
Modeling the Blood–Brain Barrier to Understand Drug Delivery in Alzheimer’s Disease

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Abstract: The blood–brain barrier is a semipermeable barrier structure that lines the walls of brain microvessels. Although the blood–brain barrier plays a key role in protecting the brain from unwanted molecules, it simultaneously challenges the delivery of drugs into the brain. In addition, the blood–brain barrier has been shown to be dysfunctional in Alzheimer’s disease, the most common cause of dementia for which there is no cure. Mouse models of Alzheimer’s disease have played a central role in investigating disease-specific changes in the blood–brain barrier, but the translation of findings from mouse models into the human system is hindered by interspecies differences. In an effort to develop new drug delivery techniques and/or understand changes in the human blood–brain barrier in Alzheimer’s disease, several human blood–brain barrier *in vitro* models have been developed. These comprise primary and immortalized human endothelial cell-based models as well as human induced pluripotent stem cell-derived brain microvascular endothelial cell models. Both two- and three-dimensional

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(2D and 3D) culture platforms have been established to better mimic the complexity of the brain. This chapter discusses the current blood–brain barrier models, their advantages and disadvantages as well as their potential to understand drug delivery in Alzheimer’s disease.

Keywords: Alzheimer’s disease; astrocyte cell culture model; blood–brain barrier; brain endothelial cell; induced pluripotent stem cell

INTRODUCTION

The blood–brain barrier (BBB), formed by tightly-sealed brain endothelial cells (BECs), serves as a selectively permeable membrane at the blood–brain interface. It allows for the delivery of oxygen and nutrients into the brain and at the same time, maintains a highly controlled brain milieu by preventing the entry of neurotoxic blood components into the brain and transporting metabolic waste products from the brain to peripheral circulation (1). Furthermore, the brain vascular system supports the activity of neuronal networks through increased blood flow and oxygen supply, a mechanism termed as neurovascular coupling (2).

Integrity of the BEC layer is critical for the barrier function of the BBB and is achieved by the presence of tight and adherens junctions (AJ) between BECs. Tight junctions (TJ) consist of claudins-3, -5, and -12, occludin, and TJ associated *zona occludens* (ZO-1 and ZO-2) proteins (3). AJ are formed by vascular endothelial cadherin (VE-cadherin) and platelet endothelial cell adhesion molecule-1 (PECAM1) (3). Synergistically, TJ and AJ proteins ensure high transendothelial electrical resistance (TEER) of the BBB (1000–2000 ohm/cm² compared to 10 ohm/cm² in peripheral capillaries) and restrict paracellular permeability to molecules under 500 Da (4). Compared to peripheral endothelial cells, BECs also have more mitochondria and less fenestrations and pinocytotic vesicles, further limiting the exchange of solutes across the BBB (4).

In addition to TJs and AJs, BECs express a unique selection of transporters, which either transport nutrients to the brain or transport molecules back to the blood. Solute carrier mediated transport (CMT) enables transendothelial exchange of organic cations and anions, carbohydrates, vitamins, fatty acids, nucleotides, amino acids, and hormones, whereas receptor-mediated transcytosis (RMT) facilitates the transport of peptides and proteins across the BBB, including insulin, transferrin, and apolipoproteins (1). ATP-binding cassette (ABC) transporters, including, P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP), protect the brain from the accumulation of xenobiotic compounds and drugs *via* their active efflux from BECs to the blood, and also greatly hinder drug delivery across the BBB (5).

Finally, to support BBB integrity, BECs are ensheathed by pericytes and astrocyte end-feet, and may create connections with microglia, neurons, and neural stem cells, which together form the so-called neurovascular unit (NVU) (Figure 1A) (1). Pericytes cover an approximate 1/3 of the BEC monolayer and can contract or relax cell membrane extensions to locally change cerebral blood flow in response to neuronal activity (6). Pericytes can also guide the polarisation of astrocyte end-feet, modulate TJ protein expression and permeability, participate in BBB immune responses, and regulate clearance of neurotoxic substances, such

