The Future of Neurological Research: Advances in Microfluidic Blood Brain Barrier (BBB) Models

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Abstract

Diseases of the central nervous system (CNS) represent a significant contributor to the global health burden. However, newly licensed medications for these conditions rank among the lowest in comparison to their counterparts. The lack of dependable and effective in vitro blood-brain barrier (BBB) models that accurately mimic in vivo barrier characteristics significantly impedes the advancement of effective therapies for central nervous system (CNS) disorders. The blood-brain barrier (BBB) serves as a natural membrane that safeguards the central nervous system (CNS) against bloodborne toxins and viruses. The presence of the blood-brain barrier complicates pharmacotherapy for central nervous system disorders, as most chemical drugs and biopharmaceuticals cannot penetrate the brain. Insufficient delivery of medication to the brain diminishes therapeutic effectiveness and increases adverse effects due to accumulation in other organs and tissues. Brain physiology and complex medical development processes render brain disorders the most debilitating health issue worldwide, contributing to increasing annual mortality rates. The blood-brain barrier (BBB) and its constituents are essential for safeguarding the brain; however, their intricate nature complicates the delivery of medications to this organ. The BBB is a primary factor in treatment failures, potentially leading to disease progression. Recent advancements in materials science and nanotechnology have resulted in a range of advanced materials with tailored structures and properties, functioning as a valuable resource for targeted drug delivery. A thorough examination of anatomical and pathological studies of the brain and blood-brain barrier supports the creation of targeted approaches to improve permeability across the blood-brain barrier. Thus, it is crucial to identify an optimal platform for predicting the behaviour of a drug delivery system in the brain during the early developmental phase. In the last twenty years, researchers have created and examined multiple in vitro blood-brain barrier models to improve comprehension of barrier properties and the degree to which these models mimic the in vivo blood-brain barrier. In vitro models of

the blood-brain barrier primarily consist of endothelial cell cultivation, frequently in co-culture with additional perivascular cells, utilising two- or three-dimensional platforms. This review offers a thorough guideline for researchers in diverse fields, highlighting advancements in brain-targeted drug delivery systems.

Keywords

Microfluidic Blood Brain Barrier (BBB) Model; Blood-Brain Barrier (BBB); Tight Junctions; Endothelial Cells; Stem Cells; Perivascular Cells; Microenvironment; In Vitro Model.

1. Introduction

Neurological illnesses constitute the majority of disability cases and are the second largest cause of mortality worldwide. In 2016, CNS illnesses accounted for 16.5% of worldwide fatalities (1). Developing therapeutics for central nervous system illnesses is crucial for their clinical, societal, and economic impact. Therapies for CNS illnesses had lower approval rates and longer approval timeframes, according to a trend study (2). Drug approval delays can be attributed to reasons such as a lack of understanding of disease biology, insufficient animal models, and unclear clinical trial goals (3). The lack of in vitro BBB models that reflect endogenous barrier characteristics is a key contributor to the CNS medication shortage. Therapeutic candidates for CNS illnesses are pre-screened for their capacity to cross the BBB before animal research or clinical trials. The neuropharmaceutical industry is looking for effective barrier models to quickly screen drug candidates for clinical trials (4). The blood–brain barrier (BBB) is a semi-permeable structure that surrounds the microvasculature of the central nervous system (CNS). The endothelial cells in capillaries are arranged in a manner that forms extensive tight junctions along the interior of the vessels (5). The barrier, in conjunction with a variety of receptors, transporters, efflux pumps, and other cellular components, regulates the entry and exit of molecules within the vascular compartment of the brain (6). The intact blood-brain barrier restricts the entry of most blood-borne substances into the brain. It is important to note that while providing brain protection, the blood-brain barrier (BBB) also prevents over 98% of small-molecule drugs and all macromolecular therapeutics from entering the brain (7). The narrow gap permits only passive diffusion of lipophilic drugs with a molecular weight below 400-600 Da. Enhancing the lipophilicity of therapeutic agents is an effective approach to augment blood-brain barrier permeability (8). Crizotinib, an oral selective small-molecular tyrosine kinase inhibitor, is an effective anti-cancer agent; however, its efficacy against brain tumour metastases is limited due to inadequate blood-brain barrier penetration (9). Treatments for central nervous system (CNS) disorders encounter several challenges, including a scarcity of therapeutics, the intricate physiology of the brain, and insufficient drug delivery mechanisms, all contributing to elevated mortality rates (10). The blood-brain barrier (BBB) serves as a protective interface between the blood circulation and the central nervous system (CNS), effectively limiting the ingress of potentially harmful substances and pathogens (11). It facilitates the exchange of essential nutrients such as glucose, iron, and blood gases, which are critical for brain function. BBB exhibits dual functions: barrier and carrier (12). The barrier function of the blood-brain barrier (BBB) is crucial for protecting the brain; however, it poses significant challenges for central nervous system (CNS) therapeutics, as over 98% of small molecules and nearly 100% of large molecules are unable to cross the BBB, resulting in ineffective CNS treatments (13). Consequently, it is essential to explore and create an optimal model that can accurately describe

