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# Advances in Blood-Brain Barrier-on-a-Chip Models for Neurological Research and Therapeutics

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**ABSTRACT**

The blood-brain barrier (BBB) is a selectively permeable gate between the brain and the central nervous system. It is essential in preserving brain function, as it protects the brain from harmful substances while permitting necessary nutrients. Traditional in vitro and in vivo models have provided fundamental insights into BBB physiology and pathology, yet they often fall short in mimicking the dynamic, multicellular, and mechanical microenvironment of the human BBB. The development of blood-brain barrier-on-a-chip (BBB-oC) platforms represents a significant degree of progress within biomedical engineering and neurobiology, leading to more physiologically relevant modeling of the neurovascular unit. These microfluidic systems utilize human-derived endothelial cells, astrocytes, pericytes, and shear flow to simulate the BBB's structure and function with unprecedented fidelity.

This study provides a comprehensive analysis of recent developments in BBB-on-a-chip (BBB-oC) technologies, focusing on novel developments in microfluidic design, materials, cellular integration, and real-time monitoring techniques. Special significance is placed on the use of these platforms to model neurodegenerative diseases, particularly Alzheimer's disease, which is characterized by early and progressive BBB dysfunction. BBB-oC systems offer encouraging new avenues for drug screening, mechanistic studies, and personalized medicine applications. Current limitations, such as scalability, cost, and integration with systemic circulation models are addressed, and emerging solutions driven by artificial intelligence, organoid fusion, and high throughput screening technologies are discussed.

By bridging neuroscience, engineering, and disease modeling, BBB-oC platforms represent a powerful tool for translational research and precision therapeutics. This study consolidates key findings, identifies knowledge gaps, and outlines directions for future development in the field.

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## Introduction

Neurological disorders are the second most common cause of worldwide deaths, killing over 10 million people every year [1]. Therefore, it is crucial to understand the intricate mechanisms of the brain and its components, one of which is the blood brain barrier. The BBB acts as a filter between brain matter and the circulating blood, ensuring homeostasis. The BBB is primarily composed of brain microvascular endothelial cells (BMECs), connected by tight junctions. Supported by astrocytes, pericytes, and the extracellular matrix, the BBB ensures homeostasis of the central nervous system (CNS) by regulating the transport of ions, nutrients, waste products, and therapeutic agents [2]. However, the strict control of the BBB creates a challenge for drug delivery, particularly in treating brain metastases and CNS disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and epilepsy [3, 4].

Traditional models of the BBB, such as static in vitro transwell assays and in vivo rodent models, have provided essential insights into its physiology. However, these systems suffer from some critical limitations. Static in vitro models often lack key physiological factors such as shear stress, while interspecies differences in in vivo models limit their relevance to solving problems that affect the human population [5]. These limitations have driven the development of BBB-oC systems-engineered microfluidic platforms that imitate the structure and microenvironment of the human BBB. Using microfabrication techniques, BBB-oC systems integrate different cell types within their channels to mirror the traditional transport mechanisms and cellular crosstalk present in the human BBB [6, 7]. Further exposure to fluidic shear stress ensures their durability and barrier integrity [8, 9].

Recent findings in organ-on-a-chip technology have led to the creation of advanced BBB models that use human-induced pluripotent stem cell (hiPSC)-derived BMECs, primary astrocytes and pericytes, and 3D matrix components within perfusable systems [10, 11]. These platforms allow for real-time monitoring of transendothelial electrical resistance (TEER), imaging of tight junction proteins, and dynamic assays of drug permeability, all within a microscale environment that mimics human physiology. Notably, many systems now integrate sensors, microelectrode arrays, and optical readouts to measure functional and molecular responses in situ [12, 13].

The urgent need to increase the efficacy of BBB models is underscored by the various neurological diseases affecting individuals around the world. Alzheimer's disease alone affects over 55 million people worldwide-the 7th leading cause of global mortality-with numbers projected to rise sharply over the coming decades [14, 15]. Emerging evidence indicates that dysfunction

within the BBB is correlated with clinical symptoms and can contribute to disease progression in Alzheimer's disease and other neurodegenerative conditions [16, 17]. Therefore, accurate human-based BBB models are necessary to study pathophysiological events and testing therapeutics in the search for cures and mitigation.

In 2024 and 2025, numerous studies demonstrated novel uses of BBB-oC platforms in disease modeling, drug testing, and mechanistic studies. These include the integration of inflammatory cytokines to mimic neuroinflammation, patient-specific cells to model personalized responses, and coculture with brain organoids to simulate neurovascular interactions [18, 19]. Moreover, the field has seen an increased emphasis on scalability, automation, and integration with other organ-on-chip systems (e.g., gut-brain axis or liver-brain models), reflecting its potential for high-throughput drug discovery and systemic toxicity testing [20].

Despite the recent progresses, challenges remain. Difficulties with reproducibility, standardization, and long-term culture stability continue to limit greater adoption of this technique. Additionally, most current platforms do not yet capture the full heterogeneity of the BBB across different brain regions or developmental stages [21, 22]. There is also a need for better integration of immune cell dynamics, circadian rhythms, and sex differences —all of which are known to affect BBB and neurologic function.

This study provides an up-to-date synthesis of BBB-oC technologies, highlighting in microfluidic design, cellular composition, sensing, and disease modeling. Particular attention is placed on studies demonstrating how these systems are transforming research in neuroscience and pharmacology. The key scientific, technical, and translational challenges that must be addressed to fully utilize the prospects of BBB-oC platforms in the coming decade are detailed.

## Current Technologies in BBB-on-a-Chip Modeling

The construction of BBB-oC systems relies on the careful integration of microfluidic engineering, biomaterials science, and cellular biology. These platforms aim to emulate the structure and function of the human BBB, including its dynamic interactions between endothelial cells, astrocytes, pericytes, and the extracellular matrix (ECM). A successful model must also mimic the biomechanical forces —particularly shear stress from blood flow-that affect BBB behavior in vivo. In this section, the core components and strategies involved in creating functional BBB-oC systems are discussed.

### *Microfluidic Design*

At the heart of any organ-on-chip device is a microfluidic system that mimics physiological fluid flow. BBB microchips often utilize two or three channels, simulating the microvessels within the barrier, with a porous membrane separating endothelial cells from glial components. The upper channel commonly contains BMECs exposed to shear stress via continuous fluid flow, while the lower channel houses astrocytes and pericytes in static or semi-dynamic conditions [6, 23].

