PARASITE TROPISM FOR THE BRAIN MICROVASCULATURE

The brain is composed of a highly diverse array of neurons and glial cells that functionally interact to support healthy cognitive and motor functions. To satisfy the high energy requirements of the brain, the cerebral parenchyma is heavily vascularized, providing continuous access of nutrients and oxygen while also facilitating the removal of waste products. This is achieved through a highly specialized vascular interface known as the blood–brain barrier (BBB) that precisely controls influx of ions and molecules into the brain while preventing the entry of toxic agents and pathogens from the blood (Obermeier et al., 2013). Despite the presence of this selective barrier, parasites have developed complex interactions with the BBB that can lead to either (a) widespread dysfunction of the brain microvasculature or (b) transmigration of parasites into the brain parenchyma.

Cerebral malaria is a paradigmatic example of parasite-induced brain microvascular pathogenesis without crossing into the brain parenchyma. It is characterized by extensive cytoadhesion of Plasmodium falciparum-infected red blood cells (iRBC) to endothelial brain receptors, such as intracellular adhesion molecule 1 (ICAM-1; Ockenhouse et al., 1992) or endothelial protein C receptor (EPCR; Turner et al., 2013). P. falciparum–iRBC interaction with EPCR has been proposed as one of the main contributors to brain microvascular dysfunction, by blocking cytoprotective, anti-inflammatory, and junctional stabilizing pathways regulated by this endothelial receptor (Bernabeu & Smith, 2017). Another barrier-disruptive mechanism that has been proposed is the release of toxic components upon P. falciparum–iRBC egress of new asexual infective parasites, known as merozoites (Gillrie et al., 2012; Moxon et al., 2020; Pal et al., 2016; Figure 1a).

Conversely, both Trypanosoma brucei and Toxoplasma gondii bypass brain barriers and infiltrate into the brain parenchyma (Matta et al., 2021; Mogk et al., 2017). While T. brucei brain tropism has been linked to the disruption of meningeal barriers or circumventricular...
organs, the presence of the parasite within cerebral capillaries suggests an alternative passage through the BBB (Frevert et al., 2012). Cytoadhesion of T. brucei to the microvasculature has been associated to endothelial receptors such as CD36, ICAM-2, E-, and P-selectins (De Niz et al., 2021), although specific receptors for brain microvessels have yet to be determined. It has been proposed that direct crossing of the BBB can be facilitated by the release of parasite-derived cytoskeletal proteases, such as brucipain (Grab & Kennedy, 2008; Masocha et al., 2007; Figure 1a). Alternatively, other studies suggest that BBB infiltration occurs following T-cell extravasation into the brain parenchyma after endothelial activation (Amin et al., 2012; Figure 1a). Similarly, T. gondii extracellular parasites have been shown to cross the BBB directly (Barragan et al., 2005). Yet, other methods of entry have been described, such as the Trojan horse mechanism, in which T. gondii-infected leukocytes become hypermigratory and extravasate microvessels (Courret et al., 2006; Figure 1a). Additionally, murine studies suggest that BBB infiltration could follow invasion, replication, and rupture of brain endothelial cells (Konradt et al., 2016; Figure 1a). Although T. gondii parasites can invade nearly all cell types, the receptors or mechanisms that provide tropism to microvessels of distinct brain regions, such as the cortex, remain unknown.

Each of these three parasites utilizes different mechanisms to disrupt or bypass the BBB throughout infection. Much of our understanding about T. brucei and T. gondii entry into the brain has been demonstrated through murine models, which lack the refined control required to elucidate and disentangle complex parasite–host interactions (Amin et al., 2012; Courret et al., 2006; Konradt et al., 2016). Conversely, animal models are not ideal to model P. falciparum–iRBC molecular interactions with the endothelium, and therefore studies have been limited to reductionist 2D cell monolayer and Transwell models (Gallego-Delgado et al., 2016; Gillrie et al., 2012; Tripathi et al., 2007). Although 2D models present advantages, such as being high throughput and easy to implement, they fail to fully recapitulate BBB architecture, function, and physiology. New emerging tools in 3D vascular engineering hold great promise in improving our understanding of how parasites interact with the brain microvasculature, as they better mimic BBB properties by incorporating additional cellular and mechanical elements. Here, we highlight design considerations that need to be considered when recreating BBB function. We outline different fabrication methodologies and enumerate their main advantages and limitations, which need to be carefully considered in terms of the biological question to be addressed. Although this review mostly focuses on current and future applications of 3D-BBB models in parasitology research, the technologies described here can be applied to research of other microbial agents with brain tropism.

