Brain organoids as models of Alzheimer's and Parkinson's diseases: a narrative review on perspectives for regenerative and personalized medicine

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ABSTRACT

For many years, two-dimensional (2D) cell culture has been used as a model to study diseases, having great importance in regenerative medicine, despite still having significant limitations. In order to circumvent these limitations, three-dimensional (3D) cell culture proposes a more complex and sustainable organization that can be produced from adult stem cells (ASCs), embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). The 3D culture enabled the cultivation of cells in an environment closer to the physiological one, leading to the formation of different organ-specific tissues. In other words, 3D cell culture makes it possible to create organic structures very similar to the organs of a human being, both structurally and functionally. In this way, we have what are called organoids. The use of organoids has grown exponentially in *in vitro* environments, allowing the analysis and observation of the various existing physiological phenomena. As an example, we can mention the brain organoids ("mini-brains") reproduced *in vitro*, seeking to delineate the peculiarities and complexities of the human brain, in order to understand some neurological dysfunctions that affect this system, such as the two main neurodegenerative diseases: Alzheimer's and Parkinson's Diseases. Therefore, brain organoids may allow a remarkable advance in regenerative medicine applied to neurodegenerative diseases, as these "mini-brains" can be produced from the patient's own cells. This will allow for personalized interventions, such as drug testing, in order to define what would be the best drug treatment. Consequently, this technology can enable more efficient and individualized therapies - which is fundamental for Personalized Medicine.

Keywords: Organoids, Alzheimer's disease, Parkinson's disease, Regenerative Medicine, Personalized Medicine.

1. INTRODUCTION

The two-dimensional (2D) cell culture has been used for years as a disease modeling tool and holds significant importance in regenerative medicine. However, this model has significant limitations, including the difficult task of isolating and cultivating cells collected from patients quickly and efficiently while maintaining their genetic originality[1, 2].

On the other hand, there are cells that multiply *in vitro* in a three-dimensional (3D) environment, with the aim of forming small groups of cells that differentiate into distinct cell types from a structural and functional organization similar to that of an *in vivo* organ. There are myriad possibilities for cultivating cells that approximate the physiology of an organ. For this reason, these cells are called mini-organs or organoids. These organoids can be constructed from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), neonatal or adult stem cells (ASCs), allowing the cultivation of distinct organ-specific tissues[3-5].

To construct an organoid, it is necessary to deposit cells (whether ASCs or ESCs) in a matrix with an abundant amount of laminin and collagen to mimic the extracellular matrix6. Thus, cells cultured in 3D become capable of organizing into more complex structures similar to an organ, both morphologically and functionally. Such organoids consist of heterogeneous cell structures with genetic stability that can be cultured in bioreactors, cryopreserved in biobanks, and subjected to various tests without losing their viability[2, 6, 7].

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The use of organoids has grown exponentially in *in vitro* environments, allowing the analysis and observation of various physiological phenomena6. The focus on 3D cells enabled the cultivation of hepatocytes for the first time[8].

As another example, cerebral organoids ("mini-brains") are reproduced *in vitro* to delineate the peculiarities and complexities of the human brain. Therefore, mini-brains enable the understanding of some neurological dysfunctions, including neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD)[9, 10]. In organoid models of AD, the most prevalent neurodegenerative disease in the world, the presence of β-amyloid plaques and structures resembling neurofibrillary tangles of tau protein - classic pathophysiological signs of AD - were identified[11, 12]. Similarly, using cerebral organoids modeling PD, the degeneration of dopaminergic neurons, mitochondrial dysfunction, and α-synuclein protein deposits were observed[13-15].

Therefore, the development of cerebral organoids represents a remarkable advancement in regenerative medicine for investigating responses that are not yet known regarding certain neurodegenerative diseases. Furthermore, these organoids can be generated from the patient's own cells, such as iPSCs[16-18], allowing for specific interventions, such as pharmacological testing and regenerative cellular therapy using cell sources to replace degenerated neurons. This will enable the development of better treatments for such diseases[19-23]. Thus, this new research approach can provide more efficient and personalized therapies, which is referred to as Personalized Medicine[24, 25]. Therefore, the aim of this study is to search for information on the organoid model in scientific publications, data indexing databases, and scientific journals, in order to gather significant indicators for the elaboration of the work.

2. METHODOLOGY

The present study follows the narrative review model, and its methodological principle is the search, analysis, and interpretation of original scientific studies related to organoids and their clinical perspectives on neurodegenerative diseases. The search period was limited to the last 10 years (2011-2021), and inclusion criteria required the studies to be in Portuguese or English.