Recent designs have moved beyond basic two-dimensional configurations to apply 3D tubular microvessels lined with endothelial cells embedded in hydrogel molds mimicking the ECM. These vessels allow radial flow, similar to in vivo conditions, and preserve tight junction morphology [11]. Computational modeling is increasingly utilized to optimize channel geometry and flow rates to ensure the presence of physiological shear stress (~1–4 dyn/cm$^2$), which is critical for tight junction integrity and gene expression, therefore enhancing the accuracy of the microvessel model [24].

### *Biomaterials and Membranes*

The choice of materials used in BBB-oC fabrication affects cell behavior, mechanical properties, and visibility. Polydimethylsiloxane (PDMS) remains one of the most widely used polymers due to its biocompatibility, gas permeability, and transparency [25]. However, drug permeability data can be skewed by the absorption of small hydrophobic molecules by PDMS. Alternatives such as cyclic olefin polymers (COP), thermoplastics, or hydrogels like gelatin methacrylate (GelMA) are being increasingly investigated [26, 27].

Porous membranes, which are typically made of polycarbonate or polyester, can act as semipermeable barriers between vascular and abluminal compartments. The optimal pore size (0.4 - 3 μm), thickness, and surface chemistry are tailored to facilitate cell to cell communication and nutrient diffusion while minimizing unintentional leakage [28]. Some devices now utilize ECM-derived membranes or electrospun nanofibers to provide more physiologically relevant substrates for cell growth.

### *Cell Sources and Co-culture Strategies*

One of the major strengths of BBB-oC systems is the ability to use human-derived cells to model species-specific properties of the BBB. Human-induced pluripotent stem cell (hiPSC) derived BMECs have emerged as one of the strongest methods for endothelial modeling due to their reproducible expression of tight junction proteins (claudin-5, occludin, ZO-1), transporters (P-gp, GLUT1), and functional barrier properties [10, 29].

Astrocytes and pericytes can be co-cultured in the abluminal compartment, often embedded in a hydrogel such as collagen I or Matrigel. This tri-culture system more closely mimics the neurovascular unit and supports the development of high TEER values, ranging from 200 to over 1,000 Ω·cm² based on the platform [12, 13, 22]. Some models further utilize microglia, neurons, or oligodendrocytes to analyze broader CNS interactions [13, 30].

Jamieson et al. [31] compared abluminal pericyte localization, direct pericyte-BMEC contact, and physiologically-low Lucifer yellow permeability to BMEC microvessels and showed the increase of suboptimal TEER in stressed monolayers by co-culturing with hiPSC-derived pericytes or conditioned media [31]. Their novel development of a BBB microvessel model with exclusively hiPSC-derived BMECs and pericytes assessed human vascular dysfunction in the CNS, which further added to the progress in co-culture strategies.

To achieve patient-specific modeling, recent systems use hiPSC-derived glial and endothelial cells from individuals with neurological diseases such as Alzheimer's or Huntington's disease. The study of inter-individual variability in BBB function and drug response are possible due to the retention of disease-relevant phenotypes in these cells [32, 33, 34].

### *Functional Assays and Sensing*

Ensuring that the BBB in chip models functions effectively is reliant on several key measurement parameters. TEER remains the gold standard for quantifying barrier integrity and is generally measured using embedded microelectrodes. Breakthroughs in electrode design now allow continuous, noninvasive monitoring of TEER over days to weeks with minimal drift [35].

Complementary information about solute transport is provided by paracellular permeability assays using fluorescent tracers like FITC-dextran (4 - 70 kDa). Additionally, labeled therapeutic molecules such as antibodies and small-molecule drugs help quantify transcytosis and efflux across the BBB. Immunostaining for tight junction proteins, efflux pumps, and adhesion molecules provides structural validation [12, 36].

Emerging systems now implement biosensors, optogenetics, and live imaging platforms to track dynamic cellular responses. Real-time measurements of oxygen tension, glucose uptake, or cytokine secretion allow for precise monitoring of barrier responses to inflammatory stimuli or therapeutic interventions [20].

***Automation and Standardization***

Scalability remains a key barrier to the widespread adoption of BBB-oC platforms. Efforts are currently underway to develop standardized, multiplexed platforms (containing multiple inputs) compatible with 96- or 384-well formats for high-throughput screening. Commercial systems such as the OrganoPlate® (MIMETAS), SynVivo, and Emulate's Brain-Chip™ provide modular solutions for academic and industrial research, each with varying levels of complexity and automation [37].

Recent studies highlight the importance of standardizing media composition, flow rates, seeding protocols, and TEER calibration to improve reproducibility across labs [27]. Artificial intelligence (AI) and machine learning (ML) tools are increasingly being used in the optimization of experimental parameters and interpretation of large datasets generated from multiplexed chips [38].

***Alzheimer's Disease Modeling Using BBB-on-a-Chip Platforms***

Alzheimer's disease (AD), the most common neurodegenerative disorder, is expected to affect twice the number of patients by 2050 [14]. AD pathology is defined by the accumulation of amyloid-beta (Aβ) plaques, tau neurofibrillary tangles, neuroinflammation, and widespread neuronal loss. However, emerging evidence suggests that BBB dysfunction is not just a consequence but an early driver of disease pathogenesis [16, 17]. BBB disruption in AD can exacerbate neurodegeneration by facilitating the entry of neurotoxic substances, impairing nutrient transport, and disturbing immune homeostasis. Therefore, accurate modeling of the BBB in the context of AD is essential for understanding disease mechanisms and evaluating therapeutic strategies.

***Pathophysiological Insights from BBB-on-a-Chip Models***

Traditional transgenic rodent models of AD often fail to replicate the human-specific aspects of BBB physiology and neurovascular unit (NVU) interactions. In contrast, BBB-oC systems allow for the controlled modeling of Amyloid beta (Aβ) exposure, tau pathology, and cytokine-induced neuroinflammation in a human-relevant microenvironment. These platforms integrate human endothelial cells, astrocytes, and pericytes under fluid flow to simulate BBB structure and function, facilitating real-time monitoring of barrier integrity and cell responses.

Grifno et al. [39] analyzed the key challenges with BBB models: cell source, cryopreservation, barrier function, and matrix stiffness. Their study with fluorescently labeled hiPSCs showed physical permeability of Lucifer yellow over six days, and their microvessels formed in collagen I hydrogels matched the stiffness of the human brain, thus demonstrating the advancing capabilities of tissue-engineered BBB models [39].

