Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/58/20241368

Advances in drug screening and toxicity testing: A review on Parkinson's disease using brain-on-a-chip

Xuejie Wang

School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, 30332

xuejiewang96@gmail.com

Abstract. Neurodegenerative diseases like Parkinson's disease (PD) pose substantial challenges to health and well-being. PD is characterized by the progressive degeneration of dopamine-producing neurons in the substantia nigra of the brain, leading to motor dysfunction, cognitive decline, and various non-motor symptoms. Advances in drug screening and toxicity testing have transformed the study of neurodegenerative diseases. Traditional methods often fall short in replicating the intricate environment of the human brain, highlighting the necessity for alternative pre-clinical models. Brain-on-a-chip technology, an innovative *in vitro* platform, offers a promising solution by simulating the intricate architecture and microenvironment of brain tissue. This microfluidic device incorporates living brain cells within a controlled setting, enabling the examination of neuronal behavior, disorder mechanisms, and pharmacological responses in a physiologically relevant context. This review examines recent advancements in brain-on-a-chip systems for PD research, emphasizes their potential to advance drug discovery and toxicity testing, also discusses the difficulty and limitations of current brain-on-a-chip technologies, for examples, challenges of specific neuron cell selection and incubation, and also restriction of organ-organ connection. By comparing *in vitro* and *in vivo* studies, brain-on-a-chip technology shows great promise for accelerating the development of effective therapies for PD, ultimately contributing to improved patient outcomes and a deeper understanding of the disease.

Keywords: Brain-on-a-Chip, Parkinson's disease, Drug Toxicity.

1. Introduction

PD pose a major challenge to people's health and well-being [1, 2]. It is marked by the gradual degeneration of dopaminergic neurons in the substantia nigra of the brain [3, 4], resulting in motor dysfunction [5], cognitive impairment [5], and various non-motor symptoms [5, 6]. A distinctive pathological feature of PD is the accumulation of Lewy bodies, which are aggregates of fibrillar α-synuclein [7]. Current medical treatments for PD primarily focus on alleviating symptoms and do not address the underlying neurodegenerative processes [7-10]. Although PD is generally regarded as a disorder of motor control and the main therapeutics of the disease target motor impairment, numerous non-motor features also need to be considered due to the degeneration of neuronal pathways [11], which present significant challenges in the development of drugs for PD.

While the precise mechanism of PD remains incompletely understood, both clinical and pre-clinical *in vivo* research have demonstrated that genetic and environmental determinants play crucial roles in PD development [12, 13]. Mutations in genes like SNCA, LRRK2, and PRKN are thought to underlie the

DOI: 10.54254/2753-8818/58/20241368

onset of PD [12]. Mutations in the SNCA (rare) and LRRK2 (most common) genes can result in autosomal dominant inheritance, and patients with these gene mutations often exhibit the classic PD phenotype [14]. Additionally, mutations in genes such as PRKN, PINK1, and DJ-1 cause recessive parkinsonism, characterized primarily by early onset (<30 years) and atypical clinical features [12, 14]. This complexity of genetic-molecular pathways makes it difficult to develop effective therapies that can target all the mechanisms. Furthermore, pathological features such as environmental agents and aging also contribute to the pathogenesis of PD [15]. Chronic exposure to neurotoxicants, including manganese, MPTP, and agricultural chemicals, could increase the risk of PD [15, 16]. Moreover, age-related mitochondrial dysfunction is another significant factor in PD progression [17]. As a result of these complex pathogenic processes, challenges in antiparkinsonian therapy development will be investigated during the drug development process.

According to the progress achieved in PD research, scientists have focused on exploring novel pharmacotherapies based on *in vitro* molecular models. Cell culture is a good choice because it develops pathology faster, easier, and cheaper [18]. One frequently employed cell line in PD research is the neuroblastoma line SH-SY5Y. Previous studies have indicated that human SH-SY5Y cells are able to acquire neurite outgrowth and branches [19]. Another typical cell line used to mimic the PD cellular environment is the PC12 line [18]. The use of various cell types offers researchers a range of *in vitro* modeling choices of PD; however, due to the lack of molecular interactions and simulation of the *in vivo* environment, simple models can not accurately replicate the full mechanism of Parkinson's pathogenesis.

