Advanced brain organoids for neuroinflammation disease modeling

Sonia Sabate-Soler[#], Henry Kurniawan[#], Jens Christian Schwamborn[*]

Brain organoids mimic closely the embryonic human brain: Over the last decade, the development of human organoid systems has evolved rapidly. Different tissues have been modeled with organoids, such as the gut, lung, liver, kidney retina and brain. These systems have a high cellular heterogeneity, with many cell types integrated into the same system. Organoids' cellular populations interact between and amongst each other in a cellular and molecular level, which represents an advantage with respects to monolayer 2D cell culture systems.

One of the limitations in human studies is obtaining human tissue with low accessibility or that requires the extensive procedure. This limitation was overcome with the development of induced pluripotent stem cell (iPSC) reprogramming technologies that allow for a somatic cell sample, for instance, fibroblasts, to be reprogrammed into stem cells. These stem cells preserve the genetic identity of the donor and can be differentiated into other cell types via the application of molecular queues. A clear example of the benefits of iPSC technology is human brain organoids. The human brain is an exclusive area, and the manipulation of the human brain comes with a risk for the patient. Therefore, iPSC-derived human brain organoids are being assessed for many biomedical applications.

Despite the fact that the first work in brain organoids was done with mouse tissues, it represented a before and after in a cell culture-based human brain modeling (Lancaster et al., 2013). Brain organoids have a high cellular heterogeneity, with many cell types integrated into the same system. Not only organoids represent an advantage for studying neurological processes in health, but more importantly in the diseased setting, especially those with complex genetic aspects that are challenging to model in animals. The pre-clinical study of human neurological disorders implies many challenges due to the diversity of genetic background, in the case of genetic conditions, the structural complexity of the central nervous system (CNS), the lack of reproducibility by animal models and the difficulties in obtaining human brain biopsies. The development of brain organoid systems has made a breakthrough in mimicking the complexity of the CNS and overcoming all these drawbacks. The cellular heterogeneity of a brain organoid consisting of various neuronal cell types that can connect and interact with each other is a great advantage. The simplicity in obtaining patient samples, reprogram them into stem cells, and use it for neurodegenerative disease modeling, enhance its translational value and a more personalized approach. iPSC-derived human brain organoids have been used to study brain infections (Qian et al., 2016), neurological disorders and neurodegenerative conditions such as Alzheimer's disease (Chen et al., 2021).

The generation of brain organoids from iPSCs is based on "unguided differentiation" approaches (**Figure 1**). These are characterized by experimental setups where iPSCs are plated in U-bottom, low attachment plates, and differentiation of neural cell types is promoted by the addition of small molecule queues. This results in 3D tissue structures that contain stem cells, neurons, and support cells. Because of the low cellular identity restriction characteristic of unguided differentiation approaches, non-ectodermal cell types have been observed in brain organoids generated via these protocols.

The unguided differentiation approaches are usually less complicated and expensive. They also give rise to brain organoids that resemble the human brain cortex. This is a very useful setup for studying brain infection and neurodegenerative diseases such as Alzheimer's disease. However, other neurological disorders take place mostly in other brain regions, which makes unguided differentiation approaches not ideal. For instance, in Parkinson's disease, specifically the dopaminergic neurons from the substantia nigra pars compacta of the midbrain, which project their axons into the striatum, degenerate. These regions cannot be modeled with whole-brain organoids since their cellular populations are far from similar to the midbrain and striatum. In such cases, following guided differentiation protocols makes more sense. In guided differentiation procedures, iPSCs, or an intermediate neural precursor stem cell population, often require a more complex small molecule setup to promote cell differentiation into specific cellular populations of a determined brain region.

iPSC-derived brain, or region-specific, organoids mimic the embryonic human brain. For instance, midbrain-specific organoids mimic the embryonic midbrain of developmental weeks 6–11 (Zagare et al., 2022). This makes them an excellent choice to study developmental disorders or neurological processes linked to brain development. On the other hand, to study adult brain processes or age-related disorders, an embryonic brain model may not be the most suitable choice. Tools to induce organoid aging are needed to better mimic the mature brain and recapitulate geriatric disorders since age is the main risk factor to develop neurodegenerative diseases.

