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Review

Blood brain barrier-on-a-chip permeation to model neurological diseases using microfluidic biosensors

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Abstract

The need to understand human body functions, monitor disease progression, and advance in drug development have consistently been major driving forces for medical innovations and advancements. Organ-on-a-Chip technology, particularly Blood-brain-barrier (BBB)-on-chip technology, creates an avenue to closely replicate the brain environment and provides real-time monitoring of cells. Located at the interface between the blood and the brain parenchyma, the blood-brain barrier is crucial for protecting the brain due to its semi-permeable nature, and is responsible for regulating the movement of molecules between the blood and the brain. Therefore its integrity and perfect functionality are essential for the unperturbed functioning of the central nervous system. The lack of effective in-vitro models to investigate these diseases due to various technical and economic limitations poses a significant challenge. Moreover, the use of in-vivo models involving other primates and rodents for experimentation further poses a challenge due to physiological variations between humans and other species and has ethical constraints. Our study explores how the BBB-on-chip overcomes a lot of these limitations posed by the other in-vitro and in-vivo models, making it a more efficient and accurate model to investigate the blood-brain barrier. The study further explains the evolution, applications, and future prospects of the BBB on-chip technologies.

Keywords

Blood-Brain Barrier, Organ-on-Chip, Microfluidics, Biosensor, Disease Modeling, Personalized Medicine

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1. Introduction

Central Nervous System (CNS) disorders and neurodegenerative disorders are a plague that have been profoundly affecting individuals and societies for decades. These disorders lead to significant decline in cognitive and motor functions and greatly impact the overall quality of life of individuals. According to a study released by The Lancet Neurology in 2021, approximately 3.4 billion individuals (corresponding to 43.1% of the world population) were found having a neurological condition which accounted for approximately 11.1 million deaths [1]. According to the World Health Organization (WHO), there has been an 18% increase in disabilities, illnesses, and premature deaths due to neurological diseases since 1990. However, scientifically, a correlation between the blood-brain barrier (BBB) dysfunction and the prevalence of these neurodegenerative diseases has been identified. Hence, the fatal nature of these diseases, coupled with the lack of effective treatments, necessitates extensive research on the BBB for disease control and drug development [2]. All extant vertebrates with a well-developed CNS have a blood-brain barrier (BBB) [3]. Its main role is to regulate the movement of ions, molecules and cells between the blood and the brain tissue and contributes to the homeostasis of the brain microenvironment. It mainly protects the brain from harmful and toxic substances present in the blood, supplies essential nutrients to the brain tissue and ejects harmful and toxic substances present in the blood, supplies essential nutrients to the brain tissue and ejects harmful substances from the brain tissue back to the bloodstream [4]. This existence of the BBB was first discovered through a series of studies conducted in the late 19th century to the early 20th century. Different dyes and compounds impermeable to the CNS were used as tracers to investigate the brain blood vessel permeability and successful results have led to the inception of the term *blood-brain barrier* [5], as shown in Figure 1.

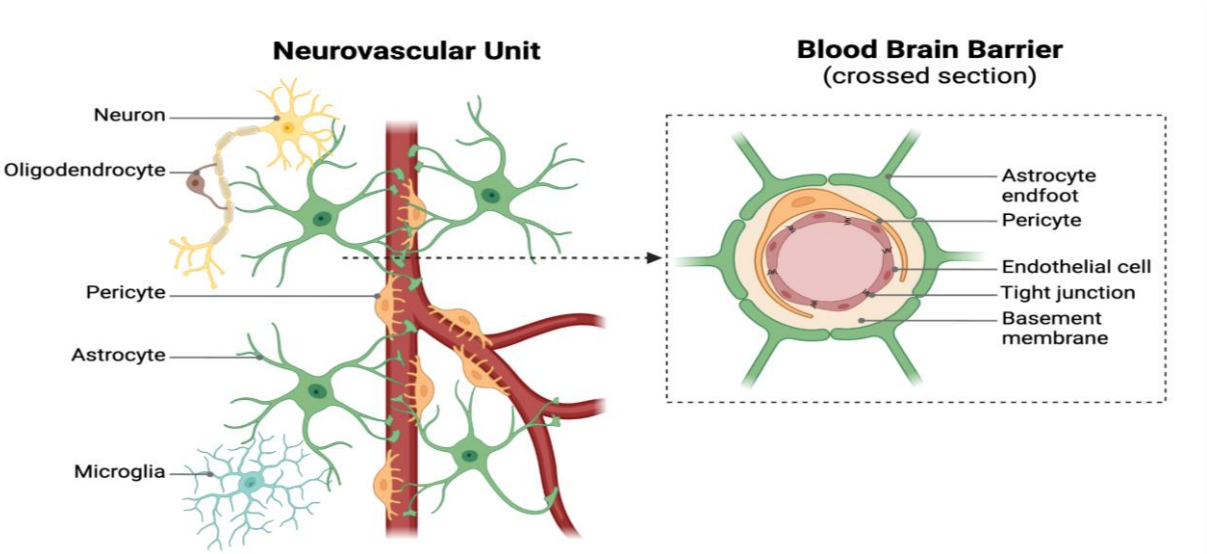

Figure 1. *Representation of blood-brain barrier structure, depicting endothelial cells (ECs), astrocytes, pericytes (PCs) and tight junctions. Illustration of blood-brain barrier structure, depicting endothelial cells (ECs), astrocytes, pericytes (PCs) and tight junctions. Adapted from "Neurovascular Unit and Blood Brain barrier", by BioRen-der.com (2024). Retrieved from https://app.biorender.com/biorender-templates.*

The BBB is made up of vascular endothelial cells in most mammals including humans but is made up of perivascular glial cells in elasmobranch fish. The combined surface area of these vessels lie in between 150 and 200 $cm^2\ g^{-1}$, making the total area for exchange in the brain of an average adult between 12 and 18 m^2 [6] [3]. Along with the specialized endothelial cells, the pericytes, astrocytes and basement membrane form the BBB. The functions of these cells are as described in Table 1.

References	Cell type	Key characteristics	Function
Daneman and Prat 2015 [7]	Endothelial cells (ECs)	They are mesodermally derived modified simple squamous epithelial cells. Form the walls of the blood vessels. They are held together by tight junctions to form a semi-permeable membrane.	Regulate the movement of molecules, ions and other cells.
Daneman and Prat 2015 [7]	Pericytes (PCs)	Present on the abluminal surface of the endothelial tube and are embedded in the vascular basement membrane.	Responsible for long cellular processes. The presence of contractile protein helps control the diameter of the capillary.
Daneman and Prat 2015[7]	Astrocytes	They completely sheath the vascular tube and contain various proteins responsible for water homeostasis.	Modulate and maintain the barrier. Relay signals to regulate blood flow upon neuronal activity.
Daneman and Prat 2015[7]	Basement Membrane (BM)	There are two types of BMs, namely, the outer parenchymal BM and the inner vascular BM.	Anchor most signaling processes at the vasculature. Provide an additional barrier and prevent molecules from entering the brain.