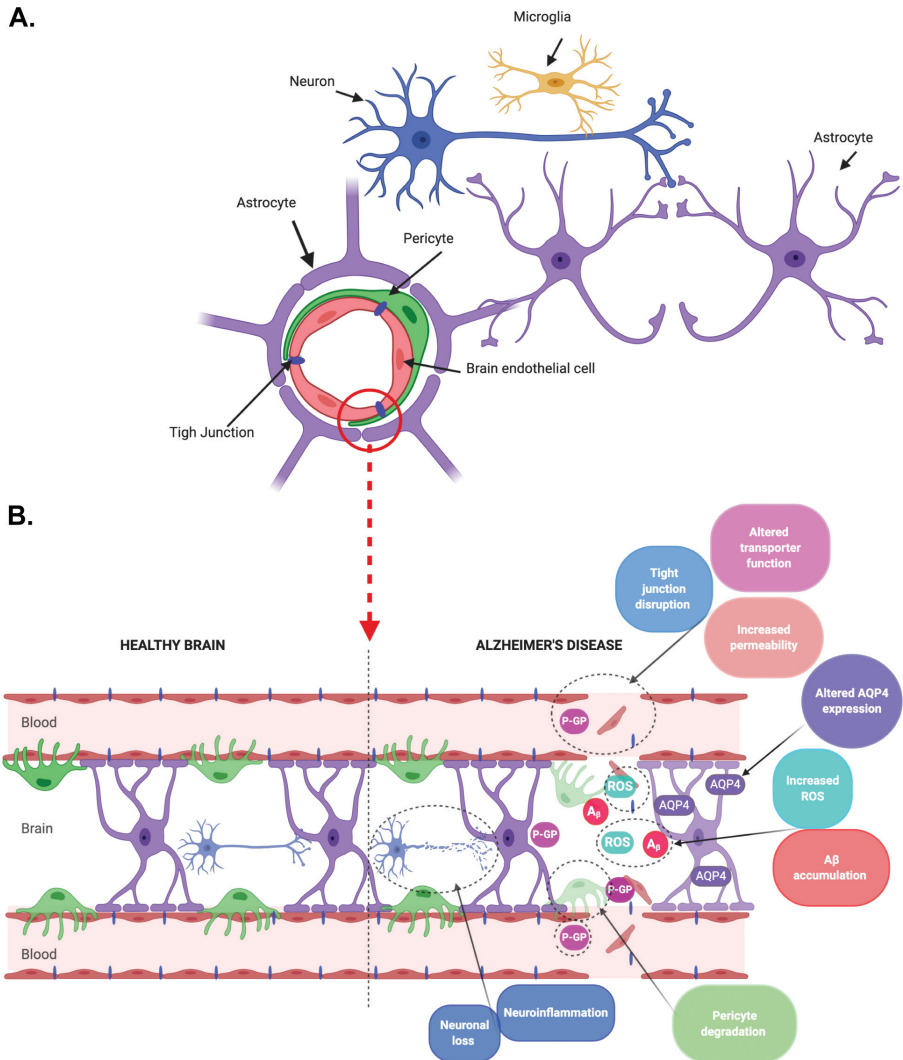


Figure 1. Cellular structure and Alzheimer’s disease (AD) specific changes in the blood–brain barrier (BBB). (A) Schematic representation of the BBB structure. The BBB is formed by brain endothelial cells (BECs) with astrocytes and pericytes functioning as key supporting cells. The BBB and other brain cells (neurons and microglia) form the neurovascular unit. (B) AD-specific changes in the BBB include altered tight junction and transporter expression in BECs, pericyte degradation, altered aquaporin-4 (AQP4) expression in astrocytes with these changes leading to increased permeability, reactive oxygen species (ROS) and neuroinflammation, and subsequent amyloid- β ($A\beta$) accumulation and neuronal loss. Created using BioRender.com.

as amyloid- β ($A\beta$) (6, 7). Astrocytes mediate innate immune responses on the brain barrier, provide nutrients from the blood to neurons, and regulate BBB barrier function by secreting protective factors, which modulate BEC TJ integrity (8). Astrocytes also play a primary role in the maintenance of parenchymal water and ionic homeostasis due to high expression of aquaporin 4 water (AQP4) channels

and ion transporters, contributing to clearance of interstitial solutes, including A β (9, 10). Together all the components comprise a highly specialized BBB unit, which plays a major role in maintaining homeostasis, demonstrates heterogeneity in disease, and poses a major hurdle for drug delivery.

ALZHEIMER'S DISEASE RELATED CHANGES IN THE BBB

Emerging evidence suggests that BBB dysfunction, together with A β plaques and hyperphosphorylated tau, is the third driving pathology underlying early Alzheimer's disease (AD) and corresponding cognitive decline (11–15). During preclinical asymptomatic stages of AD, cerebrovascular dysfunction is one of the first changes preceding any other detectable alterations characteristic of AD, including A β and tau (12, 16). The two-hit vascular hypothesis proposes that blood vessel impairment initiates the cascade of events by causing initial BBB dysfunction, decreased cerebral blood flow, and infiltration of neurotoxic molecules that in turn lead to neuronal loss (hit 1). BBB pathology subsequently causes impaired A β clearance and increased production of toxic A β species (hit 2), which synergistically with other vascular, genetic, and environmental risk factors, leads to progression of AD pathology (17).