2 | BLOOD–BRAIN BARRIER

Barrier properties of the brain microvasculature are achieved by its complex cellular and mechanical properties. The BBB is composed of a layer of endothelial cells that are wrapped by pericytes and in close physical contact with astrocytic endfeet (Figure 1b; Profaci et al., 2020). Endothelial cells in the BBB are highly specialized, as they possess complex tight junctions that limit paracellular transport, diffusion of molecules, and transmigration of cells into the brain parenchyma. This is achieved by high expression of tight junctional proteins like claudin-5 and occludin, as well as cytoplasmic scaffolding proteins such as zona occludens-1 (ZO-1), ZO-2, and ZO-3 (Figure 1c). Tight junctions are located at the apicolateral side and stitch neighboring endothelial cells together (Citi, 2019). Adherens junctions are located more basally than tight junctions and also contribute to barrier function. They are composed of proteins such as cadherins and catenins that connect to the actin cytoskeleton. Additionally, transcellular transport across brain endothelial cells is limited by high expression of selective solute transporters (GLUT-1 and LAT-1) that transport molecules towards the brain, and efflux pumps (MDR-1 and P-gp) that bring small molecules back to the blood (Figure 1c).

Further strengthening of BBB function is achieved through the molecular and biophysical crosstalk of cells that compose the neurovascular unit, which include endothelial cells, pericytes, and other brain cell types such as astrocytes and neurons. Pericytes are a heterogeneous group of cells with multiple subtypes that cover ~30% of the surface of brain microvessels in mammals (Mathiisen et al., 2010). These perivascular cells play essential roles in regulating angiogenesis and vascular tone through contractility. Additionally, they secrete basement membrane proteins (fibronectin, laminin, and type IV collagen), and vascular stabilizing factors such as angiopoietin-1 (Ang-1), essential for structural BBB formation and maintenance (Zhao & Chappell, 2019). Similarly, astrocytes are key components of the neurovascular unit and cover, almost completely, the brain microvascular interface through extension of astrocytic endfeet. They contribute to barrier formation through secretion of Sonic Hedgehog and Ang-1 (Lee et al., 2003), as well as regulating vascular flow through capillary dilation upon neuronal activity (reviewed in Cohen-Salmon et al., 2021). In addition to cellular components, the biomechanical properties conferred by the extracellular matrix (ECM) and blood flow contribute to barrier formation. Therefore, each of these components needs to be carefully considered when modeling BBB properties in vitro.

3 | DESIGN CONSIDERATIONS FOR IN VITRO BBB MODELING

Recent advances in vascular engineering have allowed for vast improvements in the generation of new in vitro 3D-BBB models. BBB models are often validated by quantifying the expression of BBB-specific markers (junctional proteins, solute transporters, and efflux pumps), as well as measuring barrier strength by trans-endothelial electrical resistance (TEER) or/and permeability rates (DeStefano et al., 2018). TEER is defined as the resistance to ionic transport across blood vessels, and is measured by applying an electric current between two electrodes located on the luminal and abluminal side of vessels. This approach is sometimes difficult to implement in
FIGURE 1 The blood–brain barrier (BBB) in disease and health. (a) Parasites have evolved complex mechanisms that result in BBB disruption. *P. falciparum*-iRBC can cause microvascular pathology and BBB breakdown either by directly releasing toxic components or via blockade of endothelial receptors, such as endothelial protein C receptor (EPCR). *T. brucei* extracellular parasites can cross the BBB following T-cell extravasation or the release of cysteine proteases, such as brucipain. *T. gondii* can enter the brain parenchyma either upon endothelial lysis following intracellular replication or paracellular infiltration as an extracellular parasite. Alternatively, *T. gondii* can cross the BBB inside infected leukocytes. (b) In healthy conditions, the BBB is composed of brain endothelial cells, astrocytes, pericytes, and basement membrane. (c) Altogether, they provide selective barrier functions through high expression of endothelial tight junction proteins or BBB transporters (P-gp, GLUT1, MRPs, LAT1).
engineered 3D-BBB models due to size constraints of the required electrodes (Srinivasan et al., 2015). Alternatively, a widely used approach is the measurement of vascular permeability as the flux of low molecular weight fluorescent tracers to an in vitro compartment representing the brain parenchyma (Fu, 2018). In vitro resistance and permeability measurements are often compared with values determined in vivo, estimated to range between 1,600 and 8,000 Ω · cm² (Butt et al., 1990; Crone & Olesen, 1982). This corresponds to permeability rates lower than $2 \times 10^{-7}$ cm/s for Lucifer yellow (3000 et al., 2014) or in a model with astrocytes that incorporates flow polarized BBB transporters, and TEER values around 500·cm².

