

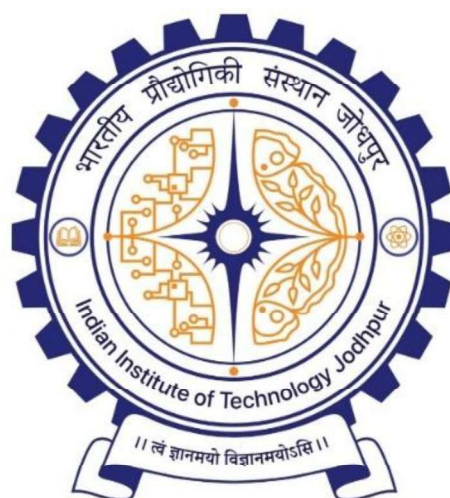
Xurography-Based Microfluidic Platform for Mimicking Neuronal Cytoarchitecture and Exploring its Application in Neurodegenerative Disease Research

A Thesis submitted by

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Chapter 6

Conclusion and Future Perspective

6.1. SUMMARY

The human brain's intricate architecture, governed by millions of interconnected neural networks, poses significant challenges to understanding its developmental processes, including axonal and dendritic growth, synaptic formation, and neuronal wiring. Traditional *in vitro* cell culture methods, while straightforward and widely used, fail to replicate the brain's highly complex and dynamic microenvironments. Recent advancements in microfluidic device-based technologies, or lab-on-chip platforms, have revolutionized the study of neural systems by offering precise control over the spatial and temporal aspects of cell culture environments. These platforms enable the reconstitution of neural cytoarchitecture, facilitating a deeper understanding of the structural and functional aspects of the central and peripheral nervous systems.

Microfluidic devices offer a significant advantage over conventional methods by mimicking the brain's microenvironment with high fidelity. This thesis explores the development and applications of a novel, cost-effective microfluidic device designed using the Xurography technique, a state-of-the-art fabrication method. The device was validated through on-chip cell culture studies with primary neurons and SH-SY5Y cells, demonstrating its cytocompatibility and utility in recreating neural systems.

One key application of the fabricated device is its use as a co-culture model for astrocytes and neurons, enabling the study of complex neuron-glia interactions. The platform also serves as an innovative drug screening system, as demonstrated by testing a multi-targeted compound in differentiated SH-SY5Y cells relevant to Alzheimer's disease. This underscores the device's potential for advancing neurotherapeutic research.

Additionally, the thesis presents the development of a Ferroptosis model within the microfluidic device, which has significant implications for studying neurodegenerative disorders. Ferroptosis, a form of regulated cell death associated with iron metabolism and oxidative stress, is increasingly recognized as a contributor to neurodegeneration. Using the fabricated device, researchers investigated the efficacy of a synthesized small molecule in modulating Ferroptosis, highlighting the device's versatility for modelling disease mechanisms and screening potential treatments.

The fabricated microfluidic device combines cost-effectiveness with high functionality, addressing a crucial gap in neuroscience research. By enabling precise control of cellular microenvironments, the platform supports the reconstitution of both the central and peripheral nervous systems, aiding the investigation of their respective dysfunctions and associated neurological disorders. It provides researchers with a robust tool for studying neurogenesis, modelling neurodegenerative diseases, and screening therapeutic compounds.

In conclusion, microfluidic lab-on-chip platforms, exemplified by the Xurography-based device developed in this study, offer transformative potential for developmental and cellular

neuroscience. They provide a bridge between simplified in vitro systems and the complex in vivo brain environment, opening new avenues for understanding neural dynamics, disease mechanisms, and therapeutic development. This advancement represents a significant step forward in unravelling the complexities of brain function and addressing the challenges posed by neurological disorders.

1. **Chapter 1** provides an overview [Figure 6.1] of i) the history of microfluidics; ii) circumstances that led to the development of microfluidic lab-on-chip platforms; iii) different methods to fabricate microfluidic devices, iv) applications of microfluidic technology in biological and chemical research. Furthermore, it critically evaluates the development of microfluidics technology in Neurobiological research which is the prime focus of the study carried out as a part of this thesis.