The human organ-on-a-chip BBB model developed by Ahn at al. [40] characterizes the physiologically relevant structures and functions of the BBB [40]. Their microfluidic parallelization technology integrating multiple devices allowed for TEER measurement, nanoparticle sampling, and fluorescent-activated cell sorting (FACS) analysis, thus presenting a new approach to 3D mapping of the BBB.

Linville et al. [41] studied the application of iBMECs in 3D microvessels to enable visualization and analysis of tight junction dynamics during homeostasis and wound repair, including increasing angiogenic and cytokine response, and provided new insights into how brain microvascular endothelial cells respond to mitosis and apoptosis events [41].

More recent studies have used BBB-oC systems to simulate Aβ-induced endothelial dysfunction. For example, Uzoechi et al. [42] reported that when $A\beta_{1-42}$ oligomers are applied to the luminal side of a tri-culture BBB chip they caused a significant accumulation of $A\beta_{1-42}$ oligomers at the BBB, disruption of the tight junctions, and subsequent leakage, mimicking early BBB impairment seen in AD patients. These findings validate the utility of BBB chips in demonstrating and better understanding the causes behind early neurovascular dysfunction.

***Personalized Modeling with Patient-Derived Cells***

One of the most drastic leaps in BBB-oC technology is the use of patient-specific hiPSC-derived cells. In a 2024 study by Ding et al. [43] endothelial cells and astrocytes were derived from AD patients with known apolipoprotein E (APOE4) genotypes—a major genetic risk factor for sporadic AD. These cells demonstrated reduced tight junction protein expression (e.g., ZO-1, claudin-5), increased oxidative stress, and impaired Aβ clearance compared to APOE3 controls. The use of such models allows researchers to understand how individual genetic backgrounds affect BBB behavior and drug response.

Another study by Raut et al. [44] used hiPSC-derived BMECs from familial AD patients to inspect barrier properties and BMEC metabolism. They discovered that the mutant Presenilin 1 (PSEN1) gene had a more impaired barrier function than PSEN2, while both showed an increase in radical oxygen species (ROS) production, pointing to a critical role of genetic mutations in AD-associated vascular pathology [44].

***Therapeutic Screening and Drug Delivery***

BBB-oC models provide a powerful tool for evaluating the permeability of compounds such as targeted drugs into the CNS, therefore demonstrating the efficacy of AD therapeutics. Traditional animal models frequently overestimate BBB permeability due to differences in efflux transporter expression and vascular structure. In contrast, human BBB-oC systems allow more reliable predictive insights into drug delivery challenges.

A 2024 study evaluated delivery of peptides and antibodies across a BBB chip using healthy endothelial cells under flow and hypoxia, demonstrating selective transcytosis [45]. Exposure to $A\beta_{1-42}$ oligomers also disrupted barrier integrity in high-throughput chip systems—supporting the platform's relevance for modeling disease-state barrier dysfunction and screening combination therapies [42].

Beyond antibody therapies, several teams have tested small molecules, nanoparticles, and exosome-based delivery systems

on BBB chips. For example, Yang et al. [46] showed that curcumin-loaded nanoparticles modified with BBB-penetrating ligands could efficiently cross the chip-based BBB and attenuate Aβ-induced inflammation in astrocytes. These data support the role of BBB-oC systems as translational platforms for nanomedicine optimization [46].

***Modeling Neuroinflammation and Immune Crosstalk***

Neuroinflammation has increasingly been recognized as a central feature of AD pathogenesis, and BBB-oC models are well-suited for studying immune-endothelial interactions. By integrating microglia or peripheral immune cells into the abluminal or luminal compartments, researchers can determine how the BBB regulates immune trafficking and cytokine signaling.

Recent studies have shown that the NVU can be modified during AD with functional changes that cause dysfunction. Migrating immune system cerebral vessel cells may act together with the modified BBB. Blocking the adhesion mechanism can inhibit Aβ deposition and tau hyperphosphorylation and reduce loss in memory. Models that can simulate vascular inflammation and leukocyte movement can lead to therapeutic delivery systems. [47]

***Biomarker Discovery and Mechanistic Studies***

BBB-oC systems are increasingly used to identify possible biomarkers of early AD pathology. Transcriptomic profiling of chip-cultured endothelial cells exposed to Aβ demonstrated upregulation of genes associated with mitochondrial dysfunction, autophagy, and senescence [33]. Moreover, metabolomic analysis of chip effluents has identified diseasespecific changes in glucose, lactate, and lipid metabolites that correlate with disease severity.

Mechanistically, BBB chips allow researchers to dissect the sequence of pathological events in AD. For instance, Campisi et al. [11] used confocal imaging and molecular assays to show that Aβ exposure first disturbs mitochondrial dynamics in endothelial cells, followed by tight junction disassembly and oxidative stress. As this form of temporal resolution is difficult to achieve in conventional models, their work may result in new therapeutic timing strategies [11, 48].

**Applications and Translational Impact**

BBB-oC systems have risen not only as a powerful research tool for basic science but also as a promising platform for translational and preclinical applications. Their capacity to mimic the NVU with high fidelity positions this technology at the forefront of drug development, toxicology testing, disease modeling, and personalized medicine. In this section, the multifaceted applications of BBB-oC technologies and their growing role in bridging the translational gap between bench and bedside are discussed.

**Drug Discovery and Development**

Delivering drugs across the BBB remains to be one of the key challenges in neurotherapeutics. Only 2% of small molecules and practically none of the large biologics are able to effectively cross the BBB, causing high attrition rates in CNS drug development [49]. BBB-oC platforms provide a human-relevant testing ground for evaluating the permeability, retention, and efficacy of investigational compounds before in vivo or clinical trials.

Studies have shown that drug permeability measured in BBB-oC devices more accurately predicts human pharmacokinetics than traditional Transwell or animal models [8, 10]. For instance, therapeutic agents such as mannitol (used for osmotic BBB disturbance), dexamethasone, or anti-amyloid antibodies have been tested on chips under shear stress conditions, revealing insights into transport kinetics, receptor-mediated transcytosis, and efflux transporter activity [50, 51, 52].

High-throughput compatible BBB-oC platforms, such as the OrganoPlate® and Brain-Chip™, enable the simultaneous screening of hundreds of candidate drugs, facilitating rapid decisions in pharmaceutical pipelines [37]. This scalability is vital for reducing cost and time in early-stage drug development.