the permeation and penetration behaviour of neurotherapeutics during the early developmental phase, aiming to establish an effective CNS therapy (14). Novel CNS entities and delivery techniques necessitate thorough assessment of in silico models, in vitro models, animal testing, and ultimately human trials (15). Research indicates that merely 50% of results from animal model testing are applicable to human responses. This discrepancy arises from inconsistent responses, inter-species variations, and differences in the expression of tight junction (TJ) proteins and transporters (16). Jamieson et al. reported that, in comparison to mice, humans exhibit a 1.85-fold higher expression of the breast cancer resistance protein (BCRP) and a 2.33-fold lower expression of P-glycoprotein (P-gp) in the blood-brain barrier (BBB) (17). Considering the aforementioned aspects, various in vitro blood-brain barrier (BBB) models have been developed and assessed by multiple research groups over recent years to predict the permeation mechanisms and measure the penetration of central nervous system (CNS) therapeutics across the BBB in vivo (18). In vitro blood-brain barrier models are not only effective instruments in drug development but also crucial for elucidating physiological and pathophysiological molecular mechanisms (19). In vitro blood-brain barrier (BBB) models have been developed to replicate the BBB, including mono- and multiple culture models, stem cell-based models, dynamic models, and microfluidic models (20). This review begins with an overview of the fundamentals and structure of the BBB. The remainder of the review provides a summary of different in vitro blood-brain barrier (BBB) models, detailing the advantages and disadvantages of each. It also discusses the associated challenges and outlines future directions for developing optimal in vitro BBB models that accurately replicate the in vivo environment.

2. Blood Brain Barrier (BBB): A Barricading for the Traffic of Brain

The blood-brain barrier (BBB) serves as a diffusional interface between brain tissue and blood vasculature, playing a crucial role in maintaining homeostasis and proper brain function (21). The absence of an energy storage system in the brain requires a continuous supply of nutrients via the vascular system at all times (22). The brain, as a highly energy-demanding organ, utilises 15–20% of total oxygen from cardiac output and 15–20% of total glucose. Consequently, to meet this elevated energy requirement consistently (23), the brain possesses a significant network of blood capillaries, encompassing a total surface area of 15–25 m^2 (24). The blood capillaries in the brain differ from other capillaries in two fundamental ways (25). Initially, they establish a stringent barrier that governs transport within the brain, and subsequently (26), they demonstrate plasticity, characterised by the constriction or dilation of capillary diameter in reaction to atypical physiological conditions (27).

