ACCSCIENCE PUBLISHING

International Journal of Bioprinting

REVIEW ARTICLE

Advances in *in vitro* blood–brain barrier models: Integrating bioprinting with microfluidic chips for compound evaluation

Yating Du[1,2], Zezhi Li[2], Xiang Li[2]*, and Jianping Xie[2]*

[1]Beijing Technology and Business University, Beijing 100048, China

[2]Beijing Life Science Academy, Beijing 102209, China

(This article belongs to the *Special Issue: Biofabrication Breakthroughs: Innovation and Application in Bioprinting, Biomaterials, and Organoid*)

Abstract

The blood–brain barrier (BBB), a vital defense interface of the central nervous system, selectively regulates molecular transport into the brain and maintains brain homeostasis. Disruption of BBB integrity contributes to various neurological diseases, making the BBB a key target for therapeutic compounds. However, traditional *in vitro* models struggle to recreate the BBB's complex structure and dynamic functions. Recent advances in microfluidics and three-dimensional bioprinting have enabled the construction of high-fidelity *in vitro* BBB models that recapitulate key aspects of the brain's vascular microenvironment. By integrating principles from materials science, microfabrication, and cell biology, these "BBB-on-a-chip" platforms support physiologically relevant shear stress, cell–cell interactions, and barrier properties, making them powerful tools for compound screening and mechanistic research. This review summarizes the advances in *in vitro* BBB models and the application of bioprinting and microfluidic technology for compound evaluation.

Keywords: Bioprinting; Blood–brain barrier; Compound evaluation; *In vitro* model; Microfluidic chip

***Corresponding authors:**
Xiang Li (lixiang@blsa.com.cn)

Jianping Xie (ztridicp@126.com)

Citation: Du Y, Li Z, Li X, Xie J. Advances in blood–brain barrier *in vitro* models: Integrating bioprinting with microfluidic chips for compound evaluation. *Int J Bioprint.* 2025;11(5):68-97. doi: 10.36922/IJB025270265

Received: July 3, 2025
Revised: July 29, 2025
Accepted: August 8, 2025
Published online: August 15, 2025

1. Introduction

The brain's vascular system stretches approximately 400 miles, with the blood–brain barrier (BBB) serving as a crucial component of the neurovascular unit (NVU) in the central nervous system (CNS). The BBB consists of brain microvascular endothelial cells (BMECs), astrocytes, microglia, pericytes, oligodendrocytes, and neurons, along with tight junction proteins and extracellular matrix (ECM) constituents.[1] Structurally, it comprises a luminal glycocalyx layer (a polysaccharide–protein complex), as well as two different basement membranes: the endothelial (vascular) basement membrane and the parenchymal basement membrane.[2] The glycocalyx is a dynamic, carbohydrate-rich network structure that coats the apical surface of endothelial cells. Composed primarily of glycolipids, glycoproteins, and mucopolysaccharides, it is often referred to as the "cell coat." This layer performs several core functions, including preserving cellular morphology, facilitating signal transduction, and regulating molecular exchange.[2,3] The BBB's barrier properties are primarily attributed to specialized endothelial

cells and are characterized by significantly reduced paracellular permeability. These cells secrete a range of adhesion molecules and tight junction proteins—such as claudin-5, occludin, zonula occludens (ZO; ZO-1, ZO-2, ZO-3), and junctional adhesion molecules—which are anchored to the actin cytoskeleton through cytoplasmic adaptors like cingulin. This protein complex stabilizes intercellular junctions and restricts the diffusion of solutes. Consequently, only a limited subset of molecules, typically those recognized by specific receptors, are permitted to cross the BBB via receptor-mediated endocytosis.[2,4]

Pericytes, which are embedded within the basement membrane, are positioned along the outer surfaces of parenchymal collateral vessels and capillaries, maintaining direct contact with adjacent endothelial cells. They play a vital role in the formation and maintenance of the basement membrane by secreting structural components such as collagen, laminin, and other ECM proteins.[5] In addition to providing mechanical support to blood vessels and regulating vascular tone, pericytes are essential for preserving the integrity of the BBB.[5] Disruption of genes specifically expressed by pericytes significantly compromises BBB function, demonstrating their critical regulatory role.[6] Astrocytes contribute to BBB stability through their end-feet, which envelop the blood vessels and provide physical scaffolding. These end-feet are involved in the regulation of water transport across the BBB, primarily mediated by the aquaporin-4 water channel. Furthermore, molecular interactions between astrocytes and endothelial cells are crucial in modulating the structural and functional integrity of the BBB.[7,8] Neurons are closely associated with astrocytes and rely on oligodendrocytes for axonal insulation. Oligodendrocytes form myelin sheaths around neuronal processes, enhancing electrical conductivity and protecting neural pathways.[2] Microglia, the resident macrophages of the CNS, typically cover distinct, non-overlapping domains but can migrate toward cerebral blood vessels during injury or inflammation. In doing so, they contribute to the maintenance and restoration of BBB integrity under pathological conditions.[2] Figure 1 presents a schematic representation of the major cellular components of the BBB structure.

The integrity of the BBB is vital for preserving brain homeostasis and normal neurological function. By tightly regulating the transport of small molecules and biological agents, the BBB maintains a stable microenvironment within the CNS.[9] Disruption of this barrier has been implicated in a range of neurological diseases, including Alzheimer's disease (AD), multiple sclerosis, stroke, and epilepsy.[10] Increased BBB permeability enables the migration of neurotoxic substances and immune cells into the brain parenchyma via the paracellular pathway, potentially inducing inflammatory responses.[11] Compromised BBB integrity is a hallmark of several pathological conditions. In acute ischemic stroke, both ischemia and reperfusion lead to the excessive generation of reactive oxygen and nitrogen radicals in the microvascular endothelium, resulting in vascular injury and BBB breakdown.[12] In brain tumors, whether primary brain cancers or metastases, BBB permeability is often elevated, facilitating tumor cell invasion into CNS tissues.[13] Sepsis-associated encephalopathy, a serious complication of systemic infection, is characterized by microglial hyperactivation, endothelial barrier dysfunction, and BBB disruption, inducing symptoms ranging from mild cognitive disturbances to severe neurological impairment or coma.[14]

Current *in vitro* models of the BBB primarily consist of two-dimensional (2D) systems, such as conventional dish cultures and Transwell platforms, as well as three-dimensional (3D) models, including spheroids, organoids, and microfluidic chips.[15,16] The emergence of bioprinting introduces new opportunities for advancing these traditional BBB models.[17,18] Bioprinting refers to the fabrication of tissue-like or organoid structures using 3D printing technologies with biological materials, enabling precise spatial control of cellular organization and extracellular architecture.[19] This level of control significantly enhances the functional fidelity and physiological relevance of BBB models, particularly in compound evaluation.[20] Reliable and physiologically representative *in vitro* BBB models are indispensable tools for disease modeling and high-throughput screening of therapeutic compounds targeting the CNS. In recent years, biomimetic BBB-on-a-chip platforms have emerged as accurate and dynamic systems for simulating *in vivo* conditions and evaluating compound permeability and toxicity. This review provides a comprehensive overview of recent advances in BBB *in vitro* modeling, with a particular focus on microfluidic chip-based platforms for compound evaluation. Additionally, we examine the contributions of bioprinting technology to the development of next-generation BBB models and discuss the current challenges and prospects in this evolving field.

2. *In vitro* blood–brain barrier models

In vitro BBB models are essential tools for recapitulating the structure and function of the BBB under controlled laboratory conditions. With continuous advancements in biomedical engineering and tissue modeling, these models have undergone significant evolution. Figure 2 illustrates the development of *in vitro* biomimetic BBB models. The earliest models were simple 2D systems, followed by the introduction of the Transwell model,[21]

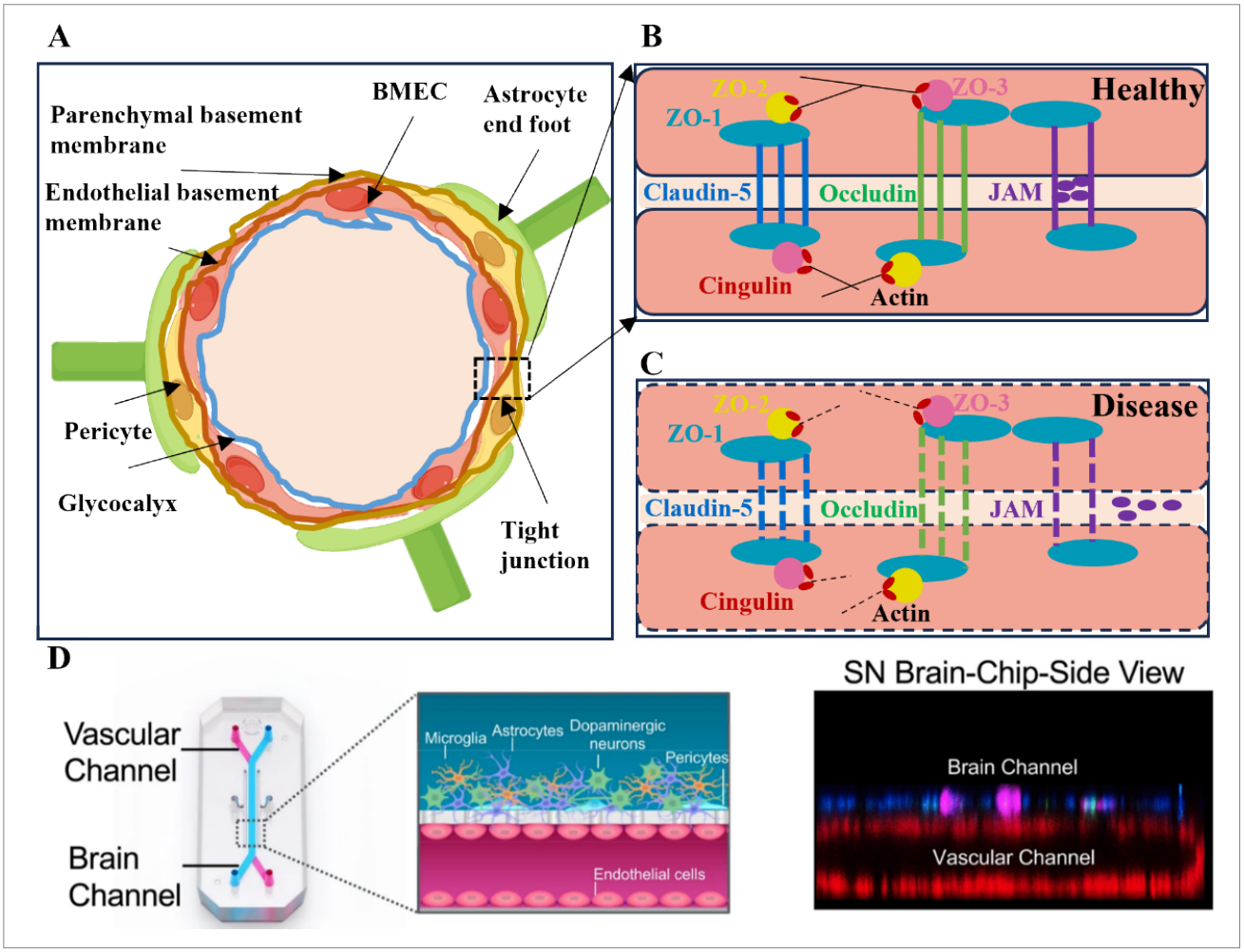

Figure 1. Schematic representation of the primary cellular components of the BBB structure. (A) Cross-sectional schematic of a brain microvessel illustrating the spatial organization of BBB key cellular components. (B) Tight junctions between BMECs under healthy physiological conditions. (C) Disrupted tight junctions in pathological states, contributing to increased BBB permeability. Illustrations (A–C) were modified from Vetter *et al.*[8] (D) Three-dimensional reconstruction of a confocal z-stack showing the organization of BMECs, pericytes, astrocytes, microglia, and neurons in an SN brain-chip model. Image adapted from Pediaditakis *et al.*[16] The selected cell images used in the figure were sourced or adapted from Servier Medical Art via the scifig.bio plugin (version 1.3.2). Abbreviations: BBB, blood–brain barrier; BMEC, brain microvascular endothelial cell; JAM, junction adhesion molecule; SN, substantia nigra; ZO, zonula occludens.

which enabled the co-culture of multiple cell types across a semipermeable membrane. Subsequently, 3D models such as spheroids[22] and organoids[23] were developed, offering improved cellular interactions and structural complexity. Currently, microfluidic chip-based models represent the most advanced form of dynamic 3D BBB models, closely mimicking *in vivo* physiological conditions.[24] As an emerging approach, bioprinting holds considerable promise for addressing the limitations of traditional models,[25] such as the static nature of conventional 3D cultures, particularly when integrated with microfluidic systems.[26] Table 1 summarizes several relevant studies on the development of *in vitro* BBB models, detailing the cell types, the apparatuses, the applications, the advantages, and the limitations associated with each model.

