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Review

Organoid and Assembloid Technologies for Investigating Cellular Crosstalk in Human Brain Development and Disease

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The biology of the human brain, and in particular the dynamic interactions between the numerous cell types and regions of the central nervous system, has been difficult to study due to limited access to functional brain tissue. Technologies to derive brain organoids and assembloids from human pluripotent stem cells are increasingly utilized to model, in progressively complex preparations, the crosstalk between cell types in development and disease. Here, we review the use of these human cellular models to study cell–cell interactions among progenitors, neurons, astrocytes, oligodendrocytes, cancer cells, and non-central nervous system cell types, as well as efforts to study connectivity between brain regions following controlled assembly of organoids. Ultimately, the promise of these patient-derived preparations is to uncover previously inaccessible features of brain function that emerge from complex cell–cell interactions and to improve our mechanistic understanding of neuropsychiatric disorders.

Highlights

Human pluripotent stem cell-derived brain organoids produce a diversity of cell types that interact with each other in a complex 3D environment.

Combining organoids resembling distinct areas into assembloids can be used to model aspects of interactions that occur between regions in the human brain.

Organoids can be supplemented with non-central nervous system-derived cell types, including microglia and endothelial cells, to study the interplay of nervous system cells with immune cells and blood vessels.

Patient-derived organoids can be genetically manipulated or infected with pathogens and subsequently used as tools for studying disease processes in a human context.

Introduction

Behavior is thought to emerge from complex cellular interactions between a cast of specialized cells in the central nervous system that are capable of receiving, processing, and sending information. However, the molecular processes underlying these interactions in the mammalian brain as well as the relative contributions of the various cell types have yet to be fully elucidated. While neurons have long been seen as the principle actors in directing behavior, other cell types including astrocytes, oligodendrocytes, and microglia are increasingly seen as playing more than just a supportive role [1,2]. For instance, astrocyte activity influences memory and behavior [3–5] and disruption of microglial development can lead to neuropsychiatric disease [6]. Recent evidence points to species specific differences in some of these cell types, including transcriptional and functional divergence in neuronal subtypes or astrocytes that may influence how these cells interact [7,8]. Therefore, recent progress in deriving *in vitro* self-organizing brain tissue preparations from human pluripotent stem cells holds promise in uncovering some of the unique features of the cellular crosstalk occurring in the nervous system [9]. Here, we describe how 3D brain organoids and combinations of regionalized brain organoids called assembloids can be used to unveil previously inaccessible aspects of neurobiology and to identify alterations in cellular interactions in disease. We also illustrate how these models can be leveraged to answer challenging questions in disease pathophysiology.

Cellular Interactions in Cerebral Cortical Development

The developing cerebral cortex is responsible for coordinating higher-brain functions and comprises an organized assembly of neurons, oligodendrocytes, astrocytes, microglia, and other cell types that interact in intricately coordinated ways [10]. Communication among cell types can occur through specialized cell-surface interactions and long-range mechanisms, including the release of secreted factors. Most of the initial work on cellular communication in the nervous system was conducted on neuronal signaling. Neurons share information by releasing neurotransmitters at synapses and through direct electrical coupling via gap junctions [11], coming together to form complex circuits [12]. During development, axons are guided to regions where they ultimately form synapses through the signaling of molecules that both attract and repel growth [13].

Neuronal activity also influences the behavior of non-neuronal cells, including stimulating astrocyte development and morphological complexity [14–16] and oligodendrocyte proliferation and myelination [17–20]. Both astrocytes and oligodendrocytes reciprocally influence neuronal behavior. Astrocytes play

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a role in both the formation and the pruning of synapses [21–24] and help to maintain active synapses by buffering ions [25] and taking up glutamate from synapses and recycling the components back to neurons [26–28]. Oligodendrocytes form layers of myelination on neuronal axons that increase the speed of signal action potential transmission [29]. Oligodendrocytes also influence neuronal function by secreting growth factors [30] and providing lactate [31]. Blocking oligodendrogenesis is thought to impact neuronal function [32,33], and the loss of myelination leads to neurodegeneration [34]. These studies suggest that these interactions are essential for human brain development and function; however, it is challenging to probe them directly. Functional human brain tissue is largely inaccessible, resulting in a reliance on animal models that may contribute to the low translational success rate of nervous system therapies [35]. Conducting complementary work in a human system may therefore be critical to capture human-specific neurobiological traits.

