A Primer on Human Brain Organoids for the Neurosurgeon

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Human brain organoids emerged in 2013 as a technology that, unlike prior in Vitro neural models, recapitulates brain development with a high degree of spatial and temporal fidelity. As the platform matured with more accurate reproduction of cerebral architecture, brain organoids became increasingly valuable for studying both normal cortical neurogenesis and a variety of congenital human brain disorders. While the majority of research utilizing human brain organoids has been in the realm of basic science, clinical applications are forthcoming. These present and future translational efforts have the potential to make a considerable impact on the field of neurosurgery. For example, glioma organoids are already being used to study tumor biology and drug responses, and adaptation for the investigation of other neurosurgery-relevant diseases is underway. Moreover, organoids are being explored as a structured neural substrate for repairing brain circuitry. Thus, we believe it is important for our field to be aware and have an accurate understanding of this emerging technology. In this review, we describe the key characteristics of human brain organoids, review their relevant translational applications, and discuss the ethical implications of their use through a neurosurgical lens.

KEY WORDS: Brain organoids, Brain repair, Disease models, Glioblastoma, Stem cells

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Human brain organoids are a growing technology that has garnered significant attention in the scientific community and public domain. These in Vitro constructs exploit the self-organizing properties of pluripotent stem cells (PSCs) to recapitulate key steps during neurodevelopment, resulting in neural tissues with a surprising degree of similarity to the human brain.[1] Two categories of brain organoids have been generated. Whole-brain organoids, dubbed "mini-brains" by the press, exhibit a variety of cerebral structures, ranging from cortical to choroid plexus to cerebellar tissues.[2] More recent work has established protocols for creating region-specific organoids that model specific brain structures, including the cortex, midbrain, hippocampus, hypothalamus, cerebellum, anterior pituitary, and retina.[3-10] These achievements have raised the possibility that human brain tissue could be wholly generated and studied in the laboratory, free from the constraints of the operating room or autopsy suite. Such a platform could enable study of human neurodevelopment and disease in previously unimaginable ways.

ABBREVIATIONS: EGFR, epidermal growth factor receptor; **ESC,** embryonic stem cell; **ETV2,** ETS variant 2; **GBM,** glioblastoma multiforme; **GEM,** genetically engineered mouse; **iPSC,** induced pluripotent stem cell; **PSC,** pluripotent stem cell

Much of the research involving brain organoids remains in the realm of basic science, but clinical applications utilizing this technology are on the horizon.[11] These applications include modeling neurosurgery-relevant diseases and disorders, such as glioblastoma, and repairing brain circuitry after damage following traumatic brain injury, stroke, and surgical resection. As such, it behooves the neurosurgical community to become familiar with these entities. This review is designed as an introduction for neurosurgeons to the subject of human brain organoids. We will cover recent developments in the field and specifically discuss what brain organoids are and are not. Subsequently, we will review ongoing and future scenarios in which brain organoids are employed for translational purposes. We conclude with a brief

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TABLE 1. Selected Works That Developed and Refined Cerebral Organoid Technology

Study	Significance
Kadoshima et al, 2013[3]	Grew cortical epithelium from human embryonic stem cells, producing self-organized, semispherical structures that developed intracortical polarity, curving morphology, and zone separations consistent with in Vivo corticogenesis. Also observed human-specific progenitors resembling outer radial glia. Thus, provided early evidence that human corticogenesis involves intrinsic programs that can be recapitulated in Vitro.
Lancaster et al, 2013[2]	Developed the whole-brain cerebral organoid. Demonstrated presence of interdependent brain regions, including cortex. Modeled microcephaly using organoids derived from patient-derived iPSCs, validating cerebral organoids as a powerful tool for studying brain disorders.
Qian et al, 2016[5]	Developed protocols for growing region-specific brain organoids, including forebrain, midbrain, and hypothalamic phenotypes, using iPSCs and a spinning bioreactor that reduced cost for upscaling organoid generation. Used forebrain organoids to study Zika virus exposure, showing preferential infection of neural progenitors and decreased organoid growth resembling microcephaly.
Paşca et al, 2015[38]	Developed a streamlined method for growing cortex-like tissue from iPSCs that, unlike prior protocols, did not require plating onto coated surfaces, embedding into extracellular matrices, or culture in complex environments. "Human cortical spheroids" generated by this method contained astrocytes in addition to layered neurons.
Birey et al, 2017[29]	Introduced methods for growing "human subpallial spheroids," which were patterned toward a ventral forebrain fate. Also described methods for combining them with human cortical spheroids (Paşca et al[38]), which resembled dorsal forebrain, to create "forebrain assembloids" that were useful for modeling interactions between glutaminergic and GABAergic neurons.
Mansour et al, 2018[84]	Transplanted human brain organoids into the adult mouse brain to provide a more physiological microenvironment for modeling purposes. Organoid grafts were vascularized and continued to survive/mature in Vivo. Electrophysiological studies showed intragraft neuronal activity and suggested graft-to-host functional integration.
Velasco et al, 2019[23]	Characterized cell compositions of forebrain organoids via single-cell RNA sequencing. Demonstrated remarkably low variability in cell type distribution among organoids that persisted over time. Showed that organoids could reproducibly capture the brain's cellular diversity despite organoid-to-organoid morphological variability, further supporting their use as brain models.

