

Chapter 1

Introduction

Miniaturization has emerged as a significant trend over the past fifty years, extending its influence beyond electronics to encompass various fields, including mechanical and biological domains. It is connected to our daily lives and chores, as the diameter of human hair typically ranges around 75 μm , while the diameter of our Red Blood Cells measures between 7.5 and 8.7 μm ¹. Advancements in micro and nanofabrication techniques have significantly contributed to the proliferation of this technology, with researchers across various fields acknowledging the substantial impact of microfluidic technology. The **motivation of this chapter** lies in providing an overview 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.

1.1. EMERGENCE OF MICROFLUIDICS TECHNOLOGY

The origins of microfluidics can be traced back to advancements in microfabrication techniques during the late 20th century, particularly the development of microelectromechanical systems (MEMS). Born in the early 1990s, the lab-on-a-chip (LOC) concept aimed to conduct all reactions and assays typically performed on a bench within a single microdevice². This approach offers improved control over liquid volumes and substantially reduces sample and reagent costs.

1.1.1. The Pioneering Foundations of Microfluidics

According to the research of George M. Whitesides, the origin of microfluidics relies on four primary areas. The foremost is molecular analysis, encompassing techniques such as gas-phase chromatography (GPC) and capillary electrophoresis. The methods, originating in the 1950s and 60s, facilitate the separation of chemical compounds or biomolecules by passing small sample volumes through narrow tubes or capillaries, achieving high sensitivity and resolution.

Microelectronics is the second most celebrated parent of microfluidics, as fabrication methods and materials are directly linked.

A lesser-known yet crucial precursor to microfluidics is military detection. Since 1994, DARPA (Defence Advanced Research Projects Agency of the USA) has made significant contributions to the advancement of MEMs and the creation of miniaturized and portable “laboratories on a chip,” primarily aimed at detecting chemical and biological weapons.

The fourth most important field that gave rise to microfluidics is molecular biology. The emergence of genomics in the 1980s and the introduction of various microanalysis fields associated with molecular biology, including high-throughput DNA sequencing, called for the evolution of microfluidics to provide greater throughput and higher sensitivity and resolution³.

1.1.2. Overview of Microfluidics Technology

Microfluidics pertains to the study of flows with characteristic length scales of the order of micrometers. A microfluidic device (μ FD) contains channels with dimensions of tens to a hundred micrometers and is designed for manipulating nanoliter (10^{-9} to 10^{-18} liters) volumes of fluids to investigate their behaviour ³.

This interdisciplinary field offers several benefits, some of which include:

- a. Microfluidics lowers application costs worldwide by enabling the analysis with smaller volumes of samples, chemicals, and reagents.
- b. Improves selectivity and sensitivity.
- c. Faster performance is possible because different forces respond differently due to lower dimensions.
- d. Offers portable devices for point-of-care applications

1.1.3. Design and theory of fluid flow at the micro level

When it comes to microfluidics, the laws of equilibrium are modified to micrometric scales. For instance, the gravitational force is negligible compared to the capillary force. To design a microculture chamber, one must understand the fluid flow behavior ⁴⁻⁶ determined by the classical Navier–Stokes equation of fluid dynamics (for noncompressible fluid) ⁷.

$$\rho (dv/dt + v \cdot \nabla v) = - \nabla p + \mu \nabla^2 v + f$$

Here, ρ represents the fluid density, v the velocity field, p the pressure drops across the channel, μ the fluid viscosity, and f the volume forces.

The equation can be viewed conceptually as a balancing of the forces acting on each fluid element:

$$\begin{array}{ccccc} \longrightarrow & & \longrightarrow & & \longrightarrow \\ \text{net inertial force} & = & \text{net pressure force} & + & \text{net viscous force} \end{array}$$

Since the inertial forces in most of the microfluidic devices (μ FDs) are negligible, the above equation can be simplified as:

$$\begin{array}{ccc} \longrightarrow & & \longrightarrow \\ \text{net pressure force} & = & - \text{net viscous force} \end{array}$$

$$\text{that is: } \nabla p = \mu \nabla^2 v$$

In fluid dynamics, a dimensionless number called the Reynolds number (Re) that describes the fluid behaviour (whether the flow regime is laminar or turbulent) is defined by the ratio of the inertial and viscous forces:

$$Re = (\text{inertial forces} / \text{viscous forces}) = (\rho v L / \mu)$$

[L represents the characteristic length scale of the system]

Laminar flow occurs when a particle's velocity in a fluid stream is not a random function of time, whereas turbulent flow is chaotic and unpredictable. All of the fluid components stay parallel to one another in a laminar flow. As is typically the case in turbulent flows, no vortex

may form. The flow is nearly always laminar due to the tiny size of the microchannels ($Re < 2000$, which implies a laminar flow). Laminar flow's primary effect is that two or more streams that come into touch with one another won't mix unless they do so by diffusion.

A perfusion system is required for long-term cell culture microculture platforms to maintain the levels of nutrients and other critical components at the appropriate level. Otherwise, the frictional force generated by the flowing media, called shear stress, can cause damage to the cultured cell lines.

It is necessary to use the convection and diffusion mass transfer equation to estimate the velocity of molecules inside the microfluidic culture chamber:

$$(\partial c / \partial t) + \nabla \cdot (-D \nabla c + cv) = 0$$

[Here, c is the molecule concentration, v represents the fluid flow velocity, and D is the diffusion coefficient.]

