermal growth factor, RA: retinoic acid, TGFfil: transforming growth factor-beta1, TEER: trans-endothelial electrical resistance, SMA: smooth muscle actin, M-CSF: macrophage colony stimulating factor, SM-MHC: smooth muscle myosin heavy chain, FBS: fetal bovine serum, MRP: multidrug resistance protein. BCRP: breast cancer resistance protein, ZO-1: zona occludens-1, P-gp: permeability glycoprotein, TFR: transferrin receptor, LAT-1: L-type amino acid transporter 1, Glut-1: glucose transporter 1, MCT-1: monocarboxylate transporter 1, LIF: Leukemia inhibitory factor, cAMP: cyclic adenosine monophosphate, Sox2: SRY Box 2, Sox1: SRY Box, Pax6: Paired box protein 6, Olig2: Oligodendrocyte transcription factor 2, CNS: Central Nervous System, CD146; cluster of differentiation 146, TAGLN: transgelin, ACTA2: Alpha actin 2, CNN1: Calponin1, SM22a: Smooth muscle 22 protein alpha, SHH: sonic hedge hog, IGF: insulin-like growth factor, IL-3: interleukin 3, FGF-2: basic fibroblast growth factor, GFAP: glial fibrillary acidic protein, ATP: adenosine triphosphate, NKX2.2: Homeobox protein Nkx2.2, CSF: Cerebrospinal fluid, GSK3: glycogen synthase kinase 3, BMP: bone morphogenetic protein, S100β:S100 calcium-binding protein B, SCF: stem cell factor, IL-34: Interleukin 34, Cx43: connexin 43, EAAT2: excitatory amino acid transporter 2.

Table 2

Current protocols to generate iPSC-derived neuronal cells

Cell Type	Sequence of major induction factors	Characterization	Reference
Motor neuron	1. All-trans RA 2. PM	• ChAT positive neurons	Sareen et al, 2012 (389)
	1. Dual SMAD Inhibition 2. SAG, FGF-2 and RA	• ChAT positive neurons • Axon response to guidance cues	Maury et al, 2015 (293)
	1. Activin Inhibitor and DMH1 2. FGF and RA	• Hb9-positive neurons • Induced and spontaneous APs	Devlin et al. 2015 (121)
	3. SB431542I, DMH1 4. CHIR99021 5. RA and PM	• ChAT positive neurons • ACh receptors in myotubes cocultures, overlapping with ChAT+ positive neurons	Du et al. 2015 (131)
Dopaminergic neuron	6. SB431542 and Noggin, 7. Shh, FGF-8, and RA	• TH positive neurons capable of dopamine release	Nguyen et al, 2011 (327)
	1. Dual SMAD Inhibition and PM 2. CHIR99021 and FGF-8	• TH positive neurons • GIRK2 positive neurons	Kriks et al, 2011(233)
	1. Dual SMAD Inhibition 2. FGF-8, RA 3. N2, BDNF, GDNF, dCAMP	• EN1, FoxA2 and NURR1 positive neurons • Induced dopamine released	Hartfield et al. 2014 (175)
	1. Dual SMAD Inhibition 2. BDNF, GDNF, dCAMP and LM511-E8	• CORIN,EN1, FoxA2 positive cells • Induced dopamine released • Ability to be transplanted in vivo and maintain DA markers	Doi et al. 2014 (126)
GABAergic neuron	1. B27 2. 5 factors a. ROCK Inhibitor b. SB431542I c. BMPRIA d. Dkk1 e. PM	• VGAT and GABA positive neurons, with GABAergic output and inhibitory APs	Nicholas et al, 2013 (329)
Cortical neuron	1. SB431542I 2. Noggin 3. FGF-2	• PSD-95, and Synaptophysin positive neurons, with mature Na+ and K+ channels, and excitatory (AMPA) action potentials.	Shi et al, 2012 (413)
	1. Dual SMAD Inhibition (with XAV939) 2. PD0325901, SU5402 and DAPT	• FOXP2 (layer V-VI), and SATB2 (layer II-III, V) positive cells • Induced and spontaneous APs	Qi et al 2017 (362)
Serotonergic neuron	1. SB431542I, DMH1, and CHIR99021 2. Shh and FGF-4	• TPH-2 (Tryptophan Hydroxylase 2) positive neurons, capable of serotoninergic release	Lu et al, 2016 (260)
Hippocampal neuron	1. SB431542I, Noggin, Dkk1, and cyclopamine 2. Wnt3a and BDNF	• PROX1 and TBR1 (dentate gyrus markers) positive neurons, and mature APs.	Yu et al, 2014 (487)
Nociceptor	1. SB431542I, LDN-193189 2. SU5402, CHIR99021, and DAPT	• ISL1 positive cells, responsive to capsaicin treatment	Chambers et al, 2012 (78)

Abbreviations: RA: retinoic acid, PM: purmorphamine, Shh: Sonic hedgehog, AP: action potential, FGF: fibroblast growth factor, GIRK2: G-protein-regulated inward-rectifier potassium channel 2, BMPRIA: bone morphogenetic protein receptor, BDNF: brain-derived neurotrophic factor, ChAT: choline acetyltransferase, TH: tyrosine hydroxylase, TPH-2: tryptophan hydroxylase 2, SAG: smoothened agonist, FGF-2: basic fibroblast growth factor, FGF-8, fibroblast growth factor 8, ROCK: Rho-associated, coiled-coil containing protein kinase, DKK1: Dickkopf-related protein 1, DMH1: dorsomorphin homolog 1, FGF-4: fibroblast growth factor 4, WNT3a: Wnt family member 3a, VGAT: Vesicular GABA Transporter, GABA: gamma-Aminobutyric acid, PSD-95: postsynaptic density protein 95, AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, PROX1: prospero homeobox protein 1, TBR1: T-box brain 1, ISL1: Insulin gene enhancer protein 1. BDNF: brain derived neurotrophic factor, GFNF: glial derived neurotrophic factor, AQP4: Aquaporin 4.