Based on existing academic research, the role of the organ-on-a-chip model in brain diseases, particularly PD will be reviewed in this article. This developing model could offer new concepts of neurodegenerative diseases for future studies.

2. Overview of brain-on-a-chip technology

2.1. Principles

The organ-on-a-chip is a microfluidic platform that cultures viable cells in micrometer-sized chambers continuously perfused to imitate the physiological roles of living tissues and organs. Rather than building a complete living organ, its design principle is to synthesize a minimal functional unit that still retains organ function in an *in vitro* environment. To create tissue-tissue interfaces more precisely, it is designed with multiple microchannels linked by a porous membrane. Different cell types are lined on either side of the membrane to reproduce cellular interactions between different tissues. By integrating physical forces, it enables the analysis of organ-specific responses to medications, poisons, or external environmental factors. Scientists utilize chips with cells from various organs to simulate physiological interactions or ascertain drug distribution [20]. Although this technology reveals production methods, the details can be modified according to the exact organ structure that scientists want to study. For example, the organ-on-a-chip that mimics the brain is the brain-on-a-chip [22].

2.2. Advantages over traditional cell culture and animal models

Despite the convenience and low cost of traditional cell culture assays, they have significant limitations. For example, it is challenging to maintain nutrient concentrations throughout the assay process due to the lack of fluid flow. Additionally, conventional cell culture models are not specifically designed for cell-cell interactions. To address these issues, scientists have also employed animal models as prevalent experimental Parkinson models. The most classical and commonly used *in vivo* model is induced by neurotoxins. By injecting neurotoxins, such as 6-OHDA and MPTP, which can result in neuronal deterioration, this typical animal model mimics the pathogenic features of PD, defined by the disruption and damage of the nigrostriatal dopaminergic pathway. In establishing the *in vivo* model of PD, multiple factors, such as different doses and variant injection methods of neurotoxins, lead to various degrees of neurodegeneration. Correspondingly, the sensitivity of animals to neurotoxins varies from strain to strain, making the establishment of animal models challenging and laborious. Although these traditional models have deepened our understanding of the development of PD, they have yet to unveil the

DOI: 10.54254/2753-8818/58/20241368

mechanisms of relationships between brain cells and other tissues in both typical or abnormal conditions [13, 21].

In recent years, to better simulate the physiological properties of tissues, organ-on-a-chip technology has come forth as a significant option to traditional cell culture and *in vivo* animal models. Taking the human brain as an example, it is a vital organ with intricate neural circuits, synapses, and neurons, along with various glial cells like astrocytes, microglia, and oligodendrocytes. Additionally, the brain's interior is shielded by a blood-brain barrier, which presents a challenge for substances, including drugs, to enter the brain. Due to the complexity of brain structure and function, it is challenging to study the respective roles of the various units of the brain. In this regard, brain-on-a-chip technology opens up a new era in the study of brain physiology due to its ability to decouple various cellular and environmental factors. This technology's advantage lies in its ability to replicate the unidirectional structure and functional connectivity of neural circuits. Since neuronal bodies and axons in the brain are spatially distinct, the microchannels in brain-on-a-chip models accurately mimic this arrangement. This allows for localized drug exposure or damage to axonal regions, facilitating axon-specific biological studies [22].

3. Design considerations of pd brain-on-a-chip models

3.1. Possible selection of cell types

3.1.1. Dopaminergic neurons

PD is marked by the death of cells in vulnerable areas of the nervous system, such as dopaminergic neurons. Dopamine, a neurotransmitter, facilitates signal transmission between neurons and is crucial for motor control and various behaviors in the human body. Dopaminergic neurons primarily project to the striatum, a brain region involved in movement regulation, and the depletion of dopaminergic neurons results in a substantial reduction in dopamine levels in the striatum, which is the main reason PD patients experience motor problems. Scientists have explored new avenues in PD research with the advancement of organ-on-chip technology, particularly by employing induced pluripotent stem cells (iPSCs). This technology generates human neuroepithelial stem cells (hNESCs) from iPSCs. They can differentiate into a variety of neural cell types, including dopaminergic neurons [23]. Consequently, microfluidic systems based on dopaminergic neurons have been developed to enhance PD research. Several studies have applied microfluidic brain-on-a-chip devices based on dopaminergic neurons [23, 24].

