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Organoid and Organ-On-A-Chip Systems: New Paradigms for Modeling Neurological and Gastrointestinal Disease

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Abstract

Purpose of review—The modeling of biological processes *in vitro* provides an important tool to better understand mechanisms of development and disease, allowing for the rapid testing of therapeutics. However, a critical constraint in traditional monolayer culture systems is the absence of the multicellularity, spatial organization, and overall microenvironment present *in vivo*. This limitation has resulted in numerous therapeutics showing efficacy *in vitro*, but failing in patient trials. In this review, we discuss several organoid and "organ-on-a-chip" systems with particular regard to the modeling of neurological diseases and gastrointestinal disorders.

Recent findings—Recently, the *in vitro* generation of multicellular organ-like structures, coined organoids, has allowed the modeling of human development, tissue architecture, and disease with human-specific pathophysiology. Additionally, microfluidic "organ-on-a-chip" technologies add another level of physiological mimicry by allowing biological mediums to be shuttled through 3D cultures.

Summary—Organoids and organ-chips are rapidly evolving *in vitro* platforms which hold great promise for the modeling of development and disease.

Keywords

Organoid; microfluidics; organ-on-a-chip; BBB-chip; personalized medicine; disease modeling; gut organoids; cerebral organoids

Introduction

Modeling disease mechanisms and testing potential therapeutics has traditionally occurred via the use of animal models or in cell culture. Animal models intrinsically exhibit complex tissue architecture with varying degrees of similarity to analogous human organs. While these models exhibit the natural complexity of multicellular, multi-organ, and multi-system interactions, numerous studies have noted the differences between human pathophysiology and the animal models used—most commonly rodents [1-5]. Furthermore, the time, cost, and ethical concerns associated with animal models add to the limitations of their use as models of disease and screening platforms for potential therapeutics. Additionally, animal models are also limited in detecting post market adverse events [6]. Traditionally, *in vitro* cell culture systems have allowed for more rapid disease modeling and drug discovery studies. Additionally, the advent of induced pluripotent stem cells (iPSCs) revolutionized the disease modeling field as it allows a more personalized and patient-specific method of modeling genetic disease [7, 8]. However, *in vitro* disease modeling relies on a somewhat reductionist approach as a single cell type is studied in relative isolation. This negates the multicellular environment and influence of other systems (i.e. lymphatic, vasculature, etc.) present in an organism. Towards achieving human-specific organ modeling, humanized rodent models have benefitted the disease modeling field as rodents have been generated which have components of human systems [9]. Notably, the majority of these studies have been carried out in the immunology field [reviewed in [10]]. In respect to the brain, chimeric mice with human glial cells were generated by transplanting human glial progenitors to transgenic mice [11-13]. Interestingly, these mice had increased synaptic plasticity and learning. While these reports primarily investigated the influence of human glial cells in respect to cell-cell interactions in the brain and behavior, future studies may use this technique for modeling diseases which affect human, but not mouse glial cells. However, while the perinatally transplanted human glial cells proliferated and "outcompeted" native mouse glia in these studies, the neuronal population remained primarily mouse-based [13]. This is expected as the majority of neurogenesis in mice and humans occurs embryonically, whereas gliogenesis occurs peri-and-postnatally in both human and mice [14, 15]. While the findings from the human glial progenitor studies referenced above are remarkable, this method requires an impressive amount of expertise in the culture of glial progenitors, animal surgeries, and the use of mice lacking many components of the immune system which allow for the xenograft.

Another more comprehensive approach is the "organo-chimeric" approach where entire organs from one species are generated in other species. In this respect, the generation of a functional rat pancreas and kidney in mice by blastocyst complementation has been reported [16, 17]. The generation of larger [porcine] organs, specifically the pancreas, was also reported using this blastocyst complementation technology [18]. The overarching goal of

this work is to obtain a source of human organs to address the shortage of organs for patients waiting for transplants. However, successful generation of human organs in porcine surrogates has not yet been reported. Furthermore, even if such a method were to successfully yield human organs (heart, kidney, etc.) within a porcine surrogate, time and cost would limit the use of this technology as a platform for personalized human disease modeling or drug screening.

The discovery that multipotent stem and progenitor cells possess the ability to natively differentiate and self-organize *in vitro* into defined structures has revolutionized the disease modeling field [19, 20]. These structures, which resemble human organs (at varying levels of accuracy), have thus been coined "organoids" and have even led to the generation of complex cell types that have not been generated *in vitro*. Over the last few years, several groups have used organoids to model human diseases which could not be modeled using animal models or traditional monolayer culture systems [reviewed in [21-23]].

Current omics approaches have contributed to the increased characterization and understanding of the complexity of human tissue types [24]. This has led to more sophisticated culture methods for generating organoids of varying tissue types [25, 26]. As these approaches are refined and adapted, new organoid types are continually added such as the recent reports of generating mammary-like organoids that can produce milk [27] and kidney organoids which model nephrogenesis [26]. In this review, we take a critical view on the use of organoids and microfluidic "organ-on-a-chip" technology for disease modeling in two fields—neurological diseases and gastrointestinal disorders (Figure 1).