Table 3

Advantages and limitations of iPSC-derived 2D, 3D, and BBB models used to model neurological diseases

	2D Cell Culture	3D Cerebral Organoids	Blood-Brain Barrier Models
Advantages	• Simple and well established (with broad literature) • Usually inexpensive and with fast production • Highly reproducible • Can be used for dissecting the effect of different factors on the individual cell and individual developmental event (e.g., NSC proliferation, neuron differentiation, or astrocyte differentiation). • Can use more complex techniques to improve the modelling ability (e.g. co-culturing, micropatterning, and neutrospheres) • Ease of downstream analysis (e.g. microscopy and immunofluorescence)	• Complex architecture and interactions • Better spatial organization (apico-basal and radial) • Highly diversity of cell types and subtypes • Able to mimic different neurodevelopmental events • A more clinically relevant model for studying disease and development mechanisms • Emergence of cell types specific to different brain regions • Intact tissue displaying dynamic growth and development over time	• Better drug action modeling with high fidelity • Able to retain heterogeneous structure and cell polarity • Cultures are viable over longer time periods • Similar transporter expression and permeability to BBB *in vivo* • Personalized medicine opportunities • Multiple cell types can be simultaneously generated from iPSCs. • Recapitulation of mechanical, structural, and electrical properties of BBB *in vivo*.
Disadvantages	• Low predictability power • Little to no cell-ECM interactions • Flat morphology and limited to side-by-side interactions • Lack of complex organization • Can lead to altered genomic and epigenomic signatures • Elimination of other cell types and structural integrity observed In the brain • Cells remain highly immature in displaying metabolic and electrophysiological activity	• High batch-to-batch variability • Slow development • Technically challenging and expensive • Hard to analyze/manipulate (interior cells) • No vasculature. Diffusion and metabolic deficits with prolonged growth in culture lead to tissue degeneration in the center • Model is yet to be employed in complex physiological studies	• High batch-to-batch variability, based on cell line and experimentery • More expensive relative to 2D monolayer approaches • Technically challenging and time consuming • Unable to accurately mimic perfusion and capillary exchange • Only a few drugs have been used to assess model efficacy
References	Centeno et al. 2018 (74), Ho et al. 2018(185), Liu et al. 2018 (260) and Miki et al. 2012 (305)	Lancaster et al. 2013 (237), Sutcliffe et al. 2017 (426), Kelava et al. 2016 (214), and Li et al. 2017 (252)	Lippmann et al. 2013 (257), Canfield et al 2017 (69), Jamieson et al 2017 (205), and Appelt-Menzel et al. 2017 (11)

Abbreviations: ECM: extracellular matrix, NSC: neural stem cells, BBB: blood brain barrier.

Table 4

Modeling Alzheimer's Disease using iPSCs

Gene	Cell type	Viability	Aβ/Tau Phenotype	Other notable phenotypes	Reference
Sporadic	βIII-tubulin+/MAP2+ neurons	Unaffected	Elevated Aβ42/Aβ40 ratio (secreted) (secreted, variable); Elevated p-tau (Thr231), variable	Increased active GSK-3pβ (variable), β-secretase inhibition reduces secreted Aβ−40, p-tau, and active GSK-3β, γ-secretase inhibition reduces secreted Aβ−40, more RAB5+ early endosomes	Israel et al, 2012 (203)
	SATB2+/TBR1 + cortical neurons	Unaffected	Detection of Aβ oligomers (variable); Tau pathology not addressed	β-secretase inhibition reduces Aβ oligomer levels, elevated ROS levels (variable)	Kondo et al, 2013 (223)
	ChAT+/Nkx2.1+/p75+ BFCNs neurons	Unaffected	Elevated Aβ42/Aβ40 ratio (secreted, variable); Tau pathology not addressed	γ-secretase inhibition alters secreted Aβ 40 levels (variable), increased susceptibility to excess glutamate	Duan et al, 2014 (132)
	MAP2+ neurons	Reduced	Elevated Aβ42 (secreted); Tau pathology not addressed	Shortened neurites, hyperexcitability, apigenin reduces disease phenotypes	Balez et al, 2016 (24)
	Mixed neuron cultures	Unaffected	Elevated Aβ42 and Aβ40 (secreted); Hyperphosphorylation of tau	Elevated APP and APP-CTF levels, increased GSK-3β activity, hypersensitivity to H_2O_2 and exogenous Aβ42	Ochalek et al, 2017 (339)
	MAP2+ neurons CD184−/CD44−/ CD24+ neurons	Not addressed Not addressed	No Aβ or tau pathology detected Not addressed	Increased ROS levels (variable), altered oxphos complex expression (variable) Retromer stabilization reduced Aβ42, Aβ40, p-tau levels	Birnbaum et al, 2018 (40) Young et al, 2018 (486)
PSEN1	βIII-tubulin+/MAP2+ neurons	Unaffected	Elevated Aβ42/Aβ40 ratio (secreted); No tauopathy detected	γ-secretase inhibition reduces Aβ42 and Aβ40 production	Yagi et al, 2011 (480)
	CD184−/CD44−/ CD24+ neurons	Unaffected	Elevated Aβ42/Aβ40 ratio (secreted, ΔE9 mutation only); No tauopathy detected	Decreased Y-secretase activity	Woodruff et al, 2013 (470)
	ChAT+/Nkx2.1+/p75+ BFCNs	Unaffected	No Aβ alterations detected; Tau pathology not addressed	Y-secretase inhibition reduces secreted Ap40 levels	Duan et al, 2014 (132)
	TBR1+/SATB2+ cortical neurons	Not addressed	Elevated Aβ42/Aβ40 ratio (secreted); Tau pathology not addressed	Increased transcript levels of the synaptic markers mGLURI and SYT1	Mahairaki et al, 2014 (281)
	MAP2+ neurons	Reduced	Aβ and tau pathology not addressed	Shortened neurites, microglia enhances viability and neurite length reduction, apigenin reverses microglia induced phenotype	Balez et al, 2016 (24)
	Mixed neuron cultures	Unaffected	Elevated Aβ42/Aβ40 ratio (secreted); Hyperphosphorylation of tau	Elevated APP and APP-CTF levels, increased GSK-3β activity, hypersensitivity to H_2O_2 and exogenous Aβ42	Ochalek et al, 2017 (339)
	NeuN+/βIII-tubulin +/NF-H+ neurons	Unaffected	Elevated intracellular and secreted Aβ42; Tau pathology not addressed	Nobiletin treatment increases neprilysin transcript and reduces Aβ42 levels	Kimura et al, 2018 (220)
APP	βlll-tubulin+/MAP2+ neurons	Unaffected	Elevated Aβ40 (secreted); Elevated p-tau (Thr231)	Increased active GSK-3β, β-secretase inhibition reduces secreted Aβ40, p-tau, and active GSK-3β, γ-secretase inhibition reduces secreted Aβ40, more RAB5+ early endosomes	Israel et al, 2012 (203)