Table 1. *Cell types of BBB, key characteristics and function.*

However, despite the impressive structure of the BBB, there is a high possibility of neuro-invading pathogens (such as bacteria, viruses, fungi, and parasites) crossing the barrier leading to many neurological and CNS-related diseases. It is worth noting that the BBB membrane disruption is characterized by the breakdown of the basement membranes by matrix metalloproteinases. This usually leads to leukocyte infiltration, which has been commonly observed in various neurological disorders [7]. Some examples include *Chlamydiophila pneumoniae* and *Borrelia burgdorferi sensu lato,* which are reportedly responsible for multiple sclerosis *and* Alzheimer's disease (AD), respectively [8] [9] [10]. However, the ambiguity in the relationship between the diseases and the pathogens necessitates further research [8].

In recent years, numerous devices have been developed to hold small amounts of body fluids or solutions known as microfluidic devices, which have been used to investigate diseases [11]. Organ on chip (OOC) also known as tissue chips, are proven to be highly informative in drug discovery [12]. These are developed from culturing microfluidic cells and they enable real-time, high-resolution imaging of in-vitro activities of cells in tissues and organs [12]. The global organ-on-chip market was valued at $103.44 million in 2020, and is projected to reach $1.6 billion by 2030, growing at a compound annual growth rate (CAGR) of 31.1% from 2021 to 2030 [2].

The blood-brain barrier-on-chip technology, a type of OOC model, is widely sought out for blood-brain barrier research. Though the conventional in vitro and in vivo animal models have been used widely, they have several limitations that are tackled by the blood-brain-barrier-on-chip models [13]. Some limitations that are associated with the animal models are as follows: they are time-consuming, face ethical issues for their use in clinical studies, require high doses of chemicals and the prime drawback is the genetic variations existing between the two species that result in differences between the data obtained from animal studies and human outcomes [13]. Moreover, after a certain period cells can lose their BBB-specific properties which can lead to a decrease in the barrier integrity. Also replication of multicellular environments along with microfabrication is quite challenging [14]. There is a great need for the human blood-brain barrier models because neither the animal models nor the conventional in vitro models have the capability to effectively replicate the barrier and transporter functions of the BBB observed in humans [15]. BBB-on-chip models help in developing CNS-targeting therapeutic technologies as they can efficiently replicate the barrier properties [16].

Here in this review study, we explored how OOC systems can be effectively utilized to study the blood-brain barrier. It covers the conception and development of this technology, including the strategies and scientific principles that enable OOC systems to support various biosystems through multiplex alignment. We aim to offer a detailed review on the blood-brain barrier-on-a-chip technologies, examine the intricate workings of BBB's selectively permeable membrane, explore its diverse applications, existing gaps, and potential advancements.

2. Discussion

2.1. Overview of Microfluidic Cell Culture

Devices

Microfluidic cell culture is a technique of modeling tissue-tissue- interfaces for curing, analyzing, maintaining, and experimenting with the cells at the microscale. In recent years, many devices have been made that hold very small amounts of body fluids or solutions known as microfluidic devices to cure diseases [11]. In recent years many microfluidic devices have been used in a wide range of fields like drug discovery, disease diagnosis, stem cell research and organ-on-chip devices, etc. Moreover, the use of traditional culturing devices involving laminar flow or rapid energy dissipation have been utilized to create cost-effective, compact, and integrated devices called microfluidic devices [17].

Microfluidic devices contain small-scale systems known as chambers that are marked with small channels. The microfluidic chips are made up of glass, polymers, and ceramics. Microfluidic devices are helpful because of their fast detection capability, small volume, and transportability [18]. Many microfluidic cell culture devices have been used in vast studies and in drug discovery. A case in cancer biology where 3D models like spheroids or organoids have been put in use to understand the mechanism of cancer development. In the past few decades, droplet microfluidics integrated with hydrogels have become prominent due to their numerous capabilities, notably minimum variation, and the ability for long term cell activation [19]. In recent times, microfluidics have been utilized to grow tissues, which significantly aided in reducing the time for drug discovery and helped minimize expenses. Ever Since, many microfluidic devices have been developed to mimic the characters and functions of organs such as Kidney-on-chip, Gut-on-chip, Brain-on-chip, Liver-on-chip, and so on. These microfluidic chip models prove to be a great tool to precisely investigate many organ-related diseases and viruses. These devices also provide an accurate profile of nutrient flow and cellular activities [20].

2.2. Conceptual framework behind organ-on-chip technology

Microfluidics enable real-time high-resolution imaging of in-vitro analysis of various genetic, biochemical, and metabolic activities of cells. This technology can make a great impact in the area of tissue development, organ physiology, and disease etiology with certain advancements [21]. The perceived translational gap between physiological processes in conventional in vitro and animal models is expected to be bridged by OOC models [22]. OOC is a product of the progressive development of microfluidic technology hence they are capable of combining microfluidic technology with cell biology and can mimic the physiological microenvironment of the in-vivo target organs. Growth and cell functions are supported by the utilization of biomimetic materials that can accurately mimic the extracellular matrix, thereby leading to an improved physiological relevance [23]. These advanced in-vitro biological models are successful in replicating the local characteristics of a disease and they can also control the environmental parameters in order for the cells to survive [24]. The most commonly used materials for the fabrication of these devices are Polydimethylsiloxane (PDMS) and polymethyl methacrylate (PMMA) due to their transparent nature and high compatibility with fluorescence microscopy that can lead to more number of images, thereby favorably giving rise to large amount of image-based data [25]. Additionally, the arrangement of multiple cell types in certain spatial arrangements to replicate the 3-D structure of the human tissues, is essential to enable complex interactions among organisms, for example, as seen in liver and lung