1.2. CONVENTIONAL CELL CULTURE VS MICROFLUIDIC DEVICE-BASED CELL CULTURE

In biological research, *in vivo*, animal models have been used as a predominant way to assess the toxicity and efficacy of a potential drug molecule against a specific disease. However, genetic heterogeneity, compromising of the immune system, and ethical concerns are some limitations of the *in vivo* animal models that led to the development of cell culture techniques in the form of two-dimensional (2D) and three-dimensional (3D) approaches ⁸. Cell cultures enable the study of cell biology, tissue morphology, disease mechanisms, drug actions, protein synthesis, and the advancement of tissue engineering.

2D cell culture models serve as effective platforms for cell morphology analysis and initial cytotoxicity screening of drugs because of the lower cost and well-standardized protocols. With the use of Petri dishes and microtiter plates, the 2D cell culture techniques are widely performed in laboratory practices. In adherent 2D cultures, cells grow in a flat monolayer on a rigid surface like the plastic flasks. However, numerous disadvantages are associated with these traditional cultural methods. Firstly, the cell-cell crosstalk is not well portrayed. It also leads to altered gene expression, cell morphology, and behavior that do not accurately mimic *in vivo* conditions, resulting in poor drug response predictability. The altered cellular morphology can affect their functions, cell secretions, and signalling pathways. Additionally, it has been noted that the 2D system alters the cell's topology, biochemistry, gene expression, and splicing ⁹.

To overcome the drawbacks of conventional cell culture techniques, the rapid development of 3D culture methods has been a boon to biological research. In contrast to the 2D approaches, 3D cultures allow the growth of cells in all three dimensions, thus providing an environment that closely mimics the *in vivo* tissue architecture. Cellular morphologies are well restored, and cellular cross-talks are well defined. The types of 3D culture methods are: **i)** suspension cultures or spheroids on non-adherent plates; **ii)** cultures in gel-like matrices; **iii)** cultures on a scaffold such as Organoid culture, microfluidic device-based cultures that provide a better biomimetic tissue model that is more physiologically relevant ⁹.

Although microfluidic cell cultures have no standardized protocols, yet they provide several benefits. It is a relatively cost-effective and less cumbersome method that involves the use of

nanolitre to picolitre volumes of fluids, low quantity of samples, and reagents ⁷. The small volume requirement allows dynamic control of temperature and gases. Stimulation with drugs and other external cues (electrical, chemical, optogenetic, etc.) as well as simultaneous imaging, is feasible inside microfluidic culture chambers. Single-cell analysis and manipulation are also accurate and high-throughput ¹⁰. Tam *et al.* (2014) showed how microfluidic technology can be used to overcome the current restriction in super-resolution microscopy that pertains to sample preparation. In their study, they first added the adherent mammalian cells and cultivated them in a sterile microfluidic chip. Then, they gave a precise quantity of a specified reagent at a predetermined point in the experiment via an automated fluid-injection system to the chosen imaging chamber ¹¹.

1.3. DIFFERENT FABRICATION METHODS OF A MICROFLUIDIC DEVICE

With the growth and development of microfluidics technology, several research studies have been focused on establishing fabrication techniques that are highly efficient, less tedious, and cost-effective.

1.3.1. Laminates

Laminate microfluidic devices are chips containing a stack of layers that are cut independently and bonded together to form microchannels. Each layer in these devices can be viewed as part of a two-dimensional flow geometry that is enclosed by the layers above and below it. The thickness of the material used to create the layer then determines the height of a channel. The four main phases in creating a laminate device are: i) Selection of material of interest, ii) Cutting of preferred microfluidic features in each layer using a knife plotter (like xurography), iii) Each layer bonded together to form the complete device. This procedure offers high scalability and a much simpler fabrication protocol. However, devices bonded with adhesives lead to uneven channel heights ¹².

➤ Xurography Technique

Researchers have developed a state-of-the-art technology called the “Xurography technique” or “razor writing” based on the principle of laminate chips. The term Xurography was first introduced in 2005 by Bartholomeusz *et al.* ¹³. This technology is based on cutting paper. It involves using a cutting plotter to carefully cut microchannel patterns out of adhesive polymer films or other appropriate materials. These patterns are then bonded together to create useful microfluidic platforms. The cutter plotter, which uses a computer-controlled cutting head to follow the blade's path on a sheet of paper or polymer, is the foundation of the xurography technology. The cut material often determines the type of blade used. The cutter's force or depth setting can be used to adjust the cut's depth. The pattern can be made with a computer-aided design (CAD) application. The material is set on a glue pad, sometimes referred to as a cutting mat, to stop it from moving while being cut. The cutting parameters – speed, force, acceleration, and offset – are tuned to produce a neat pattern. A full 3D microfluidic setup can be accomplished by designing several 2D layers and joining them via adhesive, vacuum, heat, and other methods ¹⁴.

1.3.2. Molding

The molding technique can be of three distinct types: i) Replica Molding, ii) Injection Molding, and iii) Hot Embossing.

a. Replica Molding

Also known as “Soft Lithography,” the replica molding method was first introduced by Xia and Whitesides in 1998¹⁵. The principle of this technology relies on two important steps: a photolithography process to generate silicon (Si) wafer and photoresist master and a molding process during which a liquid-set polymer such as Polydimethylsiloxane (PDMS) is poured over the master and cured [Figure 1.1]⁷.