2.1. Two-dimensional model

The Petri dish model, one of the earliest 2D cell culture systems, remains widely used due to its low cost, ease of handling, and suitability for observing and analyzing cellular characteristics. Despite its simplicity, this model has been proven effective in simulating certain structural and functional aspects of the BBB. For instance, in 1986, Audus *et al.*[27] established a monolayer BBB model using bovine BMECs, demonstrating its utility in evaluating the permeability of centrally acting drugs and other substances

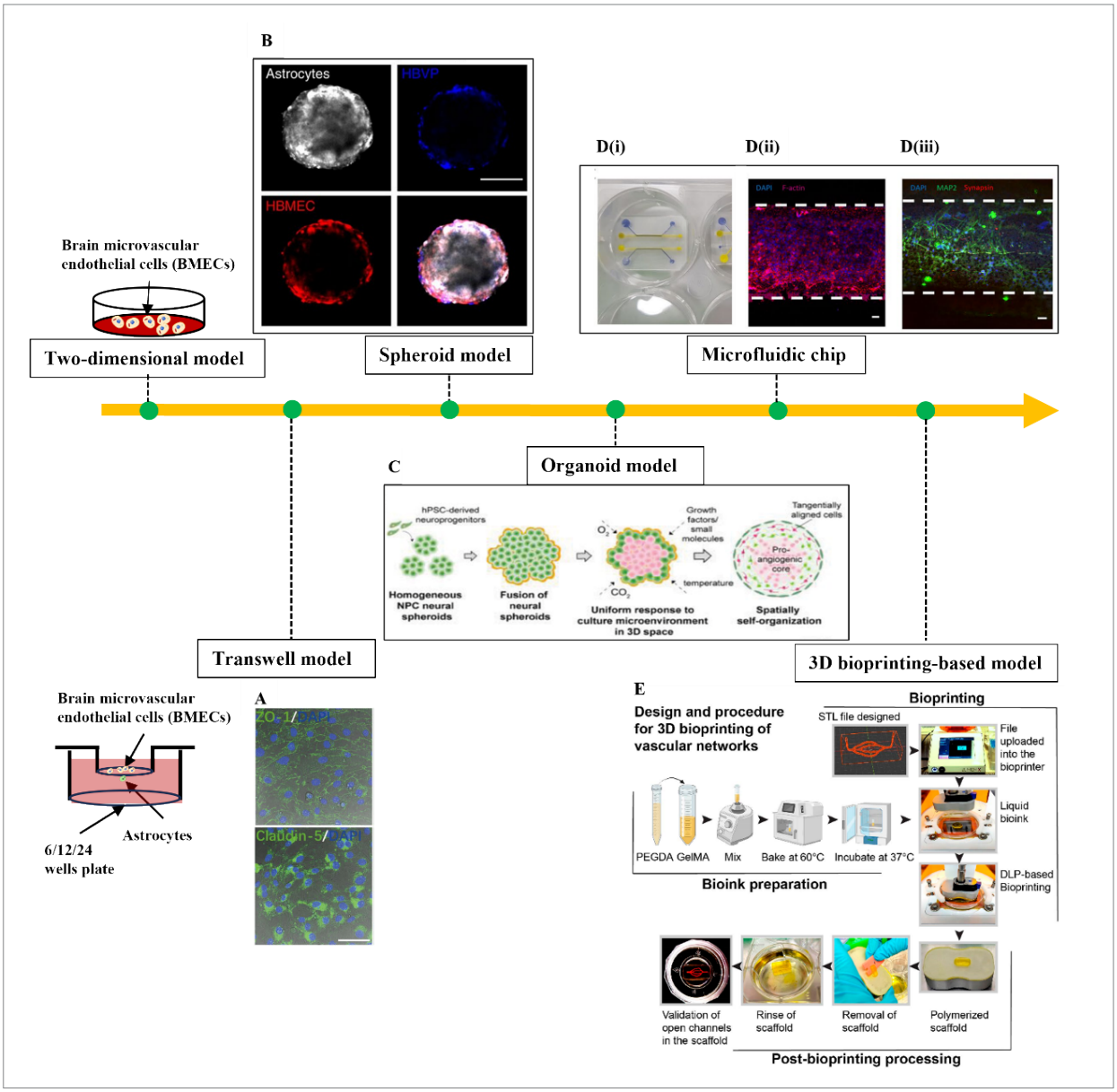

Figure 2. The development of *in vitro* biomimetic BBB models. (A) Representative immunofluorescence staining of tight junction proteins ZO-1 and claudin-5 in cultured brain endothelial cells, demonstrating tight junction formation. Scale bar: 50 μm, magnification: 63×. Image adapted from Li *et al.*[21] (B) Representative confocal images showing the organization of human astrocytes (hAs; white), HBVP (blue), and primary HBMEC (red) when co-cultured to form a spheroid. Scale bar: 100 μm. Image adapted from Cho *et al.*[22] (C) Schematics showing the concept of generating spatially self-organized neural concentroid from hPSC-derived neuroprogenitor cells. Image adapted from Chai *et al.*[23] (D) NVU-on-a-chip microfluidic model. (i) Open-top microfluidic chip integrated into a 6-well plate. The bottom channel (yellow) supports endothelial cell culture, while the open-top channel (blue) enables neuronal differentiation. (ii) Immunofluorescence staining of endothelial cell monoculture in the bottom channel on a polyester membrane showing a confluent monolayer (Blue: nuclei; red: F-actin). Scale bar: 50 μm, magnification: 10×. (iii) Immunostaining of neurons in the top channel showing neuronal markers (Blue: nuclei, green: MAP2, and red: synapsin-1/2). Scale bar: 50 μm, magnification: 10×. Image adapted from Middelkamp *et al.*[24] (E) Schematic representation of the steps for the fabrication of the scaffold with a 3D vascular network. The bioprinting steps include generating a computer-aided design file (STL) of a 3D microvascular network using a computer graphics software tool (i.e., Blender) and uploading it to the bioprinter. Bioinks were prepared by mixing PEGDA and GelMA, heating at 60°C, and then incubating at 37°C. The liquid bioink was then polymerized using DLP bioprinting to form a scaffold with channels. Red food coloring dissolved in PBS was then perfused to validate the integrity of the hollow channels. Image adapted from Don *et al.*[25] Selected cell graphics in this figure were sourced or adapted from Servier Medical Art via the scifig.bio plugin (version 1.3.2). Abbreviations: 3D, three-dimensional; BBB, blood–brain barrier; DLP, digital light-based; GelMA, gelatin methacryloyl; HBMEC, human brain microvascular endothelial cells; HBVP, human brain vascular pericytes; MAP2, microtubule associated protein 2; NVU, neurovascular unit; PBS, phosphate-buffered saline; PEGDA, polyethylene glycol diacrylate; STL, standard tessellation language; ZO, zonula occludens.

Table 1. Studies on the development of *in vitro* blood–brain barrier models

Model	Cell sources	Apparatus	Application	TEER (Ω·cm^2)	Permeability	Duration	Throughput	Advantages	Limitations	Ref.
Two-dimensional model	Bovine BMECs	100 mm petri dish	Drug delivery and metabolism	–	Leucine, propranolol, and sucrose	5 days	–	Low cost, easy to operate, easy to observe and analyze, repeatable	The physiologic environment of the 2D model differs significantly from *in vivo*; imperfect barrier structure and function	[27]
	Bovine BMECs	100 mm petri dish	Model verification	–	Fluorescein-conjugated dextrans	–	–			[28]
	Human BMECs and astrocytes	100 mm petri dish	Compound metabolism	–	Essential polyunsaturated fatty acids: 18:2n–6 and 18:3n–3	6–7 days	–			[29]
Transwell model	Primary cells: BMECs, astrocytes, pericytes, and neurons	12-well Transwell plate	Model verification	10–50	Dexamethasone	10 days	–	Simulates barrier structure and function; representative of the NVU	Limited barrier function, mainly applicable to transport mechanism studies	[31]
	Endothelial cells, astrocytes differentiated from human iPSC	12-well Transwell plate	Model verification	300–2000	70 kDa FITC dextran and lucifer yellow	10 days	–			[34]
	Mouse BMECs, astrocytes	24-well Transwell plate	Sepsis disease modeling	20–600	10 kDa FITC-dextran	24 hours	–			[36]
	b. End3-mouse BECs; rat BMSCs	24-well Transwell plate	Ischemic stroke disease modeling	–	4.4 kDa TRITC-dextran, Evans blue	24 hours	–			[21]
	Mouse BECs (bEnd.3); rat glioblastomas (C6)	24-well Transwell plate	Brain glioma disease modeling	300	–	12 hours	–			[37]
	HCMEC/D3, astrocytes (SC-1800), HBVP	24-well Transwell plate	Model verification	1200	–	24 hours	–			[38]

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doi: 10.36922/IJB025270265

Table 1. Studies on the development of *in vitro* blood–brain barrier models

Model	Cell sources	Apparatus	Application	TEER (Ω·cm²)	Permeability	Duration	Throughput	Advantages	Limitations	Ref.
Spheroid model	Primary human cells: astrocytes, pericytes, endothelial cells	ULA 24-well plates	Compound evaluation	–	FITC solution	72–120 hours	–	Simulate barrier structures; combine with Transwell models for drug delivery	Simple cellular composition lacks an accurate barrier function; internal conditions need molecular labeling and further observation	45
	Human primary BECs, primary pericytes, and primary astrocytes	24-well hanging droplet culture plates (HDCPs)	Model verification	–	–	72 hours	–			46
	Human glioma cells (U87, U118, and U251)	24-well Transwell plate	Compound evaluation	–	–	8–10 days	–			47
	bEnd.3 cells, glial cells (SVG-p12), glioblastoma multiforme (GBM8401), T98G cells	24-well Transwell plate	Compound evaluation	–	CC12 (NSC749232)- an anthraquinone tetraheterocyclic homolog with lipophilic structure	48 hours	–			48
Organoid model	Human iPSCs	ULA 96-well plates	Model verification	–	70,000 MW Texas Red dextran	13 days	–	Long-term maintenance (up to 50+ days); valuable for pathology modeling; scalable for high throughput and multi-organ fusion	Lack functional circulation and a dynamic physiological environment	50
	Human iPSCs	ULA 24-well plates	Alzheimer's disease modeling	–	Gamma-secretase inhibitor DAPT, heparin, and heparinase III	71 days	–			51
	Human PSCs	ULA 24-well plates	Alzheimer's disease modeling	–	–	10 days	–			52
	Human ESCs	Micropillar array 24-well plate	Model verification	–	–	70 days	Medium throughput			23
	Human PSCs	ULA 6-well plates	Cerebral cavernous malformation disease modeling	Around 1190.8	–	75 days	–			53
	hiPSC-derived neurons, astrocytes	12-well transparent MEA plates	Alzheimer's disease modeling	–	–	40 days	–			55