To this end, studies published and indexed in PubMed, LILACS, SciELO, and Google Scholar databases were selected. The following keywords were used for better search term definition: Organoids; Stem Cells; Neurodegenerative Diseases; Alzheimer's disease, Parkinson's disease, Regenerative Medicine, Precision Medicine; Personalized Medicine. Duplicate studies, those published before 2011, and those not related to the research topic were excluded from the search.

3. ORGANOIDS

3.1. History

The term "organoid" has been used for over a century. In 1910, the first experiment with *in vitro* organs was conducted through the dissociation and reaggregation of cells from a marine sponge (Porifera). Cells from the marine sponge were mechanically separated and then the dissociated cells were reaggregated through a chemical process. The cells that were able to reaggregate and self-organize formed tissues similar to the multicellular aggregates of marine sponges. Thus, from this experiment, various studies were developed, and it was proven that cell reaggregation could generate organically similar structures to the original[9].

In 1946, the term "organoid" was used to define an isolated mass from a human tumor, and subsequently, studies were developed on aspects of this tissue [9, 31]. The term "organoid" gained even more significance when it was discovered that embryonic and induced pluripotent stem cells had a wide capacity for self-renewal and differentiation into various types of cells. This capacity boosted studies focused on organoids, forming highly complex structures with specific organ cellular organization, exhibiting structures similar to the original tissue, including similar physiological functions. Consequently, models of pathologies affecting such organs emerged, facilitating understanding[9].

3.2. Genesis

Organoids have been constructed over many decades of biomedical research. Such tissues can be grown from a set of stem cells placed in specific containers (Petri dishes) rich in nutrients, where these cells multiply (due to their pluripotent capacity) and transform into three-dimensional (3D) structures

called organoids. These structures are capable of replicating the organic information of the body in relation to the organization, activity, and cellular function of a specific organ[26].

Several organoid models have been developed in recent years, analogous to the tissues of the intestine, kidney, eye, liver, lung, inner ear, brain, and others. These organoids (because they are derived from human pluripotent stem cells) can assist in disease modeling, studying new pharmacological interventions, and testing the application of these drugs, in addition to facilitating the use of regenerative therapies[26]. Furthermore, organoids allow for more effective cell-cell and cell-matrix interactions. Consequently, these 3D culture peculiarities more faithfully simulate signaling pathways and real cellular functions in tissues. Therefore, in many ways, mini-organs can better mimic diseases than traditional animal and/or cell culture models[27].

As previously mentioned, iPSCs have a high capacity for self-organization, which enables the culture of organoids. When iPSCs are arranged in 3D culture systems (using microcarriers and hydrogels that simulate the *in vitro* physiological extracellular matrix) associated with appropriate biochemical factors, iPSCs can develop and generate specific organoids for the cultivated tissue. Generally, the developed organoid is compatible with the stem cells employed in the culture system (containing only a single lineage). However, there is still the possibility of self-renewal of the progenitor cells that differentiate into heterogeneous cells, giving rise to different cell lineages[27-29].

Cerebral organoids are *in vitro* generated tissues derived from central nervous system stem cells and simulate human fetal neurodevelopment. This three-dimensional tissue-rich in neural progenitor cells, neurons, and to a lesser extent, glial cells (which modulate the cellular microenvironment)-enables testing and experiments on brain development and the study of physiopathological processes, such as neurodegenerative diseases[30]. Despite acknowledging that even the most advanced 3D cultures do not allow a full understanding of human organ dysfunctions, organoids have numerous potential benefits for personalized therapy[9, 31].

3.3. Brain organoids

In the brain, there is a range of neural networks that play a part in processing language, action, thinking, memory, movement, and many other functions. Therefore, understanding this organ is not an easy task. The most commonly used approach to comprehend brain function has been based on studies in animal models (such as mice). However, the information obtained from animal models has limited value, considering that the diseases that affect them are distinct from those that affect humans. In addition to animal studies, there are also two-dimensional cultures and *post-mortem* studies available. However, their applicability is directly compromised by possible irreversible changes that the brain may have undergone during the death process. Thus, considering the complexity of this organ and the different morphologies present in various pathologies, a model is needed that represents the brain in the most physiological way possible to enrich the knowledge and understanding of certain neurodegenerative diseases.[9, 27,37].

