Review

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Generation, interrogation, and future applications of microglia-containing brain organoids

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From the Contents

General Introduction to Current Brain Organoid Models, Their Applications and Advantages	1
Immune-Competent Brain Organoids	4
Characterization of Microglia in Brain Organoids	7
The Ultimate Toolbox for Characterization of Microglia-Containing Brain Organoids	10
Search Strategy	10

Abstract

Brain organoids encompass a large collection of *in vitro* stem cell–derived 3D culture systems that aim to recapitulate multiple aspects of *in vivo* brain development and function. First, this review provides a brief introduction to the current state-of-the-art for neuro-ectoderm brain organoid development, emphasizing their biggest advantages in comparison with classical two-dimensional cell cultures and animal models. However, despite their usefulness for developmental studies, a major limitation for most brain organoid models is the absence of contributing cell types from endodermal and mesodermal origin. As such, current research is highly investing towards the incorporation of a functional vasculature and the microglial immune component. In this review, we will specifically focus on the development of immune-competent brain organoids. By summarizing the different approaches applied to incorporate microglia, it is highlighted that immune-competent brain organoids are not only important for studying neuronal network formation, but also offer a clear future as a new tool to study inflammatory responses *in vitro* in 3D in a brain-like environment. Therefore, our main focus here is to provide a comprehensive overview of assays to measure microglial phenotype and function within brain organoids, with an outlook on how these findings could better understand neuronal network development or restoration, as well as the influence of physical stress on microglia-containing brain organoids. Finally, we would like to stress that even though the development of immune-competent brain organoids has largely evolved over the past decade, their full potential as a pre-clinical tool to study novel therapeutic approaches to halt or reduce inflammation-mediated neurodegeneration still needs to be explored and validated.

Key Words: 3D cell culture; brain organoids; immune response; immunocompetent model; *in vitro* model; microglia; neural organoids; neuroimmunology; neuroinflammation

General Introduction to Current Brain Organoid Models, Their Applications and Advantages

Organoids are defined as self-organizing three-dimensional (3D) cellular aggregates generated from pluripotent stem cells (PSCs) or adult stem cells, with features that resemble the structure and functions of an organ. More specifically, brain organoids have been developed – in the first instance – to reproduce the major characteristics of a developing embryonic brain, and upon long-term culture promote its transition to a more mature but still prenatal brain-like environment. Having numerous scientific applications, they have for example been used to study various physiological functions of the brain, such as the mechanisms of neuronal development and connectivity (Mariani et al., 2012; Bershteyn et al., 2017; Birey et al., 2017; Deng et al., 2018), interactions among neurons and glial cells, or evolutionary comparisons across species (Pollen et al., 2019; Benito Kwiecinski et al., 2021). Furthermore, from a biomedical point-of-view, they have contributed to the modeling of different pathologies, such as neurodegenerative diseases (Abud et al., 2017; Sabate-Soler et al., 2022), viral infections (Muffat et al., 2018; Abreu et al., 2018; dos Reis et al., 2020; Gumbs et al., 2022; Donadoni et al., 2024, Narasipura et al., 2024), congenital brain malformations (Lancaster et al., 2013; Li et al., 2017) and psychiatric disorders (Choi et al., 2016; Kathuria et al., 2020; Meng et al., 2023; Li et al., 2023). With the possibility

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to obtain large numbers of organoids in a relatively short period, expectations for future applications in compounds screening and validation are very high. Nevertheless, from an industrial point of view, their complementarity or even superiority to traditional two-dimensional (2D) culture systems still needs to be demonstrated, especially given the long-term culture and time-consuming hands-on protocols associated with brain organoid generation and maintenance (**Figure 1**). Compared to 2D cell cultures, organoids possess a long list of characteristics that make them more attractive and employable for numerous applications, the most significant of which is being more physiological to the organ of interest. In this context, while 2D cultures may have the advantage of being easy to maintain, providing rapid output, and being highly reproducible, the drawback is that monolayer cultures expand only two-dimensionally, resulting in a change in their morphology and properties. These limitations are overcome in organoids, where cells maintain their physiological shape, can grow freely in all directions and can even self-organize thereby generating cytoarchitectural features representative of the *in vivo* organ (Lu et al., 2022). Furthermore, in 2D monolayer cultures, cell-to-cell interactions are limited to side-by-side contact, typically involving only a single cell type; conversely, cell interactions in organoids are more complex and among multiple cell types in different layers. This difference in terms of cell development is because in a 2D cell culture, all cells are equally exposed to the medium and growth factors, while this is not the case in an organoid, where the medium does not reach all layers to the same extent, creating a more varied context. Furthermore, cells within an organoid mature and are frequently observed to have a slower rate of proliferation compared to 2D monolayer cell cultures, better representing the *in vivo* physiology (Bejoy et al., 2019; Abbas et al., 2023). As a downside, organoids, especially those obtained by unguided protocols, may possess high heterogeneity in terms of size, shape, and composition, making reproducibility more difficult to achieve (Hartley et al., 2017; Wang et al., 2017; Koo et al., 2019). Despite this, the variability can be reduced by using bioreactors, avoiding natural hydrogels with undefined factors, using patterning factors, and starting from iPSC-derived neural stem cells instead of iPSC (Qian et al., 2016; Wang et al., 2017; Centeno et al., 2018; Van Breedam et al 2022).

Additionally, the interaction of cells with the extracellular environment is improved in floating organoids, since they avoid interaction with 2D plastic surfaces of culture dishes, whose stiffness can affect the viability, differentiation, morphology, and gene expression of the cells (Gilbert et al., 2010; Wolfram et al., 2024).

Another experimental benefit of organoids is their complex response to mechanical and chemical stimuli. Compared to 2D cultures, organoids are considered to exhibit a more faithful response to the *in vivo* situation. Another experimental benefit of organoids is their complex response to mechanical and chemical stimuli. Multiple studies have observed differences in cellular responses to pathological stimuli and drugs between 2D and 3D cultures of the same cells. Compared to 2D cultures, cells in 3D models exhibit improved drug metabolization, higher resistance, and a greater threshold for apoptosis. In contrast, cells in 2D cultures show lower metabolization rates, reduced resistance, and increased susceptibility to apoptosis, even at low drug concentrations. This disparity suggests that conventional 2D cultures may overestimate drug efficacy, while 3D models offer a more accurate prediction of *in vivo* drug responses (Ravi et al., 2014; Imamura et al., 2015; Langhans et al., 2018).

Brain organoids as a supplement to animal models

Currently, the level of physiological complexity that a whole organism provides cannot be replicated in *in vitro* model systems yet. For this reason, animal models remain essential for the design and approval of clinical trials. Nevertheless, there is a global and increasing effort to try and reduce the use of animals for scientific studies due to ethical reasons. In this scenario, organoids appear as a promising solution, with the potential to supplement animal models in many applications.

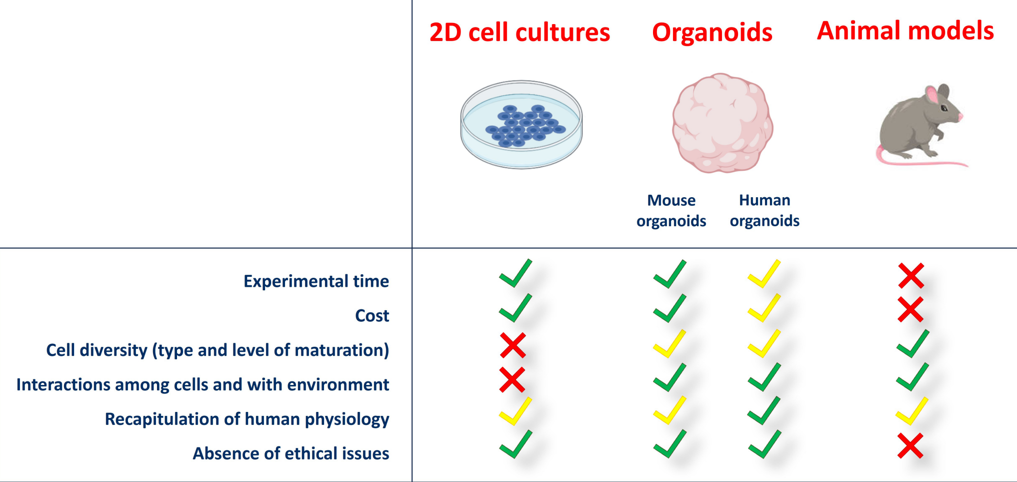

Figure 1 | Comparison of human and mouse brain organoids with 2D cell cultures and animal models.
Figure was created based on data from Kim et al. (2020). Created with BioRender.com.

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Organoids offer several unique advantages compared to traditional animal models (**Figure 1**). First, since they can be grown under standardized conditions in highly controlled bioreactor systems, their maintenance becomes easier compared to animal models, whose daily care is expensive and time-consuming. Second, depending on the protocol, organoids can be obtained on a large-scale allowing high-throughput screening, while maintaining genomic stability. This facilitates genetic manipulations and the building of biobanks (Sun et al., 2022). Finally, the biggest advantage when starting from human PSCs is the recapitulation of human features, which only human organoids can ensure. For instance, brain organoids can replicate the inner and outer layers of the subventricular zone, a subdivision that is absent in mice (Marshall and Mason, 2019). For this reason, human brain organoids are better suited to recapitulate human-specific brain structures, when compared to any other animal model (Kim et al., 2020). While non-human primates remain an invaluable *in vivo* model, with their brains more closely resembling human brains than rodent models, human brain organoids offer the ability to study features exclusive to the human brain. Nevertheless, even though at present there exists a large focus on generating human brain organoids, there is also a great need for generating PSC-derived brain organoids from other species, either from an evolutionary developmental point of view or – even more importantly – to offer the possibility to truly compare physiological and pathological processes occurring in brain organoids with actual *in vivo* brain responses. As such, PSC-derived murine brain organoids are gaining interest and will stand as an important complementary tool for human brain organoid models (Di Stefano et al., 2024). Once such profound comparisons have been made, i.e., murine brain organoids *vs*. murine brain and murine brain organoids *vs*. human brain organoids, we will have a deeper understanding of how to interpret the results coming from human organoids about the *in vivo* situation, leading to an exciting future for unraveling human brain function using human brain organoids.