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Table 1. Studies on the development of *in vitro* blood–brain barrier models

Model	Cell sources	Apparatus	Application	TEER (Ω·cm²)	Permeability	Duration	Throughput	Advantages	Limitations	Ref.
Microfluidic chip model	hiPSC-derived neurons, rat astrocytes, and human endothelial cells	PDMS microfluidic chip	Model verification	–	4 and 20 kDa FITC-dextrans, 0.5 kDa lucifer yellow	14 days	24 individual microfluidic units	Allow study of cell–cell interactions; mimic *in vivo* shear stress with high fidelity; support parallel testing of multiple stimuli	Complicated, costly design and fabrication; electrode-chip integration needs further research; multi-channel errors and cross-contamination	[24]
	Primary mouse BMECs	PDMS microfluidic chip	Model verification	–	–	38 days	–			[61]
	HCMEC/D3 and human astrocytes	PDMS multiplexing chips	Model verification	–	–	3 days	–			[62]
	BMECs (bEnd.3), astrocytes (C8D1A), pericytes (MBVP)	PDMS microfluidic chip	Model verification	220	–	12 hours	–			[63]
3D bioprinting-based model	Human astrocytes, primary human BMECs, and pericytes	Complex 3D vascular network fabricated within a hydrogel, and combined with fluid dynamics	Model development	–	40 kDa dextrans	7 days	–	More closely replicates the cellular structure and multicellular arrangement of microvessels *in vivo*	Biodegradable scaffold materials need to be developed, and more suitable cells need to be selected to improve epithelial barrier properties	[25]
	HAs, HBVPs, and HBMECs	The aqueous two-phase 3D printing (ATPP) model	Model verification	–	10 kDa dextran Texas Red	8 days	–			[69]
	Human brain endothelial cells, pericytes, and astrocytes	3D-printed serpentine channel model	Model verification	–	150 kDa FITC-dextran; ultrasound	9 days	–			[70]
	hiPSCs (IMR90-4) derived BCECs	Chip bioreactor, a membrane chip	Model verification	285–2609	–	168 hours	–			[71]

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; BECs, brain endothelial cells; BMSCs, bone marrow mesenchymal stem cells; DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; ESCs, embryonic stem cells; FITC-dextran, fluorescein isothiocyanate-dextran; HA, human astrocytes; HBMECs, human brain microvascular endothelial cells; HBVP, human brain vascular pericytes; HCMEC, human cerebral microvascular endothelial cells; iPSCs, induced pluripotent stem cells; MEA, microelectrode arrays; NVU, neurovascular unit; PDMS, polydimethylsiloxane; PSCs, pluripotent stem cells; ULA, ultra-low attachment.

doi: 10.36922/IJB025270265

(Table 1).[27] Similarly, in 1988, van Bree *et al.*[28] developed a BBB model composed of bovine cerebrovascular cells that formed tight junctions and exhibited transport characteristics consistent with those observed *in vivo* (Table 1).[28] Further advancing the approach, Bernoud *et al.*[29] co-cultured human BMECs with astrocytes in Petri dishes. Their results highlighted the critical role of astrocytes in facilitating fatty acid transport across the BBB (Table 1).[29]

However, simple Petri dish models are inherently limited in their ability to replicate the complex structure and function of the BBB under both physiological and pathological conditions. These models typically rely on monolayer or mixed-cell cultures,[30] which lack the cellular organization and dynamic intercellular signaling necessary to accurately mimic barrier properties and the exchange of soluble factors. As a result, researchers seek to develop more physiologically relevant BBB models that better emulate the *in vivo* microenvironment.

2.2. Transwell model

To address the limitations of conventional Petri dish models, researchers developed the Transwell model, which incorporates a semipermeable polymer membrane. This platform supports the establishment of single-cell or multicellular co-culture systems and enables the spatial organization of key cellular components of the BBB, including BMECs, astrocytes, pericytes, neurons, and microglia. These cells are typically cultured on the apical and basal surfaces of the porous membrane to simulate the anatomical and functional distribution found *in vivo* (Table 1).[31,32] The structural integrity and permeability of Transwell-based BBB models have been validated in multiple studies. The critical parameter for assessing barrier function is trans-epithelial electrical resistance (TEER), which reflects the permeability of the cell layer and the tightness of intercellular junctions. Higher TEER values indicate stronger cell–cell adhesion and reduced paracellular transport.[33] *In vitro* Transwell models of the BBB have been shown to express tight junction markers reliably, and fluctuations in TEER values are indicative of functional barrier properties. For example, Singh *et al.*[34] suggested that the optimal window for conducting transport studies lies between 24 and 72 hours post model establishment (Table 1).[34] Model performance can be enhanced through optimization of several culture parameters, including growth medium composition, membrane material, cell seeding density, and co-culture strategies.[32] Notably, coupling the Transwell platform with adjustable electrospun polyethyleneimine–fibronectin membranes has enabled the development of high-throughput permeability assays, further extending its application in compound screening.[35] The Transwell model has been widely applied in research on neurological diseases such as ischemic stroke and glioma, serving as a valuable tool for investigating disease mechanisms. For instance, Chen *et al.*[36] demonstrated that Maf1 protein significantly suppressed lipopolysaccharide-induced neuroinflammation and neuronal apoptosis in both *in vitro* and *in vivo* models of sepsis-associated encephalopathy (Table 1).[36] Similarly, studies on ischemic stroke have shown that caveolin-1 scaffolding proteins increase BBB permeability, whereas extracellular vesicles derived from mesenchymal stem cells can restore BBB integrity and promote neurological recovery (Table 1).[21] Additionally, the Transwell model has been used to evaluate the targeted delivery of adriamycin to glioma cells, successfully demonstrating the drug's ability to traverse the BBB and localize within tumor tissue (Table 1).[37]

However, static Transwell models are limited in their ability to replicate the dynamic physiological conditions of the *in vivo* environment—such as blood flow, shear stress, and continuous molecular signaling.[38] The absence of these mechanical and biochemical cues can result in altered cellular behavior, including aberrant proliferation and phenotypic drift. Furthermore, the lack of real-time intercellular interactions and soluble factor gradients restricts the model's ability to fully emulate the complex neurovascular microenvironment. In addition, the manual handling required for Transwell assays is time-consuming and labor-intensive, and the system's relatively low throughput poses challenges for scalability in high-throughput screening applications.

2.3. Spheroid model

Advances in materials science and bioengineering have enabled the development of novel biomaterials—such as collagen sponges—for use in constructing 3D spheroid models.[39] The spheroid model, one of the earliest forms of 3D culture, was introduced in the 1970s.[40] In 1977, Schmitz *et al.*[41] reported the spontaneous aggregation of cells into 3D spheroidal structures during *in vitro* culture, marking a foundational step in the development of spheroid modeling.[41] By the late 20th and early 21st centuries, spheroid models gained widespread application in cancer research due to their ability to replicate critical *in vivo* features, including hypoxic microenvironments and drug resistance, which are often absent in traditional 2D cultures.[42,43] More recently, spheroid models have been employed to simulate the human NVU and BBB, offering physiologically relevant platforms for studying brain function and pathology.[44] For example, Sokolova *et al.*[45] developed a multicellular 3D spheroid BBB model using primary human astrocytes, pericytes, and endothelial cells. In this configuration,

astrocytes formed the spheroid core, which was encased by pericytes, while brain endothelial cells constituted the outermost layer—successfully reconstituting a functional BBB structure (Table 1).[45] Similarly, other studies using primary human brain endothelial cells, pericytes, and astrocytes demonstrated the feasibility of assembling multicellular spheroids capable of modeling key BBB features *in vitro* (Table 1).[46]

While spheroid models offer valuable insights, they generally feature simpler cellular compositions and lack the structural and functional complexity of fully differentiated tissues. As a result, spheroid models are often integrated with other platforms—such as Transwell systems—to enhance their applicability for evaluating therapeutic agents, particularly in oncology research. For instance, iguratimod-loaded poly (lactic acid)-glycolic acid nanoparticles demonstrated inhibitory effects on tumor cell migration when tested using a combination of spheroid and Transwell tumor models (Table 1).[47] In another study, a glioblastoma multiforme spheroid model was coupled with a BBB Transwell system to evaluate the therapeutic efficacy of the lipophilic compound CC12 (NSC749232), providing a more comprehensive assessment of drug permeability and antitumor activity across the BBB (Table 1).[48]

2.4. Organoid model

Organoids are 3D culture systems derived from stem cells that differentiate under the influence of specific growth factors. These models can closely mimic the structural and functional characteristics of native organs. Organoids have been widely employed to study human organ development and disease pathogenesis—including that of the brain—due to their ability to recapitulate complex tissue architecture and cellular interactions. In the context of BBB modeling, organoids exhibit promising physiological relevance. Reported TEER values for brain organoid-based BBB models range from 1500 to 8000 $\Omega \cdot cm^2$, which is comparable to the *in vivo* BBB range from approximately 1800 to 2000 $\Omega \cdot cm^2$.[49] Additionally, organoid systems support extended culture durations, making them suitable for long-term studies. For example, Ahn *et al.*[50] successfully maintained a neural-specific vascular network for over 50 days, enabling the reconstruction of BBB-like structures and functions (Table 1).[50]

Organoids also offer the potential for constructing personalized models, making them valuable tools for both disease modeling and therapeutic development. Organoids have been applied to model the pathophysiology of neurodegenerative diseases such as AD. For example, using human induced pluripotent stem cells (hiPSCs), researchers have generated brain organoids to simulate the BBB microenvironment associated with AD. Yan *et al.*[51] demonstrated that BBB organoids derived from AD patients exhibited upregulated expression of pro-inflammatory cytokines—including interleukins and tumor necrosis factor—along with altered ECM protein profiles, providing mechanistic insights into AD pathogenesis (Table 1).[51] Additionally, Huang *et al.*[52] employed cerebrovascular organoids to investigate the role of apolipoprotein E4 in neurons and astrocytes, thereby identifying potential therapeutic targets for neurovascular dysfunction in AD (Table 1).[52] Despite their advantages, conventional brain organoids are typically composed of ectodermal cell types and lack vascular structures, limiting their ability to replicate key physiological aspects of the BBB. To address this limitation, Chai *et al.*[23] incorporated pluripotent stem cell-derived endothelial cells into organoid cultures, promoting vascularization and creating a "vascularized" brain model. This advancement provided a novel *in vitro* approach for studying angiogenesis and vascular involvement in brain disorders (Table 1).[23] Further extending this strategy, Dao *et al.*[53] developed a human BBB assemblage by combining brain and vascular organoids derived from hiPSCs. This model successfully recapitulated anatomical and functional features of cavernous hemangiomas and BBB disruption observed in patients with cerebral cavernous malformations (Table 1).[53] Taken together, organoid models are highly representative of native tissue composition, structure, and function—especially when integrated with co-culture techniques, multi-tissue interactions, biosensing platforms, bioimaging technologies, or histological analysis (Table 1).[54,55]

Despite its many advantages, the organoid model presents several notable limitations. First, the generation of organoids typically requires long incubation periods and a high level of technical expertise, which can pose challenges for widespread adoption. Moreover, variability in organoid structure and function across different batches leads to low reproducibility, thereby limiting their reliability and consistency in experimental research. Another significant drawback is the static nature of organoid models, which hinders their ability to accurately replicate the dynamic physiological conditions of the BBB, such as fluid flow, shear stress, and real-time biochemical signaling. This lack of dynamic interaction reduces the model's fidelity in mimicking *in vivo* neurovascular environments and may restrict its utility in certain applications.