Willebrand Factor (vWF), well-organized junctions, expression of endothelial tight junction formation, anti-inflammatory signaling pathways and the expression of BBB-specific transporters (reviewed in Baratchi et al., 2017). Heterogenous biomechanical conditions can be found along the hierarchical vascular tree. While blood flow in arteries exerts a WSS that ranges from 10 to 70 dyne/cm², it dampens to 1–6 dyne/cm² in capillaries and postcapillary venules (reviewed in Dessalles et al., 2021). Such differences are important when modeling the BBB in vitro. In healthy conditions, WSS is sensed by transmission through endothelial mechanosensors, such as Piezo1, caveolin-1, and VE-cadherin, leading to strengthening of endothelial tight junction formation, anti-inflammatory signaling pathways and the expression of BBB-specific transporters (reviewed in Baratchi et al., 2017). Heterogenous biomechanical conditions can be found along the hierarchical vascular tree. While blood flow in arteries exerts a WSS that ranges from 10 to 70 dyne/cm², it dampens to 1–6 dyne/cm² in capillaries and postcapillary venules (reviewed in Dessalles et al., 2021). Such differences are important when modeling parasite–vascular interactions. For example, cerebral malaria patients present preferential cytoadhesion of *P. falciparum*-iRBC in capillary and postcapillary venules (Milner et al., 2015; Spitz, 1946). Conversely, *T. brucei* presents differential tissue-specific blood vessel binding patterns with significant accumulation in capillaries of the mouse brain, and in medium and large sized vessels of the kidneys or the heart (De Niz et al., 2021).

Although the use of iPSC-derived endothelial cells is underrepresented in microbiology research thus far, we expect that their increased barrier properties will extend use in future endeavors to understand BBB–pathogen interactions. For example, iPSC-BMEC have already been used in 2D Transwell models to study bacterial interaction with the brain endothelium in meningitis (Kim et al., 2017; Martins Gomes et al., 2019). Nevertheless, the protocol developed by Lippmann and colleagues has recently faced controversy, as transcriptomic analyses have challenged the endothelial nature of differentiated cells by revealing the presence of neuroepithelial markers such as epithelial cell adhesion molecule (EPCAM; Lu et al., 2021; Vatine et al., 2019). Thus, we recommend their application to research questions that require strong barrier properties, while ideally complementing findings with alternative cell sources, such as primary cells. Furthermore, the addition of iPSC-BMEC into a BBB infection model requires validation of endothelial features proven to be necessary for specific parasite interactions with the brain microvasculature, including surface receptors, such as EPCR (Turner et al., 2013), ICAM-1 (Ockenhouse et al., 1992), ICAM-2, VCAM, and E and P-selectins (De Niz et al., 2021). Altogether, iPSC-derived models can be a worthwhile alternative to primary cells with improved barrier phenotype if a thorough characterization of the relevant cellular features is performed.

### 3.1 Cellular components

The origin and choice of cell types to be incorporated into the model need to be carefully evaluated. In past decades, immortalized and primary endothelial cells have been widely used to better understand vascular disease. However, these cell lines lose BBB-specific protein expression rapidly in culture, and possess limited physiological barrier behavior (Urich et al., 2012). Also, primary or immortalized endothelial cell monocultures present TEER values 30-fold lower than in vivo mammalian BBB measurements. The addition of primary astrocytes and pericytes to primary or immortalized endothelial cell models moderately increases barrier function to sub-physiological TEER values in multiple Transwell models (<200 Ω·cm²; reviewed in DeStefano et al., 2018; Table 1).