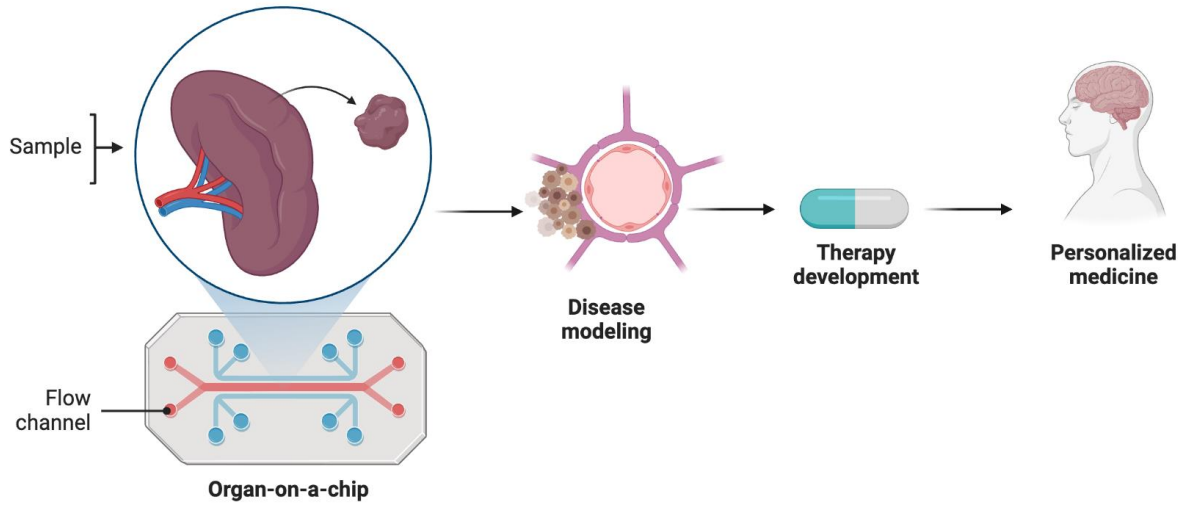

Figure 2. *Representation of OOC technology, depicting the microchannels, microchambers, and the cross sectional spatial arrangement of*

tissues. Adapted from "Complex Intestine on-a-Chip for Personalized Medicine", by BioRen-der.com (2024). Retrieved from https://app.bio-render.com/biorender-templates.

based OOC models (Figure 2). Growth and cell function can be supported through the utilization of biomimetic materials that aid in mimicking the extracellular matrix, thereby enabling improved physiological relevance. Moreover, the integration of sensors to monitor and analyze cellular behavior allows real-time observation of cellular stimuli to drugs and toxins [24].

2.3. Overview of the evolution of blood-brain barrier-on-a-chip models

Blood-brain barrier-on-a-chip (BBBoC) models have been developed to gain a comprehensive understanding on the BBB. BBB does not let therapeutic drugs used to treat neurological diseases, brain cancer, and so on, enter the brain . BBB-on-chips are considered to be a great tool to study the BBB in order to evaluate drug efficacy and delivery to CNS targets. With continuous evolution and recent advancements in other scientific techniques, new opportunities have emerged to improve the BBBoC models [26].

In the early stages of BBBoC development, static monolayer models were used but they could not effectively replicate human complexities. This facilitated a need for an advanced model due to the importance of replicating the necessary human conditions for effectively understanding its underlying properties and mechanisms. Subsequently, the transwell-based multi-space devices came into play, which were composed of endothelial monolayers with 2D cultured pericytes and astrocytes, along with a porous membrane. However, these static models lack the dynamic flow essential for regulating vascular shear stress. Additionally, they do not facilitate interactions among cells, thereby limiting their effectiveness [27].

Following that, a heterogeneous group of dynamic in vitro (DIV) models emerged and these models were also considered as the precursors to the microfluidic brain chips. They can generate physiological shear stress which enables unidirectional laminar flow along the capillaries. Nonetheless, the walls of these devices are highly thick, making it very difficult to visualize the underlying mechanisms occurring in the cells. To overcome this problem microscopes are used, however this significantly increases the cost of production of this model and makes its usage incompetent [28].

Later advancements introduced numerous devices that fall under the category of microfluidic devices. These devices provide better opportunities for studying molecular transport and nanoparticle distribution under physiologically and pathologically relevant conditions. Additionally, they provide a 3D perivascular environment that enables the direct interactions of the BBB cells [27]. These BBBoC models when integrated with different brain cells give us insights on the interactions occurring between the BBB cells and the neurons. This in turn helps in understanding of drug transport, efficacy, mechanism of action, and toxicity [29].

At present, many devices have been developed due to their recent technological advances such as induced pluripotent

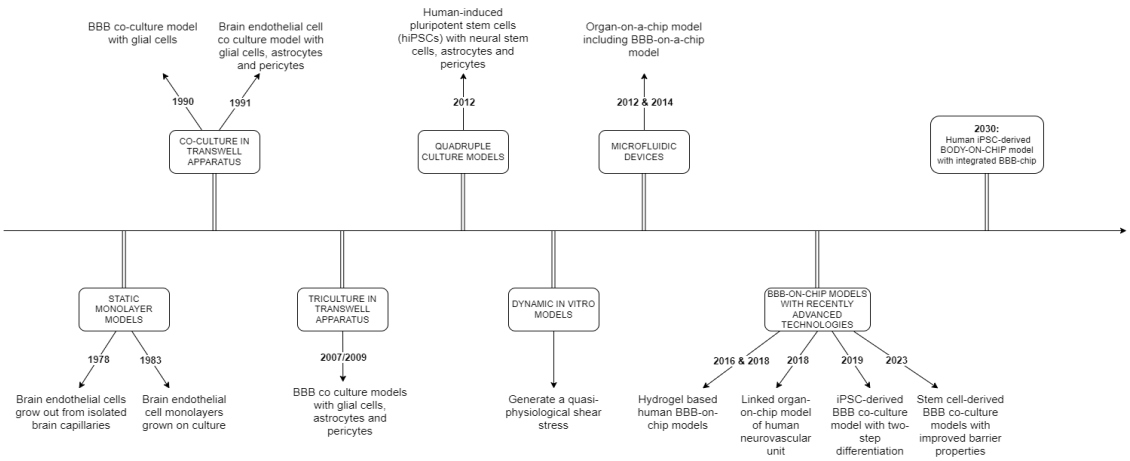

Figure 3. *Flowchart depicting the progression of BBB-on-chip models throughout the years*

stem cell (iPSC) technology, biosensors, hydrogels, soft lithography, and 3D bioprinting. iPSC models help in the development of personalized BBB chip systems. The human BBBoC system, when cultured in 3D hydrogel along with astrocytes, enables the verification of receptor-mediated transcytosis of nanoparticles. Moreover, the A2 photon lithography approach allows accurate replication of the brain capillaries [29].

2.4. Comparison of conventional models used

for blood-brain barrier research

There has been a noticeable rise in the prevalence of CNS diseases in recent years. However, the recent treatments are aimed at managing symptoms rather than cure. In comparison to other areas of drug discovery, CNS diseases pose significant challenges and have high failure rates. Therefore, developing accurate, efficient, and high-throughput in vitro models of the BBB is a critical research objective in order to maximize the discovery of effective, high-potency drugs targeting the CNS [30]. In-vitro models in comparison to in-vivo models offer benefits in practicality and have the ability to incorporate primary human cells that reflect gene and protein expression closer to that of the human brain [31]. In vivo studies require many resources and are time-consuming, and moreover the significant species-specific differences between humans and animals have minimized the transformable nature of the results to clinical application [32].

