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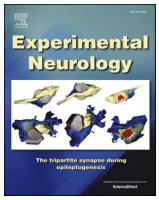

Research Paper

A review on current brain organoid technologies from a biomedical engineering perspective

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ABSTRACT

Brain organoids are 3D cytoarchitectures resembling the embryonic human brain. This review focuses on current advancements in biomedical engineering methods to develop organoids such as pluripotent stem cells assemblies, quickly aggregated floating culture, hydrogel suspension, microfluidic systems (both photolithography and 3D printing), and brain organoids-on-a-chip. These methods have the potential to create a large impact on neurological disorder studies by creating a model of the human brain investigating pathogenesis and drug screening for individual patients. 3D brain organoid cultures mimic not only features of patients' unknown drug reactions, but also early human brain development at cellular, structural, and functional levels. The challenge of current brain organoids lies in the formation of distinct cortical neuron layers, gyrification, and the establishment of complex neuronal circuitry, as they are critically specialized, developmental aspects. Furthermore, recent advances such as vascularization and genome engineering are in development to overcome the barrier of neuronal complexity. Future technology of brain organoids is needed to improve tissue cross-communication, body axis simulation, cell patterning signals, and spatial-temporal control of differentiation, as engineering methods discussed in this review are rapidly evolving.

1. Introduction

The human brain is a complex and delicate organ, making the development of a 3D model a challenging engineering problem. There are several controlled neurological mechanisms of communication among cellular types. Disruptions of any cells link to structural and physiological malfunction of the brain. Molecular, cellular, and anatomical mechanisms of human brain development have been studied by standard model organisms (Adams and Kafaligonul, 2018). However, the human brain is vastly different and unique compared to animal model brains, in terms of cellular and genetic makeup, along with processing abilities (Zhao and Bhattacharyya, 2018). Scientists are currently developing technology for specific human brain functions in diseases and disorders; and developing subsequent patient treatment strategies.

Traditional 2D cell line research has several limitations, including the absence of hierarchical structure, no dimensionality, lack of cellular diversity, and cell–cell or cell–matrix interaction failure (Edmondson et al., 2014) (Tang et al., 2022). Isolated animal tissue studies may collect relevant cell organization and interaction data, yet maintenance is difficult for long time periods *in vitro*. Tissue explants may periphrastically collect physiological cellular interactions, with expedited phenotypic disappearance. These systems rarely have any patient-customization options, in contrast to organoid models.

Methods of generating neural tissue in 3D are expanding rapidly to overcome the limitations of 2D culture systems (Andersen et al., 2020; Edmondson et al., 2014). One type of traditional cell system is the neurosphere, a 3D aggregation of several central nervous system (CNS) cell types, derived from neural progenitor cells (NPCs) (Binder et al., 2015). They contain various subtypes of neurons and glial cells. However, neurospheres lack clear organization and cytoarchitectures (Pamies et al., 2017). Additionally, the serum-free floating culture of embryoid body-like aggregates with quick re-aggregation (SFEBq) protocol has been used to generate the more complex architectures, such as sub-brain regions of the cerebral cortex and pituitary (Lancaster et al., 2013; Shevde and Mael, 2013). 3D brain organoid cultures, on the other

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hand, model patients' unknown drug reactions, early human brain development at cellular levels, creating a more efficient treatment plan (Seo et al., 2022). As single organoids are designed, several may be combined to produce a culture system of heterogeneous neural organoids, despite exhibiting limited continuity and expansion of neuroepithelial tissue. The key elements of the 3D cerebral organoid have been recently developed, such as utilizing pluripotent human stem cells, quick re-aggregation, improving model structure with biomaterials or microfluidic devices, sensor implications, brain organoids-on-a-chip, and 3D bioprinting strategies. However, the standard model scale is not practical for high throughput projects, as the new technology is not cost effective and requires specialized maintenance. Therefore, microfluidic devices, floating culture, and Matrigel suspension can benefit the technique for usage in large-scale research and improve its reproducibility (Ao et al., 2021).

Human pluripotent stem cells (hPSCs), human embryonic stem cells (hESCs), and human induced pluripotent stem cells (hiPSCs) are an encouraging advancement to modeling human brain disorders (Sun et al., 2021). hPSCs, the foundation of brain organoids, have capabilities of mimicking any cell type found in the human body under the appropriate conditions. hPSC-derived models self-assemble to form an organized architecture which are composed of progenitors, with neuronal and glial cell types, suspended in a Matrigel environment. Unlike two-dimensional cell cultures, brain organoids recapitulate the human brain; not only at the cellular level but can also create an entire system of general tissues commonly seen in the brain (Andersen et al., 2020; McDevitt, 2013). Brain organoids offer researchers a valuable tool to investigate brain disorders and the intricacies of brain development. The brain organoids' developmental trajectory is a unique model of development and function.