***Toxicology and Safety Testing***

In addition to evaluating efficacy, BBB chips are increasingly being used for neurotoxicity and CNS safety profiling. CNS toxicity accounts for a substantial percentage of drug withdrawals post-market, often due to unforeseen interactions with and at the BBB [53]. By integrating human endothelial cells with astrocytes and pericytes, BBB-oC systems can detect subtle perturbations in barrier function, such as decreased transendothelial electrical resistance (TEER), cytokine release, or apoptosis, in response to toxic agents.

Vetter et al. [36] established that chemotherapeutic agents like methotrexate and cisplatin induce dose-dependent BBB breakdown, oxidative stress, and inflammation in chip-based models—mirroring neurocognitive side effects observed in patients [36]. These findings support the use of BBB-oC devices in preclinical safety assessments mandated by regulatory bodies [54].

Importantly, BBB chips can be co-cultured with brain organoids or integrated into multi-organ-on-chip platforms to assess systemic toxicity, enabling a more holistic evaluation of a compound's safety profile [20, 55].

***Modeling Rare and Complex Diseases***

Beyond Alzheimer's disease, BBB-oC systems are being used to investigate a range of CNS conditions where the BBB plays a central role, including epilepsy, multiple sclerosis, glioblastoma, and viral encephalitis. In epilepsy research, BBB dysfunction is thought to amplify seizure activity and drug resistance. Linville et al. [13] used a microfluidic BBB model to study how repeated stimulation alters tight junctions and endothelial ion transporters-an insight difficult to achieve in vivo [13].

In multiple sclerosis (MS), immune cell infiltration across a compromised BBB is a hallmark of disease progression. El-Taibany et al. [56] have used circulating T-cells or monocytes to study their adhesion, diapedesis, and cytokine-mediated activation in response to demyelinating conditions in their chip models [56]. These models also enable testing of MS therapeutics like natalizumab or fingolimod for their ability to block immune trafficking across the BBB.

Linville et al. [57] have studied the transformation of the BBB into a 'blood tumor barrier' (BTB) using iPSCs to provide insights into physical and chemical interactions during brain metastases from primary breast cancer [57]. They find the elevation of immune cell adhesion and endothelial turnover within the metastatic BTB, ultimately leading to microvessel degeneration and subsequent loss of barrier properties, thus presenting new insights into future therapeutics.

Moreover, BBB chips are being used to study cancer metastasis and brain tumor microenvironments. By co-culturing BBB components

with glioblastoma stem cells or metastatic breast cancer cells, researchers can investigate how tumors breach the BBB, secrete proangiogenic factors, or resist chemotherapy. This has led to new insights into matrix metalloproteinase (MMP) activation, tumor cell migration, and endothelial-to-mesenchymal transition [58, 59, 60].

***Personalized and Precision Medicine***

The advent of patient-derived hiPSC technologies has expanded the possibilities of BBB-oC systems for personalized medicine. These models allow for the study of how genetic, epigenetic, and environmental factors affect BBB function at the individual level.

For example, Appelt-Menzel et al. [61] analyzed human iPSCs and multipotent fetal neural stem cells (fNSCs) to study the effects of astrocytes, pericytes, and NSCs on endothelial cell function and gene expression, and identified in vivo-like tight junction networks [61].

Jagdeesan et al. [62] developed a protocol to create patient-specific BBB chips starting with iPSC-derived iBMECs resulting in mixed neural cultures containing differentiated neurons, neural progenitors, and astrocytes [62].

In Parkinson's disease (PD) studies, using patient-specific hiPSC-BMECs have shown increased permeability and decreased efflux capacity, which may alter drug response profiles [32]. Similarly, in rare neurodevelopmental disorders such as Huntington's disease or Rett syndrome, BBB chip models have captured disease-specific endothelial dysfunction that cannot be reproduced in generic cell lines [63, 64].

Personalized BBB-oC systems are also being considered for guiding therapeutic strategies in pediatric oncology. For instance, BBB chips seeded with cells from children with diffuse intrinsic pontine glioma (DIPG) have been used to determine delivery efficiency and resistance mechanisms of novel chemotherapeutics, making tailored treatment approaches possible [65].

***Regulatory and Industry Adoption***

With increasing validation, BBB-oC platforms are gaining recognition by pharmaceutical companies and regulatory bodies. The U.S. Food and Drug Administration (FDA) has expressed interest in integrating organ-on-a-chip models into preclinical drug evaluation frameworks, particularly for analyzing BBB penetration and neurotoxicity [66]. Industry consortia such as the Innovation and Quality (IQ) Consortium and the National Center for Advancing Translational Sciences (NCATS) Tissue Chip program continue to support collaborative efforts aimed at standardization, data sharing, and regulatory alignment [67].

As these platforms become more robust and reproducible, their inclusion in Investigational New Drug (IND) applications and early-phase clinical trial planning is likely to grow. This transition represents a critical step in reducing reliance on animal testing and improving the predictive accuracy of human drug responses.

**Challenges and Future Directions**

Despite remarkable progress, BBB-oC technology faces several scientific, technical, and translational challenges that must be addressed to realize its full potential. These challenges span issues of biological fidelity, standardization, scalability, and integration with other advanced technologies. In this section, the key limitations of current BBB chip models are considered and future directions to further the field are proposed.

***Biological Complexity and Fidelity***

A major challenge in BBB chip development is achieving a physiologically accurate recreation of the NVU. While many platforms include BMECs, astrocytes, and pericytes, they often fail to fully capture the structural and functional heterogeneity of the in vivo BBB.

For instance, BMECs derived from hiPSCs may not always express appropriate levels of tight junction proteins, solute transporters, or inflammatory markers without extensive optimization [10, 13]. Moreover, regional differences in BBB properties, such as those found in the hippocampus versus the cortex, have not yet been fully reproduced in vitro.

State-of-the-art models now aim to integrate oligodendrocytes, microglia, and even vascular smooth muscle cells to better replicate NVU complexity [68]. Incorporation of 3D geometries and hydrogel scaffolds mimicking the extracellular matrix (ECM) have improved cellular polarization and alignment, but further improvement is needed to accurately model the basement membrane structure and biomechanical properties of the BBB [69].

***Standardization and Reproducibility***

The diversity of chip designs, materials, and cellular sources has led to a lack of standardization across studies. This makes it difficult to compare results or assess reproducibility across different laboratories. For example, some chips use PDMS (polydimethylsiloxane), which can absorb small hydrophobic molecules and distort drug concentration profiles [70]. Others use thermoplastics or glass, each with its own advantages and limitations.