The ability to generate and culture human induced pluripotent stem (hiPS) cells has allowed the generation of human cell types that were previously inaccessible [36], providing a useful approach complementary to animal models for studying species- and patient-specific features. hiPS cells can be generated from patients with specific disorders, potentially allowing more predictive disease modeling. Most hiPS cell-derived cultures are differentiated on adherent 2D surfaces. These cultures are useful for observing the effect of genetic and environmental manipulations on individual cell types without the confounding influence of neighboring cell types, as well as for generating large numbers of cells. However, the physiological relevance of 2D tissue culture systems is limited by the stiffness of the environment, the limited movement of secreted factors in the extracellular space, and spatially restricted cell–cell interactions. Neural progenitor cells exhibit altered migratory dynamics when grown in 2D that depend on the density of surrounding cells [37]. Growth in adherent culture can also lead to abnormal cellular morphologies, such as the flattened shape of mature oligodendrocytes in adherent culture [38–41], which complicates the study of myelination. Differences between 2D culture and the *in vivo* environment may be the cause of unusual characteristics observed in cells grown in adherent cultures and these abnormalities may contribute to differing results in drug screening trials performed in 2D cultures versus 3D tissues [42].

Organoids, or organ spheroids, are 3D cultures maintained *in vitro* that can be derived directly from primary tissue or differentiated from embryonic or induced human pluripotent stem cells (hPS cells) (Figure 1, Key Figure). Due to the greater diversity of cell composition, the lack of cellular interactions with artificial plastic substrates, and the undirected formation of complex 3D structures seen in the developing brain, organoids resembling neural tissue have the potential to create more realistic cellular environments for modeling the cell biology of the nervous system. These cultures are generated by allowing hiPS cells to differentiate spontaneously [43,44] (undirected organoids) or by adding signaling molecules to direct them to resemble particular brain regions (brain-region-specific or regionalized brain organoids [45–48]). Different organoid methodologies can be chosen for specific applications based on the complexity of cell types needed, the desired consistency of the cellular composition, and the ease of generation. Organoids, and in particular those in which fate is not guided, have some disadvantages versus 2D cultures, including variability in cellular composition and inconsistent organization between organoid regions [49], and there are questions for which it is preferable to reduce the complexity of a system and study just one or a few cell isolated cell types. However, organoids are particularly useful for studying interactions between cultured cells in a 3D environment and for ultimately asking questions about emergent properties of cellular ensembles. Additionally, organoids can be cultured *in vitro* for long periods of time, allowing studies of the prolonged human corticogenesis [50]. Moreover, cell types and organoids resembling different brain regions can also be combined in assembloids to study the interplay of cell types with differing origins (Figure 1).

Interactions between Neural Lineage Cells in Organoids

Neuronal Interactions

One of the most frequently studied cellular interactions in organoids is the interaction of neurons with other neurons. The cerebral cortex includes both glutamatergic neurons born in the dorsal forebrain