2.1 Structure of the BBB

The blood-brain barrier (BBB), integral to the neurovascular unit, consists of endothelial cells (ECs), astrocytes (ACs), pericytes (PCs), neurones, and microglia (28). Endothelial cells (ECs), specifically brain microvascular endothelial cells (BMECs) (29), are tightly interconnected via tight junctions (TJs) and play a crucial role in the restriction and regulation of molecular transport (30). Adjacent astrocytes (ACs), pericytes (PCs), and basement membranes encase the brain microvascular endothelial cells (BMECs) (Figure 1) (31), contributing to structural

support and membrane stability (32), collectively establishing the impermeable blood-brain barrier (BBB) (33, 34). Consequently, only small molecules (less than 400 Da) exhibiting high lipophilicity are able to traverse the blood-brain barrier, whereas the passage of water-soluble substances is significantly limited by tight junctions (35, 36).

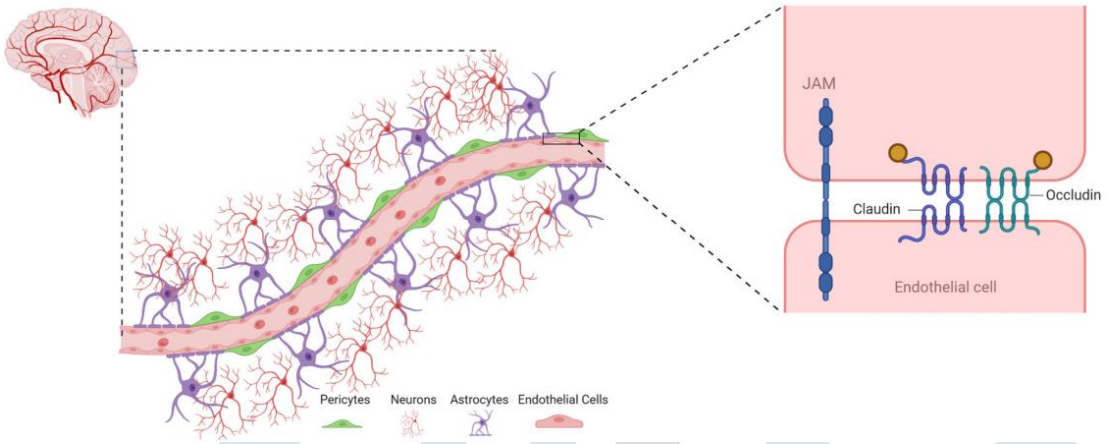

Figure 1: Structure of the BBB

The presence of the blood-brain barrier (BBB) protects the brain from damage by maintaining a stable environment (37-39). However, it also restricts the drugs that can access the central nervous system for the treatment of brain diseases, including neurodegenerative disorders and brain cancer (40, 41). Capillaries represent the primary location of the blood-brain barrier (BBB). The proximity of neural cells to capillaries, typically within 25 µm, makes transgressing the blood-brain barrier (BBB) a preferred method for drug delivery compared to alternative, longer routes (42). This case encourages researchers to formulate effective strategies for regulating blood-brain barrier permeability and developing targeted delivery systems to address the limitations imposed by the blood-brain barrier (43). Several reviews have addressed the BBB recognition, a critical factor for improved brain-targeted delivery (44, 45). To gain a deeper understanding of the interaction between delivery systems and the brain, it is essential to clarify the construction of the blood-brain barrier (BBB). The blood-brain barrier (BBB) is primarily composed of endothelial cells, astroglia, pericytes, and junctional complexes, including tight junctions and adherens junctions (46).

2.2 Physiology of the BBB

The blood-brain barrier (BBB) creates a regulated microenvironment by controlling the exchange of ions and molecules between the bloodstream and brain tissue. Multiple studies have demonstrated the physiological roles of the blood-brain barrier, including its protective function for the brain (47). The blood-brain barrier (BBB) not only restricts potentially harmful substances but also maintains homeostasis, facilitates the transport of essential molecules, and regulates inflammation (48). The BBB regulates brain homeostasis through the control of specific ion channels and transporters. Na^+, K^+, Ca^{2+}, and Cl^- are the principal ions in the central nervous system, and their optimal levels are essential for neural and synaptic signalling functions (49, 50). The ions exhibit an asymmetric distribution between luminal and abluminal membranes, with efflux and influx primarily reliant on