Brain organoids and brain-chips for modeling neurological disease

An extensive amount of knowledge has been gleaned about the nature of neural stem cells and their ability of intrinsically govern their own cellular output; this will not be discussed here [for discussion see 28, 29]. Building on this knowledge base, the Sasai group published a number of seminal studies on the self-organizing properties of neural progenitors when grown as a 3D culture [20, 19, 30]. Notably, these studies demonstrated the intrinsic ability of neural stem cells—derived from pluripotent populations—to recapitulate the 3D structure of the developing brain and other neural structures [19]. However, the stereotypical six-layer structure of the cortex remained largely elusive.

This limitation was largely surmounted in a landmark study which reported the generation of brain-like structures from human embryonic stem (ES) cells and iPSCs [31]. Knoblich and colleagues focused on improving physical growth conditions with bioreactors rather than directing organization and cell specification by extrinsically applying morphogens or ectopically misexpressing transgenes. Specifically, after directing pluripotent stem cells to neuroectoderm, cells were embedded in Matrigel to provide a supportive scaffold for tissue growth [32]. The Matrigel-coated droplets were then placed in a spinning bioreactor which encouraged nutrient penetration throughout the spheres by centripetal force. Notably, the spheres grew to their maximum size of 4mm in 2 months, though they continued to survive in culture for as long as 10 months. When assessed at various time points, they displayed tissue that resembled the neocortex, choroid plexus, retina, and meninges—though at varying consistencies. In respect to brain region specificity, markers of the forebrain,

midbrain, and hindbrain were reported. These findings along with further characterizations led to the designation of these sphere-like structures as *cerebral organoids*. Importantly, the authors did report extensive cell death at core of the organoids and plausibly attributed it to a lack of a circulatory system. This notion is supported by the finding that the endothelial system is developed and married to the developing brain during embryonic development [33, 34]. In this respect, we discuss a novel technology related to addressing the need for vasculature in organoids later in this review.

More recent studies have modified the bioreactor approach described above and reported the generation of organoids with increased neural subtypes and regional organization. In terms of creating a more scalable solution, Ming, Song and colleagues used cost-effective, miniaturized 3D-printed spinning bioreactors which they named "SpinΩ" [25]. The SpinΩ bioreactors can be 3D-printed using the blueprints provided by the authors and permit the use of (3) standard 12-well plates. This allows the growth of 36 individual samples in a profile that measures less than one square foot. The forebrain organoids generated using the SpinΩ bioreactors had enhanced region specificity as they contained cortical neural subtypes which expressed markers present in, and developed in a similar inside-out fashion, to the six-layered neocortex [35-38]. Impressively, they also contained astrocytes after ~100 days in culture. An alternative 3D method has similarly observed the generation of glial types as well as cerebral cortex-like lamination [39]. In addition to the cortex, modified protocols have been developed to generate midbrain and hypothalamic organoids [25, 40].

Due to differences in physiology, many diseases are not recapitulated in animal models [1-5]. Further, the time to generate, administer, and assess potential therapeutics in animals limits their use for high-throughput drug screening. Moreover, the ability to rapidly genetically manipulate brain organoids via CRISPR/Cas9-based gene targeting, *in vitro* electroporation, or virus is an added benefit for their use [25, 31]. Here, we review previous studies which have used new cell culture technologies (Table 1) and suggest future approaches which can use brain organoids to model developmental disorders, neurodegenerative diseases, brain tumors, and neuropsychiatric disorders.

Neurodevelopmental disorders

Neurodevelopmental disorders that have physical manifestations in respect to brain size were among the first to be modeled by the brain organoid field. Microcephaly, a condition where the brain is smaller in size, was modeled using cerebral organoids [31]. When the initial bioreactor approach was applied to iPSCs generated from a patient with compound heterozygous mutations in *CDK5RAP2* [and a loss of CDK5RAP2 protein], smaller neuroepithelial tissues were observed in the patient-derived organoids [31]. At the cellular level, the patient organoids had reduced [Sox2$^+$] progenitor and [Dcx$^+$] immature neuron populations, and increased [p-Vimentin$^+$] radial glia and [Tuj1$^+$] neuronal populations. This suggested premature neuronal differentiation at the expense of progenitor maintenance. Reintroduction of *CDK5RAP2* by electroporation into 12-day old patient organoids rescued the disease phenotype and knockdown of *CDK5RAP2* by shRNA electroporation in control cells mimicked the observed microcephaly phenotype. Due to the recent ZIKA outbreaks and resulting microcephaly in newborns [41], modeling ZIKA-induced microcephaly and

testing potential therapeutics is of increased interest to the field. In this respect, [25] infected regional forebrain-specific organoids with African or Asian ZIKV strains. The resulting organoids exhibited increased cell death, reduced proliferation, and resembled microcephaly as an overall decrease in gross morphology was observed. Combined with the cost-effective [SpinΩ] 3D-printed spinning bioreactors, this method provides a platform for the rapid screening of potential therapeutics in brain organoids for personalized medicine approaches [25, 42]. In particular, using iPSC-derived neural cells, 6,000 compounds were screened for efficacy in preventing ZIKA-induced neural progenitor death. Brain organoids generated by the SpinΩ bioreactors (described above) were used for to validate the efficacy of the most potent compounds [42].