2.4.1. In-vivo Models

Rodents can be used to study various BBB cellular components such as endothelial cells, astrocytes, pericytes, and the basement membrane [33]. Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), tracer investigations and electrophysiological are various imaging techniques that can be used to study the BBB in rodent models [34] [35]. The major advantages of this model are its ability to identify critical time periods for susceptibility and its ability to explicate regulatory mechanisms. A variety of neurological conditions, including epilepsy, multiple sclerosis, and Alzheimer's disease can be modeled with the use of rats, with BBB disruption as a predominant characteristic. Significant genetic differences between humans and rodents and their impact on the integrity of the blood-brain barrier are all noted and taken into consideration while deducing the results [36].

Due to shared evolutionary relationships and similar brain functions a variety of NHP models are particularly useful in BBB research as they are in close relationship to humans. The integrity and function of the BBB are assessed using a variety of methods that are comparable to those used on rats, such as imaging methods like PET and MRI [37]. Drug distribution and modulations in BBB can be observed through various surgical approaches, including invasive procedures like brain injections [38]. Moreover, NHP models can be used to study neuroinflammation, strokes and a variety of neurodegenerative illnesses [39]. However, due to the variations in physiology and metabolism between NHPs and humans, limitations can be found in clinical applications [40]. Hence, it can be considered useful in certain aspects such as advanced understanding of drug distribution and BBB modulations.

2.4.2. In-vitro Models

Development of in-vitro models was deemed necessary as an alternative to expensive and unvaried in-vivo models. It was developed to understand the behavior of drug delivery systems to the BBB and to also study its permeability. In the initial stages, Transwell systems were used to develop in vitro models that were based on cell cultures of brain EC (endothelial cells). These are 2-D static models [41]. However, through time, transwell systems have evolved. Transwell systems comprise two compartments separated by a semipermeable plastic membrane in which vertical diffusion occurs. Different semipermeable membrane materials have been studied in Transwell devices [42]. The Transwell model is simple in terms of complexity and fabrication, it is a simple constructional model, allows for easy detection of TEER values, and is also useful for high-throughput analysis, thereby making it appropriate for in vitro BBB studies [16] [43].However, being a simple constructible model with easy detection of TEER value it poses significant limitations. Accurate mimicry of in-vivo microenvironment is not achieved due to their 2D structures and static cell culture. As a result low barrier tightness and efflux functionality, high cell membrane permeability, and lack of 3D cellular organization and cell-to-cell contact are observed [44][42]. Moreover, the lack of fluid shear and other relevant physiological conditions does not simulate the micron-scale 3D environment [16]. Development of a more accurate model is required to mimic the in vivo conditions of the BBB more precisely.

To solve the above raised limitations dynamic cell culture systems have been developed and they have proven to pose higher throughput devices for better predictions and drug screening. Dynamic cell culture models introduce shear stress that helps in mimicking physiological conditions, significantly impacting the barrier and transport functions of the BBB, as well as the expression of tight junctions [45]. Among these, two types have received increasing attention during the last years: 1) Microfluidic devices, and 2) DIV-BBB flow-based hollow fiber models.

Most of the microfluidic chips are constructed using polymeric organosilicon (a chemically inert and stable compound) and PDMS, with various surface patterns introduced through methods such as reactive ion etching or through soft lithography techniques [16]. These models can be used to assess the morphology and intercellular interactions of the cells and can also be integrated with electrodes for TEER measurements. Further innovations in this model led to running 8 parallel experiments simultaneously in a single chip enhancing the reproducibility of the results [46]. Most of these in vitro BBB chips can only stimulate the basic structure of the fluid channels in the cerebrovascular network. Thus, hemodynamics simulations are still inconsistent with the complex, multi-stage in vivo vascular network of the BBB [16].

These models mainly consist of a dynamic perfusion bioreactor with a fluctuating number of hollow fibers (HFs) . The HFs act as capillary-like structures and permit 3D cell co-

cultures, supporting cell growth, proliferation, and differentiation until the in vitro reconstruction of the tissue [47].The constant flow of culture medium through the lumen of the HFs ensures the mass transfer and exchange of various nutrients (e.g., glucose and oxygen), and the removal of metabolites like lactate and retinoic acids also occurs providing mechanical stimuli to the cells. In addition, other studies have demonstrated the efficiency of these DIV-BBB models in vitro BBB reconstruction by reaching appropriate TEER values. A SWOT analysis identified the DIV-BBB model's ability to maintain long-term cell viability as its biggest strength, thereby allowing for multiple drug assays to be conducted within a single DIV-BBB cartridge. Through all the experimental shreds of evidence it is claimed that 3D DIV-BBB culture systems are superior to 2D transwell co-culture systems. However further improvement of morphological features is required due to 1) low cell adhesion on the HFs, and 2) an inability of the HF material to induce cell differentiation [42]. Overall it is useful in some aspects such as the investigation of shear stress effects on BBB cells and dynamic drug testing.

There have been significant advancements in blood-brain barrier models, including a move towards more complex 3D systems that enhance efficiency. BBB organoids are BBB components cultured under low-adhesion conditions which then self-assemble into multicellular structures that recapitulate the cellular heterogeneity, structure, and functions of the primary tissues at the BBB [16]. Capillary networks with surrounding lumen and BM can be formed in spherical models prepared from rat or mouse cortical tissue, although this structure is transient [48]. BBB-related transport analyses can be potentially challenging because these models do not consider fluid control [16]. However, spherical models are well-established as effective methods for screening peptide penetration of the BBB at a small scale [49].

Bioprinting has been used to develop 3D biomaterial networks that mimic blood vessels and capillaries within tissues and are more physiologically realistic and flexible than other *in vitro* models [50]. 3D bioprinting involves design modeling with the assistance of a computer and layer-by-layer printing of products in STL file format under computer control [51]. This approach combats the limitations of traditional methods, due to its accurate definition of prefusable networks and the positioning of various cell types in a high-throughput manner. Additionally, 3D bioprinting provides an important advantage in developing in vitro tumor models [52]. The model being potentially suitable still holds some limitations. Due to its complex and technically challenging nature, bioprinting research has lagged in prospective in-vitro models of BBB [16] [53].