- **Master Fabrication:** A master mold is created using photolithography, where a photoresist material is patterned on a substrate (usually silicon) to form the desired microstructure.
- **PDMS Casting:** Liquid PDMS is poured over the master mold and cured to solidify, forming a replica of the microstructure.
- **Device Assembly:** The PDMS replica is then removed from the master and sealed against another surface, often glass, using plasma treatment to create a functional microfluidic device¹⁶.

Soft Lithography offers devices with higher resolution and 3D geometries. It is one of the most preferred methods in cell culture since it produces a better biomimetic environment. However, the most prominent disadvantage of the soft lithography approach is that it requires a cleanroom facility and high-end equipment that incur a higher cost of fabrication¹².

b. Injection Molding

Microfluidics injection molding, often known as micro injection molding, was initially developed in the 1980s. It involves using thermoplastics that are first melted inside a compressible chamber. Next, a cavity is created by compressing the two halves of the mold. The thermoplastic is injected inside the mold cavity at a steady rate. Finally, the mold is cooled and the cast part is removed¹⁷.

This method is best suited for high-volume simple design production as it has fast production rates and low per-unit costs at scale. However, the higher initial entry cost and restrictions on materials for thermoplastics still need to be addressed¹⁸.

c. Hot Embossing

Sister technology of Injection molding wherein polymers or thermoplastics are converted to viscous liquids at high temperatures and shaped with the help of mold, pressure and heat. Involves heating a thermoplastic material and pressing it into a mold to create microfeatures¹⁹.

Compared to injection molding, hot embossing offers several advantages. It requires the thermoplastic to flow through a relatively small distance compared to injection molding, thus lessening the stress generated by the material. This method is highly cost-effective, allows better precision, and has widespread commercial application. Despite the advantages, the fabrication of complex 3D structures with this method is difficult¹⁸.

1.3.3. 3D Printing

3D printing, or additive manufacturing, builds microfluidic devices layer by layer from digital models. It involves slicing 3D digital models into layers that can be easily recreated using a printer¹². Different 3D printing-based technologies are:

a. Fused Deposition Modeling (FDM)

The most widely used extrusion-based 3D printing process is fused deposition modelling (FDM). Extrusion-based techniques print different layers of 2D planes on top of one another using a nozzle. FDM involves melting the material in the nozzle and then extruding it onto the layer above, to which it gets bonded before cooling. Nowadays, many companies are working on creating an optimized version of this technology to cut down the cost of fabrication²⁰.

b. Stereolithography

Stereolithography (SL), the traditional rapid prototyping technique, develops layer on layer using an optical process. When it comes to producing extremely fine features, stereolithography is highly recommended. In SL, a vat of resin polymerized using a structured light source like ultraviolet (UV) is used to develop each layer. The kind of photoinitiators combined with the photopolymer to crosslink the polymer chains is linked to this dependence on the polymerization wavelength. SL supports a range of photopolymer resins, including those with specific optical and mechanical properties tailored for various applications, such as biocompatibility for biological assays or transparency for optical applications. A primary limitation of traditional SL is the production of monolithic chips made from a single material, which can restrict the integration of multiple functionalities within a single device. This may hinder the development of more complex microfluidic systems that require diverse material properties²¹.

c. Multi Jet Modeling

Another 3D printing technique used in microfluidics is Multi Jet Modelling (MJM), commercially known as Polyjet by Stratus. A photosensitive resin that is comparable to SL is used in the procedure. An inkjet printer ejects the resin onto the preceding layer as a droplet. A light source that is connected to the travelling printhead then cures it. This method uses many materials in the same build²² and can print with excellent accuracy²³.

d. Two-Photon Polymerization

A 3D printing technique that can produce nanometer-sized features is direct femtosecond laser writing (DLW) by two-photon polymerization (2PP). This process is distinctive in its capacity to create structures by curing specific locations within the material; however, the necessary equipment is often very costly. The two-photon requirement generally necessitates two light sources, with reactions occurring in the regions where the light overlaps. The materials employed are generally photopolymers; however, the process can achieve polymerization in the absence of a photo-initiator, too²⁴.