the ion transporters present on the blood-brain barrier (BBB) (51). The influx of Na+ and the efflux of K+ are regulated by abluminal Na-K-ATPase, which operates against the concentration gradient to maintain the electrochemical gradient across the cell membrane (52). One hundred twenty-nine Co-transporters like NKCC1 maintain ion balance through the transport of Na+, K+, and Cl−. The dysfunction of ion transporters may lead to pathological alterations. Other BBB transporters, including solute carriers and ATP-binding cassette families, regulate the transport of essential molecules, metabolites, and nutrients, thereby maintaining brain homeostasis (53, 54). Pathways through the junctional complex or across cells that facilitate the transport of ions, nutrients, and other molecules may serve as potential routes for drug delivery (56).

3. IN VITRO BBB MODELS

Numerous in vitro models have been developed over the years, utilising various design strategies to mimic the barrier properties of the brain. Studies on the blood-brain barrier commenced in 1953 utilising monolayer cell cultures on transwell systems (57). The isolation of primary cerebral microvessels, initiated in the 1970s, facilitated the incorporation of these cells into in vitro blood-brain barrier models for the study of brain permeation (58). The understanding of tight junction protein formation utilising endothelial cells and astrocytes emerged in the 1980s, leading to the development of co-culture transwell models. During the 1990s and 2000s, transwell co-culture involving three distinct cell types was employed, with an increased focus on transendothelial electrical resistance (TEER) as an indicator of barrier integrity (59). During the late 2000s and early 2010s, dynamic and 3D models, including organoids and barriers on chips utilising microfluidics, emerged. Contemporary models have enhanced microfluidic design and concentrated on the vasculogenesis method to closely replicate the blood-brain barrier (60-62). The Caco-2 model, utilising transwell chambers with Caco-2 cells, represents the most straightforward in vitro blood-brain barrier (BBB) model for evaluating new molecules targeting brain diseases, effectively simulating BBB characteristics such as transendothelial electrical resistance (TEER) and permeability. The relationship between the Caco-2 model and in vivo models shows promise for small molecules transported via passive diffusion mechanisms (63).

3.1 2-Dimentional Models

Despite their limitations, 2-D models possess potential for assessing key barrier integrity functions, namely TEER and permeability. Reliability, reproducibility, simplicity, and affordability are critical characteristics of 2-D models; however, the primary limitation is their incomplete representation of the in vivo blood-brain barrier. During the initial screening and developmental phase, where shear stress and blood flow mimicry are not critical, 2-D models are favoured for their speed and popularity among researchers (64-66).

3.2 Monolayer Models

The cost-effective method of studying drug transport across the blood-brain barrier (BBB) has limitations, including the absence of two compartments, poor paracellular restrictions, and poor imitation of in vivo BBB. To

address these issues, a growing monolayer of extracellular cells (ECs) in a transwell chamber has been introduced (67). Transwell models offer several attributes, including scalability, low cost, selectivity of different membranes, and pore sizes. Primary BMECs from humans are recommended to increase barrier functions, but access to these cells is challenging, time-consuming, and costly. Primary ECs from pig, beef, mouse, and rat are considered due to their functionality, tight barrier integrity, and low permeability. However, these models face challenges in isolation and yield, as well as potential contamination in cell culture (68). To avoid isolation of primary cells, commercially available bovine BMECs and normal human ACs were adapted to establish a co-cultured BBB model. However, this model has limitations such as low TEER, high paracellular permeability, and poor expression of transporters. Monolayer models are not ideal for studying BBB integrity parameters, as only a single type of cell is present. Complex co-culture models have been developed to mimic the in vivo anatomy of the BBB (69).

3.3 Co-Culture Models

The BBB's barrier properties are maintained by neighboring cells like ACs, PCs, and microglia. Co-culture of cerebral efferent cells (ECs) with these cells enhances cellular interactions and expression of efflux transporters. In vitro models, including double and triple cultures in the transwell system, offer higher TEER values and lower permeability. However, they lack complete in vitro phenotypes due to the lack of shear stress. Both contact and non-contact methods provide different aspects of cellular interactions or communications (70, 71).

