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Organ-On-A-Chip Technologies for Advanced Blood–Retinal Barrier Models

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Abstract

The blood–retinal barrier (BRB) protects the retina by maintaining an adequate microenvironment for neuronal function. Alterations of the junctional complex of the BRB and consequent BRB breakdown in disease contribute to a loss of neuronal signaling and vision loss. As new therapeutics are being developed to prevent or restore barrier function, it is critical to implement physiologically relevant *in vitro* models that recapitulate the important features of barrier biology to improve disease modeling, target validation, and toxicity assessment. New directions in organ-on-a-chip technology are enabling more sophisticated 3-dimensional models with flow, multicellularity, and control over microenvironmental properties. By capturing additional biological complexity, organs-on-chip can help approach actual tissue organization and function and offer additional tools to model and study disease compared with traditional 2-dimensional cell culture. This review describes the current state of barrier biology and barrier function in ocular diseases, describes recent advances in organ-on-a-chip design for modeling the BRB, and discusses the potential of such models for ophthalmic drug discovery and development.

Keywords: organs-on-chip, blood–retinal barrier, microphysiological system, 3D cell culture, ophthalmic model, ocular model, ophthalmic drug discovery

Introduction

The blood–retinal barrier (BRB) helps to maintain the proper retinal environment to allow vision and is dysfunctional in several blinding eye diseases.[1] The existence of the BRB was first demonstrated using an intravenous injection of trypan blue solution and observing that the retina was impermeable to the dye.[2] We now understand the BRB as a series of molecular systems, including the junctional complex and drug efflux transporters that control the entry and exit of various molecules based on size and physicochemical properties. The retina is supplied by 2 distinct vascular beds: the retinal vasculature supporting the inner retina and the choroidal vasculature that supports the outer retina, and the BRB is composed of two separate barriers restricting influx from these two sources. The inner BRB (iBRB) is composed of specialized retinal vascular endothelial cells that have well-developed tight junctions (TJ) and limited transcellular vesicles that restrict nonspecific transport from the vascular circulation, and the outer BRB (oBRB) is made of the retinal pigmented epithelium that controls flux from choriocapillaries.[3] The BRB maintains the retina as a privileged site and controls the retinal environment to preserve neuronal function.[4–6]

In ocular diseases such as diabetic retinopathy and age-related macular degeneration (AMD), alterations of the junctional complex of the BRB and consequent BRB breakdown may contribute to a loss of neuronal signaling and neural apoptosis.[1,7] New treatments in ophthalmology are focusing on restoring the barrier function and/or limiting vascular leakage by targeting mediators of barrier dysfunction. Their successful development relies on the existence of relevant models to help disease modeling, target validation, and toxicity screening. Current *in vitro* systems are simple 2-dimensional (2D) cell monoculture devices that fail to fully recapitulate the BRB or changes associated with disease, and *in vivo* models are limited by the time and cost needed for studies. In addition, having *in vitro* models that integrate human cells would improve relevance to human diseases.[8]

Organs-on-chip are cell culture devices that integrate perfusion, 3-dimensional (3D) cell organization, and co-

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culture.[9] They provide a tissue-like architecture *in vitro* and are, therefore, more physiologically relevant than traditional 2D cell culture systems to interrogate disease pathophysiology and cellular response to treatments.[10] While capturing additional biological complexity, they remain relatively simple to use and can approach *in vivo* organization and function.[8]

Here, we review the current state of barrier biology and barrier function in ocular diseases, describe current organs-on-chip to model the BRB, and highlight their potential for ophthalmic drug development.

BRB in Health and Diseases

Anatomy and function of the BRB

The retina is a highly metabolically demanding system with the highest oxygen consumption per unit weight of tissue.[11] The outer retina requires high oxygen levels to supply the photoreceptors, while regions of the inner retina have a much lower metabolic rate. The delivery of oxygen and nutrients relies on a dual vascular system in which the inner two-thirds of the retina are nourished by the retinal circulation and the outer third, comprised by the high-energy-demanding photoreceptors, is nourished by the choroidal circulation.[12]

Like in the brain, a proper retinal neural function requires maintenance of an adequate neural environment, which is essential for phototransduction, and this is achieved, in part, by the formation of the BRB. Similar to the blood–brain barrier (BBB), the BRB is a highly specialized blood–neural barrier that is selective and restricts the flux of ions, metabolites, and harmful toxins into the retina, thus maintaining the retinal neural environment.

The BRB is present at the two distinct blood supply sites of the retina. The iBRB regulates transport across the retinal capillaries and is formed by TJ between adjacent endothelial cells, providing a physical barrier to water and hydrophilic solutes.[13] Contrary to systemic capillaries, the presence of TJ strands, lack of fenestrations, and low pinocytic activity contribute to a high transendothelial electrical resistance that maintains ionic homeostasis and low solute flux that resists albumin entry and subsequent fluid accumulation and edema, allowing a proper neuronal signal transduction, for example, by glutamate, without interference from plasma amino acids. The formation of the iBRB requires signals both from astrocytes and pericytes that promote differentiation of retinal vascular endothelial cells.[14]

The oBRB regulates the movement of solutes between the fenestrated choriocapillaris and the photoreceptor layer of the retina and is formed by the intercellular junction complex of the retinal pigment epithelium (RPE).[13] Although transepithelial resistance is much lower than transendothelial resistance observed at the iBRB, it is still sufficient to prevent the entry of water and hydrosoluble molecules from the choroid to the subretinal space.[15] RPE also maintains fluid homeostasis by active transport of water toward the choroid following an osmotic gradient.