2.5. Scalability and viability of Blood-Brain Barrier-on-a-chip

The microfluidic in-vitro model of the human blood-brain barrier (BBB), proves to be a more reliable and accurate model than its other in-vitro conventional parts and also proves to be cost effective as well as a more ethical alternative to animal testing. It accurately mimics and replicates the human brain environments, it also demonstrates technical advantages such as flexible control of the chip's fluid shear stress, it provides a dynamic simulation of blood brain barrier and also provides real time monitoring of biochemical parameters of individual cells. Moreover, the development of lithography and three-dimensional printing and use of other biomaterials such as hydrogels, further improves its efficiency [54]. Therefore, the technical advantages of BBB-on-a-chip and its diverse applications and usage across different fields, makes it more viable for research.

2.5.1. Pharmacology and Toxicology

The potency of a drug to treat certain neurodegenerative and CNS disorders depends on its ability to cross the BBB and reach the target area of the brain [55]. Therefore, considerable research is required in drug design. A study conducted in 2016 by Shao et al. used a microfluidic BBB model to investigate the permeability of CNS drugs through the BBB. This study used a model drug named Sunitinib used to treat brain metastases formed as a result of kidney cancer, to study its permeability across a BBB mimetic culture of human cerebral microvessel endothelial cells (hCMEC/D3) [56]. It is also worth noting that a 3D culture model of the BBB-on-chip was used to enable the cells to retain their properties, thereby overcoming some of the limitations of other conventional BBB-on-chip models. To check for the feasibility of the BBB-on-chip setup, the model drug, Sunitinib, was used to determine its permeability and its cytotoxicity on brain cells. The results obtained showed increased drug-induced toxicity overtime, which was in accordance with the hypothesis. Therefore, taking into consideration the results, the study also concluded that microfluidics like the BBB-on-a-chip models are not only viable but highly reliable systems for drug development [56].

Some chip models have also been used to study the adverse effects of drugs or well-known toxins on the blood-brain barrier (BBB). One such example is a study conducted by Koo et al., to deduce the effects of Organophosphate-based compounds (OPs) commonly present in pesticides and nerve agents, on neurotoxicity [57]. The experiment involved a three-dimensional microfluidic BBB system consisting of a dynamic blood-brain barrier with a membrane-free culture of endothelial layer, an extracellular matrix (ECM)-embedded tissue with microglia, astrocytes and neuroblastoma. This setup was used to study the effects of four different models of OPs on barrier integrity, viability, residual OP concentration and acetylcholinesterase (AChE) inhibition. The results showed that AChE activity was rapidly inhibited due to the

penetration of OPs through the blood-brain barrier. These in-vitro toxicity values also correlated to the in-vivo data, thereby demonstrating the potential of BBB-on-chip to replace animal testing as a cost-effective alternative [57].

2.5.2. Disease Modelling

BBB-on-chip can also be used to study various neurological and CNS diseases, since the disruption and degradation of BBB is characteristic of various diseases such as Alzheimer's disease (AD), Huntington's disease (HD), and Parkinson's disease (PD) among others [58]. Similarly, a study conducted in 2023 by Linville et al., developed a BBB-on-chip model to study metastatic brain cancer. The main challenge with metastatic brain cancer is the difficulty in treating it due to the semi-permeable nature of BBB that limits the transport of necessary drugs to the intracranial tumors. Additionally, the BBB is modified into a 'blood-tumor barrier'(BTB) as a result of primary breast cancer during the formation of brain metastases.Hence, deeming it necessary to research on the physiology of the BBB and BTB to better understand the nature of cancer across various types and stages. Here a tissue-engineered microvessel model was used that included pluripotent stem cells (iPSC) and brain derived microvascular endothelial-like cells (iBMECs) while being surrounded by human breast metastatic cancer spheroids with brain tropism. This model compared both BBB and BTB microvessels to deduce chemical and physical interactions that occur during the growth of perivascular cancer [59]. Another study conducted by Boghdeh et al., utilized the BBB-on-chip in coordination with a neurovascular unit (NVU) to understand the dire effects of neurovascular infections causing Venezuelan Equine Encephalitis Virus (VEEV) on the BBB [60]. Furthermore, the effect of omaveloxolone on counteracting the negative effects of the VEEV on BBB barrier integrity was studied, by using a human cell-based BBB-on-chip platform. A more recent study by A. Deli et al. during the advent of the SARS-CoV-2 infection, it was found out that not only the lung of the infected person was affected but also, the barrier integrity of the blood-brain barrier was severely compromised. This was found out through the use of a human brain endothelial cell-astrocyte-microglia dual-compartment biochip model [55]. Such examples of various studies clearly proves the importance and relevance of using BBBoCs for studying various diseases pertaining to the blood-brain barrier.

2.5.3. Scalability

In the recent years, there has been a surge in demand for nanomedicine to treat certain neurodegenerative diseases such as Alzheimer's, Parkinson's as well as ischemic strokes and brain tumors. Nanomedicine particles usually are in the size range of 10-1000 nm and encapsulated into 'nano-capsules' or adsorbed into 'nano-scaffolds'. Due to its small particle size they have high permeability through the blood-brain barrier, and can rapidly target different brain sites. However, the lack of reliable and accurate testing models makes it difficult to gauge the efficacy of these nanomedicines in treating brain angiogenesis-related conditions. However, with the use of blood brain barrier on a chip technology and lab on a chip technology, it is possible to investigate the role of nanomedicines and its interactions with complex human brain structure. However, there are a few short-comings, due to challenges with the replication of the complex human brain structure. However, with more advanced models of the OOC technology such as the three-dimensional microfluidic models, there still exists immense potential to make research possible in this area [61].

The controlled, dynamic microenvironments of the OOC technology can, in principle, aid in personalized medicine. It can use an individual's blood samples, primary human tissue and cells derived from induced pluripotent stem cells to emulate the physical as well as chemical parameters of the cells. This provides new opportunities for personalized medicine, wherein the drug efficacy, personalized treatment plans and disease prevention can be tailored to specific patients based on their unique medical history [62]. Similarly, the blood-brain barrier on a chip can be used for precision medicine in various conditions relating to the brain such as traumatic brain injury (TBI) and brain tumors. For example, personalized medicine can be adapted for pediatric brain tumor research. Chemotherapeutics are a fairly new option in the field of neuro-oncology and their passage through the blood-brain barrier is very crucial [63]. Similarly, BBB on chip technologies can be used for precision medicine in adult brain tumors. Some models have been successful in targeting BRAF, H3K27 demethylation, and *NTRK* fusions. It is also important to consider that temporal heterogeneity is a major challenge in the field of brain cancer and thus needs to be addressed before precision medicine can be considered for its research. However, presently, the data obtained from using BBB on chip for treatment of other types of cancer, can be leveraged to apply precision medicine for brain tumors in adults [64].