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Key Figure

Modeling Cellular Interactions in 3D Cerebral Organoid Culture

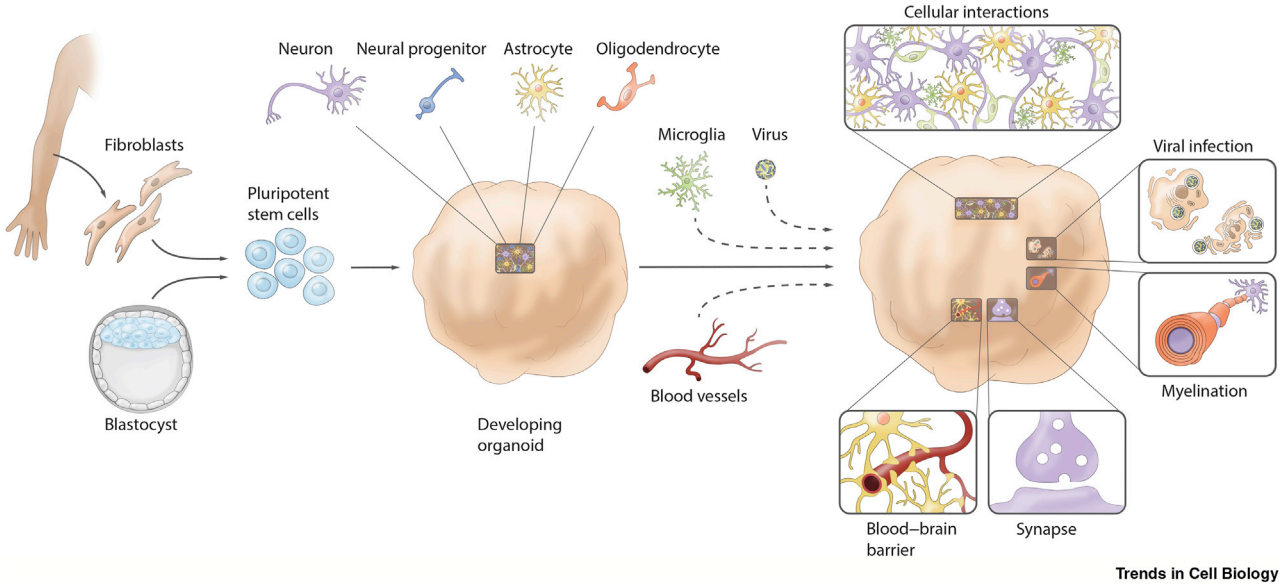

Figure 1. Organoids can be generated from human pluripotent stem cells derived from adult somatic cells or blastocysts. Cerebral organoids mimic aspects of *in vivo* human brain development to produce interacting neural progenitor cells, neurons, astrocytes, and oligodendrocytes. Non-central nervous system-derived entities including blood vessels, microglia, and viruses can be added to organoids to study their interplay with organoid-derived cells.

and GABAergic interneurons born in the ventral forebrain that then migrate into the dorsal forebrain [51–53]. Studies in forebrain organoids from our laboratory and others have demonstrated that glutamatergic neurons form synapses in organoids resembling the cerebral cortex [45,48] and that the generation of these 3D cultures is highly reliable across multiple hiPS cell lines and experiments [54]. Interactions between glutamatergic neurons and GABAergic interneurons can also be modeled by fusing organoids resembling the dorsal and ventral forebrains together into an assembloid [46,55,56]. Interneurons that migrate from the ventral organoids to the dorsal organoids formed synapses with glutamatergic neurons and, as a result of their new cellular interactions, have increased morphological complexity and integrate synaptically into a microcircuit [46]. To migrate, interneurons undergo saltations, which are stepwise cell movements through the process of nucleokinesis, or forward movement of the nucleus. Migrating interneurons in forebrain assembloids derived from patients with Timothy syndrome, a neurodevelopmental disorder caused by mutations in the $Ca_V1.2$ calcium channel, have a higher saltation frequency but overall less-efficient migration due to lower saltation length compared with controls [46]. The 3D nature of forebrain assembloids was essential for these migratory studies since neural cells in adherent 2D cultures have been shown to have altered migratory dynamics [37,46]. The assembloid technique has been used recently to study the interaction of neurons in other brain regions, such as between the cortex and thalamus [57], and can be applied to other regions in the future, including structures of the basal ganglia and midbrain. Generating striatum-like organoids and fusing them with cortical organoids would allow the study of cortical projections into the striatum and the role that they play in striatal development and in disorders impacting corticostriatal connectivity.

Organoid-derived neurons have been shown to interact not only with other organoid-derived neurons but also with host neurons when transplanted into the adult mouse brain [58]. Host neurons

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form synapses with organoid-derived neurons and thus input from the host may influence the function of the transplanted human neurons [58,59]. Organoid-derived neurons in hosts have indeed been shown to differentiate, mature, and extend axonal projections following transplantation [58,59]. Challenges remain in the transplantation of organoids into host brains, including low integration rates that may be improved by transplanting at fetal or early postnatal stages. In future studies, it will be interesting to determine whether organoid-derived cells mature differently in a rodent brain than in an organoid that remained *in vitro*, such as by comparing the transcription profiles and calcium dynamics of these neurons.