Standard culture protocols for brain organoids

Over the past decade, several protocols to generate brain organoids have been optimized and published. Although they generally follow a similar methodological and developmental pattern, they do differ in several aspects. First, the source of PSCs used to form brain organoids can be iPSCs reprogrammed from somatic cells (Takahashi et al., 2007; Lancaster et al., 2013), embryonic stem cells obtained from dissociated blastocysts (Thomson et al., 1998; Zhang et al., 2001) or adult stem cells. Regardless of the cell type used, these are typically cultured in ultra-low attachment 96 well plates, aggrewell plates, or with the hanging drop method; each of these will push the cells to tend to clump together, forming embryoid bodies. When the size of the embryoid bodies increases, these are often transferred to bigger flasks such as low-attachment 24 or 6 well plates, spinning flasks, or bioreactors (Lancaster et al., 2013; Ormel et al., 2018; Xu et al., 2021; Fagerlund et al., 2022). Here they have an abundant medium that allows them to grow further and develop into brain organoids. The growth can be supported by the use of extracellular matrix proteins, which form a network of fibrous proteins that contribute to the self-organization and differentiation of the cells forming the organoid (Simsa et al., 2021; Heo et al., 2022; Muñiz et al., 2023). Additionally, the culture can be improved by shaking the cell cultures with the use of a shaker or a bioreactor, enabling a better exchange of nutrients and a higher level of oxygenation throughout the organoid, preventing cell death and reducing the formation of a necrotic core (Qian et al., 2016; 2018; Romero-Morales et al., 2019; Cho et al., 2021; Licata et al., 2023). The culture time can highly vary from one study to another, typically ranging from 2 months (Abreu et al., 2018; Jin et al., 2022) to 6 months (Lin and Seo et al., 2018; Bodnar et al., 2021), with the longest duration being around 500 days (Kasai et al., 2020). Especially, the latter notion warrants that there is currently no clear standardization for the generation of brain organoids, and as such will make it very difficult to post-hoc compare results from different studies. Nevertheless, since the field of brain organoid generation is arriving at the stage of maturity, initiatives to establish common guidelines should be encouraged.

Limitations and possible improvements for classical brain organoid models

Even though much progress has been made in the generation and biological understanding of PSC-derived brain organoid models, there are still several limitations that need further attention. Despite this, different solutions to these problems exist and they are summarized in **Figure 2**. Currently, one of the biggest issues of brain organoids is the lack of vascularization, which inhibits the diffusion of oxygen and nutrients to the deepest layers of a brain organoid. In fact, it is estimated that sufficient delivery of oxygen in the human body is only possible when the distance of cells from a blood vessel is below 200 μm (Place et al., 2017). Therefore, the lack of vascularization within organoids limits their size and their development beyond the prenatal stage and causes cell death in the inner core upon development. Depending on the protocol and the duration of the experiment, organoids can reach a diameter size of up to 5 mm, resulting in a high percentage of dead cells within the center of the organoid. A possible solution for this would be the introduction of a perfused vascular system, either artificial or biological. Several studies have attempted to address this deficit by incorporating endothelial cells into brain organoids. This was achieved by adding endothelial cells at the moment of initial cell seeding during organoid formation, with as end-result the self-organization into an elementary microvasculature (Nashimoto et al., 2017; Pham et al., 2018; Song et al., 2019). Somewhat technologically more advanced is the use of microfluidic devices that allowed co-culture of an organoid together with umbilical vein endothelial cells resulting in the construction of a perfusable vascular network growing into the organoid (Nashimoto et al., 2017). Finally, although deviating from the concept of animal-free models, organoids can also be engrafted directly into a mouse brain, whose blood vessels will penetrate the organoid's tissues and create a true vascular network (Mansour et al., 2018; Shi et al., 2020; Schafer et al., 2023). In our opinion, the use of vascularized,

Vascularisation

- Better oxygenation
- Possibility to reach bigger size
- Reduced necrotic core

Mature organoids

- Mature cell types
- Possibility to model later neurodevelopmental stages and/or diseases

Microglia-containing brain organoids

- Better recapitulation of neurodevelopmental processes
- Allow study of neuro-immune interactions

Figure 2 | Improvements for brain organoids to solve their present limitations.
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but also perfused, brain organoids will still require many years of research before being widely adopted and applied within biomedical research.

During their initial generation, brain organoids reproduce in the embryonic stage of brain development. Consequently, another limitation is the difficulty in modeling later stages of brain development and the aging brain, which is a necessary requirement to study the development or pathogenesis of age-related neurodegenerative diseases. To overcome this, several techniques to speed the maturation and induce aging have been investigated (Studer et al., 2015). These include the induction of cellular stress through exposure to toxins and compounds that induce mitochondrial stress and the formation of reactive oxygen species (Seibler et al., 2011; Liu et al., 2012; Cooper et al., 2013), or alternatively increasing the expression of progerin or a truncated form of lamin A associated with premature aging, both of which induce aging-related features in organoids (Miller et al., 2013).

Lastly, brain organoids usually contain neurons and astrocytes but these do not always include microglia, the immune cells of the brain. Integrating microglia into brain organoids is essential to obtain an immune-competent model; nonetheless, adding this cell type can be rather challenging, due to their mesodermal origin. Microglia come from the yolk sac as primitive macrophages and subsequently migrate to the embryonic brain where they complete their maturation (Ginhoux et al., 2015). However, given the neuroectodermal specification necessary to obtain other neural cells, such as neurons, astrocytes, and oligodendrocytes, most brain organoid protocols are designed to inhibit mesodermal and endodermal differentiation. Therefore, integrating cells that originate from other germ layers such as microglia, but also endothelial or meningeal cells becomes arduous, often compromising cell diversity, a feature that is crucial to accurately reproduce the *in vivo* environment (Ormel et al., 2018; Jacob et al., 2021). Nevertheless, despite the above-described difficulties, numerous works have already successfully incorporated microglia into brain organoids. The further aim of this review is to provide a comprehensive overview of the three main categories of microglia-containing brain organoids, according to the type of protocol used.

Immune-Competent Brain Organoids

To more accurately mimic an *in vivo* neural-like environment and to allow the study of neuroinflammation and potential immunomodulation, a highly specialized subtype of brain organoids is needed, currently described as immune-competent organoids. The defining characteristic for a brain organoid to qualify as immune competent is the inclusion of relevant immune cell types. Microglia are the resident immune cells of the central nervous system (CNS) and are essential for neuronal homeostasis (Ginhoux et al., 2010 and 2013). In fact, they are involved in numerous functions, including neurite formation, synaptic homeostasis, synaptogenesis, synaptic pruning, regulation of axon fasciculation, and programmed cell death. Moreover, microglia help with the differentiation and maturation of neural and oligodendrocyte progenitor cells, promote myelinogenesis, as well as increase astrocyte activation and proliferation (Garland et al., 2022). However, the primary function of microglia is to protect the CNS against infections and chemical or physical insults. Microglia constantly scan the brain environment, actively searching for cues that signal brain damage. When cellular debris or pathogens are detected, microglia become activated and subsequently display phagocytic functions to eliminate invaders (Wright-Jin and Gutmann, 2020). Microglia exhibit different morphologies and roles according to the developmental stage and the inflammatory state of the CNS. During the fetal and early postnatal phase, microglia show the amoeboid shape and high levels of proliferation. Subsequently, in a homeostatic stage, microglia adopt a ramified shape, cease proliferation, and maintain a constant number of cells. Upon inflammation, microglia activate their immune-modulatory properties transitioning to an amoeboid shape, ready to phagocytose debris, apoptotic bodies, or pathogens. Furthermore, according to the pro- or anti-inflammatory

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cytokines they release, they can cause astrocytes to become neurotoxic, further leading to tissue damage and increased blood–brain barrier permeability, or neuroprotective, respectively (Quarta et al., 2020).

Given their important role in brain homeostasis in health and disease, integration of microglia in brain organoids is pivotal for a better recapitulation of the complexity of the CNS neural environment *in vivo*. In fact, it has been demonstrated that the presence of microglia has major beneficial effects on other cell types, including their appropriate development and function: they contribute to neuronal maturation, remodel neuronal connectivity, regulate axonal guidance and cytoskeletal organization, increase synchronization and frequency of action potentials and increase neuronal excitability (Fagerlund et al., 2022; Popova et al., 2022; Sabate-Soler et al., 2022). From their immunological point-of-view, it was shown that microglia are attracted to areas where cell death is occurring like necrotic cores, where they phagocytose apoptotic cells and cell debris. This can possibly result in visibly smaller microglia-containing brain organoids, as previously observed by Sabate-Soler et al. (2022).

As discussed further below, there are currently several strategies employed to obtain microglia-containing brain organoids. Nevertheless, the first works that introduced microglia into brain organoids required a large investment in the optimization of microglia (progenitor cell) differentiation from hPSC, as well as the characterization of the newly obtained cells. This required the analysis of microglial behavior within brain organoids to recreate and validate a complex 3D structure containing other neural cells, thus allowing for comparison with *in vivo* microglial features. These studies evaluated microglia morphology, motility, their ability to migrate and invade neural tissue (Brownjohn et al., 2018), their functional characteristics such as phagocytic activity, and their ability to respond to a physical insult (Abud et al., 2017). Subsequently, microglia-containing organoids not only served as a means of validating microglia identity, but as a complex model that allows the study of neuroinflammatory processes and consequent neuroinflammatory diseases, as well as the impact of microglia-like cells on neuronal development, functioning, and/or regeneration (Fagerlund et al., 2022).

Strategies for generating microglia-containing organoids

Despite the technical challenges associated with generating brain organoids containing microglia, there are several studies in which this was accomplished. Various approaches exist for incorporating this cell type, which can be broadly categorized into three main experimental approaches: co-culture, addition, and spontaneous development (**Figure 3**). Further discussed below, **Additional Tables 1** and **2** provide a detailed summary of recent studies applying these experimental approaches to generate microglia-containing organoids. Notwithstanding their demonstrated usefulness in solving scientific questions, each of the defined approaches has several pros and cons that are summarized in **Table 1** and will be further discussed below.

