Biology and Models of the Blood–Brain Barrier

Cynthia Hajal,1,* Baptiste Le Roi,2,* Roger D. Kamm,1,3 and Ben M. Maoz2,4,5

1Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
2Department of Biomedical Engineering, Tel Aviv University, Tel Aviv 6997801, Israel
3Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
4Sagol School of Neuroscience, Tel Aviv University, Tel Aviv 6997801, Israel; email: bmaoz@tauex.tau.ac.il
5Center for Nanoscience and Nanotechnology, Tel Aviv University, Tel Aviv 6997801, Israel

Abstract

The blood–brain barrier (BBB) is one of the most selective endothelial barriers. An understanding of its cellular, morphological, and biological properties in health and disease is necessary to develop therapeutics that can be transported from blood to brain. In vivo models have provided some insight into these features and transport mechanisms adopted at the brain, yet they have failed as a robust platform for the translation of results into clinical outcomes. In this article, we provide a general overview of major BBB features and describe various models that have been designed to replicate this barrier and neurological pathologies linked with the BBB. We propose several key parameters and design characteristics that can be employed to engineer physiologically relevant models of the blood–brain interface and highlight the need for a consensus in the measurement of fundamental properties of this barrier.

Keywords
blood–brain barrier, organ-on-a-chip, tissue engineering, self-assembly, organoids, neurological diseases
1. INTRODUCTION

The blood–brain barrier (BBB), the interface between blood and parenchyma at the brain, features a unique cellular architecture of endothelial cells lining the blood vessels, pericytes associated with the basement membrane, and astrocytes extending their endfeet to the abluminal side of the vessels. Owing to the organization of the BBB, transport across it is considerably reduced relative to other tissues. To facilitate selective transport, the BBB draws upon transcytosis, the active receptor-mediated transport of specific molecules through the endothelial cells. While this barrier protects neural tissue from toxins in the blood, it conversely limits the passage of therapeutics. The complexity of the BBB has impeded the development of models that can recapitulate its properties in health and disease. In this review, we first provide an overview of the cellular, morphological, and functional features of the BBB, and then discuss the new tools that can provide quantitative measurements of its function in health and disease. Next, we describe several BBB models with increasing levels of complexity, with a focus on the assessment of their barrier properties. Finally, we discuss models of neurological diseases in terms of current approaches and potential improvements, and we explore critical questions that remain unaddressed in the field.

2. THE BLOOD–BRAIN BARRIER

2.1. Development and Biology

Brain development and function require an effective BBB, a tight and selective interface between the blood circulation and brain parenchyma. Moreover, studies show that several brain diseases are rooted in dysfunction of the BBB. In the following subsections, we describe the development, biology, and functionality of the BBB, laying the foundation for addressing its role in the pathogenesis of brain diseases.

2.1.1. A brief history of the blood–brain barrier. In 1921, Lina Stern and Raymond Gautier (1) formally named the biological structure observed by Paul Ehrlich, Edwin Goldmann, and Max
Lewandowsky 20 years earlier “la barrière hémato-encéphalique.” Using trypan blue and potassium ferrocyanide, Ehrlich (2) had found that the blood compartment is hermetically separated from brain tissue by a barrier capable of preventing the passage of nutrients or drugs. Later, Hugo Spatz (3) observed the presence of nonfenestrated endothelial cells responsible for the properties of the blood–tissue interface. The current paradigm of the BBB was finally settled in 1967 by Reese & Karnovsky (4), following their observation, by electron microscopy, of the accumulation of horseradish peroxidase exclusively in the lumens of brain capillaries. They concluded that endothelial tight junctions (TJs) were responsible for this phenomenon and conferred on the BBB its unique barrier properties (5).

2.1.2. Embryogenesis of the blood–brain barrier. The nervous and brain vascular systems are coupled not only in their functionality but also in their developmental process. Development of the BBB occurs through two major events. First, the newly formed brain vasculature invades the future central nervous system (CNS) structures (6). Second, barrier properties are acquired via the canonical Wingless–Int1 (Wnt)/β-catenin signaling pathway, which is responsible for the upregulation of TJs in brain endothelial cells and thus the induction of barrier properties in the invading vasculature (7). Then, barrierogenesis enables endothelial cells to acquire a full BBB phenotype by increasing their expression of glucose transporter 1 (GLUT-1), claudins, and P-glycoprotein following the sustained secretion of different factors by cells of the CNS, such as platelet-derived growth factor B (PDGF-B) from pericytes (8) and retinoic acid from astrocytes (9).

2.1.3. Vasculogenesis of the blood–brain barrier. Endothelial cells of the perineural vascular plexus are the main component of the BBB and typically develop TJs during early embryonic development. Endothelial cell differentiation and BBB maintenance genes are expressed at different time points during the embryonic developmental process. They include the β-catenin gene Ctnnb1, which promotes the differentiation and maintenance of the BBB via the canonical Wnt signaling pathway (10). The development of the CNS vasculature begins via vasculogenesis, whereby mesoderm-derived angioblasts invade the head region and coalesce to form the perineural vascular plexus. This process is followed by the angiogenic formation of vessel sprouts through the secretion of vascular endothelial growth factor (VEGF) by neurons of the subventricular neuroectoderm. This angiogenic phase, characterized by interactions between vascular sprouts and neural precursor cells, is followed by the acquisition of a BBB phenotype, as evidenced by an increase in the expression of TJ proteins on endothelial cell membranes (7).

2.1.4. Perivascular cells. Astrocytes and pericytes are the two main perivascular cell types of the BBB and play key roles in its formation and properties. Both originate from the neural crest following three signaling pathways, the Delta/Notch and neuregulin/ErbB pathways (11) for astrocytes and the PDGF-B/PDGF receptor β pathway for pericytes (12). Pericytes can also derive from tissue myeloid progenitor cells or from mature macrophages in the early vascular development of the CNS (13). This double embryological origin infers the two major roles of pericytes in the BBB: barrier maturation and protection. The two cell types differ in spatial organization at the BBB. Pericytes fully wrap around the brain capillaries formed by endothelial cells, and astrocytes physically connect their endfeet to the abluminal side of the endothelium covering a small area of the vessel.

Pericytes and astrocytes have complementary functions in the BBB. Pericytes enable the expression and organization of several TJ proteins and key transporter receptors (TRs) (14) and participate in the secretion of the basement membrane surrounding BBB capillaries. Conversely, astrocytes help refine the BBB phenotype during development via their regulation of efflux TRs
VASOMOTOR CONTROL AT THE BLOOD–BRAIN BARRIER: PERICYTES, ASTROCYTES, OR SOMETHING ELSE?

Although the major elements of the BBB have been extensively studied both anatomically and histologically, their roles in the regulation of cerebral blood flow by vasomotor tuning remain ambiguous. On the one hand, Hall et al. (17) propose that pericyte contractions, resulting from smooth muscle actin contractile fibers, are activated prior to those of arteriole smooth muscle cells because neurons, which are closer to the capillaries than they are to arterioles, come in contact first with pericytes. On the other hand, Hill et al. (18) suggest that pericytes do not express smooth muscle actin, which they deem essential to generate cellular contractions. Similarly, the function of astrocytes remains debated in the context of vasomotor control in the brain. While Mishra et al. (19) demonstrated that astrocytes are a mediator of Ca\(^{2+}\)-dependent nitric oxide generation by interneurons and do not directly generate capillary contractions, others propose that caveolae expressed on astrocytes are mediators of neurovascular coupling (20) or indicate the presence of interpericyte tunneling nanotubes that mediate communication among pericytes (21). As evidenced by continuous disagreements in the field, the observed phenomena are still not fully understood at the cellular and molecular levels.

(15). In the mature BBB, astrocytes act as a filter between the contents of BBB capillaries and glial/neuronal cells in the brain parenchyma. They also maintain homeostasis between blood and brain tissue, in part via their expression of aquaporin 4 (AQP-4) (16). The full importance of pericytes and astrocytes regarding their impact on vasomotor tone at the BBB remains to be fully elucidated (see the sidebar titled Vasomotor Control at the Blood–Brain Barrier: Pericytes, Astrocytes, or Something Else?)

2.1.5. The neurovascular unit. The BBB is part of a wider structure, the neurovascular unit (NVU), which contains the BBB capillaries as well as polarized neurons and supporting cells found in the brain stroma, such as microglia, resident macrophages of the CNS, and other glial cells, along with their basement membrane. In addition to their individual functions, which involve relaying information for neurons and immune defense for microglia, these two cell types play a nonnegligible role in homeostasis, as microglial cells control proliferation and differentiation of neurons in the parenchyma and maintain BBB integrity via claudin-5 expression through their physical interactions with endothelial cells (22). Conversely, during brain injury or immunological stimulation, microglia exhibit a counterproductive response through their secretion of reactive oxygen species (ROS) to disrupt the BBB and weaken the overall integrity of the CNS (23). Neurons also play a similar dual role at the BBB. On the one hand, they regulate blood flow proportionally to their activity (24) via astrocyte engagement. On the other hand, neurons can be detrimental to the integrity of the BBB during prolonged ischemia, as they can activate astrocytes and disrupt the barrier (25).

2.2. Characteristics of the Blood–Brain Barrier

Several morphological features of the BBB inform its functional properties, ultimately governing the controlled transport of nutrients, molecules, and therapeutics from blood to brain (Figure 1).

2.2.1. Hemodynamics. Fluid flow dynamics at the BBB play an important role in determining the functional properties of this barrier. The intravascular pressure gradient between pre-BBB capillary arterioles and postcapillary venules is the primary regulator of BBB luminal flow. Dilation
of resistance arterioles increases this pressure gradient, thus increasing BBB capillary flow (30). In addition, owing to the abundance of caveolae, neurovascular coupling in arteriolar endothelial cells begins with increased neural activity and ends with smooth muscular cell relaxation surrounding the arterioles. This process ultimately causes arteriolar vasodilation and increased capillary blood flow (20). Red blood cell velocity in the BBB of rodents is remarkably high (0.5–2.0 mm/s), owing primarily to the small diameters of BBB capillaries (around 4 μm in rodents) and brain arteriolar vasodilation (31). These rapid responses and changes in blood flow in BBB capillaries are especially critical for the regulation of gas transport across the barrier and for overall brain function (20).