Several protocols have been developed for generating cerebral organoids from three-dimensional (3D) cultures. However, to reproduce the aspects of the human brain, initially, two-dimensional (2D) stem cell culture models were used. However, neuronal development was limited, and it was difficult to create biological models precisely similar to the brain. Among these limitations was the lack of interaction between cells and the deficit in neuronal self-organization capability[9].

The development of 3D culture brought greater capacity for cellular interaction and high self-organizational ability. The result was the development of miniature brains, called "mini-brains," produced from stem cells. A proposal for personalized medicine is the production of organoids derived from patients with specific dysfunctions, for example, those with genetic mutations and neurodegenerative disorders. This would allow a better understanding of the pathophysiology, evolution, and, consequently, new perspectives on the development of therapies, drug applicability, and prognosis[9, 27].

Several *in vitro* procedures are necessary to facilitate stem cells' multiplication and self-organization without artificially inducing this process. The 3D culture protocol for neural tissue growth aims to provide a suitable environment for the development of intrinsic cellular factors. Firstly, neuroectoderm is generated from embryoid bodies - the neuroectoderm represents the layer of cells from which the nervous system derives, and embryoid bodies are the result of the uniform growth of embryonic stem cells. Subsequently,

neuroectodermal tissues are incorporated into 3D culture immersed in Matrigel droplets[37].

This gel provides the necessary support for iPSCs' self-organization and subsequent nervous tissue growth. To improve and increase oxygen and nutrient exchange between cells, Matrigel droplets are transferred to a rotating bioreactor. Within eight to ten days after transferring the Matrigel to the bioreactor, cells already display neuronal conformity. In approximately twenty to thirty days, this neural tissue is capable of forming well-defined brain regions, such as the cerebral cortex, retina, meninges, choroid plexus, hippocampus, and ventral prosencephalon. These regions can reach up to 4mm in structure and can remain alive for up to ten months while retained in the rotating bioreactor[9, 27, 28].

3.4. Neurodegenerative diseases

Neurological diseases such as PD and AD have a significant impact on human life and impose a heavy financial burden on families and society. The mechanisms and pathogenesis of these diseases are complex and their symptoms are diverse, which makes early diagnosis and effective treatments challenging. The current understanding of neurological diseases is largely based on animal models and post-mortem examination. However, not all animal models will produce these factors, as the etiology of some neurological diseases is linked to multigenic mutations. Moreover, it is noteworthy that neurological diseases affect multiple brain regions and are more related to lesions of multiple cell types. Therefore, it is unlikely that traditional cell culture technology can model neurological diseases[9, 27, 29].

An efficient way to study neurological diseases is through brain organoids, considering their diverse cell types, multiple brain regions, and ability to recapitulate the distinct essential characteristics in human cortical development[9, 27, 29].

The use of cerebral organoids is a significant advancement in science, but there is still a long way to go for them to become competent tools in modeling and studying neurological diseases. Techniques that allow genetic editing in association with 3D cerebral organoid technology are fundamental for future studies on more complex brain disorders. It is also noteworthy that cerebral organoids can be cultivated from patient-derived somatic cells, enabling assistance in the discovery of new drugs and their use in evaluating the individualized patient response to different medication possibilities in the treatment of neurodegenerative diseases[9, 27, 29].

To understand the development of neurodegenerative diseases, it is necessary to explain the functions of microglia. Microglia are immune cells that act at the level of the central nervous system, whose function is related to the performance of synapses, neuronal plasticity, and neuronal circuit maturation. These cells are activated when there is a degenerative process at the neuronal level and release neurotoxic substances to act generating an inflammatory response. Therefore, it is possible to infer that microglia are related to the genesis and progression of neurodegenerative diseases[9, 29].

The ability to recruit microglia within organoids would be a possibility to understand the development and progression of these diseases. In a study based on the simultaneous development of microglia and neurons in 3D culture, it was observed that the microglia that emerged within the brain organoids were very similar to the microglia present in the adult organism and that these were capable of producing an inflammatory response when stimulated by lipopolysaccharides (an exogenous pyrogen that acts on macrophages, stimulating them to produce pro-inflammatory cytokines), thus demonstrating the importance of microglia in modeling the inflammatory response. In this way, important information has emerged regarding the interactions between neurons and microglia and the development of diseases at the cerebral level[9, 29].

3.4.1. Alzheimer's Disease

One of the main neurodegenerative diseases is Alzheimer's disease (AD), which affects over 46.8 million people worldwide and represents one of the leading causes of death worldwide. The main risk factors for developing this disease include advanced age, exposure to aluminum (which can accumulate in brain tissue), and head trauma[38]. Alzheimer's disease can manifest in two distinct forms: sporadic, a late-onset form that results in a demented state, and an early-onset familial form, which usually arises before the age of 65 and is characterized by rapid cognitive impairment[10,39].