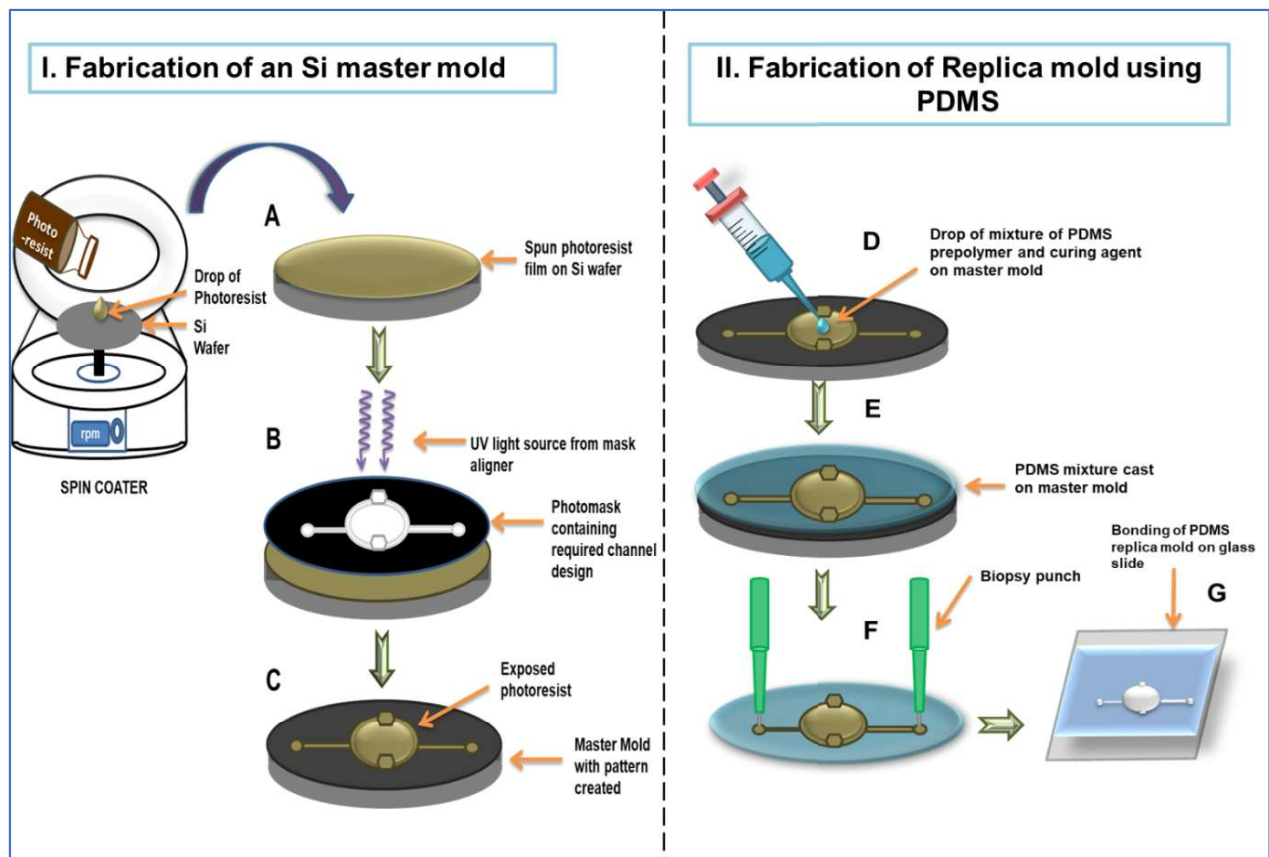


Figure 1.1: Schematic representation of the steps involved in PDMS chip fabrication following the Soft Lithography Approach.

I. Fabrication of a-Si master mold: (A) Photoresist is spin-coated onto a silicon (Si) wafer and subsequently soft baked at a specified temperature for several minutes, (B) Photoresist-coated wafers are exposed to UV light in a mask aligner utilizing a designed transparency photomask, (C) Si master mold is developed using a photoresist developer.

II. Fabrication of a replica mold using PDMS: (D) PDMS is mixed with a curing agent in a defined ratio, (E) The PDMS elastomer is poured and spin-coated onto the master mold, (F) The cured PDMS is removed from the master and cut; holes are formed on the replica mold using a biopsy punch, (G) The surface-treated PDMS block is then bonded to a glass slide by oxygen plasma treatment²⁵.

1.4. APPLICATIONS OF MICROFLUIDIC DEVICE

Microfluidic devices exhibit diverse applications across multiple disciplines, utilizing their ability to manipulate small fluid volumes rapidly. Some key areas where microfluidics is making a significant impact are [Figure 1.2]:

1.4.1. Biomedical Applications:

a. Point-of-care Diagnostics:

As the name suggests, Point-of-care diagnostics (POC) refers to medical analyses conducted near the site of patient care, and hence, it is also referred to as 'bedside testing'. Microfluidic devices, particularly those based on polymers or paper, possess disposability and cost-effectiveness, which are essential in this domain. Microfluidic applications in POC settings are currently utilized for pregnancy testing, HIV diagnosis, glucose biosensing, and drug abuse screening, although widespread adoption of such devices remains limited²⁶.

The application of microfluidics offers several advantages, including speed, increased throughput, and reduced sample consumption, which are essential for point-of-care applications. Microfluidic-based point-of-care devices are straightforward to handle and manufacture, making them an effective medical tool. The POC paper-based microfluidics can also be used to detect hormones, especially within home pregnancy tests. Walgama *et al.* 2021 developed a novel hybrid microfluidic device consisting of a disposable paper electrode and a three-dimensional (3D) printed plastic chip for electrochemical detection of magnetic bead-silver nanoparticles (MB-AgNP) bioconjugate²⁷.

b. Cell culture and analysis:

The development of microfluidic organs or tissues-on-a-chip is gaining popularity for two primary reasons: the ethical limitations of direct experimentation on humans and the inadequacy of animal models in accurately recapitulating human physiology. Furthermore, these devices have the potential to decrease investments and expedite the processes of drug discovery and testing. Ren *et al.* created a μ FD utilizing a variable number of multi-constriction channels to distinguish between the human breast cancer cell line MDAMB-231 and the nontumorigenic human breast cell line MCF-10A based on the differences in their mechanical properties²⁸. Another notable advantage of utilizing microfluidic cell cultures is the enhanced capability for single-cell analysis. The enhanced control over the cellular microenvironment in these systems significantly improves live-cell imaging analysis. Dynamic phenomena, including cell movements and migration, cell-cell, and cell-extracellular-matrix (ECM) interactions, and subcellular and molecular events, can be detected⁷.

c. Organ-on-chip Platforms:

Using organs-on-a-chip [Figure 1.3] eliminates the space and effort required for animal testing, allowing multiple tests to be conducted simultaneously on a single platform²⁶. Recently, research is going on to mimic different organs like lungs-on-chip, heart-on-chip, and brain-on-chip, focusing on developing the human body on-chip²⁹.