Increased blood flow velocities in BBB capillaries correspond to large wall shear stresses (20–40 dyn/cm²) (32), which maintain the BBB phenotype of brain capillary endothelial cells through junctional protein upregulation and transport regulation. In cerebral arteries, however, this pulsatility is typically damped once fluid reaches the BBB, which then translates to reduced interstitial flow in the parenchyma (33, 34). In addition, the absence of endothelial cell fenestrations and highly controlled transport at the brain suggest that BBB capillaries must employ an alternate system to regulate fluid flow in the interstitial space. Indeed, the discovery of the meningeal lymphatic system surrounding BBB capillaries, through which astrocytes regulate cerebrospinal and interstitial fluid flows via their AQP-4 channels, has elucidated some of the mechanisms employed by the BBB to regulate flow in the brain parenchyma (see section 1 of the Supplemental Material) (35).

### 2.2.2. Functionality

The upregulation of several TJ, adherens junction (AJ), and TR proteins at the BBB governs its reliance on selective modes of transport and informs its restricted endothelial
TRANSCELLULAR MODES OF TRANSPORT

Transcellular transport is governed primarily by the size and chemical composition of the solute:

1. Passive diffusion. Ions, gases, and other lipophilic molecules passively cross the BBB by diffusing through the lipid bilayer and cytosol of endothelial cells (46). Highly soluble lipids with low molecular weight are better able to diffuse through the barrier (37, 47).

2. Active ABC transporter efflux. Lipophilic substances that cannot passively diffuse through the BBB, such as cholesterol and long-chain fatty acids, rely instead on energy-dependent ABC transport through specific efflux pumps (P-glycoprotein, breast cancer resistance protein, multidrug resistance–associated protein).

3. Active solute carrier transport or carrier-mediated transport. Specific solute carriers expressed on BBB endothelial cells (e.g., GLUT-1, monocarboxylic acid transporters, L-type amino acid transporter 1) can actively bind and transport solutes (e.g., glucose, monocarboxylates, amino acids) into the brain parenchyma (37, 46).

4. Active transcytosis with receptor- or adsorptive-mediated transport (RMT or AMT). Although the majority of large molecules are significantly impeded in their transport across the BBB, some enter the brain via active vesicular transcytosis (46). In RMT, surface receptors bind to macromolecules, form caveolae, and exocytose the compounds on the opposite pole of the cell (46). AMT occurs in the case of nonspecific interactions between solutes and cell surfaces, such as electrostatic interactions (38).

2.2.2.1. Junctions and modes of transport. The maintenance of homeostasis at the BBB via solute transport is unique in that it is highly regulated via junctional surface proteins (36). These proteins can be categorized as TJs connecting the plasma membranes of cells and regulating paracellular transport, AJs joining the actin filaments of neighboring cells and stabilizing TJs, or TRs governing transcellular shuttling across the barrier (Supplemental Table 1) (37). Transport across the BBB adopts two major routes: paracellular and transcellular. Owing to smaller gaps between endothelial cells, paracellular transport at the BBB is impeded in comparison to other endothelial barriers (38). A few small molecules, such as osmotic or biologically active agents, transiently relax junction regulation in order to passively cross the BBB (38, 39). In general, molecules that adopt the paracellular route are smaller than ~70 kDa, as evidenced by low permeability measurements for 70 kDa dextran at the BBB (40, 41). The production of ROS and certain proteins, such as cytochalasin, also increases barrier permeability in intact microvessels, suggesting that several factors can influence transport. Limited paracellular transport at the BBB has resulted in the reliance on regulated modes of transcellular transport to maintain homeostasis (42).

During inflammation, diapedesis of mononuclear cells, such as leukocytes and monocytes, is highly upregulated across the BBB through the opening of TJs following interleukin-8 secretion (43). Tumor cells from various primary sites have also been observed to extravasate from the BBB via the paracellular route following secretion of serine proteases that disrupt TJs and AJs (44). Importantly, although transcellular exchange across the BBB is believed to be crucial, the limited resolution offered by animal models and current imaging techniques has hindered the ability to clearly discern transcellular from paracellular transport at the brain. Advances in in vitro technologies have attempted to address these challenges via high-resolution spatiotemporal imaging of transport phenomena (37, 45).
2.2.2.2. **Barrier permeability.** The unique morphological features of the BBB, such as cellular organization and TJ, AJ, and TR expression profiles, inform its distinctive functions, particularly in terms of regulated transport and toxin clearance between blood and parenchyma. To establish a direct link between architecture and transport functionality, key transport measurement parameters, such as barrier permeability and transendothelial resistance (TEER), have been employed to compare the BBB with other blood–tissue barriers.

Barrier permeability is commonly measured with the help of contrast agents or fluorescent tracers transported from blood to tissue over time (Figures 2 and 3). The choice of tracer (type, size, charge, lipophilicity, and propensity to bind to cells) and imaging technique plays a significant role in the permeability value obtained. The ability to label particles with fluorescent tags has allowed various molecules to be assessed using classical methods of permeability measurement (37). While overall transport across the endothelial cell monolayer can be assessed with the use of fluorescent tracers, different methods should be implemented to distinguish between the different mechanisms or pathways of exchange. Modifying the fluid flow characteristics or temperature of the system has been shown to influence the permeability of physiological compounds across the BBB, confirming the role of these factors in vascular permeability in vivo (45). Transport across the BBB by diffusion and convection via transmural flow can be described by permeability to solute...
Comparison of barrier permeability to solutes and TEER in terms of the type of endothelial cells employed (nonbrain, primary, immortalized, and iPSC-derived) and the type of BBB model (cell culture insert, 2D chip, 3D vessel-like, 3D self-assembled). Human iPSC-derived endothelial cells in 3D microfluidic chips exhibit the lowest permeability and highest TEER values. (a) Two methods to assess BBB permeability can be employed (the two equations, and the equivalent circuit, are extensively described in section 4.1 of the Supplemental Material). (b) Graph of permeability and TEER measurements of four cell types used as brain endothelial cells. Each data point represents a different model. In vivo TEER values are from Reference 57, and in vivo permeability values are from Reference 51 (see Supplemental Table 3). (c,d) Heat maps of the highest TEER and lowest permeability values measured in different models (up to 3 days of culture, 4–20 kDa dextrans). Red corresponds to experimental values reaching physiological values (the data, references, and rationale used to plot the graphs are presented in sections 4.2 and 4.3 of the Supplemental Material). Abbreviations: BBB, blood–brain barrier; iPSC, induced pluripotent stem cell; TEER, transendothelial resistance.

Supplemental Material

$P$, given by

$$P = P_0 + L_p (1 - \sigma)(\Delta p - \sigma \Delta \pi),$$

where $L_p$ is the hydraulic conductivity, $\Delta p$ is the pressure differential across the endothelium, $\sigma$ is the reflection coefficient, $\Delta \pi$ is the osmotic pressure differential, and $P_0$ is the diffusive permeability.

TEER, which defines barrier impedance to the passage of small ions through barriers, has also been employed as a quantitative metric to assess BBB permeability to charged solutes. Briefly, one
**Table 1  Solute permeability and TEER: What do they measure?**

| Principle                      | Solute permeability                                                                 | TEER                                                                 |
|-------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------|
|                               | Net exchange of molecules, typically of higher molecular weight, across the endothelial barrier | Transport of charged molecules or ions across the endothelial barrier |
| Biological measurement        | Diffusion and convection of molecules                                               | Paracellular transport of ions                                       |
| Typical in vitro values in human BBB models | $10^{-7}$ cm/s for 4–20 kDa dextrans                                              | 1,500 Ω/cm$^2$                                                       |
| Measured in vivo values in animals | 4 × 10$^{-7}$ cm/s [rat (51)]                                                       | 1,300 Ω/cm$^2$ [frog (52)]                                          |
| Availability                  | Most in vivo and in vitro models                                                   | In vivo and in vitro models for which the area of the endothelium is well defined |
| Correlation/translation       | Although both solute permeability and TEER measure transport of different types of solutes across the barrier, they are negatively correlated. Low permeabilities indicate reduced paracellular transport, which often correlates with restricted ionic transport and high TEER values. |                                                                     |

Abbreviations: BBB, blood–brain barrier; TEER, transendothelial resistance.

can use the voltage and current through a BBB capillary to compute the electrical resistance of the cellular barrier via Ohm’s law. One thus obtains TEER as the product of the cellular layer resistance and area; high values indicate restricted ionic transport through the BBB and optimal barrier function (Table 1). Shortly after its first implementation in frog mesentery, the TEER technique was used in rat BBB capillaries, yielding values close to 6,000 Ω/cm$^2$, significantly higher than those found in in vitro BBB models (48). These discrepancies partly reflect the relatively poor barrier function of in vitro BBB monolayer systems, where ionic transport cannot be fully recapitulated. More importantly, voltage levels applied across the BBB endothelia can play a significant role in the resulting TEER values, as shown by Sharabi et al. (49). These authors found that voltages as low as 10 V led to an ∼40% decrease in TEER due to electroporation of the cellular membrane. In addition, probe design, positioning, and shape, as well as anatomical configurations, can result in nonuniform current distributions across the barrier, reducing its effective area and overestimating its TEER (50). Finally, similarly as for solute permeability, TEER values do not provide information about transcellular transport of charged compounds. TEER measurements should be interpreted with caution, since several parameters can significantly affect the results.

3. MODELS OF THE BLOOD–BRAIN BARRIER

3.1. In Vivo Models

Much of our basic knowledge regarding the BBB first came from animal models, which elucidated the fundamental mechanisms of regulated transport, barrier permeability, cellular architecture, and associated morphological features (53–55). The clear advantage of these models is their physiological relevance, including not only all the structures forming the BBB but also the effects of blood flow or biochemical mediators of function. Thus, animal models have offered tremendous insight into the physiology of brain tissue, particularly in the context of disease modeling. However, challenges remain in the translation of animal findings to human patients. The underlying genetic, molecular, and immunologic differences between human and animal BBBs have limited the use of in vivo models as effective platforms for the development of therapeutics. This is clearly evident in the high failure rates (∼80%) in clinical trials of drugs validated in animals (37).
limitation, in combination with increased ethical concerns and high costs, has spurred the development and use of in vitro human BBB systems.

### 3.2. Cell Source

The choice of cell source is key to the development of in vitro BBB models. Important considerations include availability, human relevance, cost, robustness, reproducibility, batch-to-batch variability, capability for disease modeling, and barrier properties. Decisions regarding the species (e.g., murine, bovine, porcine, simian, or human) and cells used [e.g., cell lines, induced pluripotent stem cells (iPSCs), primary cells, or direct/transdifferentiated cells] are critical to the success of a BBB model. Here, we focus on endothelial cells employed to design in vitro BBB models, although other cells (e.g., pericytes, astrocytes, and to a lesser extent neurons) are also important.