3.1.2. Astrocytes

Astrocytes are present in all brain regions and possess the capability to mediate both physiological and pathological states of neurons. For example, astrocytes can release metabolic substrates such as the antioxidant glutathione (GSH) against reactive oxygen species (ROS) produced by aerobic metabolism in mitochondria. Oxidative stress generated by large amounts of ROS is thought to be one of the causative mechanisms of neurodegenerative diseases such as PD. Cleavage of γ-glutamyltranspeptidase in the plasma membrane of astrocytes generates precursors for the synthesis of neuronal GSH, thus ensuring neuronal survival and normal function. Additionally, astrocytes can provide neuroprotection to dopaminergic neurons by eliminating excessive extracellular toxic α-synuclein and reducing its accumulation in the brain [25]. In Alzheimer's disease, scientists have constructed a BoC platform to observe the interactions between neurons and astrocytes through microchannels [22].

3.1.3. Microglia

As crucial immune regulatory factors in CNS, microglia safeguard the nervous system against pathological effects such as neurodegenerative diseases, injury, and ischemia. In inflammatory states, microglia are activated to monitor the CNS and secrete a large number of cytokines, such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α). In the pro-inflammatory state, they secrete CCL2, while in the anti-inflammatory state, they secrete IL-10, IL-4, and transforming growth factor beta (TGF-β) [26]. Persistent microglia activation and neuroinflammation also appear to be causative factors

DOI: 10.54254/2753-8818/58/20241368

in PD, with some studies suggesting that aggregation of α-synuclein (aSyn) may be associated with microglia activation, leading to chronic neuroinflammation [27]. To delve deeper into PD mechanisms, Fernandes et al. performed microfluidic platform experiments on aSyn delivery between cell populations and the effects of activated microglia on neuron-like cells [27].

3.2. Incorporation of relevant PD pathology

Recent researches have indicated that PD patients are diagnosed with immune system. The primary pathological marker of PD is misfolded α-synuclein, also recognized as the central component of Lewy bodies. The protein exists within the central nervous system in monomeric and oligomeric forms, and all three forms of this protein trigger a central immune response coordinated by microglia. Scientists believe that misfolded α-synuclein produces neuroinflammation, and many studies have reported that PD is also associated with a chronic inflammatory response [28]. Moreover, the actions of microglia and monocytes induced by mutant α-synuclein monomers heighten cytotoxic immune reactions [29]. Pediaditakis and his team have developed a brain organ-on-chip to replicate the nigrostriatal region of the human brain using organ-on-chip technology. This microfluidic platform contains dopaminergic neurons, astrocytes, microglia, and microvascular cerebral endothelial cells. They simulated it by adding αSyn fibers to the fluidic channels, which leads to αSyn accumulation in the substantia nigra. With this novel brain-on-chip platform, scientists could better study the cell interactions in the PD brain, and also provide new target identification and novel therapeutic approaches for PD [30].

4. Drug screening using pd brain-on-a-chip models

A significant development in PD research is the use of brain-on-a-chip models for drug screening. This novel microfluidic device can better mimic the complex cellular and molecular environment of the human brain, providing a more accurate platform for studying PD compared to traditional animal models or cell cultures [31]. Brain-on-a-chip technology integrates various cell types that play key roles in PD, such as dopaminergic neurons, astrocytes, and microglia. These cells, cultured in a three-dimensional environment, can more accurately mimic the structure and role of the brain's neural networks and the main pathological features of PD like α-synuclein aggregation, dopaminergic neuronal degeneration, and chronic neuroinflammation.