2. Human stem cell derived brain organoids

hPSCs have capabilities of differentiating any cell type found in the human body under the appropriate conditions. Early prototypes of the 3D brain organoid were generated by combining astroglia with other neuronal types (McDevitt, 2013). These astroglia may be further differentiated into an organized architecture, composed of progenitors of neuronal and glial cell types, suspended in a Matrigel environment, similar to neurospheres with more effective organization. The resulting original brain organoids recapitulated the human brain at the cellular level as an accurate system of brain tissues (McDevitt, 2013). Brain organoids originally using hPSCs are closely related with the concept of spheroids.

Spheroid models are cultured *via* astrocyte lineage cells in a three-dimensional cytoarchitecture, with a substructure of human cerebral cortical spheroids (hCSs) derived from pluripotent stem cells (Sloan et al., 2017). Astrocytes have collocation with neuronal synapses, allowing interstitial fluid control, neuronal energy substrate supply, biosynthesis precursors, neurotransmitter recycling, and scavenger oxidation (Weber and Barros, 2015). These vital functions provide the basis of spheroid structure, later developed into brain organoid study, as spheroids group together to form the initial type of brain organoid. From the neurosphere, a structureless cluster of neural stem cells, to spheroids, brain organoids have evolved at an exponential rate. Currently, brain organoids are derived by a single embryoid body alone, without the use of astroglia. Single embryoids are now formed by dissociating hPSCs, which self-assemble into the organoid structure. There are several current brain organoid development methodologies.

2.1. Quickly aggregated floating brain organoid culture approach

Serum-free floating culture of a single embryoid, containing body-like aggregates with quick re-aggregation, (SFEBq) has been used to generate more complex architectures, such as the cerebral cortex and pituitary (Shevde and Mael, 2013). As single organoids are designed for SFEBq protocol, several may be combined to produce a culture system of heterogeneous cerebral organoids. This method has been described to exhibit limited continuity and expansion of neuroepithelial tissue, in contrast to its ease of multi-organoid capability. First, induced pluripotent stem cells (iPS) are dissociated and implanted in a low-adhesion 96-well plate, with standard culture media. Aggregation is then performed and neuroectoderm is added to increase growth of central nervous system neurons and glia, demonstrated in Fig. 1 below. After aggregation, hypothalamic progenitor and several genetic transcription factors are added with an induced signal to create the three-dimensional floating culture. Extracellular matrices may support the organoid if needed, depending on experimental conditions set by the research type.

2.2. Biomaterials: Matrigel and hydrogel approaches

Animal-derived extracellular matrices are largely used as a support material in 3D organoids. Matrigel is a type of support enriched in various growth factors, laminin, and type 4 collagen, enhancing the self-assembling capacity of the iPSC and facilitate neuronal differentiation (Cruz-Acuña et al., 2018). However, immunogenicity is a major setback in clinical application of animal-derived matrix-assembled brain organoids (Serban et al., 2008). Due to a lack of control over the mechanosensitive signals and composition, cells often assemble into heterogeneous organoids in terms of viability, shape, and size.

To create the Matrigel using this approach, hPSCs are cultured in hES media containing low bFGF, basic fibroblast growth factor; for approximately 6 days. At this point, embryoid bodies start to form due to the self-assembling capabilities mentioned. Cells are collected and placed into a micro centrifuge tube with neural induction media. This allows the embryoid bodies to clump and form what is known as a neuroectoderm. After 5 days, the media containing neuroectoderm is carefully transferred to a Matrigel droplet in a petri dish containing differentiation media, where the cells will be allowed to form as a premature organoid for 4 days. After cells have been allowed time to seat properly into the Matrigel droplet, the structure is placed into a spinning bioreactor to form the final cerebral tissue. Using the spinning method, essential fluid transport cavities are naturally formed, and are seen to be larger and more continuous compared to previous methods.

As alternatives, biomaterials have been widely used in tissue regenerative medicine, since they mimic the microenvironment that enhances cell survival and grafting success. Moreover, biomaterials could control the cellular network assembly spatially and temporally, representing a significant step forward for organoid development.

An alternative approach engineered brain organoids from human PSCs is the hydrogel composed of Sodium hyaluronan (HA-Na) and Chitosan (CT) material. HA has been widely recognized as an important niche component in neural tissue engineering. The organoids were capable of the neural rosette and neural tube-like structure formation, and demonstrated early corticogenesis (Lindborg et al., 2016). These organoids demonstrated protein and gene expression representative of forebrain, midbrain, and hindbrain development. Appropriate mechanical stiffness of the support material, along with cell-matrix interaction, are important for the efficient formation of brain organoids (Zhang et al., 2022). Soft and micropatterned support material promoted the differentiation of hPSCs to motor neurons when compared to those cultured in usual culture discs (Lindborg et al., 2016). Therefore, coupling of the biomechanical and biophysical properties of the materials are potent regulators for neuronal lineage formation and overall organoid formation.

