1521-0111/99/4/256–265$35.00
MOLECULAR PHARMACOLOGY

https://doi.org/10.1124/molpharm.120.000142
Mol Pharmacol 99:256–265, April 2021

MINIREVIEW

The Emerging Role of Neuronal Organoid Models in Drug Discovery: Potential Applications and Hurdles to Implementation

Laura A. Struzyna and Marla L. Watt

Merck & Co., Inc., Kenilworth, New Jersey

Received October 29, 2020; accepted January 15, 2021

ABSTRACT

The high failure rate of drugs in the clinical pipeline is likely in part the result of inadequate preclinical models, particularly those for neurologic disorders and neurodegenerative disease. Such preclinical animal models often suffer from fundamental species differences and rarely recapitulate all facets of neurologic conditions, whereas conventional two-dimensional (2D) in vitro models fail to capture the three-dimensional spatial organization and cell-to-cell interactions of brain tissue that are presumed to be critical to the function of the central nervous system. Recent studies have suggested that stem cell–derived neuronal organoids are more physiologically relevant than 2D neuronal cultures because of their cytoarchitecture, electrophysiological properties, human origin, and gene expression. Hence there is interest in incorporating such physiologically relevant models into compound screening and lead optimization efforts within drug discovery. However, despite their perceived relevance, compared with previously used preclinical models, little is known regarding their predictive value. In fact, some have been wary to broadly adopt organoid technology for drug discovery because of the low-throughput and tedious generation protocols, inherent variability, and lack of compatible moderate-to-high-throughput screening assays. Consequently, microfluidic platforms, specialized bioreactors, and automated assays have been and are being developed to address these deficits. This mini review provides an overview of the gaps to broader implementation of neuronal organoids in a drug discovery setting as well as emerging technologies that may better enable their utilization.

SIGNIFICANCE STATEMENT

Neuronal organoid models offer the potential for a more physiological system in which to study neurological diseases, and efforts are being made to employ them not only in mechanistic studies but also in profiling/screening purposes within drug discovery. In addition to exploring the utility of neuronal organoid models within this context, efforts in the field aim to standardize such models for consistency and adaptation to screening platforms for throughput evaluation. This review covers potential impact of and hurdles to implementation.

Neurologic conditions, including neurodegenerative diseases, neurodevelopmental disorders, and injury to the nervous system, remain a significant, unmet burden to the US healthcare system. In 2014 alone, these conditions affected over 100 million Americans and cost nearly 800 billion dollars (Gooch et al., 2017; Jensen et al., 2018; Papariello and Newell-Litwa, 2020). Specifically, in 2017 it was estimated that Parkinson's disease (PD) affected 1 million Americans and cost 52 billion dollars (Yang et al., 2020). In 2020, Alzheimer's disease (AD) affected 5.8 million Americans and, along with other dementias, was estimated to cost 305 billion dollars (Alzheimer's Association, 2020). Furthermore, because the size of the country's elderly population will nearly double by 2050, these numbers are predicted to increase substantially (Gooch et al., 2017). Despite the large demand for improved neurologic care, the greater than 90% failure rate of drugs in the clinical pipeline has resulted in a dearth of pharmaceutical treatments for these conditions (Jensen et al., 2018; Hingorani et al., 2019; Papariello and Newell-Litwa, 2020). These clinical failures often occur after billions of dollars and years of development have been invested in a potential new drug (Breslin and O'Driscoll, 2013; Hung et al., 2017).

The poor translatability of promising preclinical compounds to the clinic is partially the consequence of inadequate preclinical models of neurologic disease. Typical preclinical compound evaluation involves in vitro assessment of the target followed by in vivo assessment of pharmacodynamic effect in at least one nonhuman species. In the case of neurologic conditions, researchers have struggled to reliably recapitulate disease pathology within historical in vitro and

This material was not previously presented elsewhere.
This work received no external funding.
The authors declare there is no conflict of interest.
https://doi.org/10.1124/molpharm.120.000142.

ABBREVIATIONS: AD, Alzheimer's disease; 2D, two-dimensional; 3D, three-dimensional; EB, embryoid body; ECM, extracellular matrix; FLSM, fluorescent light sheet microscopy; Hit ID, hit identification; IHC, immunohistochemistry; LID, lead identification; LO, lead optimization; MEA, multielectrode array; PD, Parkinson's disease; ROP, research operating plan; SAR, structure activity relationship.

in vivo models in part because of limited understanding of the underlying pathologic mechanisms (Charvin et al., 2018). Furthermore, conventional models of human origin in a monolayer are not representative of the physiologic environment of the brain: they lack the relevant cytoarchitecture, spatial organization, and cell-to-cell interactions that are crucial to the function of the nervous system. Therefore, they have shown limited efficacy in the exploration of disease mechanisms and in the screening of neuroprotective agents (Schüle et al., 2009; Baden et al., 2019). As with in vitro models, in vivo models have also failed to appropriately recreate neurologic disorders. Modest conservation of genome sequences across species as well as fundamental differences in brain development and function have resulted in animal models that often only reproduce a few facets of the human disease (Dawson et al., 2018). Furthermore, the limited lifespans of many animals may prevent the development of pathologic mechanisms that are associated with age-related neurodegenerative diseases, including PD and AD (Dawson et al., 2018).

Recent studies suggest that three-dimensional (3D) tissue models derived from human embryonic stem cells and induced pluripotent stem cells reproduce relevant complex physiology that may make them superior to both in vivo and conventional in vitro preclinical models for the interrogation of human neurologic disorders (Zhang et al., 2014; Jo et al., 2016; Lee et al., 2016; Centeno et al., 2018; Liu et al., 2018; Trujillo et al., 2019). Such 3D models include organoids, spheroids, tissue-engineered constructs, bioprinted tissue, microfluidic systems, and organ-on-a-chip technologies. For the purposes of this review, we will focus on the potential utility of organoid technology in drug discovery; the use of other 3D tissue models has been reviewed elsewhere (Sanders et al., 2014; Fang and Eglen, 2017; Haring et al., 2017; Thomas and Willerth, 2017; Langhans, 2018; Antill-O'Brien et al., 2019; Frimat and Luttge, 2019). We also want to discriminate between neuronal organoid and neuronal spheroid models, which are frequently confused (Fig. 1A). Although both are 3D models generated through the controlled differentiation of stem cells, organoids are larger (up to 4 mm in diameter), and they demonstrate self-organization of tissue into discrete cellular layers. Furthermore, as described below, organoid fabrication protocols frequently use externally applied extracellular matrices (ECMs) to support tissue outgrowth. Spheroids, on the other hand, are small (~500 μm in diameter), do not exhibit self-organization, and do not require the use of ECMs (Zhuang et al., 2018).

Briefly, a general neuronal organoid fabrication protocol involves first aggregating stem cells into a cell mass called an "embryoid body" (EB). Next, a series of growth factors and/or small molecules are applied to differentiate the EB toward the appropriate lineage. Finally, the growing organoid is encapsulated within an ECM, which promotes further tissue outgrowth and maturation over months in vitro (Fig. 1B). Through their own self-assembly, neuronal organoids develop complex three-dimensional structures, including proliferative zones that closely imitate the cytoarchitecture of the neural tube during development (Lancaster and Knoblich, 2014a,b; Wang, 2018). Such structures may encourage cell morphology, signaling, and electrophysiological properties within the organoids that are more representative of brain tissue than corresponding features in traditional 2D cultures (Breslin and O'Driscoll, 2013; Paşca et al., 2015; Jo et al., 2016; Tekin et al., 2018; Wang, 2018; Ao et al., 2020). Neuronal organoids are also capable of proactively producing and self-organizing distinct neuronal subtypes as well as astrocytes and oligodendrocytes that are crucial for intrinsic neuronal function (Lancaster and Knoblich, 2014a; Dezonne et al., 2017; Kim et al., 2019b; Marton et al., 2019). It should be noted, however, that current neuronal organoid models do not intrinsically develop microglia or vascular systems (Lee et al., 2017). Furthermore, analyses demonstrate that neuronal organoids more closely resemble human brain tissue compared with traditional 2D induced pluripotent stem cell–derived neuronal cultures (Jo et al., 2016). However, with that said, gene expression analyses show that neuronal organoids most closely align with prenatal brain tissue rather than adult brain tissue, which is not ideal when using organoids to study age-related neurodegenerative diseases (Jo et al., 2016). Thus, to "age" these models for greater applicability to such diseases, researchers are exploring the application of oxidative stress to accelerate pathogenic mechanisms associated with age (Grenier et al., 2020).