Cellular competent organoid model: integration of cell compartments: As the notion of "immune privilege" has dissipated over the past decades, numerous studies have revealed that the CNS contains a rich population of immune cells consisting of innate and adaptive cells in the surrounding dura and meninges area (Buckley and McGavern, 2022). Because of its distinct compartmentalization, the immune homeostasis in the brain has a specialized regulation, to ensure sufficient protection against infection while limiting damages to constant postmitotic neuronal cells and CNS remodeling. In fact, altered immune function in the brain has been associated with various pathological conditions, ranging from cancer, autoimmunity, and even mental disorders.

With further evidence showing inflammation being one of the common denominators in the majority of CNS pathology, however, the use of brain organoids from the currently established protocols suffers a major setback due to the lack of the immune compartment. Thus, a setup that incorporates different parts of the brain, as well as the immune cells, is needed to better resemble the physiological features of the brain organoid in modeling neuroinflammation.

The reprogramming of iPSC into innate cell repertoires such as macrophages, microglia, monocytes, granulocytes, natural killer cells and dendritic cells has been reported (Bernareggi et al., 2019). The iPSC-derived cells share similar characteristics compared to their hematopoietic progeny counterparts, such as comparable surface markers and functionality, e.g., the ability to

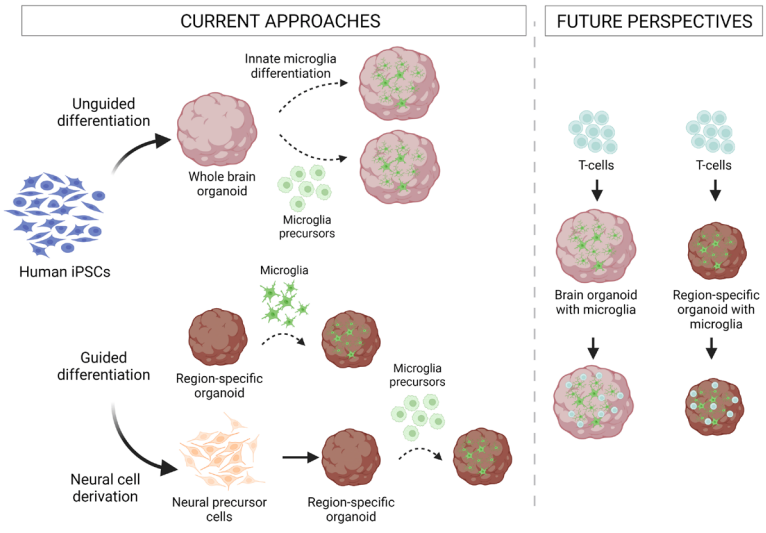

Figure 1 | **Approaches to obtain immunocompetent organoids.**
On the left side, current approaches being applied to integrate microglia into brain organoids. Human induced pluripotent stem cells (iPSCs) can be used to generate whole-brain organoids following an unguided differentiation (Lancaster et al., 2013). Microglia can innately develop within those organoids (Ormel et al., 2018). Furthermore, the integration of microglia can be achieved by co-culture with precursor cells (Xu et al., 2021). iPSCs can undergo guided differentiation to obtain brain region-specific organoids, that can be co-cultured with microglia (Song et al., 2019). Alternatively, region-specific organoids can be generated from a neural precursor cell population, and microglia precursors can be integrated to the system in order to obtain a microglia-containing organoid (Sabate-Soler et al., 2022). On the right side, future perspectives further increase the immune capabilities of brain organoids. T-cells, but also other circulating immune cells (such as monocytes) could be integrated to whole brain or region-specific organoids with microglia, allowing the study of immune cell interactions within the system. Created with BioRender.com.

perform phagocytosis, antigen presentation and effector cytokine secretion. Microglia is one of the most important residents innate immune cells in the brain where they are involved not only in the immune response but also in the development and homeostasis of the neurons. As microglia is exclusively present in the CNS, obtaining primary microglia from the brain requires extensive surgery, and is most likely done post-mortem. Therefore, the use of iPSC technology to study microglia biology is significant.