2.3. Standard and dynamic microfluidic device approach

The standard microfluidic approach of brain organoid development is considered as efficient as the method of a well plate format, which was discussed in the SFEBq protocol. A challenge to brain organoid cultures includes their preference to merge with each other during the incubation

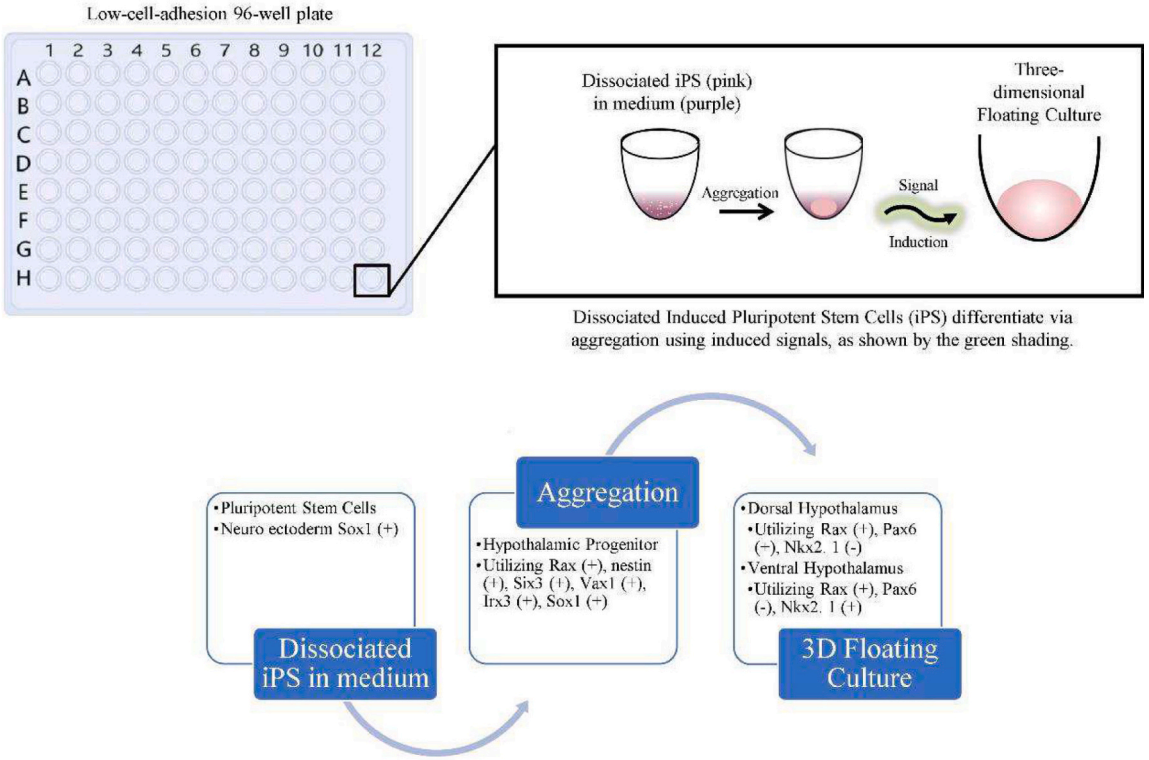

Fig. 1. Serum-free floating culture of a single embryoid, containing body-like aggregates with quick re-aggregation protocol summary. Fig. 1 is created by Lokai et al., based on the procedure detailed by Shevde and Mael, 2018.

process, with inconsistent shape and size. Microfluidic technology can create a platform where the organoid can obtain a biochemical environment with controlled conditions. Moreover, gas and media exchanges are continuously occurring such that the organoid can be in a relatively native environment. Precise media perfusion control and mechanical shaking can mimic the human body's condition (Cho et al., 2021). Microfluidics also model oxygen supply and nutrient/waste exchange, extending brain organoid life (Cho et al., 2021). Additionally, a multi-layered chamber microfluidic device is to precisely generate controlled media flow. More specifically, microfluidic devices provide the option of a gravity-driven flow with a laboratory rocker that imitates cerebrospinal and interstitial spaces.

Alternatively, micropillar array devices are also used for the study of *in situ* generations of human brain organoids (Zhu et al., 2023). Using a multilayered microfluidic chip, which is composed of a micropillar array in a bottom layer and a complementary microhole array in a movable upper layer, the organoids microcapsules can be introduced and settled into microholes with specific patterns. This array device, under microfluidic conditions, can adjust to large-scale organoid cultures and high-throughput assays and screening (Zhu et al., 2023). Brain organoids are provided with dynamic flow *in vitro*, thus fusing different brain region-specific organoids and forming assembloids (Zhu et al., 2023). This modeling of interactions between various brain regions is useful for wide biomedical applications.