discussion of ethical issues related to brain organoids. It is our hope that this material sparks additional engagement of neurosurgeons with the burgeoning and highly promising field of human brain organoids.

A BRIEF HISTORY OF HUMAN BRAIN ORGANOID DEVELOPMENT

Organoids are defined as collections of organ-specific cells derived from stem cells that self-organize in a manner similar to in Vivo development.[1] Efforts to generate brain organoids were motivated by the perceived inadequacies of prior in Vitro cellular techniques for modeling the brain. Aggregates of PSCs, often called neurospheres, lacked architectural features of the developing brain.[12] Differentiation of PSCs along neural lineages succeeded in inducing the spontaneous radial organization of cells into characteristic developmental structures such as neural rosettes. However, these planar techniques did not capture the complex interplay of cells within a 3-dimensional environment.[13] Several developments in cellular biology paved the way for what we currently know as brain organoids.[3] First, steady sources of PSCs became more readily available with the advent of fetus-derived embryonic stem cells (ESCs) and then induced pluripotent stem cells (iPSCs) created via the de-differentiation of adult somatic cells.[14,15] Second, techniques were optimized for using PSCs to form embryoid bodies, large multicellular aggregates that possessed similar developmental potential to that of an embyro.[16] Lastly, efficient protocols for producing neurons from PSCs were distilled.[17,18]

The modern era of human brain organoids began in 2013 with descriptions of cortical organoids with stratified cortical neuroepithelium by Sasai and colleagues[19] and whole-brain organoids by Lancaster et al and Kadoshima et al[2,3] (Table 1). The former represented the culmination of efforts to use patterning factors to engage specific differentiation programs in embyroid body-like cellular aggregates.[20,21] Remarkably, these cortical organoids recapitulated the spatial aspects of neurodevelopment in Vivo. The organoid neuroepithelium formed distinct layers found in the developing brain, including the ventricular, subventricular, and intermediate progenitor zones as well as the subplate, cortical plate, and Cajal-Retzius neuronal cell layers.[10] In contrast, whole-brain organoids relied upon default intrinsic differentiation pathways to generate multiple heterogeneous brain regions reminiscent of the forebrain, midbrain, and hindbrain within one organoid unit.[2] Additional structures such as the meninges and choroid plexus were also observed, and areas consistent with the lobes of the cerebral cortex could be identified.

The next wave of studies focused on enhancing the architectural fidelity of cortical organoids, using techniques ranging from custom spinning bioreactors to polymeric cores for shaping the organoids and dissolved extracellular matrix proteins as media