Interestingly, major genetic risk factors of AD, including mutations in *APP* and *PSEN1* as well as *APOE* $\epsilon 4$ polymorphism, have been shown to contribute to BBB leakage, brain microbleeds, BEC degeneration, pericyte injury, and abnormal A β clearance, linking multiple pathways of vascular- and neurodegeneration (13, 14, 18, 19). Increased BBB leakage in AD has been shown in both brain imaging studies as well as post-mortem brain tissue demonstrating an accumulation of blood-derived proteins in the brain (13, 20–23). Leakage of blood-borne factors further contributes to multiple pathological pathways in the AD brain, including BBB breakdown, pericyte dysfunction, neuronal death, neuroinflammation, and increased oxidative stress (24–27).

Defective function of nutrient and efflux transporters in the BBB has also been identified in human and animal studies of AD. Impaired glucose transport and reduced glucose transporter 1 (GLUT1) expression has been shown at the BBB of individuals with mild cognitive impairment (MCI) and in transgenic murine models of AD (28–30). The brain-to-blood efflux transporter P-gp has been implicated in AD pathology with clinical studies showing decreased P-gp activity in patients with mild AD, suggesting P-gp dysfunction and corresponding xenobiotic and A β build-up in the brain could contribute to AD pathogenesis (31, 32). Altered expression of efflux transporters in AD has further been demonstrated in a human induced pluripotent stem cell (hiPSC)-derived BEC model of familial AD (fAD) (33).

Cell-specific changes in the NVU have also been shown in AD. Human post-mortem and cell model studies of AD have identified reduced BEC integrity, altered BEC TJ and AJ protein expression, and reduced expression in the brain endothelium of low-density lipoprotein receptor-related protein 1 (LRP1), the main receptor facilitating removal of A β (11, 33–35). Pericyte loss and decreased pericyte coverage of brain capillaries has also been observed in brain samples from AD individuals—an effect being amplified in *APOE* $\epsilon 4$ carriers (11, 13, 36).

In addition, pericytes contribute to the clearance of A β from the brain, and their loss has been shown to decrease the age of onset of AD and accelerate the development of A β and tau pathology in transgenic mice models (37). Astrocyte dysfunction is also implicated in AD, with altered APQ4 expression and localization as well as increased inflammatory and oxidative stress responses reported in human and mouse models of AD (38–40).

AD-specific changes in the BBB are summarized in Table 1 and Figure 1B. These alterations highlight severe AD-specific effects on the BBB with likely important consequences on disease progression and drug delivery.

TABLE 1

Alzheimer's disease related changed in the BBB

Change	Details	Model	Ref
BBB breakdown	<ul style="list-style-type: none"> Increased albumin ratio in CSF/blood Increased leakage in the hippocampus Increase in blood-borne factors in the brain 	Human brain imaging (MCI and APOE4) Human post-mortem tissue	(11, 13, 16, 36)
Vascular pathology	<ul style="list-style-type: none"> Reduced cerebral blood flow Cerebral amyloid angiopathy Infarcts Haemorrhages Abnormal blood vessels Increased fibrin(ogen) deposition in cortical vessels 	Human brain images (LOAD) Human post-mortem tissue Transgenic (<i>PSEN1</i>) AD mouse model	(11, 12, 14, 15, 18, 19, 21)
Brain endothelium dysfunction	<ul style="list-style-type: none"> Reduced integrity Reduced LRP1 expression Altered TJ and AJ protein expression Accumulation of CypA and MMP-9 	Human post-mortem tissue Human fAD iBEC model	(11, 33–35)
Transporter dysfunction	<ul style="list-style-type: none"> Reduced P-gp activity Reduced GLUT-1 expression Reduced glucose metabolism Altered efflux transporter expression 	Transgenic (<i>APP</i>) AD mouse model Human brain imaging (MCI and AD) Human fAD iBEC model	(28–33)
Pericyte dysfunction	<ul style="list-style-type: none"> Loss of pericyte number and coverage in the hippocampus Increased PDGFβ in the CSF Accumulation of CypA and MMP-9 Upregulated calcineurin signalling 	Human brain imaging (MCI) Human post-mortem tissue Human APOE4 iBEC model	(11, 13, 16, 36, 64)
Astrocyte dysfunction	<ul style="list-style-type: none"> Increased AQP4 expression Altered AQP4 distribution Altered inflammatory response Altered calcium signalling Increased oxidative stress 	Human post-mortem tissue Transgenic (<i>APP</i>) AD mouse model Human fAD iPSC-derived astrocytes	(38–40)

AJ, adherens junction; APOE4, apolipoprotein E allele $\epsilon 4$ carrier; APP, amyloid precursor protein; AQP4, aquaporin 4; BBB, blood–brain barrier; CSF, cerebrospinal fluid; CypA, cyclophilin A; fAD, familial Alzheimer's disease; GLUT1, glucose transporter 1; iBEC, induced brain endothelial cell; iPSC, induced pluripotent stem cell; LRP1, low density lipoprotein receptor-related protein 1; LOAD, late onset Alzheimer's; MCI, mild cognitive impairment; MMP9, matrix metalloproteinase-9; PDGF β , platelet-derived growth factor β ; PSEN1, presenilin 1; TJ, tight junction.