To Engineer a different type of brain organoid supporting structure, a 3D brain-mimetic microenvironment may be utilized. This support features a human-derived brain extracellular matrix (BEM) with a dynamic microfluidic system (Cho et al., 2021). BEM is a newer development, simulating neural and glial differentiation for brain organogenesis, which would be commonly lacking in the non-neuronal matrix, such as Matrigel alone. Based on results shown in Fig. 2, specific extracellular matrix (ECM) cues and improved nutrient and oxygen exchange simulation will support cell expansion, neuronal differentiation, and functional maturation. In part 2a, this figure demonstrates the cerebral organoid BEM hydrogel culture system supported with a microfluidic device. The brain organoid culture protocol uses iPSCs, similar to the Matrigel procedure discussed previously. BEM process results in precise, duplicable features of human embryonic cortical development, providing features compatible with microfluidic devices. This data demonstrates how dynamic BEM-infused microfluidic device flow provides reduced formation of the necrotic region, decreasing apoptotic cell death, aiding in metabolic states, and enhancing cell proliferation. These specific cerebral organoids engineered with BEM-microfluidic devices also improve structural, phenotypic, and functional maturation.

Microfluidic devices, such as the one utilized in Fig. 2, use a standard soft lithography technique. Polydimethylsiloxane (PDMS) pre-polymer solution 2.2 mm thick is first cured in a patterned master wafer. Next, 8-mm diameter holes are punched, and the layers are oxygen plasma bonded to create a seal. Once the organoids are cultured with procedures detailed previously, they are added to a 24-well Matrigel plate and combined with the microfluidic device, which is shown in Fig. 2. Table 1 summarizes microfluidic devices, their approaches, and applications.

2.4. Utilizing sensors in brain organoids

Another method builds SERS microsensors (SERS-MS) that have the ability to sense pH in the lumen and extra cellular matrix (ECM) of patient-derived organoids. Although this is a brand-new method and not specifically categorized as a brain organoid sensor, it is a very promising tool for the future of cerebral organoids. In short, SERS-MS evade endosomal uptake pathways because of size comparable to a cell (Campbell et al., 2023). SERS-MS were incorporated into 3D airway organoid cell cultures with suspensions of basal epithelial cells in liquefied ECM. The ECM is set into a gel, inducing the basal cell to differentiate into airway organoids (AOs). As the organoids formed and