The use of AI in various organ-on-chip technologies has been researched and studied in recent years. The integration of AI provides certain advantages such as use of automation, low cost, high quality imaging and recognition of minute factors that would otherwise go unnoticed. The AI learns to recognise patterns using the unstructured data presented to it, this process is a part of machine learning. However, the drawback of this is the large set of data required to train the AI model, however, the advantages far outweigh the limitations [65]. For example Blasi et al. demonstrated and constructed an AI model to determine the DNA content of the cell, using dark-field and brightfield images that were obtained through conventional imaging flow cytometry. It is also worth noting that this technology is also capable of detecting the different phases of mitosis. Due to this feature, this AI model was used

to investigate the regenerative properties of the liver and was also used to determine if some patients with severe liver damage were eligible for a liver transplant [66]. Similarly, the glial cells in the brian increase their DNA content to maintain the blood-brain barrier [67], thereby conducting further research for the integration of such AI models can be extremely beneficial. Another study conducted by Chu et al. developed a machine learning model for real time monitoring of the quality of microfluidic microencapsulation using spheroid images. This technology due to its 3D printing platform, can be extremely useful to detect abnormal spheroids and can be very beneficial for drug screening purposes [65]. Therefore, the integration of AI in microfluidics such as blood-brain barrier-on-chip technologies can be revolutionary and can play a pivotal role in drug development and neurological research.

2.6. Engineering and Design of Blood-Brain-Barrier

2.6.1. Cellular Complexity

The BBB is the main access point into the brain parenchyma for the transport of native or remedial molecules alike. The blood-brain barrier (BBB) is composed of complex cellular structures. At its core are endothelial cells (ECs) surrounded by pericytes. Pericytes support the ECs and control the size and permeability of the blood vessels [68]. The intricate multicellular structure and characteristics effects of diseases cannot be accurately studied in in-vitro conditions and the functional mechanism cannot be verified as well. Therefore, researchers over the years have developed a microfluidic BBB on-chip model which is treated with supporting cells such as EC's, astrocytes, pericytes, and microglial co-culture which innovatively reestablishes the physiological and pathological behaviors when combined with particular hydrogels.

Important considerations should be taken while designing such an intricate model, including cell types, extracellular matrix (ECM) material(s), and perfusion/flow. When it comes to the selection of material or fabric of the model it is imperative to note that living systems require oxygen and carbon dioxide for several factors such as permeability, diffusivity and molecular exchange. The best sustainable option is Polydimethylsiloxane (PDMS) due to its low cost and high-fidelity curing. The diffusivity of oxygen on PDMS along with glass substrate reached a desirable mark of 3.4 * 1/10^9 m^2s [69]. The dynamically curated microfluidic device should be ideally composed of two compartments namely, 1) a vascular compartment mainly composed of Brain Microvascular Endothelial Cells (BMVECs) and 2) the brain compartment surrounded by glial cells, astrocytes, pericytes and neurons. ECs with glia and pericytes are established to produce fluid shear stress to mimic the effects of blood flow in the vessels which creates tight gaps between the endothelial cells activating the barrier [69].

The advancements in in-vivo BBB model can be summarized under three different designs starting with 1) The Sandwich design which produces a separation between the upper vascular channel and the lower brain channel by a layer of Basement membrane (BM) (figure 4); 2) The parallel design includes the parallel vascular and brain channels connected together using microchannels at the bottom of the chip; and 3) The 3rd Tubular structure design which involves the act of injecting hydrogels containing pericytes and glia into chambers containing the material PDMS followed by the insertion of cylindrical objects into it to form the two vascular channels in a parallel fashion. Out of all the three designs, hydrogels stand out because of their excellent biocompatibility. This in turn stimulates the Extracellular matrix (ECM) which improves cell adhesion from pascals to megapascals and this promotes cell differentiation [70]. Even

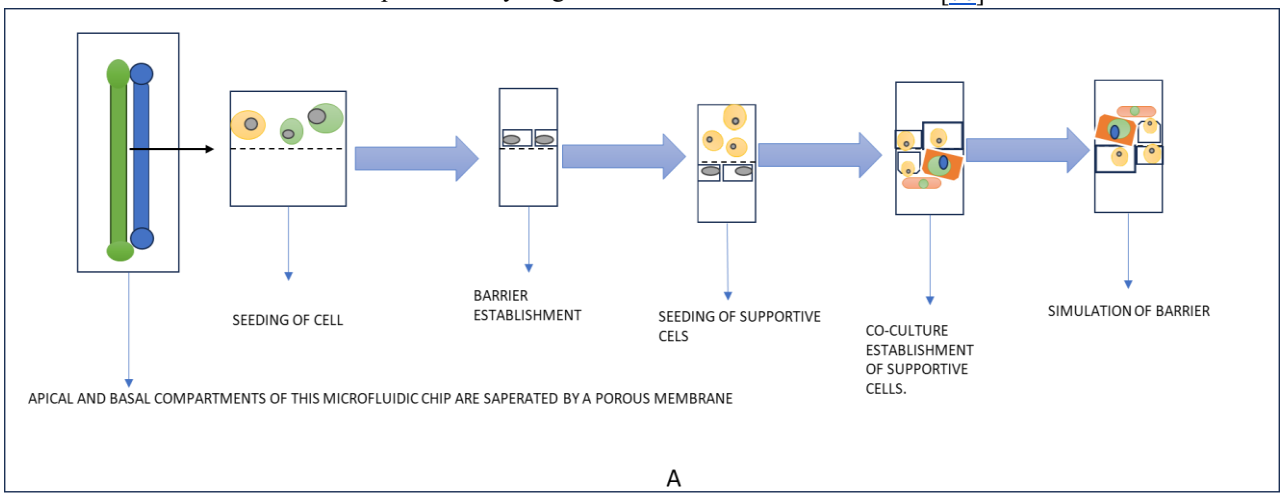

Figure 4. The Sandwich design produces a separation between the upper vascular channel and the lower brain channel by a layer of Basement membrane (BM) 2) The parallel design includes the parallel vascular and brain channels connected together using microchannels at the bottom of the chip.

though animal models and other in-vitro models have given a huge amount of awareness regarding BBB physiology, the advanced BBB models provide more effective principles for studying and researching neurodegenerative disease and drug development [68]. There are three types of BBB models: the Transwell model, the Animal model, and the Parallel Artificial Membrane Permeability Assay (PAMPA).

2.6.2. Evolution of BBB model on chip

At first, the plot of BBB on-chip devices focuses on how to add fluid flow in the models. So, the transwell structure is placed in the microfluidic device to develop a vertical structure that forms different channels. The porous membrane separated these vertical channels. ECs cultured in the upper chamber form the barrier, Pericytes, and the Astrocytes cultured in the lower chamber, mimic cell interactions. Although these models have been widely used in BBB, this model had some drawbacks. Due to its vertical shape, the cells would settle towards the bottom of the channel, because gravity and vertical structure make it difficult to study the cell processes [69].