DIFFERENCES BETWEEN ANIMAL AND HUMAN BBB AND IMPLICATIONS TO DRUG DELIVERY

Due to the challenges in studying the BBB in humans, animal models have played a central role in understanding AD-specific changes in the BBB and modeling delivery of brain targeting therapeutics (1, 41). Animal models have allowed for the in-depth investigation of BBB structure and biology, which is mostly only possible in humans using post-mortem tissue. The limitation of post-mortem tissue is that it does not allow for the analysis of BBB structure and function in a living person and at early stages of disease, critical for understanding the role of the BBB at different stages of disease progression.

Although extensive research has been conducted in animal models to identify novel therapeutics for AD, successful pre-clinical studies rarely translate into humans. In fact, over 99% of AD clinical trials with drugs which provided promising results in model animal systems, have not been successfully translated into humans (41, 42). The underlying problem is that although animal models of AD express key pathological hallmarks, including A β and hyper-phosphorylated tau, these models do not necessarily exhibit other biological features of AD and can be considered to not have AD (42). In addition, there is a lack of animal models for sporadic forms of AD, the most common type of AD in humans (42). Finally, interspecies differences are a central hindrance to translation of therapies from animal models to humans.

The mouse is one of the most commonly used animal models in BBB research and has been central to understanding BBB development and biology (43). Structurally mouse and human brains and BBB contain the same cell types, but distinct differences between mouse and human have been reported in properties of cells located in the neocortex, such as differential morphology and gene expression (43, 44). In addition, the human neocortex is vastly larger and more complex compared to that of the mouse, which complicates drug delivery in humans. The human neocortex also contains proportionally more astrocytes than the mouse cortex (45), which could potentially affect BBB formation and subsequent drug delivery.

Differences between the rodent and human BBB have been reported in TJ protein (TJP) and transporter expression and function. Key TJPs including claudin-5, occludin, and ZO-1 have been reported to have higher mRNA expression in mouse BECs compared to human BECs (46). In addition, comparison of protein level expression of transporters in brain microvessels identified a clearly higher expression of some transporters, including ABC (P-gp and MRP4) and solute carrier transporters (monocarboxylate transporter 1, L-type amino acid transporter, and organic anion transporter 3) in rats compared to humans (47). Real-time brain imaging also revealed differences in P-gp-dependent uptake of drugs between rat and human brains with brain concentrations of P-gp substrates found to be higher in humans than rats (48). These results indicate higher BBB permeability in humans compared to rodents and suggest that drug delivery experiments cannot be directly translated from rodents to humans. Interestingly, differences between humans and other primates have been shown to be smaller (47), with non-human primates potentially providing a more accurate model for human drug delivery than rodents.

Interspecies differences have also been found in other cell components of the NVU. Human astrocytes are larger and exhibit differences in process complexity than corresponding rat astrocytes (49). AQP4, the main water channel in the brain expressed by astrocytes, is polarized in astrocyte end-feet surrounding the BBB to a lesser extent in human astrocytes compared to mouse (50). Mislocalization of AQP4 has been linked to AD in humans (51), thus the differential expression and localization of AQP4 between mouse and human could have implications to how AD features in mouse models. Finally, primates are unique in terms of the presence of interlaminar astrocytes, which are not found in rodents (49). The disruption of processes of interlaminar astrocytes in AD has been reported (52), highlighting an important characteristic of AD humans that cannot be replicated in rodent models.

BBB IN VITRO CELL MODELS

To overcome species differences in BBB modeling, *in vitro* models of the human BBB are central to enhancing our understanding of BBB biology at a cellular level in health and disease. BBB cell models have traditionally been limited to primary and immortalized BECs (53), with hiPSC-derived BBB cells emerging as a novel approach for BBB modeling (54).

Primary and immortalized BECs

Human and mouse primary BEC isolation has been described from both fetal and adult brain tissue (Figure 2A) (55, 56). Primary BECs are reported to express BBB markers, such as TJPs and transporters (57), providing a tool for *in vitro* modeling of the BBB. BEC isolation from AD patient post-mortem tissue has also been described, revealing disease-specific differences compared to healthy BECs (56), suggesting a potential for using primary BECs for disease modeling.