In addition to microcephaly, macrocephaly (a condition where the head is larger than normal) was modeled in organoids from hESCs wherein a CRISPR/Cas9-mediated PTEN loss-of-function (LOF) mutation was induced [43]. Notably, PTEN loss-of-function mutations are associated with human macrocephaly [44, 45]. The PTEN LOF organoids displayed enhanced AKT signaling, increased proliferation, delayed neuronal differentiation, and an increase in the neural progenitor population and overall surface area of the organoids. The PTEN LOF organoids also exhibited increased cortical folding, characteristics not observed when organoids where generated from mouse cells exhibiting PTEN LOF or in mouse models of PTEN LOF [46, 47]. When the PTEN LOF organoids were exposed to ZIKV, the organoids displayed a decrease in expansion and folding. This report speaks to the ability of organoids to not only model disease in human-specific manner, but to also build on earlier hypotheses about the relationships of increased neural progenitor proliferation and the folding observed in the human cortex [35].

Neurodegenerative diseases

Though neurodegenerative diseases have been modeled for several decades in culture, several characteristics of 3D cultures have the potential to enhance the precision with which these diseases are modeled and used as model systems for drug discovery and personalized medicine. These revolve around the necessity to generate the post-mitotic neuronal subtypes impacted *in vitro* from stem and progenitor cells as they cannot be isolated and grown in culture due to their intricate phenotype.

Firstly, certain neuronal subtypes, such as corticospinal motor neurons (CSMNs) which reside in layer 5 of the cortex, have not been efficiently generated from human cells *in vitro*. These neurons are among the neuronal subtypes which degenerate in ALS [48, 49]; thus, testing therapeutics which may preserve their function is of increased interest. Furthermore, the directed differentiation of iPSCs from *C9ORF72* familial ALS patients to spinal motor neurons (spMNs), another cell type impaired in ALS patients [48]; has uncovered pathophysiological processes related to their degeneration [50]. Thus, the generation of CSMNs from familial ALS patient iPSCs may provide insight into the degeneration and preservation of these cells. It should be noted that upon careful examination, monolayer systems appear to exhibit stalled maturation of cortical subtypes in terms of inappropriate expression of laminar and postmitotic markers [51]. Impressively, the forebrain organoids generated by [25] harbor distinct cortical organization; and though an in-depth assessment

regarding the existence of bona fide CSMNs in these organoids is yet to be reported, this culturing system provides promise towards the generation of this intricate cell type. However, progress is being made on other disease fronts. For example, a key neuronal subtype lost in Parkinson's disease are classes of dopaminergic projection neurons. Towards modeling Parkinson's disease in a personalized manner within a niche composed of multiple cellular subtypes; Ng, Je, and colleagues generated midbrain organoids which possessed electrically active and functionally mature dopamine-producing neurons [40]. Furthermore, these midbrain organoids produced neuromelanin-like granules which were organizationally similar to those seen in post-mortem substantia nigra tissue.

Secondly, the maturity of neuronal populations generated *in vitro* has been reported to be far from similar to those of the *in vivo* populations being mimicked. Specifically, a transcriptomic analysis of spinal motor neurons (spMNs) generated *in vitro* revealed that the cells were more similar to fetal spinal tissue than adult spMNs [52]. While it would not be practical to elongate culture times for many years, the use of organoids (and the "organ-on-a-chip" platforms discussed below) may provide increased maturity due to the presence of a more physiological microenvironment. Additionally, combining organoid culture methods with methods to externally regulate gene expression in a developmentally-relevant temporal fashion may allow for the enhanced generation and maturation of complex cell types [53].

Glioma

Comprehensive modeling of the spectrum of brain cancers such as glioma has remained an challenging task to achieve [reviewed in [54]]. Traditional patient-derived xenograft (PDX) models rely on transplanting human cells into rodents; this may yield confounding results due the interspecies interaction afforded when human cells are transplanted into the mouse brain. As discussed above, the transplantation of human glial cells into the mouse brain elicited behavioral changes related to learning and synaptic plasticity [11]. Furthermore, the natural progression of the tumor growth and interaction with surrounding environment during tumorigenesis is not recapitulated in transplant models as the animal immediately transitions from being completely healthy to receiving a large implanted tumor mass. Viral models of tumorigenesis allow for the targeted genetic manipulation of certain cell-types and have expanded the glioma field. However, experimenter safety is of utmost concern when using highly pathogenic viruses with high grade driver mutations (e.g. Ras). The use of postnatal electroporation for modeling pediatric glioma allows for the rapid and stable insertion of oncogenic drivers to stem and progenitor cells in the murine brain [55]. Combining this technique with dual recombinase-mediated cassette exchange allows for site-specific insertion of oncogenic drivers at the single copy level, adding to the physiological mimicry of patient glioma [56]. While all of the abovementioned techniques lend to a better understanding of pathophysiological tumor progression in mice, the differences pertaining to human vs mouse neural development speak to the need of human-specific models. In this respect, the ability to combine clinically-relevant oncogenic drivers [57] with electroporation techniques that allow for the genetic manipulation of organoids [25] would allow for personalized and patient-specific models of human glioma *in vitro* in a rapid fashion. Additionally, for instances where PDX models must be used, applying 3D culture techniques to PDX models of glioma may provide a more representative model of

the disease as it was observed that human glioma cells grown in 3D culture before transplantation (in contrast to being grown as a monolayer) pathologically represented the parent tumor to a higher degree [58] .