2.5. Microfluidic chip model

The advent of microfluidic technology has opened new avenues for the development of *in vitro* biomimetic BBB models.[56] Traditional static 3D models lack critical physiological features, such as blood flow, shear stress, and dynamic intercellular interactions, which are essential for

accurately simulating the complex *in vivo* microenvironment of the BBB.[56] To address these limitations, Kawakita *et al.*[57] developed dynamic BBB organoid models using microfluidic platforms, aiming to more precisely recreate the human brain microenvironment.[57] Microfluidic BBB models integrate microfluidics, cell culture technology, and in some cases, 3D printing, to construct miniaturized *in vitro* systems that replicate both the structure and function of the BBB. These models enable spatial and temporal control over key cell types, including endothelial cells, pericytes, and astrocytes, which are cultured according to their native anatomical distribution and functional interactions. This approach facilitates the formation of highly biomimetic barrier structures with enhanced physiological relevance.[58] Currently, common types of microfluidic chips for the human brain include compartmentalized chips with microchannel separation, compartmentalized chips with membrane separation, and gradient-inducing chips.[59] For instance, Sonninen *et al.*[60] co-cultured hiPSC-derived endothelial cells and astrocytes on AKITA plates (a commercially available organ-on-a-chip microfluidic platform) and applied a shaking-induced flow of 0.6 dyne/cm^2. Permeability assays and protein expression analyses confirmed the successful construction of a functional microfluidic BBB chip (Table 1).[60] In another study, Middelkamp *et al.*[24] demonstrated that culturing cells in a microfluidic environment induced significant changes in gene expression compared to conventional static plates. Specifically, endothelial cells exhibited reduced proliferation, while neurons and astrocytes showed enhanced adhesion and neuronal maturation (Table 1).[24] These findings highlight the potential of microfluidic chips for disease modeling and compound screening. Furthermore, Jeong *et al.*[61] employed numerical simulations to predict shear stress within microfluidic BBB chip channels. Their results showed that shear stress increased with decreasing channel size and porosity, and the simulation error was maintained within 2.17%, establishing a quantitative framework for optimizing microfluidic organ-on-a-chip systems to more closely replicate *in vivo* physiological conditions (Table 1).[61]

In addition, multichannel microfluidic chips enable the parallel testing of multiple experimental conditions, thereby improving experimental throughput and reproducibility. For example, Zakharova *et al.*[62] developed a polydimethylsiloxane (PDMS)-based chip featuring eight parallel microchannels, allowing simultaneous analysis of permeability under varying conditions. Their study revealed significant differences in permeability coefficients among molecules of different sizes, demonstrating the chip's sensitivity and versatility.[62] In short, biomimetic BBB microfluidic chips represent a transformative platform for the evaluation of BBB-permeable compounds. Their ability to closely mimic physiological conditions, combined with high-throughput and modular design capabilities, makes them highly valuable for both basic neuroscience research and translational drug development.[63]

2.6. Bioprinting-based model

3D bioprinting is an additive manufacturing technology that enables the precise layer-by-layer deposition of biomaterials and living cells under computer-aided design control to construct tissue-like structures.[64] Bioink (the printable formulation) is a key component of this process; it is typically composed of biocompatible hydrogels combined with live cells.[65] The choice of cell types included in the bioink depends on the target tissue. For neural tissue or BBB models, common cell types include astrocytes, pericytes, endothelial cells, and neurons. During the printing process, the bioink is rapidly solidified or crosslinked upon deposition to ensure cell encapsulation and structural stability. Crosslinking can occur through various mechanisms, such as ultraviolet light exposure in the case of photosensitive bioinks, or through the addition of crosslinking agents.[65,66] Bioprinting technologies can be broadly categorized into several types, each with distinct advantages and limitations.[64,67] For example, inkjet bioprinting is suitable for low-viscosity bioinks and offers high-speed printing, but may struggle with cell density and resolution. Extrusion-based bioprinting can accommodate higher-viscosity materials and cell densities but may expose cells to shear stress during printing. Laser-assisted bioprinting provides high resolution and avoids nozzle clogging, but it requires complex and costly optical systems. Regardless of the specific method, all bioprinting techniques construct 3D structures through the sequential deposition and curing of bioink layers based on pre-defined models.[64,65,67]

Traditional 2D cell culture methods are limited in their ability to replicate the 3D architecture and biomechanical cues—such as shear stress—present in the native capillary microenvironment. In contrast, 3D bioprinting offers a promising strategy for constructing physiologically relevant BBB models. This technology enables the spatial organization of multiple cell types—such as endothelial cells, pericytes, and astrocytes—into layered, biomimetic structures that closely resemble the vascular architecture of the blood–brain interface.[68] For example, Dona *et al.*[25] used digital light processing-based 3D bioprinting to construct a human brain microvascular model embedded with primary human astrocytes. The vascular component was formed using human BMECs and pericytes, and continuous perfusion was performed to promote vascular maturation. The

printed model was functionally evaluated for immune-endothelial interactions, permeability, and barrier integrity, demonstrating key BBB characteristics (Table 1).[25] Similarly, Oh *et al.*[69] introduced an aqueous two-phase printing approach to construct a BBB microstructure model in a liquid-phase environment. This technique overcame the limitations associated with air–liquid interface printing and then resulted in improved barrier functionality. The model incorporated three different human cell types: astrocytes, endothelial cells, and vascular pericytes, and enabled precise control over nutrient supply and drug concentrations during experimentation (Table 1).[69] In another study, Royse *et al.*[70] applied stereolithography-based 3D bioprinting to fabricate a BBB model, which is used as a drug screening platform. They printed a cylindrical hydrogel-based vascular network populated with human brain endothelial cells, pericytes, and astrocytes in an anatomically relevant arrangement. The model exhibited proper cell adhesion, expressed tight junction proteins, and was perfused to assess compound transport. Functional assays confirmed the presence of intact barrier properties and expression of key efflux transporters, including P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), highlighting the model's utility in CNS drug delivery research (Table 1).[70]

Additionally, the integration of 3D bioprinting with microfluidic technology offers a powerful approach for constructing physiologically and dynamically relevant *in vitro* BBB models. This hybrid strategy enables the simulation of fluidic conditions and precise architectural control that closely resemble the *in vivo* microenvironment. For example, Choi *et al.*[71] developed a bioreactor system designed to assess BBB integrity under dynamic flow conditions (Table 1).[71] The bioreactor was fabricated using a combination of 3D printing techniques and PDMS casting, and subsequently evaluated using a hiPSC-derived BBB model over a 7-day culture period. The system successfully supported real-time barrier monitoring and dynamic tissue culture, demonstrating its applicability for advanced BBB modeling studies.[71]

3. Structural and functional characterization of *in vitro* blood–brain barrier models

The structural and functional integrity of *in vitro* BBB models is fundamental to their reliability and relevance. Structural integrity serves as the foundation for functional performance, providing the necessary cellular architecture to support intercellular communication and maintain the physical barrier properties of the model. Conversely, functional integrity reflects the model's ability to preserve physiological homeostasis, regulate selective permeability, and respond appropriately to pathological stimuli. Structural integrity is typically assessed by evaluating cellular morphology and the expression and localization of tight junction proteins, such as claudin-5, occludin, and ZO-1, which are essential for maintaining paracellular barrier function. Functional integrity is commonly evaluated using several key parameters, including TEER, permeability to molecular tracers, and the expression or activity of transporter proteins, such as P-gp and BCRP. Together, these structural and functional assessments are critical for validating the fidelity of BBB models and ensuring their suitability for applications in disease modeling, compound evaluation, and neurovascular research.

3.1. Structural integrity

Structural integrity is a fundamental criterion for evaluating the physiological relevance of *in vitro* BBB models. Fluorescence imaging is commonly employed to visualize cellular morphology, spatial arrangement, and intercellular interactions, allowing researchers to assess whether the architecture of the model mimics that of the *in vivo* BBB. Astrocytes, which reside between capillaries and neurons, play a crucial role in BBB structure by forming a basement membrane surrounding BMECs. These glial cells are essential for maintaining brain homeostasis by regulating the balance of ions, amino acids, neurotransmitters, and water. Serving as a bridge between the vascular and neural systems, astrocytes are key contributors to the integrity and function of the BBB.[7] Ohbuchi *et al.*[72] used fluorescence imaging to examine the structural organization of brain endothelial cells and pericytes within a BBB model. Their findings showed that the cellular arrangement closely resembled *in vivo* conditions and exhibited selective permeability, providing strong evidence for the structural fidelity of the model.[72]

Tight junction proteins and adhesion complexes between endothelial cells play a central role in the formation and maintenance of the BBB. These tight junctions are composed of both transmembrane and intracellular proteins, including zonula occludens (ZO-1, ZO-2, ZO-3), claudin-5, occludin, and junctional adhesion molecules, which are anchored to the actin cytoskeleton via adaptor proteins such as cingulin.[2] The proper assembly of tight junctions is essential for establishing the structural integrity and selective permeability of the BBB. However, tight junction integrity is highly susceptible to disruption under pathological conditions. Studies have shown that diseases such as ischemic stroke,[73] major depressive disorder,[74] and encephalitis[75] can significantly impair the expression and organization of tight junction proteins, compromising BBB function. A variety of analytical

techniques have been used to assess tight junction protein expression and localization in BBB models. These include transmission electron microscopy for ultrastructural analysis,[76] immunofluorescence staining for spatial distribution,[77] western blotting for protein identification and quantification,[78] and quantitative real-time polymerase chain reaction for gene expression analysis.[79] Although maintaining BBB integrity remains a key challenge in the development of *in vitro* models, optimization of critical culture parameters, such as medium composition, cell seeding density, and substrate material, can improve the physiological relevance and structural fidelity of these models.[80]