Moreover, variations in hiPSC differentiation protocols, endothelial cell sources, flow rates, and co-culture conditions can drastically affect barrier function and data interpretation [71]. There is an urgent need for consensus guidelines on quality control, functional assays (e.g., TEER thresholds, permeability coefficients), and validation criteria.

Organizations like the National Institute of Health (NIH) Tissue Chip Consortium and the Innovation and Quality Microphysiological Systems (IQ MPS) Affiliate are working to establish benchmarking standards, inter-laboratory reproducibility studies, and validation datasets that can lead to greater adoption in industry and regulatory environments [67].

***Integration with Other Organ Systems***

The BBB does not operate in isolation—it is affected by systemic physiology, including liver metabolism, kidney clearance, and immune surveillance. Current BBB-oC models rarely include these interactions, limiting their ability to predict in vivo pharmacokinetics or toxicity profiles.

Multi-organ-on-chip (MOC) platforms that connect BBB chips to liver, gut, or kidney modules via microfluidic circulation systems are now being developed to simulate inter-organ crosstalk. For example, a connected gut-BBB chip can be used to study how gut microbiota-derived metabolites impact BBB integrity and brain inflammation, a growing area of interest in Alzheimer's and Parkinson's disease research [35, 72].

However, maintaining the functional viability of multiple tissues under shared media and flow conditions remains a major engineering and biological challenge. Improvements in

microfluidic design, organ-specific media, and real-time sensing technologies are needed to further develop these integrated models [73].

***Technical and Operational Limitations***

While BBB-oC platforms provide a lot of advantages over traditional models, they still require specialized equipment, cleanroom environments, expertise, state-of-the-art imaging systems, and time-intensive protocols that limit their accessibility. Furthermore, chips have a limited lifespan, generally ranging from several days to a few weeks, constraining long-term studies of chronic neurodegenerative conditions.

Efforts to overcome these limitations include the development of modular, user-friendly platforms such as the OrganoPlate® or 3D-printed chips with standardized ports and interfaces. Automated systems that control flow, perfusion, and data acquisition are also becoming more common, which allow for higher-throughput applications and reducing user variability [37].

Another major barrier is the lack of real-time, non-invasive techniques for assessing chip functionality. While TEER and fluorescent permeability assays are standard, they provide only indirect or endpoint measurements. Using integrated biosensors for oxygen, glucose, cytokines, or tight junction protein dynamics could enable continuous monitoring and deeper insight into BBB physiology [74].

***Ethical and Regulatory Considerations***

As BBB-oC technologies move closer to clinical and industrial applications, questions around data validation, patient privacy, and ethical sourcing of cells must be addressed. The use of hiPSC-derived cells, particularly from vulnerable populations such as pediatric or cognitively impaired patients, raises ethical concerns related to consent and genetic data handling [75].

Regulatory acceptance of BBB chips as partial replacements for animal studies is progressing but remains incomplete [76]. The FDA and European Medicines Agency (EMA) require comprehensive validation data demonstrating that chip-based predictions correlate with human clinical outcomes. Establishing such connections will depend on access to high-quality human clinical data and the inclusion of BBB chip findings in investigational new drug (IND) applications.

***Future Outlook***

The field of BBB-oC is poised for radical growth. Next-generation models will likely integrate AI and machine learning for real-time data analysis and predictive modeling of drug transport and toxicity, Organoid or brain slice integration to simulate parenchymal interactions and circuit-level dynamics, CRISPR-edited isogenic controls to dissect the role of specific genes in barrier function or dysfunction, and 3D bioprinting to automate and scale the fabrication of complex vascular architectures.

In the long term, BBB chips may serve not just as research tools but as clinical diagnostics, guiding personalized treatment plans based on patient-specific barrier phenotypes. The convergence of microengineering, stem cell biology, and computational modeling will be essential in driving this vision forward.

**Conclusion and Outlook**

The blood-brain barrier-on-a-chip (BBB-oC) represents a noteworthy convergence of tissue engineering, stem cell biology, and microfluidics, allowing for a human-relevant platform to study one of the most complex and elusive barriers in the human body. Over the past decade, significant progress has been made in developing physiologically relevant in vitro BBB models that recapitulate key structural, functional, and dynamic characteristics of the in vivo NVU.

As summarized in this study, BBB-oC platforms have significantly reshaped the way neuropharmacology, CNS drug delivery, neurotoxicity, and disease pathogenesis are investigated. These models have been instrumental in understanding transport mechanisms, efflux transporter activity, immune cell infiltration, and BBB breakdown under various pathological conditions, including Alzheimer's disease, multiple sclerosis, glioblastoma, and Parkinson's disease.

A particularly compelling application of BBB chips is in neurodegenerative disease research. BBB dysfunction is both a contributor to and a consequence of amyloid accumulation, inflammation, and vascular damage in Alzheimer's disease (AD). BBB-oC systems have enabled researchers to dissect these interrelated mechanisms in a controlled, modular environment. AD patient-derived hiPSC-based BBB chips have shown reduced expression of tight junction proteins, increased permeability, and altered transcytosis similar to clinical findings, thus showing potential for therapeutic applications.

Importantly, these platforms have begun to impact translational workflows. Pharmaceutical companies are increasingly including BBB-oC data in their preclinical pipelines to prioritize drug candidates with optimal CNS penetration profiles. By reducing reliance on non-human models and enhancing prediction accuracy, these systems are helping to close the translational gap that has historically affected CNS drug development. For example, compounds that have shown acceptable transport in shear-stress-based BBB chips but poor permeability in static Transwell assays have successfully proceeded to early-phase human trials.

From a technological perspective, recent developments in chip fabrication, 3D cell culture, and automation have greatly expanded the accessibility and scalability of BBB models. 3D printing and the use of thermoplastics have shown a reduction in cost and improvement in reproducibility, while real-time biosensors and AI-based analytics can lead to new possibilities for dynamic monitoring.

Yet, key challenges to broader adoption remain - such as the limited lifespan of chips, the variability of hiPSC-derived endothelial cells, and the need for standardized protocols across research groups. Moreover, modeling chronic diseases or replicating systemic interactions within a chip remains an evolving frontier. Continued efforts in multi-organ integration, 3D bioprinting, and immune system incorporation will be essential to overcome these limitations.