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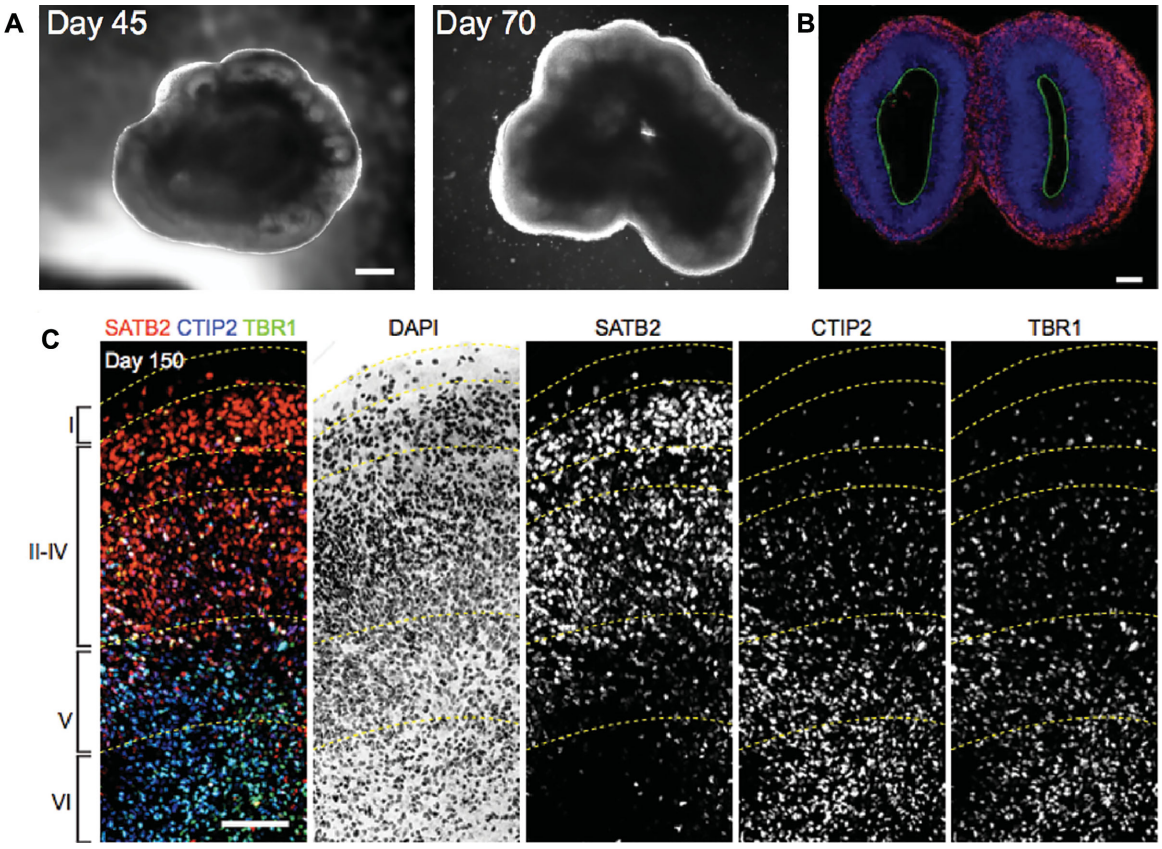

FIGURE 1. *Structure of human brain organoids.* **A**, *Bright-field images of cerebral organoids. Reprinted from Cell Stem Cell, in press; corrected proof available online March 5, 2020, Qian et al,*[104] *Copyright 2020, with permission from Elsevier.* **B**, *A forebrain organoid with 2 cortical structures. The ventricular surface is deliniated by staining for adherens junction marker (green). Reprinted by permission from Copyright Clearance Center: Springer Nature, Nature Protocols, Qian et al,*[49] *copyright 2018.* **C**, *Immunostaining confocal images of an organoid with cortical neuron markers SATB2, CTIP2, and TBR1. The distribution and layer-dependent expression patterns of markers resemble the layers I-VI in human late gestation embryonic cortex. The fluorescent image for DAPI was inverted to white background to help visualize the nuclei density in each layer. Reprinted from Cell Stem Cell, in press; corrected proof available online March 5, 2020, Qian et al,*[104] *Copyright 2020, with permission from Elsevier.*

components.[5,22] These studies demonstrated further maturation of neuroepithelial layers. In particular, the cortical plate began exhibiting rudimentary laminar structure with segregation of upper (layers II/III) and lower (layer V) layers in the proper temporal sequence[23] (Figure 1). Also observed was the generation of distinct populations of outer radial glial cells, which are hypothesized to be crucial for the increased cortical size and complexity seen in human cortex.[5,24,25] Again mirroring normal developmental, astrocytes and possibly oligodendrocytes appeared at later time points.[26,27]

There has been growing interest in creating region-specific organoids with cell types other than glutamatergic cortical neurons. Few interneurons are found in standard cortical organoids as they originate from the ventral ganglionic eminences rather than the ventricular and subventricular zones. Organoids containing predominantly GABAergic neurons have been generated using protocols that ventralize neural fate and have proved useful in studying interneuron migration patterns.[28-30] Exploiting knowledge of other normal developmental programs has led to creation of a host of additional region-specific organoids, including the midbrain,[4] hippocampus,[6] anterior pituitary,[31] hypothalamus,[5] and cerebellum.[8]

WHAT IS A BRAIN ORGANOID NOT?