The use of primary BECs is, however, associated with multiple limitations. Isolation of BECs from brain tissue is challenging as the proportion of BECs from all brain cell types is low (approximately 1–2%), easily resulting in contamination by unwanted cell types (55). In addition, the availability of tissue from patients is limited with donor-to-donor variability and ethical considerations posing challenges (57). Withdrawing BECs from their *in vivo* tissue and culturing them *in vitro* has also been reported to result in the loss of TJ markers and reduced transporter expression (58, 59). Passaging primary BECs has been reported to result in reduced TEER and an unstructured monolayer with loss of localized TJP expression (53), limiting the time that these cells can be utilized for experiments.

Immortalized human BEC lines (such as the hCMEC/D3 line) have helped overcome some of the challenges associated with primary BECs (Figure 2B) (60). Advantages of immortalized BEC lines include their ability to maintain cell properties over multiple passages, high viability, and expression of brain endothelium-specific transport systems, making them an ideal model to perform high-throughput screening of new drugs targeting specific transporters and/or receptors (61). However, immortalization has been shown to affect the cell phenotype compared to primary BECs, including highly upregulated expression of genes related to

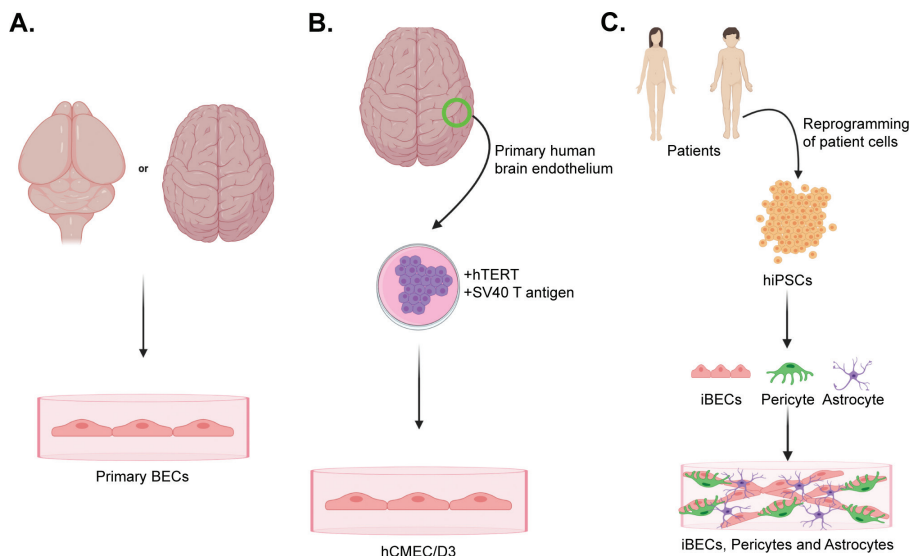


Figure 2. Brain endothelial cell (BEC) sources for blood–brain barrier (BBB) modeling. (A) Primary BECs are isolated directly from the brain with (B) immortalized BEC lines (e.g., hCMEC/D3) generated via the introduction of immortalization factors. (C) An alternative source of BECs are human induced pluripotent stem cells (hiPSC), which are generated by reprogramming patient-derived cells. hiPSCs can be used to generate all BBB cell types, including iBECs, pericytes and astrocytes. Created using BioRender.com.

nucleic acid processing and repair as well as interferon signaling (46). In addition, differential cell growth and altered gene expression of TJPs, receptors and transporters has been reported between immortalized and primary BECs (46). A major limitation of immortalized BECs is that they do not allow for the study of AD-specific changes. The overexpression of *fAD* mutations in *APP* and *PSEN1* in human immortalized neural progenitor cells has been described, resulting in the production of $A\beta$ -plaques and pathological tau *in vitro* (62). A similar approach in human immortalized BECs could be possible, however, how this would alter BEC properties is unknown.

Human-induced pluripotent stem cell derived BECs

Due to the limitations associated with primary and immortalized BEC models, hiPSC-derived models have arisen as a promising approach for *in vitro* BBB modeling (Figure 2C) (54, 63). BECs can be generated from iPSCs (iBECs) with relative ease and AD patient-derived hiPSCs allow for the study of disease-specific differences (33, 63). iBECs exhibit key characteristics of BECs, including high TEER, high expression of BBB-specific TJPs as well as expression and function of efflux transporters (33, 63). Importantly, hiPSCs from the same patient can be differentiated into other cell types of the NVU, including pericytes and astrocytes, enabling the generation of an isogenic multi-cell BBB model (Figure 2C) (64, 65). In addition, CRISP/Cas9 gene editing allows for the further examination of the

contribution of AD risk genes on BBB function (33, 64). iBECs generated from patients with familial and sporadic AD have revealed key cellular differences giving insights into AD-specific effects on the BBB and potential implications to drug delivery (33, 64).