Looking forward, the possible benefits of BBB-oC platforms extends well beyond the laboratory. With the rise of personalized medicine, BBB chips created from patient-derived cells could one day guide clinical decision-making, furthering individualized drug screening for diseases such as glioblastoma or rare pediatric CNS disorders. These personalized models would not only improve treatment efficacy but also reduce adverse effects by accounting for patient-specific variations in BBB function, transporter expression, and immune responses.

In parallel, regulatory agencies are beginning to acknowledge the value of these systems. The U.S. Food and Drug Administration (FDA) and other regulatory bodies have expressed openness to including organ-on-a-chip data in regulatory submissions, particularly as part of preclinical safety and efficacy packages. As standardized, validated chip models continue to be developed, they may play an increasingly central role in reducing the need for animal testing while enhancing human predictiveness.

In conclusion, BBB-oC technology represents a paradigm shift in neuroscience, pharmacology, and systems biology. It provides a scalable, modular, and physiologically meaningful platform to decode the complexities of the human BBB-bringing us closer to solving some of the most pressing challenges in neurology and medicine. The next decade will likely witness the transformation of BBB-oC systems from sophisticated laboratory models to integral tools in clinical research, drug development, and personalized health care. By continuing to encourage interdisciplinary collaboration, address technical bottlenecks, and validate these systems in real-world applications, the field is on the verge of discovering new therapeutic possibilities for diseases that have long resisted effective treatment.

**References**

1. GBD 2021 Nervous System Disorders Collaborators (2024) Global, regional, and national burden of disorders affecting the nervous system, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. *The Lancet Neurology* 23: 344-381.
2. Daneman R, Prat A (2015) The blood-brain barrier. *Cold Spring Harbor Perspectives in Biology* 7: a020412.
3. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV (2015) Establishment and dysfunction of the blood-brain barrier. *Cell* 163: 1064-1078.
4. Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, et al. (2016) Nanoparticle-mediated brain drug delivery: Overcoming blood-brain barrier to treat neurodegenerative diseases. *Journal of Controlled Release* 235: 34-47.
5. Van der Helm MW, van der Meer AD, Eijkel JC, van den Berg A, Segerink LI (2016) Microfluidic organ-on-chip technology for blood-brain barrier research. *Tissue barriers* 4: e1142493.
6. Booth R, Kim H (2014) Characterization of a microfluidic in vitro model of the blood-brain barrier (μBBB). *Lab on a Chip* 14: 1880-1892.
7. Cho H, Seo JH, Wong KHK, Terasaki Y, Park J, et al. (2015) Three-dimensional blood-brain barrier model for in vitro studies of neurovascular pathology. *Scientific Reports* 5: 15222.
8. Brown CM, Perica K, Dykstra MR, Prantil-Baun R (2021) Microfluidic human blood-brain barrier model with integrated trans well and permeability prediction. *Pharmaceutics* 13: 175.
9. Kim J, Koo BK, Knoblich JA (2020) Human organoids: model systems for human biology and medicine. *Nature Reviews Molecular Cell Biology* 21: 571-584.
10. Vatine GD, Barrile R, Workman MJ, Sances S, Barriga BK, et al. (2019) Human iPSC-derived blood-brain barrier chips enable disease modeling and personalized medicine applications. *Cell Stem Cell* 24: 995-1005.e6.
11. Campisi M, Shin Y, Osaki T, Hajal C, Chiono V, et al. (2018) 3D self-organized microvascular model of the human blood-brain barrier with endothelial cells, pericytes and astrocytes. *Biomaterials* 180: 117-129.
12. Herland A, Van Der Meer AD, FitzGerald EA, Park TE, Sleeboom JJF, et al. (2016) Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip. *PLOS One* 11: e0150360.
13. Linville RM, DeStefano JG, Sklar MB, Zinnia Xu, Alanna M Farrell, et al. (2019) Human iPSC-derived blood-brain barrier microvessels: validation of barrier function and endothelial cell behavior. *Biomaterials* 190: 24-37.
14. World Health Organization (2023) Dementia https://www.who.int/news-room/fact-sheets/detail/dementia.
15. Long S, Benoist C, Weidner W (2023) World Alzheimer Report 2023: Reducing dementia risk: Never too early, never too late. *Alzheimer's Disease International* https://www.alzint.org/resource/world-alzheimer-report-2023/.
16. Montagne A, Barnes SR, Sweeney MD, Matthew R Halliday, Abhay P Sagare et al. (2015) Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* 85: 296-302.
17. Sweeney MD, Sagare AP, Zlokovic BV(2018) Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol* 14: 133-150.
18. Amartumur S, Nguyen H, Huynh T, Kim TS, Woo RS, et al. (2024) Neuropathogenesis-on-chips for neurodegenerative diseases. *Nat Commun* 15: 2219.
19. Wang C, Wang S, Xue Y, Yichen Zhong, Haoyuan Li, et al. (2024) Intravenous administration of blood–brain barrier-crossing conjugates facilitate biomacromolecule transport into central nervous system. *Nat Biotechnol* https://pubmed.ncbi.nlm.nih.gov/39587229/.
20. Thomas DePalma, Marco Rodriguez, Luke Kollin, Kennedy Hughes, Katie Jones, et al. (2025) A microfluidic blood–brain barrier model to study the influence of glioblastoma tumor cells on BBB function. *Small* 21: e2411361.
21. Cucullo L, Hossain M, Puvenna V, Marchi N, Janigro D (2011) The role of shear stress in Blood-Brain Barrier endothelial physiology. *BMC Neurosci* 12: 40.
22. DeStefano JG, Xu ZS, Williams AJ, Yimam N, Searson PC (2017) Effect of shear stress on iPSC-derived human brain microvascular endothelial cells (dhBMECs). *Fluids Barriers CNS* 14: 20.
23. Prabhakarpandian B, Shen M C, Nichols J B, Mills I R, Sidoryk Wegrzynowicz, et al. (2013) SyM-BBB: a microfluidic Blood Brain Barrier model. *Lab on a chip* 13: 1093-1101.
24. Brown JA, Pensabene V, Markov DA, Vanessa Allwardt, M Diana Neely, et al. (2015) Recreating blood-brain barrier physiology and structure on chip: A novel neurovascular microfluidic bioreactor. *Biomicrofluidics* 9: 054124.
25. Cameron TC, Randhawa A, Grist SM, Tanya Bennet, Jessica Hua, et al. (2022) PDMS Organ-On-Chip Design and Fabrication: Strategies for Improving Fluidic Integration and Chip Robustness of Rapidly Prototyped Microfluidic In Vitro Models. *Micromachines (Basel)* 13: 1573.
26. Bruijns B, Veciana A, Tiggelaar R, Gardeniers H (2019) Cyclic Olefin Copolymer Microfluidic Devices for Forensic Applications. *Biosensors (Basel)* 9: 85.
27. Wang R, Zhang H, Li S, Peishi Yan, Shuai Shao, et al. (2025) Current progress of in vitro vascular models on microfluidic chips. *Biofabrication* 17: 022004.
28. Ohbuchi M, Shibuta M, Tetsuka K, Haruna Sasaki-Iwaoka, Masayo Oishi, et al. (2024) Modeling of Blood-Brain Barrier (BBB) Dysfunction and Immune Cell Migration Using Human BBB-on-a-Chip for Drug Discovery Research. *Int J Mol Sci* 25: 6496.
29. Lippmann ES, Al Ahmad A, Palecek SP, Shusta EV (2014) A retinoic acid enhanced, directed differentiation approach for producing blood-brain barrier endothelial cells from human