3.4 Stem Cell Models

HiPSCs, an alternative to primary and immortalized BMECs, have the potential to overcome limitations in developing in vitro BBB models. These models exhibit biological resistance and can be improved by collecting ACs and PCs from the same source of HiPSCs (72). A recent publication showed that HiPSCs differentiated into a polarized monolayer with TEER values greater than 2500 Ω cm2, producing an excellent barrier phenotype without the need for co-culture. The minimum TEER threshold for studying brain transport is 500 Ω cm2 for sodium fluorescein and 900 Ω cm2 for IgG (73). However, the TEER threshold for one molecule is not statistically enough to represent other molecules considering molecule structure, interaction, and disease conditions. BBB dysfunction is closely related to pathological conditions like cerebral ischemia, Alzheimer's, and Parkinson's disease (74). HiPSCs have great potential to promote physiological and medical studies, as demonstrated by Kokubu et al.'s in vitro BBB disease model in cerebral ischemia, which showed that oxygen-glucose deprivation disrupted barrier function and was restored by resupply of oxygen and glucose (75).

3.5 Microfluidic Models: The Future of neurological research

A microfluidic method was introduced to address the limitations of the DIV model, as it facilitates processing with a low cell load and enhances cellular communication through the presence of a thin membrane (76). This model includes semi-porous polycarbonate membranes that are sandwiched and sealed between two polydimethylsiloxane (PDS) channel networks, orientated vertically and horizontally (77). One channel functions as a vascular component for the growth of endothelial cells, while the second channel serves as a parenchymal

section for the cultivation of perivascular cells (78). As a culture medium passes through the membrane, shear stress is generated, and integrated electrodes measure the transendothelial electrical resistance (TEER) (79). The design of this model necessitates maintaining the thickness of the PDS membrane below 50 µm to enhance compatibility with the native brain microvasculature. Furthermore, the parallel design of the PDS channel facilitates improved cellular cross-talk (80). Adriani et al. created an innovative three-dimensional neurovascular microfluidic model that incorporates primary rat astrocytes and neurones alongside endothelial cells (81). The TEER was not assessed in this model. The functionality of the endothelial cells was validated through permeability testing of fluorescent dextran, which illustrated the selectivity of the endothelial monolayer. Additionally, neural functionality was evidenced by calcium imaging (82).

Recent developments in micro-electro-mechanical systems (MEMS) technology enable the fabrication of microfluidic devices that replicate biological microenvironments in vitro (83). Microfluidics is recognised as both a scientific discipline and a technological field that examines the behaviour and manipulation of fluids at the micrometre scale (84). Microfluidic chips necessitate micro-scale engineering techniques for the fabrication of channels, chambers, and valves on materials such as silicon, glass, quartz, or macromolecule polymers, resulting in sub-micrometer sized mechanical channel structures (85). Precise and complex operations with sub-millimeter scale fluids can be executed in these microstructures utilising micropumps or microvalves. Multiple functional units can be integrated to facilitate purification, separation, and detection, enabling a series of experiments and analyses on a single chip (86). A microfluidic chip exhibiting these characteristics is referred to as a micro-total-analytical system or a lab-on-a-chip device (87). Microfluidic chips possess potential applications across various fields, including chemistry, physics, biology, and medicine.

3.5.1 Design of Microfluidic Models

Many microfluidic models employ porous membrane segmentation to create sandwich structures within the chip, analogous to those utilised in transwell systems (88). Endothelial cells and additional cell types are cultured on both sides of a membrane positioned at the interface of two microchannels, creating a neural chamber and a vascular chamber (89). Additional studies have employed micro-gaps, trapezoidal structures, or porous tubular structures to isolate epithelial cells from adjacent cell types (90, 91). The designs for microfluidic blood-brain barrier models exhibit significant variability (Figure 2).