This disease is characterized by excessive deposition of β-amyloid peptide in the brain, leading to the formation of intracellular neurofibrillary tangles primarily composed of hyperphosphorylated Tau protein[10, 32]. The accumulation of β-amyloid aggregates

and hyperphosphorylated Tau protein can trigger an inflammatory immune response mediated by the release of pro-inflammatory cytokines (IL-6 and TNF-α) and the production of reactive oxygen species. In addition, an increase in lactate dehydrogenase activity has been detected. All these factors culminate in increased cytotoxicity and neuronal loss, contributing to the progression of AD. This pathological process leads to a decline in cognitive ability and severe memory impairment.

Several risk factors are attributed to this disease, such as advanced age, family history, and environmental factors. Genetic mutations are also described as a risk factor, specifically involving some precursor proteins (β-amyloid, presenilin 1, and presenilin 2)[10, 32].

There are numerous research studies focused on understanding the pathogenic mechanism of AD, with the aim of finding ways to prevent or cure the disease. However, no means has been discovered yet to achieve this purpose. In 2014, AD was studied for the first time in a 3D organoid model[32]. When incorporated into matrigel, the organoids presented neuro-inflammatory markers, extracellular matrix remodeling, altered synaptic functioning, increased aggregation of β-amyloid peptide and hyperphosphorylated Tau protein, and also overexpressed β-amyloid and presenilin 1 proteins with mutations similar to those found in AD[10, 32].

The cleavage of amyloid precursor protein (APP) through the β-secretase enzyme gives rise to the β-amyloid peptide. Therefore, understanding the APP processing is important for raising new therapeutics that can reduce β-amyloid levels. Under normal conditions, the α and γ-secretase enzymes cleave APP and give rise to soluble APP fragments (sAPPβ). But, in patients with AD, this cleavage of APP occurs via β and γ-secretase, resulting in sAPPβ fragments and β-amyloid peptides, the main component of amyloid plaques present in AD[10, 32].

An important finding during the study of these alterations was the effective result obtained with the use of secretase inhibitors, which were able to block the proteolytic enzyme action involved in the β-amyloid formation, as well as reduce Tau protein phosphorylation. It was believed that the inhibition of β and γ-secretase enzymes would be the therapeutic target for reducing β-amyloid peptide production[9, 10, 32].

However, despite demonstrating a reduction in plasma levels of β-amyloids in cerebrospinal fluid, there was an increase in the incidence of skin cancer and impairment of cognitive scores in patients who participated in clinical trials[32, 33]. The failure of these studies may be related to the diverse substrates and important functions that these enzymes have outside the brain, such as the formation of the muscle spindle. A possible solution to these failures could be the use of therapies targeted at inhibiting brain-specific β and γ-secretases, thereby reducing extra-cerebral adverse effects[9, 10].

Therefore, it is evident that brain organoid models are excellent means to validate these attempts. Although the intervention from using secretase inhibitors was not safe, this fact confirmed the hypothesis that AD pathology could be recapitulated from 3D organoids and that they are capable of expressing mutations (pathogenic processes) associated with AD, making it easier to conduct studies for the discovery and development of new interventions for AD, whether pharmacological or not. More studies with brain organoids are necessary to evaluate the enzymatic functions, neuronal changes, and synaptic activity that occur in AD [9, 10, 32].

3.4.2. Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative disease with a prevalence of 2 in every 1,000 individuals. It is classically considered an age-associated disorder and is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra of the midbrain and accumulation of α-synuclein (a protein that composes neurons) mainly in presynaptic terminals, with cytoplasmic inclusions (Lewy bodies). The impairment of α-synuclein (α-syn) autophagic clearance is one of the main causes of α-syn accumulation in PD. α-Synuclein plays a role in the process of formation and fusion of presynaptic vesicles, thereby regulating the release of neurotransmitters. Additionally, it has the ability to alter the composition and viscosity of lipid membrane fatty acids, contributing to neuronal plasticity[9, 34, 35].

The gradual degeneration of dopaminergic neurons and accumulation of α-synuclein cause tremors, hypertonia, and oligokinesis characterized by a reduction in spontaneous motor activity, postural instability, bradykinesia, muscular rigidity, and resting tremors[9, 35].