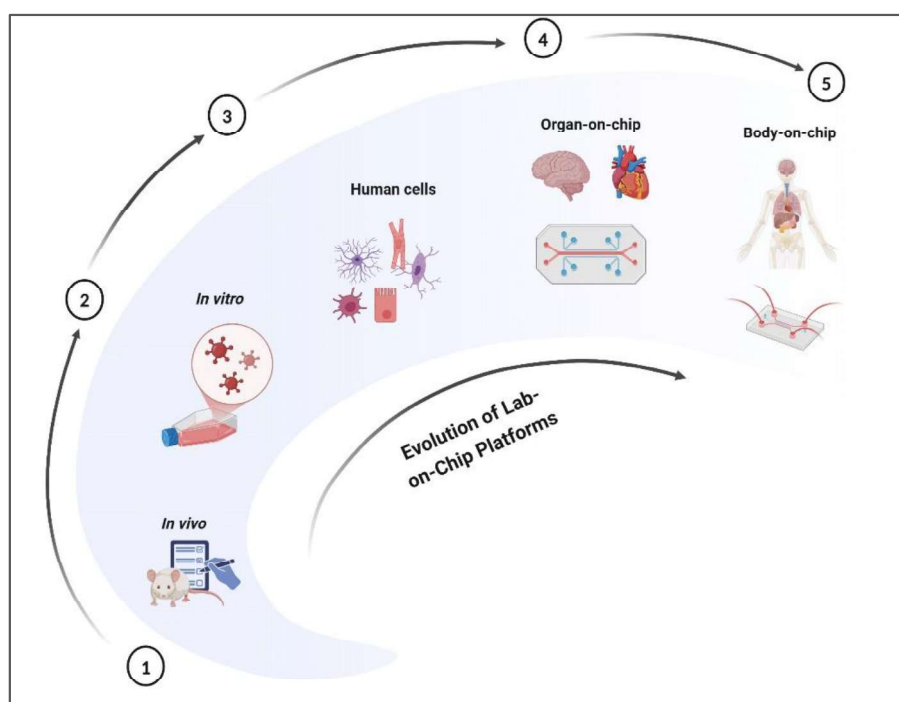


Figure 6.1. Schematic Representation of Chapter 1. Some icons of this illustration are adopted from Biorender.com

2. **Chapter 2** underscores the potential of microfluidic devices to replicate the central and peripheral nervous systems on a chip [Figure 6.2]. By mimicking the brain's complex cytoarchitecture, these systems allow researchers to study neuronal networks' structural and functional aspects and the pathological mechanisms underlying neurological disorders. Additionally, the chapter reviews the screening of drugs on these platforms, demonstrating their effectiveness for evaluating therapeutic candidates in a controlled, high-throughput manner. In conclusion, microfluidics-based lab-on-chip technologies offer a promising avenue for advancing neuroscience research. They provide innovative platforms for disease modelling, neurodegenerative disorder studies, and drug discovery, paving the way for novel therapeutic strategies and enhancing our understanding of the human brain's microenvironment.

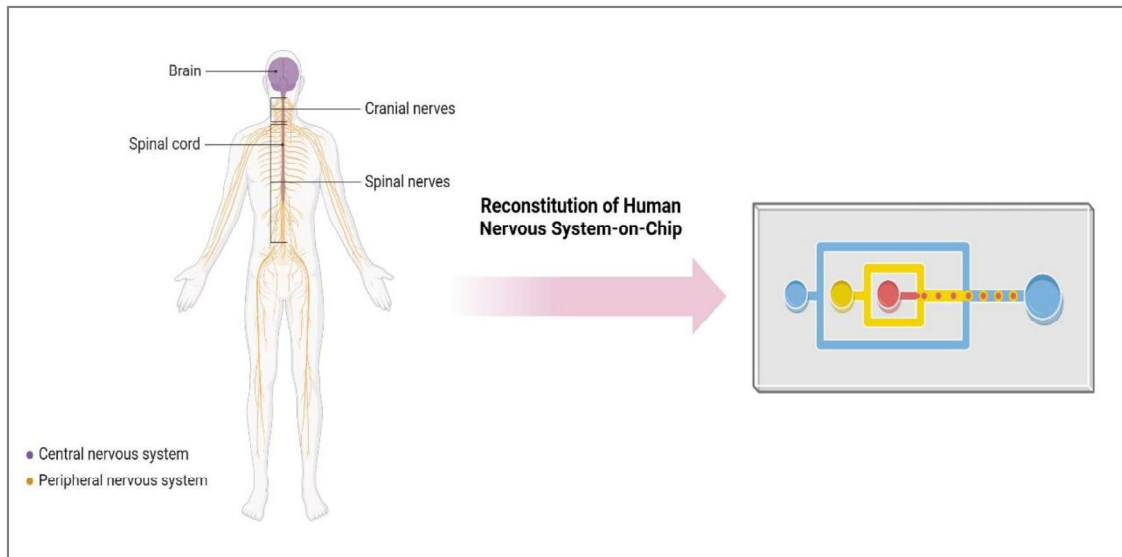


Figure 6.2: Schematic Representation of Chapter 2. Some icons of this illustration are adopted from Biorender.com

3. Microfluidic devices offer precise spatiotemporal control over compartmentalized cell cultures, enabling researchers to recreate the intricate cytoarchitecture of neurons within a regulated environment. *Chapter 3 [Figure 6.3]* highlights the use of microfluidic platforms for modelling neuronal development and the strategies employed to mimic neurogenesis on a chip. It also reviews the application of brain-on-chip models for studying the blood-brain barrier and neurodegenerative diseases. These systems allow researchers to replicate complex brain functions and pathological mechanisms, providing a robust platform for high-throughput drug screening. By mimicking the brain's microenvironment, lab-on-chip technologies offer valuable insights into fundamental brain processes. These models hold great promise for advancing our understanding of neurological disorders and serve as innovative platforms for developing novel neurotherapeutics.