Neuropsychiatric disorders

Given the current limitations of organoids and *in vitro* models in terms of producing mature circuits (i.e. lack multi-region structures, lack of microglia and oligodendrocytes, lack of spines, etc.), the use of these models appears limited for the immediate future. Nevertheless, neuropsychiatric disorders are often complex and involve polygenic mutations; for these reasons and others mouse models are often similarly limited in their utility. However, 3D cultures may allow a reductionist platform in which cell biological manifestations associated with these diseases may become uncovered. For a thorough review on the use of organoids for neuropsychiatric disorders, we direct our readers to [59].

BBB Modeling and Brain-Chips

BBB Modeling

In terms of the potential influx of compounds and microorganisms into the brain, the human central nervous system (CNS) is effectively sheltered by complex control of vascular diffusion known as the blood brain barrier (BBB) [60]. While evolutionary advantageous, these stringent barrier mechanisms remain a formative challenge for delivering therapeutic compounds to the CNS [61]. An interplay of cell types known as the neurovascular unit (NVU) form this barrier and include brain microvascular endothelial cells (BMECs), pericytes, astrocytes, microglia, and neural tissue [62]. The complete cohort of NVU cell types are not present in existing brain organoid models, limiting their ability to accurately model the delivery of neurotherapeutics. Traditional BBB models *in vitro* have utilized immortalized human and animal BMEC lines in a transwell system to assay for the transmission of novel drugs. Assays for drug resistance through efflux transporters, such as permeability glycoprotein (P-gp), have been exhaustively tested with relatively poor predictive power [63]. Trans-endothelial cell electrical resistance (TEER), a measurement of electrical resistance across a monolayer of cells, can also be used to determine overall barrier competency [64]. TEER values in rat brain microvasculature range from 1500-5900$\Omega*cm^2$ [65]; in contrast, human transformed primary BMEC (pBMEC) lines do not achieve TEER above 140 $\Omega*cm^2$ [66]. Far from adequate, most preclinical delivery and efficacy studies are conducted in animals, which have had limited success in modeling human brain pathophysiology.

Microfluidic devices known as organs-on-chips [reviewed in [67]] aim to better mimic organ physiology *in vitro* by mimicking the geometric and functional interactions of relevant cell types. These devices are composed of an upper and lower chamber separated by a flexible porous polydimethylsiloxane membrane. This concept has been adapted by many groups to better recreate the function of the BBB (known as BBB-chip) by seeding multiple NVU cell types in distinct chambers and perfusing with the constant media flow [reviewed in [68]]. The mechanical sheer stress experienced by the vasculature *in vivo* is also of particular interest to BBB-chip platforms which aim improve physiological relevance in culture. Flow

applied to transformed primary BMECs in a microfluidic device has shown to increase tight junction expression and TEER [69] as well as resist elongation, a property distinct to endothelial cells [70]. However, due to the immortalized nature of these cell types, longevity of immortalized cell line-based systems is a major challenge; and even under flow, near-physiological TEER values are not approached. Isolated primary rat BMECs have been shown to increase up to 1298 $\Omega*cm^2$ in a BBB-chip model of metastatic brain tumors [71]; however, these observations were relatively short-lived (up to 3 days). Methods to generate BMECs from iPSCs [72] have shown a superior ability to maintain upwards of 3000 $\Omega*cm^2$ TEER, reproduce known drug permeability in transwells, and resemble the transcriptomic profile of human brain microvasculature. Impressively, iPSC-derived BMECs seeded into a BBB-chip reported a maximum of 4000 $\Omega*cm^2$, stabilizing to 2000 $\Omega*cm^2$ for 10 days [73]. As more groups report human physiological TEER values, there is an increasing need to include additional characteristics of the BBB physiology, both in the natively occurring and diseased state. More relevant modeling of BBB physiology and molecule transport can be assayed live in BBB-chip effluent over time through paracellular permeability assays and mass spec analysis. For example, the inflammatory response to lipopolysaccharide and other cytokines in a BBB-chip was found to elicit unique metabolomic signatures that may shed light on developmental inflammatory disorders [74]. Further adoption of BBB-chip models for use in neurotherapeutic drug development will require accurate validation of a panel of established BBB penetrant and non-penetrant drugs. Due to the proprietary nature of the pharmaceutical development, the assembly of this panel will require academic-industry collaborative discussion. If BBB-chips are indeed sufficiently predictive of BBB function, assays conducted in micro-volumes in human tissue could accelerate future drug development in a cost-effective manner with enhanced accuracy.

Brian Chips

In addition to the opportunity to model BBB function with supplementary cell types, organ-on-chip platforms offer distinct culture parameters and assays to cultivate and evaluate neuronal function *in vitro* (these chips will be referred to herein as Brain-chips). As with barrier function, transgenic animal models of human neurological diseases are lacking due to significant species differences in brain anatomy. Utilizing iPSCs to generate neural tissue from patients with neurological diseases at scale can provide a better avenue with which to screen drugs targeting specific human diseases [75]. However, as mentioned above neurons produced in monolayers in the culture dish often lack developmental maturity [76, 77, 51]. Brain-chips taking advantage of unique co-culture geometries have successfully co-cultured human ES cell-derived neural progenitor cells, endothelial cells, mesenchymal stem cells, and microglia/macrophage precursors, allowing complex interactions reminiscent of the developing CNS [78]. In this particular study, the neural cultures were then assayed through optically clear polyethylene glycol (PEG) or polydimethylsiloxane (PDMS) material for neural function and toxicity, indicating the platform's utility for drug development. These co-culture systems may incite unappreciated maturation effects that are only now being uncovered. As mentioned earlier, organoids also suffer from the maturation roadblock due to the lack of perfusion; this has also been attributed to the development of a necrotic core [31]. Micro-volume conditioning of media under laminar flow has unique mass transport

characteristics that could increase neural development and maturation in Brain-chips beyond what has been reported with the use of spinning bioreactors [31, 25].