Some risk factors have been described, such as exposure to pesticides or brain injury during pre/

perinatal ages, which would lead to dysfunction of the dopaminergic neuron system in adulthood[9, 35]. There are many genetic variants involved in α-synuclein that contribute to susceptibility to PD, including the PARK1 gene (which encodes for α-synuclein protein), PARK2 (encodes for parkin), PARK6 for PINK1 (PTEN-induced kinase 1), PARK7, and PARK8 (which encodes for LRRK2 protein). Among these genes, the most common genetic factor for early and late PD is the LRRK2 mutation (leucine-rich repeat kinase 2 gene locus). However, it is still a challenge to understand the role of LRRK2 in PD, and for a long time, models that could accurately transmit disease associated with LRRK2 mutations were sought. Initially, the most commonly used models were based on 2D cultures, but these did not accurately recapitulate the protein and cellular functions and actions of PD. However, 3D organoid technology has demonstrated greater potential for modeling α-synuclein pathogenesis and cellular maturation[9, 35].

In 2019, a study generated midbrain organoids derived from induced pluripotent human stem cells with LRRK2 gene mutations. After 60 days, these midbrain organoids already had an accumulation of neuromelanin and clustering of genes characteristically expressed in the midbrain of an older human[35]. Additionally, these LRRK2 mutant organoids showed pathology similar to PD, including abnormal localization of phosphorylated α-synuclein, changes observed in patients with PD associated with LRRK2 gene mutations, corroborating results from other brain organoid studies that, in addition to these same findings, found mitochondrial defects and oxidative stress in dopaminergic neuronal cells[9, 15, 35].

An important breakthrough during these studies was the discovery of treatment with a specific LRRK2 kinase inhibitor, which significantly reduced neuronal cell death in the organoids and also decreased α-synuclein accumulation[35], suggesting that the 3D mesencephalic organoid model is more accurate in recapitulating PD and may be useful for screening potential drugs to be used in this disease, with great potential not only in understanding the molecular factors of pathogenesis but also in the search for new therapies[9, 15].

3.5. Organoids in Regenerative and Personalized Medicine

In comparison with existing traditional methods, it is evident that brain organoid technology surpasses animal-based models or other strategies in humans, facilitating the understanding of the gap in knowledge of organic and cellular processes, bringing significant advantages in biological research applications[27].

The application of iPSC-derived brain organoids can be efficient in studying brain development, pharmacological therapeutic possibilities for disease improvement, or even for transplantation at the site of brain injury aimed at repairing damaged tissues as regenerative medicine therapy[27].

As mentioned, organoids are derived from stem or progenitor cells, which facilitates organ development. Thus, another breakthrough that organoids provide is the supply of a source of cells and tissues that may be viable for transplantation. While there are no reports of brain organoids used for replacement therapy, it is known that these organoids possess neural progenitor cells and various other types of neurons that are a great hope for cell replacement therapies in neurodegenerative diseases[27].

Normal brain organoids and organoids that mimic a specific disease have high potential in personalized medicine applications through personalized drug response testing. The introduction of a miniaturized rotating bioreactor, for example, may contribute to the discovery of drug therapy through high-throughput screening to test drug efficacy and toxicity[27].

Regenerative medicine is an important field in modern medicine that aims to replace or regenerate cells, tissues, or organs in order to restore or establish normal function that was previously compromised. Stem cells have the ability to self-renewal and differentiation into various cell lineages, and stem cell-based strategies are promising in the healing of injuries caused by neurodegenerative diseases[27].

Many studies have been conducted in an attempt to identify therapeutic targets and neuroprotective drugs capable of acting on diseases that compromise brain function, and it is the progress in stem cell research that enhances the development of regenerative medicine[27].

A major breakthrough was recently made with the cultivation of mesencephalic organoids containing dopaminergic neurons. When compared with normal cell culture, it was observed that mesencephalic organoids were able to generate a significantly higher amount of dopaminergic neurons in a shorter period of time, as well as produce neural precursor cells with higher levels of survival and greater

longevity in growth and progression of nerve fibers. Therefore, tissues derived from brain organoids not only provide a platform for testing new drugs and their toxicity, but they may also be part of studies aimed at developing therapeutic strategies for tissue and neuronal promotion and regeneration, as well as providing a means for studying new replacement therapies in PD and AD, which are essential contributions to regenerative medicine[27].