For better monitoring parallel channels have been designed. PDMS channels were designed to replace the Transwell design. For instance; to separate blood from the brain-mimicking chambers, micropillars of size 3 μm were used; similarly, to examine cancer, tumor cells were seeded. The construction of this model has made device fabrication easy and has improved the observation of cell behavior [69]. Recently, 3D spheroid models have been developed to mimic brain tissues. By gathering brain ECs, Pericytes, and BBB spheroids can be created [68]. In recent BBB models, hydrogels have been used to provide a 3D environment for the co-culture system. These gel mesh models were assembled within the basal membrane of the BBB in-vivo [69]. Hydrogels have excellent biocompatibility and stimulate the extracellular matrix which helps in improving cell adhesions from pascal to megapascals, thereby promoting cell differentiation [70].

2.6.3. Detection of minute interactions of cells in a neurodegenerative environment

Microfluidic BBB cells simulate the movement of tumor cells in the microenvironment which facilitates understanding of the interactions between the BBB cancer cells and also aids in recording its response to the treatment. The cause of neurodegenerative disorders is usually characterized by BBB injury or death of neurons [71]. The use of BBB on chip promotes an efficient way to restore a functional BBB in-vitro. Early drug screening can be considered an effective application of this scientific model due to its optimal design.

2.7. Assessing Barrier Integrity using Biosensors

The integrity of the blood-brain barrier is being investigated using in-vitro BBB on chip models with the implementation of biosensors. Biosensors act as an important tool for real-time monitoring of the barrier's permeability and disruption due to diseases and drug interactions. Standard core structures for such biosensors could be a bioreceptor specific against markers for barrier integrity, a transducer that will convert this bioreceptor–analyte interaction to a measurable signal, and, finally, an electronic system processing this signal for analysis [72]. Preclinical drug testing on animals has been a conventional method to run drug screening for neurodegenerative disorders due to many reasons and the important ones being high cost and highly inaccurate results. Therefore, researchers developed various kinds of biosensors with different functionality on BBB on chip.

2.7.1. Importance of Biosensors on BBB-on-chip

Real-Time Monitoring: Biosensors provide real-time data about the integrity of the barrier, without any interruption. With that ability, disruptions or changes in the integrity will then be instantaneously detected. This would be important in studies of dynamics where barrier function may be altered rapidly [73]. 1) Improved Accuracy and Sensitivity: High sensitivity and specificity are main merits of biosensors, leading to the possibility of detecting minute changes in barrier integrity that are sometimes invisible to other methods. This precision will help in the correct assessment of health and functionality of the BBB [73]. 2) Simulation of Human-Like Conditions: It is through the incorporation of biosensors that the BBB-on-chip models will get closer to human brain physiological conditions, hence bearing data relevant to in vitro drug testing and diseases, unlike the traditional models [73]. 3) Testing Drug Delivery: The functioning of biosensors allows assessment of how far drugs or therapeutic agents can diffuse through the blood-brain barrier. This is very important information in the development of treatments against disorders in the CNS, since this would assure that the drug may reach the targeted point without side effects [73]. 4) Understanding Pathologies: Biosensors help in studying how diseases affect the BBB and pinpoints changes in barrier function associated

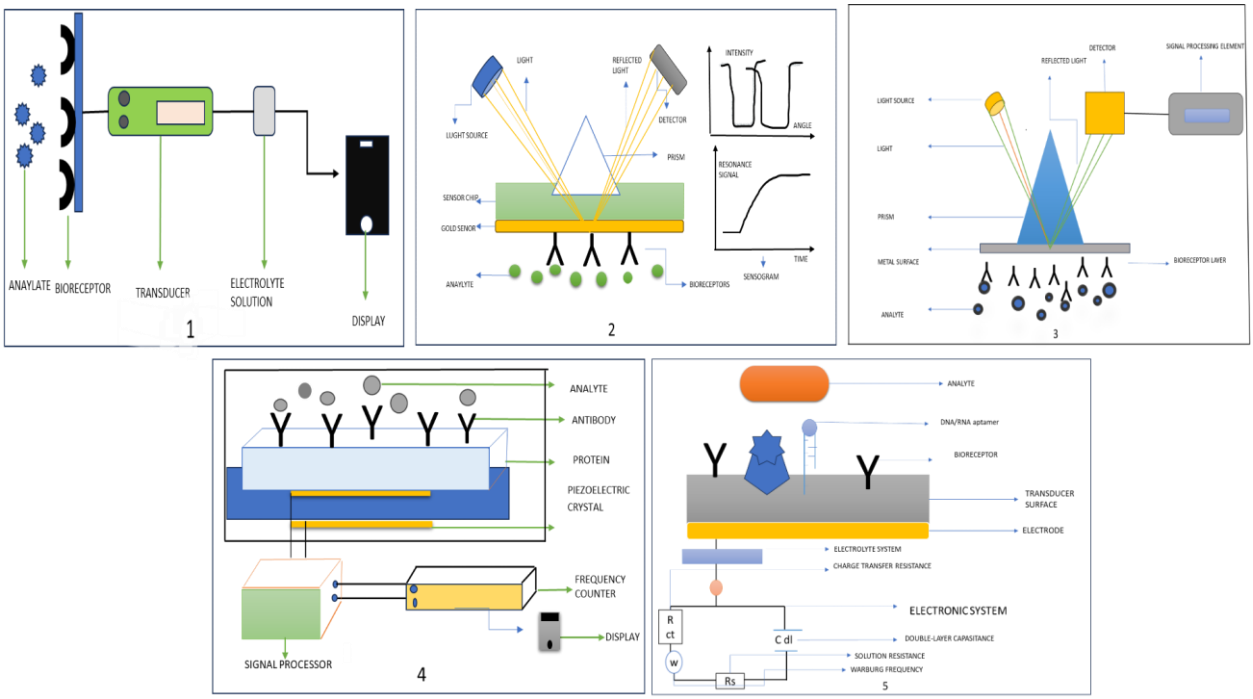

Figure 5. *Schematic representation of the different types of Biosensors used in detection of barrier integrity in the blood-brain-barrier-on-chip technologies. 1) Electrochemical Biosensor; 2) Optical Biosensors; 3) Surface Plasmon Resonance (SPR) Biosensor; 4) Piezoelectric Biosensor; 5)Impedimetric Biosensors*

with neurological disorders. Further, such insights may provide a better understanding of mechanisms of disease and possible therapeutic targets [73]. 5) Ethical and practical advantages: the biosensors reduce animal testing through the delivery of accurate in-vitro data, thus addressing ethical concerns and solving problems related to species differences that may affect the translatability of results [73].