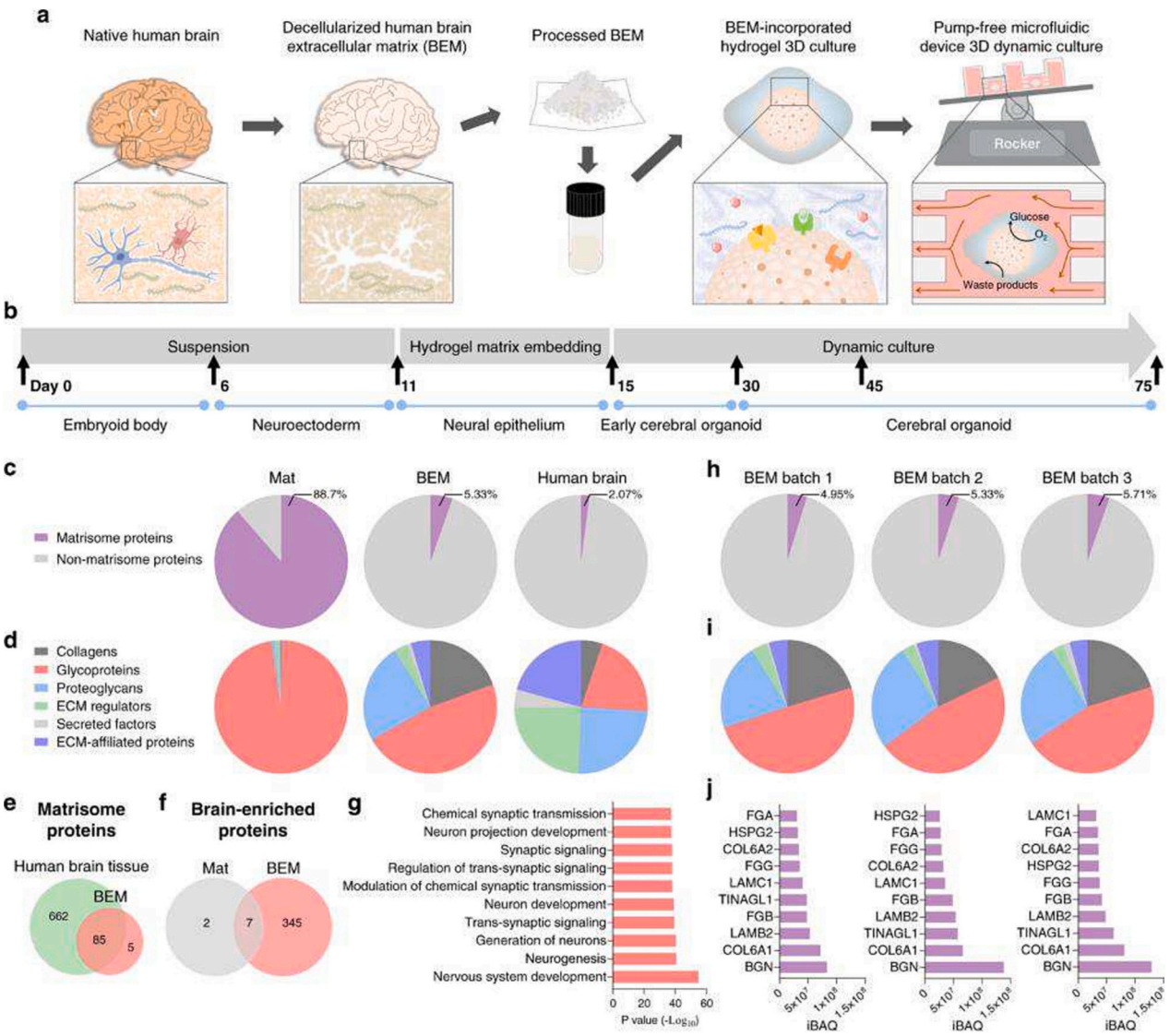

Fig. 2. A summary of the microfluidic processing of brain organoid models discussed. a. Schematic of the cerebral organoid culture system with a combination of 3D BEM hydrogel culture and the microfluidic device. b. Schematic of the culture protocol for generating cerebral organoids from human induced pluripotent stem cells (iPSCs) using a brain-mimetic culture system. The developmental stage of organoids is shown at the bottom, the timeline for each developmental stage is shown in the middle, and the culture condition is shown at the top. c–j Proteomic analysis for the identification of extracellular matrix (ECM) components in human BEM. The percentages of (c) matrisome proteins out of total proteins, and (d) the subtypes of matrisome proteins identified in BEM, Matrigel (Mat), and human brain tissue ($n = 1$ for Mat, $n = 3$ for BEM). e Total numbers of matrisome proteins detected in the human brain tissue and BEM. f The number of the brain-enriched proteins by at least 4-fold compared to other organs. g The top 10 biological process terms ordered by p value after gene ontology enrichment analysis of brain-enriched proteins exclusively present in BEM, which are not detected in Mat. The compositions of (h) matrisome proteins relative to total proteins and (i) the subtypes of matrisome proteins in three batches of BEM (biological replicates = 3). j Most abundant matrisome proteins found in each batch of BEM. Source data are provided as a Source Data file. Reproduced with permission from reference (Cho et al., 2021).

expanded, SERS-MS was internalized into surrounding ECM. This 3D cell culture spread throughout the AOs' extracellular environment and lumen-based SERS-MS (Campbell et al., 2023). This is one of the main procedures for microsensors that deliver probes to the organoid lumen. Leveraging the solid phase of SERS-MS allows organoid expansion to engulf the sensors and avoids the need for the traditional technical probe microinjection, according to Campbell et al. (2023).

This concept of microsensors can be applied in disease study such as for Parkinson's disease, an analysis of midbrain organoids. Microfluidic platforms are integrated with multiple sensors for dynamic long-term cultivation. This specific organoid-on-a-chip platform (with an optical, electrical, or electrochemical microsensor added) consists of three interconnected chambers with a unidirectional medium flow; as a hydrogel-based, gravity-driven flow restrictor simulates interstitial flow profiles (Spitz et al., 2022). A multi-electrode array non-invasively monitors the electrophysiological neuronal activity, promoting functional cell coupling and tissue maturation. An amperometric sensor is also introduced to monitor neurotransmitter dopamine, directly reflecting the maturation and pathological status of midbrain tissues (Spitz et al., 2022).

2.5. *Brain organoids-on-a-chip*

Microfluidic advantages as an *in vitro* culture platform can be applied to brain organoids-on-a-chip as well. Contrasting microfluidic chambers that are easily adjusted for larger-scale study, this organoid-on-a-chip system is not practical for high throughput projects, as the pump requires extra materials and maintenance. Brain-organoid-on-a-chip

Table 1
Microfluidic device summary of approaches and applications.