The recent development of protocols to differentiate brain microvascular endothelial cells from induced pluripotent stem cells (iPSC-BMEC) has led to a renewable endothelial cell source with increased physiological barrier rates. Additional advantages in the use of iPSC-derived cells include the ability to create patient-specific isogenic BBB models, and to genetically engineer cell types with knock ins or knock outs. Lippman and colleagues have developed a robust and widely used protocol in which differentiated cells exhibit endothelial markers, such as von Willebrand Factor (vWF), well-organized junctions, expression of polarized BBB transporters, and TEER values around 500 Ω · cm² (Lippmann et al., 2012). Further increases in barrier function have been achieved with differentiation under retinoic acid in a static BBB Transwell model with pericytes (1,500 Ω · cm²; Lippmann et al., 2014) or in a model with astrocytes that incorporates flow (3000 Ω · cm²; Wang et al., 2017). A recent key advance on this differentiation method includes a short period of hypoxia to replicate the microenvironment during brain development, before cells are introduced into a 3D-BBB model. The resultant iPSC-BMEC show an enhancement of TEER by two orders of magnitude for a sustained period of 7 days and expression of functional efflux pumps that recapitulate peptide and antibody trafficking observed in vivo (Table 1; Park et al., 2019).

**3.2 Biomechanical components**

#### 3.2.1 Flow

Endothelial cells are constantly exposed to blood flow-induced hydrodynamic forces, including wall shear stress (WSS), the frictional force exerted by flow acting tangentially to the cell surface. Therefore, flow is an essential element that must be considered when modeling the BBB in vitro. In healthy conditions, WSS is sensed by transmission through endothelial mechanosensors, such as Piezo1, caveolin-1, and VE-cadherin, leading to strengthening of endothelial tight junction formation, anti-inflammatory signaling pathways and the expression of BBB-specific transporters (reviewed in Baratchi et al., 2017). Heterogenous biomechanical conditions can be found along the hierarchical vascular tree. While blood flow in arteries exerts a WSS that ranges from 10 to 70 dyne/cm², it dampens to 1–6 dyne/cm² in capillaries and postcapillary venules (reviewed in Dessalles et al., 2021). Such differences are important when modeling parasite–vascular interactions. For example, cerebral malaria patients present preferential cytoadhesion of *P. falciparum*-iRBC in capillary and postcapillary venules (Milner et al., 2015; Spitz, 1946). Conversely, *T. brucei* presents differential tissue-specific blood vessel binding patterns with significant accumulation in capillaries of the mouse brain, and in medium and large sized vessels of the kidneys or the heart (De Niz et al., 2021).