Despite these achievements, it is important to keep in mind what human brain organoids can model and what they cannot, as well as their limitations as a model system. As a blunt reminder, brain organoids are not actual human brains, especially in the domains of size, architecture and cellular composition, maturity, and activity. The limits of diffusion constrain organoid growth to a maximum of 3 to 4 mm, after which necrosis within

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the organoid core prevents further growth.[32] Bioreactors that promote media agitation and hyperoxic conditions somewhat mitigate the problem of mass transport,[2,3,5] but growth of organoids beyond current size limits will require microfluidic systems and similar technologies to promote organoid perfusion or transplantation into host animals that function as in Vivo bioreactors.

Many aspects of the structure and composition of brain organoids differ significantly from the brain itself. At the most superficial level, brain organoids do not possess gyrencephalic folds, although introducing PTEN deletions leads to surface contortions reminiscent of folding.[33] Moreover, architecture within a brain organoid is often distorted, as evidenced by the finding of multiple hemisphere-like cortical zones. Most research efforts focus on region-specific brain organoids, as opposed to whole-brain organoids, to minimize interorganoid variability. A byproduct of this approach has been the simplification of organoid structure and loss of interactions among different brain regions. Fusing together different types of organoids and engineering axon tracts may restore some systems-level complexity, but these strategies remain to be explored more fully.[28-30,34] Finally, brain organoids lack microglia, and other immune cells, as current protocols direct cellular differentiation along exclusively ectodermal pathways. Classically, they have also lacked endotherial cells but recently embryonic stem cells have been engineered to ectopically express human ETS variant 2 (ETV2) that contributed to forming a complex vascular-like network in organoids.[35]

Separate from organoid structure is the issue of organoid maturity. Studies demonstrate a high degree of genomic similarity between current brain organoids and human fetal brains through the end of the second trimester.[36,37] Further maturation, especially to postnatal time points, has not yet been achieved, which brings into question the suitability of using brain organoids to model disorders that arise in adolescence and adulthood.

Related to the maturity of organoids is the complexity of their neural activity. Slow neuronal calcium waves, postsynaptic potentials, and induced action potentials have been reported in brain organoids.[2,5,38,39] Only 2 studies of spontaneous action potentials have been reported.[39,40] Data supporting the formation of neural networks within brain organoids are limited. Stimulation of light-sensitive retinal cells in whole-brain organoids attenuates the activity of a subpopulation of distant neurons, indicating interdependency of neural activity, but there has been no direct evidence of communication across multiple network nodes.[39] A recent study reported synchronized bursting of organoid neurons and purported nesting of delta and gamma activity.[40] However, these recordings were performed after partially dispersing organoids on a planar microelectrode array, and the relevance of the recorded activity (eg, "nonoscillatory gamma activity") to normal oscillations in the brain is not clear. Generally speaking, there is the larger question of what degree of complexity of neural activity can be achieved in current brain organoids, given that most second-trimester human neurons in Vivo are immature with a limited capacity for spontaneous firing.

MODELING NEUROSURGERY-RELEVANT DISORDERS WITH BRAIN ORGANOIDS

Even with the above caveats, brain organoids are appealing model systems for investigating the human brain and its disorders (Figure 2). The principles of self-organization that enable brain organoids to arise in Vitro mirror the same drivers of normal neurodevelopment in Vivo.[41] Thus, organoids lend themselves as useful models for human-specific neurodevelopment. Their 3-dimensional architecture and multiple cell types provide significant advantages over planar cultures of dissociated neurons. Finally, the human origin of brain organoids bypasses problems with animal models arising from species differences.

Brain organoids have already proven themselves to be quite valuable in the study of cortical neurogenesis and modeling of congenital human brain disorders[2,25,42-45] (Table 2). Perhaps most notably, brain organoids played a key role in elucidating the pathogenesis of Zika virus infections and associated cases of microcephaly.[5,46-50] Attempts have been made to model other diseases with brain organoids, including autism spectrum disorder, schizophrenia, other neuropsychiatric diseases, and neurodegenerative disorders such as Alzheimer disease.[51,52] Additionally, the possibility of employing brain organoids for the purposes of high-throughput drug testing, development, and validation has been suggested.[33,53,54] The progress and challenges of these various applications have been covered in a number of excellent review articles and will not be discussed further here.[1,11,55-57] Instead, we will explore an area in which brain organoid modeling has the potential to make a significant impact in neurosurgery: glioblastoma multiforme (GBM).