3.2. Functional integrity

Barrier function is a fundamental characteristic of the BBB and a critical criterion for assessing the validity of *in vitro* BBB models. A functionally competent BBB is essential for protecting the brain by blocking the entry of neurotoxic substances, regulating the selective transport of nutrients, and facilitating the clearance of metabolic waste products.[81] These barrier properties are tightly associated with the presence and organization of tight junction proteins, which form the structural basis for paracellular restriction. TEER measurement is one of the most widely used and reliable methods for evaluating the integrity and permeability of endothelial monolayers in *in vitro* BBB models.[82,83] TEER provides a quantitative, non-invasive, and real-time assessment of tight junction formation and is particularly useful for monitoring intracellular signaling events and barrier dynamics. Although TEER values of *in vitro* models are generally lower than those observed *in vivo* (typically 1800–2000 $\Omega \cdot cm^2$), recent advances have led to significant improvements. For example, Chang *et al.*[63] developed a microfluidic chip-based BBB model with a TEER value of approximately 220 $\Omega \cdot cm^2$ (Table 1).[63] Co-culture systems, particularly those combining endothelial cells with astrocytes, have been shown to markedly enhance barrier function, achieving TEER values as high as 1200 $\Omega \cdot cm^2$ (Table 1).[38] In addition to TEER, the use of permeability assays with hydrophilic tracers is another common approach for evaluating barrier function. Tracers such as fluorescein isothiocyanate-dextran (FITC-dextran), glucose, sucrose, mannitol, fluorescein sodium, and fluorescein yellow are commonly employed due to their low permeability across an intact BBB. These compounds enable the quantification of paracellular leakage and provide complementary evidence of tight junction integrity in microfluidic BBB models.[84,85] Evans blue dye, which binds tightly to serum albumin, is another established tracer for assessing BBB permeability.[86] Under normal physiological conditions, albumin does not cross the BBB. However, the presence of Evans blue-albumin complexes in the brain parenchyma serves as a clear indicator of tight junction disruption and paracellular leakage.[87]

Selective permeability is another hallmark feature of the BBB and a critical functional parameter in evaluating *in vitro* BBB models. Under physiological conditions, large hydrophilic tracers such as FITC-dextran are unable to cross the BBB. However, smaller hydrophilic molecules may traverse the barrier via transcellular or paracellular pathways, with permeability generally decreasing as molecular weight increases, which demonstrates the size-selective permeability of the model.[77] Alterations in permeability often correlate with BBB dysfunction, particularly in pathological states such as ischemic stroke, where endothelial permeability is markedly increased. Therefore, permeability assays that combine exogenous tracers with other functional measurements, such as TEER and immunostaining, can provide valuable insights into the mechanisms of BBB disruption in neurological disorders. For instance, TEER measurements, FITC-dextran permeability assays, and immunofluorescence staining have been widely used to evaluate the BBB integrity of *in vitro* ischemic stroke models.[88] However, assessing selective permeability based solely on non-permeable compounds is insufficient. A more robust evaluation requires testing a range of molecules with varying permeability characteristics and comparing the results to those obtained from Transwell systems or *in vivo* data. For example, Shi *et al.*[77] assessed BBB permeability using three compounds with distinct transport properties: (i) caffeine, a lipophilic small molecule that crosses the BBB via passive diffusion and carrier-mediated transport, (ii) cimetidine, a moderately permeable compound subject to efflux transporter activity, and (iii) adriamycin, a compound that does not cross the BBB. The permeability coefficients obtained from the *in vitro* model closely matched those observed *in vivo*, supporting the model's ability to replicate the physiological barrier properties of the human BBB.[77]

Furthermore, the BBB is characterized by high expression of various functional proteins and transporters that are critical for regulating the selective transport of nutrients and xenobiotics. These include P-gp, BCRP, multidrug resistance-associated proteins, glucose transporters (e.g., glucose transporter 1), and amino acid transporters.[89] These transporters collectively contribute to the homeostatic and protective functions of the BBB by facilitating the uptake of essential molecules and actively excluding potentially harmful compounds. For instance, P-gp plays a key role in limiting the distribution of many CNS drugs by actively effluxing them back into the bloodstream. In *in vitro* BBB models, the functional expression of P-gp has been validated using rhodamine

123—a known P-gp substrate—along with its inhibitor verapamil, which demonstrated the transporter's activity and regulatory capacity.[77] Additionally, Ito *et al*.[90] confirmed the presence and functionality of multiple BBB-specific transporters, including P-gp, BCRP, and other adenosine triphosphate-binding cassette transporters, in their *in vitro* model system. Their results emphasized not only the intercellular barrier properties but also the functional transporter activity necessary for maintaining BBB fidelity and physiological relevance.[90] These transporters are indispensable for preserving the functional integrity of the BBB and are essential parameters for evaluating the accuracy and utility of *in vitro* BBB models in drug screening and neurological research.

3.3. Characterization of the blood–brain barrier model integrating microfluidics and bioprinting

In vitro BBB models that integrate microfluidic technology with bioprinting offer advanced platforms for replicating physiologically and dynamically relevant neurovascular environments. The characterization of such models typically involves a combination of assessments, including cell viability and morphology, barrier permeability, junctional protein expression, receptor-mediated transport, and response to dynamic stimulation. For instance, in the study by Oh *et al*.,[69] cell viability and morphology of endothelial and glial cells within 3D-printed scaffolds were evaluated under flow conditions using viability dyes with confocal laser scanning microscopy.[69] Barrier permeability was commonly assessed by perfusing fluorescent dextrans of various molecular weights through the microchannels, such as 10 kDa Texas Red or 40 kDa FITC-dextran. Real-time monitoring and calculation of apparent permeability coefficients enabled quantification of barrier integrity within the 3D-printed BBB models.[25,69] Notably, Royse *et al*.[70] conducted permeability studies and ultrasound studies through FITC-dextran in a 3D-printed *in vitro* BBB model. Under continuous perfusion, FITC-dextran was used as a tracer while fluorescence imaging was conducted in parallel with ultrasound application. Fluorescence signals were collected through molecular channels in real time, and the permeability coefficient values were calculated to evaluate the effects of mechanical stimuli on barrier permeability.[70] In addition to permeability assessments, immunofluorescence staining of key proteins provided insights into the structural and functional fidelity of the models. Junctional markers such as ZO-1 and claudin 5 (tight junctions), vascular endothelial cadherin (adherens junctions), and efflux transporters, including P-gp and BCRP, were used to verify cell–cell connectivity and transporter expression. Transcytosis receptors, such as the transferrin receptor and low-density lipoprotein receptor-related protein 1, were also analyzed to confirm the presence of receptor-mediated uptake pathways vital for CNS drug delivery.[25,70] Collectively, these characterization strategies demonstrate that the performance and reliability of bioprinting-microfluidic integrated BBB models depend heavily on how well they recapitulate key structural and functional features of the *in vivo* BBB. Therefore, rigorous evaluation against these parameters is essential for validating the model's application in compound evaluation and neurovascular research.

4. Principles of blood–brain barrier chip design

Figure 3 shows the graphical abstract of BBB-on-a-chip construction and application. The development of a BBB microfluidic chip typically involves three key components: material and fabrication, cell sources, and physiological conditions simulation. From early BBB models based on static culture dishes to Transwell-based platforms and, ultimately, dynamic microfluidic chips, the reliability and physiological relevance of *in vitro* BBB models have progressively improved. Recent advances suggest that the integration of 3D bioprinting technology may represent the next frontier in organ-on-chip development, offering greater spatial control and enhanced tissue complexity.[91] Table 2 summarizes the applications of 3D bioprinting for developing organ-on-chip models. The application trajectory of BBB microfluidic chips has evolved over time. Initially, these models were primarily used for validating barrier properties and model fidelity. Subsequently, their use expanded into disease modeling, enabling mechanistic research of neurological disorders under more physiologically relevant conditions. At present, the most prominent application of BBB microfluidic chips is in compound evaluation, particularly for assessing compound permeability, transport mechanisms, and neurotoxicity. This progression highlights the growing utility of BBB-on-a-chip systems as versatile and powerful tools in both basic research and translational biomedical applications.

4.1. Materials and fabrication

The selection of materials for constructing BBB microfluidic chips must balance several critical criteria, including biocompatibility, chemical stability, optical transparency, and compatibility with cell culture. PDMS is the most widely used material due to its favorable properties, such as gas permeability, flexibility, ease of molding, and optical clarity. However, PDMS also presents several limitations, including incompatibility with non-polar organic solvents, high water vapor permeability, hydrophobic surface characteristics, and a strong tendency to adsorb biomolecules, which may interfere with experimental reproducibility and sensitivity.[92] Alternative

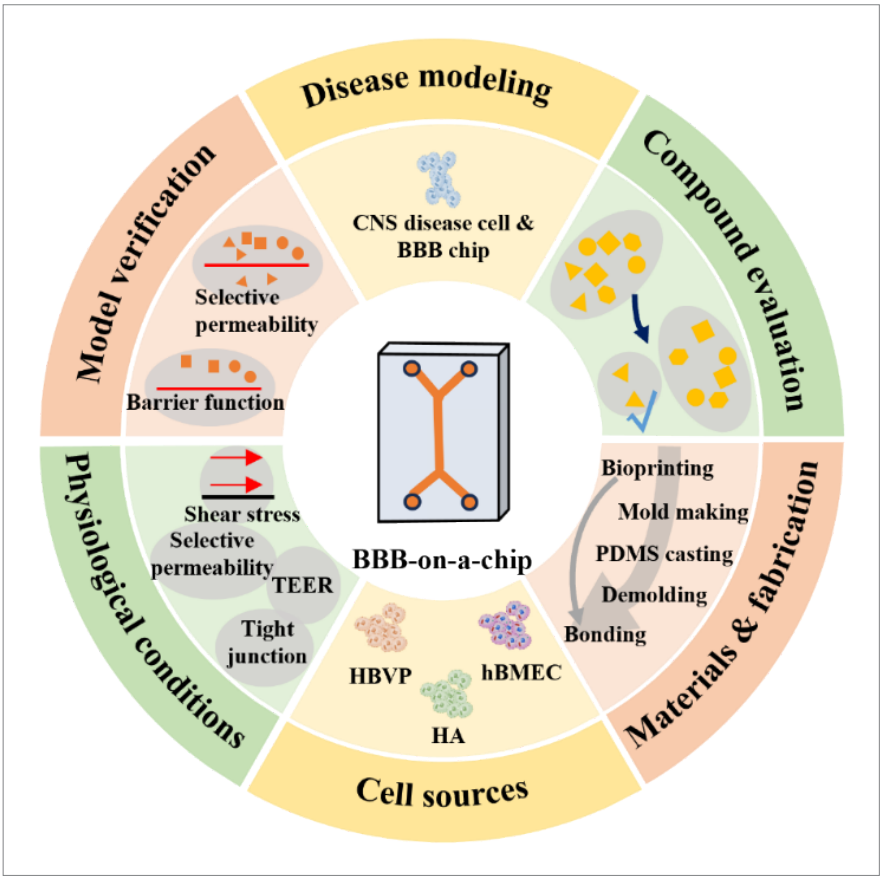

Figure 3. Graphical abstract of BBB-on-a-chip construction and application. The construction of a BBB-on-a-chip system involves three primary components: materials and fabrication, cell sources, and simulation of physiological conditions. The key application areas include model verification, disease modeling, and compound evaluation. Selected cellular elements shown in the figure were sourced or adapted from Servier Medical Art via the scifig.bio plugin (version 1.3.2). Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; HA, human astrocytes; hBMEC, human brain microvascular endothelial cells; HBVP, human brain vascular pericytes; PDMS, polydimethylsiloxane; TEER, transepithelial electrical resistance.

materials have been explored to overcome these limitations. Polymethylmethacrylate has gained attention for its rigidity, optical transparency, low refractive index, and minimal autofluorescence, making it suitable for optical imaging and analytical applications. Polycarbonate membranes are frequently employed as porous interfaces due to their permeability, enabling the simulation of cell–cell interactions and transport across barriers.[93] Polystyrene, a well-established material in traditional cell culture, offers high biocompatibility and supports robust cell adhesion and proliferation.[94] Additionally, fiber-based ECM membranes have shown promise in facilitating cell–cell communication and migration, making them ideal for studies focused on intercellular signaling and barrier remodeling.[95] Despite the availability of alternative materials, PDMS remains the predominant material used in BBB chip fabrication due to its superior mechanical and chemical processing characteristics. While polymethylmethacrylate has been employed in a limited number of studies, approximately 50% of BBB microfluidic models incorporate porous membranes made from various materials to physically separate fluidic channels and mimic basement membranes.[96]