Gene	Cell type	Viability	Aβ/Tau Phenotype	Other notable phenotypes	Reference
	SATB2+/TBR1 + cortical neurons	Unaffected	Elevated Aβ42/Aβ40 ratio (secreted, V717L mutation only), Detection of Aβ oligomers (E693Δ mutation only); Tau pathology not addressed	β-secretase inhibition reduces Aβ oligomer levels, elevated ROS levels (E693Δ mutation only), DHA treatment reduces ROS levels (E693Δ mutation only)	Kondo et al, 2013 (223)
	Cux1 +/TBR1 + cortical neurons	Unaffected	Elevated Aβ42/Aβ40 ratio (secreted); Elevated p-tau (Ser262)	γ-secretase inhibition reduces Aβ levels, increased co-localization of APP and EEA1 (early endosomal marker), reduction of Aβ with anti-Aβ antibodies reduces tau levels	Muratore et al, 2014 (318)
	CD184−/CD44−/ CD24+ neurons	Not addressed	Not addressed	Retromer stabilization decreases Aβ42, Aβ40, and p-tau levels	Young et al, 2018 (486)
PSEN2	βIII-tubulin+/MAP2+ neurons	Unaffected	Elevated Aβ42/Aβ40 ratio (secreted); No tauopathy detected	γ-secretase inhibition reduces Aβ42 and Aβ40 production	Yagi et al, 2011 (480)

Abbreviations: Aβ: amyloid-β, BFCNs: basal forebrain cholinergic neurons, MAP2: Microtubule-associated protein 2, GSK-3β: glycogen synthase kinase 3, ROS: reactive oxygen species, SATB2: SATB Homeobox 2, TBR1: Tbox brain 1, ChAT: Choline acetyltransferase, Nkx2.1: NK2 Homeobox 1, CD184: C-X-C chemokine receptor type 4, PSEN1: presenilin 1, NeuN: neuronal nuclei, NF-H: neurofilament H, Cux1: cut like homeobox 1, p-tau: phosphorylated tau, APP-CTF: amyloid precursor protein-carboxyterminal fragments, H_2O_2: hydrogen peroxide, Rab Ras-related protein Rab-5, DHA: Docosahexaenoic Acid, EEA1: Early Endosome Antigen 1.

Table 5

Modeling Parkinson's Disease using iPSCs

Gene/PARK ID	Cell viability	Protein aggregation or mislocalization	Other notable phenotypes	Reference
SNCA/PARK1	Not affected	Not addressed	Increased α-synuclein levels, increase in oxidative stress, increase in protein-aggregation genes	Beyers et al, 2011 (61)
	Not affected	Not addressed	Increased α-synuclein levels	Devine et al, 2011 (120)
	Not addressed	Not addressed	Increased α-synuclein levels, impaired neuronal differentiation, delayed neuronal maturation, decreased neurite outgrowth, lowered neuronal activity, increased autophagic flux	Oliveira et al, 2015 (343)
	Not affected	α-synuclein	Distorted neurites, synaptic defects, decreased neurite length	Kouroupi et al, 2017 (227)
Parkin/PARK2	Not affected	Not addressed	Increased spontaneous DA release, decreased DA uptake, increased oxidative stress	Jiang et al, 2012 (209)
	Not addressed	Not addressed	Decreased neurite length and branching, reduced microtubule stability, phenotype rescue with overexpression of WT parkin or taxol treatment	Ren et al, 2014 (372)
	Not affected	α-synuclein	Impaired dopaminergic neuronal differentiation, increased α-synuclein levels, lowered mitochondrial content, abnormal mitochondria, increased susceptibility to MTT	Shaltouki et al, 2015 (406)
	Not affected	α-synuclein	Abnormal mitochondria, increased mitochondrial stress, susceptibility to mitochondrial stress, and α-synuclein levels, upregulation of dopamine	Chung et al, 2016 (90)
	Not affected	Not addressed	Impaired mitophagy, disrupted mitochondrial quality control	Shiba-Fukushima et al, 2Q17 (415)
	Not affected	Not addressed	Reduced Complex I activity, increased mitochondrial branching, fragmented mitochondria	Zanon et al, 2017 (493)
	Not affected	Not addressed	Activating D1 receptors cause oscillatory activities, overexpressing WT parkin rescues	Zhong et al, 2017 (500)
PINK1/PARK6	Not affected	Not addressed	Reduced mtDNA, increase in PGCIα expression	Seibler et al, 2011 (398)
	Not affected	Not addressed	Increased susceptibility to cellular stress, increased oxidative stress, impaired mitochondrial respiration	Cooper et al, 2012 (98)
	Not affected	α-synuclein	Abnormal mitochondria, increased mitochondrial stress, susceptibility to mitochondrial stress, and α-synuclein levels, upregulation of dopamine	Chung et al, 2016 (90)
	Not affected	Not addressed	Disrupted mitochondrial quality control	Shiba-Fukushima et al, 2017 (415)
	Not affected	Not addressed	Increased LRRK2 expression, increased mitochondrial fragmentation, WT PINK1 overexpression reduces LRRK2 induction	Azkona et al, 2018 (18)
LRRK2/PARK8	Not affected	Not addressed	Increased α-synuclein levels, increased oxidative stress, increased susceptibility to H_2O_2 and 6-OHDA	Nguyen et al, 2011 (327)
	Not affected	Not addressed	Increased susceptibility to cellular stress, impaired mitochondrial respiration, dysfunctional mitochondrial mobility	Cooper et al, 2012 (98)
	Reduced	α-synuclein	Decreased neurite length, impaired autophagy, defective autophagosome clearance	Sánchéz-Danes et al, 2012 (387)
	Not affected	Not addressed	Decreased neurite outgrowth, increased sensitivity to 6-OH-Dopa and Rotenone, increased levels of α-synuclein and tau, ZFN-mediated gene correction reverses	Reinhardt et al, 2013 (371)