Although hiPSC-based technologies hold enormous potential for the development of preclinical BBB models, there are a few hindrances that need to be considered. hiPSC lines often exhibit a high level of variability and their generation and maintenance is expensive (66). The genetic editing of these cells also often results in loss of patient-specific epigenetic signatures and genetic instability. Other limitations include the lack of relevant genome matched-controls and lack of maturity in iPSC-derived cells, particularly important for modeling late onset diseases (67, 68). Finally, models based on hiPSC-derived iBECs allow only for a narrow experimental window, since cells tend to de-differentiate rapidly (54). Despite these limitations, continuous advancements in hiPSC research and commercially available reprogramming kits and cell culture reagents have the ability to ensure standardized culture conditions and the production of high quality hiPSC lines for BBB research.

IN VITRO CULTURE PLATFORMS FOR MODELING OF BBB STRUCTURE AND DRUG DELIVERY

In vitro platforms of the BBB are central to modeling the human BBB and to screening and developing BBB permeable drugs. BBB *in vitro* culture systems can broadly be divided into static 2D cultures and static or microfluidic 3D cultures.

2D models of the BBB

Static monolayer (i.e., 2D) culture systems are, to date, the most commonly used BBB *in vitro* model platforms. Most commonly, BECs (primary, immortalized or iPSC-derived) (69–71) are seeded inside a Transwell insert, in which cells are cultured on a permeable support as opposed to solid plastic (Figure 3A). The inside of the insert represents the luminal (blood) side, whereas the surrounding well, in which the insert is placed, represents the abluminal (brain) side (Figure 3A). The Transwell model allows for measurement of integrity as well as permeability of compounds through the BEC monolayer, from the luminal to the abluminal side (72).

BEC barrier integrity in the Transwell system can be increased via co-culture with pericytes, astrocytes, and other cells of the NVU usually resulting in increased TEER, higher TJP and transporter expression, promoting *in vivo*-like BBB phenotype (70, 73, 74). Co-cultures are achieved by culturing other BBB cells, such as pericytes or astrocytes, in the surrounding well, in which the BEC containing Transwell insert is placed, or on the underside of the BEC containing Transwell insert (Figure 3A) (63, 69). In this formation, cells are not in direct physical contact, as they are separated by a membrane, but will regulate each other via secreted factors. Direct contact Transwell models have also been described, where BECs and astrocytes, for example, are layered directly on top of each other, with this reported to result to higher TEER than indirect co-cultures (64, 70).

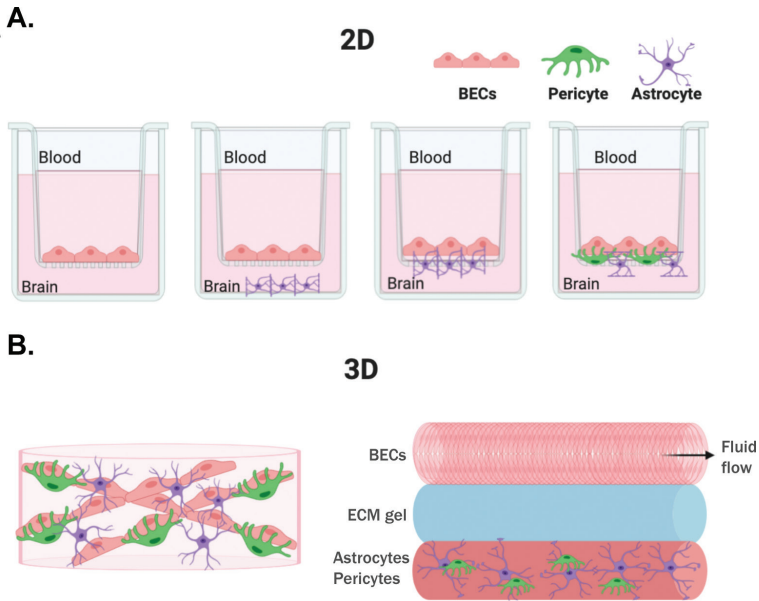


Figure 3. Two- (2D) and three- (3D) dimensional model platforms of the blood–brain barrier (BBB). (A) The Transwell system is the most common 2D platform for BBB modeling. In the Transwell system, brain endothelial cells (BECs) are cultured inside the Transwell insert with BBB co-cultures established by culturing other cell types (such as pericytes or astrocytes) in the surrounding well or on the underside of the Transwell insert. (B) 3D cultures can be established by embedding BBB cell types in a scaffolding matrix and allowing them to grow in a 3D conformation. In 3D and microfluidic models of the BBB, BECs are cultured in a tubular formation and the culture medium is allowed to flow through the BEC tube mimicking blood flow. Other BBB cell types (pericytes and astrocytes) are embedded in an extracellular matrix and allowed to grow adjacent to the BEC tube. *Created using BioRender.com.*