- **Lung-on-chip:** Devices mimicking human lung-on-chip are valuable tools for assessing the toxicity of various nanoparticles and elucidating the pulmonary diseases associated with the obstruction of small airways³⁰. Chips designed to mimic the essential functional alveolar-capillary liquid/air interface are created by cultivating alveolar epithelial cells and microvascular endothelial cells on opposing sides of a perforated silicone membrane²⁹.

In 2015, Huh *et al.* presented a proof-of-concept human “breathing lung-on-a-chip” microdevice that offers distinct abilities to restore the three-dimensional microarchitecture, dynamic mechanical properties, and cohesive physiological function of the alveolar-capillary interface³¹.

- **Heart-on-a-chip:** represents a feasible modelling approach, facilitating the examination of contractility and electrophysiological behaviours *in vitro*. Additionally, cardiac tissue contractility can be quantified under the conditions of health, disease, and exposure to chemical agents³².

Abulaiti *et al.* reported a Heart-on-a Chip microdevice system in 2020 comprising dynamic culture-based 3D cardiac microtissues derived from human induced pluripotent stem cells (iPSCs) and microelectromechanical system (MEMS)-based microfluidic chips. The device facilitated the visualization of cardiac microtissue pulsation kinetics through particle

displacement monitoring, quantifying physiological parameters such as fluidic output, pressure, and force ³³.

- **Liver-on-a-chip:** Liver-on-a-chip platforms replicate *in vivo* conditions by recreating the sinusoidal architecture of the organ, ensuring high cell viability and preserving cellular phenotypes while also simulating the functions of native tissues ³⁴. Several models have been developed to study liver disease progression, aiding in drug discovery and toxicity testing³⁵.

Bavli *et al.*, in 2016, introduced a liver-on-chip device that sustains human tissue *in vitro* for over one month under physiological conditions. They also evaluated mitochondrial respiration in real-time through two-frequency phase modulation of phosphorescent microprobes embedded in tissue ³⁶.

- **Kidney-on-a-chip** platforms have garnered considerable interest in microfluidics by simulating the real environment of renal tubular cells ³⁷.

For instance, Yin *et al.* developed a three-layer microfluidic kidney chip incorporating PDMS microchannels and porous membranes alongside a supporting microfluidic culture platform that facilitated the long-term culture of renal cells ³⁸.

Even brain-on-chip models have gained popularity in recent years, which will be discussed in detail in the later sections.

1.4.2. Chemical analysis and drug screening

Microfluidic systems can perform multiple analyses simultaneously, significantly speeding up the screening process for drug discovery and chemical reactions. This is achieved through parallelization and automation of assays. For example, Pandya *et al.* reported a 3D microfluidic platform that may be utilized for real-time deterministic assessment of the efficacy of a chemotherapeutic agent within 12 hours. The platform (66 mm×50 mm; L×W) incorporates microsensors (interdigitated gold electrodes with a width and spacing of 10 μm) capable of measuring alterations in the electrical response of cancer cells cultured in a 3D extracellular matrix when a chemotherapeutic agent is introduced adjacent to the matrix ³⁹.

Shin *et al.* have developed an Alzheimer's disease model by co-culturing human neural progenitor cells with brain microvascular endothelial cells in a three-dimensional microfluidic system. Utilizing this as a drug screening platform, they demonstrated that etodolac considerably reduced the permeability of the blood-brain barrier (BBB) compared to beclomethasone, concluding that the restoration of BBB integrity may serve as a valuable therapeutic target for Alzheimer's disease (AD) ⁴⁰.

1.4.3. Multianalyte Monitoring

A multi-analyte monitoring system can focus on extracellular and intracellular (bio)molecules, as well as physical parameters such as pH, oxygen levels, micronutrients, and cellular characteristics, to assess the status of cell shape and proliferation status in cultures or organoids. The development of human-based 3D organ models is crucial for accurately replicating their biological counterparts' essential physiology and functionality. Organ-on-Chip (OoC) technology is a micro-engineered model that simulates the complex biological processes involved in cellular interactions, thereby effectively predicting the cellular microenvironment. This technology provides an innovative drug development platform characterized by high throughput, cost-effectiveness, and efficiency.

Improving the accuracy of preclinical predictions is crucial in pharmaceutical research and development, as existing drug development methods often fall short in creating effective treatments for various human diseases. Although animal models remain the standard for preclinical drug testing, they frequently fail to predict human responses reliably. This limitation highlights the need for more advanced and accurate model platforms. Furthermore, ethical concerns, high costs, and the challenges of extrapolating results from animals to humans present significant obstacles to relying on animal models as a primary standard. Consequently, alternative platforms incorporating both in vitro and in vivo experimental models are essential for studying human drug responses ^{41,42}.