Gene/PARK ID	Cell viability	Protein aggregation or mislocalization	Other notable phenotypes	Reference
	Not affected	Not addressed	Increased mtDNA damage, ZFN-mediated genome correction reverses	Sanders et al 2014 (388)
	Not affected	Not addressed	Increased neurite branching	Borgs et al 2016 (47)
	Not addressed	Not addressed	Delayed mitochondrial arrest, delayed axonal mitophagy, miro accumulation	Hsieh et al, 2016 (194)
	Not affected	Not addressed	Increased α-synuclein levels, LRRK2 knockdown decreases α-synuclein protein levels, altered NFκB activity	López de Maturana et al, 2016 (270)
	Not affected	Not addressed	Alterations in mitochondrial content, distribution, velocity, movement, and respiration, LRRK2 kinase inhibition does not reverse phenotypes, increased sirtuin expression, decreased sirtuin activity, decreased NAD+ levels	Schwab et al, 2017 (396)
Sporadic	Reduced	None	Decreased neurite length, impaired autophagy, defective autophagosome clearance	Sánchez-Danés et al, 2012 (387)
	Not affected	Not addressed	Decreased neurite outgrowth, LRRK2 kinase inhibition does not rescue	Marrone et al, 2018 (290)

Abbreviations: DA: Dopamine, SNCA: synuclein alpha, PARK1, 2, 6, 8: Parkinson's disease associated genes 1, 2, 6, 8, mtDNA: mitochondrial DNA, WT: wild type, PINK1: PTEN-induced putative kinase 1, D1: dopamine receptor D1, ZFN: zinc finger nuclease, NAD: Nicotinamide adenine dinucleotide, PGCIα: Proliferator-Activated Receptor-Gamma Coactivator-1α, LRRK2: leucine-rich repeat kinase 2, H2O2: hydrogen peroxide, 60HDA: 6-hydroxydopamine.

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Table 6

Modeling Huntington's Disease using iPSCs

Repeats	Cell type	Viability	Protein Aggregation	Other notable phenotypes	Reference
42/44 39/43 17/45	MAP2+/GABA neurons	Unaffected (basal)	Not addressed	Increased lysosomal and autophagosome content	Camnasino et al, 2012 (66)
72	MAP2+ neurons	Reduced (basal)	Not addressed	Decreased neurite length, increased expression of pro-antioxidant proteins, decreased expression of cytoskeletal proteins	Chae et al, 2012 (76)
1S0 109 60	DARPP-32+ MSNs	Reduced (BDNF withdrawal)	None detected	Enhanced vulnerability to exogenous stressors	HD iPSC Consortium, 2012 (97)
>60 (exact number not provided)	DARPP-32+ MSNs	Reduced (BDNF withdrawal)	Not addressed	Decreased neurite length, increased ROS, decreased ATP, reduction of phenotypes with inhibition of mitochondrial fission	Guo et al, 2013 (167)
180 109	DARPP-32+ MSNs	Reduced (BDNF withdrawl)	Not addressed	ATM inhibition rescues BDNF withdrawal-induced death	Lu et al, 2014 (272)
43	DARPP-32+ MSNs	Reduced (H_2O_2 treatment)	Not addressed	Increased DNA damage after H_2O_2 treatment, reduced adenosine 2A receptor expression, adenosine 2A receptor agonists reduce phenotype	Chiu et al, 2015 (87)
180 109 60	DARPP-32+ MSNs	Unaffected	Not addressed	Persistent NPC population that is susceptible to BDNF withdrawal, knockdown of mHtt reduces this death	Mattis et al, 2015 (292)
70 47	DARPP-32+ MSNs	Reduced (BDNF withdrawal)	Not addressed	Activity reduction of the striatal-enriched GPCR Gpr52 reduces mHtt levels	Yao et al, 2015 (485)
109 90	DARPP-32+ MSNs	Unaffected (basal)	Not addressed	RNA-seq reveals alterations in genes related to neuronal development and function, knock down of mHtt with ASOs reverse these changes	HD iPSC Consortium, 2017 (96)
180	MAP2+/GABA+ neurons	Reduced (BDNF withdrawal)	None detected	No changes in electrical activity, alterations in gene expression (in NPC cultures), CRISPR correction reverses these changes	Xu et al, 2017 (479)

Abbreviations: MSNs: medium spiny neurons, BDNF: brain derived-neurotrophic factor, mHtt: mutant Huntingtin, ROS: reactive oxygen species, MAP2: microtubule associated protein, GABA: gamma-Aminobutyric acid, DARPP-32: Dopamine and cAMP-regulated phosphoprotein Mr 32,000, H_2O_2: hydrogen peroxide, ATP: adenosine triphosphate, ATM: ataxia-telangiectasia mutated, NPC: neural progenitor cell, GPCR: G-protein coupled receptor, GPR52: G Protein-Coupled Receptor 52, ASO: antisense oligonucleotide, CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat

Table 7

Modeling amyotrophic lateral sclerosis using iPSCs

Gene	Viability	Protein Aggregation or Mislocalization	Other notable phenotypes	Reference
SOD1	Unaffected	SOD1 and neurofilament	Neurite degeneration	Chen et al, 2014 (79)
	Reduced	Not addressed	Hyperexcitability, CRISPR correction and retigabine treatment rescues excitability and viability alterations	Wainger et al, 2014 (453)
	Not addressed	Not addressed	Downregulation of genes that are associated with age, mimicking primary MN samples from ALS patients	Ho et al, 2016 (186)
	Unaffected	Not addressed	Hypoexcitability, 4AP treatment rescues	Naujock et al, 2016 (324)
	Reduced	SOD1	Morphological alterations, ER stress, CRISPR correction reverses phenotypes	Bhinge et al, 2017 (35)
	Unaffected	SOD1 and optineurin	Increased HspB8 levels, no enhanced stress granule formation	Seminary et al, 2018 (401)
C9orf72	Unaffected	GP dipeptide repeat protein	RNA foci, lowered variant 2 mRNA levels, enhanced vulnerability to autophagy inhibition	Almeida et al, 2013 (8)
	Unaffected	None detected	RNA foci, altered expression of genes related to cell adhesion, synaptic transmission, and neural differentiation, hypoexcitability, knockdown of C9orf72 reduces phenotypes	Sareen et al, 2013 (390)
	Not addressed	Not addressed	Hyperexcitability, retigabine treatment rescues	Wainger et al, 2014 (453)
	Unaffected	Not addressed	Hyperexcitability, decreased ability to fire APs with time	Devlin et al, 2015 (121)
	Unaffected	GR, PR, GA, and GP dipeptide repeat protein	RNA foci, elevated ER calcium, mitochondrial abnormalities, elevated pro-apoptotic markers, elevated stress granule formation	Dafinca et al, 2016 (109)
	Unaffected	GA, GR, and GP dipeptide repeat proteins	RNA foci, no alterations in excitability, increased expression of GluA1, enhanced vulnerability to AMPA, CRISPR correction reverses phenotypes	Selvaraj et al, 2018 (400)
	Unaffected	SOD1 and optineurin	No basal heat shock response induction, no enhanced stress granule formation	Seminary et al, 2018 (401)
TDP-43	Reduced	TDP-43	Enhanced vulnerability to PI3K inhibition	Bilican et al, 2012 (38)
	Unaffected	TDP-43	Shortened neurites, altered expression of RNA metabolism related genes and neurofilament genes, anacardic acid reverses disease phenotypes	Egawa et al, 2012 (137)
	Unaffected	Not addressed	Hyperexcitability, decreased ability to fire APs with time	Devlin et al, 2015 (121)
	Unaffected	TDP-43	Increased Bag3 levels, inability to induce the heat shock response after acute heat shock, no enhanced stress granule formation	Seminary et al, 2018 (401)
FUS	Not addressed	Not addressed	Hyperexcitability, retigabine treatment rescues	Wainger et al, 2014 (453)
	Reduced	FUS	Enhanced vulnerability to arsenite, inclusions increase with aging	Japtok et al, 2015 (208)
	Unaffected Not addressed	FUS FUS	FUS localization to stress granules DNA damage, FUS localization to stress granules	Lenzi et al, 2015 (244) Higelin et al, 2016 (184)
	Unaffected	Not addressed	Hypoexcitability, 4AP treatment rescues	Naujock et al, 2016 (324)

Gene	Viability	Protein Aggregation or Mislocalization	Other notable phenotypes	Reference
	Unaffected	Not addressed	Transcriptional alterations in genes relating to cell-cell adhesions, among others, and downregulation of miR-375	De Santis et al, 2017 (112)
	Unaffected	FUS (trend at baseline, significant after exogenous stress)	FUS localizes to SGs under stress, induction of autophagy reduces phenotypes	Marrone et al, 2018 (291)
	Unaffected	FUS	Hypoexcitability, axonal swelling, distal axon degeneration, reduced mitochondrial and lysosomal movement distally, increased DNA damage, CRISPR correction reverses phenotype	Naumann et al, 2018 (325)
Sporadic	Not addressed	TDP-43	Hyperphosphorylation of TDP-43 aggregates, cardiac glycosides decrease aggregates	Burkhardt et al, 2013 (60)
	Not addressed	Not addressed	Transcriptional alterations in genes related to mitochondrial function	Alves et al, 2015 (9)

Abbreviations: MN: motor neurons, SGs: stress granules, SOD1: superoxide dismutase 1, APs: action potentials, C9orf72: chromosome 9 open reading frame 72, TDP-43: TAR DNA-binding protein 43, FUS: Fused in Sarcoma/Translocated in Sarcoma, CRISPR: Clustered Regularly Interspaced Short Palindromic Repeat, 4AP: 4-Aminopyridine, ER: endoplasmic reticulum, HSpB8: heat shock protein beta 8, GluA1: Anti-AMPA Receptor 1, AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, PI3K: Phosphoinositide 3-kinase, BAG3: BAG family molecular chaperone regulator 3.