Microfluidic model	Approach	Application
Standard, single microfluidic device	Soft Photolithography	Maintaining uniform size and shape of merging organoids
Multi-layered microfluidic chambers	Biochemical environment with continuous gas and media exchange	Precise fluid flow for large-scale organoid cultures, high-throughput assays, and screening
Dynamic microfluidic system	BEM-infused microfluidic device with iPSCs	Decreased apoptotic cell death, aids metabolic states, and enhances cell proliferation
Micropillar Array Device	Micropillar array bottom layer with a complementary microhole array patterned in a movable upper layer, while the organoid microcapsules are cultured into the microholes.	*In situ* generations of human brain organoids, assembloids

cultures typically use a 5-channel chip, applied in modeling neuro-developmental disorders under chemical exposure. To do so, a syringe pump-based perfusion method is performed, represented by Fig. 3 (Berger et al., 2018). Four QV900 circuits connect to one peristaltic pump, containing one hMO each, depicted in Fig. 2Ai and Aii. The schematic drawing of one QV900 six-chamber circuit in Aii represents Bi and Biii, the latter being Hoechst stained (white). hMO sections are then derived from three different WT NESC lines and are cultured under fluidic or shaking conditions. Yellow dotted lines indicate the area of the "dead core", whereas the yellow dashed lines indicate the area of the entire hMOs, in Image Bi. Quantification of area measurements as ratio of "dead core" area and total hMO area is then analyzed for patient study. Although the chips' short-term cultures were demonstrated to have cell population, cortical layers and radial organization was not observed (Berger et al., 2018). Hence this chip model is well-suited for individual study rather than a high throughput model.

2.6. 3D printing approach

Advanced microfluidic chips for 3D cell culture can be developed using 3D printing methods. This rapid prototyping mechanism makes bio scaffold and microfluidic optimization extremely efficient compared to the previous methods (Liu et al., 2016). 3D bioprinting is an especially powerful tool used for precise biocompatible scaffold production. The use of 3D printing makes 3D cell culture platforms a versatile and cost-effective method for organoid development. Due to the specific requirements for the formation of organoids, it is imperative to develop precise microfluidic systems to support and deliver proper nutrients to the organoid, as discussed. 3D printing methods can improve many functions of an organoid environment, such as mechanical fluid flow, nutrient delivery, and self-organization (Chen et al., 2021). Using a 3D printing method, controlled environments can be catered for specific organoid models, being an innovative approach to organoid development. With the current research being done, 3D printing organoids can take place using inkjet-based, laser-assisted, extrusion-based, and photocuring applications (Ren et al., 2021). Some of the current developments include the forebrain organoids, using pluripotent stem cell derived organoid in a 3D culture system, which enhanced the lifespan of the organoid. There has also been a recent discovery to help support the self-organization function through photocrosslinkable bioink and a therapeutic support bath (Ren et al., 2021). The potential of utilizing bioprinted organoids could lead to a reproducible mechanism to be used for drug screening and a variety of other applications, to help further the treatment of diseases such as Alzheimer's, Parkinson's, and several types of cancers. Induced pluripotent stem cells can be bioprinted using the extrusion based or laser-based printing methods (Salaris and Rosa, 2019). iPSCs differentiation can be done either before printing where the neural model's cytoarchitecture can be controlled, or after printing where the position and number of cells in the model are out of control.