3.6. Limitations in the use of brain organoids

Given all the theoretical apparatus previously addressed regarding brain organoids, it is noteworthy that they are part of technological advancement with distinct advantages compared to traditional methods. However, it is important to highlight that despite being good instruments for regenerative medicine, they still have many disadvantages. A large portion of organoids has partial organ components, which does not allow for fully effective control over their development regarding the types of cells and growing structures9. Therefore, brain organoids may not always be able to simulate neurodevelopmental situations, particularly when involving more complex conditions such as schizophrenia[27].

The main disadvantages of brain organoids include high cost, lack of aging, cell heterogeneity, and variability of brain regions within each organoid. Cell heterogeneity is caused by the lack of spontaneous self-organization of stem cells, which hinders the conduct of controlled experiments with viable correlations9. The formation of an organoid depends on the self-organizing process that is dependent on stem cells. One strategy to control the growth and differentiation of stem cells *in vitro* is to treat organoids with signaling molecules at different growth stages. Such a strategy would likely lead to less heterogeneity and more similarity in organoid size[27].

The protocol for growing brain organoids is still a difficult task since there are very specific brain regions involved in neurodegenerative diseases. A brain organoid can reach up to 4mm in diameter and can have a long lifespan, surviving for a long time if kept in a rotating bioreactor. Thus, it is believed that the use of rotating bioreactors improves organoid reproducibility, increases yield, lifespan, and maturation, and improves organoid growth conditions, thereby optimizing protocols [9, 27].

Another disadvantage of brain organoids is related to the absence of vascularization. For example, cells composing the core of the organoid undergo apoptosis after 100 days of initial culture and are unable to differentiate into any other cell type. This is due to the absence of blood vessels that transport nutrients and perform the necessary gas exchange for new cell growth [27]. As a result, there is a significant impairment in organoid growth and maturation. One strategy to reduce this failure is the integration of functional and connectable vascular networks, which would allow for much more efficient morphogenesis and considerable growth of these organoids[9, 31].

Another factor affecting the use of brain organoids is the lack of comprehensive studies on the chemical and physical evolution of the brain. Although many characteristics present in the human brain are represented in brain organoids, there are still structures and cells that are not well developed. In other words, brain organoids can represent the development of a fetus's brain for up to three months, but it is still incomplete and does not encompass all the structures formed after birth[9, 27].

Regarding practical issues, it is important to note that the use of the human brain is subject to restrictions in scientific research due to ethical and legal issues. Therefore, sharing and maintaining *in vitro* brain tissue involves many restrictions, with the processes subject to various guidelines and regulations[9].

Currently, the most commonly used methods for studying the brain are animal models and *post-mortem* examinations, which allow for a close understanding of vertebrate and mammalian brain development. However, due to structural differences, studies using animal models are not always successful. An example of such structural differences is the absence of the internal fiber layer and external subventricular zone, which play fundamental roles in human brain development but are absent in the brains of rats[9, 27, 31].

Despite the challenges associated with 3D organoid technology, single-cell RNA sequencing is a useful method for identifying different cell types and lineage trajectories during cellular differentiation. This method provides single-cell gene expression data and information on the age of the cell lineage, which facilitates organoid expression across the genome. Therefore, it would be a useful tool in identifying different cell types and distinct lineage trajectories that occur during cellular differentiation[31]. Another possibility is the use of brain assembloids,

which are self-organizing 3D cellular systems formed by the combination of neurospheres and capable of recapitulating interactions between GABAergic and glutamatergic neurons, for example[36].

In any case, new technologies are needed to facilitate the automated production of brain organoids. High yield in the production and development of organoids through more advanced systems is essential for the routine generation and application of organoid models for human diseases, paving the way for large-scale studies of possible pharmaceutical applications and personalized therapies[31].

4. CONCLUSION

In vitro cultured brain organoids generated through the 3D method represent the most promising model for understanding the complex processes of neurodevelopment in the human brain, disease progression, and pathogenesis, facilitating the development of intervention strategies that were previously impossible to study in humans. Organoids can develop many of the characteristics of the human brain that are difficult to develop through traditional methods in cells or animals.

So far, the use of brain organoids has focused on studies of physiological and pathogenic development (neurodegenerative diseases) and drug testing that can be used as an intervention for specific diseases. However, due to the limitations that organoids still face, they do not yet represent a standard model for expressing brain development in its entirety, as well as its neurodegenerative dysfunctions. Therefore, many studies are still necessary to advance the reproducibility and maturity of brain organoids.

Despite the need for further progress in realizing the full clinical potential of brain organoids, they have considerable potential to assist in prognostication and more efficient therapeutic options for neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

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