The Transwell assay has been widely used to study BBB integrity, permeability, and drug delivery (71, 72, 75). Although the Transwell assay is relatively easy to set-up and thus, widely used in BBB research, its limitations are that it lacks the complex 3D structure of the BBB *in vivo*, and BECs are cultured as a monolayer instead of a tubular structure, lacking complex cell interactions.

3D models of the BBB

Three-dimensional model systems of the BBB are emerging to better mimic the complexity of the BBB *in vivo*. In the brain, the BBB is a tubular structure, which is not accurately replicated using traditional 2D culture settings. Other features of the *in vivo* BBB include the complex interaction of BECs and other cells of the NVU with each other and with the extracellular matrix. In the body the BBB is also exposed to blood flow, which causes shear stress in BECs (4).

A central component of scaffold-based 3D models is using a supporting matrix (e.g., Matrigel or collagen I) that forms a gel, in which cells are able to grow in 3D conformation (Figure 3B) (76). In 3D BBB modeling, a central aim is to allow BECs to grow in a tubular formation using extracellular matrix (ECM) support (64, 77, 78).

Other cells, such as astrocytes and pericytes are then ideally cultured in direct contact with BECs, mimicking cell interactions in the body (64, 77). Vascular networks of both the healthy and AD BBB have been achieved *in vitro* by allowing BECs to self-assemble in an extracellular matrix containing 3D culture environment in co-culture with astrocytes and pericytes (64, 79). When combining microfluidic technology with a 3D growth environment, it is possible to achieve tubular *in vitro* models of the BBB that also mimic blood flow (Figure 3B). These “organ-on-a-chip” models have shown promising outcomes in understanding cellular interactions and modeling drug transport, often allowing for minimal use of cells and culture reagents (79–81). Three-dimensional and microfluidic culture conditions have been used to model the AD BBB using immortalized human BECs and neural progenitor cells, with this model revealing AD-specific BBB dysfunction (78).

The limitation of many 3D and microfluidic BBB models is that they are complex in-house made platforms, often utilising proprietary materials, which are difficult to replicate in other laboratories. Commercial 3D and microfluidic platforms are emerging, providing a means for off-the-shelf systems for BBB modeling (82, 83). Other limitations include that most of the 3D and microfluidic BBB models have been established using cell lines, such as immortalized BECs. To be able to accurately model AD- or other neurodegenerative disease-specific effects, patient-derived cells, such as primary BECs or iPSC-derived iBECs would be ideal. Likely the challenge of culturing patient-derived BECs in co-culture with other cells as well as the poor long-term survival has hindered the development of patient-derived 3D and microfluidic models of the BBB.

BBB organoids provide an additional platform of studying BBB function in a 3D format without the need for a complex device. Previously generated BBB spheroids have consisted of primary or iPSC-derived BECs and other NVU cell types which spontaneously assemble under low-attachment conditions into a multicellular 3D structure (84, 85). These spheroids have been shown to demonstrate direct cell-to-cell contacts, enhanced TJ and AJ expression, higher efflux transporters expression and reduced paracellular permeability, thus more closely mimicking the *in vivo* BBB when compared to traditional 2D cultures (84, 85). Human cortical spheroids containing BECs, pericytes, microglia, astrocytes, oligodendrocytes and neurons were shown to exhibit high expression of BBB markers as well as high viability, making them an attractive platform for drug discovery, disease modeling and long-term neuro- and cytotoxicity testing (85). The resemblance of organoid-like spheroids to the *in vivo* environment make them a promising platform for high-throughput screening of BBB penetrating drugs. Limitations of organoid research, however, include inter-sample variability, high processing time and technical difficulties in TEER measurements and permeability studies (86).