Lab-on-chip (LOC) technologies have recently gained significant importance in detecting and monitoring metabolites, particularly concerning the central nervous system and its associated disorders. These metabolites include intermediate and final byproducts of cellular metabolism, such as short-chain fatty acids, carbohydrates, amino acids, steroids, polypeptides, vitamins, and metal ions. Integrating these technologies into biopharmaceutical processes enhances our understanding of cellular behaviour and facilitates drug discovery and disease modelling advancements.

Over the past two decades, microphysiological systems (MPS), commonly known as "organ-on-a-chip", "body-on-a-chip," or "human-on-a-chip," have gained prominence as valuable tools for studying how the human body responds to drugs and other compounds. These systems are particularly effective in predicting human reactions before clinical drug testing or complementing clinical trials to explore underlying mechanisms. MPS offers several advantages, including replicating tissue architecture, simulating drug, and signalling molecule diffusion kinetics, and mimicking physiological flow conditions. Microfluidics, a key component of MPS, finds applications in micro propulsion, microthermal technologies, DNA chips, and LOC platforms. By enabling the manipulation of pico- to microliter volumes of fluid, microfluidics significantly reduces sample volume, reagent consumption, analysis time, and costs ⁴³.

Traditional techniques such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, gas chromatography, and high-performance liquid chromatography (HPLC) are widely used for identifying and separating cellular metabolites. UV-visible spectrophotometers and fluorescence microscopes or spectrophotometers are commonly employed for colorimetric and fluorometric assays, respectively. These established methods offer high sensitivity and repeatability for detecting target molecules. However, they rely on expensive equipment, are labour-intensive, resource-demanding, and generally unsuitable for continuous monitoring systems ⁴⁴.

Developing MPS models requires a microfluidic chip to replicate an organ-specific microenvironment's unique characteristics. Key factors to consider include the spatial arrangement of cells, the presence of biomechanical stresses, and appropriate tissue characterization techniques. OoC platforms, which are microfabricated cell culture systems, offer a simplified yet effective way to mimic the structure and function of human organs. Recent advancements in OoC technology have successfully demonstrated models of various human organs, including the heart, lungs, liver, brain, and skin.

1.4.4. Environmental Monitoring

Microfluidics technology can be used to detect contaminants such as heavy metals, bacteria, or pesticides in water samples. It can also monitor air quality and detect harmful microorganisms or pollutants in real-time ⁴⁵.

Apart from these, microfluidics technology can be used as miniaturized chemical reactors to enable faster reactions with high precision and yield, which is useful for synthetic chemistry and can be deployed as biosensors.

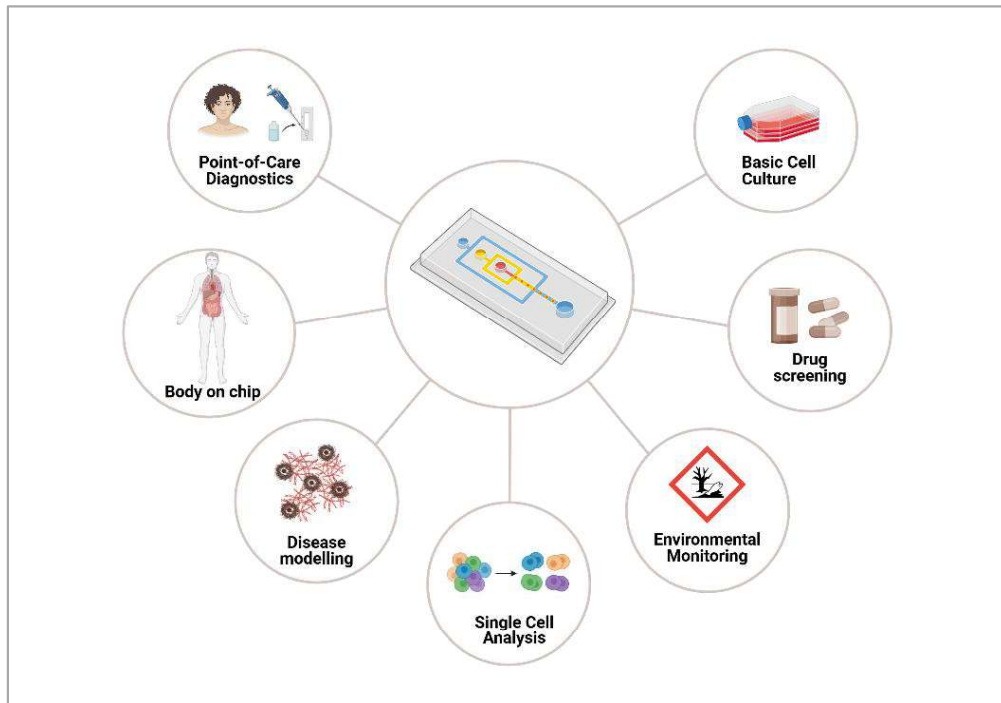


Figure 1.2. Schematic Representation of the Applications of Microfluidics Technology in various fields of research (Some icons of the illustration are adapted from and created with BioRender.com. <https://www.biorender.com/>).