Progenitor Cell Interactions

An advantage of organoid models is that they can model early stages of brain development, including the interactions of neural progenitors and their progeny. During brain development, radial glial cells (RGCs) in the ventricular zone extend processes to the pial surface. RGCs can divide both to self-replicate and to give rise to neurons that migrate along these processes to their final position in the developing cortical plate [60–62]. Direct contact in the form of adherens junctions between RGCs is essential to establish the cytoarchitecture and control proliferation [63,64] and gap junctions are necessary for neuronal migration along RGC processes [65]. Capturing these interactions in human brain organoids allows the study of disorders linked to alterations in early neural development, such as Miller–Dieker syndrome (MDS). MDS, a brain disorder caused by a deletion in chromosome 17p, is characterized by lissencephaly, microcephaly, developmental delays, and intractable epilepsy. Two groups utilized patient-derived hiPS cells to study developmental mechanisms in MDS-derived organoids [66,67]. Both studies reported a reduction in MDS-derived organoid size and alterations in ventral RGC division. These abnormal divisions resulted in increased production of intermediate progenitors [66,67]. Additionally, both research groups identified alterations in cellular interactions that may contribute to the phenotypes seen in MDS-derived organoids. The first group observed non-cell-autonomous changes in N-cadherin/β-catenin signaling in adherens junctions between RGCs that altered ventricular niche structure in MDS-derived organoids [66]. The second group observed a migration defect of neurons along progenitor processes and a defect in outer RGC mitosis [67]. Outer RGCs are enriched in primates [68,69], emphasizing the utility of a human cell-derived model compared with rodent models. A combination of the abnormal intercellular interactions identified using MDS-derived organoids may contribute to lissencephaly in MDS patients and future studies using MDS-derived organoids may identify methods to correct these alterations.

In addition to modeling genetic disorders [70,71], organoids can be used to study injuries that affect early brain development. The premature birth of infants with underdeveloped lungs can result in hypoxic episodes and a condition called hypoxic encephalopathy of prematurity [72]. Due to the limited availability of functional fetal tissue, the underlying mechanisms and human cell types most affected by hypoxia are unknown. The temporary exposure of cerebral cortical organoids to hypoxic conditions resulted in altered gene expression profiles in both neuronal and non-neuronal markers [73] and a defect in intermediate progenitors [74]. While studying hypoxia in 3D culture has its challenges, this method can be used to generate predictions about the mechanisms leading to reduced cortical gray matter and potential strategies to reverse cell-specific defects.

Astrocyte Interactions

Glial cells are gaining increasing recognition for playing important roles in neuronal development and disease. Astrocytes in particular interact extensively with neurons to influence their development, maturation, synapse formation, and survival [21–24]. As occurs during brain development *in vivo*, astrogenesis follows neurogenesis in 3D culture and increases in abundance and maturity over time [50]. The ability to maintain organoids in long-term culture for months to years allows astrocytes to reach advanced maturation stages that would be difficult to achieve in 2D culture [50]. For example, cortical organoid-derived astrocytes promoted the survival and neurite growth of mouse embryonic cortical neurons [75]. Human organoid-derived astrocytes were also able to induce synapse formation, take up glutamate and synaptosomes, and increase the amplitude of calcium dynamics in human neurons [16,50]. Co-culture with postnatal-like organoid-derived astrocytes promoted a higher amplitude of

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calcium rise in human glutamatergic neurons than co-culture with fetal-like organoid-derived astrocytes. The properties of postnatal astrocytes that make them better able to produce mature calcium responses in human neurons and whether this is due to direct cell–cell interactions or secreted factors remains a mystery. Currently, it is difficult to visualize or measure some of these behaviors of mature astrocytes in intact 3D organoids, requiring astrocytes to be isolated from organoids and plated in tissue culture plates. Advances in imaging capabilities may allow the capture of such behaviors in organoids directly, making them a more powerful tool for discerning the unknown mechanisms by which astrocytes modulate neuronal development.

The production of functional astrocytes in organoids has allowed the study of astrocyte interactions in the context of disease. Thomas *et al.* used cerebral cortical organoids to study Aicardi–Goutières syndrome (AGS), a rare childhood inflammatory disorder that results in severe neurological damage and microcephaly [76]. AGS can be caused by mutations in three-prime repair exonuclease 1 (*TREX1*). Organoids generated from TREX1-deficient hiPS lines were smaller in size and had higher rates of neuronal apoptosis. Astrocytes lacking TREX1 secreted more neurotoxic type 1 interferon and culture of healthy cortical organoids in TREX1-deficient astrocyte-conditioned media resulted in increased neuronal cell death and reduced organoid size [76]. Future studies can utilize this model to study the effect of astrocyte dysfunction on neurons in AGS and other neurological disorders.