Glioma Organoid Models

Glioblastoma is thought to arise from glioma initiating cells capable of self-renewal and multilineage differentiation.[58] These precursors were first isolated nearly 20 yr ago, demonstrating pluripotency and clonal expansion in culture.[59,60] However, neurospheres and planar cultures of these stem cells do not fully recapitulate the heterogeneity and 3-dimensional cellular architecture of glial tumors.[61] Mouse xenograft models better maintain key molecular and histologic characteristics of human glioblastoma but are less suitable for studying tumor initiation and are limited by inherent differences between human and murine brain cells, variability in tumor latencies, and restrictions to real-time experimental manipulation.[62] Genetically engineered mouse (GEM) models provide insights into genetic alterations and disruptions in key signaling pathways responsible for tumor initiation and progression, but their development is both time-consuming and expensive and they fail to capture the intratumoral heterogeneity of GBM.[63]

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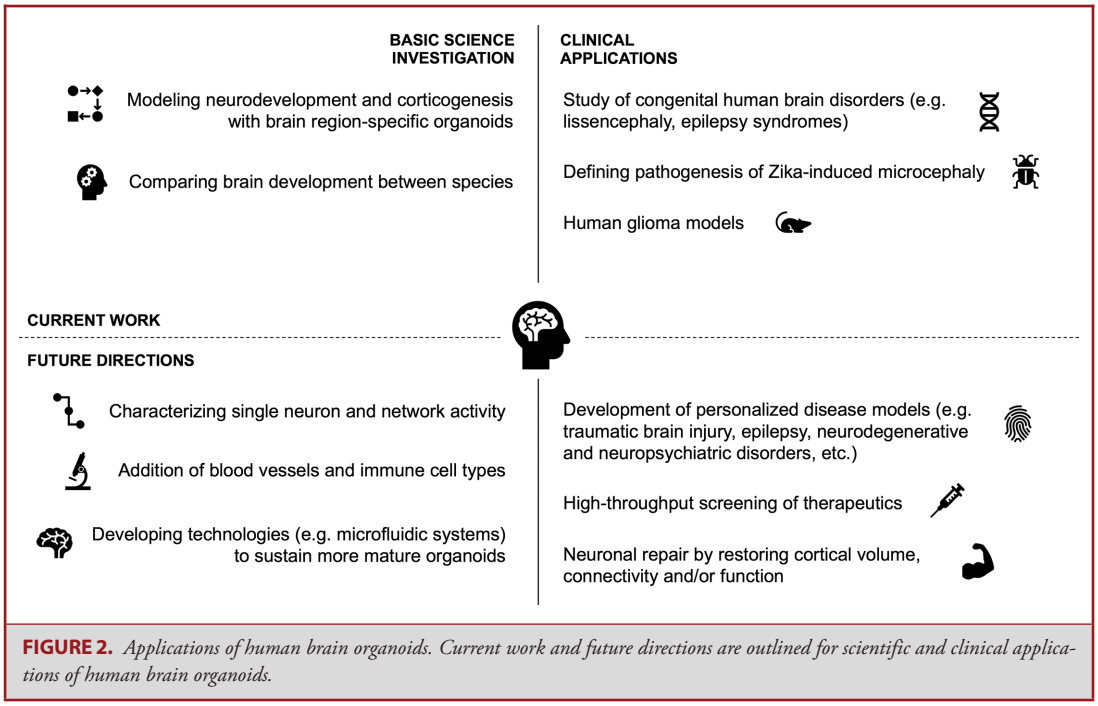

FIGURE 2. *Applications of human brain organoids. Current work and future directions are outlined for scientific and clinical applications of human brain organoids.*

TABLE 2. Selected Works That Applied Cerebral Organoid Technology to the Study of Disease