Recent studies have described over dozens of different methods to incorporate microglial cell lines, mature microglia or microglia precursor cells into brain organoids (Zhang et al., 2023). The brain organoids-microglia assembly could respond to inflammatory stimuli and were able to secrete various cytokines and chemokines (Ao et al., 2021). The introduction of microglia also led to increased neuronal maturation and functionality, e.g., higher electrophysiological properties and synaptic transmission of the remaining synapses (Sabate-Soler et al., 2022). The roles of microglia in immune surveillance (phagocytosis, pinocytosis and receptor-mediated endocytosis), as well as in neural development and maturation, synaptic formation and plasticity, and neural pruning have been described. Due to the indispensable roles of microglia in brain development and homeostasis, the microglia containing brain organoids undoubtedly provide new avenues to better model neurodegenerative diseases. This model represents a first step to advance the use of brain organoids to model neuroinflammation *in vitro*.

Neuroimmune organoids: contribution of the innate and adaptive immune cells: While studying the role of microglia in neuroinflammatory diseases is inarguably important, the lack of other immune cells in this model may obscure the involvement of these cells in the disease pathology. Myeloid-derived suppressor cells are innate cells specialized with the ability to suppress immune responses. They can expand depending on environmental cues, such as the presence of antigens or other immune cells, and cytokine gradient. Myeloid-derived suppressor cells have been associated with Alzheimer's disease and Parkinson's disease, where they are observed in high numbers in the early stage of the diseases. It is likely that myeloid-derived suppressor cells are involved in suppressing the aberrant microglia activity during early acute inflammation, but are not able to sustain the immunosuppressive activity, which leads to disease progression. Granulocytic cells such as neutrophils and eosinophils are also major sources of cytokines, where their abnormal function is also related to neuroinflammation. Taking these points into account, it is worth noted that combining more innate cells into brain organoids will undoubtedly help to better understand the innate contribution to the neuroinflammatory pathways leading to neurodegeneration *in vitro*.

The second arm of the immune system, the adaptive immunity, mounts a much stronger immune reaction and forms immunological memory upon the resolution of the immune reaction. While beneficial, infiltration and chronic activation of T cells have been associated with neuronal cell death due to their ability to induce Fas ligand activation through cell-cell contact (Dai and Shen, 2021). Increased T cell pools also promote cross-presentation between T cells and microglia, which in turn elevate inflammatory cytokines such as interleukin-6, tumor necrosis factor-α, and interleukin-1β, further exacerbating neuroinflammation. T cell-derived cytokines were even shown to influence cognitive function (Buckley and McGavern, 2022). Considering the prominent roles of T cells in neuroinflammation and overall CNS health, modulating lymphocytic function is one major way to mitigate its neuronal toxicity effect.

To date, the incorporation of other immune cells except for microglia in the brain organoid has not been reported. In fact, obtaining peripheral immune cells from the blood is rather straightforward process. These immune cells can be integrated into the brain organoid generated from the same donors, providing a more personalized disease modeling system. Though the generation of iPSC-derived immune cells such as T cells has been described, the process requires significant time and resources. Thus, obtaining peripheral immune cells is a more efficient option to pursue. In the context of the innate-adaptive interaction, T cells can be incorporated into microglia-containing brain organoids, where the interaction between the brain and the innate/adaptive immune system can be explored (**Figure 1**). Although this co-culture system has not been established, it is worth investigating as this model will give a closer representation of the neuroinflammatory processes.

Over the past years, tremendous breakthroughs have been made in brain organoid technologies that have become a promising model to study neurodegenerative diseases. Although brain organoids recapitulate a number of key features of the human brain, they are not a perfect replica. Several limitations of this model remain to be overcome: the degree of variability and batch-to-batch effect, the standardization of the various protocols in generating brain organoids to improve reproducibility, and the absence of surrounding tissues. The latter is a major setback compared to animal models. Thus, the incorporation of different cells and tissues, such as the immune cells and a vasculature system, is necessary to better mimic the real human brain. More complex brain organoids will certainly warrant their use in clinical research. Nevertheless, the challenges in combining two or more populations into one culture system entail extensive testing and optimizations, since different media, growth factors, chemokines and/or cytokines may be required. Moreover, whether they can establish the interactions as in a physiological setting remain to be evaluated. However, with the advancement of 3D cell culture technologies, the co-culture and fusion of different brain region-specific organoids have been achieved, giving rise to brain assembloids that allow the study of inter-region communication (Miura et al., 2022). Furthermore, organoids that mimic different human organs have been co-cultured *in vitro*, showing that a multi-organ cell culture approach is possible. This evidence supports the idea that building a more complex brain assembloids system that consists of diverse support cells and tissues is not anymore hypothetical and is becoming a reality.

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