Oligodendrocyte Interactions

While initial brain organoid methods produced progenitors, neurons, and astrocytes, they lacked oligodendrocytes. This precluded the study of myelination and white matter disorders in organoid cultures. Several methods have been developed to generate oligodendrocytes from hiPS cells in 2D culture [77–79]. While these methods have been useful for comparing oligodendrocytes derived from different hiPS cell lines, there are aspects of oligodendrocyte function, such as myelination, that are difficult to study in 2D culture. Additionally, adherent differentiation methods are often optimized for maximal oligodendrocyte production and therefore are not useful for studying interactions of heterogeneous populations of oligodendrocytes, neurons, and astrocytes as they develop together. As an alternative, protocols have been developed in our laboratory and others to generate oligodendrocytes in addition to astrocytes and neurons in organoid culture [80,81], allowing interaction with neuronal processes and the formation of compact myelin [80,81]. The transcriptional profile of oligodendrocyte-lineage cells in these organoids closely resembled primary adult oligodendrocyte progenitor cells and myelinating oligodendrocytes, and these more mature stages of the lineage developed on an accelerated timescale relative to human development *in vivo*, allowing shorter culture periods [46]. These organoids can be applied to model diseases in which myelin formation or interactions between oligodendrocytes and other cell types of the brain are disturbed, such as vanishing white matter disease (VWMD). VWMD is caused by mutations in any of the five *EIF2B* genes (*EIF2Bα*, *EIF2Bβ*, *EIF2Bε*, *EIF2Bδ*, *EIF2Bγ*), which are expressed ubiquitously and form a complex that controls translation rate by acting as a guanine exchange factor for eIF2 [82–84]. In VWMD patients, significant cellular abnormalities are usually found only in the astrocyte and oligodendrocyte lineages [85]. Previous studies suggest that oligodendrocytes develop abnormally due to altered astrocyte development, making this an interesting disease to study in organoids in which the two cell types develop concurrently [86–88]. Combinations of control and patient-derived cells derived in organoids can be used to elucidate the cell-autonomous and non-cell-autonomous effects of *EIF2B* gene mutations on astrocyte and oligodendrocyte development.

Cancer Cell Interactions

Malignant brain tumors are often devastating and difficult to treat, resulting in low survival rates of brain cancer patients versus cancers of other organs [89]. Tumor invasion in organoid culture has been studied both by adding glioma cells to organoids and by introducing cancer-causing mutations in organoids. Studies that added glioblastoma cells directly to cortical organoids either as individual cells or by fusing glioblastoma and normal organoids were able to model the invasion of cancer cells into healthy tissue [90,91], the proliferation of glioma stem cells, and the formation of microtubes that allow tumor growth [90]. The induction of oncogenic mutations in organoids likewise allowed the

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study of tumor cells invading surrounding tissue [92,93]. The generation of tumor cells directly in organoids is useful for studying the earliest stages of disease or developmental cancers and this method can be used to examine the expression of invasion-related genes and can potentially serve as a platform for drug discovery [92]. An advantage of organoid models is the ability to live image repeatedly over periods of time, and these methods can be used in the future to study the migrational dynamics of tumor cells, including the velocity and directness of movement as well as the presence of calcium waves, or local changes in intracellular calcium concentrations that propagate across cells.