We note that BBB endothelial cells have critical morphological and functional differences from other endothelial cells, as they possess tighter cell–cell junctions, are highly polarized, and generally exhibit lower values of permeability or higher TEER values compared with capillaries found in other tissues (56, 57). TRs, such as P-glycoprotein and GLUT-1, as well as TJ proteins, such as claudins and occludins, are key elements involved in barrier function of the BBB and need to be taken into account when selecting endothelial cells to engineer an in vitro BBB model.

#### 3.2.1. Primary cells.

Primary endothelial cells can be easily isolated from animals or human biopsies and cultured in vitro. These cells are accessible, widely used, and well documented in the literature (58). However, animal primary cells are not fully representative of human cells in either composition or function despite their common characteristics, such as TJ and TR protein expressions. Human primary cells collected from donors and used either in single-cell monolayers or in coculture models have thus been the preferred choice. Although they express high levels of platelet endothelial cell adhesion molecule 1 (PECAM-1), claudin-5, and zonula occludens 1 (ZO-1) (59), human primary cells paradoxically present low TEER values, usually under 100 Ω/cm² (Figure 3) (60). Finally, two major drawbacks of human primary cells are that they often lose their BBB properties through cell passage (61) and the source heterogeneity significantly reduces the reproducibility of experiments.

Human umbilical vein endothelial cells (HUVECs) have been grown in coculture with pericytes, astrocytes, and even neurons. Under these conditions, expression of key BBB markers, such as ZO-1 and VE-cadherin, is often upregulated. HUVECs generate stable channels or vascular networks (62). They also exhibit strong barrier function, even for 3 kDa dextran, with permeability values as low as $2 \times 10^{-7}$ cm/s, even though they are not specific to the brain microvasculature (45, 63). These observations suggest that endothelial cells alter their phenotype depending on their coculture conditions and the stromal cells employed in the model.

#### 3.2.2. Immortalized cells.

Owing to their low cost and ease of culture, immortalized cell lines are now extensively employed in in vitro BBB models. They retain their properties following cell passage and are genetically identical to one another, thus improving reproducibility in BBB models. Despite these advantages, cell lines generally present poor BBB properties and barrier function and may not be suitable for molecular transport studies (64). In addition, rodent BBB cell lines have been observed to lose their in vivo features, such as the expression of GLUT-1 (65).

Human cells lines, in contrast, can present inconsistent protein levels. For example, the TY10 cell line expresses high levels of claudin-5 and VE-cadherin, while the hCMEC/D3 line has the highest expression level of ZO-1 but no claudin-5 (66). These inconsistencies imply that different cell lines might be optimal, depending on the application of the study (67, 68). Because of low TJ
or TR expression levels, immortalized cell lines are not capable of creating a tight cell layer and may not be relevant for general BBB modeling but only for specific studies such as drug absorption or pathogen transmigration across the BBB.

A promising cell source derived from stem cells has garnered increasing attention over the last decade. This includes cells differentiated from embryonic or mesenchymal stem cells (ESCs or MSCs) and iPSCs, which are stem cells generated from a differentiated tissue. Stem cells are usually defined by their capacities to self-renew and to generate multiple different cell types.

### 3.2.3. Embryonic and mesenchymal stem cells
ESC are pluripotent stem cells (PSCs) with the ability to differentiate into all cell types. ESCs from the blastocyst can be employed to recapitulate the embryonic development of brain microvascular endothelial cells (BMECs), and several embryoid differentiation protocols have been generated for this purpose. Generally, 3D embryoid differentiation protocols enable direct differentiation into the three embryonic layers. Yet the yield with these protocols is low, and there is a need for further isolation and expansion. In contrast, 2D monolayer differentiation protocols offer a high yield but generally result in the generation of a single cell type (69).

Various types of human ESC lines can be employed, depending on the desired protein expression of the resulting differentiated cells. For example, H1 and H9 are used for PECAM-1 expression (70), and HES3 and HES4 are employed for VE-cadherin expression (71). ESCs are also used in the context of modeling development in vitro. For example, Weidenfeller et al. (72) demonstrated that undifferentiated neural progenitor cells do not express BBB properties but differentiated cells do. In addition to ESCs, MSCs, which give rise to embryonic connective tissue, can improve the stabilization of the resulting BBB models.

### 3.2.4. Induced pluripotent stem cells
Since their development by Yamanaka and colleagues (73), human iPSCs have found application in numerous in vitro models. Yet, there is still no consensus in terms of a single differentiation protocol to produce BMECs from iPSCs (iBMECs). Protocols vary in length, number of steps, and differentiation factors employed, such as retinoic acid and Wnt/β-catenin agonist (74) or hypoxia (75). However, common to all protocols is a succession of steps including iPSC culture, endothelial induction, BMEC specification, and purification (76).

iPSCs have the considerable advantage that they can, in principle, produce all the cells composing an organ while maintaining genetic consistency among all the differentiated cells, thus enabling the generation of a patient-specific BBB model. In addition, they present excellent barrier properties, with strong expression of BBB markers such as ZO-1, claudin-5, and occludin as well as cell activity markers such as GLUT-1 and P-glycoprotein. Finally, their TEER and permeability values are comparable to those measured in vivo (Figure 3). However, in contrast to cell lines and primary cells, they often have a limited life span in vitro, and processes ranging from dedifferentiation to redifferentiation are complex, time consuming, and very sensitive to experimental conditions.

As mentioned above, iPSCs show promise for personalized medicine applications, wherein a model based on the patient’s own genotype is constructed (77). For example, Vatine et al. (78) modeled psychomotor retardation with iPSCs from patients deficient in monocarboxylate transporter 8. In addition, such models need not focus solely on inducing BMECs but can include all relevant components of the NVU (79). However, one needs to keep in mind several key elements when inducing iBMECs, such as the expression of canonical endothelial cell markers (cadherin-5, PECAM-1, VEGF receptor 2, apelin receptor, endothelial nitric oxide synthase), ability to form...
tubular networks, low-density lipoprotein uptake, response to angiogenic stimuli, high TEER/low permeability, and expression of critical efflux transporters (80).

While human primary cells appear to be the best source of cells for BBB models, they tend to lose their BBB properties over time and present weak barrier properties. In order to overcome these limitations, iPSCs are being employed to build complex BBB models including different cell types with a common genome. Note, however, that iPSCs have a nonnegligible tumorigenic and teratogenic potential. As a result, one must take care to eliminate nonusable or potentially tumorigenic differentiated cells.

3.3. In Vitro Models

The development of BBB models with physiological transport properties has been a major challenge since the 1980s. Cells were first cultured in two dimensions by use of a single cell type to mimic the BBB. Gradual increases in the complexity and functionality of in vitro BBB models enabled the generation of sophisticated platforms including multiple interacting cell types that can be dynamically stimulated, as well as the presence of hydrogels to mimic the 3D brain extracellular matrix (ECM). Some of these elements have been included in Transwell® systems and microfluidic platforms. Here, we classify systems as either 2D or 3D; 3D models are defined as those with hydrogels at least 100 μm thick.

3.3.1. 2D static models. In 2D static models, cells are cultured on hard plastic (Figure 2a) or in Transwell inserts without the application of flow (Figure 2b). Historically, the first models of the BBB consisted of monocultures of endothelial cells isolated from brain capillaries. These allowed for the observation of TJ structures via freeze fracture (86). In order to recapitulate the BBB microenvironment and reduce the lack of neighboring cell stimuli, coculture models were developed, notably with a full NVU composition of endothelial cells, astrocytes, pericytes, and neurons (87). Culturing cells on plastic offers several advantages in terms of ease of imaging and environmental control, as well as the presence of several standard protocols. However, hard plastics do not recapitulate the interface between vascular and brain parenchymal compartments, the major aspect of the BBB, thus limiting the utility of these models to assess BBB functionality through metabolic, diffusion, or permeability studies. Finally, the stiffness of tissue culture plastics, their flat geometry, and the lack of ECM in 2D layer systems all limit cell development, alter cell phenotype, and result in poor models with limited potential for clinical translation (88).

Transwell systems can overcome these challenges, as they offer the ability to perform permeability assays, observe cell migration across a membrane, study multicellular interactions, and promote epithelial cell polarity (89). Transwell systems have progressively improved and are now widely used owing to their broad commercial availability. While these systems are static, several parameters can be optimized to improve the resulting models, such as the size of the inserts, their membrane porosity, their thickness, and the type of material (polymer) employed, with or without coating (90). Transwell systems allow for the introduction of at least two cell types, usually (a) endothelial cells on top and (b) astrocytes, pericytes, and sometimes neurons either in direct contact with the endothelial cells, when cultured on the opposite side of the membrane, or in indirect contact, when cultured at the bottom of the well (81). Transwell models of the BBB have been generated with all types of cells (primary cells, cell lines, and iPSCs), and they enable measurement of barrier properties such as TEER and solute permeability (56). However, their flat geometry limits cellular interactions that could promote barrier properties or the polarization of astrocytes, resulting in subpar BBB models. Hybrid devices containing 3D gels on Transwell inserts have recently been developed and are referred to as chip-on-a-Transwell systems (91). Nevertheless, the
presence of a Transwell insert introduces artifacts and limits the natural interactions between cells and the underlying ECM, resulting in poor recapitulation of the natural morphology of the BBB.

**3.3.2. 2D organs-on-a-chip.** Standard in vitro models lack cell–cell interactions and do not permit accurate control of hemodynamics (92) or appropriate organization of the microenvironment represented. These limitations have been mitigated by the introduction of organ-on-a-chip (OoC) models in which cells can be compartmentalized in several channels, often separated by a membrane. These systems facilitate cell–cell interactions, the application of fluid flow and controlled shear stresses, and real-time readouts (93). This technology can be used to generate multiorgan systems by linking different OoCs together, producing so-called human-on-a-chip platforms (94–96). Recent developments in microfluidic chip technologies have enabled the integration of sophisticated sensors (e.g., microelectrode arrays, TEER electrodes, oxygen tension probes) and measurements (e.g., mass spectroscopy analyses) (97, 98). Given the complexity of these additions, several OoC systems lack sensors and rely on the use of Transwell systems to perform parameter measurements such as TEER. The results obtained in Transwell systems might not be representative of those in OoCs, however, and they are sometimes mathematically corrected, which can introduce a degree of error (99). TEER values, for example, vary according to the cell type and BBB model employed. Currently, iPSC models (79) present the lowest permeability values and the highest resistance, where TEER values reach or exceed physiological values of 1,300 Ω/cm² (Figure 3c) (52).