Alterations in flow dynamics as a consequence of infection can promote inflammation, via transcriptional factors such as KLF-2, NF-kB, and YAP (Baratchi et al., 2017), which can lead to changes
| Endothelial cell origin | Model | Perivascular cells | Flow/wall shear stress (dyn/cm²) | TEER (Ω · cm²) | Permeability (cm/s) | References |
|------------------------|-------|--------------------|---------------------------------|---------------|---------------------|------------|
| In vivo                | NA    | NA                 | 1–70                            | 1,500–8,000   | Lucifer yellow: 2 × 10⁻⁷ 10 kDa dextran <1 × 10⁻⁷ | Reviewed in (DeStefano et al., 2018) |
| Immortalized and primary HBMEC | Transwell | Not included | NA | 40–50 | 2.4 × 10⁻⁶ | Reviewed in (DeStefano et al., 2018) |
| iPSC-BMEC            | Transwell | Not included | NA | 500 | | Lippmann et al. (2014) |
| iPSC-BMEC            | Transwell | Pericytes | NA | 1,500 | | Lippmann et al. (2014) |
| iPSC-BMEC            | Flow chamber | Astrocytes | 0.25 | 3,000 | 10 kDa dextran: ~10⁻⁶ 70 kDa dextran: ~10⁻⁸ | Wang et al. (2017) |
| Immortalized HBMEC   | Spheroids | Pericytes, Astrocytes | NA | 70 kDa dextran: Normalized. Not comparable | | Cho et al. 2017; Urich et al., 2013 |
| iPSC-BMEC            | Organoid | Pericytes, Astrocytes, Neurons, Progenitor cells | 300 | Not tested | | Cakir et al. (2019) |
| iPSC-BMEC hypoxia    | Pre-patterned PDMS-based | Pericytes, Astrocytes | 6 | 3 kDa dextran: 8.9 × 10⁻⁸ 10 kDa dextran: 8.9 × 10⁻⁸ 70 kDa dextran: 2.2 × 10⁻⁹ Lucifer yellow: Not tested | | Park et al. (2019) |
| iPSC-BMEC            | Pre-patterned hydrogel-based needle | Astrocytes | 4 | 10 kDa Dextran: below detection limit Lucifer yellow: 2.5 × 10⁻⁷ Rhodamine 123: 2.84 × 10⁻⁷ | | Linville et al. (2019) |
| Primary HUVEC        | Pre-patterned hydrogel-based injection molding | Pericytes | 20 | 70 kDa dextran: 0.15 × 10⁻⁶ 332 fluorescein: 7 × 10⁻⁶ | | Zheng et al. (2012) |
| iPSC-EC              | Self-assembled | Pericytes, Astrocytes | Yes, unknown | 10 kDa dextran: 2.2 × 10⁻⁷ | | Campisi et al. (2018) |
| Primary HBMEC        | Self-assembled | Pericytes, Astrocytes | Yes, unknown | 10 kDa dextran: 0.86 × 10⁻⁶ 70 kDa dextran: 0.31 × 10⁻⁶ | | Lee et al. (2020) |
in endothelial cell morphology and reorganization of the cytoskeleton (Chiu & Chien, 2011), as well as a decrease of tight junction and BBB transporter expression (Garcia-Polite et al., 2017). Whether changes in flow dynamics downstream of cytoadhesion sites of *P. falciparum*-iRBC trigger signaling pathways associated with inflammation remains unknown. Overall, engineered microfluidic 3D-BBB models offer the opportunity to fine-tune blood flow biomechanical parameters to replicate healthy and pathological flow regimes.

### 3.2.2 ECM and basement membrane

The basement membrane is a 20–200 μm-thick layer of ECM that underlies endothelial cells (DeStefano et al., 2018), consisting mainly of structural proteins such as collagen IV, fibronectin, heparan sulfate, proteoglycans, chondroitin sulfate proteoglycans, and laminins (Sanes, 1989). The ECM not only provides scaffolding to endothelial cells but also contributes to microvascular barrier properties. Brain endothelial cells sense mechanical cues exerted by ECM components through interactions with integrins expressed at the basal side. Blockade of β1-integrin interaction with the ECM decreases the expression of the tight junction protein claudin-5, and increases microvascular permeability both in vitro and in the mouse brain (Osada et al., 2011). Therefore, the mechanical properties of the cell substrate should be considered when engineering brain microvascular models. Previous in vitro studies focused on endothelium–pathogen interactions have mostly been performed on stiff surfaces such as glass or plastic. Conversely, bioengineered microvascular models are fabricated with hydrogels, such as type I collagen and fibrin, which are compatible with different fabrication techniques. The mechanical properties of hydrogels can be modulated by the polymer concentration or the gelation temperature and time (Kohn et al., 2015). Recent studies have shown that compared with brain primary endothelial cells grown in glass, cells grown in hydrogels under in vitro stiffnesses equal to those found in the brain show significantly increased cell area and cell coverage, as well as greater proportion of continuous tight junctions (Gray et al., 2019). Therefore, recreating the proper mechanical support provided by the ECM is of great relevance when mimicking brain microvascular properties in vitro and should be taken into account when modeling parasite-mediated breakdown and transmigration through the BBB.

## 4 3D-BBB MODELS

The use of engineered 3D microvascular models offers a unique alternative to study host–pathogen interactions in assays where both biochemical and biomechanical properties can be individually defined and controlled. Recent advances in the development of 3D-BBB models include three different fabrication methods: (a) spheroids and organoids, (b) pre-patterned, and (c) self-assembled microvascular models.