Such attractive properties have prompted researchers to generate organoid models of many neurodevelopmental disorders and neurodegenerative diseases, including microcephaly, macrocephaly, autism, schizophrenia, AD, PD, and dementia (Lancaster et al., 2013; Mariani et al., 2015; Li et al., 2017; Seo et al., 2017; Ye et al., 2017; Amin and Paşca, 2018; Park et al., 2018; Wang, 2018; Kim et al., 2019a). However, despite their improved physiologic relevance, the utility of organoids to screen preclinical compounds and potentially better predict clinical efficacy remains unclear. Poor reproducibility, low-throughput generation, and a lack of amenable 3D assays have limited their widespread adoption in the context of drug discovery. These hurdles must be fully recognized and addressed to ascertain their predictive value for compound profiling.

As with other complex 3D models, organoids suffer from both within-batch as well as between-batch variability in size and cellular composition (Quadrato et al., 2017; Yakoub and Sadek, 2018). Yakoub and Sadek (2018) showed that organoid diameter within a batch can differ by several millimeters, whereas Quadrato et al. (2017) used single-cell RNA sequencing to determine that organoids from separate batches produced varying neuronal subtypes. Because neuronal organoid self-assembly is highly dependent upon the precise application of small molecules and growth factors in appropriate intervals, minute variations in any of these factors can potentially affect stem cell differentiation and lead to downstream inconsistencies. In addition to growth media, extracellular matrix composition, environmental factors (i.e., temperature and humidity), and the initial number of cells seeded can influence both organoid size and maturation (Booij et al., 2019). This variability presents a major hurdle to the implementation of organoids for both traditional and phenotypic screening applications as well as target validation efforts. For example, compounds may penetrate organoids of different sizes at different rates and to different depths, potentially leading to confounding assay outcomes depending on the readout. Since drug discovery research operating plans (ROPs) are built upon the premise that in vitro assay readouts have sufficient relative predictive resolution regarding a compound-mediated effect to guide structure activity relationships

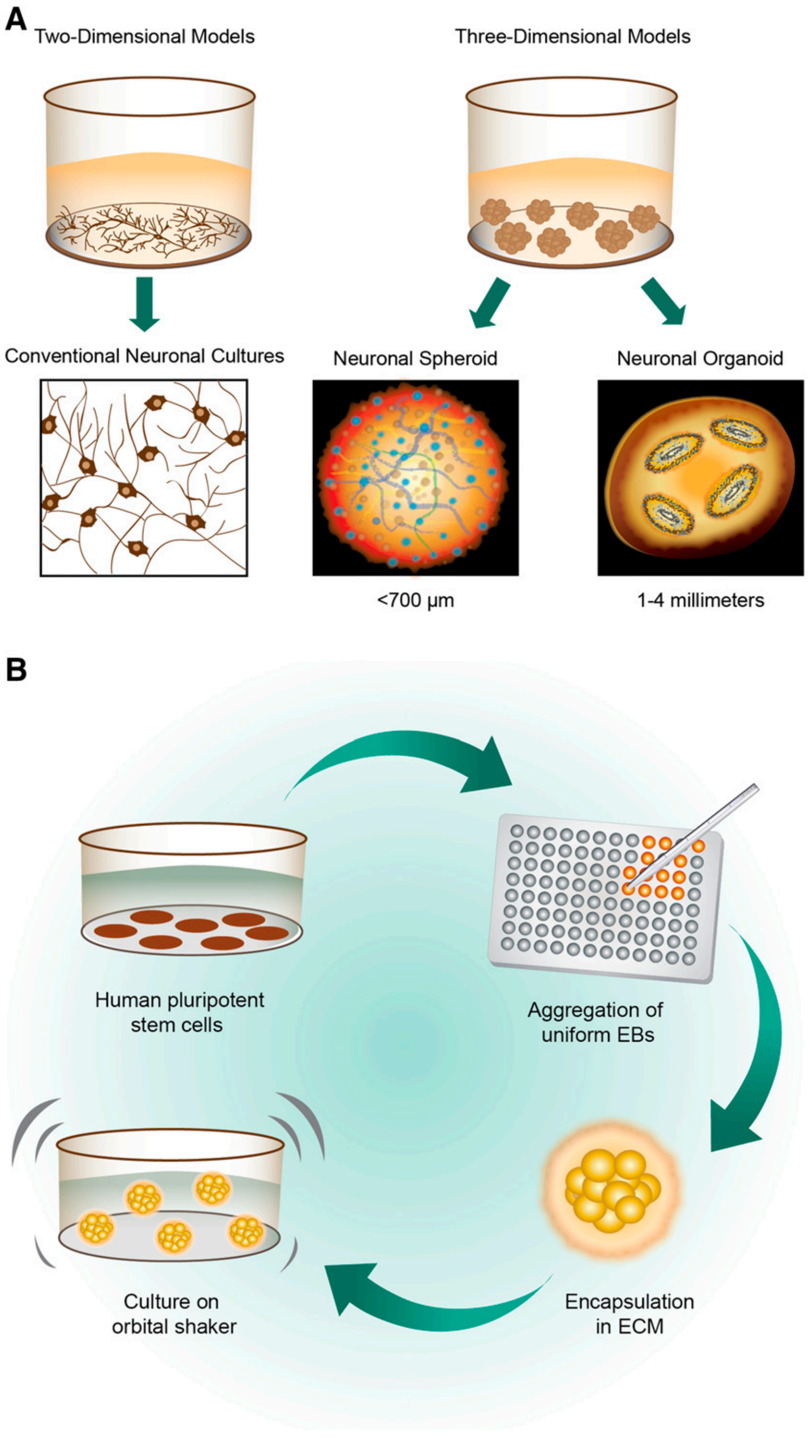

Fig. 1. Neuronal organoid model and generation protocol. (A) Neuronal organoids and spheroids are two of the most common 3D in vitro models, and they represent an alternative to conventional 2D monolayer cultures. Although both neuronal organoids and spheroids contain multiple cell types, organoids are much larger in size, and they develop organized cell layers representative of neurodevelopmental structures. Spheroids, on the other hand, are typically less than 700 μm in diameter and demonstrate a homogenous cytoarchitecture. (B) The standard steps for generating neuronal organoids include 1) culture of stem cells, 2) aggregation of stem cells into embryoid bodies, 3) encapsulation of embryoid bodies within an extracellular matrix, and 4) long-term culture with shaking/spinning for improved mass transport. Throughout these steps, the organoid media is supplemented with small molecules and/or growth factors in phases to promote differentiation toward the appropriate neuronal lineage.

(SARs), organoid generation must be standardized prior to their use as screening tools to reduce the likelihood of unreliable results.

Automation, fate-restricting differentiation protocols, 3D bioprinting, and tissue-engineering technologies could potentially assist with improving organoid homogeneity (Table 1). Because the size of the initial EB cell mass plays a crucial role in organoid development, many researchers are no longer relying upon spontaneous cellular aggregation to form their EBs. Instead, the centrifugation of stem cells into patterned microwells and V-shaped well plates is being used to improve EB consistency between batches (Ungrin et al., 2008). In particular, Ungrin et al. (2008) found that the centrifugation method reduced their coefficient of variation for EB size from 0.72 to ~0.09. Similarly, several groups have built microfluidic platforms that confine growth volume to generate organoids of more uniform size (Zhu et al., 2017; Ao et al., 2020). Ao et al. (2020) achieved a coefficient of variation of 0.087 for mature organoid size by restricting their perfusable growth chamber to 2 mm in diameter.