2D BBB-on-a-chip (BBBoC) models are defined as linear systems in microfluidic chips that incorporate two or more BBB cells with hydrogels no more than 100 μm thick. Compared with Transwell platforms, BBBoC systems offer greater physiological relevance and complexity and can facilitate the observation of cell migration/protrusion (100) and angiogenesis (85). They have been employed to recapitulate the effects of hypoxia on the BBB by use of both primary cells and iPSC models (Figure 2c) (75, 82). BBBoCs are a powerful tool for studying BBB physiology in terms of membrane protein expression (TJs, AJs, and TRs) or cell–cell interactions such as the formation of astrocyte endfeet (100). Recent studies have incorporated other CNS cells into BBBoC models in order to produce an NVU platform to uncover metabolic pathways between the vasculature and brain parenchyma in health and disease (101, 102). More complex models including five (brain, intestine, liver, kidney, and skeletal muscle) or eight organs (i.e., human-on-a-chip models) have been designed and employed in pharmacological studies (94, 95).

Despite these advances in 2D OoC technologies, several challenges remain regarding miniaturizing these systems and increasing their throughput to perform large-scale drug screens (103). Finally, there has been a major push toward 3D models because of their ability to better recapitulate organ form and function.

**3.3.3. 3D models.** Researchers’ attention is shifting to 3D brain models, as they can better mimic the architecture and function of the BBB while offering greater physiological relevance. Several platforms have been proposed to generate human-like brain models, particularly with the use of biocompatible hydrogels, relevant cell types, and techniques drawing from the process of embryogenesis.

**3.3.3.1. Monolayer of cells in a gel system.** As mentioned above, we define 3D BBB models as those for which the BBB cells are cultured adjacent to a hydrogel with a thickness of at least 100 μm. One example is the model developed by Ahn et al. (104), who created a platform with human brain pericytes and endothelial cells in a microfluidic device with astrocytes on the bottom, seeded in Matrigel (Figure 2f). The astrocytes extended their AQP-4-expressing endfeet to
establish contact with the pericytes and endothelial cells, similar to the behavior observed in vivo (104). The simple design and ease of use of 3D monolayer brain models, coupled with their increased physiological relevance, have been instrumental in the generation of high-throughput in vitro brain models. With the help of multichannel microfluidic chips, investigators have engineered identical BBB platforms for use as functional assay platforms, notably for studies of metastasis or drug transport (105). Importantly, when compared with 2D BBB models, brain endothelial cells cultured in 3D platforms exhibit improved TJ expression and increased TEER values, suggesting that the addition of appropriate matrices plays a fundamental role in the integrity and function of the resulting BBB model (106). Additionally, the application of physiological levels of fluid flow and resulting wall shear stresses improve TJ protein expression as well as solute permeability and TEER values (105). In comparison to 2D systems, the ability of 3D brain models to recapitulate physiological BBB hemodynamics is paramount in the function of the barrier.

### 3.3.3.2. Vessel-like structures in gels.

Microfluidic technology has played a significant role in the development of BBB models with increased complexity and realism, giving rise to a new approach to in vitro 3D BBB models: 3D channels embedded in or adjacent to hydrogel compartments. These platforms possess several desirable features in terms of morphology, cellular organization, high-throughput capabilities, and improved barrier function. Importantly, they better recapitulate important aspects of BBB morphology consisting of hollow tubular structures lined with endothelial cells and variably cocultured with neural stromal cells, all embedded in a 3D hydrogel. For example, Marino et al. (83) used two-photon lithography to generate porous tubular structures with an average diameter of 10 μm, which were subsequently coated with mouse BBB endothelial cells (Figure 2d).

Similar platforms with increasing levels of complexity have been designed. They incorporate hydrogels with improved physiological relevance (107) and additional BBB cell types such as pericytes and astrocytes. Both Brown et al. (108) and Adriani et al. (109) employed multichannel microfluidic chips in which one of the channels was lined with primary human brain endothelial cells, with combinations of pericytes, astrocytes, and neurons in an adjacent collagen hydrogel. Although collagen type 1 is commonly used in cell culture systems, it is not usually found in the brain parenchyma; the use of more physiological matrices, such as hyaluronic acid– or proteoglycan-based hydrogels, could improve the BBB properties of these models (110). In addition, the morphological attributes and cellular organization of these platforms do not fully recapitulate the BBB, either because of their large diameter (typically approximately 500 μm) or because of the lack of stromal cells with appropriate spatial distribution. Herland et al. (84) addressed some of these challenges by generating hollow cylindrical structures in a collagen gel via a pressure-driven viscous fingering method (Figure 2e). The channel lumens (still large, with diameters of 600–800 μm) were perfused with pericytes or astrocytes followed by human BMECs, which resulted in a decrease of barrier permeability to solutes in comparison to platforms without brain stromal cells (84). To address the remaining challenge of reducing vessel diameter and improving vascular morphology, several groups have generated self-assembled vascular systems that recapitulate in vitro the inherent ability of endothelial cells to self-assemble into vascular networks with small-diameter lumens.

### 3.3.3.3. Self-assembled vasculatures and the role of emergence.

To better recapitulate the morphological and functional features of the BBB, researchers have focused on harnessing the natural processes of vascular development in vivo, whereby endothelial precursors coalesce and organize into complex microvascular networks (vasculogenesis) and endothelial cells sprout from preexisting blood vessels (angiogenesis) (111). This inherent ability of endothelial cells to form tubular
structures has been extensively employed to engineer 3D vascular networks with HUVECs and lung stromal cells or placental pericytes; the resulting structures exhibit improved morphology, TJ protein expression, and solute transport machinery in comparison to other 3D tubular structures or 2D models (112). Extrapolating from these models, Bang et al. (113) incorporated astrocytes and neurons into a HUVEC–fibroblast platform to mimic the BBB in three dimensions. While this model includes non-brain-specific cell types, it offers valuable insight into the role astrocytes play in promoting the expression of BBB properties (113).

Challenges in isolating tissue-specific human endothelial cells and ensuring that they retain their properties when cultured in vitro have hindered the development of 3D OoC self-assembled models, particularly BBB microvascular platforms. However, certain commercial human BMEC lines isolated from brain biopsy samples do retain their ability to self-lumenize when cultured in appropriate 3D hydrogels. For instance, Lee et al. (85) used human BMECs, primary brain pericytes and astrocytes, and lung fibroblasts to create via angiogenesis in a microfluidic chip a 3D self-assembled BBB model that exhibited improved barrier permeability and increased expression of TJ proteins. In another model (29), self-assembled BBB vasculatures were generated via vasculogenesis using human iPSC-derived endothelial cells, as well as primary brain pericytes and astrocytes embedded simultaneously in a fibrinogen–thrombin gel, without the need for cell types from different organs for vascular support (e.g., lung fibroblasts). The self-assembled BBB vessels exhibited physiological morphologies with diameters as small as 20 μm and decreased solute permeability, comparable to levels measured in rat BBB (Figure 3) (Supplemental Tables 2 and 3).

These self-assembled vascular systems have revolutionized the field of in vitro BBB models, significantly improving their physiological relevance and allowing for high-resolution spatiotemporal imaging of key events at the BBB, such as cell diapedesis or solute transport. Despite these advances, challenges regarding the choice of hydrogels in these systems remain. The common use of fibrinogen gels for 3D angiogenic and vasculogenic BBB models does not fully recapitulate the in vivo brain ECM, which comprises mostly hyaluronic acid and other proteoglycans. In addition, fluid flow, which is not included in most in vitro self-assembled BBB models, exhibits specific dynamic patterns at the brain that promote the upregulation of TJ and transporter proteins (31, 114). Recapitulating BBB hemodynamics, and thus improved transport of gases and nutrients, would be paramount for the maintenance of long-term cultures in vitro, since the current engineered BBB vessels are known to regress after a couple of weeks.

### 3.3.3.4. Cerebral organoids and the blood–brain barrier.

The last ten years have witnessed the development of 3D brain organoids from the differentiation of PSCs, such as iPSCs or ESCs. These systems harness the entire development process to generate structures that recapitulate specific brain regions, such as midbrain organoids or cerebral cortex organoids that house BBB capillaries (115). Cerebral organoids that model neurological diseases such as Alzheimer’s disease (AD) have also been created through the use of patient-derived iPSCs (116).

Despite successes in generating brain organoids, these structures still lack appropriate vasculature. Pham et al. (117) addressed this limitation by engineering discrete vascular sprouts in their patient-derived iPSC cerebral organoids by using differentiated endothelial cells from the same iPSCs. Although a few CD31-positive blood vessel sprouts were observed, they were not connected, and perfusion was not achieved in these structures (117). In another cerebral organoid model, Cakir et al. (118) engineered ESCs to express human ETS variant 2, which reprograms human dermal fibroblasts into endothelial cells, promoting vascularization. However, BBB vessels were observed only at the organoid surface. Homan et al. (119) employed a similar approach, based on the application of fluid flow, to enhance the vascularization of kidney organoids.
High-shear-stress flow resulted in the most effective vessel formation in the surrounding ECM in comparison to static conditions and low-shear-stress flow. These results obtained in kidney organoids could be extrapolated to cerebral structures to enhance vascularization, confirming the importance of hemodynamics in BBB modeling and function. These models attest to the significance of design considerations, such as the application of fluid flow, the choice of 3D matrix, and the use of appropriate growth factors in the vascularization of cerebral organoids. Recapitulating organ development at the brain in these structures also offers insight into the development of the brain cortex, particularly in the context of developmental neurological disorders.

3.3.3.5. 3D-printed models. Microfabrication of OoCs can be a long, expensive process that requires clean-room facilities and fabrication engineers and offers little flexibility in modifying the design of the chip. Moreover, OoCs are not truly 3D organ models. 3D printing is now a well-established technique that overcomes these issues. 3D printing, or additive manufacturing, consists of layer-by-layer production of a computer-aided design file. In order to print organ models, hydrogels are often used as bioinks, which are networks of polymeric and hydrophilic molecules such as gelatin (collagen) derivatives like gelatin methacryloyl (120), hyaluronic acid, or alginate (121). These bioinks integrate cells such as neurons, astrocytes, HUVECs, or glioblastoma cells (122). In order to print different types of cells, either multinozzle printers can be used simultaneously (123) or a single-nozzle printer can be employed to sequentially print multilayered bioinks in which each layer contains a different cell type (124).