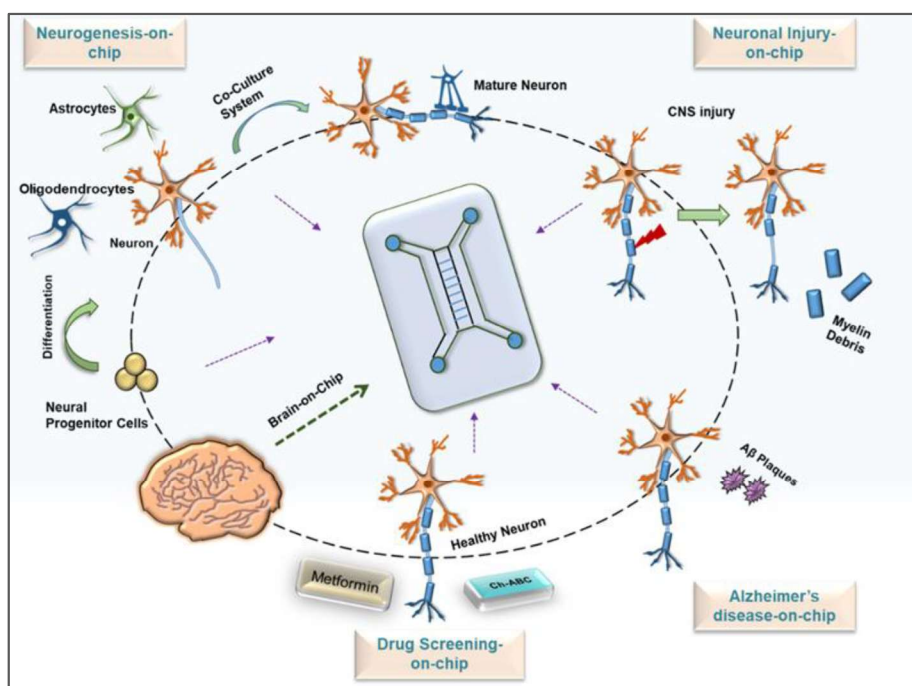


Figure 6.3: Schematic Representation of Chapter 3. Some icons of this illustration are adopted from Biorender.com

4. In *Chapter 4*, we have introduced a novel, cost-effective microfluidic device designed and fabricated using the innovative Xurography technique [Figure 6.4]. The device was tested for its ability to support primary neuron cultures, enabling the formation of neurospheres and single cells at various seeding densities within its microchannels. To validate its compatibility with disease modelling, SH-SY5Y cells were cultured and differentiated inside the device, showcasing its utility in replicating neuronal conditions. Additionally, the platform was demonstrated as a co-culture model using astrocytes and primary neurons, further expanding its potential for drug screening applications.

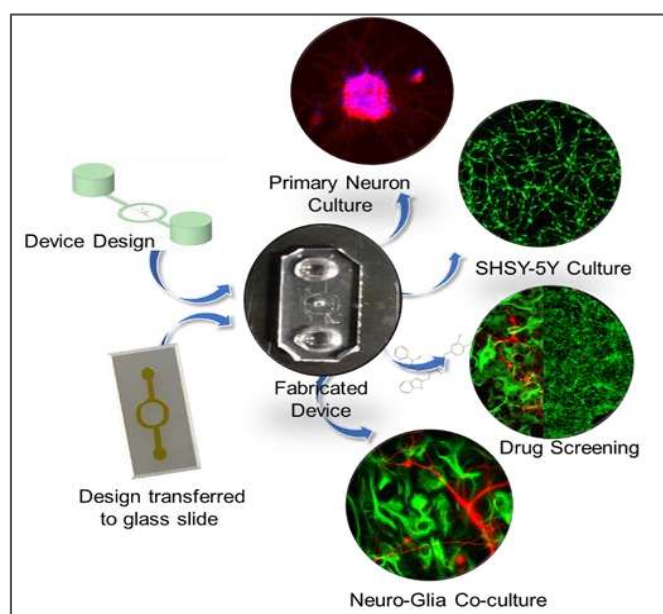


Figure 6.4: Schematic Representation of Chapter 4. Some icons of this illustration are adopted from Biorender.com