To reduce inconsistencies in cellular composition between neuronal organoids, numerous (>15) differentiation

TABLE 1
Potential approaches to improve neuronal organoid reproducibility

Solution	Potential Application	References
Patterned microwells	Improve consistency in embryoid body size	Ungrin et al. (2008)
Physical growth restriction	Improve consistency in organoid size	Zhu et al. (2017); Ao et al. (2020)
Restriction of cell lineage fate	Reduce tissue heterogeneity	Schuldiner et al. (2001); Qian et al. (2016); Monzel et al. (2017)
3D bioprinting	Improve organoid cytoarchitecture consistency	Cui et al. (2012); Hinton et al. (2015); Jang et al. (2018); Silva et al. (2019)
Spatiotemporally defined differentiation cues	Improve organoid cytoarchitecture consistency	Mahoney and Saltzman (2001); Raeber et al. (2005); Kloxin et al. (2009); Yoshikawa et al. (2011); Przybyla et al. (2016); Uzel et al. (2016); Lancaster et al. (2017); Sun et al. (2018); Karzbrun and Reiner (2019); Silva et al. (2019)
Bioreactors	Improve environmental consistency	Quadrato et al. (2017)
Purchase of ECM/media components in bulk	Improve media consistency	
Differentiation protocols using only small molecules	Improve media consistency	Reinhardt et al. (2013)
Synthetic hydrogels	Improve ECM consistency	Langhans (2018)

protocols have been generated that restrict cell lineage fate. For example, Qian et al. (2016) demonstrated that prepatterning their EBs with fate-restricting dual SMAD inhibitors decreased tissue heterogeneity. The dual SMAD inhibition functioned to prevent differentiation of EBs toward a non-neural fate and to push them toward a neuroectodermal lineage (Qian et al., 2016). Retinoic acid has also commonly been used to promote differentiation toward a neuronal lineage (Schuldiner et al., 2001). Along these same lines, Monzel et al. (2017) used fate-restricted neuroepithelial stem cells rather than pluripotent stem cells to initiate organoid production for more efficient differentiation toward the midbrain.

Three-dimensional bioprinting has been proposed as a method to regulate organoid cytoarchitecture and self-assembly via precise placement of extracellular matrix hydrogels and/or individual cells (Silva et al., 2019). For example, hydrogel scaffolds that impose complex 3D structures for tissue-engineering applications have been produced by nozzle, laser, and inkjet printing systems (Cui et al., 2012; Hinton et al., 2015; Jang et al., 2018; Silva et al., 2019). In addition to printing biomaterials, 3D printers have the capacity to deposit individual cells. Although not in the context of a neuronal model, Fedorovich et al. (2012) used 3D fiber deposition to generate osteochondral tissue by printing chondrocytes and osteogenic progenitors in separate compartments of an alginate scaffold. The separate compartments developed into distinct tissue types and expressed different protein markers (Fedorovich et al., 2012). These existing 3D printing capabilities could be adapted to guide consistent cytoarchitecture organization within embryoid bodies.

In addition to automation, fate restriction, and 3D bioprinting, the use of spatiotemporally defined differentiation cues has also been proposed as a means to control neuronal organoid fate more closely (Silva et al., 2019). Morphogen gradients generated by microfluidic injection, slow-releasing microbeads, and bioactive scaffolds have all been considered (Mahoney and Saltzman, 2001; Uzel et al., 2016; Lancaster et al., 2017; Sun et al., 2018; Karzbrun and Reiner, 2019; Silva et al., 2019). Uzel et al. (2016) previously demonstrated they could establish linear biochemical concentration gradients within their microfluidic chambers to closely control the neuronal differentiation of 3D tissue constructs. Their device relies upon diffusion of molecules across inlet and outlet ports to generate gradients, which are naturally occurring during development and direct differentiation of developing tissues (Uzel et al., 2016). Likewise, Mahoney and Saltzman (2001) incorporated microparticles within cell aggregates transplanted into the brain for the purpose of establishing a "synthetic micro-environment." The microparticles released nerve growth factor over time to generate a local concentration gradient and promote targeted differentiation (Mahoney and Saltzman, 2001). Finally, Wylie et al. (2011) used light patterning to immobilize growth factors at certain locations within a 3D hydrogel to direct stem cell differentiation. This technique utilizes two-photon irradiation to expose reactive chemical groups within the hydrogel that are then able to bind and immobilize growth factors (Wylie et al., 2011). In addition to morphogen gradients, mechanical cues may be used to regulate stem cell differentiation within neuronal organoids (Silva et al., 2019). For example, Przybyla et al. (2016) showed that matrix stiffness influenced activation of differentiation pathways in human embryonic stem cells, with more compliant matrices enhancing mesodermal differentiation. Many ECM attributes can be adjusted and/or incorporated to control mechanical cues in a spatiotemporal manner, including cross-linking density, photodegradable hydrogels, and enzymatic cleavage points (Raeber et al., 2005; Kloxin et al., 2009; Silva et al., 2019). Yoshikawa et al. (2011) generated a pH-sensitive hydrogel that could be exploited to tune the elasticity of cell-laden scaffolds. Therefore, spatiotemporal control of differentiation cues could also be employed to direct uniform differentiation of stem cells within organoids.

Finally, to eliminate inconsistencies in environmental conditions across preparations, the use of regulated bioreactors to closely control temperature, humidity, and mechanical forces has been suggested (Quadrato et al., 2017). Because of the significant lot-to-lot variations in composition and concentration observed for naturally derived growth factors, serum, and extracellular matrices, it also may be advisable to buy such components in bulk. Such a practice could reduce inconsistencies in organoid growth media across batches. Alternatively, differentiation protocols that eliminate the need for growth factors are also being developed. For example, Reinhardt et al. (2013) established a protocol to generate neural precursor cells from stem cells using only small molecules. Likewise,

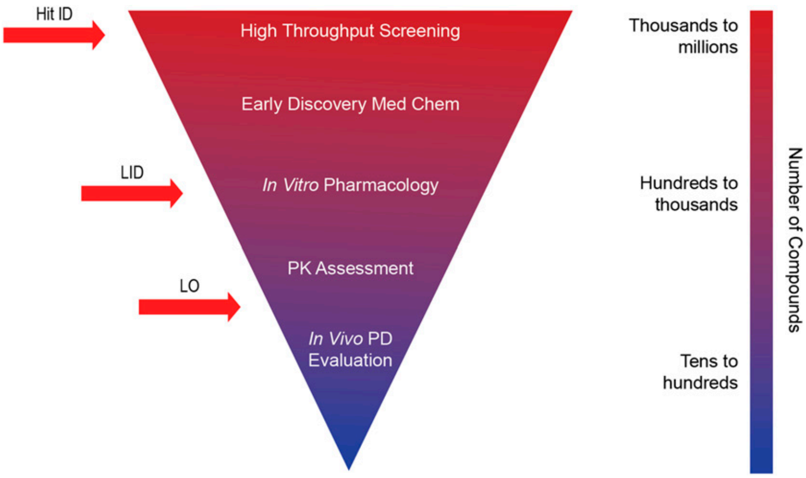

Fig. 2. Incorporation of neuronal organoids into the hit identification, lead identification, and lead optimization efforts. Assuming identified gaps to implementation are bridged, neuronal organoids could potentially be incorporated into several different stages of early drug discovery, including Hit ID, LID, and LO efforts. Organoids could serve as a theoretically more physiologically relevant platform to identify meaningful high-throughput screening hits. Within the SAR ROP, organoids could be used as an alternative or orthogonal assay to 2D cell-based assays to assess on-target activity. Finally, organoids could be incorporated toward the bottom of the ROP to help facilitate selection of appropriate preclinical candidates. Implementation of organoids at the various phases (arrows) would have unique requirements for at-scale generation and automation because of the number of compounds being tested. PK, pharmacokinetics.

synthetic hydrogels could potentially replace biologic hydrogels within the organoid fabrication process to avoid between-batch variability (Langhans, 2018).