Several groups have generated BBBoC models of glioblastoma using 3D printing of compartmentalized concentric tumor/vascular compartment structures in order to study the effect of drugs and radial oxygen gradients on tumor progression (123). The cells were surrounded by polydimethylsiloxane to ensure chip integrity (123). Other researchers have built tubular printed vessels-on-a-chip with excellent properties (e.g., TEER of 1,500 Ω/cm²) (125). Finally, bioinspired synthetic scaffolds have been designed for the coculture of endothelial cells and neurons via two-photon lithography (83). 3D-printed models differ from microfluidic ones in terms of tissue maturation and self-assembly. Two techniques are generally used to print vessels: indirect printing, which involves the use of a sacrificial material employed to generate the vessel lumens (126), and direct printing, where lumens are assembled without sacrificial structures. Vascular lumens can be formed by seeding cells either inside the preformed, multilayer tubes (127) or within porous materials (128).

4. BLOOD–BRAIN BARRIER–RELATED DISEASES: CURRENT MODELS AND IMPROVEMENTS

Multiple neurological or brain diseases are known to affect the BBB through the secretion of inflammatory cytokines that degrade TJs (129). Because of the limited spatiotemporal resolution of in vivo models and the challenge of translating results from animal to human studies, several groups have shifted their efforts to the development of in vitro human BBB disease systems. These platforms also enable the generation of high-throughput identical models that can be used for studies pertaining to drug delivery across the BBB.

4.1. Alzheimer’s Disease Models

According to the two-hit vascular hypothesis of AD, BBB damage is thought to be the initial insult that results in diminished brain perfusion and neuronal injury (the first hit), followed by amyloid-β (Aβ) accumulation due to faulty clearance and increased antibody production (the second hit) (130). In an early 2D Transwell model of AD with patient-derived BBB endothelial cells,
Mackie et al. (131) uncovered the role of the endothelial cell receptor for advanced glycation end products and scavenger receptor in the transcytosis of Aβ-40 into the brain parenchyma. More recently, Robert et al. (132) used a 3D in vitro vessel-like structure of brain endothelial cells, lined with smooth muscle cells and astrocytes, to show that apolipoprotein E2 (APOE2) can reduce the accumulation of amyloid plaques. While these models provide useful insight into the mechanisms of accumulation and clearance of Aβ, they do not fully recapitulate AD pathology in the context of neuron–BBB interaction and BBB disruption.

Microfluidic technologies have addressed some of these challenges. Recently, Shin et al. (133) developed a 3D microfluidic model with a vessel-like structure consisting of primary brain endothelial cells adjacent to neural progenitor cells. In this model, mutations in the amyloid precursor protein and presenilin-1 genes in neurons resulted in their robust secretion of Aβ as well as an observable deposition at the endothelial surface, corresponding to an increase in barrier permeability. In another 3D AD BBB model of endothelial cells, pericytes, and astrocytes differentiated from the same iPSCs in Matrigel, APOE4 pericytes exhibited upregulated calcineurin signaling, which is associated with cerebral amyloid angiopathy, a common precursor to AD (134). Despite their improved throughput and physiological relevance, 3D AD BBB systems, which are cultured for only a few weeks, remain limited in their ability to recapitulate barrier dysfunction and plaque deposition, which occur over the course of multiple years in humans. The emergence of cerebral organoid models that can be cultured for several months might provide a way to model and study long-term progressing neurological disorders such as AD.

4.2. Stroke Models

Strokes, primarily ischemic in nature, are characterized by disruptions of the BBB wherein hypoxia and ROS production during the ischemic insult lead to TJ breakdown and subsequent ionic shifts in the brain parenchyma (135). Hundreds of drugs have been reported to effectively improve outcomes in experimental animal models. However, almost all of them have failed to translate into successful therapies for humans (136). Several groups have thus developed in vitro human BBB models of stroke to better recapitulate this pathology. For instance, Chen et al. (137) created a BBB stroke Transwell model with primary brain endothelial cells and astrocytes subjected to oxygen and glucose deprivation, showing that the increase of Sirtuin-1 following stroke and ischemia was correlated with ROS production and subsequent inflammation and cell damage. Similarly, Chaitanya et al. (138) subjected a Transwell BBB model with primary brain endothelial cells, astrocytes, and neurons to various metabolic stressors, such as hypoxia, glucose deprivation, and reoxygenation. These stressors induced brain capillary tube formation and angiogenesis in vitro and promoted lymphocyte adhesion via increased expression of adhesion markers on the BBB endothelial cells (138). Although these models could be employed as high-throughput platforms for drug screening, they remain severely limited in their ability to mimic the architecture and physiology of the BBB. More importantly, current human in vitro stroke models primarily mimic the lack of oxygen and glucose at the brain, disregarding other key features of strokes such as inflammatory cytokine production and microglial activation (135).

4.3. Brain Cancer Models

Given the poor prognoses of primary brain cancers, the prevalence of metastatic tumors in the brain, and the lack of in vitro brain tumor platforms that incorporate BBB vessels, there is a significant need for models that can aid in the understanding of cancer progression and drug delivery across the BBB. In one recent example, Plummer et al. (139) designed a high-throughput glioblastoma–neuron–astrocyte tumor platform to test drug transport and found that therapeutics
selectively target the glioblastoma cells. More recently, in vitro primary brain cancer models that integrate vasculature and tumor have been designed. Ngo & Harley (140) developed a coculture model of glioma cells with HUVECs and lung fibroblasts in gelatin and hyaluronic acid and observed that the addition of cellular and matrix elements led to a decrease in the response of glioma cells to therapeutics. Similarly, Ozturk et al. (141) designed a long-term 3D in vitro model of glioblastoma tumor spheroids surrounded by bioprinted HUVEC gelatin channels to study drug delivery over time. Although these models are useful to screen for therapeutic toxicity, the use of HUVECs or lung fibroblasts does not fully recapitulate cell types found in the brain, and the 3D architecture of these systems could be improved to replicate the in vivo BBB vasculature in contact with tumors.

Given the prevalence of metastatic brain tumors originating from various primary sites, models have been designed to elucidate the mechanisms employed by cancer cells to cross the BBB. For example, Bos et al. (142) observed that increased expression of the sialyltransferase gene ST6GALNAC5 promotes extravasation in a HUVEC–astrocyte Transwell system. In another study using a high-throughput microfluidic platform with brain endothelial cells and astrocytes in collagen, Xu et al. (105) found that lung and breast tumor cells exhibit greater transmigration potential in comparison to melanoma and liver cancer cells. While these high-throughput models allow for high-resolution spatiotemporal imaging of tumor extravasation, their physiological relevance remains limited, particularly in terms of the cell types employed and the 3D architecture of the BBB capillaries.

4.4. Infectious Disease Models

Although the BBB is a highly restrictive endothelial barrier, bacteria and viruses are still able to enter the parenchyma. Two general mechanisms are thought to be responsible for their transport: increased barrier permeability following infection and active transport via receptor-mediated transport (143). Infectious diseases known to target the BBB include Zika virus disease, malaria, and, more recently, coronavirus disease 2019 (COVID-19), yet in vitro models of these pathologies are limited. The use of patient data revealed that SARS-CoV-2, the virus that causes COVID-19, employs the S1 unit of its spike protein to attach to angiotensin-converting enzyme 2 receptor on cells and infect the patient (144). Buzhdygan et al. (145) used primary brain endothelial cells to engineer 2D and 3D BBB models showing that the spike protein increases inflammation, barrier permeability to dextran, and expression of adhesion molecules on brain endothelial cells, suggesting cellular activation and BBB breakdown. Despite its simple architecture and lack of brain stromal cells, the COVID-19 in vitro platform offers useful and important information about this globally significant disease.

4.5. Other Diseases: In Vitro and Computational Models

Several other neurological diseases are known to predominantly affect the BBB (129, 146, 147). Although not discussed extensively in this review, these include neurodegenerative disorders such as Parkinson’s and Huntington’s diseases, as well as epilepsy and mental disorders such as schizophrenia, clinical depression, and anxiety disorder. In vivo animal models, although useful to understand the genetic and phenotypic profiles of these diseases, often fail in their translation to meaningful clinical results. Yet, the use of in vitro platforms to recapitulate these pathologies remains limited. Lim et al. (148) designed a 2D in vitro model of Huntington’s disease by using patient iPSC-derived endothelial cells. They uncovered BBB deficits in terms of angiogenic signaling, transcytosis protein expression, and overall barrier permeability. Similar models that recapitulate other neurological diseases are necessary to bridge the gap between in vivo models and clinical outcomes.
Despite significant advances in in vitro technologies, the complexity of the BBB and its transport characteristics cannot be entirely captured by these systems. Several scientists have employed computational models to address these limitations and investigate various BBB pathologies such as brain tumors, AD, and traumatic brain injury (149). These computational approaches, ranging from molecular dynamics to partial differential equation–based models, finite-element methods, and pharmacokinetics, are used to investigate drug design and delivery at the brain, as well as BBB pathologies. For instance, Adhikari et al. (150) designed an in silico model of traumatic brain injury and its effect on lipid bilayer damage at the BBB by using a coarse-grained molecular dynamics simulation. Their simulation showed that low-intensity shock waves in the presence of nanobubbles can break claudin–TJ interactions between adjacent endothelial cells of the BBB, thus damaging the barrier. While current simulations are relatively straightforward to modulate in comparison to complex cellular systems, they do not fully recapitulate all the relevant properties of the BBB, particularly in the context of neurological diseases. Large data sets of readily available 3D images of the BBB and information about its properties have certainly promoted the generation of improved computational models of the brain in health and disease, notably through the use of machine learning and artificial intelligence technologies. These novel techniques will foster the development of more complex BBB simulations that can incorporate patient-specific pathologies, thus allowing for the evaluation of disease progression or drug delivery with greater ease.