Moreover, brain-on-a-chip models enable high-throughput screening of potential therapeutic compounds. Due to the high attrition rates, only one viable drug may emerge from the millions of compounds screened. To address the various reasons of attrition, it is imperative to enhance discovery methodologies and initiatives [32]. This requires the screening of larger libraries of compounds with advanced *in vitro* models, allowing researchers to test multiple drugs or drug combinations simultaneously, thus greatly accelerating the drug discovery process. Additionally, brain-on-a-chip modeling allows real-time monitoring of cellular responses to treatment and a dynamic understanding of drug efficacy and mechanisms. Compared to *in vivo* experiments using animals, brain-on-a-chip models significantly improve the reliability and timeliness of drug screening.

Furthermore, the brain-on-a-chip model helps acquire understanding of the intricate relationships between diverse cell types and signaling pathways in PD. By examining neuron-glia interactions and the impact of neuroinflammatory processes, these models reveal the multifaceted nature of PD pathogenesis. This comprehensive understanding can contribute to the development of multi-targeted therapeutic strategies that address not only the symptoms but also the underlying causes of the disease.

5. Toxicity testing using pd brain-on-a-chip model

Brain-on-a-chip technology, this third dimension of cell culture technology, is particularly important for the toxicity testing of drugs, as it closely mimics the human body's *in vivo* situation compared to conventional cell culture. For example, the EU-funded integrated project "ACuteTox" utilizes the cell-on-chip technique with the aim of predicting the acute systemic toxicity of orally administered drugs. The project utilized approximately 50 endpoints and various cell models. This approach robustly supports cell-cell interactions necessary for mechanistic investigations [33].

DOI: 10.54254/2753-8818/58/20241368

In drug toxicology testing, organ-on-a-chip technology can significantly reduce reliance on animal experimentation by replicating human organ functions on microchips, offering clear ethical advantages aligned with the 3R principles (Replacement, Reduction, Refinement). Firstly, this technology can substitute many animal experiments, particularly in toxicological studies, by providing more relevant data through the use of human cells to construct organ models. Secondly, it substantially decreases the number of animals needed, as preliminary experiments can be conducted on-chip, with only a few animal tests performed when absolutely necessary. Finally, organ-on-a-chip technology improves experimental design, making experiments more precise and efficient, thereby minimizing animal suffering. By more accurately mimicking the human physiological environment, organ-on-a-chip technology not only improves the accuracy of experimental data but also improves the clinical relevance of toxicological results. This advancement propels biomedical research forward while significantly reducing the use and suffering of animals [34].

6. Conclusion

This review discusses the rationale and basic use of organ-on-a-chip technology in PD research. This project also examines working principles, the necessity for drug development, and the benefits of utilizing this technology for drug toxicity screening. Addressing remaining challenges in accurately simulating brain tissue structure and physiology is also a focal point [35].

Human cell sources are essential for replicating human brain physiology and for the development of personalized drugs. As mentioned earlier in this article, iPSCs are a good cultivation technique for human cells because they can be induced and differentiated into other cell types. However, not all diseases can utilize iPSC technology effectively. For example, iPSC populations from Pompe disease are generated less efficiently than from healthy cells. Additionally, iPSCs from patients with some diseases exhibit poor proliferation capacity and low cloning efficiency [36]. In these cases, iPSCs are not the best choice for cell cultivation, and scientists need to find more convenient *in vitro* cell cultivation techniques to select human cells.

Region-to-region or organ-to-organ interactions should also be closely studied to better understand brain diseases. The brain consists of more than 250 distinct regions, each with a unique microenvironment and function. In recent studies, there is a shortage of detailed descriptions of the distinctive composition of each brain region, and not much research has been conducted on connecting different regions to create a multifunctional unit [37]. Simultaneously, the scarcity of organ-organ interactions severely restricts the ability to study the brain's role as a regulator of all body functions and to comprehend the feedback loops that release hormones from the endocrine system [37]. Multi-unit interactions are integral considerations in the future development. Despite the shortcomings of organ-on-a-chip, such as challenges in sourcing cells and other technological factors, this technology remains an important innovation. It has made significant progress and holds the potential to replace animal models and traditional cell culture models.