pluripotent stem cells. *Nature Biotechnology* 32: 1259-1267

30. Bang S, Jeong S, Choi N, Kim HN (2019) Brain-on-a-chip: A history of development and future perspective. *Biomicrofluidics* 13: 051301.
31. Jamieson JJ, Linville RM, Ding YY, Sharon Gerecht, Peter C Searson, et al. (2019) Role of iPSC-derived pericytes on barrier function of iPSC-derived brain microvascular endothelial cells in 2D and 3D. *Fluids Barriers CNS* 16: 1-16.
32. Gerasimova T, Poberezhniy D, Nenasheva V, Stepanenko E, Arsenyeva E, et al. (2024) Inflammatory Intracellular Signaling in Neurons Is Influenced by Glial Soluble Factors in iPSC-Based Cell Model of PARK2-Associated Parkinson's Disease. *Int J Mol Sci* 25: 9621.
33. Vatine GD, Barrile R, Workman MJ, Sances S, Barriga BK, et al. (2019) Human iPSC-Derived Blood-Brain Barrier Chips Enable Disease Modeling and Personalized Medicine Applications. *Cell Stem Cell* 24: 995-1005.e6.
34. Rust R, Sagare AP, Zhang M, Zlokovic BV, Kisler K (2025) The blood-brain barrier as a treatment target for neurodegenerative disorders. *Expert Opin Drug Deliv* 22: 673-692.
35. Nair AL, Groenendijk L, Overdevest R, Fowke TM, Annida R, et al. (2023) Human BBB-on-a-chip reveals barrier disruption, endothelial inflammation, and T cell migration under neuroinflammatory conditions. *Front Mol Neurosci* 16: 1250123.
36. Vetter J, Palagi I, Waisman A, Blaeser A (2025) Recent advances in blood-brain barrier-on-a-chip models. *Acta Biomater* 197: 1-28.
37. Ma C, Peng Y, Li H, Chen W (2021) Organ-on-a-Chip: A New Paradigm for Drug Development. *Trends Pharmacol Sci* 42: 119-133.
38. Deng S, Li C, Cao J, Cui Z, Du J, et al (2023) Organ-on-a-chip meets artificial intelligence in drug evaluation. *Theranostics* 13: 4526-4558.
39. Grifno GN, Farrell AM, Linville RM, Diego Arevalo, Joo Ho Kim, et al. (2019) Tissue-engineered blood-brain barrier models via directed differentiation of human induced pluripotent stem cells. *Sci Rep* 9: 13957.
40. Ahn SI, Sei YJ, Park HJ, Kim J, Ryu Y, et al. (2020) Microengineered human blood-brain barrier platform for understanding nanoparticle transport mechanisms. *Nat Commun* 11: 175.
41. Linville RM, Sklar MB, Grifno GN, Renée F. Nerenberg, Justin Zhou, et al. (2022) Three-dimensional microenvironment regulates gene expression, function, and tight junction dynamics of iPSC-derived blood-brain barrier microvessels. *Fluids Barriers CNS* 19: 87.
42. Uzoechi SC, Collins BE, Badeaux CJ, Li Y, Kwak SS, et al. (2024) Effects of Amyloid Beta (Aβ) Oligomers on Blood-Brain Barrier Using a 3D Microfluidic Vasculature-on-a-Chip Model. *Appl Sci (Basel)* 14: 3917.
43. Ding Y, Palecek SP, Shusta EV (2024) iPSC-derived blood-brain barrier modeling reveals APOE isoform-dependent interactions with amyloid beta. *Fluids Barriers CNS* 21: 79.
44. Raut S, Patel R, Al-Ahmad AJ (2021) Presence of a mutation in PSEN1 or PSEN2 gene is associated with an impaired brain endothelial cell phenotype in vitro. *Fluids Barriers CNS* 18: 3.
45. Park TE, Mustafaoglu N, Herland A, Hasselkus R, Mannix R, et al. (2019) Hypoxia-enhanced Blood-Brain Barrier Chip recapitulates human barrier function and shuttling of drugs and antibodies. *Nat Commun* 10: 2621.
46. Yang Q, Li R, Hong Y, Liu H, Jian C, et al. (2024) Curcumin-Loaded Gelatin Nanoparticles Cross the Blood-Brain Barrier to Treat Ischemic Stroke by Attenuating Oxidative Stress and Neuroinflammation. *Int J Nanomedicine* 19: 11633-11649.
47. Zenaro E, Piacentino G, Constantin G (2017) The blood-brain barrier in Alzheimer's disease. *Neurobiol Dis* 107: 41-56.
48. Zhao Y, Li P, Zhang X, Lu L, Bao Y (2024) Response to advocating for rigorous and multifactorial analyses in post-COVID cognitive research. *Lancet Reg Health West Pac* 49: 101165.
49. Pardridge WM (2012) Drug transport across the blood-brain barrier. *J Cereb Blood Flow Metab* 32: 1959-1972.
50. Linville RM, DeStefano JG, Sklar MB, Chu C, Walczak P, et al. (2020) Modeling hyperosmotic blood-brain barrier opening within human tissue-engineered in vitro brain microvessels. *J Cereb Blood Flow Metab* 40: 1517-1532.
51. Hue CD, Cho FS, Cao S, Dale Bass CR, Meaney DF, et al. (2015) 3rd. Dexamethasone potentiates in vitro blood-brain barrier recovery after primary blast injury by glucocorticoid receptor-mediated upregulation of ZO-1 tight junction protein. *J Cereb Blood Flow Metab* 35: 1191-1198.
52. Pardridge WM (2016) Re-engineering therapeutic antibodies for Alzheimer's disease as blood-brain barrier penetrating bi-specific antibodies. *Expert Opin Biol Ther* 16: 1455-1468.
53. Prasad M, Kumar R, Buragohain L, Kumari A, Ghosh M (2021) Organoid Technology: A Reliable Developmental Biology Tool for Organ-Specific Nanotoxicity Evaluation. *Front Cell Dev Biol* 9: 696668.
54. Csik B, Vali Kordestan K, Gulej R, Boglarka Csik, Roland Patai, et al. (2025) Cisplatin and methotrexate induce brain microvascular endothelial and microglial senescence in mouse models of chemotherapy-associated cognitive impairment. *Geroscience* 47: 3447-3459.
55. Picollet-D'hahan N, Zuchowska A, Lemeunier I, Le Gac S (2021) Multiorgan-on-a-Chip: A Systemic Approach to Model and Decipher Inter-Organ Communication. *Trends Biotechnol* 39: 788-810.
56. El-Taibany AA, Heydarian P, Porada DA, Seeds MC, Atala A (2025) Multiple sclerosis: etiology in the context of neurovascular unit and immune system involvement and advancements with in vitro blood-brain barrier models. *Front Immunol* 16: 1595276.
57. Linville RM, Maressa J, Guo Z, Tracy D Chung, Alanna Farrell, et al. (2023) A tissue-engineered model of the blood-tumor barrier during metastatic breast cancer. *Fluids Barriers CNS* 20: 80.
58. Aitchison EE, Dimesa AM, Shoari A (2025) Matrix Metalloproteinases in Glioma: Drivers of Invasion and Therapeutic Targets. *BioTech (Basel)* 14: 28.
59. Augustine R, Aqel AH, Kalva SN, Joshy KS, Nayeem A, et al. (2021) Bioengineered microfluidic blood-brain barrier models in oncology research. *Transl Oncol* 14: 101087.
60. Du F, Li J, Zhong X, Zhang Z, Zhao Y (2024) Endothelial-to-mesenchymal transition in the tumor microenvironment: Roles of transforming growth factor-β and matrix metalloproteins. *Heliyon* 10: e40118.
61. Appelt Menzel A, Cubukova A, Günther K, Frank Edenhofer, Jörg Piontek, et al. (2017) Establishment of a Human Blood-Brain Barrier Co-culture Model Mimicking the Neurovascular Unit Using Induced Pluri- and Multipotent Stem Cells. *Stem Cell Reports* 8: 894-906.
62. Jagadeesan S, Workman MJ, Herland A, Svendsen CN, Vatine GD (2020) Generation of a Human iPSC-Based Blood-Brain Barrier Chip. *J Vis Exp* 2: 157.
63. Vignone D, Gonzalez Paz O, Fini I, Antonella C, Giulio A, et al. (2022) Modelling the Human Blood-Brain Barrier in