Each modeling approach has intrinsic pros and cons (Table 2). Perhaps by combining approaches and allowing monocultures to develop 3D characteristics, the 3D tissue-specific physiology achieved in organoids can be increasingly reproduced in a fluid controlled manner that can be more readily observed compared to dense organoid clusters. It is increasingly apparent that the advantages of self-organization inherent in organoid cultures may be combined with Brain-chip and BBB-chip platforms to enhance organoid growth, provide increased interactions with distinct cell types, and generate an array of assays for personalized disease modeling and *in vitro* tissue generation.

Gut organoids

The intestinal epithelium is composed of a single layer of columnar cells which forms the innermost lining of the intestinal tract. The differentiated cells found in this epithelium are absorptive enterocytes, anti-microbial producing Paneth cells, mucus producing goblet cells and hormone producing enteroendocrine cells. It functions not only as a physical barrier which separates the luminal contents from the host, but it determines intestinal permeability and plays multiple roles in regulating the mucosal immune system [reviewed in [79]]. Given the importance of this tissue, it is not surprising that it is implicated in numerous diseases including inflammatory bowel disease [80], coeliac disease [81], cystic fibrosis [82] and graft versus host disease [83] among others. Despite its fundamental role both in health and disease, studies into this tissue have been hampered given that primary human intestinal epithelial cells rapidly undergo apoptosis when cultured *ex vivo* [84, 85]. To overcome such difficulties, adenocarcinoma lines such as Caco2 cells, have been used extensively to study this tissue [86, 87] but while certainly useful, have limitations in that they do not possess all the various intestinal epithelial subtypes nor do they contain the genetic variants associated with numerous diseases.

A substantial breakthrough in the intestinal epithelial field was reported when it was found that "intestinal" organoids, which contained all the intestinal epithelial subtypes, could be generated from murine intestinal epithelial cells [88]. This process involved harvesting stem cells from the intestinal crypts and subsequently culturing them with pro-intestinal growth factors including various Wnt agonists, noggin and EGF. This system was subsequently adapted such that similar intestinal structures could be generated from human biopsy samples [89, 90]. Interestingly, both groups reported that biopsy derived organoids retained region specific characteristics from where they were originally obtained from. An alternative method for this involved directing human iPSCs to form intestinal organoids by administering various growth factors and small molecules to mimic the developmental cues that occur *in vivo* [91]. These iPSC-derived intestinal organoids also possessed all the epithelial subtypes, but in contrast to biopsy-derived organoids, also possessed mesenchymal cells.

Given that the intestinal epithelium is at the interface between the microbiome and mucosal immune system and is known to be highly influenced by both of these factors [92], there is enormous interest in studying epithelial-microbial and epithelial-immune cell interactions. A

benefit of gut organoid technology is that the milieu in which these organoids grow can be tightly regulated. The sole culture of these organoids may permit an examination of the intrinsic properties of the tissue, while the controlled addition of various microbes and/or immune cells may permit an examination of epithelial-microbial and epithelial-immune cell interactions. However, despite this tantalizing potential, there are substantial technical challenges associated with the aforementioned studies. Firstly, organoid cultures are heterogeneous both in shape and size which will inevitably lead to variability in results. Secondly, organoids, whether derived from biopsy samples or iPSCs, are polarized towards the lumen, therefore requiring that microbes must be microinjected into the lumen of the organoid so as to mimic the *in vivo* paradigm. Thirdly, permeability assays, which are routinely carried out in 2D transwell cultures are technically more demanding in a 3D paradigm. Finally, the feasibility of carrying out immune cell-epithelial interactions is low given that the organoids themselves are embedded in Matrigel.

Gut-chips

While a number of groups have overcome such technical challenges by microinjecting Salmonella enterica serovar Typhimurium [93], rotavirus [94] and Clostridium difficile [95] into the lumen of organoids, one potential way to simplify the process and overcome the previously mentioned barriers is to combine organoid technology with the microfluidic organ-on-chip technology described above. Indeed it was reported that Caco2 cells spontaneously gave rise to villous-like structures similar to those found *in vivo* while cultured under these conditions [96]. A further benefit of the continuous media flow present in these chips is that it permitted the co-culture of Lactobacillus rhamnosus GG (a typical intestinal microbe) for up to 6 days which was substantially longer that the aforementioned studies. Given that these villous-like structures were polarized, microbes and microbial metabolites could be administered to the upper channel which is representative to the luminal aspect of the intestine, while immune cells or cytokines could be added to the lower channel which is representative of the host's aspect of the intestine. Indeed, a recent report showed that the addition of peripheral blood mononuclear cells to the lower channel and the addition of enteroinvasive *E. coli* bacteria to the upper channel together stimulated epithelial cells to produce a number of proinflammatory cytokines that induce villus injury and compromise intestinal barrier function [97]. Furthermore, given that dextran FITC efflux could easily be measured in this system, the intrinsic permeability of intestinal tissue could be measured along with its response to any exogenous pathogen added to the luminal or host side of the chip.