References

[1] Manrique de Lara A., Soto-Gómez L., Núñez-Acosta E., Saruwatari-Zavala G., Rentería M.E. Ethical issues in susceptibility genetic testing for late-onset neurodegenerative diseases. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 180(8):609–21 (2019).

[2] Cupidi C., Realmuto S., Coco G.L., Cinturino A., Talamanca S., Arnao V., et al. Sleep quality in caregivers of patients with Alzheimer's disease and Parkinson's disease and its relationship to quality of life. International Psychogeriatrics. Nov;24(11):1827–35 (2012).

[3] Lo Bianco C., Ridet J.L., Schneider B.L., Déglon N., Aebischer P. α-Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. Proceedings of the National Academy of Sciences. Aug 6;99(16):10813–8 (2002).

[4] Surmeier D.J. Determinants of dopaminergic neuron loss in Parkinson's disease. FEBS J. Oct;285(19):3657–68 (2018).

[5] Fang C., Lv L., Mao S., Dong H., Liu B. Cognition Deficits in Parkinson's Disease: Mechanisms and Treatment. Parkinsons Dis. Mar 24;2020:2076942 (2020).
[6] Aarsland D., Batzu L., Halliday G.M., Geurtsen G.J., Ballard C., Ray Chaudhuri K., et al. Parkinson disease-associated cognitive impairment. Nat Rev Dis Primers. Jul 1;7(1):1–21 (2021).
[7] Wakabayashi, K., Tanji, K., Odagiri, S. et al. The Lewy Body in Parkinson's Disease and Related Neurodegenerative Disorders. Mol Neurobiol 47, 495–508 (2013).
[8] Jankovic J., Tan E.K. Parkinson's disease: etiopathogenesis and treatment. J Neurol Neurosurg Psychiatry. Aug 1;91(8):795–808 (2020).
[9] Ntetsika T., Papathoma P.E., Markaki I. Novel targeted therapies for Parkinson's disease. Molecular Medicine. Feb 25;27(1):17 (2021).
[10] Lee TK, Yankee EL. A review on Parkinson's disease treatment. Neuroimmunology and Neuroinflammation. 8: 222 (2021).
[11] Pires, A. O., Teixeira, F. G., Mendes-Pinheiro, B., Serra, S. C., Sousa, N., & Salgado, A. J. Old and new challenges in Parkinson's disease therapeutics. Progress in Neurobiology, 156, 69–89 (2017). doi:10.1016/j.pneurobio.2017.04.006
[12] Gasser, T. Identifying PD-causing genes and genetic susceptibility factors. Recent Advances in Parkinson's Disease: Basic Research, pp.2–20 (2010).
[13] Koszła, O.; St ˛epnicki, P.; Zi ˛eba, A.; Grudzi ´nska, A.; Matosiuk, D.; Kaczor, A.A. Current Approaches and Tools Used in Drug Development against Parkinson's Disease. Biomolecules,11, 897 (2021).
[14] Singleton, A. B., Farrer, M. J., & Bonifati, V. The genetics of Parkinson's disease: Progress and therapeutic implications. Movement Disorders, 28(1), 14–23 (2013).
[15] Di Monte, D. A., Lavasani, M., & Manning-Bog, A. B. Environmental Factors in Parkinson's Disease. NeuroToxicology, 23(4-5), 487–502 (2002).
[16] Drechsel, D. A., & Patel, M. Role of reactive oxygen species in the neurotoxicity of environmental agents implicated in Parkinson's disease. Free Radical Biology and Medicine, 44(11), 1873–1886 (2008).
[17] Linchi Rani, Manas Ranjan Sahu, Amal Chandra Mondal. Age-related Mitochondrial Dysfunction in Parkinson's Disease: New Insights Into the Disease Pathology, Neuroscience, Volume 499, pp.152-169 (2022).
[18] Koszła, O., Stępnicki, P., Zięba, A., Grudzińska, A., Matosiuk, D., & Kaczor, A. A. Current Approaches and Tools Used in Drug Development against Parkinson's Disease. Biomolecules, 11(6), 897 (2021).
[19] Fernanda Martins Lopes, Rafael Schröder, et al. Comparison between proliferative and neuron-like SH-SY5Y cells as an in vitro model for Parkinson disease studies, Brain Research, Volume 1337, pp.85-94 (2010).
[20] Bhatia, S. N., & Ingber, D. E. Microfluidic organs-on-chips. Nature Biotechnology, 32(8), 760–772 (2014). Pediaditakis, I., Kodella, K.R., Manatakis, D.V. et al. Modeling alpha-synuclein pathology in a human brain-chip to assess blood-brain barrier disruption. Nat Commun 12, 5907 (2021).
[21] Bang, S., Lee, S., Choi, N., & Kim, H. N. Emerging Brain-Pathophysiology-Mimetic Platforms for Studying Neurodegenerative Diseases: Brain Organoids and Brains-on-a-Chip. Advanced Healthcare Materials, 2002119 (2021). doi:10.1002/adhm.202002119.
[22] Kane, K. I. W., Moreno, E. L., et al. Automated microfluidic cell culture of stem cell derived dopaminergic neurons. Scientific Reports, 9(1) (2019).
[23] Moreno, E. L., Hachi, S., Hemmer, K., et al. Differentiation of neuroepithelial stem cells into functional dopaminergic neurons in 3D microfluidic cell culture. Lab on a Chip, 15(11), 2419–2428 (2015).
[24] Rappold, P. M., & Tieu, K. Astrocytes and therapeutics for Parkinson's disease. Neurotherapeutics, 7(4), 413–423 (2010). . doi:10.1016/j.nurt.2010.07.001