The co-culture approach

At first, the co-culture approach requires the differentiation of neural precursor cells and microglia progenitor cells separately using pre-defined culture protocols. Subsequently, when both cell types reach the maturation level needed, they are combined in a specific ratio to form the organoids and they mature during further co-culture (**Figure 3**). For example, in the study by Xu et al. (2021), neural precursor cells (NPC) and primitive macrophage progenitor cells were cultured in parallel, for 21 days each. Subsequently, 7×10^3 NPC and 3×10^3 macrophage progenitor cells were co-cultured in 96 well plates in a 1:1 ratio of their respective culture media (Xu et al., 2021). By day 50 of co-culture, approximately 8% of cells were positive for microglia markers, consistent with the average percentage of microglia in the human brain (Mittelbronn et al., 2001). Microglia were able to promote neural differentiation,

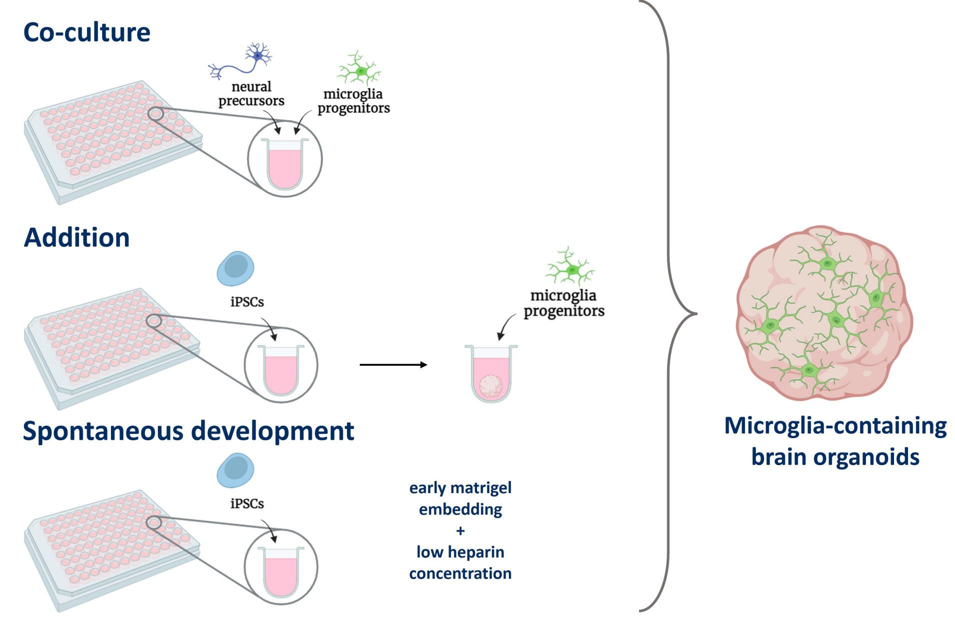

Figure 3 | Three most common approaches to adding microglia to brain organoids.
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Table 1 | **Pros and cons of the three main techniques to obtain microglia-containing brain organoids**

	Co-culture	Addition	Spontaneous
Pros	Starting numbers of microglia progenitors and neural precursor cells can be controlled, leading to more precision and less variability between organoids	All cell types are already developed and mature when they are added together, making it easier to co-culture them	No need for separate, elaborate and time-consuming differentiation protocols
Cons	Difficult to find culture conditions that suit the requirements of different cell types	Limited time window for optimal microglia integration into organoids and support of neuronal development microglia can become reactive when handled for the addition	High heterogeneity of cell types and impossibility to control it (due to the absence of SMAD inhibition) microglia in brains are not arising from spontaneous development

SMAD: Small body size-mothers against decapentaplegic.

phagocytose apoptotic cells, and foreign bodies, and perform synaptic pruning. While this model was initially utilized to study Zika virus infection, with a similar protocol, Jin et al. (2022) studied microglial functions in Down syndrome.

A major advantage of the co-culture approach is that the starting number of the individual cell types can be strictly controlled, reducing the variability between organoids (**Table 1**). In fact, the abundance of microglia varies considerably among different brain regions. In the human brain, microglia constitute between 0.3% in the cerebellum and 11% in the medulla oblongata (Mittelbronn et al., 2001), while in the mouse brain, their proportion ranges from 5% in the cerebral cortex or corpus callosum to 12% in the substantia nigra (Lawson et al., 1990). Therefore, being able to add the desired amount of microglia to obtain region-specific microglia to neural cell ratios seems highly attractive. However, this co-culture approach may come with the disadvantage that the different types of cells need different media and growth factors to fully differentiate and mature (**Table 1**). Subsequently, long-term co-cultures starting from different cell types in an immature stage, can lead to problems in finding the right culture conditions (Xu et al., 2021).

The addition approach

Second, the addition approach to establish immune-competent brain organoids is based on the addition of microglia (progenitor) cells to already pre-generated organoids, after which a period of co-culture allows further co-maturation. With the addition approach, organoids are first cultured until they have reached a certain level of growth and development. Subsequently, microglia progenitor cells are added to the culture medium, whereafter they progressively migrate into the organoid, colonizing the whole structure (**Figure 3**). At present, this is the most commonly used technique, with numerous studies employing several variations of this approach (**Additional Tables 1** and **2**). One of the first protocols described by Abud et al. (2017) in which they differentiated microglia-like cells from human iPSC, while separately culturing brain organoids containing neurons and astrocytes. After 38 days of maturation, 5×10^5 microglia progenitors were added to 12-week-old brain organoids. Following migration into the organoids, microglia expressed numerous specific markers and were able to respond to physical injury (Abud et al., 2017). Similarly, most of the protocols based on the microglia addition method were able to obtain organoids containing neurons, astrocytes, and microglia (Abreu et al. 2018; Lin et al. 2018; Sabate-Soler et al., 2022). Brain organoids were kept in culture for different periods, starting from the shortest period of 14 days (Muffat et al., 2018) to 213 days (Fagerlund et al., 2022). Most of these works modeled neuroinflammation by adding pro-inflammatory stimuli, but these were also used within specific disease contexts such as Alzheimer's disease (Abud et al., 2017; Lin et al., 2018), Parkinson's disease (Smits et al., 2019), Zika virus and Dengue virus infections (Abreu et al., 2018; Muffat et al., 2018), frontotemporal dementia-like syndrome and Nasu-Hakola disease (Brownjohn et al., 2018). Lastly, although the above-referenced studies refer to hiPSC-derived immune-competent brain organoids, a similar approach has also been reported recently for the generation of murine iPSC-derived immune-competent brain organoids (Di Stefano et al., 2024). The importance of studying both murine and human immune-competent brain organoid models has been discussed above.

Highly advantageous for the addition approach, is that all cell types are already committed to a specific cellular fate and developing when they are put together, making it easier to co-culture them (**Table 1**). This however does not reflect the development *in vivo*, where immature neural precursor cells and primitive microglia interact with each other, differentiating and maturing together. The timing of microglia integration into organoids may impact the course of neuronal differentiation and maturation; the timeframe is therefore crucial for both populations to have the correct maturation stage (Xu et al., 2021; **Table 1**). Furthermore, microglia handling and transfer for the addition could cause their activation (Cadiz et al., 2022), potentially affecting the other neural cells. Nevertheless, the addition approach allows researchers to carefully choose the developmental stage of both the neural organoid as well as the microglial progenitor cells, thereby mimicking both the developmental stage as well as the timing of yolk-sac microglia progenitor cell migration into the developing brain (Di Stefano et al., 2024). Furthermore, both the co-culture and the addition approach are highly convenient as they employ exogenous microglia, thereby allowing the evaluation of the behavior of iPSC-derived microglia with specific genetic mutations, or even primary microglia directly isolated from brain tissue that may have retained most of their *in vivo* characteristics, such as transcriptomic profile and physiologic functions.

The spontaneous development approach

Third, the spontaneous development approach for generating microglia-containing brain organoids is largely based on the protocols of Lancaster and Knoblich (2014) and Ormel et al. (2018), utilizing matrigel embedding and a low concentration of heparin during the culture procedure. Here, microglia cells

develop spontaneously during organoid development, as evidenced by immunocytochemical analyses for the presence of Iba1^{+} cells (**Additional Table 2**). This approach was repeated and adapted in different works by various groups, undergoing further optimization and characterization (Bodnar et al., 2021), and was utilized to model SARS-CoV-2 (Samudyata et al., 2022; **Additional Table 2**).

The most important advantage of this approach is that there is no need for separate differentiation protocols, making this approach less time-consuming. Furthermore, given this all-at-once approach, it better replicates the co-development between neurons, astrocytes, and microglia that takes place *in vivo* (Xu et al., 2021; **Table 1**). However, the spontaneous development of microglia in these brain organoids does not accurately reflect the *in vivo* situation, where microglia progenitor cells originate from the yolk sac and migrate into the developing brain (Ginhoux et al., 2010 and 2013). Furthermore, due to the absence of SMAD inhibition, brain organoids generated with spontaneous formation may have the disadvantage of having a high degree of heterogeneity among the differentiated cell types, both in type as well as maturity (Quadrato et al., 2017; **Table 1**).