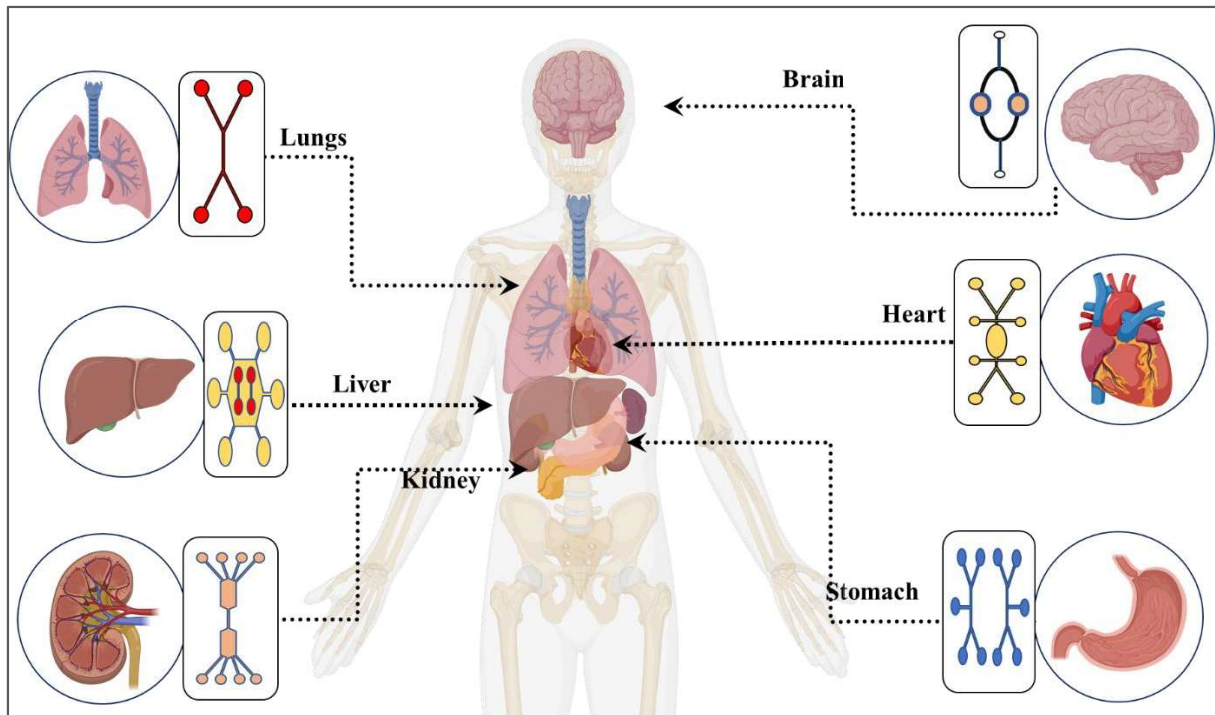


Figure 1.3. Schematic Representation of research adopted to mimic different organs of the human body on a chip (Some icons of the illustration are adapted from and created with BioRender.com. <https://www.biorender.com/>).

1.4.5. Microfluidics Technology: An Innovative Tool for Neurobiological Research

Microfluidics plays a vital role in neurobiological research since it enables precise spatiotemporal control of cells, molecules, and microenvironments that are critical for studying complex neural systems ⁴⁶. The following section highlights how microfluidics technology acts as a cosset for neurobiology:

a. Neural cell culture and co-culture:

Neurons are susceptible to various environmental factors compared to other cell types. They respond dramatically to cues such as substrate roughness, temperature, the composition of the culture medium, pH levels, and calcium ion (Ca^{2+}) concentration. As a result of this sensitivity, maintaining long-term cultures of neurons in vitro can be pretty challenging.

In line with this, Cui *et al.* conducted a study on the adherence and viability of Nigral cells by culturing them on silicon wafers with varying roughness levels. They observed that the cells could survive for more than five days while maintaining normal morphology when the surfaces had an average roughness (Ra) between 20 and 50 nm. In that case, the cells also expressed neuronal tyrosine hydroxylase (TH). As opposed to this, cell adherence was negatively impacted when smooth surfaces with Ra values below 10 nm or rough surfaces with Ra values exceeding 70 nm were used as substrates ⁴⁷.

b. Neuron Manipulation

Microfluidics technology can be used to manipulate neurons, an essential phenomenon in studying isolated axons and somata at microscale levels. In neuron manipulation research, emerging techniques, including hydraulic and air pressure systems, optical traps, laser

tweezers, and microstructures, have gained popularity. These approaches are often enhanced by combining them with surface chemistry for greater precision and effectiveness.

c. Neural stem cell differentiation

Neural stem cells (NSCs) are self-renewing and multipotent, capable of generating differentiated functional progeny such as neurons and glial cells. They have been successfully isolated and cultured from the developing and adult central nervous systems (CNS) of various mammalian species, including humans.

Chung *et al.* used a gradient-generating microfluidic device to study the proliferation and differentiation of human cortical neural stem cells by precisely controlling the growth factor density gradient ⁴⁸.

d. Neuropharmacology

Neuropharmacology focuses on how drugs can affect cellular functions within the nervous system. Traditional techniques, such as patch clamp and microelectrophoresis, have significantly advanced the field at cellular and molecular levels. Nevertheless, considerable challenges persist in conventional neuropharmacology, including the precise control of microenvironments, the targeted delivery of micro- and nano-scale drug volumes to specific neurons, and the isolated study of neuronal somata and axons, among others.