Soft lithography is the most commonly used method for fabricating microfluidic chips, particularly when working with PDMS. This technique involves a series of steps, including photolithography to define microchannel features, mold fabrication, PDMS casting, demolding, and bonding of the PDMS structure to a substrate (e.g., glass or polymer). However, the tendency of PDMS to adsorb biomolecules and its relatively poor long-term structural stability present challenges for durability and reproducibility in extended culture systems.[97]

To address the limitations associated with traditional soft lithography and PDMS-based fabrication, researchers have

Table 2. Applications of three-dimensional bioprinting for developing organ-on-chip models

Organ/Tissue	Cells	Bioprinting technology	Bioink	Fabrication	Apparatus	Finding	Ref.
Blood–brain barrier for neurodegenerative diseases	Neuroblastoma (SH-SY5Y) and glioblastoma (U87) cell; human FPA	Syringe extrusion printhead on a Cellink BIOX 3D bioprinter	Mixtures of hydrogel and cells	Extrusion printing with a syringe printhead on a Cellink BIOX 3D bioprinter	Modular hyaluronan-based hydrogel system	Enhanced interaction of FPA with hyaluronan-based gels	[101]
Blood–brain barrier	Mouse brain endothelial cells (bEnd.3)	Co-printed resins using the EDEN 260V 3D printing system	Vero White PlusFullCure 835 resin, PVA	3D-printed frame, insert microneedles, apply collagen mixture in the square opening, remove microneedles, seed cells in the collagen channel	Engineered brain microvessel array	The disruption of the barrier function of the model and its functional recovery demonstrated its effectiveness	[102]
Blood–brain barrier	Outer: astrocytes (C8-D1A), inner: pericytes (MBVP), endothelial cells (bEnd.3), mouse hippocampal neurons (HT22)	Coaxial extrusion bioprinting with the inner and outer layer nozzle (EFL-BP-6602)	GelMA, GMHA, and LAP, photocurable bioink	Layer-by-layer deposition, followed by UV-induced cross-linking of the photocurable components	Hollow vessel with inner and outer layers	The device exhibits perfusion capacity and barrier function, making it a promising platform for compound evaluation	[103]
Cardiac for thrombosis research	HUVECs, human dermal fibroblasts (CRL-2522, ATCC)	Hydrogel printing, bioprinter (BioBots, Inc.) equipped with a 27G blunt needle	Alginate/GelMA	Bioprinting channels and walls, printing the GelMA scaffold, curing with UV, dissolving the sacrificial layer, and obtaining the final GelMA block	The final construct with hollow channels	A powerful model for studying thrombosis, thrombolysis, and fibrosis, as well as the interactions among different components. It may be extended to other vascularized models	[105]

Abbreviations: BCECs, brain capillary-like endothelial cells; FPA, fetal primary astrocytes; GelMA, gelatin methacrylate; hiPSCs, human induced pluripotent stem cells; HUVECs, human umbilical vein endothelial cells; LAP, lithium phenyl-2,4,6-trimethylbenzoylphosphinate; PDMS, polydimethylsiloxane; PEGDA, poly(ethylene glycol) diacrylate; PVA, poly-vinyl alcohol; UV, ultraviolet

doi: 10.36922/IJB025270265

explored alternative techniques such as laser engraving and 3D printing. Laser engraving involves directing a laser beam onto the material surface to selectively ablate regions and create microfluidic channels. This method is particularly effective for post-processing tasks, such as punching access ports or removing excess material.[98] However, the high cost of laser engraving equipment and the risk of thermal damage to heat-sensitive substrates present notable challenges. The integration of 3D printing technology with microfluidic platform design has opened new avenues for simulating complex biological environments, including multi-organ interactions. This advancement significantly enhances the applicability of microfluidic models in compound evaluation and translational research.[99] For instance, Cenhrang *et al.*[100] incorporated biologically relevant ECM materials, such as collagen, into 3D-printed microfluidic devices to generate high-barrier-function BBB models. The integration of ECM materials with microfluidics provided a physiologically relevant matrix environment, supporting cell–matrix interactions and mimicking the native BBB structure more effectively.[100] Moreover, 3D printing offers substantial advantages in the rapid prototyping and customization of chip components, making it a powerful tool in the construction of BBB-on-a-chip systems. Its flexibility, cost-effectiveness, and ability to fabricate complex geometries position it as a promising approach for next-generation *in vitro* BBB modeling.

In bioprinting, material selection is a critical factor, particularly the choice of bioink. Customizing ECM-mimicking hydrogels that are compatible with astrocytes enables additive biofabrication of advanced CNS tissues and disease models.[101] For example, Matthiesen *et al.*[101] developed a modular hydrogel system based on hyaluronic acid using 3D bioprinting to study interactions among neuroblastoma (SHSY5Y), glioblastoma (U87) cell lines, and human fetal primary astrocytes. They found that bioprinting enhanced astrocyte interactions with the hyaluronan-based matrix, thereby facilitating the development of physiologically relevant CNS disease models (Table 2).[101] Similarly, Kim *et al.*[102] utilized microneedles and 3D-printed frameworks to construct a functional *in vitro* brain microvascular hydrogel model. Mouse brain endothelial cells (bEnd.3) were seeded onto the luminal surface of cylindrical collagen microchannels. This model successfully demonstrated barrier function, which could be disrupted by hypertonic mannitol and subsequently restored—highlighting its suitability for simulating both normal and pathological BBB conditions (Table 2).[102] Wang *et al.*[103] introduced a coaxial extrusion bioprinting technique to fabricate triple-layered tubular blood vessels that mimic the BBB structure. In their model, photopolymerizable bioink was used to form the inner and outer vessel walls, with endothelial cells seeded between two layers. Following crosslinking and removal of uncured materials, the resulting hollow vessel was perfused with culture medium. Functional testing revealed that the bioprinting vessels allowed the passage of small neuroprotective molecules while restricting larger compounds, effectively replicating the selective permeability of the physiological BBB (Table 2).[103] These examples underscore the ability of 3D bioprinting to generate hollow, cell-lined channels with capillary-like properties, including layer-specific architecture, fluid perfusion, and molecular selectivity. Collectively, these advances demonstrate that 3D-bioprinting constructs can successfully recapitulate key structural and functional features of the BBB, such as multi-layered cellular organization, flow dynamics, and selective permeability, which makes them valuable platforms for compound screening and neuropharmacological research.[68]

Bioprinting tissues can be seamlessly integrated into microfluidic organ-on-a-chip platforms to more accurately simulate the dynamic physiological environment of living organs. Microfluidic chips offer precise control over fluid flow and shear stress, which are critical for promoting vascular maturation and enhancing BBB functionality. Notably, shear stress has been shown to strengthen tight junction formation between endothelial cells, thereby improving barrier integrity.[104] One practical approach involves directly bioprinting the tissue construct and coupling it with a perfusion system for dynamic cell culture. For example, Dona *et al.*[25] utilized digital light processing-based 3D bioprinting to fabricate a human brain microvascular model surrounded by embedded astrocytes. Using computer-aided design software, they designed a microvascular scaffold composed of crosslinkable photosensitive bioinks, including polyethylene glycol diacrylate and gelatin methacrylate. The successful formation of perfusable channels was verified by infusing red dye. Human BMECs were then seeded into the channels to achieve endothelialization, and the construct was enclosed with a PDMS cap and cultured under gradually increasing flow rates via perfusion.[25] In another example, Zhang *et al.*[105] applied bioprinting technology to precisely position both cells and matrix materials within a prefabricated microfluidic chip (Table 2).[105] They developed a sacrificial bioprinting method to produce vascularized hydrogels: Pluronic F127 was printed as a sacrificial template and dried before being transferred onto a PDMS substrate. The template was then embedded in gelatin methacrylate, which was crosslinked under ultraviolet light. Following the dissolution of the sacrificial material, a hollow channel network remained. Human umbilical vein endothelial cells were seeded into

these channels and cultured under perfusion to establish a functional endothelialized microvasculature. While this specific model was heart-related, the same fabrication principle can be extended to create bioprinted-BBB systems by embedding NVUs within perfusable scaffolds. Overall, the integration of bioprinting tissues with microfluidic platforms, known as "bioprinting organ-on-a-chip" systems, enables physiological fluid flow, real-time monitoring, and high-throughput experimental capabilities. For BBB modeling, such systems allow the construction of multicellular, flow-responsive barrier structures that closely mimic the architecture and function of the NVU. These next-generation platforms hold significant promise for applications in CNS disease modeling, compound permeability testing, and neuropharmacological compound evaluation.[25,104]

4.2. Cell sources

The choice of cell source is a critical determinant of the physiological relevance, functionality, and reproducibility of *in vitro* BBB models. Among the available options, primary cells offer high fidelity to native tissue, as they retain key phenotypic characteristics, differentiation status, and metabolic activity. Moreover, primary human cells pose a relatively low risk of oncogenic transformation. In dynamic microfluidic cultures, primary cells have been shown to maintain functional activity for several days to over a week, underscoring their growing importance in advanced *in vitro* BBB models.[106]

Immortalized cell lines offer practical advantages such as ease of acquisition, simple handling, and high proliferative capacity, enabling repeated passaging and high-throughput applications. Although they may not fully replicate the properties of native cells, immortalized cell-based BBB models are widely used for evaluating compound permeability and performing large-scale screening assays. For instance, Yang *et al.*[107] co-cultured immortalized human cerebral microvascular endothelial cells (CMECs) with pericytes and astrocytes to assess the permeability of 10 pharmacological agents, including caffeine, carbamazepine, and desipramine. Their results revealed that permeability trends in the *in vitro* model closely mirrored those observed *in vivo*, supporting the model's utility for drug evaluation.[107] Immortalized cell lines are also instrumental in studying the mechanisms underlying neuroimmune responses and neurodegenerative diseases. Ohbuchi *et al.*[72] demonstrated that brain endothelial cells formed microvascular-like structures *in vitro*, while pericytes enhanced barrier integrity. This system effectively modeled BBB dysfunction and immune cell infiltration, providing insights into the pathophysiology of CNS disorders.[72]

hiPSCs, which can differentiate into various cell types, are increasingly utilized in BBB models for disease modeling and compound evaluation, particularly in the context of rare or patient-specific neurological disorders.[96] For example, Ferreira *et al.*[108] developed BBB models using hiPSCs derived from patients with neurodegenerative diseases to enhance understanding of disease mechanisms and support the development of improved therapeutic strategies.[108] Although the combination of hiPSC-derived endothelial cells, pericytes, and astrocytes within fibrin hydrogels has enabled the construction of vascularized chip models, challenges remain. Notably, in some 3D configurations, endothelial cells fail to integrate fully into the hydrogel scaffold, thereby limiting the acquisition of key BBB-like properties such as tight junction formation and selective permeability.[109] In recent years, significant progress has been made in developing human *in vitro* BBB models. This includes the differentiation of NVU cell types from stem cells, improved replication of the native ECM environment, and the application of BBB-on-a-chip platforms to better mimic *in vivo* physiology and function.[108] In summary, hiPSC-based BBB models offer a personalized and scalable approach for investigating individual differences in BBB characteristics, disease phenotypes, and drug responses, thereby holding great promise for both basic research and precision medicine applications.