Characterization of Microglia in Brain Organoids

Microglia are highly specialized immune cells able to sense changes in the surrounding environment and to rapidly respond to these. Phenotypical and functional characterization of microglia can be achieved through a range of assays, including assessments of morphological changes, motility activity, phagocytic activity, cytokine release, and increased expression of complement receptors and histocompatibility complex molecules (**Figure 4**). Many of these microglia evaluation protocols include the participation of other cell types, underscoring the importance of utilizing multicellular models such as brain organoids, which better recapitulate these characteristics. The first and most classical way to characterize microglia is the analysis of their gene/protein expression profile (**Figure 4A**). However, finding specific markers to identify microglia can be challenging. For instance, the expression profile varies between fetal and mature microglia, between the homeostatic or activated state, between human or murine cells, as well as according to the culture conditions (Butovsky et al., 2014; Muffat et al., 2016; Abud et al., 2017; Douvaras et al., 2017; Haenseler et al., 2017; Pandya et al., 2017; Brownjohn et al., 2018; Ormel et al., 2018; McQuade et al., 2018; Reitboeck et al., 2018; Song et al., 2019; Elise Vankriekelsvenne et al., 2022). Furthermore, microglia and macrophages carry out the same functions, but the first in the CNS and the latter in the periphery. When macrophages infiltrate the brain, they even adopt a morphology that resembles microglia. Therefore, these two cell types share many markers, making their distinction challenging, even when using specific reporter mouse models (Le Blon et al., 2014, 2016; Dooley et al., 2016; Guglielmetti et al., 2016; Hamzei et al., 2018; Quarta et al., 2019). In 2014, Butovsky et al. identified six main genes to define human primary microglia: P2Ry12, C1QA, GPR34, PROS1, MERTK, and GAS6. Furthermore, TMEM119 and FCRLS are often referred to as microglia-specific markers, even though the first one is only expressed by homeostatic microglia in healthy CNS, while the second one is specific for murine models (Butovsky et al., 2014; Vankriekelsvenne et al., 2022). Nevertheless, many subsequent papers refer to these markers when they characterize their hiPSC-derived microglia (Muffat et al., 2016; Abud et al., 2017; Douvaras et al., 2017; Haenseler et al., 2017; Pandya et al., 2017; Brownjohn et al., 2018; McQuade et al., 2018; Ormel et al., 2018; Reitboeck et al., 2018; Song et al., 2019). An overview of the microglia-specific markers is represented in **Figure 4A**.

Two additional aspects that are commonly used to characterize microglia are their morphological properties and motility (**Figure 4B**). These features can provide valuable information, since microglia display very distinctive characteristics according to their developmental stage and activation state. In fact, they show an amoeboid shape when they enter the brain parenchyma; however, over time they acquire a ramified morphology, that is maintained in the adult brain. During the homeostatic state, microglia appear with a small cell body with extending motile processes, which are essential to scan the surrounding environment. These changes in morphology that happen during the development of microglia are observed in brain organoids too, where microglia migrate from the borders to the deeper layers of the organoids and slowly change morphology over time. These modifications in morphology are further described by Fagerlund and colleagues, who identified four different microglia morphology types: ramified, intermediate, rod-shaped, and spheric, outlining how the portion of ramified cells increases with time (Fagerlund et al., 2022). Furthermore, microglia morphology undergoes changes following an injury. When the brain experiences an injury or an infection, microglia become activated, retract their branches, and adopt a rounded morphology. These changes are also mirrored in the brain organoids. A mechanical stimulus like piercing an organoid with a needle, causes microglia to migrate and cluster around the injury site, where they acquire an amoeboid morphology (Abud et al., 2017). Similarly, virus exposure profoundly alters microglial morphology, with cells displaying fewer endpoints and shorter processes, suggesting microglial activation (Xu et al., 2021). The formation of a necrotic core that increases over culturing time, was shown to activate and attract microglia, that adopted a round morphology (Schafer et al., 2023). Finally, microglia-like cells harboring the APOE4 variant – the greatest genetic risk factor for sporadic Alzheimer's disease – exhibit altered morphologies with fewer and shorter processes (Lin et al., 2018).

Phagocytic activity is another important aspect of microglial behavior that undergoes changes according to their state (**Figure 4B**). Following activation, they acquire an amoeboid morphology, whereby the cells become mobile and able to phagocytose pathogens and damaged tissue. This functional activity appears to be maintained in brain organoids, where protrusions of the plasma membrane called phagocytic cups were observed (Popova et al., 2022). Different articles show how organoid-resident microglia can phagocytose apoptotic cells (Popova et al., 2022; Xu et al., 2022), but also small accidental foreign debris such as fragments from a glass pipette tip (Xu et al., 2022).

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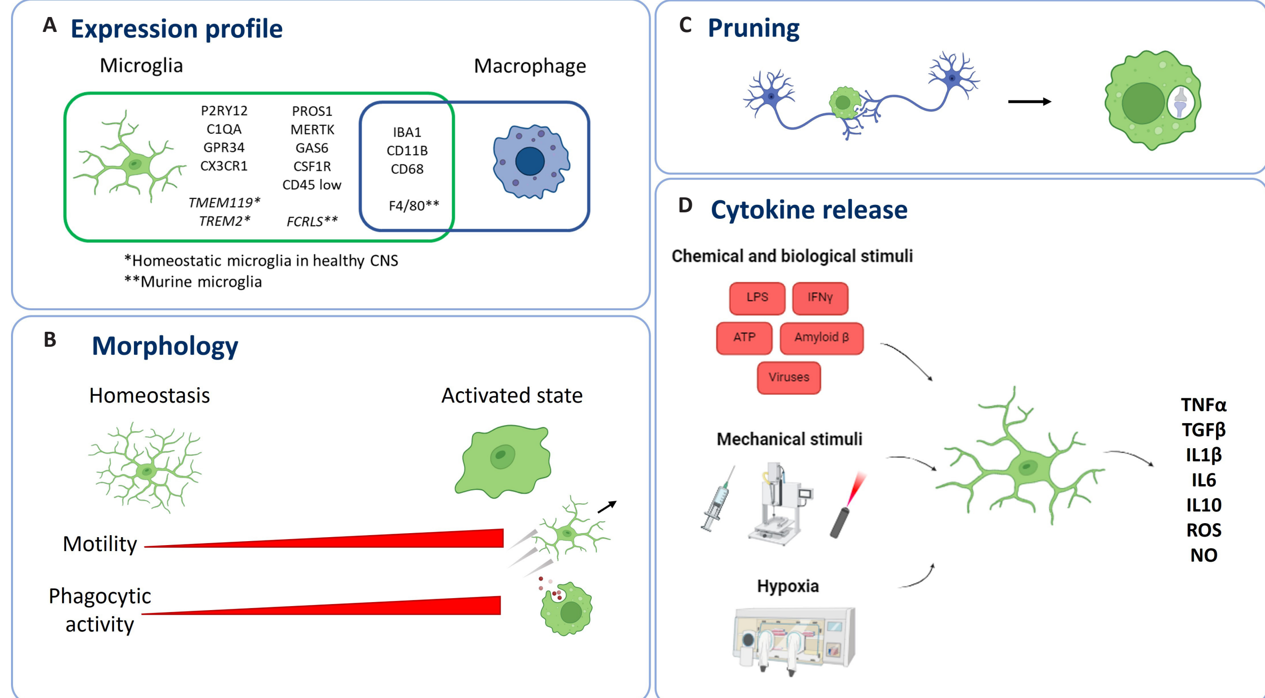

Figure 4 | Microglia-specific features used for characterization.
(A) Microglia-specific markers, (B) morphology, motility, and phagocytic activity of microglia during homeostasis and activated state, (C) microglia pruning synapses, (D) release of cytokines and other factors following noxious stimuli. Note that only a subset of all possible secreted factors is visualized in the picture. Created with BioRender.com. ATP: Adenosine triphosphate; C1QA: complement c1q A chain; CD11B: cluster of differentiation molecule 11B; CD68: cluster of differentiation 68; CNS: central nervous system; F4/80: EGF-like module-containing mucin-like hormone receptor-like 1; FCRLS: Fc receptor-like molecules; GAS6: growth arrest – specific 6; IBA1: ionized calcium-binding adapter molecule 1; IFNγ: interferon gamma; IL10: interleukin 10; IL1β: interleukin 1 beta; IL6: interleukin 6; LPS: lipopolysaccharide; MERTK: MER proto-oncogene, tyrosine kinase; NO: nitric oxide; P2RY12: purinergic receptor; P2Y12GPR34: G-protein coupled receptor 34; PROS1: protein; S1ROS: reactive oxygen species; TGFβ: transforming growth factor beta; TMEM119: transmembrane protein 119; TNFα: tumor necrosis factor alpha.

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Lastly, microglia also carry out important pruning functions in brain organoids (**Figure 4C**). With combined staining for CD68, a marker for the phagolysosome, Iba1 and post-synaptic marker PSD95, different groups reported the engulfment of postsynaptic structures (Popova et al., 2022; Samudyata et al., 2022; Xu et al., 2022). Additionally, the colocalization of synapsin1, PSD95, and CD68, indicates whole synapses pruned by the microglia in organoids (Xu et al., 2022). The synaptic pruning function can be altered during pathological conditions. Exposure to Zika virus (ZIKV) or Dengue virus DENV increases PSD95 engulfment in $CD68^+$ microglial phagolysosome compartments, with similar effects seen following SARS-CoV-2 infection (Samudyata et al. 2022; Xu et al., 2022).

Apart from their morphological and phenotypical features, functional properties of microglia need to be considered as well when aiming to achieve full characterization of microglia in brain organoids. Just as observed *in vivo*, microglia within brain organoids respond to noxious stimulation by releasing inflammatory cytokines (**Figure 4D**). The most popular agent being added to brain organoid cultures to activate microglia is lipopolysaccharide (LPS), being used in multiple studies with similar results. Abreu et al. (2018) observed increased gene expression of interleukin (IL)6, IL10, and IL1β with maximum expression at 6–12 hours post-exposure, while Ormel et al. (2018) saw an increased release of IL6 and tumor necrosis factor alpha (TNFα) after 24 and 72 hours, indicating both an acute and a long-term effect, coupled with increased mRNA levels of IL6 and TNFα after 72 hours of LPS stimulation. Additionally, cell viability decreased after LPS treatment (Abreu et al., 2018). Similarly, using an alternative activation approach, in a model of Alzheimer's disease, Song and colleagues investigated the immune response of microglia-containing brain organoids to $Aβ_{42}$ oligomers. They observed differential expression of the TNFα, prostaglandin E2, and vascular endothelial growth factor-A genes, along with an increase in the production of reactive oxygen species (Song et al., 2019).