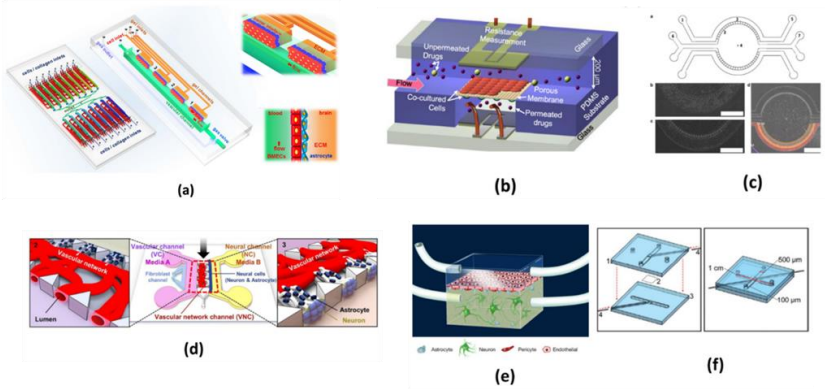

Figure 2: Typical design of microfluidic blood-brain barrier (BBB) in vitro models

3.5.2 Assessment of Microfluidic Models

3.5.2.1 Determining Specific Tight Junction Markers

In the blood-brain barrier, cells exhibit extensive tight junctions and adhesion molecules at interendothelial cell-cell junctions, which are essential for maintaining barrier integrity (92, 93). Immunofluorescence and western blots are techniques utilised to assess the expression of specific markers, including zona occludens-1 (ZO-1), claudin-5, and occludin. The P-glycoprotein efflux pump is a membrane transporter that plays a crucial role in preventing hydrophobic molecules from crossing the blood-brain barrier (94). The expression of P-glycoprotein serves as an indicator for assessing blood-brain barrier characteristics in microfluidic models.

3.5.2.2 Trans-Epithelial Electric Resistance (TEER) Measurement

TEER serves as a critical metric for assessing and monitoring the integrity and tightness of barriers. Tighter packing of epithelial cells decreases the number of gaps in the barrier, thereby reducing the movement of ions and charged species, which leads to increased resistance. TEER quantitatively measures the resistance across cell layers and cultured cell membranes (69). TEER measurement can be conducted in real time if a microfluidic chip incorporates Ag/AgCl pellet electrodes, platinum electrodes, or alternative devices capable of functioning as electrodes (94). The TEER value of an in vitro model should closely approximate the in vivo TEER, generally falling within the range of 1800 to 2000 Ω·cm2. However, most reported in vitro models exhibit values significantly below this range, with 150 to 200 Ω·cm2 regarded as the minimum acceptable TEER value in functional models. TEER provides a rapid, label-free, and real-time evaluation of barrier tightness; however, it is inadequate for assessing barrier selectivity. TEER measurements on a chip are influenced by various confounding factors. The origin of cells and the degree of cell confluence may influence resistance. The distribution of current across the membrane interface in a chip may be non-uniform, resulting in overestimates of TEER. TEER exhibits sensitivity to both temperature and the ionic composition of the culture medium. Consequently, it is essential to maintain these parameters at a constant level throughout the measurement process (97).

3.5.2.3. Permeability Assessment

The blood-brain barrier is a highly selective structure that allows the passage of only a limited number of molecules. Small ions, including K+ and Cl−, are capable of traversing the blood-brain barrier via ion channels. Small lipophilic molecules, including ethanol and nicotine, are capable of passive transport across the barrier. Small polar molecules, including glucose, lactate, and pyruvate, traverse the blood-brain barrier via carrier-mediated transport mechanisms. Large molecules, including insulin, transferrin, leptin, albumin, and tumour necrosis factor alpha (TNFα), traverse the blood-brain barrier (BBB) via receptor-mediated transport, adsorption-mediated transcytosis, and active efflux transporters (98). Permeability must be evaluated to assess barrier function in a BBB model. A high-quality model must exhibit permeability characteristics akin to those observed in vivo. In the evaluation of permeability, the selection of an appropriate molecule is crucial. An effective marker must be

inert and should not influence the physiology and function of the blood-brain barrier (BBB). Fluorescein isothiocyanate (FITC) labelled dextrans are commonly utilised to assess permeability (99). FITC-dextrans with molecular weights of 4, 10, 40, and 70 kDa are commercially available. 14C-D-mannitol (182 Da) and 14C-urea (60 Da) are utilised in permeability experiments (100). Typically, permeability analyses utilise a single marker. Different molecules cross the barrier through various mechanisms; therefore, it is essential to evaluate both hydrophilic and lipophilic molecules for permeability.