5. CHALLENGES AND FUTURE DIRECTIONS

As the gatekeeper of the brain, the BBB has attracted tremendous interest for its role in both health and disease. This requires the design of relevant in vitro human models that recapitulate its key morphological and functional properties. Despite significant recent advances in the ability to engineer novel in vitro systems, major challenges remain, such as incorporating all the relevant cell types of the BBB, employing an appropriate 3D hydrogel to recapitulate the brain ECM, and achieving vascularization in vitro to emulate transport across this barrier, notably for therapeutics in the context of neurological diseases. Future efforts and advances in the vascularization of cerebral organoids that incorporate all the relevant BBB cells in a brainlike matrix will certainly provide platforms that can accurately recapitulate the human BBB. More importantly, there is a need to further improve permeability measurement techniques in order to capture changes in barrier integrity in health and disease. Next-generation in vitro BBB models with tunable transport properties will certainly provide new avenues to modulate barrier function and recapitulate neurological pathologies with greater accuracy. In addition, connecting in vitro brain models with other OoCs and perfusing the interconnected systems with patient plasma or blood will recapitulate upstream effects from other organs on the BBB or systemic effects from perfused blood on the multi-OoCs. Yet, prior to engineering these complex physiological BBB models, it will be crucial to characterize the cells of the brain and their specific markers and genotypes. Current techniques rely mostly on in vitro characterization tools following isolation, sorting, and culture of cells obtained from fresh brain tissue, where loss of function and markers is inevitable. The development of novel mapping techniques for gene expression in vivo will allow for the generation of accurate baseline characteristics of the different cell types of the BBB. These can then be employed as benchmarks for in vitro BBB models, notably for the generation of stem cell–derived brain-specific cells that can be engineered to overexpress relevant BBB-specific genes.

DISCLOSURE STATEMENT

R.D.K. is a cofounder of AIM Biotech, a company that markets microfluidic systems, and receives research support from Biogen, Amgen, and Gore. The other authors are not aware of any
affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to our colleagues whose research we could not cite or discuss owing to space limitations. C.H. is supported by a Ludwig Center for Molecular Oncology Graduate Fellowship. C.H. and R.D.K. acknowledge funding from the National Cancer Institute (U01 CA202177) and the National Science Foundation (CBET-0939511). B.L. is supported by a PhD fellowship from the Biomedical Engineering Department of Tel Aviv University. B.L. and B.M.M. acknowledge funding from the Azrieli Foundation, the Israel Science Foundation (2248/19), and the European Research Council (SweetBrain 851765).

LITERATURE CITED

1. Stern L, Gautier R. 1921. Récherches sur le liquide céphalo-rachidien. I. Les rapports entre le liquide céphalo-rachidien et la circulation sanguine. Arch. Int. Physiol. 17:138–92
2. Ehrlich P. 1885. Das Sauerstoff-Bedarfss des Organismus: Eine farbenanalytische Studie. Berlin: Hirschwald
3. Spatz H. 1934. Die Bedeutung der vitalen Färbung für die Lehre vom Stoffaustausch zwischen dem Zentralnervensystem und dem übrigen Körper. Arch. Psychiatrie Nervenkrankh. 101:267–358
4. Reese TS, Karnovsky MJ. 1967. Fine structural localization of a blood-brain barrier to exogenous peroxidase. J. Cell Biol. 34:207–17
5. Saunders NR, Dreifuss JJ, Dziegielewska KM, Johansson PA, Habgood MD, et al. 2014. The rights and wrongs of blood-brain barrier permeability studies: a walk through 100 years of history. Front. Neurosci. 8:404
6. Risau W. 1997. Mechanisms of angiogenesis. Nature 386:671–74
7. Daneman R, Agalli D, Zhou L, Kuhnert F, Kuo CJ, Barres BA. 2009. Wnt/β-catenin signaling is required for CNS, but not non-CNS, angiogenesis. PNAS 106:641–46
8. Lindahl P, Johansson BR, Leåen P, Betsholtz C. 1997. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 277:242–45
9. Mizsei MR, Wooldrik D, Lakeman KAM, van het Hof B, Drexhage JAR, et al. 2013. Retinoic acid induces blood-brain barrier development. J. Neurosci. 33:1660–71
10. Hupe M, Li MX, Kneitz S, DavydoVA D, Yokota C, et al. 2017. Gene expression profiles of brain endothelial cells during embryonic development at bulk and single-cell levels. Sci. Signal. 10:eaag2476
11. Levison SW, de Vellis J, Goldman JE. 2005. Astrocyte development. In Developmental Neurobiology, ed. MS Rao, M Jacobson, pp. 197–222. New York: Springer. 4th ed.
12. Ormestad M, Astorga J, Carlsson P. 2004. Differences in the embryonic expression patterns of mouse Foxf1 and -2 match their distinct mutant phenotypes. Dev. Dyn. 229:328–33
13. Yamamoto S, Muramatsu M, Azuma E, Ikutani M, Nagai Y, et al. 2017. A subset of cerebrovascular pericytes originates from mature macrophages in the very early phase of vascular development in CNS. Sci. Rep. 7:3855
14. Dore-Duffy P, Cleary K. 2011. Morphology and properties of pericytes. In The Blood–Brain and Other Neural Barriers, ed. S Nag, pp. 49–68. New York: Humana
15. Nag S. 2011. Morphology and properties of astrocytes. Methods Mol. Biol. 686:69–100
16. Armulik A, Genové G, Måe M, Nisancioglu MH, Wallgard E, et al. 2010. Pericytes regulate the blood-brain barrier. Nature 468:557–61
17. Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, et al. 2014. Capillary pericytes regulate cerebral blood flow in health and disease. Nature 508:55–60
18. Hill RA, Tong L, Yuan P, Murikinati S, Gupta S, Grutzendler J. 2015. Regional blood flow in the normal and ischemic brain is controlled by arteriolar smooth muscle cell contractility and not by capillary pericytes. Neuron 87:95–110
19. Mishra A, Reynolds JP, Chen Y, Gourine AV, Rusakov DA, Attwell D. 2016. Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. *Nat. Neurosci.* 19:1619–27
20. Chow BW, Núñez V, Kaplan L, Granger AJ, Bistrong K, et al. 2020. Caveolae in CNS arterioles mediate neurovascular coupling. *Nature* 579:106–10
21. Alarcon-Martinez L, Villafranca-Baughman D, Quintero H, Kacerovsky JB, Dotigny F, et al. 2020. Interpericyte tunnelling nanotubes regulate neurovascular coupling. *Nat. Commun.* 10:5816
22. Haruwaka K, Ikegami A, Tachibana Y, Ohno N, Konishi H, et al. 2019. Dual microglia effects on blood brain barrier permeability induced by systemic inflammation. *Nat. Commun.* 10:5816
23. Hanisch UK, Kettenmann H. 2007. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10:1387–94
24. Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, et al. 2003. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat. Neurosci.* 6:43–50
25. Li YN, Pan R, Qin XJ, Yang WL, Qi Z, et al. 2014. Ischemic neurons activate astrocytes to disrupt endothelial barrier via increasing VEGF expression. *J. Neurochem.* 129:120–29
26. Charabati M, Rabanel JM, Ramassamy C, Prat A. 2020. Overcoming the brain barriers: from immune cells to nanoparticles. *Trends Pharmacol. Sci.* 41:42–54
27. Weiss N, Miller F, Cazaubon S, Couraud PO. 2009. The blood-brain barrier in brain homeostasis and neurological diseases. *Biochem. Biophys. Acta Biomembr.* 1788:842–57
28. Rodriguez-Baeza A, Reina-De La Torre F, Ortega-Sanchez M, Sahuquillo-Barris J. 1998. Perivascular structures in corrosion casts of the human central nervous system: a confocal laser and scanning electron microscope study. *Anat. Rec.* 252:176–84
29. Campisi M, Shin Y, Osaki T, Hajar C, Chiono V, Kamm RD. 2018. 3D self-organized microvascular model of the human blood-brain barrier with endothelial cells, pericytes and astrocytes. *Biomaterials* 180:117–29
30. Cipolla MJ. 2016. *The Cerebral Circulation*. San Rafael, CA: Morgan & Claypool. 2nd ed. Colloq. Ser. Integr. Syst. Physiol. Mol. Funct.
31. Follain G, Osmani N, Azevedo AS, Allio G, Mercier L, et al. 2018. Hemodynamic forces tune the arrest, adhesion, and extravasation of circulating tumor cells. *Dev. Cell* 45:33–52
32. Destefano JG, Jamieson JJ, Linville RM, Searson PC. 2018. Benchmarking in vitro tissue-engineered blood-brain barrier models. *Fluids Barriers CNS* 15:32
33. Zarrinkoob L, Ambarki K, Wahlin A, Birgander R, Carlberg B, et al. 2016. Aging alters the dampening of pulsatile blood flow in cerebral arteries. *J. Cereb. Blood Flow Metab.* 36:1519–27
34. Webb AJ, Simoni M, Mazzucco S, Kuker W, Schulz U, Rothwell PM. 2012. Increased cerebral arterial pulsatility in patients with leukoaraiosis: Arterial stiffness enhances transmission of aortic pulsatility. *Stroke* 43:2631–36
35. Jessen NA, Munk ASF, Lundgaard I, Nedergaard M. 2015. The glymphatic system: a beginner's guide. *Neurochem. Res.* 40:2583–99
36. Andreone BJ, Chow BW, Tata A, Lacoste B, Ben-Zvi A, et al. 2017. Blood-brain barrier permeability is regulated by lipid transport–dependent suppression of caveolae-mediated transcytosis. *Neuron* 94:581–94
37. Hajar C, Campisi M, Mattu C, Chiono V, Kamm RD. 2018. *In vitro* models of molecular and nanoparticle transport across the blood-brain barrier. *Biomicrofluidics* 12:042213
38. Barar J, Rafi MA, Pourseif MM, Omidi Y. 2016. Blood-brain barrier transport machineries and targeted therapy of brain diseases. *BioImpacts* 6:225–48
39. Rapoport SI. 2000. Osmotic opening of the blood-brain barrier. *Cell. Mol. Neurobiol.* 20:217–30
40. Yuan W, Lv Y, Zeng M, Fu BM. 2009. Non-invasive measurement of solute permeability in cerebral microvessels of the rat. *Microvasc. Res.* 77:166–73
41. Shi L, Zeng M, Sun Y, Fu BM. 2014. Quantification of blood-brain barrier solute permeability and brain transport by multiphoton microscopy. *J. Biomech. Eng.* 136:031005
42. Schaad MB, Houbaert D, Mece O, Agostinis P. 2019. Autophagy in endothelial cells and tumor angiogenesis. *Cell Death Differ.* 26:665–79
43. Pieper C, Pieloch P, Galla HJ. 2013. Pericytes support neutrophil transmigration via interleukin-8 across a porcine co-culture model of the blood-brain barrier. *Brain Res.* 1524:1–11
44. Fazakas C, Wilhelm I, Nagyoszi P, Farkas AE, Haskó J, et al. 2011. Transmigration of melanoma cells through the blood-brain barrier: role of endothelial tight junctions and melanoma-released serine proteases. PLOS ONE 6:e20758
45. Offeddu GS, Possenti L, Loessberg-Zahl JT, Zunino P, Roberts J, et al. 2019. Application of transmural flow across in vitro microvasculature enables direct sampling of interstitial therapeutic molecule distribution. Small 15:1902393
46. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. 2010. Structure and function of the blood-brain barrier. Neurobiol. Dis. 37:13–25
47. Banks WA. 2009. Characteristics of compounds that cross the blood-brain barrier. BMC Neurol. 9:S3
48. Butt AM, Jones HC, Abbott NJ. 1990. Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study. J. Physiol. 429:47–62
49. Sharabi S, Bresler Y, Ravid O, Shemesh C, Atrakchi D, et al. 2019. Transient blood–brain barrier disruption is induced by low pulsed electrical fields in vitro: an analysis of permeability and trans-endothelial electric resistivity. Drug Deliv. 26:459–69
50. Elbrecht DH, Long CJ, Hickman JJ. 2016. Transepithelial/endothelial electrical resistance (TEER) theory and applications for microfluidic body-on-a-chip devices. J. Rare Dis. Res. Treat. Open 1:46–52
51. Deosarkar SP, Prabhakarpandian B, Wang B, Sheffield JB, Krynska B, Kiani MF. 2015. A novel dynamic neonatal blood-brain barrier on a chip. PLOS ONE 10:e0142725
52. Crone C, Olesen SP. 1982. Electrical resistance of brain microvascular endothelium. J. Gen. Physiol. 77:349–71
53. Wiranowska M, Wilson TC, Beneze KS, Prockop LD. 1988. A mouse model for the study of blood-brain barrier permeability. J. Neurosci. Methods 26:105–9
54. Rapoport SI, Bachman DS, Thompson HK. 1972. Chronic effects of osmotic opening of the blood-brain barrier in the monkey. Science 176:1243–44
55. Sohet F, Daneman R. 2013. Genetic mouse models to study blood-brain barrier development and function. Fluids Barriers CNS 10:1
56. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ. 2015. TEER measurement techniques for in vitro barrier model systems. J. Lab. Autom. 20:107–26
57. Crone C, Christensen O. 1981. Electrical resistance of a capillary endothelium. J. Gen. Physiol. 77:349–71
58. Helms HC, Abbott NJ, Burek M, Cecchelli R, Couraud PO, et al. 2015. In vitro models of the blood-brain barrier: an overview of commonly used brain endothelial cell culture models and guidelines for their use. J. Cereb. Blood Flow Metab. 36:862–90
59. Lacombe O, Videau O, Chevillon D, Guyot AC, Contreras C, et al. 2011. In vitro primary human and animal cell-based blood–brain barrier models as a screening tool in drug discovery. Mol. Pharm. 8:651–63
60. Gaston JD, Bischel LL, Fitzgerald LA, Casick KD, Ringeisen BR, Pirlo RK. 2017. Gene expression changes in long-term in vitro human blood-brain barrier models and their dependence on a Transwell scaffold material. J. Healthc. Eng. 2017:5740975
61. Cecchelli R, Berezowski V, Lundquist S, Culot M, Renftel M, et al. 2007. Modelling of the blood–brain barrier in drug discovery and development. Nat. Rev. Drug Discov. 6:650–61
62. Bertassoni LE, Cecconi M, Manoharan V, Nikkhah M, Hjortnaes J, et al. 2014. Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. Lab Chip 14:2202–11
63. Offeddu GS, Haase K, Gillrie MR, Li R, Morozova O, et al. 2019. An on-chip model of protein paracellular and transcellular permeability in the microcirculation. Biomaterials 212:115–25
64. Watanabe T, Dohgu S, Takata F, Nishioku T, Nakashima A, et al. 2013. Paracellular barrier and tight junction protein expression in the immortalized brain endothelial cell lines bEND.3, bEND.5 and mouse brain endothelial cell 4. J. Biomed. Eng. 36:492–95
65. Rist RJ, Romero IA, Chan MW, Couraud PO, Roux F, Abbott NJ. 1997. F-actin cytoskeleton and sucrose permeability of immortalised rat brain microvascular endothelial cell monolayers: effects of cyclic AMP and astrocytic factors. Brain Res. 768:10–18
66. Eigenmann DE, Xue G, Kim KS, Moses AV, Hamburger M, Oufir M. 2013. Comparative study of four immortalized human brain capillary endothelial cell lines, hCMEC/D3, hBMEC, TY10, and BB19, and optimization of culture conditions, for an in vitro blood-brain barrier model for drug permeability studies. Fluids Barriers CNS 10:33
88. Shafaie S, Hutter V, Brown MB, Cook MT, Chau DY. 2017. Influence of surface geometry on the culture of human cell lines: a comparative study using flat, round-bottom and v-shaped 96 well plates. *PLOS ONE* 12:e0186799