Study	Significance
Mariani et al, 2015[45]	Applied cerebral organoids to the study of autism spectrum disorder (ASD). iPSCs from idiopathic ASD patients were cultured into organoids and studied for neurodevelopmental aberrations. Notably, they displayed overproduction of GABAergic neurons, and overexpression of FOXG1 was found to cause this phenomenon. Demonstrated the utility of organoids in studying disease pathophysiology.
Cugola et al, 2016[50]	Provided direct in Vivo and in Vitro proof that Brazilian ZIKV caused birth defects. Key experiment using human brain organoids showed that infection resulted in suppressed proliferative zones and disrupted cortical layers
Bian et al, 2018[65]	Applied cerebral organoids to the study of brain tumors. Generated and characterized neoplastic brain organoids resembling CNS-PNET and GBM via CRISPR/Cas9-mediated mutagenesis. Demonstrated variance in invasiveness and response to therapy based on mutation profile, validating organoid use in studying tumor biology and drug screening.
Jacob et al, 2020[103]	Generated a biobank of glioblastoma organoids (GBOs) cultured from fragments of resected glioblastomas. GBOs demonstrated retention of cellular and mutational diversity of parental tumors and were amenable to cryopreservation, transplantation, and therapeutic testing with pharmacological and cellular therapies. GBOs were thus useful for investigating patient-specific treatment strategies, and the biobank presented a valuable resource for basic and translational GBM research.
Bhaduri et al, 2020[71]	Demonstrated that GBMs contained a stem cell subpopulation resembling outer radial glia (oRG). In a key experiment, oRG-enriched tumor cells were transplanted into cortical organoids and were found to engraft and invade. Use of organoids enabled isolation of transplant-derived cells via fluorescence activated cell sorting, and subsequent transcription analysis showed expansion of a diverse array of cell types present in the original tumor.

Gliomas organoids are 3-dimensional in Vitro tumor models that are generated using the same principles and protocols as brain organoids. Without the constraints of animal hosts, organoid models retain the biological relevance (specifically intratumoral heterogeneity and invasive phenotypes) and experimental manipulability necessary for study of tumor pathophysiology and high-throughput screening of patient-specific therapeutics.[64] Thus far, glioma organoids have been generated directly from patient tumor samples or via transposon- and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas)-mediated mutagenesis of standard brain organoids and are thus genetically modified organoids.[65,66] The latter approach has

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been used effectively to study GBM tumorigenesis with shorter timeframes than GEM models. Specific combinations of genetic alterations induced either GBM- or PNET-like neoplasms in brain organoids by histologic and transcriptional analyses.[65]

Tumor heterogeneity is remarkably maintained in glioma organoids derived from cut pieces of patient tumor samples.[67] A combination of histologic, transcriptomic, and exomic data demonstrated significant similarity between glioma organoids and parent tumor samples. Within a single glioma organoid, a broad variety of cell types were identified, mirroring the parent tumor. Interestingly, glioma organoids derived from different geographic regions of the same tumor exhibited a great deal of interorganoid variability, suggesting that a single organoid line is not sufficient to understand the global biology of GBM.

Glioma organoids offer novel approaches for assessing the influence of the tumor microenvironment. Tumor organoids organized in response to resource diffusion in culture. Cells with a higher proliferative index and more stem-like characteristics were located more densely near the organoid periphery, closer to oxygen, nutrients, and growth factors, while the organoid core was comprised of lineage committed, quiescent cells.[68] The microenvironment engendered in 3-dimensional tumor tissue is critical for recapitulating features of human disease in culture.[69] While glioma initiating cells grown in planar cultures were susceptible to alkylating agents and ionizing radiation, tumors derived from invasion of these cells into brain organoids demonstrated significant treatment resistance.[69]

Organoid models provide a unique platform for assessing cellular mechanisms of glioma infiltration. Mutagenesis experiments in normal brain organoids show that the organoid is almost entirely overtaken by tumor cells, which display a highly proliferative and invasive phenotype by histology and an expression profile similar to the mesenchymal subtype of GBM.[66] Co-culture techniques between glioma initiating cells or glioma organoids and standard brain organoids have been used to study the dynamics of GBM infiltration.[69-71] This approach helped build support for the idea that GBM infiltration is facilitated by networks of tumor microtubules.[69] Transplantation of glioma organoids either into the kidneys or brains of immunodeficient mice resulted consistently in invasion of the host animal on the order of a few months.[65,67,68] Infiltrative cells exhibited greater expression of invasion-related genes.[65]