5. **Chapter 5:** Alzheimer's disease (AD), a leading neurodegenerative disorder, is closely linked to the deposition of amyloid plaques, exacerbated by copper ions and reactive oxygen species (ROS). To address these interconnected issues, a multi-targeted compound, TDSB, was designed. TDSB integrates three key functionalities: amyloid fibril inhibition (via a tryptophan residue), copper ion chelation (using 1-hydroxy-2-naphthaldehyde), and ROS management (through a dopamine moiety). Biophysical and in vitro assays demonstrated TDSB's ability to interact with A β peptides, reduce metal-induced ROS, inhibit A β -Cu (II) cytotoxicity, and cross the blood-brain barrier. Validated in 2D assays, TDSB was further tested in a cost-effective, Xurography-based microfluidic device. The device supported differentiated SH-SY5Y cells and co-culture models of primary neurons and astrocytes, effectively demonstrating its utility in modelling AD and screening neurotherapeutics. Additionally, the device facilitated the study of a Ferroptosis model, which links oxidative stress and cell death to various neurodegenerative disorders [Figure 6.5].

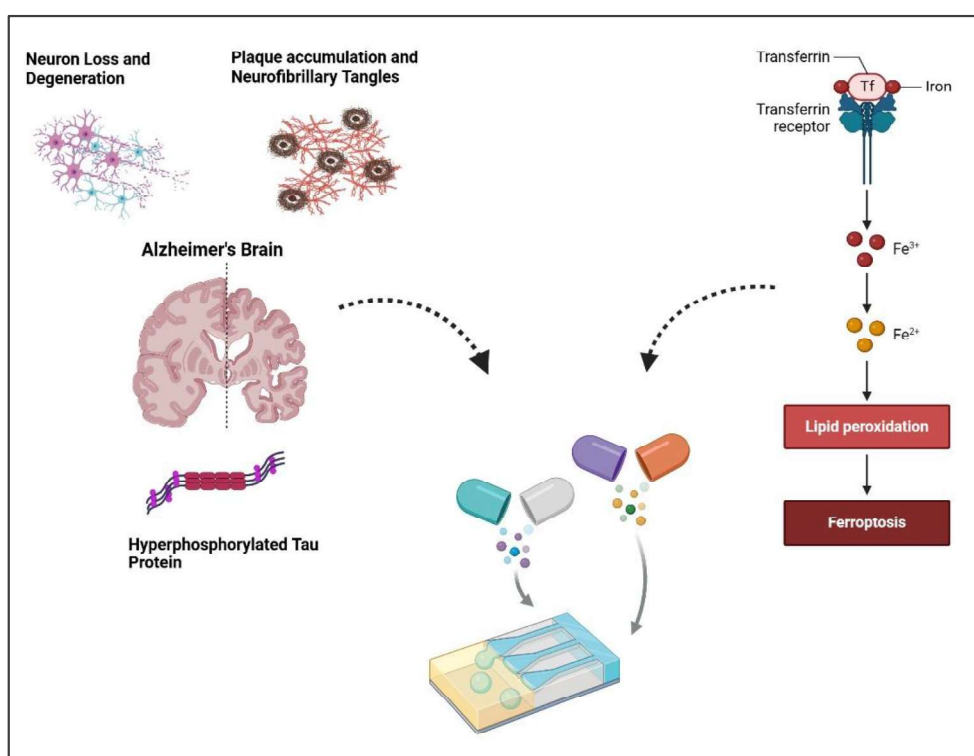


Figure 6.5: Schematic Representation of Chapter 5. Some icons of this illustration are adopted from Biorender.com

6. Chapter 6 provides an overall summary of the work presented and also outlines future directions for using microfluidic platforms.

6.2. FUTURE PERSPECTIVE

Future advancements in microfluidic technology will focus on creating more realistic brain models that would have the ability to replicate the structural, functional, and biochemical aspects of the brain. Integrating diverse cell types, such as neurons, astrocytes, oligodendrocytes, and microglia, into 3D architectures will enable researchers to study complex interactions and processes like synaptogenesis, myelination, and neuroinflammation. Incorporating vascular components will also allow for the recreation of the blood-brain barrier (BBB) and its role in brain physiology and pathology. The advent of patient-specific brain-on-chip models using induced pluripotent stem cells (iPSCs) will revolutionize personalized medicine. The convergence of microfluidic platforms with technologies like artificial intelligence (AI), machine learning, and big data analytics will provide new dimensions for data interpretation. Future designs might aim to connect multiple brain regions on a single chip to study inter-regional communication and functional connectivity. As these devices continue to evolve, they will deepen our understanding of brain function and dysfunction, accelerate therapeutic discoveries, and pave the way for personalized treatment approaches, ultimately transforming the landscape of neurobiology and medicine.