89. Dehouck M, Meresse S, Delorme P, Fruchart J, Cecchelli R. 1990. An easier, reproducible, and mass-production method to study the blood–brain barrier in vitro. *J. Neurochem.* 54:1798–801

90. Bischel LL, Coneski PN, Lundin JG, Wu PK, Giller CB, et al. 2016. Electrospun gelatin biopapers as substrate for in vitro bilayer models of blood-brain barrier tissue. *J. Biomed. Mater. Res. A* 104:901–9

91. Sip CG, Bhattacharjee N, Folch A. 2014. Microfluidic Transwell inserts for generation of tissue culture-friendly gradients in well plates. *Lab Chip* 14:302–14

92. Wang X, Xu B, Xiang M, Yang X, Liu Y, et al. 2020. Advances on fluid shear stress regulating blood-brain barrier. *Microsc. Res. 128:103930

93. Zhang B, Korolj A, Lai BFL, Radisic M. 2018. Advances in organ-on-a-chip engineering. *Nat. Rev. Mater.* 3:257–78

94. Vernetti L, Gough A, Baetz N, Blutt S, Broughman JR, et al. 2017. Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. *Sci. Rep.* 7:42296

95. Novak R, Ingram M, Marquez S, Das D, Delahanty A, et al. 2020. Robotic fluidic coupling and interrogation of multiple vascularized organ chips. *Nat. Biomed. Eng.* 4:407–20

96. Herland A, Maoz BM, Das D, Somayaji MR, Prantil-Baun R, et al. 2020. Quantitative prediction of human pharmacokinetic responses to drugs via fluidically coupled vascularized organ chips. *Nat. Biomed. Eng.* 4:421–36

97. Junaid A, Mashaghi A, Hankemeier T, Vulto P. 2017. An end-user perspective on organ-on-a-chip: assays and usability aspects. *Curr. Opin. Biomed. Eng.* 1:15–22

98. Esch EW, Bahinski A, Huh D. 2015. Organs-on-chips at the frontiers of drug discovery. *Nat. Rev. Drug Discov.* 14:248–60

99. Odijk M, Van Der Meer AD, Levner D, Kim HJ, Van Der Helm MW, et al. 2015. Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems. *Lab Chip* 15:745–52

100. Brown TD, Nowak M, Bayles AV, Prabhabakarpandian B, Karande P, et al. 2019. A microfluidic model of human brain (µHuB) for assessment of blood brain barrier. *Bioeng. Transl. Med.* 4:e10126

101. Maoz BM, Herland A, FitzGerald EA, Grevesse T, Vidoudez C, et al. 2018. A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. *Nat. Biotechnol.* 36:865–74

102. Herland A, Maoz BM, FitzGerald EA, Grevesse T, Vidoudez C, et al. 2020. Proteomic and metabolomic characterization of human neurovascular unit cells in response to methamphetamine. *Adv. Biosyst.* 4:1900230

103. Probst C, Schneider S, Loskill P. 2018. High-throughput organ-on-a-chip systems: current status and remaining challenges. *Curr. Opin. Biomed. Eng.* 6:33–41

104. 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

105. Xu H, Li Z, Yu Y, Sizdahkhani S, Ho WS, et al. 2016. A dynamic in vivo–like organotypic blood-brain barrier model to probe metastatic brain tumors. *Sci. Rep.* 6:36670

106. Kart ME, Linville RM, Mayo LN, Xu ZS, Searson PC. 2018. Functional brain-specific microvessels from iPSC-derived human brain microvascular endothelial cells: the role of matrix composition on monolayer formation. *Fluids Barriers CNS* 15:7

107. Rauti R, Renous N, Maoz BM. 2019. Mimicking the brain extracellular matrix in vitro: a review of current methodologies and challenges. *Isr. J. Chem.* 60:1141–51

108. Brown JA, Pensabene V, Markov DA, Allwardt V, Neely MD, et al. 2015. Recreating blood-brain barrier physiology and structure on chip: a novel neurovascular microfluidic bioreactor. *Biomicrofluidics* 9:054124

109. Adriani G, Ma D, Pavesi A, Kamm RD, Goh EL. 2017. A 3D neurovascular microfluidic model consisting of neurons, astrocytes and cerebral endothelial cells as a blood-brain barrier. *Lab Chip* 17:448–59

110. Ruoslahti E. 1996. Brain extracellular matrix. *Glycobio: Glycobiology 6:489–92*
111. Peak CW, Cross L, Singh A, Gaharwar AK. 2015. Microscale technologies for engineering complex tissue structures. In Microscale Technologies for Cell Engineering, ed. A Singh, AK Gaharwar, pp. 3–25. New York: Springer

112. Chen MB, Whisler JA, Fröse J, Yu C, Shin Y, Kamm RD. 2017. On-chip human microvasculature assay for visualization and quantification of tumor cell extravasation dynamics. Nat. Protoc. 12:865–80

113. Bang S, Lee SR, Ko J, Son K, Tähk D, et al. 2017. A low permeability microfluidic blood-brain barrier platform with direct contact between perfusable vascular network and astrocytes. Sci. Rep. 7:8083

114. Wang YI, Abaci HE, Shuler ML. 2017. Microfluidic blood–brain barrier model provides in vivo–like barrier properties for drug permeability screening. Biotechnol. Bioeng. 114:184–94

115. Qian X, Song H, Ming GL. 2019. Brain organoids: advances, applications and challenges. Development 146:dev166074

116. Raja WK, Mungenast AE, Lin YT, Ko T, Abdurrob F, et al. 2016. Self-organizing 3D human neural tissue derived from induced pluripotent stem cells recapitulate Alzheimer’s disease phenotypes. PLOS ONE 11:e0161969

117. Pham MT, Pollock KM, Rose MD, Cary WA, Stewart HR, et al. 2018. Generation of human vascularized brain organoids. NeuroReport 29:588–93