Table 8

Summary of directed reprogramming strategies to study neurodegenerative disease

Induced cell type	Starting cell type	Genotypes	Disease phenotypes	Reference
Neurons	Fibroblasts	AD (*APP, PSEN1*)	Elevated Aβ40, Aβ42, Aβ42/Aβ40 ratio, and p-tau levels (variable), no alteration in GSK-3β activity	Hu et al, 2015 (197)
	Fibroblasts	AD (*APOE3/E4*)	Increase in neurons expressing Ap42	Kim et al, (218)
NPCs	Fibroblasts	AD (*APOE4/E4, PSEN1*)	Neurons derived from iNPCs secrete higher levels of Aβ40 and Aβ42 (*PSEN1* lines) and have higher levels of p-tau (APOE4 line), GSK3β inhibition reduces p-tau levels	Hou et al, 2017 (192)
DA neurons	Fibroblasts	PD (*SNCA, Parkin*)	Not addressed	Caiazzo et al, 2011 (63)
	Fibroblasts	Control	Not addressed	Pfiesterer et al, 2011 (356)
	Fibroblasts	Control	Transplantation into a rat PD model alleviates some symptoms	Liu et al, 2014 (264)
DA NPCs	Fibroblasts	Control	NPCs can be further differentiated into DA neurons	Mirakhori et al, 2015 (310)
"Neuron-like" (DARPP-32+)	Fibroblasts	HD (68Q, 86Q) Control (16Q)	Reduced viability, Htt+ inclusions	Liu et al, 2014 (266)
NPCs	Fibroblasts	HD (41Q, 41Q) Control	Neurons derived from iNPCs exhibit elevated DNA damage that is decreased with $A_{2A}R$ agonist treatment	Hou et al, 2017 (192)
MSNs	Fibroblasts	HD (40Q, 43Q, 44Q) Control (17Q, 18Q, 19Q)	Detectable Htt aggregates, proteostasis collapse, DNA damage, reduced viability, increased ROS levels, decreased mitochondrial membrane potential, converted fibroblasts from pre-symptomatic patients exhibit fewer phenotypes	Victor et al, 2018 (451)
NPCs	Fibroblasts	ALS (*SOD1, C9orf72,* sporadic) Controls	Astrocytes derived from iNPCs decrease cell survival of mouse ESC-derived MNs	Meyer et al, 2014 (303)
Neurons	Fibroblasts	ALS (*FUS*) Controls	Mislocalization of FUS to the cytoplasm, localization of FUS to SGs upon arsenite stress	Lim et al, 2016 (254)
Motor Neurons	Fibroblasts	ALS (*FUS*) Controls	FUS mislocalization, smaller soma size, reduced survival, hypoexcitability	Liu et al, 2016 (262)
	Fibroblasts	Controls (young and old)	Direct reprogramming maintains aging-related marks that are reversed in iPSC reprogramming	Tang et al, 2017 (430)

Abbreviations: Aβ: amyloid-β, iNPCs: induced neural progenitor cells, DA neurons: dopaminergic neurons, DA: dopamine, Htt: huntingtin, mtHTT: mutant huntingtin, $A_{2A}R$: adenosine 2A receptor, MSNs: medium spiny neurons, MNs: motor neurons, SGs: stress granules, APOE: apolipoprotein E, AD: Alzheimer's disease, PD: Parkinson's disease, ALS: Amyotrophic Lateral Sclerosis, HD: Huntington's disease, ROS: reactive oxygen species, ESC: embryonic stem cell, p-tau: phosphorylated tau, DARPP-32: Dopamine And CAMP-Regulated Neuronal Phosphoprotein 32, APP: amyloid precursor protein, PSEN1: Presenilin-1, SNCA: alpha-synuclein, c9orf72: c9orf72: chromosome 9 open reading frame 72, FUS: Fused in Sarcoma/Translocated in Sarcoma, GSK-3β: Glycogen synthase kinase 3 beta.

Table 9

iPSC-derived 3D models to study neurodegenerative diseases

Applicable Disease	3D/Cell lines Model Used	Model Details	Reference
AD	NPCs embedded into Matrigel and differentiated into neurons and glia/control NPCs overexpressing AD mutant APP and/or PSEN1	Mixed neuron and glial cells, extracellular amyloid-p deposits, insoluble amyloid-β and pTau aggregates, β- and γ-secretase inhibition reduces phenotypes	Choi et al, 2014 (89)
	Cortical spheroids/Sporadic AD iPSCs	Mixed neurons and glial cells, high levels of secreted Aβ42 and Aβ40, reduced with γ-secretase inhibition, reduced efficacy of γ-secretase inhibition compared to 2D models	Lee et al, 2016 (240)
	Brain organoids/Control and AD (*APP, PSEN1*) iPSCs	Initially generate organized NPC regions, mature into unorganized neuronal regions, evidence of necrosis in the middle with extended time in culture, AD organoids show evidence of Aβ and pTau aggregates that are reduced with β- and γ-secretase inhibition	Raja et al, 2016 (368)
	Forebrain cortical organoids/Control and AD (*PSEN1*) iPSCs	AD organoids are smaller, have elevated levels of Aβ42 and pTau, exhibit increased cell death, elevated inflammation markers, altered ECM protein expression, heparin, heparinase III, and Y-secretase inhibitor treatment ameliorates phenotypes	Yan et al, 2018 (483)
PD	Midbrain engineered neural tissue/Control stem cells (ESCs and iPSCs)	Midbrain ENTs contain DA neurons	Tieng et al, 2014 (434)
	Midbrain-like organoids/Control stem cells (ESCs and iPSCs)	Form layers similar to murine embryonic midbrain floor plate, MLOs contain DA neurons that are electrically active, expression of neuromelanin detected	Jo et al, 2016 (210)
	Midbrain organoids/Control iPSCs	Midbrain organoids contain DA neurons	Qian et al, 2016 (364)
	Midbrain organoids/NESCs derived from control iPSCs	Spatially patterned tissue, contain DA neurons, cell death in core of organoid, small percentage of astrocytes present, evidence of myelination, electrically active neurons	Monzel et al, 2017 (314)
HD	Cortical organoids/Control (21Q, 28Q) and HD (60Q, 109Q) iPSCs	HD organoids lack embryonic ventricle-like structures, disruption of TBR1 and CTIP2 expression and organization, immature transcriptional profile, knockdown of mtHtt reduces developmental defects	Conforti et al, 2018 (93)
ALS	NSC-derived MN spheroids grown with iPSC-derived endothelial cells in a microfluidic device/ Control NSCs and iPSCs	MN spheroids contain electrically active MNs and astrocytes, MNs make connections with vasculature, microfluidic device generates interstitial flow	Osaki et al, 2018 (346)