Lastly, glioma organoids show promise for screening and testing targets for therapeutic intervention. The efficacy of specific chemical compounds has been tested on predefined genetic aberrations introduced into organoids.[65] Organoids that demonstrated epidermal growth factor receptor (EGFR) overactivation were susceptible to afatinib, an EGFR inhibitor. Modification of an organoid system to include fluorescent measurements of tumor size enabled simultaneous comparison of the efficacy of several novel EGFR inhibitors on tumor growth. Glioma organoids also have been adapted as a method for testing targeted therapeutics, including chimeric antigen receptor (CAR) T-cell immunotherapy.[67] The generation of glioma organoid biobanks, which mirror efforts for other cancers, could facilitate more effective screening, testing, and implementation of personalized treatment in GBM.[72]

Prospects for Modeling Other Neurosurgery-Relevant Diseases

As organoid technology progresses, other disorders in neurosurgery will likely become amenable to modeling with brain organoids. Traumatic brain injury is as much a problem of axonal damage as it is of neuronal soma injury. Models that build upon recent work developing more robust axon tracts from brain organoids will enable injury of both grey and white matter structures to be investigated.[34,73] In Vitro devices that have been employed for injuring cultured cells provide a natural starting point for such endeavors.[74] Congenital epilepsy syndromes (eg, Miller-Dieker Syndrome[25] and Rett Syndrome[75]) have already been modeled using brain organoids. However, these studies have focused on molecular and cellular derangements without examining changes in electrical activity. Generation of organoid-based epilepsy models will require better understanding of organoid activity at both the single cell and network levels and how this activity changes with experimental manipulations. Inclusion of vasculature, associated blood-brain barrier characteristics and immunological features in future iterations of organoids will create opportunities for investigating neurovascular diseases.[76,77] While these applications are all bound by the limitations of brain organoids enumerated above, they represent exciting possibilities for acquiring new insights into neurosurgical diseases and developing novel interventions for these problems.

ORGANOIDS AS SUBSTRATES FOR REBUILDING BRAIN CIRCUITRY

Injury to the brain from stroke, traumatic injury, and other similar conditions often leads to a wide range of clinical deficits. The high burden of disability in neurological injury is largely due to the limited repair capacity of the adult brain. Processes of brain regeneration exist, including plasticity, neurogenesis, and axon regeneration,[78-80] but these mechanisms are often not robust enough to restore meaningful clinical function. There remains a critical need to develop novel approaches for brain repair. Reconstruction of the brain using new neurons remains one of the most promising strategies.

While the majority of neural transplantation studies, including recent human clinical trials, have relied upon injections of dissociated cells, some of the most compelling transplantation data come from the fetal cortical graft literature.[81,82] These studies showed that pieces of fetal cortical tissue inserted into the cortex of host animals projected axons widely into the animal's brain and adopted higher order network function such as limb motor activity and receptive fields in visual cortex.[77-81]

Ethical concerns associated with obtaining human fetal tissue have precluded translation of fetal cortical grafts. Brain organoids

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are an intriguing alternative that offers many of the same benefits as fetal grafts, particularly brain-specific architecture. Whole-brain organoids transplanted into the cortex of adult mice maintain their structural features with rapid ingrowth of host vasculature, superior to that observed with transplanted dissociated neural progenitor cells.[83,84] Organoid grafts send widespread projections into the host brain with evidence of synaptic formation and functional integration with local cortex.[84] Although a variety of repair paradigms are conceivable from various region-specific organoids, the few published reports of brain organoid transplantation have described only cortical grafts.[76,83,84]

Future clinical applications of organoid transplantation could come in a number of forms. Organoids could be inserted into the cortex as supplementary cortical columns, increasing local computational capacity in the case of cortical volume loss, as has been observed after traumatic brain injury.[85-88] Alternatively, transplantation could replace lost neuronal tissue in parenchymal cavities caused by stroke, hemorrhagic contusions, or surgical resection. Organoid-derived axon tracts could act as "jumper cables" to rewire areas of the brain that had lost connectivity.[34,73,76] Transplantation of autologous tissue may not be financially or logistically feasible, but patient-matched organoids that alleviate the issues of immune rejection could be derived from "universal" iPSC lines[89] or pre-established, patient-matched lines.[90,91] A final point to make is that iterative refinements of organoid technology will be necessary before translation along the above lines is clinically feasible.