Interactions between CNS- and Non-CNS-Lineage Cells in Organoids

Microglial Interactions

Patterning organoids toward the ectodermal lineage results in the nearly exclusive production of neural cells. This precludes the study of non-ectodermal cell types that play an important role in brain function, such as microglia, the resident immune cells of the brain. Microglia originate in the yolk sac, migrate through the vasculature, and mature in the developing brain [94]. The presence of microglia in organoids would be useful for studying the roles microglia play in phagocytosing damaged cells, releasing chemokines and cytokines, and initiating [95] and eliminating synapses [96]. Microglial development is difficult to reproduce in culture [97] and two approaches have been taken to study the interaction of microglia and neural cells in brain organoids. One technique generates microglia from hiPS cells separately and then integrates them into brain organoids to form multilineage assembloids [98,99] (Figure 1). This method allows for the study of microglial morphology, migration, and response to injury in a complex, human 3D cellular environment [98]. In this preparation, hiPS cell-derived microglia are able to infiltrate organoids, distribute themselves in a tiled pattern, and extend ramified processes [98]. Interestingly, the migratory behavior, calcium dynamics, and response to proinflammatory stimuli differed in microglia transferred to dorsal versus ventral forebrain organoids [100]. In the second approach, microglia are derived alongside neural cell types in minimally patterned organoids that contained multiple germ layers derivatives. Utilizing an undirected method to produce organoids, Ormel *et al*. demonstrated that mesodermal progenitors gave rise to microglia-like cells that physically interacted with neurons and are able to phagocytose synaptic structures [101]. Work remains in demonstrating that culture-derived microglia are representative of primary cells. Improving microglia generation *in vitro* will permit analysis of the pathophysiology of neuroimmunological disorders such as multiple sclerosis and autoimmune encephalitis.

Vascular Cell Interactions

Endothelial cells and pericytes are major non-ectodermal components of the brain vasculature and play a critical role in controlling the movement of molecules from the blood into the brain parenchyma [102]. Incorporating blood vessels into brain organoids may allow the production of larger, more complex organoids by allowing the transport of nutrients internally, reducing the levels of cell death and stress that may occur in organoids. Blood vessels may also be used to model the blood–brain barrier (BBB) and the intricate interplay of the response of blood flow to neural activity.

Previous attempts to model the BBB have utilized organ-on-a-chip methods that layer combinations of endothelial cells, astrocytes, neurons, and pericytes on a microfluidic platform to create a microphysiological system that mimics the cellular interactions that occur *in vivo* [103]. Attempts to recreate similar interactions in organoids have been conducted in two ways. First, transplantation of neural organoids into a mouse host results in the invasion with host blood vessels of the human organoid [58]. These blood vessels appear to support the viability of the organoid graft and allow the study of interactions that occur between the cells forming blood vessels and the surrounding neural organoid tissue [58]. Second, hiPS cell-derived blood vessel-like structures can be incorporated into brain organoids *in vitro* to investigate the interactions of endothelial cells and neural cells (Figure 1). Recently, endothelial cells have been introduced to organoid-like cultures by fusing clusters of hiPS cell-derived endothelial cells and neural progenitor cells along with human mesenchymal stem cells (hMSCs) that may secrete factors that aid in angiogenesis and neural development [104]. These mixed-lineage assembloid cultures expressed markers of deep cortical layer neurons as well as enrichment of genes associated with the formation of the BBB [104]. Endothelial cells have also

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been generated in organoids by genetically modifying some hiPS cells to express *ETV2* [105]. The *ETV2*-expressing cells give rise to vasculature-like structures. Tight junction-related genes were also expressed in these organoids and transendothelial electrical resistance (TEER), a hallmark of BBB formation, was elevated compared with other organoids but was not as high as in other BBB models.

One of the challenges of building a BBB in organoids is the production of brain microvascular endothelial cells (BMECs) *in vitro*. BMECs have unique properties, including the high TEER observed in the BBB due to specialized tight junctions and the expression of selective transporters [106,107]. Due to these specialized features, generating BMECS from hPS cells is challenging and further work is necessary to accurately recreate their development [108]. In addition to the incorporation of blood vessels, organoids may be adapted to study the BBB by coating organoid surfaces with BMECs and pericytes. With the development of reliable methods to produce BMECs, organoids and assembloids can become a useful tool for studying the formation and breakdown of the BBB in the context of disease.

Pathogen Interactions

In addition to studying interactions between human cells, organoids can be used to study host-pathogen interactions (Figure 1). At the height of the Zika outbreak in 2016 and 2017, a number of studies used brain organoids to study the devastating effect of Zika virus (ZIKV) infection on early brain development. There has been previous debate about whether ZIKV primarily infects progenitor cells, mature neurons, or astrocytes in the brain to exert its teratogenic effects [109]. ZIKV specifically infected progenitor cells in organoids, which resulted in smaller organoids and disrupted cortical structure [48,110–113]. The smaller size of ZIKV-infected organoids may be caused by increased Caspase3/7-mediated cell death, which appeared to be ZIKV specific as it was not induced by infection with the virus that causes dengue fever [113]. In previous studies in non-neuronal cell types, the AXL adhesion factor was predicted to allow ZIKV entry, and modulation or elimination of AXL reduced viral entry [114–116]. A study in organoids did not replicate these findings in neural tissues, suggesting the importance of conducting future research in disease-relevant human cell types [117]. Other evidence suggests that AXL may play a role in ZIKV entrance in astrocytes; however, the role of AXL in ZIKV infection, and which cell types it most impacts, remains unclear [118].