The methodologies to generate intestinal tissue from either biopsies or iPSCs have been paradigm shifting in terms of how the intestinal epithelium can be studied (Table 3). However, given the technical difficulties associated with the organoid structures (Table 4), a move towards combining this technology with microfluidics may make this technology more amenable to study. While there are advantages and disadvantages to using biopsy-derived organoids versus iPSC-derived organoids, one benefit that may accrue from using iPSC-derived organoids is that the parent iPSCs could also be used to generate other cell types such as macrophages [98], dendritic cells [99] and neutrophils [100], all of which are implicated in various intestinal diseases. Given that organoids from either source retain the

host's genetic profile *in vitro,* fusion of these technologies may ultimately lead to significant advances which may usher in a new era of personalized medicine.

Organoid Challenges and Potential Solutions for Personalized Medicine

The organoid field has the potential to rapidly enhance the current state of personalized medicine. As expected, with the conception of any field, novel challenges await and will need to be addressed (Figure 2A). In respect to translating the use of organoids and organs-on-chips for personalized medicine at the population level, these challenges relate to improving the reproducibility of results between groups, establishing standards for characterization of the cell types generated, adding missing components to improve maturation and pathophysiological mimicry, and enhancing the scalability for which organoids can be used for drug testing and regenerative approaches. Below, we suggest possible solutions to address these challenges (Figure 2B).

Reproducibility and Characterization of Organoids

As organoids rely on the inherent ability of cells to self-organize, culture paradigms (frequency of media changes, etc.), which may normally seem mundane, may have a more significant impact in 3D culture (as compared to cells differentiated using morphogens or by the ectopic expression of transgenes). Thus, we propose the following standards to increase consistency between research groups (Figure $2B_1$): 1) The efficiency by which tissues and cell types are generated be accurately reported within the specific organoid and across all the organoids generated for the experiment, 2) Results be independently reproduced by other scientists (or an independent company) and in multiple cell lines, and 3) Visualized experiments (e.g. videos, graphics, etc.) of treatment paradigms, bioreactor assembly, passaging techniques, and methods used for downstream analysis be included.

In respect to an established standard for characterization (Figure $2B_2$), it is imperative that studies quantify cellular markers associated with images in a publication. Additionally, a more accurate characterization would involve analyses at the single-cell level using multiplexed approaches; an example is the combination of single-cell RNA sequencing with other single-cell approaches, such as index sorting [101]. Unbiased proteomic approaches at the single-cell level may also be available in the near future and would provide a much-needed addition to single-cell RNA sequencing. [For a more in-depth analysis of RNA sequencing approaches and integrating Multi-Omic approaches, we direct our readers to [102, 103]].

Pathophysiological Mimicry and Scalability

As discussed above, the addition of other cell types (such as BMECs to mimic the vasculature as well as glial, immune, and epithelial subtypes) to 3D cultures may provide enhanced maturity and accuracy in respect to disease modeling (Figure $2B_3$). The addition of pathogens to gut chips may also increase the pathophysiological capacity of 3D systems. Additionally, applying extracellular matrix components and mechano-stimulation may improve maturation and provide more physiological mechano-transduction (signal

transduction as a result of physical stress); the microfluidic chips discussed herein have made strides in relation to starting to achieve this.

In respect to scaling for personalized disease modeling and regenerative approaches, several critical matters will need to be addressed (Figure 2B$_4$): 1) Banking strategies for 3D cultures to access a heterogeneous population will need to be achieved. Due to the intricate nature of organoids and elongated growth paradigms associated with some protocols (>100 days), cryogenic storage of mature organoids may be challenging. 2) Automating the overall 3D culture process will enhance scalability while also reducing variability. The includes all steps in 3D culture protocols, from the initial patterning of iPSCs, to the seeding/induction of sphere formation, to the construction of scalable bioreactors/chips; the construction of the miniaturized [SpinΩ] 3D-printable bioreactors discussed above demonstrates an example of increasing automation [25, 104]. 3) Scaling of technology for commercialization and wide-spread use; this will include reducing costs, developing GMP conditions and applying clinical grade standards.

Conclusion

In a relatively short period of time, organoids have lent to our knowledge of human-specific development, our ability to more accurately model human diseases *in vitro*, and provide a platform to test therapeutics in human-specific tissue. They also have the potential to limit the use of animal models while also providing modeling systems for diseases which animal models do not exist. However, while excellent for studying neural precursor types, significant improvements in these approaches are necessary to allow for the more physiological study of neuronal function as the stage of development remains rather immature. Brain-chips represent an intriguing complementary technology that might allow for the efficient bridging of multiple cell types in a neurovascular niche-type structure. Gut organoids and chips similarly present tantalizing possibilities but require optimization and continued innovation. Nevertheless, a new era of personalized medicine based on the combination of these technologies with iPSCs and multi-OMICs appears to be dawning.