Going beyond classical pro-inflammatory stimulation of microglia within brain organoids, viral infection studies have recently also emerged using microglia-containing brain organoid models. Studies involving ZIKV showed increased expression of the IL6, IL1β, TNFα, and CCL2 genes (Abreu et al., 2018), the release of CCL2 and IL8 (Muffat 2018), along with higher expression of inflammatory cytokines IL6, IL1β, and TNFα by qRT-PCR (Xu et al., 2022). Additionally, mRNA expression of INFAR1 and INFAR2 was also found to be increased after ZIKV exposure (Xu et al., 2022). Similarly, DENV caused increased expression of the IL6, IL1β, TNFα, and CCL2 genes (Abreu et al., 2018). When infected with DENV, microglia increased the release of cytokines and chemokines CCL5, C5, and CXCL10, while human immunodeficiency

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virus (HIV) elicited the release of TNFα and IL 1β (Dos Reis et al., 2020). In contrast, following SARS-CoV-2 exposure, no upregulation of pro-inflammatory cytokines was detected, neither at 24 hours post-infection nor at 72 hours post-infection (Samudyata 2022).

More recently, our group performed untargeted transcriptomic and proteomic analyses of microglia-containing miPSC-derived brain organoids under steady-state and pro-inflammatory stimulation (LPS + interferon γ). Using a multi-omics approach, this study demonstrated a higher level of (i) downstream inflammatory responses, (ii) impairment of homeostatic and developmental processes, and (iii) activation of cell death processes in immune-stimulated microglia-containing brain organoids, as compared to immune-stimulated brain organoids without microglia. Furthermore, a large number of downstream pathways identified in this study go beyond inflammatory responses pointing to new avenues to study developmental and homeostatic features of brain organoids (Di Stefano et al., 2024).

Promising Current and Future Applications of Microglia-Containing Brain Organoids

Beyond the microglia characterization stage, as discussed above, brain organoids' newer applications encompass (i) the assessment of microglia effects on the efficacy of neuronal excitability and the development of neuronal networks, (ii) the analysis of the immune-modulating properties of microglia, (iii) and their use as a screening tool for the validation of potential therapeutic anti-inflammatory compounds.

The influence of microglia on neuronal differentiation and network formation

Microglia play a crucial role in influencing neuronal differentiation through their interactions with neuronal populations, modulating key processes such as neurogenesis, migration, and synaptic connectivity (Cunningham et al., 2013; Squarzoni et al., 2014).

The importance of microglia in the formation of an efficient neuronal network is evident when analyzing microglia-containing brain organoids, using a variety of electrophysiological measurement tools, in comparison to brain organoids without microglia. Looking at whole-cell patch-clamp recordings from neurons in organoid slices, it has been demonstrated that these exhibit K^+ and Na^+ voltage-gated currents, spontaneous action potentials, and spontaneous post-synaptic currents, proving the presence of functional neurons in organoids containing microglia (Ormel et al., 2018; Xu et al., 2021). When compared to their counterpart without microglia, neurons from microglia-containing organoids show larger K^+ and mean Na^+ current density, larger prevalence of repetitive action potential firing neurons, and are more likely to express active ionic currents. Additionally, the spontaneous excitatory post-synaptic currents were only present in neurons from microglia-containing organoids but were absent in organoids without microglia (Fagerlund et al., 2022). Furthermore, the voltage threshold for the action potential generation was more negative in the group with microglia, indicating the increased neuronal excitability of the mature neurons (Sabate-Soler et al., 2022). Using an alternative approach, analyses with a multi-electrode array applied on the surface of organoids or organoids slices, demonstrated that neurons in microglia-containing organoids display a lower interspike interval (Sabate-Soler et al., 2022) and increased synchronization and frequency of oscillatory bursts compared to organoids without microglia (Fagerlund et al., 2022; Popova et al., 2022).

Microglia also have been shown to regulate the ratio of NSCs/NPCs (Cunningham et al., 2013; Miyamoto et al., 2016; Weinhard et al., 2018); in fact, they can promote neurogenesis from NPCs by releasing factors such as insulin growth factor 1 and IL1β (Ueno et al., 2013; Casano and Peri, 2015) but also avoid premature neurogenesis through the expression of notch receptors. The detection of these cytokines in microglia-containing organoids (Xu et al., 2021), together with the upregulation in notch receptors expression, suggest that there is a regulation in the balance between the progenitor pool and the neuron pool (Bejoy et al., 2019).

Concluding, the presence of microglia in brain organoids thus increases the development and the maturation of neuronal properties and network formation and stimulates more diverse neuronal phenotypes in organoids with microglia (Bodnar et al., 2021; Fagerlund et al., 2022).

The influence of physical stress on microglia-containing brain organoids

To study the immune functions related to microglia, brain organoids need to undergo a stress-inducing event able to activate inflammatory responses. While the direct addition of pro-inflammatory stimuli (such as LPS, interferon γ, ATP, amyloid β42 oligomers, and viruses) have been discussed above, several other types of organoid injury can be applied. The most evolved belong to the category of mechanical stimuli, such as needle piercing, cortical impact, and focal laser injury. Given their small size, the physical injury has to be delivered in a precise and controlled way. Abud and colleagues used a 25-gauge needle to pierce their immune-competent brain organoids, inducing migration and clustering of microglia around the injury site, which showed an amoeboid morphology (Abud et al., 2017). In another approach, 2-photon-induced focal laser injury was applied to brain organoids grafted into mice brains. This elicited a response in the surrounding microglia which migrated and extended their processes towards the injured site (Schafer et al., 2023). An alternative option is to perform controlled cortical impact on brain organoids. In a study by Ramirez et al., they used a controlled cortical impact device and observed astrogliosis. Even though microglia cells were not yet present in their model, this work represents a potential direction for future research on immune-competent organoids (Ramirez et al. 2021). Lastly, in several recent studies, a condition of oxygen and glucose deprivation was applied to hiPSC-derived neurospheroids and cortical organoids to simulate hypoxic brain injury or stroke-like events (Paşca et al., 2019; Van Breedam et al., 2022; De Paola et al., 2023). Here, additional organoid interrogation tools were presented, including live cell bioluminescence imaging, and the determination of glial

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fibrillary acidic protein and neurofilament release, as markers for organoid damage after an ischemic insult. Although no microglia were incorporated in either of these studies, they have laid the groundwork for replicating the same type of experiment on immune-competent brain organoids.

The Ultimate Toolbox for Characterization of Microglia-Containing Brain Organoids

This review aims at summarizing some of the latest advancements in the development of immune-competent brain organoids. The most recent works using immune-competent brain organoids were collected and divided into three main groups, according to the method of microglia insertion inside the organoids: co-culture, addition, and spontaneous development. While the technique of addition of microglia is the most commonly found in literature so far, there is no definitive method for obtaining microglia-containing brain organoids. In fact, each approach has its own set of advantages and disadvantages (summarized in **Table 1**). Nevertheless, these models have been applied to various types of immune stimulation, including direct and indirect microglial stimulation, which were used to reproduce and study numerous neurodegenerative diseases. Some examples include Alzheimer's disease (Abud et al., 2017; Lin et al., 2018; Jin et al., 2022), Zika or dengue virus (Abreu et al., 2018; Muffat et al., 2018; Xu et al., 2021), Down syndrome (Jin et al., 2022), frontotemporal dementia–like syndrome and Nasu-Hakola disease (Brownjohn et al., 2018), HIV (Dos Reis et al., 2020; Gumbs et al., 2022; Donadoni et al., 2024, Narasipura et al., 2024), and SARS-CoV-2 (Samudyata et al., 2022).

Using a variety of experimental tools, brain organoids have been interrogated, however, there is currently no real consensus regarding the ultimate characterization toolbox for brain organoids that allows comparison of different studies. Ending this work, we would like to propose a generalized workflow to fully understand neuronal, astrocyte, and microglial behavior in (immune) stressed brain organoids (**Figure 5**). We therefore present a comprehensive brain organoid interrogation toolbox, including live-cell Ca^{2+}-imaging and multi-electrode array recordings (to determine electrophysiological network behavior), multi-omics analyses (including transcriptomics, proteomics, and metabolomics), multiplex cytokine assay for secretome profiling, as well as intensive microscopic imaging (to validate multi-omics findings). Using the latter, we believe that brain organoid research, especially those that contain microglia and eventually also endothelial cells, will arrive at a stage of maturity and will truly be able to accurately mimic pathological events from a wide range of neurological diseases and trauma in a multicellular context.

Search Strategy

The studies mentioned in this review were primarily searched using PubMed and Google, and the most commonly used search terms likely included: "microglia-containing brain organoids," "brain organoids," and "immune-competent brain organoids." No specific limits or filters were applied during the search.

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Data availability statement: *All relevant data are within the manuscript and its Additional files.*

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Additional files:

Additional Table 1: *Details of papers that used co-culture or addition (Pt. 1) approaches.*

Additional Table 2: *Details of papers that used addition (Pt. 2) or spontaneous development approaches.*

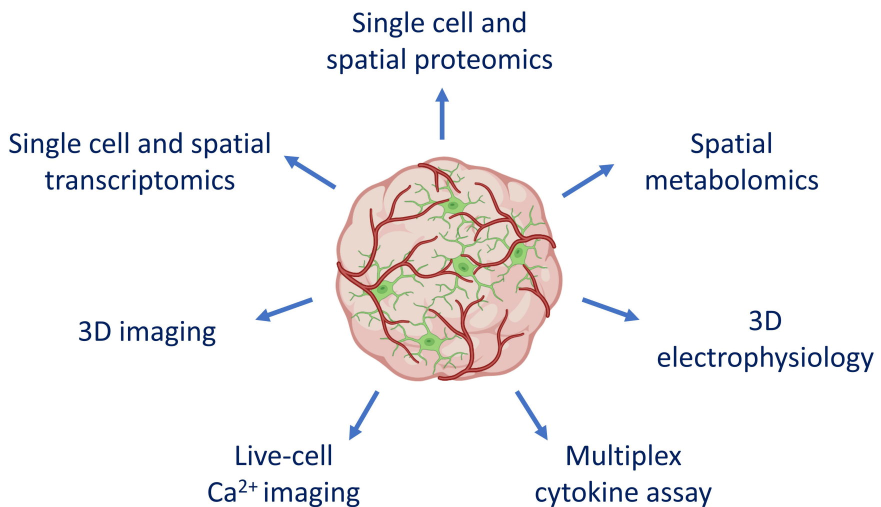

Figure 5 | **The future brain organoid with microglia and vasculature and the relative interrogation toolbox.**
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References

Abbas ZN, Al-Saffar AZ, Jasim SM, Sulaiman GM (2023) Comparative analysis between 2D and 3D colorectal cancer culture models for insights into cellular morphological and transcriptomic variations. Sci Rep 13:18380.