118. Cakir B, Xiang Y, Tanaka Y, Kural MH, Parent M, et al. 2019. Engineering of human brain organoids with a functional vascular-like system. Nat. Methods 16:1169–75

119. Homan KA, Gupta N, Kroll KT, Kolesky DB, Skylar-Scott M, et al. 2019. Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat. Methods 16:255–62

120. Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayo A, Annabi N, Khademhosseini A. 2015. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. Biomaterials 73:254–71

121. Zhang Z, Jin Y, Yin J, Xu C, Xiong R, et al. 2018. Evaluation of bioink printability for bioprinting applications. Appl. Phys. Rev. 5:041304

122. Bajaj P, Schweller RM, Khademhosseini A, West JL, Bashir R. 2014. 3D biofabrication strategies for tissue engineering and regenerative medicine. Annu. Rev. Biomed. Eng. 16:247–76

123. Yi HG, Jeong YH, Kim Y, Choi YJ, Moon HE, et al. 2019. A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. Nat. Biomed. Eng. 3:509–19

124. Chen N, Zhu K, Zhang YS, Yan S, Pan T, et al. 2019. Hydrogel bioink with multilayered interfaces improves dispersibility of encapsulated cells in extrusion bioprinting. ACS Appl. Mater. Interfaces 11:30585–95

125. Grifno GN, Farrell AM, Linville RM, Arevalo D, Kim JH, et al. 2019. Tissue-engineered blood-brain barrier models via directed differentiation of human induced pluripotent stem cells. Sci. Rep. 9:13957

126. Yang L, Shridhar SV, Gerwitz M, Soman P. 2016. An in vitro vascular chip using 3D printing-enabled hydrogel casting. Biofabrication 8:035015

127. Pi Q, Maharjan S, Yan X, Liu X, Singh B, et al. 2018. Digitally tunable microfluidic bioprinting of multilayered cannular tissues. Adv. Mater. 30:1706913

128. Lee JB, Kim DH, Yoon JK, Park DB, Kim HS, et al. 2020. Microchannel network hydrogel induced ischemic blood perfusion connection. Nat. Commun. 11:615

129. Rosenberg GA. 2012. Neurological diseases in relation to the blood–brain barrier. J. Cereb. Blood Flow Metab. 32:1139–51

130. Sweeney MD, Sagare AP, Zlokovic BV. 2018. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat. Rev. Neurol. 14:133–50

131. Mackie JB, Stins M, McComb JG, Calero M, Ghiso J, et al. 1998. Human blood-brain barrier receptors for Alzheimer’s amyloid-β 1-40 asymmetrical binding, endocytosis, and transcytosis at the apical side of brain microvascular endothelial cell monolayer. J. Clin. Investig. 102:734–43

132. Robert J, Button EB, Yuen B, Gilmour M, Kang K, et al. 2017. Clearance of β-amyloid is facilitated by apolipoprotein E and circulating high-density lipoproteins in bioengineered human vessels. eLife 6:e29595

133. Shin Y, Choi SH, Kim E, Bylykbashi E, Kim JA, et al. 2019. Blood–brain barrier dysfunction in a 3D in vitro model of Alzheimer’s disease. Adv. Sci. 6:1900962
134. Blanchard JW, Bula M, Davila-Velderrain J, Akay LA, Zhu L, et al. 2020. Reconstruction of the human blood–brain barrier in vitro reveals a pathogenic mechanism of APOE4 in pericytes. Nat. Med. 26:952–63
135. Yang C, Hawkins KE, Doré S, Candelario-Jalil E. 2019. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. Am. J. Physiol. Cell Physiol. 316:C135–53
136. Xiong Y, Mahmood A, Chopp M. 2013. Animal models of traumatic brain injury. Nat. Rev. Neurosci. 14:128–42
137. Chen T, Dai SH, Li X, Luo P, Zhu J, et al. 2018. Sirt1–Sirt3 axis regulates human blood–brain barrier permeability in response to ischemia. Redox Biol. 14:229–36
138. Chaitanya GV, Minagar A, Alexander JS. 2014. Neuronal and astrocytic interactions modulate brain endothelial properties during metabolic stresses of in vitro cerebral ischemia. Cell Commun. Signal. 12:7
139. Plummer S, Wallace S, Ball G, Lloyd R, Schiapparelli P, et al. 2019. A human iPSC-derived 3D platform using primary brain cancer cells to study drug development and personalized medicine. Sci. Rep. 9:1407
140. Ngo MT, Harley BA. 2019. Perivascular signals alter global gene expression profile of glioblastoma and response to temozolomide in a gelatin hydrogel. Biomaterials 198:122–34
141. Ozturk MS, Lee VK, Zou H, Friedel RH, Intes X, Dai G. 2020. High-resolution tomographic analysis of in vitro 3D glioblastoma tumor model under long-term drug treatment. Sci. Adv. 6:eaaay7513
142. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, et al. 2009. Genes that mediate breast cancer metastasis to the brain. Nature 459:1005–9
143. Chaudhuri JD. 2000. Blood brain barrier and infection. Med. Sci. Monit. 6:1213–22
144. Baig AM, Khaleeq A, Ali U, Syeda H. 2020. Evidence of the COVID-19 virus targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. ACS Chem. Neurosci. 11:995–98
145. Buzhdygan TP, DeOre BJ, Baldwin-Leclair A, McGary H, Razmpour R, et al. 2020. The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in vitro models of the human blood–brain barrier. bioRxiv 150912. https://doi.org/10.1101/2020.06.15.150912
146. Shimizu F, Nishihara H, Kanda T. 2018. Blood–brain barrier dysfunction in immuno-mediated neurological diseases. Immunol. Med. 41:120–28
147. Jacobs BM. 2014. Stemming the hype: What can we learn from iPSC models of Parkinson’s disease and how can we learn it? J. Parkinson’s Dis. 4:15–27
148. Lim RG, Quan C, Reyes-Ortiz AM, Lutz SE, Kedaigle AJ, et al. 2017. Huntington’s disease iPSC-derived brain microvascular endothelial cells reveal WNT-mediated angiogenic and blood-brain barrier deficits. Cell Rep. 19:1365–77
149. Shityakov S, Förster CY. 2018. Computational simulation and modeling of the blood–brain barrier pathology. Histocemb. Cell Biol. 149:451–59
150. Adhikari U, Goliaei A, Berkowitz ML. 2016. Nanobubbles, cavitation, shock waves and traumatic brain injury. Phys. Chem. Chem. Phys. 18:32638–52
Contents

Vascular Mechanobiology: Homeostasis, Adaptation, and Disease
Jay D. Humphrey and Martin A. Schwartz .................................................1

Current Advances in Photoactive Agents for Cancer Imaging
and Therapy
Deanna Broadwater, Hyllana C.D. Medeiros, Richard R. Lunt, and Sophia Y. Lunt ..........29

Signaling, Deconstructed: Using Optogenetics to Dissect and Direct
Information Flow in Biological Systems
Payam E. Farahani, Ellen H. Reed, Evan J. Underbill, Kazuhiro Aoki, and Jared E. Toettcher .................................................................61

Therapeutic Agent Delivery Across the Blood–Brain Barrier Using
Focused Ultrasound
Dallan McMahon, Meaghan A. O’Reilly, and Kullervo Hynynen ..........................89

Procedural Telementoring in Rural, Underdeveloped, and Austere
Settings: Origins, Present Challenges, and Future Perspectives
Juan P. Wachs, Andrew W. Kirkpatrick, and Samuel A. Tisherman ......................115

Engineering Vascularized Organoid-on-a-Chip Models
Venktesh S. Shirure, Christopher C.W. Hughes, and Steven C. George ..................141

Integrating Systems and Synthetic Biology to Understand and
Engineer Microbiomes
Patrick A. Leggieri, Yiyi Liu, Madeline Hayes, Bryce Connors, Susanna Seppälä,
Michelle A. O’Malley, and Ophelia S. Venturelli ........................................169

Circadian Effects of Drug Responses
Yaakov Nahmias and Ioannis P. Androulakis .............................................203

Red Blood Cell Hitchhiking: A Novel Approach for Vascular Delivery
of Nanocarriers
Jacob S. Brenner, Samir Mitragotri, and Vladimir R. Muzykantov .......................225
Quantitative Molecular Positron Emission Tomography Imaging Using Advanced Deep Learning Techniques  
Habib Zaidi and Issam El Naqa .................................................. 249

Simulating Outcomes of Cataract Surgery: Important Advances in Ophthalmology  
Susana Marcos, Eduardo Martinez-Enriquez, Maria Vinas, Alberto de Castro, Carlos Dorronsoro, Seung Pil Bang, Geunyoung Yoon, and Pablo Artal .......... 277

Biomedical Applications of Metal 3D Printing  
Luis Fernando Velásquez-García and Yosef Kornbluth .......................... 307

Engineering Selectively Targeting Antimicrobial Peptides  
Ming Lei, Arul Jayaraman, James A. Van Deventer, and Kyongbum Lee .......... 339

Biology and Models of the Blood–Brain Barrier  
Cynthia Hajal, Baptiste Le Roi, Roger D. Kamm, and Ben M. Maoz .......... 359

In Situ Programming of CAR T Cells  
Neha N. Parayath and Matthias T. Stephan ................................... 385

Vascularized Microfluidics and Their Untapped Potential for Discovery in Diseases of the Microvasculature  
David R. Myers and Wilbur A. Lam .......................................... 407

Recent Advances in Aptamer-Based Biosensors for Global Health Applications  
Lia A. Stanciu, Qingshan Wei, Amit K. Barui, and Noor Mohammad ........... 433

Modeling Immunity In Vitro: Slices, Chips, and Engineered Tissues  
Jennifer H. Hammel, Sophie R. Cook, Maura C. Belanger, Jennifer M. Munson, and Rebecca R. Pompano ........................................ 461

Integrating Biomaterials and Genome Editing Approaches to Advance Biomedical Science  
Amr A. Abdeen, Brian D. Cosgrove, Charles A. Gersbach, and Krishanu Saha ...... 493

Cell and Tissue Therapy for the Treatment of Chronic Liver Disease  
Yaron Bram, Duc-Huy T. Nguyen, Vikas Gupta, Jiwoon Park, Ch Nabul Richardson, Vasuretha Chandar, and Robert E. Schwartz ....................... 517

Fluid Dynamics of Respiratory Infectious Diseases  
Lydia Bourouiba ................................................................. 547

Errata  
An online log of corrections to Annual Review of Biomedical Engineering articles may be found at http://www.annualreviews.org/errata/bioeng