Various models of biosensors used in BBBoCs are Electrochemical Biosensors; Optical Biosensors; Surface Plasmon Resonance (SPR) Biosensors; Piezoelectric Biosensors and Impedimetric Biosensors as shown in Figure 5. Electrochemical biosensors aid in monitoring the changes in electrical signals due to ionic flux across the BBB and offer high sensitivity with real-time monitoring. On the other hand, Impedance-based biosensors can estimate the resistance and reactance across the barrier; therefore, they could continuously inform about its tightness or, inversely, the permeability of endothelial cell junctions. In the case of marker passage across the BBB, Optical biosensors use fluorescence or surface plasmon resonance and thus allow both visual and quantitative measurements. While Piezoelectric biosensors measure mechanical changes caused by cell interactions, these are rather less applied in BBB models. Among these, Electrochemical and Impedance-based biosensors have been shown to be highly useful tools for assessing the integrity of the bloodstream barrier due their high sensitivity, availability of features like real-time monitoring, and are also relatively easy to integrate into microfluidic devices [74].

2.7.2. Advancements in BBB-on-chip model after the usage of biosensors

Biosensors have integrated drug-testing and development processes by providing more accurate and reliable data on BBB permeability. This has implications for the development of treatments against neurological disorders, in that researchers can now better predict which therapeutic agents may effectively cross the blood-brain barrier. Furthermore, the more efficient control of the BBB in real time has benefited the study of mechanisms of diseases, such as the role of neuroinflammation or amyloid-beta accumulation in the course of Alzheimer's disease. The integration of biosensors into BBB on-chip technology also appears to be a quantum leap in neurobiological research since this enables the study of the detailed function of the BBB and its interactions with various biological and chemical agents at higher resolution, accuracy, and dynamics [75].

3. Conclusion

This study highlights the significance of the blood-brain barrier-on-chip, its recent advancements, features, and design. The blood-brain barrier plays a crucial role in protecting the brain from harmful substances. The revolutionary Organ-on-chip technology, aims at simulating the human body and has been extensively researched in the past couple of decades.

Numerous conventional models are used for blood-brain barrier research. However, the design and features of the BBB-on-chip model distinguish it significantly from the other in vitro models. The BBB-on-chip models use a combination of brain microvascular endothelial cells (BMVECs), microglia, astrocytes and pericytes to precisely mimic the complex structure of the blood brain barrier. The extracellular matrix (ECM) that is incorporated with hydrogels, enhances biocompatibility, cell viability and control transport of molecules across the barrier [76]. It also plays a vital role in researching various neuropathogenic and neurodegenerative diseases, thereby also aiding in drug development. Furthermore it is also known to be extremely cost effective and makes mass production possible. Although BBB-on-chip overcomes some of these limitations, it can be stated that if important advancements concerning the lagging of perspective in 3-D bioprinting models are made, it can turn out to be a more physiologically realistic and accurate model.

In the last few decades, advancements in technology have also given rise to the integration of biosensors in the BBB-on-chip technologies, which have proven to be time-saving and have efficiently aided in monitoring the BBB. But there are still some challenges involved in biosensor progress such as, the lack of efficiency in capturing biorecognition and transforming it into electrochemical and electric signals, enhancing transducer performance i.e, not being able to increase the sensor sensitivity, less reproducibility, shorter time responses, reduced capability to detect the individual molecules and the need to shorten biosensing devices using micro and macro fabrication technologies. However, these challenges can be overcome through various procedures such as attachment of sensing technology and integrating nanomaterials which possess a high surface-to-volume ratio, good conductivities, shock bearing capability, and color tunability. These sensors additionally possess other issues like, issues in sustainability of nanostructures in sensors, difficulty in the fabrication of nanostructures and toxicity, which gets replaced according to physical properties of the material type [77]. In the future, more research is required in this area and there is a need for the production of nanostructure based biosensors at affordable cost that also provide rapid accurate results with a user friendly interface.

Our study, including other numerous studies previously conducted, are stepping stones for future advancements in the OOC and BBB-on-chip technologies. The evolution of BBB-on-chip technologies from the static monolayer model to the current stem-cell-derived, BBB co-cultured model with biosensor integration is a testament to the role and importance of innovative research. Further research can give rise to future advancements such as, increased BBB application in studying nanomedicine, development of precision medicine, incorporation of artificial intelligence, and other automation technologies for greater accuracy [61] [62]. However, certain challenges within the blood-brain-barrier-on-a-chip are to be addressed to enhance the advantages offered by these devices. These limitations include; lack of standardized equipment, difficulty in replicating the blood-brain barrier structure, and the limitations of the biosensors [78]. Therefore, further investigation can help to dissolve these critical gaps and can significantly increase the applicability of the BBB-on-chip models.

Over the past few decades, many blood-brain models, organ-on-chips, microfluidic devices, and in vitro culture technologies have been created for the development of drugs to treat neurodegenerative and CNS-related diseases considering factors such as time, cost, and ease. Evidence from previous studies shows that blood-brain barrier-on-chip models have overcome the limitations of its predecessor models and have also successfully aided in biomedical research [16][79]. This study conducted a comprehensive literature review on Organ on chip technologies, particularly on blood-brain barrier on chip technologies, highlighting its evolution, advantages, design, and applications. However, Organ-on-Chip technologies and blood-brain barrier research are vast fields that require extensive future research and investigation. This includes understanding the complex cellular interactions, optimizing microenvironment conditions, and developing accurate models that replicate human physiology. Such efforts are essential for advancing our knowledge and improving applications in personalized medicine and the treatment of neurological disorders. Furthermore, including statistical insights through quantitative meta-analysis can provide a comprehensive understanding of the data, identify trends, and highlight potential gaps in current research. This approach can also enhance the reproducibility and reliability of findings, ultimately accelerating the advancement of Organ-on-Chip technologies in blood-brain barrier research. Additionally, the study about materials, cost in manufacturing and ethical implications can give rise to more intriguing insights into the practical aspects of blood-brain-barrier-on-chips. Therefore, the future of BBB research is highly encouraging and promising, and in the upcoming years innovations in the field of BBBoC models can occur driven by the need for more effective CNS disease treatments and drug delivery.

Author Contributions

SV, KD- conceptualization and illustrations. FNV- illustrations. PP, KK, YSM, FNV, MM, SV, KD- resources, formal analysis, writing- original draft, writing- review and editing.

Conflicts of Interest

The authors declare no competing financial interests or conflicts of interest.

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