[25] Osaki T., Shin Y., Sivathanu V., Campisi M., Kamm R.D. In Vitro Microfluidic Models for Neurodegenerative Disorders, pp. 7 (2018).
[26] Fernandes, J. T. S., Chutna, O., Chu, V., Conde, J. P., & Outeiro, T. F. A Novel Microfluidic Cell Co-culture Platform for the Study of the Molecular Mechanisms of Parkinson's Disease and Other Synucleinopathies. Frontiers in Neuroscience, pp.10 (2016).
[27] Pajares, M., I. Rojo, A., Manda, G., Boscá, L., & Cuadrado, A. Inflammation in Parkinson's Disease: Mechanisms and Therapeutic Implications. Cells, 9(7), 1687 (2020).
[28] White, A. J., Wijeyekoon, R. S., Scott, K. M., et al. The Peripheral Inflammatory Response to Alpha-Synuclein and Endotoxin in Parkinson's Disease. Frontiers in Neurology, pp.9 (2018).
[29] Pediaditakis, I., Kodella, K.R., Manatakis, D.V. et al. Modeling alpha-synuclein pathology in a human brain-chip to assess blood-brain barrier disruption. Nat Commun 12, 5907 (2021) .
[30] Miccoli, B., Braeken, D., & Li, Y.C. E. Brain-on-a-chip devices for drug screening and disease modeling applications. Current Pharmaceutical Design, pp.25 (2019).
[31] Aldewachi, H., Al-Zidan, R. N., Conner, M. T., & Salman, M. M. High-Throughput Screening Platforms in the Discovery of Novel Drugs for Neurodegenerative Diseases. Bioengineering, 8(2), 30 (2021).
[32] Pamies, D., Hartung, T., & Hogberg, H. T. Biological and medical applications of a brain-on-a-chip. Experimental Biology and Medicine, 239(9), 1096–1107 (2014).
[33] Schmerbeck, S., Schulze, F., Scheinpflug, J., & Ebrahimi, L. Organ-on-chip systems and the 3Rs. Science in School, 54(2021).
[34] Bang, S., Jeong, S., Choi, N., & Kim, H. N. Brain-on-a-chip: A history of development and future perspective. Biomicrofluidics, 13(5), 051301(2019).
[35] Escribá, R., Ferrer-Lorente, R. & Raya, Á. Inborn errors of metabolism: Lessons from iPSC models. Rev Endocr Metab Disord 22, 1189–1200 (2021).
[36] Maoz, B. M. Brain-on-a-Chip: Characterizing the next generation of advanced in vitro platforms for modeling the central nervous system. APL Bioengineering, 5(3), 030902 (2021).