These models can potentially be used as a drug screening method to identify compounds that reduce the effects of ZIKV infection on neural development [119].

Concluding Remarks

Brain development and function are dependent on complex interactions between many cell types distributed across multiple brain regions that can have severe consequences on human health when perturbed by genetic mutations, environmental injury, or tumorigenesis. Organoids allow the study of these interactions in a 3D, human cellular context that mimics aspects of the *in vivo* environment that are often lacking in 2D cultures. Alongside future disease studies, brain organoids and assembloids can be used to study injury, such as the effect of repetitive forces over time on cellular contact and communication relevant to the modeling of traumatic encephalopathy. Additionally, transmission of prions between cells [120] and the accumulation of extracellular aggregates are strongly implicated in the pathophysiology of several severe neurological disorders. Organoids have previously been used to model the accumulation of Aβ peptides *in vitro* [121] and future studies can be conducted to study the transmission of protein aggregates and prions between cells and between assembled organoids to investigate cell-specific effects and the movement of prions across brain regions, such as the movement of Creutzfeldt–Jakob disease PrP^{Sc} prions through nanotubes between neurons [122].

Remaining limitations of organoid methodologies include the variable proportions of cell types and internal cytoarchitectures that do not necessarily correspond to the full cellular composition and complex structure of the human brain, making it difficult to locate and visualize, in real

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time, specific cell types in organoids. Additionally, it is challenging to distinguish cell-autonomous versus non-cell-autonomous effects in organoids due to their diverse cellular compositions. This problem is compounded by the presence of cell types not found in the brain that can arise in organoids, especially when derived using undirected methods, that may influence neural cell types of interest. Future studies may be facilitated by creating regionally patterned organoids, such as forebrain organoids patterned with a Sonic Hedgehog protein gradient [123], to more carefully control differentiation, to know where key internal structures are spatially located, and to create more precise assembloids between brain regions (see Outstanding Questions). One application of standardized organization and assembloid formation is an investigation of long-range interactions such as axonal guidance. Such assembloids can be used to study the basic neurodevelopment mechanisms of axonal guidance between brain regions similar to previous work in synthetic 3D scaffolds [124].

In addition to designing methods to more precisely control organoid development, approaches to accelerate maturation would significantly expand the applications of brain organoids. While 3D cultures may be best suited for neural developmental disorders, age-related neurodegenerative diseases are more challenging to study in organoid culture. In addition to promoting maturation, organoid analysis tools can be improved. As organoids can be variable, careful consideration needs to be applied not only to choosing the number of hiPS cell lines and organoids per line used in disease-modeling studies, but also to the timepoints and assays selected for analysis. Advances in imaging techniques in 3D tissues with two-photon or light-sheet microscopy and the identification of human cell type-specific reporters by recognizing unique chromatin states will both allow more accurate data collection in organoid culture. Use of CRISPR-generated isogenic control lines for disease-modeling applications can be useful in reducing variability between lines. The generation of chimeric organoids comprising both control and disease hPS cell lines will also help to identify cell-autonomous effects of individual mutations versus the effects caused by interactions between cell types. Collectively organoids, assembloids, animal models, and technological advances in cell biology will reveal the intimate crosstalk among cell types in human brain development and disease.

Outstanding Questions

Are there human-specific interactions that occur between progenitors, neurons, astrocytes, and oligodendrocytes during brain development?

Can complex assembloids comprising multiple brain regions be combined and maintained over time?

How can blood vessels best be incorporated into organoid culture?

How can technological developments in imaging be applied to advance the study of interactions in organoids?

Can organoids be patterned to create reproducible internal organization?

Can organoids be induced to reliably produce cell types of ectodermal and non-ectodermal lineages concurrently?

What unique properties of human microglia shape their interactions with cells of the developing cerebral cortex?

What aspects of pathogen infection can be best captured in organoid culture?

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