In the research by Ravula *et al.*, an innovative experimental apparatus that integrates a microfluidic divider with a multi-electrode array substrate, enabling the independent monitoring of injury-induced electrical activity from dorsal root ganglion (DRG) cell bodies and axons while isolating them in distinct culture microenvironments. At specific concentrations of vincristine (a neurotoxin that induces distal axonal degeneration and peripheral neuropathy), the cell body vincristine did not result in morphological neurodegeneration or sustained hyperexcitability.

Conversely, it was observed that treating the distal axon with an equivalent amount of vincristine first resulted in a reduction of axonal excitability, subsequently leading to axonal degeneration in a dying-back pattern. Furthermore, vincristine exposure to axons resulted in an early hyperexcitability phase in the cell bodies. This indicates that a signal is transmitted from the distal axon to the soma during vincristine-induced axonal degeneration. The data asserts that vincristine exerts a direct neurotoxic effect on the axon ⁴⁹.

e. Neuroelectrophysiology

The recent implementation of microfluidic devices in neuroelectrophysiology has significantly enhanced their efficacy. Integrating microfluidic devices with patch-clamp technology facilitates the modulation of the biochemical environment at the cellular level while monitoring the electrophysiological characteristics of reconstructed rat embryonic cortical networks, enabling effective recording of membrane potential and/or current variations through voltage/current clamp modes.

Morin *et al.* constructed a PDMS microfluidic system and utilized it alongside both bespoke and commercially available planar microelectrode arrays (pMEAs). Cortical neurons derived from chick and mouse embryos were effectively integrated into this system, with a successful recording of extracellular electrical signals from all cell types positioned within the patterns, and this bioelectrical activity persisted for several weeks ⁵⁰.

f. Analyte sensing in neurodegenerative disorders

Microtechnological advancements have led to the development of microsystems, including OoC and cell chips, that recreate in vivo-like microenvironments⁵¹. Additionally, 3D culturing techniques using microfabrication technology have been introduced, allowing the simulation of interstitial flow effects on cultured cells. Recent studies have demonstrated microfluidic systems delivering interstitial-level flow to cells in vitro, replicating the brain's extremely slow interstitial flow, which ranges between 0.1 and 0.3 liters per minute (L/min)⁵²⁻⁵⁸.

Micro total analysis systems (μ TASs), which integrate multiple functions into a single platform, are products of advancements in sensing technology miniaturization—fabricating disposable electrodes with thick films on ceramics or thin films on silicon substrates. Miniaturizing electrochemical sensors to the microscale significantly improves analyte diffusion behaviour from the electrode surface, transitioning from semi-infinite to radial diffusion. This results in enhanced signal strength, a higher signal-to-noise ratio, better molecule distribution to the electrode surface, lower detection limits, increased sensitivity, and higher current density than macro electrodes.

The ability to achieve low detection limits for neurotransmitters such as dopamine (DA) is paramount, underscoring the value of these advancements in sensor technology. The hallmark features of Parkinson's disease and related synucleinopathies include the loss of dopaminergic neurons, gliosis in the substantia nigra, and the pathological accumulation of misfolded alpha-synuclein. Additionally, both clinical studies and in vitro research suggest that the blood-brain barrier (BBB) is disrupted in Parkinson's disease, although the precise mechanisms underlying endothelial dysfunction remain unclear⁵⁹.

Accurate and sensitive monitoring of dopamine (DA) in brain extracellular fluid is critical for understanding its role in normal and pathological processes. A significant challenge in DA analysis lies in reducing the detection period to gain deeper insights into DA dynamics, particularly considering its pivotal role in neurotransmission⁶⁰.

Senel *et al.* introduced an innovative and highly precise electrochemical sensing platform for DA detection using a 2D culture of neuroblastoma cells. This platform employs a gold-coated MPy array (Au-MPy) to measure DA released into the culture medium following stimulation of the cells. The Au-MPy sensor demonstrated excellent performance, with a remarkably low detection limit of 0.50 ± 0.08 nM, a wide linear range of 0.01 to 500 μ M, and a high sensitivity of 0.18 ± 0.01 A/M. Furthermore, the sensor exhibited exceptional selectivity for DA over interfering substances such as ascorbic acid, uric acid, KCl, and glucose⁶¹.

This approach holds significant potential for advancing the investigation and diagnosis of various neurological and psychiatric disorders, including schizophrenia and Alzheimer's disease (AD).

1.5. CONCLUSION

The need to better understand the architecture and functions of brain networks has fuelled a growing body of research into the application of microfluidics in neurobiology. In particular, understanding how individual cells within the human brain function is essential to figuring out the principles behind memory, learning, and disorders. Despite the challenges faced by contemporary microfluidic devices in neurobiology, a future where microfluidic devices exhibit superior performance—incorporating multifunctional modules within a single apparatus and automation for enhanced speed and high throughput, is foreseeable.

Therefore, the following chapters of this thesis essentially deal with the need to develop a brain-on-chip platform using a low-cost microfluidic device. The device was further exploited to screen a molecule of interest that had been designed during the research against Alzheimer's Disease.

1.6. REFERENCES

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