ETHICAL CONSIDERATIONS FOR BRAIN ORGANOIDS

The concept of brain organoids has evoked ethical debate, conjuring the image of a "brain in a vat"—a disembodied entity capable of thought and perception but trapped in a dehumanizing existence.[92,93] At present, brain organoids are far from the functional sophistication of the human brain, but this may not always be the case. Given the rapid evolution of organoid technology, considering the ethical implications is warranted. Two interdisciplinary workshops sponsored in part by the National Institutes of Health were held in May 2017 and March 2018 to explore, in part, the ethics of using brain organoid models.[94] A number of issues have been examined including the moral status of brain organoids, the generation of chimeric animals through organoid transplantation, and issues pertaining to social-legal governance of organoids.

The moral status of brain organoids is contentious. Depending on the moral status of a research entity, ranging from the status granted to nonhuman cells to that granted to human beings, investigators must ensure sufficient research protections for that entity. This topic is highly controversial in reproductive and regenerative medicine, with extensive discussion on the criteria used to delineate the moral status of embryos in human research.[95] Historically, moral status has been attributed to human embryonic cells based on 3 characteristics: human origin, potential to generate human beings, and potential to feel pain or develop rational decision-making abilities. These principles have been applied to brain organoids.[96] It has been argued that brain organoids have moral value simply because they are of human origin, which would afford them some degree of legal protection.[96,97] Whether brain organoids deserve moral status because of their perceptual or cognitive potential is an interesting and challenging question. It is highly unlikely that current brain organoids possess such brain functions given their small size and limited functional activity. However, methods for detecting such function and defined thresholds of concern remain underdeveloped. The potential development of sentience in organoids is viewed as particularly problematic.[98] Traditional paradigms of human consciousness have been used to assess which levels of consciousness within brain organoids would require moral consideration, but this strategy remains controversial as there is no consensus understanding of consciousness and ranking forms of consciousness is a matter of opinion.[93,99]

Transplantation of brain organoids into animals adds an additional layer of complexity to these questions. There is inherent difficulty in quantifying the threshold at which point chimeric animals are "humanized". It may be more instructive to examine the ethical implications of enhancing specific brain functions, such as vision, learning capacity, or self-awareness, and how these enhancements reframe the moral status of chimeric animals.[100,101] Others have suggested borrowing concepts and tools from the study of disordered consciousness (eg, coma and persistent vegetative state) to assess the moral significance of organoid models.[92,93] Regardless of which approach is taken, further discussion among scientists and engagement with the broader public will be required.

For now, most experts argue for a pragmatic approach toward ethical use of organoid technology. All experiments that involve the engraftment of human cells into animals are expected to adhere to the ethical standards for animal research, including minimizing suffering in animal hosts while monitoring for unexpected changes in behavior or cognition. Going forward, it will be important to consider whether creating chimeras from human brain organoids alters our traditional perception of stewardship, ownership, and postresearch handling of these animals.[94] Moreover, there are legitimate questions about how consent processes for the acquisition of human cells and tissues might need to be adjusted to take into account the implications of brain organoid research and biobanking of these entities.[102,103]

FUTURE DIRECTIONS

The future of brain organoids holds exciting promise across multiple domains. As a platform, they have already been valuable in the study of cortical neurogenesis and modeling of brain disorders. Further work will continue to broaden

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understanding human-specific normal and aberrant neurodevelopment. Additionally, if true presence and increasing complexity of neuronal networks is achieved, it will enable analysis and experimental manipulation of neural networks in ways previously not possible.

In the field of brain tumors, continued organoid work holds hope for a better understanding of tumorigenesis, infiltration, and progression, as well as a platform for high-throughput screening of patient-specific therapeutics. Another exciting avenue of brain organoid research is their potential as a novel approach for brain repair—a field which is currently desperately lacking. Organoids could be inserted as supplementary cortical columns to increase computational capacity after traumatic brain injury, or could be used to replace lost neuronal tissue after stroke, contusions, or surgical resections. While all of these domains will require continued iterative refinements before clinical translation, they hold much promise for the future of this exciting technology.

CONCLUSION

Brain organoids have created much enthusiasm in the scientific community as novel 3-dimensional platforms for modeling human brain development and disease and developing personalized therapies. These applications are beginning to filter into our field of neurosurgery. Using brain organoids and their derivatives as structured neural tissues for brain repair is a particularly exciting prospect that falls squarely in the domain of neurosurgeons. Overall, brain organoids hold much promise for many avenues of research, but much work is still needed to bring these translational indications to fruition. Ongoing ethical discussions also will be imperative as the field progresses forward.

Disclosures

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