Because of the many sources of variability within neuronal organoids, the field could benefit from the creation of a committee capable of providing guidelines and best practices for both the generation and use of neuronal organoids. At a recent panel discussion on “Organoid Quality Control” at a European Molecular Biology Laboratory Symposium focused on organoid technology, academic and industry experts stressed the need for standardization and transparency of protocols across the burgeoning organoid field (https://www.embo-embl-symposia.org/symposia/2020/EES20-11/). For example, many scientists incorrectly use the terms “spheroid” and “organoid” interchangeably, and default metrics for organoid quality control are unclear. Additionally, there is confusion over what constitutes a biologic versus technical organoid replicate and whether isogenic controls are necessary. A guidance committee could help normalize organoid work across institutions by creating defined criteria for nomenclature, publishing optimized differentiation protocols, and even potentially providing organoid standards for external comparison. Such normalization across the field would help enhance the quality of research by allowing for increased transparency and improved communication.

Along with reproducibility, throughput generation remains a major hurdle to the implementation of organoids in a drug discovery setting. There are several phases within early drug discovery efforts in which organoids could be used, which would require higher-throughput organoid production for sufficiently powered experiments (Fig. 2). Typically, a preclinical drug discovery ROP is used to triage and refine targeted compounds of interest and encompasses hit identification (Hit ID), lead identification (LID), and lead optimization (LO) efforts. Hit ID helps narrow the population of active chemistries. During this process, moderate-to-high-throughput screening campaigns ultimately deliver a package containing active compounds (hits) derived from a library. Such campaigns may involve single-point hit identification followed by multiple-point titration confirmation efforts among other evaluations. Because even relatively small compound libraries are typically comprised of thousands to tens of thousands of compounds, one can begin to see how the numbers of organoids required to support even a small screening campaign would add up quickly. Therefore, although it is theoretically applicable for screening campaigns, organoid implementation in LID efforts would be far more practical currently. This phase of the ROP explores SAR tractability within various chemical classes, allowing for determination of the most promising lead series. Here specifically, organoids could replace or serve as orthogonal assays to 2D cell-based assays, assessing on-target activity in a theoretically more physiologically relevant system. Adequate organoid numbers would be required to support the compound flow at this stage in the ROP—likely at least hundreds if not thousands of compounds over the course of a program. Alternatively, if their value as a complex human model for predicting preclinical to clinical translation can be established, organoids could be placed toward the bottom of an ROP to facilitate LO efforts. This phase of the ROP allows for establishment of an optimized preclinical candidate, in which organoid models may add value orthogonal to traditional in vivo preclinical evaluation. Unfortunately, the majority of current protocols to generate organoids are low-throughput and expensive, requiring time-intensive manual intervention. Furthermore, because screens have historically been performed with 2D monolayer or suspension cultures, the infrastructure to operate with organoids may be lacking. For example, it is more difficult to program standard liquid handlers to manage 3D cultures, and such instrumentation is generally not ideal for working with viscous solutions like the ECM hydrogels used to encapsulate EBs (Booij et al., 2019).

Specialized bioreactors and, again, microfluidic platforms could be the ideal tools to increase organoid production capacity and reduce costs (Table 2). Several researchers are already utilizing microfluidic platforms to streamline organoid production and obviate the need for manual intervention. Wang et al. (2018) adapted technology previously devised for organ-on-a-chip platforms to continuously perfuse growing organoids with media, thus cutting the time required for media changes. Ao et al. (2020) used microfluidic technology to seed stem cells, form embryoid bodies, induce differentiation, and encapsulate developing neuronal organoids in extracellular matrix. This platform required minimal manual manipulation of organoids and no organoid transfers. It was also capable of producing 169 neuronal organoids in one six-well plate, which is relatively high-density compared with traditional manual protocols (Ao et al., 2020). Specialized

TABLE 2
Potential methods to enable higher-throughput neuronal organoid generation

Solution	Potential Application	References
Microfluidics	Automation of embryoid body seeding, media changes, and encapsulation with ECM	Wevers *et al.*, 2016; Wang *et al.*, 2018; Ao *et al.*, 2020; Mimetas, 2020
3D liquid handlers	Automation of embryoid body seeding, media changes, and encapsulation with ECM	Formulatrix, Inc. (2020)
Bioreactors	Miniaturization for reduced media consumption, cost, and footprint	Qian et al. (2016); Wang et al. (2018)
Differentiation kits	Streamlined media production	Stemcell Technologies, Inc. (2020)

bioreactors are also being developed to minimize the volume of expensive growth media required to support organoid production. Qian et al. (2016) developed the “Spin Omega” bioreactor to provide suspension culture for organoids as an alternative to large spinning flasks. These bioreactors feature a small footprint, allow multiple test conditions to be investigated at once, and require minimal media. In fact, compared with the previously used spinning flasks that require 100 ml of media, each Spin Omega bioreactor well only requires 3 ml of media to support neuronal organoid growth (Qian et al., 2016). Wang et al.’s (2018) organ-on-a-chip technology (described above) was able to maintain organoid cultures at a perfusion rate of 25 μl of growth media per hour. As an alternative to bioreactors with intrinsic mass transport support, researchers are also utilizing orbital shakers and shaking incubators to promote diffusion of media into organoid cultures (Lancaster and Knoblich, 2014a).

In addition to microfluidic platforms and specialized bioreactors, high-throughput generation of organoids is also becoming more feasible because of the increased interest of laboratory supply manufacturers that are beginning to develop and commercialize 3D cultureware. For example, Stemcell Technologies, Inc. offers a six-well plate that can produce an estimated 42,000 EBs at one time (Stemcell Technologies, Inc., 2020). They also offer neuronal organoid differentiation kits that allow researchers to efficiently mix all necessary growth media components. Given the large number of growth factors and small molecules that must be precisely aliquoted and added to the organoid media at various maturation time points, these differentiation kits offer a mechanism to streamline media production. MIMETAS, B.V.’s OrganoPlate is a commercially available microfluidic platform for perfusion culture of ECM-encapsulated 3D tissues, including organoids. The OrganoPlate enables in situ gelation of cell-laden ECM and can support up to 96 pump-free perfusion cultures (Mimetas, 2020). It has successfully been used to culture 3D gels containing both neurons and glial cells (Wevers et al., 2016). Although such 96-well technology may not be of sufficient density to support high-throughput screening, such platforms could aid in secondary pharmacology profiling efforts supporting SAR ROPs. Finally, liquid handlers capable of generating as well as maintaining 3D cultures are being developed. For example, FORMULATRIX’s MANTIS is capable of encapsulating stem cells in biologic hydrogels and performing media changes without disturbing these 3D cultures (Formulatrix, Inc., 2020). Conventional industrial liquid handlers can, however, be used for injection of synthetic hydrogels (as opposed to biologic hydrogels) under shear-flow conditions because of their physical properties (Worthington et al., 2017).

The final barrier to the implementation of neuronal organoids in drug discovery is the current lack of assays to measure organoid physiology and function in a high-throughput fashion. Without compatible screening assays of complementary throughput, generation of standardized organoids at-scale for high- or even moderate-throughput compound evaluation is not worthwhile. Unfortunately, although the intrinsic three-dimensionality of organoids is crucial to their improved physiologic relevance, it is currently unfeasible to adapt many conventional 2D screening assays to this 3D platform; organoids more closely resemble tissue than cells in a monolayer. Poor diffusion of antibodies into the organoid tissue prevents the use of standard immunocytochemical techniques, whereas light scattering and/or absorption due to organoid thickness necessitates altered sample preparation. Visualization of proteins within the organoid interior requires low-throughput immunohistochemical techniques, such as tissue sectioning followed by 2D microscopy or tissue clearing followed by 3D microscopy. Both tissue sectioning and tissue clearing demand time-consuming manual intervention, whereas 3D microscopy necessitates long acquisition times, image analysis with high computational demands, and large data sets (Booij et al., 2019). Because of the inaccessibility of the organoid interior, functional readouts, such as electrophysiology and calcium imaging, have typically been performed in slices or dissociated organoid cultures rather than in whole organoids (Mariani et al., 2015; Paşca et al., 2015; Qian et al., 2016). Such analyses do not take advantage of the cytoarchitecture of the organoids, and the implications of dissociating organoid tissue are unclear. Finally, extended treatment times required for compounds to enter intact organoid structures and exert their effects must also be taken into account during such functional assay development (Booij et al., 2019).