Another common strategy for constructing physiologically relevant BBB models involves the use of multiple cell types, including a combination of primary cells and hiPSCs. This hybrid approach enables the preservation of specific functional properties inherent to primary cells while leveraging the scalability and patient specificity offered by hiPSC-derived components, thereby enhancing the biological realism and applicability of the model. For example, Noorani *et al.*[84] demonstrated that essential BBB characteristics could be faithfully reproduced by co-culturing hiPSC-derived BMECs with primary human pericytes and astrocytes within a microfluidic chip platform, such as low paracellular permeability, active efflux transporter function, and appropriate osmotic responsiveness.[84] This integrated BBB-on-a-chip model effectively mimicked the structural and functional properties of the human NVU. Such composite models, combining the strengths of both cell sources, not only provide a more physiologically accurate platform but also serve as valuable tools for therapeutic screening and mechanistic studies of brain diseases. As shown in recent work, these hybrid BBB models offer insights into disease pathophysiology and compound transport, supporting their use in preclinical evaluation and personalized medicine.[110]

4.3. Simulation of physiological conditions

The validity of *in vitro* BBB modeling depends on its ability to replicate the physiological structure and dynamic microenvironment of the *in vivo* BBB. Effective BBB models must accurately mimic local microenvironmental conditions, support cellular communication, and simulate organ interactions.[59] BBB microfluidic chips were proven effective in compound screening by replicating endothelial glycocalyx structure and dynamics, which provided effective protection to endothelial cells under shear stress and enhanced transendothelial transport efficiency.[111]

Fluid shear stress is a critical biomechanical regulator of BBB function. It plays a key role in inducing the expression of tight junction proteins by BMECs, thereby enhancing the barrier's selective permeability. This selectivity allows lipid-soluble molecules, such as theophylline, to accumulate outside the lumen, while restricting the passage of hydrophilic or larger compounds like morphine and sucrose, thus preserving BBB integrity.[112] Cucullo *et al.*[113] demonstrated that human BMECs cultured under dynamic flow conditions exhibited permeability profiles closely resembling those of the *in vivo* BBB, even in the absence of astrocytes. Their model achieved a high correlation coefficient (r = 0.93) with *in vivo* data, underscoring the importance of shear stress in maintaining BBB-like function.[113] Physiologically, fluid shear stress is generated by blood flow within cerebral vessels, acting directly on the endothelial surface and subsequently influencing surrounding neurons and glial cells. In the human cerebral vasculature, shear stress ranges from 10–70 dyn/cm^2 in arteries and 2.8–95.5 dyn/cm^2 in capillaries, with an average capillary shear stress typically between 5 and 23 dyn/cm^2.[114] However, several *in vitro* models fall short of reproducing these physiological flow conditions. For instance, models developed by Chim *et al.*[110] and Griep *et al.*[115] produced shear stress values ranging only from 0.01 to 0.06 dyn/cm^2, which are considerably lower than those observed *in vivo* and may be insufficient to induce appropriate endothelial responses.[110,115] Notably, Ceccarelli *et al.*[116] addressed this limitation by employing a dual syringe pump system to drive fluid through a BBB microfluidic chip at a flow rate of 40 μL/min. This setup generated shear stress values of approximately 11–13 dyn/cm^2, successfully replicating physiologically relevant conditions and supporting the development of a more accurate and functional *in vitro* BBB model.[116]

5. Application of blood–brain barrier chips in compound evaluation

In vitro biomimetic BBB chip models offer several significant advantages for compound evaluation and drug development. These models closely replicate the physiological structure and functional characteristics of the native BBB, including selective permeability, active transport of nutrients and neurotransmitters, and barrier responses to various stimuli.[81] This physiological relevance makes BBB chips well-suited for evaluating drug candidates and other bioactive compounds under both normal and pathological conditions. One of the major strengths of microfluidic BBB chips is the ability to dynamically control the cellular microenvironment. Parameters such as shear stress, flow rate, and biochemical gradients can be precisely modulated, enabling more accurate simulation of *in vivo* conditions and allowing compounds to be tested under disease-specific or stress-inducing settings. Importantly, BBB chip models can reduce reliance on traditional animal models, thereby aligning with the principles of the 3Rs (Replacement, Reduction, and Refinement) in ethical research. By minimizing animal usage and circumventing inter-individual variability inherent to animal studies, these models improve data reproducibility while addressing ethical concerns associated with *in vivo* experimentation.[117] In addition, microfluidic platforms support parallelized culture conditions and real-time monitoring, enabling high-throughput screening of a large number of compounds during the early stages of drug discovery. This capability significantly reduces the time and cost associated with conventional drug development pipelines.[118,119] Furthermore, the high degree of standardization and environmental control afforded by BBB chip models reduces the confounding influence of systemic metabolic variability seen in whole animal studies. As a result, these platforms are particularly valuable for mechanistic studies and basic research aimed at elucidating BBB transport, barrier integrity, and cellular interactions under defined conditions.[120] Table 3 summarizes the application of BBB microfluidic chips in compound evaluation.

In vitro biomimetic BBB chips have emerged as a rational and versatile platform for investigating the effects of hazardous compounds on the CNS (Table 3). By integrating microfluidic technologies with advanced cell culture techniques, these chips enable the replication of key structural, functional, and mechanical characteristics of the *in vivo* BBB. For example, Xu *et al.*[121] utilized a microfluidic-based BBB chip to culture cells and tissues in a dynamic environment that closely mimicked the native NVU, enabling reliable assessments of compound permeability, toxicity, and therapeutic efficacy.[121] This platform has proven particularly valuable in preclinical drug development, safety testing, and evaluating the risk of clinical trial failure. Recent studies have demonstrated the effectiveness of BBB chip models in elucidating toxicity mechanisms and supporting compound evaluation.

Table 3. Application of blood–brain barrier microfluidic chips in compound evaluation

Compound name	Compound usage	Chip materials	Chip characteristics	Cell sources	Evaluation parameters	Ref.
Methamphetamine	Neuroactive, used in drug and medicine production	PDMS	Three coupled chips simulate BBB inflow, brain parenchyma, and BBB outflow	Primary human BMECs, pericytes, astrocytes, neurons	BBB permeability increased; immunofluorescence micrographs of cells showed integrity; untargeted metabolomics showed metabolite production altered	[122]
Organic phosphate malathion	Neurotoxic effects, organophosphorus malathion	Two-lane OrganoPlate	3D microfluidic brain-on-a-chip model	Neurons and astrocytes derived from human iPSCs	Confocal imaging of lanes and immunofluorescence images of cells showed integrity; cell viability decreased	[123]
Organophosphate chlorpyrifos (CPF)	Neurotoxic effects, agricultural pesticides	PDMS, PET membrane	Two-chamber device made with three layers separated by a membrane	Primary human BMECs, astrocytes (SVG p12), pericytes	Combined with MS and electrochemical analysis; CPF and primary metabolites distribution; acetylcholine levels increased; microscopy images showed contracted cells	[124]
Citronellol	Neurotoxicity, used in cosmetics, perfumes, and furniture	Not reported	Two parallel microchannels separated by a porous membrane	Human BMECs, pericytes (HBVP), astrocytes (NHA)	Neurotoxic effects sustained; neurochemical alterations; permeation measurement of citronellol	[125]
Sunitinib	Model drugs	PDMS sheets and porous PC membrane	Microfluidic platform connecting two separate microchips	Human CMEC/D3, glioma cells U251	Determination of permeation by MS; cell viability decreased; structural and functional characterization by immunofluorescence imaging	[127]
Caffeine, cimetidine, and coxorubicin	Model drugs	PDMS, silicone sheets, and a sandwiched porous polycarbonate membrane	Three-layer model: lid layer, chamber layer, and media perfusion layer	Human iPSCs-derived brain endothelial cells (IMR90-4), rat primary astrocytes	Shear stress simulation; TEER value measurement; evaluation of permeability coefficients	[99]
Paclitaxel and carboplatin-loaded biomimetic hybrid liposomes	Drugs for the treatment of glioblastoma	Microfluidic devices integrated with 3D printing	Two different T-shaped devices from a 3D-printed, digital-light processing AsigaMax UV (Asiga, Alexandria, Australia) printer, PlasCLEAR resin, resolution of 0.0025 mm	Human glioblastoma U87-MG cells, human CMEC/D3	Hybrid liposomes increased *in vitro* uptake and BBB penetration ability of drugs; tumor cell viability decreased; drug-loaded biomimetic hybrid liposomes increase the efficacy of paclitaxel and carboplatin	[126]

(*Continue*)

doi: 10.36922/IJB025270265

Table 3. Application of blood–brain barrier microfluidic chips in compound evaluation

Compound name	Compound usage	Chip materials	Chip characteristics	Cell sources	Evaluation parameters	Ref.
Dexamethasone	Glucocorticosteroid (corticosteroid drug)	PDMS, porous absorbent paper tube	PDMS chips with siphon-driven flow control, flow rate limited by porous absorbent paper tube	Primary rat BECs, pericytes, astrocytes	Immunofluorescence images of cells showed integrity; relative gene expression altered; dextran dye leakage reduced; measurement of TEER value changed; inflammatory activation and treatment	[128]
Nanoparticles-gold nanorods	Gold nanorods-PEG-Ang2/D1 for β-amyloid fibrinolysis	PDMS	BBB organ-on-a-chip with resistive permeability sensor	Human CMEC/D3, hippocampal astrocytes, cerebral perivascular cells	Optical images of cells and fluorescence imaging of tight junction proteins showed integrity; permeability assays of model and compound; cell viability assay	[130]
Nanoparticles-PLGA polymers	Ferulic acid encapsulated PLGA nanoparticles	PDMS	Three parallel microchambers: a central main chamber and two side channels	Immortalized human CMEC/D3), pericytes, astrocytes	Permeability assays of model and compound; cellular internalization of nanoparticle	[131]
Ang2 functionalized lipid cubes loaded with cisplatin and temozolomide	Cisplatin and temozolomide delivery for glioblastoma	PDMS	Microfluid-based BBB-GBM model for drug permeability and cellular uptake	Human CMEC/D3, human glioblastoma U87 cell line	Physicochemical characterization and permeability coefficients measurements of the compound; TEER and transport ratio measurements of the model; confocal imaging of tight junction proteins showed integrity	[132]
Matrine, wogonin, osthole, paeoniflorin, resveratrol, and quercetin	Anti-glioma components in traditional Chinese medicine	PDMS	Chip with four parallel channels	Primary human BMECs, human astrocytes, and human brain vascular pericytes	Immunofluorescence staining of cells showed integrity; TEER values measurements and permeability assay of the chip	[77]

Abbreviations: 3D, three-dimensional; Ang2, angiopoietin-2; BBB, blood–brain barrier; BECs, brain endothelial cells; BMECs, brain microvascular endothelial cells; CMEC, cerebral microvascular endothelial cells; GBM, glioblastoma multiforme; iPSCs, induced pluripotent stem cells; MS, mass spectrometry; PC, polycarbonate; PDMS, polydimethylsiloxane; PEG, polyethylene glycol; PET, polyethylene terephthalate; PLGA, poly(lactic-co-glycolic acid); TEER, transepithelial electrical resistance.

doi: 10.36922/IJB025270265

Maoz *et al.*[122] successfully used a microfluidic BBB chip to simulate the penetration of the psychoactive drug methamphetamine, which comprised primary human BMECs, pericytes, astrocytes, and neurons. Their findings revealed that methamphetamine exposure increased BBB permeability and altered cellular metabolite profiles, highlighting its neurotoxic effects and providing mechanistic insight into drug transport across the NVU (Table 3).[122] Similarly, Liu *et al.*[123] employed a BBB chip model incorporating neurons and astrocytes derived from hiPSCs to assess the neurotoxicity of the organophosphate compound malathion. Confocal imaging, cell viability assays, and enzymatic activity measurements demonstrated a significant reduction in total acetylcholinesterase and butyrylcholinesterase activity, validating the model as a platform for neurotoxicity screening.[123] In addition, combining BBB-on-a-chip systems with targeted mass spectrometry and electrochemical analysis has enabled quantitative assessment of compound exposure. For example, this approach was used to study the neurovascular effects of organophosphate compounds, further supporting the application of BBB chips in toxicology testing (Table 3).[124] Furthermore, citronellol, as a fragrance compound commonly found in cosmetics and personal care products, was shown to cross the BBB and induce neurotoxic effects, including inflammation and apoptosis. This finding highlights the utility of BBB microfluidic models for evaluating consumer product safety and identifying potential CNS hazards.[125] Overall, BBB chip models represent a powerful tool for investigating compound-induced CNS toxicity. Their ability to recapitulate neurovascular physiology, support high-throughput testing, and provide mechanistic insights makes them invaluable for both basic research and translational applications in neurotoxicology.