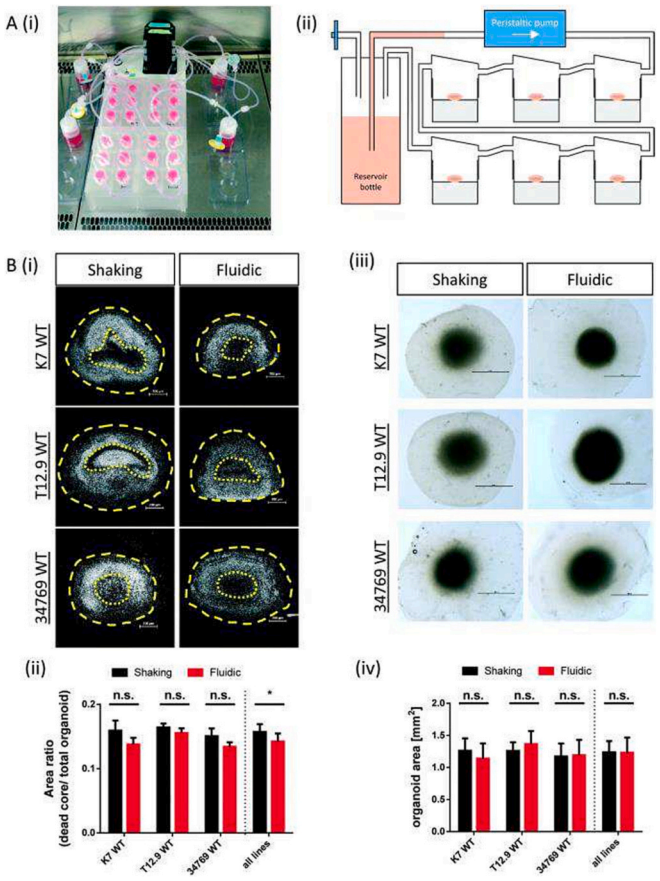

Fig. 3. Reduced "dead core" size in hMO cultured in the millifluidic device. (Ai) Experimental setup for culturing hMO in the millifluidic device. The picture shows four QV900® circuits connected to one peristaltic pump. All chambers contain one hMO each. (Aii) Schematic drawing of one QV900® six-chamber circuit. (Bi) Hoechst staining of nuclei (white) of representative hMO sections derived from three different WT NESC lines and cultured under fluidic or shaking conditions. Yellow dotted lines indicate the area of the "dead core", whereas the yellow dashed lines indicate the area of the entire hMOs (scale bar = 200 μm). (Bii) Quantification of area measurements as ratio of "dead core" area and total hMO area ($n = 3$ per line and culture condition, $^*p < 0.05$). (Biii) Brightfield images of fixed (unsectioned) hMOs with surrounding halos of matrix (scale bar = 1 mm). (Biv) quantification of hMO area measurements ($n = 6$ per line and culture condition). Reproduced with permission from reference (Berger et al., 2018). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Further organoid development

As the current approaches of Section 2 have been addressed, including sFEBq, biomaterials, microfluidic devices, utilizing possible brain organoid sensors, brain organoids-on-a-chip, and 3d bioprinting, this new field requires additional aspects to be addressed as well. Lack of vascularization in early models of the brain organoid limited the 3D model in terms of accuracy to the human brain; and this element is now developing rapidly. As vascularization progresses, so does genome engineering, which aids in customization of a patient's brain organoid *via* the addition of lineage studies and guiding organoids' aggregation to improve the model's efficiency. Lastly, stimulation of the organoid is also applicable in terms of electrical, optogenetic, and magnetic types. These examples of development are exponentially improving the brain

organoid's functions and may aid in the future formation of more complex organoid matrices and cross-communications.

3.1. Capillary theory and vascularization

Another innovative feature of organoid development includes vascular system integration. In early development, capillaries were grown with an iPSC organoid in Matrigel-based substrate (Pham et al., 2018). Additional iPSCs were then differentiated into endothelial cells. As the vascularized organoids were grown *in vitro* for 3–5 weeks, Human CD31-positive blood vessels were found inside and in-between rosettes within the center of the organoids (Pham et al., 2018). This development would be beneficial to modern platforms, obtaining a functional organoid in terms of physiology, shape, and size.

Vascularization is a vital part of brain organoids, as lack of blood vessel representation limits an organoids' size. Without vascularization, apoptosis of inner organoid cell layers is likely, caused by insufficient oxygen and nutrients (Giandomenico et al., 2019). In contrast, orbital shakers may provide supply to an organoid's surface layers. To further supply inner layers of brain organoid with oxygen and nutrients, slice culture is beneficial, which is a type of organoid-specific 3D structure (Qian et al., 2020).

In addition to oxygen and nutrient supply, embryonic human FLVCR2 gene impair vascularization has the capability to create a new pathway in genetic study. This gene impairing type of vascularization aids in the research of glomeruloid vasculopathy, a thin cerebral cortex and hydrocephalus (Birey et al., 2017). The success of the vascularization of 3D organoids could be a significant advancement in mimicking human organs, including the way to access nutrition, releasing factors as way of interactions, and expanding knowledge of genetic study applications.

3.2. Genome engineering

As the complexity of brain organoids expands its applications such as vascularization above, a current challenge in personalized medicine is expanding human genetic diversity knowledge for disease pathogeneses and drug responses (Lu et al., 2014). Brain organoid models now have the ability to integrate genetic engineering into high-throughput single cell transcriptomics. Hence human pluripotent culture systems have now created *in vitro* patient-centered models for genetic variant studies (Zhu and Huangfu, 2013). This revolutionizes patient care, as most vital regions of the brain are inaccessible through direct tissue experimentation. The *in vitro* system is split into two types, guided and unguided (Benito-Kwiecinski and Lancaster, 2020). Unguided brain organoids rely on spontaneous morphogenesis and intrinsic differentiation capacities within stem cells aggregates; and guided organoid methods require supplementation of external patterning factors to induce hPSCs to differentiate towards desired lineages (Lancaster et al., 2017). Differentiated protocols using external factors vary, as unguided and guided approaches depend on the project goal, considering diversity and consistency. Table 2 below presents a summary of skin biopsy-based cell line research used for disease study, genome editing, vascularization, and microglia additions, alongside neurodevelopment and xenotransplantation.

3.3. Brain organoid stimulation: electrical, magnetic, and optogenetic

Besides disease and genetics, alongside chemical topographical and chemical inducers in neuronal development, other physical factors also can modulate the development. It has been reported that optogenetics, electrical stimulation, and magnetic stimulation also regulate physiological signals. The stimulating of neurons with light-driven opsins is widely used to study cell responses in optogenetic stimulation techniques (Dagher et al., 2022; Lee et al., 2016). This technique is non-invasive and highly selective of neurons specific to opsins integrated

Table 2
Summary of Section 4, genome engineering and vascularization.