Emerging microfluidic, imaging, and electrophysiological platforms may be the solution to enabling the high-throughput assessment of intact neuronal organoids (Table 3). Along with generating and maintaining organoids, researchers have shown that microfluidic and organ-on-a-chip technologies are capable of in situ characterization of organoid physiology. For example, Zhang et al. (2017) incorporated real-time biosensors into their microfluidic platform that measured the concentration of soluble proteins as well as imaged the morphology of maturing organoids over days in vitro. For biomarker detection, they functionalized gold electrodes with antibodies to capture and measure relevant antigens, such as albumin. These electrodes could be multiplexed to measure several biomarkers at a time and also could be regenerated with fresh antibodies upon saturation with antigen (Zhang et al., 2017). Such integrated electrochemical biosensors could be used for automated compound profiling in organoids with the caveat that thus far they have been limited to the detection of secreted proteins.

Electrophysiological platforms also hold promise for throughput assessment of organoid function. Several researchers

TABLE 3
Potential techniques for higher-throughput assessment of neuronal organoids

Solution	Potential Application	References
Microfluidics	Automated biosensing	Zhang et al. (2017)
Multielectrode arrays	Automated measurement of electrophysiological properties	Axion Biosystems, Inc., 2020; Kathuria *et al.*, 2020
Gelatin embedding en masse	Throughput sample preparation prior to imaging	Nagamoto-Combs et al. (2016)
Automated immunohistochemistry	Throughput sample preparation prior to imaging	(Biosystems, 2020)
Automated tissue clearing	Throughput sample preparation prior to imaging	Chen et al. (2016); Logos Biosystems, Inc. (2019)
Restriction of organoid size and/or density	Throughput sample preparation prior to imaging	Durens et al. (2020); Nickels et al. (2020)
Confocal high-content microscopy	Throughput analysis of histologic markers and probes	Boutin et al. (2018)
Fluorescent light sheet microscopy	Throughput analysis of histologic markers and probes	Eismann et al. (2020)
Multiphoton microscopy	Throughput analysis of histologic markers and probes	Rausch and Peker (2020)
Microplate readers	Throughput analysis of histologic markers and probes	Vergara et al. (2017); Nzou et al. (2018)

have already shown that neither dissociation nor slicing of organoids is necessary to record from organoid neurons. Whole-cell patch-clamp recordings performed by Mariani et al. (2015) demonstrated equivalent current amplitudes between neurons located in the exterior of retinal organoids and neurons in dissociated retinal organoid cultures. Likewise, Quadrato et al. (2017) were able to record extracellularly in neuronal organoids using high-density silicon electrodes. In this instance, the electrode shank was inserted into the intact organoid and enabled assessment of spike rate and neuronal network dynamics without the need to dissociate and/or slice the cultures. Although neither of these examples inherently allows for increased-throughput assessment, they provide proof of concept for extracellular recordings and for recordings in whole organoids.

Such findings support the use of multielectrode arrays (MEAs) for throughput evaluation of organoid function in intact organoids via extracellular recordings. Kathuria et al. (2020) used ECM coatings to attach 6-month-old cerebral organoids to a 24-well MEA plate, after which they were able to record spontaneous neuronal activity as well as firing rate. Furthermore, MEA technology is commercially available in platforms, such as AXION BioSystem, Inc.'s Maestro Pro. This system allows for multiple recordings over months within 96-well plates and provides analysis software to calculate firing rate, neuronal synchronization, and network oscillations within organoids (Axion Biosystems, Inc., 2020).

In addition to microfluidic and electrophysiological platforms, several emerging technologies are increasing the plausibility of analyzing organoids using imaging techniques. In terms of histologic techniques, Nagamoto-Combs et al. (2016) used a mold to embed eight whole mouse brains in gelatin for simultaneous sectioning on the cryostat. If followed by immunohistochemistry (IHC) on an automated IHC staining platform (Biosystems, 2020) and imaged using an automated slide scanner, the application of this technique to organoids could significantly reduce the time and bench work associated with conventional IHC processes.

Tissue clearing is a relatively common histologic method that enables immunohistochemical labeling and imaging of 3D specimens without the need for tissue sectioning (Chung et al., 2013). This method involves removing lipids from a 3D specimen to enable penetration of antibodies into otherwise inaccessible tissue. Once the tissue has been cleared and labeled, refractive index matching is performed to allow light penetration into the sample during imaging. Although tissue-clearing typically involves time-consuming incubation steps, several automated clearing platforms have recently been commercially developed to streamline this process. These platforms, such as Logos Biosystems's X-CLARITY system, can process multiple specimens at a time and often use electrophoresis to accelerate lipid removal (Logos Biosystems, Inc., 2019). Similarly, Chen et al. (2016) built a microfluidic platform to rapidly clear and label 3D spheroids. Rather than electrophoresis, their system utilizes pressure-driven flow to overcome the limitations of passive diffusion and is capable of boosting fluid exchange by 567-fold (Chen et al., 2016).

Tissue clearing as well as other emerging tech and protocol adaptations enable high-throughput image acquisition of intact organoid models using conventional high-content confocal microscopes. Although such platforms are capable of imaging 3D structures several millimeters in thickness, light scattering limits the visualization of the inner tissue beyond a depth of ~50–100 μm within unprocessed tissue. For example, Boutin et al. (2018) used high-content microscopy to image both cleared and uncleared spheroids in a 384-well plate and reported superior image quality in the cleared spheroids. As an alternative to laborious tissue clearing, Durens et al. (2020) instead used cell culture inserts to limit organoid growth to 100 μm and enable high-content imaging in 96-well plates. Similarly, Nickels et al. (2020) reduced organoid cell density to allow for antibody penetration within intact organoids. However, as with dissociation of organoids, the downstream consequences of altering physical properties on organoid physiologic relevance are unknown. Finally, the emerging technique of "expansion microscopy" uses swellable hydrogels to expand tissue isotropically. Chen et al. (2015) showed that this technique is capable of generating transparent specimens for improved light penetration. Although cell density reduction and expansion microscopy have not yet been used in conjunction with whole mount organoid staining followed by high-content imaging, such methods could potentially be employed in the future to enable image acquisition using high-content microscopy as well as the other microscopy techniques discussed later.

Sample compatibility aside, several high-content confocal microscopes also have software to facilitate rapid image acquisition of 3D structures. For example, PerkinElmer, Inc.'s high-content software supports a low-magnification prescan to locate spheroids and/or organoids within a well followed by a high-magnification scan of only the organoid area to reduce acquisition times (PerkinElmer, Inc., 2020). On the other hand, high-content software capable of single-cell segmentation in 3D cultures is still in its infancy (Booij et al., 2019). Existing software is open-source rather than integrated, and many researchers are choosing to develop their own 3D analysis software (Booij et al., 2019). For example, Boutin et al. (2018) developed an internal nuclear segmentation script to accurately identify subpopulations of fluorescently labeled cells.

Because of minimal light bleaching and improved light penetration, fluorescent light sheet microscopy (FLSM) has recently emerged as a popular alternative to confocal microscopy for imaging-cleared 3D specimens. Although typically low-throughput, Eismann et al. (2020) recently used FLSM for simultaneous imaging of groups of 38 spheroids in 3D. They captured 260 slices at 0.5-μm intervals for each spheroid and were able to image all 38 individual organoids with these parameters in 5 minutes (Eismann et al., 2020). Although their spheroids were not thick enough to necessitate tissue clearing, this technique could theoretically be adapted to cleared neuronal organoids. It should also be noted that imaging 228 spheroids in this fashion at 5-minute intervals over 24 hours generated 10.06 TB of raw data (Eismann et al., 2020). Such large data volumes are not uncommon in 3D microscopy. To decrease data usage and maintain throughput, it has been suggested that analysis pipelines in the future use automated, real-time phenotypic analyses on low-magnification images followed by automated higher-magnification acquisition of areas of interest (Booij et al., 2019). Automated recognition of the area of interest may be necessary to enable such a workflow.