Biomimetic BBB chips offer considerable advantages in the screening of biologically active compounds, particularly for research related to neurological diseases such as glioma and neuroinflammation (Table 3). These models replicate essential features of the human BBB, enabling precise evaluation of compound transport, efficacy, and barrier disruption in physiologically relevant contexts. The integration of 3D bioprinting technology has further simplified the fabrication of microfluidic BBB models. For instance, Arduino *et al.*[126] utilized 3D bioprinting to develop an *in vitro* BBB microfluidic model, which was used to assess the delivery of paclitaxel and carboplatin encapsulated in liposomes for glioblastoma treatment. The results demonstrated that the liposomal formulations successfully crossed the BBB while retaining therapeutic efficacy, underscoring the utility of the bioprinting platform for brain tumor drug screening (Table 3).[126] Shao *et al.*[127] developed a BBB chip by co-culturing human CMEC/D3 with glioma cells (U251) to study the BBB penetration of sunitinib, a model therapeutic compound. Using electrospray ionization quadrupole time-of-flight mass spectrometry, they quantified drug transport and observed that glioblastoma cells exhibited higher viability in the 3D culture compared to traditional 2D conditions, highlighting the physiological relevance of the microfluidic environment (Table 3).[127] In another study, Wang *et al.*[99] constructed a BBB chip using BMECs differentiated from hiPSCs and primary rat astrocytes. By monitoring TEER and shear stress, they confirmed the functional integrity of the model. Permeability assays for three model drugs (caffeine, cimetidine, and doxorubicin) demonstrated that their transport profiles closely matched *in vivo* data, validating the model for compound screening applications (Table 3).[99] Furthermore, a microfluidic BBB chip was developed by Yu *et al.*[128] to simulate neuroinflammation-induced barrier dysfunction. The model, composed of primary rat brain endothelial cells, pericytes, and astrocytes, showed high expression of cell-type-specific genes, confirming cellular maturity and *in vitro* differentiation. Upon induction of neuroinflammation and treatment with dexamethasone, the chip exhibited measurable therapeutic responses, demonstrating its applicability for validating biologically active compounds in neuroinflammatory disease contexts (Table 3).[128] Together, these studies illustrate the growing utility of BBB-on-a-chip platforms in translational neuroscience, offering a physiologically relevant, high-throughput approach to evaluate compound transport, neurotoxicity, and therapeutic potential in CNS-related diseases.

Based on recent *in vitro* BBB chip studies, nanoparticles have emerged as promising drug delivery vehicles capable of overcoming biological barriers and enabling efficient transport of therapeutic agents across the BBB.[129] These nanoformulations offer targeted delivery, improved drug bioavailability, and enhanced therapeutic outcomes, particularly for CNS disorders. As summarized in Table 3, Palma *et al.*[130] evaluated the permeability of peptide-functionalized gold nanorods using a BBB organ-on-a-chip platform. Their study demonstrated that gold nanorods were able to cross the BBB and degrade β-amyloid aggregates, highlighting their potential for the treatment of AD.[130] In a separate study, ferulic acid-loaded poly(lactic-co-glycolic acid) nanoparticles were shown to traverse the BBB and exert antioxidant effects, suggesting a novel therapeutic strategy for combating oxidative stress-related neurological disorders.[131] For glioblastoma therapy, Cai *et al.*[132] developed lipid-based nanoparticles functionalized with vascular endothelin ligand, angiopoietin-2, and co-loaded with cisplatin

and temozolomide. These nanoparticles successfully penetrated the BBB and enhanced drug accumulation within glioblastoma cells, demonstrating improved therapeutic efficacy compared to free drugs (Table 3).[132] Collectively, these findings underscore the value of BBB chip models in evaluating the transport behavior, targeting efficiency, and therapeutic potential of nanoparticle-based drug delivery systems. Such platforms not only facilitate mechanistic insights but also accelerate the development of advanced nanomedicines for CNS applications.

Natural compounds derived from plants, animals, and microorganisms remain a vital source for the discovery of new therapeutic agents. These substances are often associated with low toxicity and are particularly suitable for the treatment of mild or chronic diseases. However, the quality control of the compounds remains challenging due to variability in raw material sources and environmental factors, along with high production costs.[133] In recent years, there has been growing interest in the potential of traditional Chinese medicine and its active constituents for treating CNS disorders.[134–136] Karthika *et al.*[137] further synthesized evidence regarding the efficacy of various traditional Chinese medicine-derived compounds in the treatment of neurological diseases.[137,138] In this context, *in vitro* BBB chip models offer a valuable platform for elucidating the mechanisms of action of herbal medicines and bridging the gap between modern pharmacology and traditional herbal therapies.[139] For example, Shi *et al.*[77] developed a BBB-glioma microfluidic chip (referred to as the BBB-U251 chip) using a co-culture of primary human BMECs, pericytes, astrocytes, and glioma (U251) cells. The model was validated using immunofluorescence imaging, TEER measurements, and permeability assays with FITC-dextran and model drugs, demonstrating barrier properties closely resembling *in vivo* conditions. This platform was then employed to screen anti-glioma compounds derived from traditional Chinese medicine using high-performance liquid chromatography combined with ultraviolet detection. Active components, such as baicalein and naringenin, were identified for their antitumor properties. Notably, the study also revealed that the presence of an intact BBB significantly limited the effectiveness of these compounds on glioma cells, underscoring the importance of incorporating barrier functions into compound evaluation platforms (Table 3).[77] These findings highlight the utility of BBB microfluidic chip models in advancing the mechanistic understanding and pharmacological screening of traditional Chinese medicines. Such platforms are instrumental in identifying bioactive compounds, evaluating BBB permeability, and supporting the development of novel therapeutics derived from natural products.

6. Perspectives

6.1. Challenges for bioprinting and microfluidic integrated systems

The integration of 3D bioprinting with microfluidic technology offers great promise for developing physiologically relevant *in vitro* BBB models, but also presents a series of unique technical challenges. The shear stress serves as a double-edged sword. Moderate shear stress generated by perfusion enhances tight junction formation and improves BBB integrity,[140] whereas excessive shear stress during the bioprinting process can compromise cell viability, particularly in extrusion-based systems.[141] Nozzle design further complicates the balance between print resolution and cell safety.[142,143] Microfluidic integration introduces additional design complexities. Seamless bonding between the printed scaffold and microfluidic materials must be achieved to ensure sealed fluid flow and maintain optical transparency for real-time imaging. Under continuous perfusion, it is essential to preserve vascular lumen patency and ensure that printed structures can withstand the resulting fluidic pressures without collapsing or delaminating.

Despite the challenges mentioned above, recent advancements have led to the successful development of hybrid platforms that integrate 3D bioprinting with microfluidic technology, enabling the creation of increasingly complex BBB-on-a-chip systems. For example, Xu *et al.*[142] designed a sophisticated human NVU chip using 3D bioprinting to simulate the pediatric brain microenvironment.[144] The model's integrated, automated perfusion system mimics physiological blood flow, while specialized fluidic channels in the brain region facilitate comprehensive metabolite analysis. Another particularly promising area is the development of high-throughput screening platforms. 3D bioprinting offers a scalable and efficient manufacturing method for generating multiplexed vascularized models. For example, Ahmad *et al.*[143] proposed a bioprinting strategy that created six perfusable hollow channels embedded in hydrogel; this multi-channel design supports the parallel evaluation of multiple compounds or concentration gradients.[145] These innovations pave the way for customizable, scalable, and multifunctional *in vitro* brain models.

6.2. The future of biomimetic blood–brain barrier chips

The biomimetic BBB chip is poised to make substantial contributions to the future of compound evaluation and CNS drug development. With ongoing developments in biomaterials science, new ECM-mimicking materials are expected to emerge that better replicate the mechanical and biochemical cues of the native brain microenvironment.[146,147] These materials would enhance cell viability, differentiation,

and functionality, further improving the physiological relevance of *in vitro* BBB models.

Moreover, the integration of biomimetic BBB chips with emerging analytical and sensing technologies represents a critical frontier in next-generation model development. Recent studies have shown that combining metabolomic analysis with microfluidic organ-on-a-chip models enables detailed characterization of metabolic pathway shifts associated with neuroinflammatory damage and BBB integrity loss.[148] Collectively, the integration of biomimetic BBB chips with biosensing,[149] proteomic,[150] and metabolomic technologies is expected to uncover complex interaction networks at the neurovascular interface, delivering predictive insights into compound behavior.

The serial integration of BBB microfluidic chips with other organ-on-a-chip systems presents additional opportunities for comprehensive compound evaluation. For example, Koenig *et al.*[149] developed a closed microfluidic system that interconnected a hiPSC-derived BBB model with cortical brain and hepatospheroid models, all derived from the same donor.[151] Such interconnected systems enable researchers to study compounds in a more holistic context, mimicking systemic circulation and inter-organ interactions. By replicating physiologically relevant inter-organ communication, these platforms represent a promising step toward more predictive preclinical testing and personalized drug development strategies.

7. Conclusion

The biomimetic BBB microfluidic chip represents a powerful and promising platform for replicating the structure and function of the human BBB *in vitro*. Constructed using microfluidic technology, these chips co-culture key neurovascular cell types, such as BMECs, astrocytes, pericytes, and neurons, within a controlled environment that closely mimics the physiological microenvironment of cerebral vasculature and brain tissue. This system enables dynamic simulation of intercellular interactions, shear stress, and nutrient exchange, thereby providing an advanced and physiologically relevant tool for evaluating the permeability of candidate compounds, offering significant advantages over traditional static and animal-based models.[152,153] As a result, biomimetic BBB microfluidic chips hold significant potential for accelerating CNS drug development, reducing reliance on animal testing, and deepening our understanding of neurovascular pathophysiology.

Acknowledgments

Schematic illustrations of cells shown in the graphical abstract or figures were used from or adapted from pictures provided by Servier Medical Art based on the plugin scifig.bio (version 1.3.2), licensed under a Creative Commons Attribution 4.0 Unported License.

Funding

This work was supported by the Beijing Life Science Academy (2024400CB0060) and (2023600CA0080).

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Xiang Li, Yating Du
Funding acquisition: Xiang Li
Investigation: Yating Du, Zezhi Li
Supervision: Jianping Xie
Visualization: Yating Du
Writing–original draft: Yating Du
Writing–review & editing: Xiang Li

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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