### 4.1 Spheroids and organoids

Spheroids and organoids have become popular tools in the tissue engineering and disease modeling field. Spheroids are spherical cellular units that self-assemble when cells are placed in suspension. Several spheroid-based BBB models have been generated, in which primary or immortalized human brain microvascular endothelial cells (HBMEC) and brain pericytes form an outer layer that surrounds an inner core of astrocytes (Cho et al., 2017; Urich et al., 2013). Other models include additional cells of the neurovascular unit such as iPSC-derived neurons, oligodendrocytes, and microglia (Nzou et al., 2018). These spheroids have been shown to express tight junction markers as well as BBB-specific transporters (Cho et al., 2017; Nzou et al., 2018). Their barrier properties have been assessed by measuring the influx of low molecular weight dextran fluorescent signal into the spheroid core at a single time point. However, this methodology prevents the calculation of permeability rates, and hence the comparison with other in vitro models or in vivo physiological conditions (Cho et al., 2017; Urich et al., 2013). Additional limitations of the system include the complexity of flow perfusion, as well as the non-physiological convex shape of spheroids compared with the concavity of blood vessels. As microvessel curvature influences endothelial orientation and cytoskeleton organization (Dessalles et al., 2021), endothelial cells in spheroid models may present different cellular behavior, promoting atypical interactions with parasites. Despite the disadvantages, spheroids are relatively simple to culture and high throughput (Cho et al., 2017), and hence have already been utilized in the parasitology field. Recently, a study performed on BBB spheroids has shown parasite internalization and increased permeability associated to cytoadhesion of *P. falciparum*-iRBC with dual binding to EPCR-ICAM-1 (Adams et al., 2021). Similarly, 3D neurospheres have been used to model *T. gondii* invasion of the brain parenchyma (Correa Leite et al., 2021).

Brain organoids are generated through differentiation of embryonic or adult iPSC into specific cerebral regions. Their complex 3D cellular interactions and architecture recreate embryonic and fetal brain development, making them a popular and emerging tool in the developmental biology field (Marton & Pasca, 2020). Brain organoid models have been utilized to study *T. gondii* infection and cyst formation, revealing parasite-induced increases in inflammatory type-I interferon signaling pathways (Seo et al., 2020). In addition, cortical brain organoids have been used to study malaria pathogenesis due to heme, a byproduct of infection that crosses the BBB. This experimental model showed that the increase in pro-inflammatory markers associated to heme was attenuated by addition of neuregulin-1 (Harbuzar et al., 2019). However, caution should be taken when modeling infectious diseases in brain organoids as one of their
current limitations is the lack of a perfusable vasculature. This often results in the development of a necrotic core and prevents the study of molecular mechanisms of parasite-mediated BBB breakdown. Although vascularization of organoids represents one of the biggest challenges in the tissue engineering field and several attempts are being pursued (Cakir et al., 2019; Ham et al., 2020; Shi et al., 2020), the formation of perfusable vessels in vitro organoids has not yet been achieved. In the following sections, we will cover alternative fabrication methods that can be used to build 3D microvessel models.

4.2 | Pre-patterned microvascular models

4.2.1 | Polydimethylsiloxane (PDMS) based

Pre-patterned models possess perfusable microfluidic networks of defined sizes and flow properties. These models are often created using negative molds made of polydimethylsiloxane (PDMS), a transparent and versatile silicon-based polymer widely used to create microfluidic devices with micron-scale dimensions and geometries. The Ingber group has pioneered the use of pre-patterned PDMS-based models, by developing a device with two parallel channels separated by a porous membrane that has been used to build several organ-on-chip models including lung, gut, bone marrow vasculature, and BBB (Huh et al., 2010, 2013; Figure 2a). The 3D-BBB model is composed of an upper channel harboring primary human astrocytes and pericytes that form an interface with iPSC–BMEC in the lower channel (Figure 2a; Park et al., 2019). Within this model, iPSC–BMEC grown under flow conditions develop complex junctions, show functional BBB transporters and deposition of basement membrane proteins. The model also presents low permeability coefficients (8.9 × 10⁻⁸ cm/s to 3 kDa dextran; Table 1), with selective BBB-specific transcytosis of cargos and therapeutic agents across the barrier. The ability to quantify molecule diffusion to a second compartment representing the brain parenchyma, and the addition of electrodes to measure real-time readouts of TEER, makes this model specially tailored to study molecular trafficking across the BBB and understanding temporal disruptions in its barrier function.