Imaging platforms that avoid the need for tissue sectioning and clearing of 3D models also exist. Multiphoton microscopy technology allows light to penetrate into thick tissues without sample preparation and is therefore ideal to use with cell-type-specific fluorescent reporters to assess proteins of interest. As with FLSM, multiphoton imaging is conventionally low-throughput, and upright multiphoton microscopes are often incompatible with multiwell plates. However, Rausch and Peker (2020) recently developed an inverted two-photon microscope for compound screening, with the inverted lens promoting sample accessibility within 24-well plates. Using this set-up, they were able to image 40 spheroids per hour in multiwell plates (Rausch and Peker, 2020).

Aside from microfluidic, electrophysiological, and imaging platforms, it has been shown that several assays conventionally used for high-throughput examination of 2D cultures are amenable to use with 3D organoids. For example, Nzou et al. (2018) successfully measured ATP production as a proxy for cell viability and metabolic activity within intact neuronal organoids. This assay was performed in a 96-well plate using Promega's CellTiter-Glo Luminescent Cell Viability Assay with luminescent readouts taken using a standard microplate luminometer (Nzou et al., 2018). Likewise, Vergara et al. (2017) used a 3D automated reporter quantification platform to measure oxidative stress and mitochondrial membrane potential using standard assays in whole retinal organoids within 96-well plates. The 3D automated reporter quantification platform necessitates the use of a plate reader that can focus in the z-direction and uses background subtraction of the signal generated by a ubiquitous fluorescent reporter to normalize for organoid size. Vergara et al. (2017) estimated that this platform could be used to assess over 200,000 samples per day. Such technology could also be used with reporters that look at the expression of specific proteins relevant to the neurologic condition over time (Vergara et al., 2017).

Although the superior physiologic relevance of neuronal organoids has generated a lot of interest in using these models for compound screening in drug discovery, high variability, low-throughput generation, and lack of readouts with compatible throughput have limited their utility in drug discovery. Nevertheless, automated technologies are increasingly addressing these hurdles to neuronal organoid implementation in drug discovery. Specifically, organoid production via microfluidic platforms, next-generation liquid handlers, and specialized bioreactors represent steps toward miniaturization for higher-throughput standardized generation, reduced costs, and the need for minimal manual bench work. Likewise, growth restriction, careful regulation of differentiation lineage, and controlled application of biologic and mechanical cues has reduced both size and cell type variability between organoids. Finally, integrated biosensors, MEA electrophysiological platforms, automated histology, and high-content microscopy hold promise for sufficient throughput evaluation of neuronal organoid physiology and function.

Despite the significant progress that has been made, the generation and evaluation of reproducible organoids on the order required for routine use in early drug discovery may not be readily achievable in the near future. Regardless, the knowledge gleaned from these efforts could still inform the use of slightly less complex 3D models in the context of drug discovery. For example, if the size and heterogeneous nature of neuronal organoids prove to be prohibitive, the innovative technologies and assays developed to support increased-throughput neuronal organoid production and assessment could be adapted for use with neuronal spheroids. The smaller size of spheroids as well as their less involved fabrication protocol, could potentially make them more amenable for use in compound screening, assuming the desirable attributes achieved in neuronal organoids can be recapitulated in the spheroid model. Furthermore, as neuronal organoid models continue to progress, allowing for their broader application, researchers should be mindful not to presume an exaggerated value of these models in neuronal drug discovery just yet; there is currently little evidence to support that they are superior to conventional in vitro models in their ability to predict clinical compound success with regard to endpoint achievement. To this end, retrospective screens comparing the performance of successful (or unsuccessful) clinical compounds in neuronal organoids versus conventional in vitro models would be an ideal method to evaluate the predictive power of neuronal organoids (Booij et al., 2019). Such an evaluation could help indicate whether the potential of neuronal organoids for drug discovery is truly worthy of the further investment in the technology required to enable their use.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Struzyna, Watt.

References

Alzheimer's Association (2020) 2020 Alzheimer's disease facts and figures. *Alzheimers Dement*, doi: 10.1002/alz.12068 32157811.

Amin ND and Paşca SP (2018) Building models of brain disorders with three-dimensional organoids. *Neuron* **100**:389–405.