Abreu CM, Gama L, Krasemann S, Chesnut M, Odwin-Dacosta S, Hogberg HT, Hartung T, Pamies D (2018) Microglia increase inflammatory responses in iPSC-derived human brainspheres. Front Microbiol 9:2766.

Abud EM, et al. (2017) iPSC-derived human microglia-like cells to study neurological diseases. Neuron 94:278-293.

Bejoy J, Yuan X, Song L, Hua T, Jeske R, Sart S, Sang QA, Li Y (2019) Genomics analysis of metabolic pathways of human stem cell-derived microglia-like cells and the integrated cortical spheroids. Stem Cells Int 2019:2382534.

Benito-Kwiecinski S, Giandomenico SL, Sutcliffe M, Riis ES, Freire-Pritchett P, Kelava I, Wunderlich S, Martin U, Wray GA, McDole K, Lancaster MA (2021) An early cell shape transition drives evolutionary expansion of the human forebrain. Cell 184:2084-2102.

Bershteyn M, Nowakowski TJ, Pollen AA, Di Lullo E, Nene A, Wynshaw-Boris A, Kriegstein AR (2017) Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia. Cell Stem Cell 20:435-449.

Birey F, Andersen J, Makinson CD, Islam S, Wei W, Huber N, Fan HC, Metzler KRC, Panagiotakos G, Thom N, O'Rourke NA, Steinmetz LM, Bernstein JA, Hallmayer J, Huguenard JR, Paşca SP (2017) Assembly of functionally integrated human forebrain spheroids. Nature 545:54-59.

Bodnar B, Zhang Y, Liu J, Lin Y, Wang P, Wei Z, Saribas S, Zhu Y, Li F, Wang X, Yang W, Li Q, Ho WZ, Hu W (2021) Novel scalable and simplified system to generate microglia-containing cerebral organoids from human induced pluripotent stem cells. Front Cell Neurosci 15:682272.

Brownjohn PW, Smith J, Solanki R, Lohmann E, Houlden H, Hardy J, Dietmann S, Livesey FJ (2018) Functional studies of missense TREM2 mutations in human stem cell-derived microglia. Stem Cell Reports 10:1294-1307.

Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B, Wu PM, Doykan CE, Fanek Z, Liu L, Chen Z, Rothstein JD, Ransohoff RM, Gygi SP, Antel JP, Weiner HL (2014) Identification of a unique TGF-β-dependent molecular and functional signature in microglia. Nat Neurosci 17:131-143.

Cadiz MP, Jensen TD, Sens JP, Zhu K, Song WM, Zhang B, Ebbert M, Chang R, Fryer JD (2022) Culture shock: microglial heterogeneity, activation, and disrupted single-cell microglial networks in vitro. Mol Neurodegener 17:26.

Casano AM, Peri F (2015) Microglia: multitasking specialists of the brain. Dev Cell 32:469-477.

Centeno EGZ, Cimarosti H, Bithell A (2018) 2D versus 3D human induced pluripotent stem cell-derived cultures for neurodegenerative disease modelling. Mol Neurodegener 13:27.

Cesarz Z, Tamama K (2016) Spheroid culture of mesenchymal stem cells. Stem Cells Int 2016:9176357.

Cho AN, et al. (2021) Microfluidic device with brain extracellular matrix promotes structural and functional maturation of human brain organoids. Nat Commun 12:4730.

Choi H, Song J, Park G, Kim J (2017) Modeling of autism using organoid technology. Mol Neurobiol 54:7789-7795.

Cooper LL, Li W, Lu Y, Centracchio J, Terentyeva R, Koren G, Terentyev D (2013) Redox modification of ryanodine receptors by mitochondria-derived reactive oxygen species contributes to aberrant Ca2+ handling in ageing rabbit hearts. J Physiol 591:5895-5911.

Cunningham CL, Martínez-Cerdeño V, Noctor SC (2013) Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33:4216-33.

De Paola M, Pischiutta F, Comolli D, Mariani A, Kelk J, Lisi I, Cerovic M, Fumagalli S, Forloni G, Zanier ER (2023) Neural cortical organoids from self-assembling human iPSC as a model to investigate neurotoxicity in brain ischemia. J Cereb Blood Flow Metab 43:680-693.

Deforest CA, Sims EA, Anseth KS (2010) Peptide-functionalized click hydrogels with independently tunable mechanics and chemical functionality for 3D cell culture. Chem Mater 22:4783-4790.

Deng B, Li Q, Liu X, Cao Y, Li B, Qian Y, Xu R, Mao R, Zhou E, Zhang W, Huang J, Rao Y (2019) Chemoconnectomics: mapping chemical transmission in drosophila. Neuron 101:876-893.

Di Stefano J, et al. (2024) Transcriptomic and proteomic profiling of bi-partite and tri-partite murine iPSC-derived neurospheroids under steady-state and inflammatory condition. Brain Behav Immun 121:1-12.

Discher DE, Janmey P, Wang YL (2005) Tissue cells feel and respond to the stiffness of their substrate. Science (New York, NY) 310:1139-1143.

Donadoni M, Cakir S, Bellizzi A, Swingler M, Sariyer IK (2024) Modeling HIV-1 infection and NeuroHIV in hiPSCs-derived cerebral organoid cultures. J Neurovirol 30:362-379 .

Dooley D, Lemmens E, Ponsaerts P, Hendrix S (2016) Interleukin-25 is detrimental for recovery after spinal cord injury in mice. J Neuroinflammation 13:101.

Dos Reis RS, Sant S, Keeney H, Wagner MCE, Ayyavoo V (2020) Modeling HIV-1 neuropathogenesis using three-dimensional human brain organoids (hBORGs) with HIV-1 infected microglia. Sci Rep 10:15209.

Douvaras P, Sun B, Wang M, Kruglikov I, Lallos G, Zimmer M, Terrenoire C, Zhang B, Gandy S, Schadt E, Freytes DO, Noggle S, Fossati V (2017) Directed differentiation of human pluripotent stem cells to microglia. Stem Cell Reports 8:1516-1524.

Dunn JC, Tompkins RG, Yarmush ML (1992) Hepatocytes in collagen sandwich: evidence for transcriptional and translational regulation. J Cell Biol 116:1043-1053.

Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, Elvassore N, Piccolo S (2011) Role of YAP/TAZ in mechanotransduction. Nature 474:179-183.

Edmondson R, Broglie JJ, Adcock AF, Yang L (2014) Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol 12:207-218.

Evans M (1981) Origin of mouse embryonal carcinoma cells and the possibility of their direct isolation into tissue culture. J Reprod Fertil 62:625-631.

Fagerlund I, Dougalis A, Shakirzyanova A, Gómez-Budia M, Pelkonen A, Konttinen H, Ohtonen S, Fazaludeen MF, Koskuvi M, Kuusisto J, Hernández D, Pebay A, Koistinaho J, Rauramaa T, Lehtonen Š, Korhonen P, Malm T (2021) Microglia-like cells promote neuronal functions in cerebral organoids. Cells 11:124.

Garcia-Reitboeck P, Phillips A, Piers TM, Villegas-Llerena C, Butler M, Mallach A, Rodrigues C, Arber CE, Heslegrave A, Zetterberg H, Neumann H, Neame S, Houlden H, Hardy J, Pocock JM (2018) Human induced pluripotent stem cell-derived microglia-like cells harboring TREM2 missense mutations show specific deficits in phagocytosis. Cell Rep 24:2300-2311.

Garland EF, Hartnell IJ, Boche D (2022) Microglia and astrocyte function and communication: what do we know in humans? Front Neurosci 16:824888.

Gilbert PM, Havenstrite KL, Magnusson KE, Sacco A, Leonardi NA, Kraft P, Nguyen NK, Thrun S, Lutolf MP, Blau HM (2010) Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science 329:1078-1081.

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841-845.

Ginhoux F, Lim S, Hoeffel G, Low D, Huber T (2013) Origin and differentiation of microglia. Front Cell Neurosci 7:45.

Guglielmetti C, Veraart J, Roelant E, Mai Z, Daans J, Van Audekerke J, Naeyaert M, Vanhoutte G, Delgado YPR, Praet J, Fieremans E, Ponsaerts P, Sijbers J, Van der Linden A, Verhoye M (2016) Diffusion kurtosis imaging probes cortical alterations and white matter pathology following cuprizone induced demyelination and spontaneous remyelination. NeuroImage 125:363-377.

Gumbs SBH, Berdenis van Berlekom A, Kübler R, Schipper PJ, Gharu L, Boks MP, Ormel PR, Wensing AMJ, de Witte LD, Nijhuis M (2022) Characterization of HIV-1 infection in microglia-containing human cerebral organoids. Viruses 14:829.

Haenseler W, Sansom SN, Buchrieser J, Newey SE, Moore CS, Nicholls FJ, Chintawar S, Schnell C, Antel JP, Allen ND, Cader MZ, Wade-Martins R, James WS, Cowley SA (2017) A highly efficient human pluripotent stem cell microglia model displays a neuronal-co-culture-specific expression profile and inflammatory response. Stem Cell Reports 8:1727-1742.