3.5.3 Application of Microfluidic Models

Microfluidic chip models offer several advantages in brain blood barrier (BBB) research. They are easy to design and fabricate, customizable to meet specific experimental requirements, and can generate independent and closed environments similar to microvascular structures in vivo (101). These models allow for more physiological information and prediction data by observing cell behavior from 2D to 3D. Additionally, they can combine and integrate various functional units, allowing for real-time monitoring of TEER in real time (102). Several microfluidic models have been developed to answer specific research questions, such as studying BBB function, screening drug candidates, and predicting pharmaceutical clearance by the BBB (103). These models provide a reliable platform to observe the influence of one or more molecules on BBB characteristics. For example, Brown used the microfluidic model to study how the BBB responds to inflammatory stimuli, such as lipopolysaccharide or a cytokine cocktail (104). Stimulation by a cytokine mix resulted in the loss of VE-cadherin and ZO-1 expression, indicating disruption of the endothelial barrier (105). Microfluidic models can also be used to assess the permeability of different compounds, including free forms, binding to nanomaterials, or functionalized (106). Falanga used a microfluidic model to evaluate a new nano drug delivery vector for the CNS, finding that nanoparticles pass more easily through the BBB barrier when combined with the membranotropic peptide GH625 (107). Bonakdar found sub-electroporation pulsed electric field can disrupt the integrity of the BBB and increase permeability (108). Papademetriou's research found that shear flow impacted the binding and internalization of angiopep-2 coupled liposome nanoparticles by brain endothelial cells in microfluidic models (109, 110).

4. Conclusions

This review discusses the structure and categories of in vitro BBB models, including monoculture, double co-culture, and triple co-culture with transwell and 3-D approaches. These models are crucial for target identification, lead optimization, and high-throughput screening in CNS drug development. Understanding the BBB's anatomical arrangement helps design an ideal model that closely mimics in vivo BBB microvasculature. However, there is no ideal model, and each type has its pros and cons. Microfluidic models are not suitable for commercial use due to their complexity and the need for intricate systems. Simulation of the BBB microenvironment requires complex microchannel networks with multiple elements, making fabrication more difficult. Minor changes can produce chips with drastically different properties. Currently, most academic research uses PDMS as the chip material, which is unfavorable for large-scale production in commercial applications. Thermoplastics may be superior for

industrial purposes but it is more difficult to obtain complicated and meticulous microstructures using this material. Despite advances in chip design and fabrication, there remains a large gap between microfluidic models and the in vivo environment. For example, a porous membrane used in experiments is over 300 times thicker than the naturally occurring basement membrane in endothelial cells, making it difficult for different types of cells to establish direct contact. To enable direct contact between co-cultured cells, microchannels can be filled with hydrogels as an extracellular matrix, but rigid extracellular matrix substrates have stiffness values orders of magnitude higher than those observed in living brain microvessels. Cell manipulation on a microfluidic chip requires substantial labor, with six orders of magnitude differences between microfluidic chips and conventional laboratory equipment. Small operation details can affect model properties and experimental reproducibility, and unintended variations may result in widely different results. To improve experimental reproducibility, more attention will be required to standardize details such as chip fabrication, cell seeding, cell localization, and other design factors. Standardizing the measurement of parameters is also necessary for comparison between in vitro models and in vivo experiments. Understanding and comparing microfluidic model designs can help select the most suitable ones for data adjustment and promote BBB research. Further goals include optimizing microfluidic models as screening platforms for drugs and studying neurological disease pathogenesis, thereby enhancing BBB research.

5. Conflict of Interest

None

6. References

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