Antill-O'Brien N, Bourke J, and O'Connell CD (2019) Layer-by-layer: the case for 3D bioprinting neurons to create patient-specific epilepsy models. *Materials (Basel)* **12**: 3218.
Ao Z, Cai H, Havert DJ, Wu Z, Gong Z, Beggs JM, Mackie K, and Guo F (2020) One-stop microfluidic assembly of human brain organoids to model prenatal cannabis exposure. *Anal Chem* **92**:4630–4638.
Axion Biosystems, Inc. (2020) Maestro Pro, c.
Baden P, Yu C, and Deleidi M (2019) Insights into GBA Parkinson's disease pathology and therapy with induced pluripotent stem cell model systems. *Neurobiol Dis* **127**:1–12.
LeicaBiosystems (2020) BOND RX fully automated research stainer.
Booij TH, Price LS, and Danen EHJ (2019) 3D cell-based assays for drug screens: challenges in imaging, image analysis, and high-content analysis. *SLAS Discov* **24**: 615–627.
Boutin ME, Voss TC, Titus SA, Cruz-Gutierrez K, Michael S, and Ferrer M (2018) A high-throughput imaging and nuclear segmentation analysis protocol for cleared 3D culture models. *Sci Rep* **8**:11135.
Breslin S and O'Driscoll L (2013) Three-dimensional cell culture: the missing link in drug discovery. *Drug Discov Today* **18**:240–249.
Centeno EGZ, Cimarosti H, and Bithell A (2018) 2D versus 3D human induced pluripotent stem cell-derived cultures for neurodegenerative disease modelling. *Mol Neurodegener* **13**:27.
Charvin D, Medori R, Hauser RA, and Rascol O (2018) Therapeutic strategies for Parkinson disease: beyond dopaminergic drugs. *Nat Rev Drug Discov* **17**:804–822.
Chen F, Tillberg PW, and Boyden ES (2015) Optical imaging. Expansion microscopy. *Science* **347**:543–548.
Chen YY, Silva PN, Syed AM, Sindhwani S, Rocheleau JV, and Chan WCW (2016) Clarifying intact 3D tissues on a microfluidic chip for high-throughput structural analysis. *Proc Natl Acad Sci USA* **113**:14915–14920.
Chung K, Wallace J, Kim SY, Kalyanasundaram S, Andalman AS, Davidson TJ, Mirzabekov JJ, Zalocusky KA, Mattis J, Denisin AK, et al. (2013) Structural and molecular interrogation of intact biological systems. *Nature* **497**:332–337.
Cui X, Boland T, D'Lima DD, and Lotz MK (2012) Thermal inkjet printing in tissue engineering and regenerative medicine. *Recent Pat Drug Deliv Formul* **6**:149–155.
Dawson TM, Golde TE, and Lagier-Tourenne C (2018) Animal models of neurodegenerative diseases. *Nat Neurosci* **21**:1370–1379.
Dezonne RS, Sartore RC, Nascimento JM, Saia-Cereda VM, Romão LF, Alves-Leon SV, de Souza JM, Martins-de-Souza D, Rehen SK, and Gomes FCA (2017) Derivation of functional human astrocytes from cerebral organoids. *Sci Rep* **7**:45091.
Durens M, Nestor J, Williams M, Herold K, Niescier RF, Lunden JW, Phillips AW, Lin YC, Dykxhoorn DM, and Nestor MW (2020) High-throughput screening of human induced pluripotent stem cell-derived brain organoids. *J Neurosci Methods* **335**:108627.
Eismann B, Krieger TG, Beneke J, Bulkescher R, Adam L, Erfle H, Herrmann C, Eils R, and Conrad C (2020) Automated 3D light-sheet screening with high spatiotemporal resolution reveals mitotic phenotypes. *J Cell Sci* **133**:jcs245043.
Fang Y and Eglen RM (2017) Three-dimensional cell cultures in drug discovery and development. *SLAS Discov* **22**:456–472.
Fedorovich NE, Schuurman W, Wijnberg HM, Prins HJ, van Weeren PR, Malda J, Alblas J, and Dhert WJA (2012) Biofabrication of osteochondral tissue equivalents by printing topologically defined, cell-laden hydrogel scaffolds. *Tissue Eng Part C Methods* **18**:33–44.
Formulatrix, Inc. (2020) Scaling up organoid cultures using MANTIS liquid handler: a case study with MIT.
Frimat J-P and Luttge R (2019) The need for physiological micro-nanofluidic systems of the brain. *Front Bioeng Biotechnol* **7**:100.
Gooch CL, Pracht E, and Borenstein AR (2017) The burden of neurological disease in the United States: a summary report and call to action. *Ann Neurol* **81**:479–484.
Grenier K, Kao J, and Diamandis P (2020) Three-dimensional modeling of human neurodegeneration: brain organoids coming of age. *Mol Psychiatry* **25**:254–274.
Haring AP, Sontheimer H, and Johnson BN (2017) Microphysiological human brain and neural systems-on-a-chip: potential alternatives to small animal models and emerging platforms for drug discovery and personalized medicine. *Stem Cell Rev Rep* **13**:381–406.
Hingorani AD, Kuan V, Finan C, Kruger FA, Gaulton A, Chopade S, Sofat R, MacAllister RJ, Overington JP, Hemingway H, et al. (2019) Improving the odds of drug development success through human genomics: modelling study. *Sci Rep* **9**:18911.
Hinton TJ, Jallerat Q, Palchesko RN, Park JH, Grodzicki MS, Shue HJ, Ramadan MH, Hudson AR, and Feinberg AW (2015) Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Sci Adv* **1**:e1500758.
Hung SSC, Khan S, Lo CY, Hewitt AW, and Wong RCB (2017) Drug discovery using induced pluripotent stem cell models of neurodegenerative and ocular diseases. *Pharmacol Ther* **177**:32–43.
Jang TS, Do Jung H, Pan HM, Han WT, Chen S, and Song J (2018) 3D printing of hydrogel composite systems: recent advances in technology for tissue engineering. *Int J Bioprinting* **4**:126.
Jensen G, Morrill C, and Huang Y (2018) 3D tissue engineering, an emerging technique for pharmaceutical research. *Acta Pharm Sin B* **8**:756–766.
Jo J, Xiao Y, Sun AX, Cukuroglu E, Tran HD, Göke J, Tan ZY, Saw TY, Tan CP, Lokman H, et al. (2016) Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. *Cell Stem Cell* **19**:248–257.
Karzbrun E and Reiner O (2019) Brain organoids-a bottom-up approach for studying human neurodevelopment. *Bioengineering (Basel)* **6**:9.
Kathuria A, Lopez-Lengowski K, Vater M, McPhie D, Cohen BM, and Karmacharya R (2020) Transcriptome analysis and functional characterization of cerebral organoids in bipolar disorder. *Genome Med* **12**:34.
Kim H, Park HJ, Choi H, Chang Y, Park H, Shin J, Kim J, Lengner CJ, Lee YK, and Kim J (2019a) Modeling G2019S-LRRK2 sporadic Parkinson's disease in 3D midbrain organoids. *Stem Cell Reports* **12**:518–531.
Kim H, Xu R, Padmashri R, Dunaevsky A, Liu Y, Dreyfus CF, and Jiang P (2019b) Pluripotent stem cell-derived cerebral organoids reveal human oligodendrogenesis with dorsal and ventral origins. *Stem Cell Reports* **12**:890–905.
Kloxin AM, Kasko AM, Salinas CN, and Anseth KS (2009) Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science* **324**: 59–63.
Lancaster MA, Corsini NS, Wolfinger S, Gustafson EH, Phillips AW, Burkard TR, Otani T, Livesey FJ, and Knoblich JA (2017) Guided self-organization and cortical plate formation in human brain organoids. *Nat Biotechnol* **35**:659–666.
Lancaster MA and Knoblich JA (2014a) Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc* **9**:2329–2340.
Lancaster MA and Knoblich JA (2014b) Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* **345**:1247125.
Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, and Knoblich JA (2013) Cerebral organoids model human brain development and microcephaly. *Nature* **501**:373–379.
Langhans SA (2018) Three-dimensional in vitro cell culture models in drug discovery and drug repositioning. *Front Pharmacol* **9**:6.
Lee CT, Bendriem RM, Wu WW, and Shen RF (2017) 3D brain Organoids derived from pluripotent stem cells: promising experimental models for brain development and neurodegenerative disorders. *J Biomed Sci* **24**:59.
Lee HK, Velazquez Sanchez C, Chen M, Morin PJ, Wells JM, Hanlon EB, and Xia W (2016) Three dimensional human neuro-spheroid model of Alzheimer's disease based on differentiated induced pluripotent stem cells. *PLoS One* **11**: e0163072.
Li Y, Muffat J, Omer A, Bosch I, Lancaster MA, Sur M, Gehrke L, Knoblich JA, and Jaenisch R (2017) Induction of expansion and folding in human cerebral organoids. *Cell Stem Cell* **20**:385–396.e3.
Liu C, Oikonomopoulos A, Sayed N, and Wu JC (2018) Modeling human diseases with induced pluripotent stem cells: from 2D to 3D and beyond. *Development* **145**: dev156166.
Logos Biosystems, Inc. (2019) X-CLARITY systems and reagents for tissue clearing.
Mahoney MJ and Saltzman WM (2001) Transplantation of brain cells assembled around a programmable synthetic microenvironment. *Nat Biotechnol* **19**:934–939.
Mariani J, Coppola G, Zhang P, Abyzov A, Provini L, Tomasini L, Amenduni M, Szekely A, Palejev D, Wilson M, et al. (2015) FOXG1-Dependent dysregulation of GABA/glutamate neuron differentiation in autism spectrum disorders. *Cell* **162**: 375–390.
Marton RM, Miura Y, Sloan SA, Li Q, Revah O, Levy RJ, Huguenard JR, and Paşca SP (2019) Differentiation and maturation of oligodendrocytes in human three-dimensional neural cultures. *Nat Neurosci* **22**:484–491.
Mimetas B.V. (2020) OrganoPlate 3-lane.
Monzel AS, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuellen T, Jarazo J, Walter J, Brüggemann I, Boussaad I, et al. (2017) Derivation of human midbrain-specific organoids from neuroepithelial stem cells. *Stem Cell Reports* **8**:1144–1154.
Nagamoto-Combs K, Manocha GD, Puig K, and Combs CK (2016) An improved approach to align and embed multiple brain samples in a gelatin-based matrix for simultaneous histological processing. *J Neurosci Methods* **261**:155–160.
Nickels SL, Modamio J, Mendes-Pinheiro B, Monzel AS, Betsou F, and Schwamborn JC (2020) Reproducible generation of human midbrain organoids for in vitro modeling of Parkinson's disease. *Stem Cell Res (Amst)* **46**:101870.
Nzou G, Wicks RT, Wicks EE, Seale SA, Sane CH, Chen A, Murphy SV, Jackson JD, and Atala AJ (2018) Human cortex spheroid with a functional blood brain barrier for high-throughput neurotoxicity screening and disease modeling. *Sci Rep* **8**:7413.
Papariello A and Newell-Litwa K (2020) Human-derived brain models: windows into neuropsychiatric disorders and drug therapies. *Assay Drug Dev Technol* **18**:79–88.
Park J, Wetzel I, Marriott I, Dréau D, D'Avanzo C, Kim DY, Tanzi RE, and Cho H (2018) A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. *Nat Neurosci* **21**:941–951.
Paşca AM, Sloan SA, Clarke LE, Tian Y, Makinson CD, Huber N, Kim CH, Park JY, O'Rourke NA, Nguyen KD, et al. (2015) Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat Methods* **12**:671–678.
PerkinElmer, Inc. (2020) PreciScan intelligent acquisition.
Przybyla L, Lakins JN, and Weaver VM (2016) Tissue mechanics orchestrate wnt-dependent human embryonic stem cell differentiation. *Cell Stem Cell* **19**:462–475.
Qian X, Nguyen HN, Song MM, Hadiono C, Ogden SC, Hammack C, Yao B, Hamersky GR, Jacob F, Zhong C, et al. (2016) Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell* **165**:1238–1254.
Quadrato G, Nguyen T, Macosko EZ, Sherwood JL, Min Yang S, Berger DR, Maria N, Scholvin J, Goldman M, Kinney JP, et al. (2017) Cell diversity and network dynamics in photosensitive human brain organoids. *Nature* **545**:48–53.
Raeber GP, Lutolf MP, and Hubbell JA (2005) Molecularly engineered PEG hydrogels: a novel model system for proteolytically mediated cell migration. *Biophys J* **89**:1374–1388.
Rausch M and Peker B (2020) Bridging the gap between high-resolution live cell microscopy and 3D cellular models – a breakthrough method. *Microsc Anal (Am Ed)* **34**:15–17.
Reinhardt P, Glatza M, Hemmer K, Tsytsyura Y, Thiel CS, Höing S, Moritz S, Parga JA, Wagner L, Bruder JM, et al. (2013) Derivation and expansion using only small molecules of human neural progenitors for neurodegenerative disease modeling [published correction appears in *PLoS One* (2013) 8]. *PLoS One* **8**:e59252.
Sanders LH, Laganière J, Cooper O, Mak SK, Vu BJ, Huang YA, Paschon DE, Vangipuram M, Sundararajan R, Urnov FD, et al. (2014) LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: reversal by gene correction. *Neurobiol Dis* **62**:381–386.
Schuldiner M, Eiges R, Eden A, Yanuka O, Itskovitz-Eldor J, Goldstein RS, and Benvenisty N (2001) Induced neuronal differentiation of human embryonic stem cells. *Brain Res* **913**:201–205.
Schüle B, Pera RAR, and Langston JW (2009) Can cellular models revolutionize drug discovery in Parkinson's disease? *Biochim Biophys Acta* **1792**:1043–1051.