Hamzei Taj S, Le Blon D, Hoornaert C, Daans J, Quarta A, Praet J, Van der Linden A, Ponsaerts P, Hoehn M (2018) Targeted intracerebral delivery of the anti-inflammatory cytokine IL13 promotes alternative activation of both microglia and macrophages after stroke. J Neuroinflammation 15:174.

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Hartley BJ, Brennand KJ (2017) Neural organoids for disease phenotyping, drug screening and developmental biology studies. Neurochem Int 106:85-93.

Heo JH, Kang D, Seo SJ, Jin Y (2022) Engineering the extracellular matrix for organoid culture. Int J Stem Cells 15:60-69.

Imamura Y, Mukohara T, Shimono Y, Funakoshi Y, Chayahara N, Toyoda M, Kiyota N, Takao S, Kono S, Nakatsura T, Minami H (2015) Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. Oncol Rep 33:1837-1843.

Jacob F, Schnoll JG, Song H, Ming GL (2021) Building the brain from scratch: Engineering region-specific brain organoids from human stem cells to study neural development and disease. Curr Top Dev Biol 142:477-530.

Jacob L, de Brito Neto J, Lenck S, Corcy C, Benbelkacem F, Geraldo LH, Xu Y, Thomas JM, El Kamouh MR, Spajer M, Potier MC, Haik S, Kalamarides M, Stankoff B, Lehericy S, Eichmann A, Thomas JL (2022) Conserved meningeal lymphatic drainage circuits in mice and humans. J Exp Med 219:e20220035.

Jin M, Xu R, Wang L, Alam MM, Ma Z, Zhu S, Martini AC, Jadali A, Bernabucci M, Xie P, Kwan KY, Pang ZP, Head E, Liu Y, Hart RP, Jiang P (2022) Type-I-interferon signaling drives microglial dysfunction and senescence in human iPSC models of Down syndrome and Alzheimer's disease. Cell Stem Cell 29:1135-1153.

Kasai T, et al. (2020) Hypothalamic contribution to pituitary functions is recapitulated in vitro using 3D-cultured human iPS cells. Cell Rep 30:18-24.

Kim J, Koo BK, Knoblich JA (2020) Human organoids: model systems for human biology and medicine. Nat Rev Mol Cell Biol 21:571-584.

Koo B, Choi B, Park H, Yoon KJ (2019) Past, present, and future of brain organoid technology. Mol Cells 42:617-627.

Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA (2013) Cerebral organoids model human brain development and microcephaly. Nature 501:373-379.

Lancaster MA, Knoblich JA (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. Science 345:1247125.

Langhans SA (2018) Three-dimensional in vitro cell culture models in drug discovery and drug repositioning. Front Pharmacol 9:6.

Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39:151-170.

Le Blon D, Hoornaert C, Daans J, Santermans E, Hens N, Goossens H, Berneman Z, Ponsaerts P (2014) Distinct spatial distribution of microglia and macrophages following mesenchymal stem cell implantation in mouse brain. Immunol Cell Biol 92:650-658.

Le Blon D, Guglielmetti C, Hoornaert C, Quarta A, Daans J, Dooley D, Lemmens E, Praet J, De Vocht N, Reekmans K, Santermans E, Hens N, Goossens H, Verhoye M, Van der Linden A, Berneman Z, Hendrix S, Ponsaerts P (2016) Intracerebral transplantation of interleukin 13-producing mesenchymal stem cells limits microgliosis, oligodendrocyte loss and demyelination in the cuprizone mouse model. J Neuroinflammation 13:288.

Li Y, Muffat J, Omer A, Bosch I, Lancaster MA, Sur M, Gehrke L, Knoblich JA, Jaenisch R (2017) Induction of expansion and folding in human cerebral organoids. Cell Stem Cell 20:385-396.

Li C, Fleck JS, Martins-Costa C, Burkard TR, Themann J, Stuempflen M, Peer AM, Vertesy Á, Littleboy JB, Esk C, Elling U, Kasprian G, Corsini NS, Treutlein B, Knoblich JA (2023) Single-cell brain organoid screening identifies developmental defects in autism. Nature 621:373-380. Erratum in: Nature 623:E20.

Licata JP, Schwab KH, Har-El YE, Gerstenhaber JA, Lelkes PI (2023) Bioreactor technologies for enhanced organoid culture. Int J Mol Sci 24:11427.

Lin YT, et al. (2018) APOE4 causes widespread molecular and cellular alterations associated with Alzheimer's disease phenotypes in human iPSC-derived brain cell types. Neuron 98:1141-1154.

Lu X, Yang J, Xiang Y (2022) Modeling human neurodevelopmental diseases with brain organoids. Cell Regen 11:1.

Mansour AA, Gonçalves JT, Bloyd CW, Li H, Fernandes S, Quang D, Johnston S, Parylak SL, Jin X, Gage FH (2018) An in vivo model of functional and vascularized human brain organoids. Nat Biotechnol 36:432-441.

Mariani J, Simonini MV, Palejev D, Tomasini L, Coppola G, Szekely AM, Horvath TL, Vaccarino FM (2012) Modeling human cortical development in vitro using induced pluripotent stem cells. Proc Natl Acad Sci U S A 109:12770-12775.

Marshall JJ, Mason JO (2019) Mouse vs man: organoid models of brain development & disease. Brain Res 1724:146427.

Mayer S, et al. (2019) Multimodal single-cell analysis reveals physiological maturation in the developing human neocortex. Neuron 102:143-158.

McQuade A, Coburn M, Tu CH, Hasselmann J, Davtyan H, Blurton-Jones M (2018) Development and validation of a simplified method to generate human microglia from pluripotent stem cells. Mol Neurodegener 13:67.

Meng X, Yao D, Imaizumi K, Chen X, Kelley KW, Reis N, Thete MV, Arjun McKinney A, Kulkarni S, Panagiotakos G, Bassik MC, Pașca SP (2023) Assembloid CRISPR screens reveal impact of disease genes in human neurodevelopment. Nature 622:359-366.

Miller JF, Neufang M, Solway A, Brandt A, Trippel M, Mader I, Hefft S, Merkow M, Polyn SM, Jacobs J, Kahana MJ, Schulze-Bonhage A (2013) Neural activity in human hippocampal formation reveals the spatial context of retrieved memories. Science 342:1111-1114.

Mittelbronn M, Dietz K, Schluesener HJ, Meyermann R (2001) Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. Acta Neuropathol 101:249-255.

Miyamoto A, Wake H, Ishikawa AW, Eto K, Shibata K, Murakoshi H, Koizumi S, Moorhouse AJ, Yoshimura Y, Nabekura J (2016) Microglia contact induces synapse formation in developing somatosensory cortex. Nat Commun 7:12540.

Muffat J, Li Y, Omer A, Durbin A, Bosch I, Bakiasi G, Richards E, Meyer A, Gehrke L, Jaenisch R (2018) Human induced pluripotent stem cell-derived glial cells and neural progenitors display divergent responses to Zika and dengue infections. Proc Natl Acad Sci U S A 115:7117-7122.

Muñiz AJ, Topal T, Brooks MD, Sze A, Kim DH, Jordahl J, Nguyen J, Krebsbach PH, Savelieff MG, Feldman EL, Lahann J (2023) Engineered extracellular matrices facilitate brain organoids from human pluripotent stem cells. Ann Clin Transl Neurol 10:1239-1253.

Narasipura SD, Zayas JP, Ash MK, Reyes A, Shull T, Gambut S, Schneider JR, Lorenzo-Redondo R, Al-Harthi L, Mamede JI (2024) HIV-1 infection promotes neuroinflammation and neuron pathogenesis in novel microglia-containing cerebral organoids. bioRxiv [Preprint]. doi: 10.1101/2024.06.13.598579.

Nashimoto Y, Teraoka Y, Banan Sadeghian R, Nakamasu A, Arima Y, Hanada S, Kotera H, Nishiyama K, Miura T, Yokokawa R (2018) Perfusable vascular network with a tissue model in a microfluidic device. J Vis Exp doi: 10.3791/57242.

Ormel PR, Vieira de Sá R, van Bodegraven EJ, Karst H, Harschnitz O, Sneeboer MAM, Johansen LE, van Dijk RE, Scheefhals N, Berdenis van Berlekom A, Ribes Martínez E, Kling S, MacGillavry HD, van den Berg LH, Kahn RS, Hol EM, de Witte LD, Pasterkamp RJ (2018) Microglia innately develop within cerebral organoids. Nat Commun 9:4167.

Pandya H, Shen MJ, Ichikawa DM, Sedlock AB, Choi Y, Johnson KR, Kim G, Brown MA, Elkahloun AG, Maric D, Sweeney CL, Gossa S, Malech HL, McGavern DB, Park JK (2017) Differentiation of human and murine induced pluripotent stem cells to microglia-like cells. Nat Neurosci 20:753-759.

Pașca AM, Park JY, Shin HW, Qi Q, Revah O, Krasnoff R, O'Hara R, Willsey AJ, Palmer TD, Pașca SP (2019) Human 3D cellular model of hypoxic brain injury of prematurity. Nat Med 25:784-791.

Pham MT, Pollock KM, Rose MD, Cary WA, Stewart HR, Zhou P, Nolta JA, Waldau B (2018) Generation of human vascularized brain organoids. Neuroreport 29:588-593.

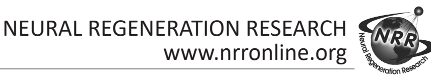
Place TL, Domann FE, Case AJ (2017) Limitations of oxygen delivery to cells in culture: An underappreciated problem in basic and translational research. Free Radic Biol Med 113:311-322.

Pollen AA, et al. (2019) Establishing cerebral organoids as models of human-specific brain evolution. Cell 176:743-756.

Popova G, Soliman SS, Kim CN, Keefe MG, Hennick KM, Jain S, Li T, Tejera D, Shin D, Chhun BB, McGinnis CS, Speir M, Gartner ZJ, Mehta SB, Haeussler M, Hengen KB, Ransohoff RR, Piao X, Nowakowski TJ (2021) Human microglia states are conserved across experimental models and regulate neural stem cell responses in chimeric organoids. Cell Stem Cell 28:2153-2166.