Approaches	Methods	Models	Applications
Genome Editing	CRISPR/Cas-based gene editing	Neuro-connectivity, Neurological Disorder, Epigenetics	Xenotransplantation, Neurodevelopment
Mutations	Disease-causing Mutations	Neurological Disorder	Research on disease related mutations and disease models
Vascularization	Introduction of mesenchymal stem cells or iPSC-derived endothelial cells	Neurological Disorders, nutrient transport, genetic impairment, Blood-brain barrier	Increased size to create a more accurate mature organoid model

cells. Studies have shown that optogenetic stimulation of dorsal root ganglion cells exhibited increased neurite growth (Park et al., 2015; Tiwari et al., 2022). This highlights the use of optogenetic stimulation being beneficial to achieve improved neural activities of organoids. The intensity, location and stimulation parameters of electrical fields influence cellular behavior during neuronal development (Bertucci et al., 2019). Additionally, magnetic stimulation induces the functional and structural plasticity of brain components (Jog et al., 2023; Vlachos et al., 2012). With three-dimensional models, patients can save time and use a more individualized pain treatment due to the creation of a brain organoid using their own stem cells. Since the organoid would be stimulated prior to treatment, better stimulation parameters and techniques can be optimized for the individual patient. However, it is common to require a trial period of the exact concentration of these stimulation types are effective, and exactly which areas of the brain should be treated. This confirms exactly how individual patients' stem cells would react to stimulation and provide a solid foundation for treatment plans. This was not possible with two-dimensional, traditional models since no patient customization is involved. Hence, it is optimistic collaborating the brain organoid current technologies with external stimuli can step forward to brain organoid development and post-organoid studies.

One promising method of 3D brain organoid stimulation and electrical monitoring includes mesh electrodes utilized for chronic electrophysiological interfaces with brain tissue (Li et al., 2022). Unlike traditional multi-electrode arrays detailed earlier, mesh electrodes are interconnections of flexible conductive material encapsulated by insulating polymeric materials. Mesh electrodes achieve a stable long-term interface with a low bending stiffness, minimal volume compared to solid electrodes, and can be made thinner than one micrometer. The mesh's thin layers are shown to conform to neural tissue more easily, minimizing foreign body interactions.

4. Conclusions and future perspectives

For neurological diseases treated today, the most prevalent hurdle of two-dimensional models is a perfectly identical treatment plan for all diagnoses. Although these two-dimensional models have continued to make revolutionary strides, these treatment options are the first to be exhausted by treatment prognosis. Now that brain organoids exist, drug screening and treatment methods will be more practically tested on the patients' own cultured stem cells before patient administration, to predict patient response and success rate more accurately. This not only provides a safer option for the patient, but also a more promising glimpse of hope without taking unnecessary trial-and-error and valuable time loss (Qian et al., 2019).

Despite these incredible advances, there are several areas of brain

organoids to be improved. The future technology of cerebral organoids must develop a solution for tissue cross-communication and body axis simulation. This and other shortcomings will be developed in time, *via* biomedical engineering approaches. Surrounding tissues of the brain are currently being addressed by patterning factors. Cell patterning signals may use customized scaffolds with immobilized signals, or a signal gradient formation using control-released particles, improving tissue organization. The current models are not suited to emulate brain shape and structure *in vivo*. Culture systems without patterning factors improved developed brain organoid-controlled organization, although not accurately enough for cross-communication tissue study. So, although a safer patient option in theory, there are still many specifications to be improved.

Lastly, current limitations also include spatial-temporal control of differentiation; however, spheroids have potential to bridge the gaps of this patterning. These will all have potential engineering differentiation and patterning guidance of specific neural regions in future applications of organoids. With so much potential of current organoids, there are also several discrepancies that are vital to future success, such as the vascular and cellular diversity factors, as discussed. This will aid in concepts such as myelination induction, to regenerate neuronal damage, which will have the ability to help medical professionals understand their patients' neurological disorders for drastically improved treatment. Additionally, the integration of host brain networks for brain injuries can aid different brain centers such as visual and motor. This concept is achieved *via* transplanting an organoid into the brain site, a translational strategy to restore function after brain damage (Jgamadze et al., 2023). However, this has not been well established and needs vast improvement.

The organoid field is relatively young, even so is using the bioengineering approaches on brain organoids. But as more about the factors that play roles on brain development are further developed, it is expected that biomedical engineering will play a more important role in future organoid development, including brain organoids.

Declaration of Competing Interest

The authors have no conflicts of interest.

Data availability

No data was used for the research described in the article.

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