Seo J, Kritskiy O, Watson LA, Barker SJ, Dey D, Raja WK, Lin YT, Ko T, Cho S, Penney J, et al. (2017) Inhibition of p25/Cdk5 attenuates tauopathy in mouse and iPSC models of frontotemporal dementia. *J Neurosci* **37**:9917–9924.
Silva TP, Cotovio JP, Bekman E, Carmo-Fonseca M, Cabral JMS, and Fernandes TG (2019) Design principles for pluripotent stem cell-derived organoid engineering. *Stem Cells Int* **2019**:4508470.
Stemcell Technologies, Inc (2020) Control EB or spheroid size with AggreWell.
Sun AX, Ng HH, and Tan EK (2018) Translational potential of human brain organoids. *Ann Clin Transl Neurol* **5**:226–235.
Tekin H, Simmons S, Cummings B, Gao L, Adiconis X, Hession CC, Ghoshal A, Dionne D, Choudhury SR, Yesilyurt V, et al. (2018) Effects of 3D culturing conditions on the transcriptomic profile of stem-cell-derived neurons. *Nat Biomed Eng* **2**:540–554.
Thomas M and Willerth SM (2017) 3-D bioprinting of neural tissue for applications in cell therapy and drug screening. *Front Bioeng Biotechnol* **5**:69.
Trujillo CA, Gao R, Negraes PD, Gu J, Buchanan J, Preissl S, Wang A, Wu W, Haddad GG, Chaim IA, et al. (2019) Complex oscillatory waves emerging from cortical organoids model early human brain network development. *Cell Stem Cell* **25**:558–569.e7.
Ungrin MD, Joshi C, Nica A, Bauwens C, and Zandstra PW (2008) Reproducible, ultra high-throughput formation of multicellular organization from single cell suspension-derived human embryonic stem cell aggregates. *PLoS One* **3**:e1565.
Uzel SGM, Amadi OC, Pearl TM, Lee RT, So PTC, and Kamm RD (2016) Simultaneous or sequential orthogonal gradient formation in a 3D cell culture microfluidic platform. *Small* **12**:612–622.
Vergara MN, Flores-Bellver M, Aparicio-Domingo S, McNally M, Wahlin KJ, Saxena MT, Mumm JS, and Canto-Soler MV (2017) Three-dimensional automated reporter quantification (3D-ARQ) technology enables quantitative screening in retinal organoids. *Development* **144**:3698–3705.
Wang H (2018) Modeling neurological diseases with human brain organoids. *Front Synaptic Neurosci* **10**:15.
Wang Y, Wang L, Zhu Y, and Qin J (2018) Human brain organoid-on-a-chip to model prenatal nicotine exposure. *Lab Chip* **18**:851–860.
Wevers NR, van Vught R, Wilschut KJ, Nicolas A, Chiang C, Lanz HL, Trietsch SJ, Joore J, and Vulto P (2016) High-throughput compound evaluation on 3D networks of neurons and glia in a microfluidic platform. *Sci Rep* **6**:38856.
Worthington P, Drake KM, Li Z, Napper AD, Pochan DJ, and Langhans SA (2017) Beta-hairpin hydrogels as scaffolds for high-throughput drug discovery in three-dimensional cell culture. *Anal Biochem* **535**:25–34.
Wylie RG, Ahsan S, Aizawa Y, Maxwell KL, Morshead CM, and Shoichet MS (2011) Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. *Nat Mater* **10**:799–806.
Yakoub AM and Sadek M (2018) Development and characterization of human cerebral organoids: an optimized protocol. *Cell Transplant* **27**:393–406.
Yang W, Hamilton JL, Kopil C, Beck JC, Tanner CM, Albin RL, Ray Dorsey E, Dahodwala N, Cintina I, Hogan P, and Thompson T (2020) Current and projected future economic burden of Parkinson's disease in the U.S. *NPJ Parkinsons Dis* **6**:15.
Ye F, Kang E, Yu C, Qian X, Jacob F, Yu C, Mao M, Poon RYC, Kim J, Song H, et al. (2017) DISC1 regulates neurogenesis via modulating kinetochore attachment of ndel1/nde1 during mitosis [published correction appears in *Neuron* (2017) 96: 1204]. *Neuron* **96**:1041–1054.e5.
Yoshikawa HY, Rossetti FF, Kaufmann S, Kaindl T, Madsen J, Engel U, Lewis AL, Armes SP, and Tanaka M (2011) Quantitative evaluation of mechanosensing of cells on dynamically tunable hydrogels. *J Am Chem Soc* **133**:1367–1374.
Zhang D, Pekkanen-Mattila M, Shahsavani M, Falk A, Teixeira AI, and Herland A (2014) A 3D Alzheimer's disease culture model and the induction of P21-activated kinase mediated sensing in iPSC derived neurons. *Biomaterials* **35**:1420–1428.
Zhang YS, Aleman J, Shin SR, Kilic T, Kim D, Mousavi Shaegh SA, Massa S, Riahi R, Chae S, Hu N, et al. (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. *Proc Natl Acad Sci USA* **114**:E2293–E2302.
Zhu Y, Wang L, Yin F, Yu Y, Wang Y, Liu H, Wang H, Sun N, Liu H, and Qin J (2017) A hollow fiber system for simple generation of human brain organoids. *Integr Biol (camb)* **9**:774–781.
Zhuang P, Sun AX, An J, Chua CK, and Chew SY (2018) 3D neural tissue models: from spheroids to bioprinting. *Biomaterials* **154**:113–133.

Address correspondence to: Dr. Marla L. Watt, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486. E-mail: Marla.Watt@Merck.com