Such models have already been used to study viral-induced vascular disruption and to test antimicrobial therapies (Si et al., 2021). Future applications could include the study of potential parasite-mediated vascular pathogenesis or BBB transmigration mechanisms. However, the current model is composed by a single endothelialized channel and therefore only one flow condition can be tested at a time, limiting the study of complex host-parasite interactions under different physiological vessel sizes, WSS, or flow regimes, such as those of P. falciparum (Arapawa et al., 2020; Bernabeu et al., 2019) or T. gondii (Harker et al., 2014). Another limitation of the model is the planar, 2D disposition of pericytes and astrocytes along the microvessel which complicates the study of how pathogens alter perivascular cell morphology and architecture.

4.2.2 | Hydrogel based

Hydrogel-based pre-patterned microvascular models take advantage of the possibility of casting hydrogels around a mold that can be removed after gelation. These models create circular microvessels surrounded by a hydrogel where perivascular cells can be embedded.

Subtractive molding with a needle

The creation of a channel using a thin metallic needle represents a simple yet effective technique in which a type I collagen solution is poured around a needle that crosses a housing chamber (Figure 2b; Chrobak et al., 2006). After hydrogel gelation, the needle is removed leaving a hollow circular 120–180 µm diameter channel that is seeded with endothelial cells (Figure 2b). This approach has given the lowest permeability rates yet evaluated in an in vitro 3D-BBB model by seeding a device with iPSC–BMEC (Table 1; Katt et al., 2018; Linville et al., 2019). Furthermore, recreation of the cellular architecture of the BBB is possible by seeding pericytes and iPSC–BMEC in subsequent days (Jamieson et al., 2019), or the addition of astrocytes in the hydrogel bulk (Partyka et al., 2017). The high barrier levels achieved by this model make it suitable to quantify BBB breakdown after perfusion of parasites such as P. falciparum, T. gondii, and T. brucei. However, as with PDMS-based pre-patterned 3D models, the main drawback is the linear geometry and fixed vessel size of the network. As an alternative, complex vascular network geometries can be fabricated using other hydrogel-based pre-patterned fabrication methods, including soft-lithography, collagen photoablation, or 3D bioprinting.

Soft lithography fabrication and multi-photon ablation

This fabrication methodology consists of lithographic patterning of a type I collagen hydrogel by a micro-patterned positive PDMS mold (Zheng et al., 2012). After gelation and removal of the mold, the patterned hydrogel is assembled against a flat collagen piece, creating complex networks of channels within the collagen scaffold. The 50–200 µm diameter networks are seeded with endothelial cells, while perivascular cells are embedded in the collagen bulk (Figure 2c). The first model generated by this fabrication method consisted of primary human umbilical vein endothelial cells (HUVEC) in the presence of brain pericytes, and presented modest permeability rates (Zheng et al., 2012; Table 1). Nevertheless, the creation of a complex network with controlled and heterogenous microfluidic properties represents a key advantage to understand parasite tropism for the brain microvasculature. Recent iterations of this model seeded with primary HBMEC have been used to study of P. falciparum–iRBC interactions with endothelial cells at a wide range of WSS, revealing that P. falciparum–iRBC interactions are shear stress dependent. The parasite cytoadhesion pattern was shown to change after endothelial activation due to variations in the surface expression of ICAM-1 and EPCR (Bernabeu et al., 2019).

The main drawback of this method is that it requires a time-consuming and complex fabrication steps. Additionally, vessels
with a diameter lower than 50 µm cannot be generated. To address this limitation, this fabrication method has been coupled with high-resolution multi-photon ablation. This procedure consists of precise laser destruction of the collagen hydrogel, which generates capillary-sized microvessels down to 5–20 µm diameters (Arakawa et al., 2020). Perfusion experiments of *P. falciparum*-iRBC through...
microvessels recreating the arteriole-capillary-venule transition, recapitulated parasite cytoadhesion patterns seen in cerebral malaria autopsy samples in capillaries and postcapillary venules (Arakawa et al., 2020). Recent advances in this technique include the generation of microvessels with complex 3D architecture, including a human glomerulus or alveolar-capillary unit which could be exploited to understand parasite cytoadhesion in the microvasculature of different organs (Rayner et al., 2021). While technically challenging, combining pre-patternning with soft lithography and multi-photon ablation represents a good system to model microvessel interaction with parasites and other blood components in a wide range of microfluidic conditions present in the human microvasculature.