Abbreviations: AD: Alzheimer's disease, PD: Parkinson's disease, HD: Huntington's disease, ALS: amyotrophic lateral sclerosis, NPCs: neural progenitor cells, Aβ: amyloid-β, ENT: engineered neural tissue, mtHtt: mutant huntingtin, MN: motor neurons, APP: amyloid precursor protein, PSEN1: Presenilin-1, ESC: embryonic stem cell, MLO: midbrain-like organoids, NSCs: neural stem cells, p-tau: phosphorylated tau, ECM: extracellular matrix, DA: dopamine, TBR1: t-box brain 1, CTIP2: Chicken ovalbumin upstream promoter transcription factor-interacting protein 2

Table 10

Modeling neurodevelopmental disorders using iPSCs

Disease/Condition	Stem cell model	Experimental procedure	Major findings	Reference
Cytomegalovirus infection	iPSC-derived neural stem cells, neural progenitor cells, and neurons	Viral transduction of AD169 strain from infected human fibroblasts	• iPSCs and neurons did not allow viral replication • NSCs display impaired differentiation • NPCs allow viral replication	D'Aiuto et al, 2012 (107)
	iPSC-derived neural stem cells and neural progenitor cells	Viral infection of Towne strain	• Apoptosis in neural stem cells and neural progenitors infected with cytomegalovirus	Nakamura et al, 2013 (322)
Zika virus infection	iPSC-derived cortical neurons, motor neurons, and astrocytes	Transduction of cells with African and Asian Zika viral strains	• Cell damage in cortical neurons • Cell damage in motor neurons • Cell damage in astrocytes • Greater ability of Asian virus to replicate in different brain models	Lanko et al, 2017 (238)
	iPSC-derived neural progenitor cells and cerebral organoids	Infection of cells with Puerto Rican Zika viral strain	• NPCs were vulnerable to viral infection • Cerebral organoid size reduction	Wells et al, 2016 (240)
	iPSC-derived cerebral organoids	RNA-seq to assess Zika virus receptor abundance	• Enriched Zika virus receptor AXL in radial glial NSCs	Nowakowski et al, 2016 (333)
	iPSC-derived cerebral organoids	Infection of cells with American and Asian Zika viral strains	• Premature differentiation of NPCs	Gabriel et al, 2017 (151)
	iPSC-derived forebrain cerebral organoids	Infection of cells with African and Asian Zika viral strains	• Microencephaly of neural cell layer • Infection of NPCs	Qian et al, 2016 (364)
Autism spectrum disorder	iPSC-derived neurons from patients with a copy number variation associated with autism iPSC-derived olfactory placodal neurons from autistic patients	Immunohistochemistry, Whole-cell patch clamping Neurite outgrowth assay, Synaptic puncta measurement	• Altered neuronal morphology • Differences in electrophysiological current in copy number variant neurons • Reduced synaptic density • Reduced synapse formation in iPSC-derived neurons from autistic patients • Increased neurite branching in iPSC-derived neurons from autistic patients • SHANK3 overexpression provided neuroprotection	Deshpande et al, 2017 (118) Kathuria et al, 2018 (211)
	iPSC-derived neurons and astrocytes from autistic patients	• Multielectrode array • Quantification of synaptic puncta • Reactive oxygen species assay • Co-culture between neurons and astrocytes • Glutamate release assay	• iPSC-derived neurons from autistic patients showed a reduction in synaptic markers, glutamate release, and electrical activity • iPSC-derived astrocytes from autistic patients contributed neuronal consequences	Russo et al, 2017 (380)
Rett syndrome	iPSC-derived neurons and neural progenitor cells from Rett syndrome patients	• Calcium imaging • Whole-cell patch clamping	• Rett syndrome neurons showed reduced glutamatergic synapses • Reduced calcium release in Rett syndrome neurons • Attenuation of post synaptic current in Rett syndrome neurons	Marchetto et al, 2011 (287)
	iPSC-derived Astrocytes	• Immunocytochemistry • Methylation Profiling	• Rett-derived astrocytes decreased neurite length and number	Williams et al, 2014 (468)

Disease/Condition	Stem cell model	Experimental procedure	Major findings	Reference
		• Electrophysiology		
AHDS	iPSC-derived Neurons and BMECs	• Immunocytochemistry • qRT-PCR • Electrophysiology • Permeability	• AHDS-derived BMECs display appropriate barrier properties but are defective in Ts transport contributing to reduced neuronal maturation.	Vatine et al, 2017 (449)

Abbreviations: AHDS: Allan-Herndon-Dudley syndrome, BMECs: brain microvascular endothelial cells, NPCs: neural progenitor cells, NSCs: neural stem cells, AXL: tyrosine-protein kinase receptor UFO, SHANKS: SHS and multiple ankyrin repeat domains 3.

Table 11

Modeling neuropsychiatric brain diseases using iPSCs

Disease/ Condition	Stem cell model	Experimental procedure	Major findings	Reference
Schizophrenia	iPSC-derived glutamatergic and dopaminergic neurons from schizophrenic patients	• High-pressure liquid chromatography to assess • amino acids and metabolites Mitochondrial respiration measurements • Mitochondrial membrane potential measurements	• Dopaminergic cells displayed impaired differentiation into neurons • Glutamatergic cells could not mature • Loss of mitochondrial membrane potential in dopaminergic and glutamatergic cells	Robicsek et al, 2013 (377)
	iPSC-derived neurons from schizophrenic patients	• GFP-reporter assay to measure neurites • Immunostaining of synaptic markers • Whole-cell patch clamping • Calcium imaging • Genome scan array	• Reduced neuronal connectivity • Altered neurite morphology • Decreased glutamate receptor abundance • Loxapine attenuated schizophrenic phenotypes	Brennand et al, 2012 (53)
Bipolar disorder	iPSC-derived neurons from bipolar disorder patients	• Microarray for gene expression profiles • Calcium imaging	• Bipolar disorder neurons displayed altered transcriptional signature • Bipolar disorder neurons showed an increased abundance of ventral differentiation markers • Lithium decreased calcium transient of bipolar disorder neurons	Chen et al, 2014 (80)
	iPSC-derived neural progenitor cells and neurons from bipolar disorder patients	• Global transcriptome analysis • BrdU assay to assess proliferation	• Altered differentiation of neural progenitor cells • Altered gene expression of markers related to neurogenesis and neuroplasticity	Madison et al, 2015 (278)
	iPSC-derived hippocampal dentate gyrus-like neurons	• RNA sequencing • Calcium imaging • Whole-cell patch clamping • Response to lithium treatment	• Mitochondrial abnormalities in bipolar disorder patients • Over-activity in action potential firing and calcium release • Treatment with lithium attenuated hyperexcitability	Mertens et al, 2015 (302)

Abbreviations: BrdU: Bromodeoxyuridine, GFP: green fluorescent protein.