Acknowledgments

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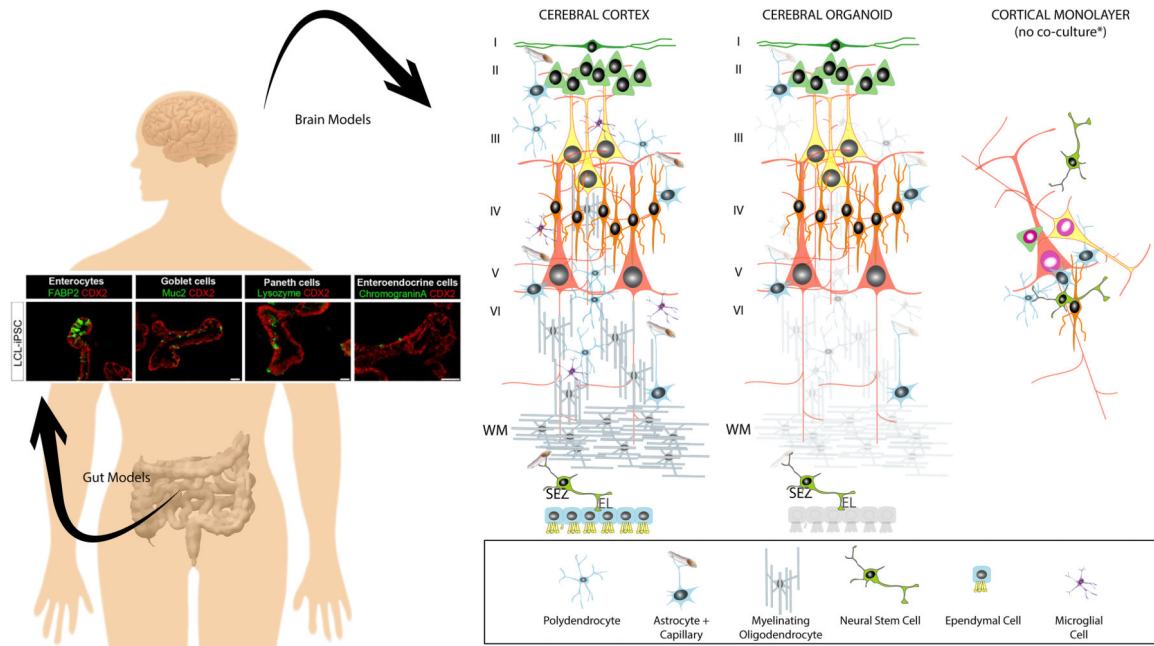
Figure 1. Histogenesis in organoid models

Gut organoids generate appropriate cell types *in vitro* based on the expression of cell type markers, including the intestinal transcription factor CDX2, the enterocyte marker FABP2, the goblet marker MUC2, the Paneth cell Lysozyme label, and the enteroendocrine marker ChromograninA. (LCL-iPSC panel adapted with permission from [105]). Compared with the native cerebral cortex, current cerebral organoid approaches exhibit comparable lamination but lack many of the critical cell types present *in vivo*, including oligodendrocyte lineage cells (polydendrocytes and myelinating oligodendrocytes), vascular cells (endothelial and pericytes), ependymal cells, and microglia. These can be co-cultured in monolayers but monolayer cultures exhibit "stalled" phenotypes when compared with *in vivo* neurons (see text for discussion).
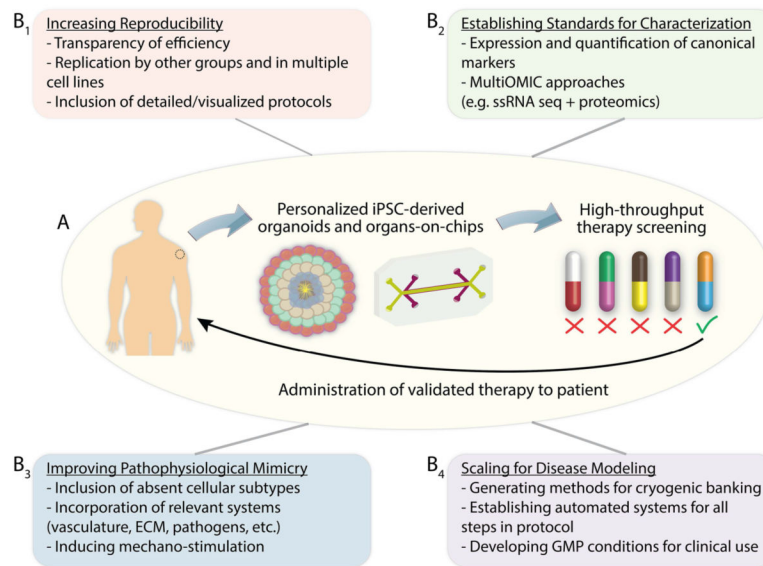
Figure 2. Organoid Challenges and Potential Solutions for Personalized Medicine
Recent advancements in organoid and organ-on-chip technologies have enhanced the efficacy and accuracy of personalized medicine. **(A)** Organoids and organ-on-chips can be used to generate various tissues from patient-specific induced pluripotent stem cells (iPSCs). High throughput screens of candidate therapies can then be carried out on these personalized 3D cultures, allowing the effective therapy to be validated and administered in rapid fashion. **(B$_{1-4}$)** Several sets of challenges await in respect to the widespread use of 3D culture methods for personalized medicine at the population level. We propose plausible solutions to address these challenges.