3D bioprinting
Another manufacturing method that achieves spatial control over vessel network geometry is 3D bioprinting, which involves the controlled placement of bioink, a solution composed of cells in a hydrogel, to create highly customizable and reproducible 3D constructs. Extrusion-based bioprinting has been employed to generate hydrogel scaffolds containing vessel-shaped sacrificial inks that upon thermal modification or chemical reaction can be removed resulting in hollow vessels in which endothelial cells can be seeded (Kinstlinger et al., 2020; Kolesky et al., 2016). Yet, the resolution of extrusion-based bioprinting depends on nozzle size and cannot generate vessels smaller than 100 μm. Therefore, high-definition two photon polymerization-based bioprinting has been used to achieve a resolution of ~10 μm (Dobos et al., 2021). This technique has been employed to create HUVEC or mouse endothelial cell capillary-sized microvessels in a variety of complex 3D shapes and microfluidic networks (Dobos et al., 2021; Marino et al., 2018). However, one of the main caveats of this technique is the fabrication time and the requirement of expensive equipment. Although this technology is still in its infancy, future applications to study infectious biology will likely be explored as it becomes more widely accessible.

4.3 | Self-assembled microvascular models
Endothelial cells self-organize into tubular-like structures when placed in a hydrogel in the presence of growth factors and perivascular cells, after several days in culture (Figure 2d; Nakatsu & Hughes, 2008). In the majority of self-assembled models, endothelial and perivascular cells are placed in a fibrin hydrogel in the central channel of a PDMS device (Kim et al., 2013; Moya et al., 2013; Whisler et al., 2014). This fabrication method has been used to generate 3D-BBB models using either primary HBMEC (Lee et al., 2020) or non-brain-specific iPSC-endothelial cells (Campisi et al., 2018) in combination with primary human pericytes and astrocytes. The generated microvessels present an average diameter of 30 μm and recapitulate BBB structural interactions found in the adult brain, such as wrapping of microvessels by pericytes and astrocytic endfeet-microvessel contact. These models presents improved barrier function and permeability rates to 10 kDa dextran, reaching values of $2 \times 10^{-7}$ cm/s (Campisi et al., 2018) and $0.86 \times 10^{-6}$ cm (Lee et al., 2020; Table 1), as well as increased expression of tight junction proteins. Furthermore, addition of adjacent microfluidic channels in the device allows for the generation of models that incorporate neurons, better mimicking the neurovascular unit (Adriani et al., 2017).

Because these devices possess complex microvascular branching and architecture, self-assembled models can be used to study vessel remodeling after *P. falciparum*-iRBC sequestration. Likewise, the improved barrier properties of the model can be leveraged to study *T. brucei* or *T. gondii* transport across the BBB. However, the main limitation of the model is the variability and heterogeneity of the microfluidic network, as self-organization impairs the ability to control vessel geometry and flow conditions. Therefore, sophisticated microfluidic simulations need to be generated to understand flow-driven mechanisms behind parasite-microvessel interactions. Overall, self-assembled models enable the study of the molecular mechanisms of parasite interaction with self-formed microvessels that recapitulate the architecture and function of the BBB, without the ability to easily control flow conditions and vasculature geometry.

5 | CONCLUSIONS AND FUTURE DIRECTIONS
The last decade has witnessed great progress in the development of perfusable 3D engineered systems (Haase & Kamm, 2017). This is exemplified by the development of several 3D-BBB models that recapitulate BBB morphology and function. These systems are already being exploited to model diseases, such as cancer or neurodevelopmental and degenerative disorders. In this review, we discussed future applications to study parasite interactions with BBB vasculature that can be widely extended for the study of other infectious agents. We expect that in the near future, these emerging approaches will enable the generation of more accurate and tunable disease models.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
Data sharing not applicable to this article as no datasets were generated or analysed during the current study.
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