Table 12

Screening drug toxicity using iPSCs

Disease/ condition	Stem cell model	Experimental procedure	Major Findings	Reference
Anesthetic exposure	iPSC-derived neurons	• Exposure of iPSC-derived neurons to ketamine • Apoptosis assay • Reactive oxygen species production • Adenosine triphosphate concentration • Mitochondrial membrane potential	• Increased apoptosis • Reduction in mitochondrial membrane potential • Increased reactive oxygen species production • Reduction in adenosine triphosphate concentration	Ito et al, 2015 (204)
	iPSC-derived neural progenitor cells	• Exposure of iPSC-derived neural progenitors to propofol • Apoptosis assay • Microarray	• Propofol did not elicit apoptosis in neural progenitors • Propofol did not alter neural progenitor proliferation • High doses of propofol induced cytotoxicity and altered gene abundance	Long et al, 2017 (269)
General addiction	iPSC-derived dopaminergic neurons from individuals with polymorphism in nicotine receptor gene iPSC-derived glutamatergic neurons from opioid-dependent individuals	• Nicotine exposure • RNA sequencing • Whole-cell patch clamping	• Polymorphism in nicotine receptor gene was observed in conjunction with increased dopaminergic neuron activity • Altered gene profiles between polymorphic and wild type iPSC-derived neurons • Nicotine increased glutamatergic neuron firing in iPSC-derived neurons with the genetic polymorphism	Oni et al, 2016 (344)
Opioid addiction	iPSC-derived dopaminergic neurons from individuals with polymorphism in nicotine receptor gene	• Valproic acid exposure • RT-PCR to assess gene abundance	• Polymorphism in dopamine receptor gene reduced receptor expression • Valproic acid changed gene expression of genes related to dopaminergic neuron function	Sheng et al, 2016 (409)

Abbreviations: BrdU: Bromodeoxyuridine, GFP: green fluorescent protein, RT-PCR: reverse transcription polymerase chain reaction.

Table 13

Examples of personalized medicine using iPSCs

Disease state	Patient-derived cells	Experimental procedure	Major findings	Reference
Bipolar disorder	iPSC-derived hippocampal dentate gyrus-like neurons	• RNA sequencing • Calcium imaging • Whole-cell patch clamping • Response to lithium treatment	• Mitochondrial abnormalities in bipolar disorder patients • Over-activity in action potential firing and calcium release • Treatment with lithium attenuated hyper-excitability	Mertens et al, 2015 (302)
General neuropsychiatric disorder model	iPSC-derived serotonergic neurons	• Assessment of serotonergic neuron release • Whole-cell patch clamping	• Neurons showed essential molecules required for serotonergic specificity • Neurons displayed characteristic electrical activity characteristic of serotonergic neurons • Expected response to FDA-approved pain, depression, anxiety drugs	Lu et al, 2016 (271)
Zika virus	iPSC-derived forebrain neural progenitor cells, astrocytes, and cerebral organoids	• Infection of cells with MR766 viral strain • Screening of ~6,000 potential Zika inhibitors	• Zika virus increased caspase-3 activity in neural progenitor cells and cerebral organoids • Successful inhibitors of Zika replication were cyclin-dependent kinase inhibitors and Niclosamide	Xu et al, 2016 (478)
Parkinson's/Gaucher disease	iPSC-derived dopaminergic neurons from Parkinson's disease patients with GBA1 mutation	• Measurement of autophagy • Calcium imaging • Assessment of levels of secreted factors within CNS	• GBA1 mutation led to increased intracellular calcium and deficient autophagy • Correction of GBA1 mutation attenuated adverse phenotypes	Gurwitz, 2016 (168) and Schondorf et al, 2014 (395)

Abbreviations: GBA1: glucocerebrosidase 1, CNS: central nervous system, FDA: Food and drug administration; RNA: ribonucleic acid.

Table 14.

Gene editing in personalized medicine using iPSCs

Disease state	Patient-derived cells	Experimental procedure	Major findings	Reference
General neurodevelopmental disorder	Patient with GTDC1 mutation	• DNA sequencing for genetic variants • Assessment of iPSC pluripotency • Differentiation into neural progenitors and neurons • Comparison of iPSC findings to other model systems	• Reduced neural progenitor proliferation in MS patient cells • Impaired neurogenesis and migration in MS patient cells • MS neurons showed similar phenotype to hESCs and zebrafish with GTDC1 knock-down	Aksoy et al, 2016 (5)
Rare neurological disorders	Healthy patients	• Differentiation of fibroblast-derived iPSCs into midbrain and forebrain organoids • Transfection with CRISPR/Cas9 in iPSCs • qPCR validation of genotype	• Generation of multiple clonal lines of iPSCs • Individual knock down efficiency varied, with a mix of homozygous and heterozygous knock outs	Bell et al, 2017 (30)
Schizophrenia	Schizophrenic patients with DISC1 mutation	• Establishment of isogenic cell lines for DISC1 mutation • DISC1 knockout and rescue by TALEN. • Measurement of synaptic markers	• DISC1 mutations result in reduced synaptic vesicle release • DISC1 rescue attenuates adverse synaptic phenotypes	Wen et al, 2014 (466)

Abbreviations: GTDC1: Glycosyltransferase Like Domain Containing 1, DISC1: Disrupted in schizophrenia 1, TALEN: Transcription activator-like effector nucleases, MS: multiple sclerosis, hESC: human embryonic stem cell.