Table 1

Organoids, and Brain-chip studies for modeling neurobiological systems

	Disease/System Modeled	Mutation/cell type(s) used	Citation
Brain organoids studies			
	Microcephaly	*CDK5RAP2* iPSCs, shRNA knockdown of *CDK5RAP2*	[31]
	ZIKA-induced microcephaly	iPSCs	[25]
	Macrocephaly; cortical folding	PTEN-LOF hESCs	[43]
Brain-Chip studies			
	Model of developing brain for neurotoxicity screening	NPCs, endothelial cells, MSCs, microglial precursors	[78]
	Neurovascular Unit (NVU) Blood Brain Barrier (BBB) model	Endothelial cells, pericytes, astrocytes, cortical neurons	[74]

Table 2

Pros and Cons of animal models, organoids, and monolayer cell cultures for modeling brain development and disease

Animal Models	
PROS	**CONS**
Intact Blood Brain Barrier	Genetic mutations may not give phenotype (eg *C9ORF72*)
Intact immune systems (except for xenograft studies)	Time-consuming breeding and husbandry is needed for conditional genetic manipulation
Allows assessment of behavioral phenotypes	Does not always recapitulate human pathophysiology
Observe secondary phenotypes of diseased cell	Expensive to maintain
Assess systemic influence of disease	Considerable engineering needed to generate germline genetic knockout/ins
Monolayer Cultures	
PROS	**CONS**
Inexpensive	Cannot isolate primary neurons
Ease of use	Neurons differentiated from stem cells *in vitro* lack maturity
Enhanced imaging (including real-time imaging)	Aging-related phenotypes largely not observed *in vitro*
Scalable and higher throughput	Poor longevity (cannot keep cultures indefinitely)
Co-culture simpler when compared with organoids	Cannot generate certain cell types in vitro (eg CSMNs)
Readily Amenable to Genetic manipulation	Cell-line variability
	Deficient cell diversity (no secondary cell types) unless co-cultured
	Varying results according to media/growth conditions
Organoid	
PROS	**CONS**
Cell organization mimics brain	Need spinning bioreactors
Generates distinct cell types not readily generated in vitro (eg CSMNs)	More sample-sample variability
3-dimensional architecture	Increased processing time
Human-specific disease modeling	Regional specificity precludes longer-range targets within organoids (e.g. CSMNs won't find spinal cord in a cortical organoid)
More physiological microenvironment	Many critical cell types not present (vasculature, glia, microglia, oligodendrocytes, etc.)
Tissue architecture promotes enhanced cell maturity	Maturation of neurons hindered by timescale of human development, lack of microglia, lack of regional inputs, myelination, etc.
Electroporation allows for facile genetic lineage tracing in human cells	
Microfluidic Chips	
PROS	**CONS**
Introduce multiple cell types with distinct organization	Difficult to manufacture at scale
Biomarker discovery from effluent	PDMS absorption of molecules (inhibit use of current chip designs for drug screening)
Flow through physiological mediums/blood	Poor cell attachment on chip polymers
	Difficult to compare amongst different designs
	Complex lamination seen in organoids not yet demonstrated

Table 3

Organoids, and Gut-chip studies for modeling gut physiology

	Interaction Modeled	Pathogen used	Citation
Gut organoids studies			
	Host-microbes interactions	Salmonella enterica serovar Typhimurium	[93]
	Host-microbe interactions	Clostridium difficile	[95]
	Host-microbe interactions	Rotavirus	[94]
Gut-Chip studies			
	Host-microbe interactions	Lactobacillus rhamnosus GG	[96]
	Host-microbe-Immune cell interactions	Peripheral blood mononuclear cells and enteroinvasive *E. coli* bacteria	[97]

Table 4

Pros and Cons of animal models, organoids, and monolayer cell cultures for modeling gut development and gastrointestinal disorders

Animal Models	
<u>**PROS**</u>	<u>**CONS**</u>
Widely used-standard robust protocols are in place	Epithelial studies are difficult to interpret due to confounding influence of microbes and mucosal immune system
Various components of IBD (eg innate immune response etc) can modeled in mice	Genetic variations found in humans are not recapitulated in animal models
Role of various genes in IBD can be examined using knockout/in mice	Different housing facilities can influence results by affecting microbial composition
Monolayer Cultures	
<u>**PROS**</u>	<u>**CONS**</u>
Widely used-standard robust protocols are in place	Overwhelming majority of studies have used cell lines which do not contain intestinal epithelial subtypes nor genetic variants of interest
Amenable to permeability experiments	Not amenable to host-microbe interactions
	Not amenable to immune cell-epithelial studies
Organoid	
<u>**PROS**</u>	<u>**CONS**</u>
Organoids contains all the epithelial subtypes	Difficult to carry out permeability experiments
Organoids faithfully mimic the genetic variants found in the host	Difficult to assess host-microbe interactions
Epithelial tissue can be studied in isolation away from confounding influence of microbes and mucosal immune system	Difficult to assess epithelial-immune cell interactions
Microfluidic Chips	
<u>**PROS**</u>	<u>**CONS**</u>
Amenable to permeability experiments	Only cells lines have been cultured in these devices-protocols to utilize organoid tissue in microfluidic chips not yet published
Amenable to host-microbe interactions	
Amenable to immune cell-epithelial interactions	