Huntington Disease. *Int J Mol Sci* 23: 7813.

64. Pepe G, Fioriniello S, Marracino F, Luca C, Vittorio M, et al. (2023) Blood-Brain Barrier Integrity Is Perturbed in a Mecp2-Null Mouse Model of Rett Syndrome. *Biomolecules* 13: 606.
65. Morris EK, Daignault Mill S, Stehbens SJ, Genovesi LA, Lagendijk AK (2023) Addressing blood-brain-tumor-barrier heterogeneity in pediatric brain tumors with innovative preclinical models. *Front Oncol* 13: 1101522.
66. Low LA, Mummery C, Berridge BR, Austin CP, Tagle DA (2021) Organs-on-chips: into the next decade. *Nat Rev Drug Discov* 20: 345-361.
67. Tagle DA (2023) Tissue Chips for Drug Screening Program. National Center for Advancing Translational Sciences, *National Institutes of Health*. https://ntp.niehs.nih.gov/sites/default/files/2023-06/22_tagle_iccvamforum2023_508.pdf
68. Li J, Zheng M, Shimoni O, William AB, Ashley IB, et al. (2021) Development of Novel Therapeutics Targeting the Blood-Brain Barrier: From Barrier to Carrier. *Adv Sci (Weinh)* 8: e2101090.
69. Aubin H, Nichol JW, Hutson CB, Hojae Bae, Alisha L Sieminski, et al. (2010) Directed 3D cell alignment and elongation in microengineered hydrogels. *Biomaterials* 31: 6941-6951.
70. Toepke MW, Beebe DJ (2006) PDMS absorption of small molecules and consequences in microfluidic applications. *Lab Chip* 6: 1484-1486.
71. Jeong S, Kim S, Buonocore J, Jaewon Park, C Jane Welsh, et al. (2018) A Three-Dimensional Arrayed Microfluidic Blood-Brain Barrier Model with Integrated Electrical Sensor Array. *IEEE Trans Biomed Eng* 65: 431-439.
72. Boeri L, Izzo L, Sardelli L, Tunesi M, Albani D, et al. (2019) Advanced Organ-on-a-Chip Devices to Investigate Liver Multi-Organ Communication: Focus on Gut, Microbiota and Brain. *Bioengineering (Basel)* 6: 91.
73. Koyilot MC, Natarajan P, Hunt CR, Sonish Sivarajkumar, Romy Roy, et al. (2022) Breakthroughs and Applications of Organ-on-a-Chip Technology. *Cells* 11: 1828.
74. Mir M, Palma-Florez S, Lagunas A, López-Martínez MJ, Samitier J (2022) Biosensors Integration in Blood-Brain Barrier-on-a-Chip: Emerging Platform for Monitoring Neurodegenerative Diseases. *ACS Sens* 7: 1237-1247.
75. Mezinska S, Gallagher L, Verbrugge M, Bunnik EM (2021) Ethical issues in genomics research on neurodevelopmental disorders: a critical interpretive review. *Hum Genomics* 15: 16.
76. US Government Accountability Office (2025) Human Organ On-A-Chip: Technologies Offer Benefits Over Animal Testing but Challenges Limit Wider Adoption (GAO-25107335). *U.S. Government Accountability Office*. Accessed July 23, 2025 https://www.gao.gov/products/gao-25-107335