Qian X, et al. (2016) Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell 165:1238-1254.

Qian X, Jacob F, Song MM, Nguyen HN, Song H, Ming GL (2018) Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. Nat Protoc 13:565-580.



Quadrato G, Nguyen T, Macosko EZ, Sherwood JL, Min Yang S, Berger DR, Maria N, Scholvin J, Goldman M, Kinney JP, Boyden ES, Lichtman JW, Williams ZM, McCarroll SA, Arlotta P (2017) Cell diversity and network dynamics in photosensitive human brain organoids. Nature 545:48-53.
Quarta A, Le Blon D, D'Aes T, Pieters Z, Hamzei Taj S, Miró-Mur F, Luyckx E, Van Breedam E, Daans J, Goossens H, Dewilde S, Hens N, Pasque V, Planas AM, Hoehn M, Berneman Z, Ponsaerts P (2019) Murine iPSC-derived microglia and macrophage cell culture models recapitulate distinct phenotypical and functional properties of classical and alternative neuro-immune polarisation. Brain Behav Immun 82:406-421.
Quarta A, Berneman Z, Ponsaerts P (2021) Functional consequences of a close encounter between microglia and brain-infiltrating monocytes during CNS pathology and repair. J Leukoc Biol 110:89-106.
Ramirez S, Mukherjee A, Sepulveda SE, Gherardelli C, Becerra-Calixto A, Bravo-Vasquez N, Soto C (2021) Protocol for controlled cortical impact in human cerebral organoids to model traumatic brain injury. STAR Protoc 2:100987.
Ravi M, Paramesh V, Kaviya SR, Anuradha E, Solomon FD (2015) 3D cell culture systems: advantages and applications. J Cell Physiol 230:16-26.
Romero-Morales AI, O'Grady BJ, Balotin KM, Bellan LM, Lippmann ES, Gama V (2019) Spin∞: an updated miniaturized spinning bioreactor design for the generation of human cerebral organoids from pluripotent stem cells. HardwareX 6:e00084.
Sabate-Soler S, Nickels SL, Saraiva C, Berger E, Dubonyte U, Barmpa K, Lan YJ, Kouno T, Jarazo J, Robertson G, Sharif J, Koseki H, Thome C, Shin JW, Cowley SA, Schwamborn JC (2022) Microglia integration into human midbrain organoids leads to increased neuronal maturation and functionality. Glia 70:1267-1288.
Sakaguchi H, Kadoshima T, Soen M, Narii N, Ishida Y, Ohgushi M, Takahashi J, Eiraku M, Sasai Y (2015) Generation of functional hippocampal neurons from self-organizing human embryonic stem cell-derived dorsomedial telencephalic tissue. Nat Commun 6:8896.
Samudyata, Oliveira AO, Malwade S, Rufino de Sousa N, Goparaju SK, Gracias J, Orhan F, Steponaviciute L, Schalling M, Sheridan SD, Perlis RH, Rothfuchs AG, Sellgren CM (2022) SARS-CoV-2 promotes microglial synapse elimination in human brain organoids. Mol Psychiatry 27:3939-3950.
Schafer ST, Mansour AA, Schlachetzki JCM, Pena M, Ghassemzadeh S, Mitchell L, Mar A, Quang D, Stumpf S, Ortiz IS, Lana AJ, Baek C, Zaghal R, Glass CK, Nimmerjahn A, Gage FH (2023) An in vivo neuroimmune organoid model to study human microglia phenotypes. Cell 186:2111-2126.
Schmid CD, Sautkulis LN, Danielson PE, Cooper J, Hasel KW, Hilbush BS, Sutcliffe JG, Carson MJ (2002) Heterogeneous expression of the triggering receptor expressed on myeloid cells-2 on adult murine microglia. J Neurochem 83:1309-1320.
Schwartz MP, Hou Z, Propson NE, Zhang J, Engstrom CJ, Santos Costa V, Jiang P, Nguyen BK, Bolin JM, Daly W, Wang Y, Stewart R, Page CD, Murphy WL, Thomson JA (2015) Human pluripotent stem cell-derived neural constructs for predicting neural toxicity. Proc Natl Acad Sci U S A 112:12516-12521.
Seibler P, Graziotto J, Jeong H, Simunovic F, Klein C, Krainc D (2011) Mitochondrial Parkin recruitment is impaired in neurons derived from mutant PINK1 induced pluripotent stem cells. J Neurosci 31:5970-5976.
Shi Y, Sun L, Wang M, Liu J, Zhong S, Li R, Li P, Guo L, Fang A, Chen R, Ge WP, Wu Q, Wang X (2020) Vascularized human cortical organoids (vOrganoids) model cortical development in vivo. PLoS Biol 18:e3000705.
Simsa R, Rothenbücher T, Gürbüz H, Ghosheh N, Emneus J, Jenndahl L, Kaplan DL, Bergh N, Serrano AM, Fogelstrand P (2021) Brain organoid formation on decellularized porcine brain ECM hydrogels. PLoS One 16:e0245685.
Sloan SA, Darmanis S, Huber N, Khan TA, Birey F, Caneda C, Reimer R, Quake SR, Barres BA, Paşca SP (2017) Human astrocyte maturation captured in 3D cerebral cortical spheroids derived from pluripotent stem cells. Neuron 95:779-790.
Sloan SA, Andersen J, Pașca AM, Birey F, Pașca SP (2018) Generation and assembly of human brain region-specific three-dimensional cultures. Nat Protoc 13:2062-2085.
Smits LM, et al. (2019) Modeling Parkinson's disease in midbrain-like organoids. NPJ Parkinsons Dis 5:5.
Song L, Yuan X, Jones Z, Vied C, Miao Y, Marzano M, Hua T, Sang QA, Guan J, Ma T, Zhou Y, Li Y (2019) Functionalization of brain region-specific spheroids with isogenic microglia-like cells. Sci Rep 9:11055.
Squarzoni P, Oller G, Hoeffel G, Pont-Lezica L, Rostaing P, Low D, Bessis A, Ginhoux F, Garel S (2014) Microglia modulate wiring of the embryonic forebrain. Cell Rep 8:1271-1279.
Studer L, Vera E, Cornacchia D (2015) Programming and reprogramming cellular age in the era of induced pluripotency. Cell Stem Cell 16:591-600.
Sun BB, Kurki MI, Foley CN, Mechakra A, Chen CY, Marshall E, Wilk JB, Chahine M, Chevalier P, Christé G, Palotie A, Daly MJ, Runz H (2022) Genetic associations of protein-coding variants in human disease. Nature 603:95-102.
Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861-872.
Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145-1147.
Trujillo CA, Gao R, Negraes PD, Gu J, Buchanan J, Preissl S, Wang A, Wu W, Haddad GG, Chaim IA, Domissy A, Vandenberghe M, Devor A, Yeo GW, Voytek B, Muotri AR (2019) Complex oscillatory waves emerging from cortical organoids model early human brain network development. Cell Stem Cell 25:558-569.
Tung TC, Kü SH (1944) Experimental studies on the development of the pronephric duct in anuran embryos. J Anat 78:52-57.
Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, Yamashita T (2013) Layer V cortical neurons require microglial support for survival during postnatal development. Nat Neurosci 16:543-551.
Van Breedam E, et al. (2022) Luminescent human iPSC-derived neurospheroids enable modeling of neurotoxicity after oxygen-glucose deprivation. Neurotherapeutics 19:550-569.
Vankriekelsvenne E, Chrzanowski U, Manzhula K, Greiner T, Wree A, Hawlitschka A, Llovera G, Zhan J, Joost S, Schmitz C, Ponsaerts P, Amor S, Nutma E, Kipp M, Kaddatz H (2022) Transmembrane protein 119 is neither a specific nor a reliable marker for microglia. Glia 70:1170-1190.
Veenvliet JV, Bolondi A, Kretzmer H, Haut L, Scholze-Wittler M, Schifferl D, Koch F, Guignard L, Kumar AS, Pustet M, Heimann S, Buschow R, Wittler L, Timmermann B, Meissner A, Herrmann BG (2020) Mouse embryonic stem cells self-organize into trunk-like structures with neural tube and somites. Science 370:eaba4937.
Wang Z, Wang SN, Xu TY, Miao ZW, Su DF, Miao CY (2017) Organoid technology for brain and therapeutics research. CNS Neurosci Ther 23:771-778.
Weinhard L, di Bartolomei G, Bolasco G, Machado P, Schieber NL, Neniskyte U, Exiga M, Vadisiute A, Raggioli A, Schertel A, Schwab Y, Gross CT (2018) Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction. Nat Commun 9:1228.
Weiss P, Taylor AC (1960) Reconstitution of complete organs from single-cell suspensions of chick embryos in advanced stages of differentiation. Proc Natl Acad Sci U S A 46:1177-1185.
Wolfram L, Gimpel C, Schwämmle M, Clark SJ, Böhringer D, Schlunck G (2024) The impact of substrate stiffness on morphological, transcriptional and functional aspects in RPE. Sci Rep 14:7488.
Wörsdörfer P, Dalda N, Kern A, Krüger S, Wagner N, Kwok CK, Henke E, Ergün S (2019) Generation of complex human organoid models including vascular networks by incorporation of mesodermal progenitor cells. Sci Rep 9:15663.
Wright-Jin EC, Gutmann DH (2019) Microglia as dynamic cellular mediators of brain function. Trends Mol Med 25:967-979.
Xu R, Boreland AJ, Li X, Erickson C, Jin M, Atkins C, Pang ZP, Daniels BP, Jiang P (2021) Developing human pluripotent stem cell-based cerebral organoids with a controllable microglia ratio for modeling brain development and pathology. Stem Cell Reports 16:1923-1937.
Zhang SC, Wernig M, Duncan ID, Brüstle O, Thomson JA (2001) In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat Biotechnol 19:1129-1133.
Zhong X, Gutierrez C, Xue T, Hampton C, Vergara MN, Cao LH, Peters A, Park TS, Zambidis ET, Meyer JS, Gamm DM, Yau KW, Canto-Soler MV (2014) Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. Nat Commun 5:4047.

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