Permeability and drug delivery assays

Integrity of *in vitro* models of BBB is usually assessed via the permeability to fluorescently-conjugated molecules, such as dextran or sodium fluorescein, which have both been used in 2D Transwell and 3D microfluidic models of the BBB (33, 72, 83). For modeling drug delivery, various methods have been studied to transiently open the BBB *in vitro* cell model. Mannitol can be used to reversibly open the BBB, based on hyperosmosis, and has been used in an *in vitro* hiPSC-derived 3D BEC model to increase paracellular permeability (87). In the clinic, mannitol

has been used to deliver antibodies to treat brain tumors (88), with its use otherwise not widely described due to possible side-effects. Human patient cell based AD BBB models may be important to allow the in-depth investigation of mannitol-dependent BBB opening (87), to develop its use for the delivery of therapeutic antibodies, such as to treat AD. Another reversible means to open the BBB is focused ultrasound (FUS) applied in-conjunction with gas-filled microbubbles (MB) (89). The safety of FUS+MB treatment in AD patients has been demonstrated, opening an avenue for potential A β clearance or therapeutic drug delivery (90). The effects of FUS+MB have also been investigated in a patient iPSC-derived fAD iBEC *in vitro* model, which demonstrated a differential response to FUS+MB treatment between patient and control cells, such as in FUS+MB-mediated permeability and A β clearance (33). These results highlight the importance of using patient-derived cell models to identify potential patient-specific differences that could affect drug delivery in the clinic.

ENHANCING DRUG DELIVERY IN ALZHEIMER'S DISEASE THROUGH *IN VITRO* MODELS OF BBB—CHALLENGES AND FUTURE PERSPECTIVES

BBB dysfunction in AD not only underlies disease pathogenesis and progression but also serves as the main burden for successful drug delivery. Additionally, BBB impairment in AD and subsequent physiological changes lead to disruption in drug delivery by diffusion, with pathological changes in transporters contributing to minimal (or no) bioavailability of the drug in the brain (1, 91). Furthermore, infiltrating toxic blood-derived products, reactive oxygen species and increased neuroinflammation may change the tightly controlled brain milieu and lead to undesired metabolism and/or interactions of delivered drugs (Figure 1B) (1). As such, to overcome challenges associated with drug delivery in AD, an in-depth understanding of AD-related changes at the BBB and subsequent effects on drug delivery are needed, which can be addressed using accurate model systems.

The basis of an accurate AD BBB cell model is that the cells, as closely as possible, recapitulate the disease phenotype. With challenges associated with all the described BEC sources, it is important to consider the benefits and limitations of each model. AD patient-derived primary BECs provide an opportunity to examine cells obtained directly from a patient (56), but patient brain tissue is difficult to obtain with these cells often only capturing late-stage of the disease. In addition, the lifespan of primary BECs *in vitro* is short, limiting their use for large-scale experiments (53). Patient-derived iPSCs on the other hand provide a scalable approach to generate BECs *in vitro* and one patient line can be used to generate all components of the NVU, providing an isogenic patient-specific BBB model (33, 64). In addition, using CRISPR/Cas9 gene editing, the role of specific AD mutations on BBB dysfunction can be investigated (33, 64). To ensure the translatable use of iPSC-derived cells, it is important to generate standardized differentiation protocols and utilize defined xeno-free reagents to minimize variability associated with iPSC culture.

Following selection of a good BBB cell source, it is vital to consider the culture environment for accurate BBB modeling. Successful screening for drug delivery

across the BBB will be best achieved in an environment that as closely as possible mimics the brain environment in complexity, structure and chemistry. It has been shown that following a long-term culture (>3 months), AD-patient derived iPSC cerebral organoids exhibit hallmarks of AD, including A β plaques and accumulation of hyper-phosphorylated tau (92). Thus, to replicate physiological changes characteristic of AD, long-term cell culture models of the BBB are likely needed. Furthermore, to capture the complexity of the brain, standardized platforms that enable both 3D and microfluidic culture conditions are likely the future means to establishing an accurate BBB *in vitro* model. The challenge that still exists is developing a reproducible culture platform that is easy and cost-effective for laboratories to use, with emerging commercial platforms providing a potential solution (82, 83).

Finally, to accelerate drug discovery in AD, high-throughput testing in BBB *in vitro* models is important. Scaling down reagent and cell use (i.e., minimizing culture platform size) and scaling up the number of replicates, would help to achieve testing of a large number of drugs in a cost-effective manner. For this reason, small-scale “organ-on-a-chip” type platforms may be ideal (93). It is also vital to consider how easily drug delivery efficiency and downstream effects can be measured. Ideally, a BBB *in vitro* model that enables the simultaneous observation of drug delivery efficiency as well as effects on brain cell types and AD pathologies (A β and tau) would be a key step in AD drug discovery.

CONCLUSION

BBB dysfunction is associated with AD, likely playing a central role in AD progression and drug delivery. Considering inter-species differences, accurate human BBB *in vitro* models are needed to understand AD-specific changes on the BBB and subsequent effects on drug delivery. Human BBB cell models of AD, in particular using patient-derived cells in a culture environment that accurately mimics the AD brain, are an important step to enhance drug discovery in AD.

Conflict of Interest: The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this chapter.

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