Transport across the BRB is highly regulated and exists in two main forms, the paracellular pathway between adjacent endothelial or epithelial cells, which is regulated by the intercellular junction complex, and the transcellular pathway, that occurs through the endothelial cells and RPE, and involves specialized transport vesicles, including caveolae and receptor-mediated transport.

The intercellular junctions are composed of TJ, adherens junctions, and gap junctions, where TJ are responsible for the rate-limiting step in the paracellular diffusion of ions and polar solutes.[16] TJ are present at the most apical side of the highly polarized epithelial cells, whereas in endothelial cells they form a more intricate complex with adherens junctions. TJ consist of a multiprotein complex, including a series of transmembrane proteins embedded in the plasma membrane. Transmembrane TJ proteins include: junctional adhesion molecules, 24 claudin isoforms, and the MAL and related proteins for vesicle trafficking and membrane link (MARVEL) protein family, including occludin and tricellulin. These transcellular proteins are linked to the cytoskeleton and are connected laterally through associated cytoplasmic adaptors, such as the zonula occludens (ZO) proteins.[17] Besides providing a gate function at the paracellular space, TJ also act as a fence, regulating the lateral diffusion of proteins and lipids in the plasma membrane throughout the surface of the cell.[18,19] TJ have also been implicated in regulating cell proliferation and morphology, as well as in establishing and maintaining cell polarity.[20–22]

Given the low permeability of the intercellular junctions, cells at the BRB rely on a highly regulated transcellular movement of certain ions and macromolecules by active membrane transporters and vesicular transport. The expression of many of these transport proteins is polarized such as the secretion and transport of soluble factors by endothelial and epithelial cells.[23,24]

The transport of metabolic substrates, such as lipids, amino acids, and glucose, is mediated by specific transporters at the cell membrane, whereas water and small solutes are preferentially transported via the paracellular pathway. As mentioned before, endothelial cells from the mature iBRB have low levels of transcytosis, and the repression of transcytosis observed during retinal vessel development enhances barrier properties.[25]

Changes in barrier properties, that is, permeability, occur when cellular transport is altered, and a number of mediators involved in the pathophysiology of retinal diseases have been shown to alter either or both paracellular and transcellular transport. This will be discussed in further detail in the following section.

BRB breakdown in retinal diseases

Given the central role of the BRB in maintaining a proper retinal function, it is not surprising that major retinal diseases that affect visual function, such as diabetic retinopathy, including diabetic macular edema (DME), central retinal vein occlusion, and AMD, are characterized, to varying extents, by BRB dysfunction. Typically, BRB loss, characterized by increased permeability, leads to fluid accumulation, edema formation, leakage of harmful neurotoxic substances, and infiltration of inflammatory cells, which can all directly or indirectly impair visual function.[1] The most concerning retinal diseases that affect visual function, DME and wet AMD, are characterized by breakdown of the inner and outer BRB, respectively.

Several mediators such as hyperglycemia, inflammation, and hypoxia have been implicated in retinal diseases, where BRB dysfunction is a major cause of vision loss. The

activation of vascular endothelial growth factor (VEGF) pathway secondary to both hyperglycemia and hypoxia is thought to be one of the major contributors for BRB permeability, and to date the only approved medical therapies are local injections of anti-VEGF molecules and corticosteroids, which are used to control ocular inflammation.[26]

Local inflammatory mediators such as proinflammatory cytokines have been shown to directly affect paracellular permeability by altering the integrity of TJ complex.

Tumor necrosis factor-α alters both the content and the subcellular distribution of ZO-1 and claudin-5 cells through protein kinase Cζ (PKCζ)-mediated NF-κB activation, leading to increased retinal endothelial monolayer permeability.[27] *In vivo*, interleukin-1β has been shown to induce increased BRB permeability by disrupting endothelial junctions and possibly by increasing transendothelial vesicular transport.[28,29] Elevated levels of transforming growth factor-β have also been linked to increased vascular permeability, inducing occludin degradation via matrix metalloproteinase-9.[30,31]

The loss of barrier properties also leads to the infiltration of circulating inflammatory cells that will further exacerbate BRB breakdown by secreting more inflammatory mediators.[32]

At the iBRB, VEGF induces occludin phosphorylation via the activation of PKCβ, which stimulates endocytosis of the TJ complex, resulting in increased endothelial cell permeability.[33,34] VEGF also downregulates occludin through the β-catenin pathway.[35] The activation of atypical PKC also seems to contribute to VEGF-induced TJ complex breakdown and increased permeability through a still unknown pathway.[36] Additionally, phosphorylation and internalization of VE-cadherin, a major constituent of the adherens junctions, also contributes to VEGF-induced permeability.[37] Together, these results show that VEGF signaling at the endothelial cells of the iBRB induces alterations of the junctional complex, with the most prominent effects being vesicle-mediated internalization of the complex and increased vascular permeability.

The VEGF pathway is also involved in the increase of transcellular permeability in retinal endothelial cells. VEGF stimulates caveolae-mediated transcytosis in a nitric oxide synthase-dependent manner, accompanied by a shift in caveolae distribution, from a predominantly abluminal localization to a predominantly luminal localization.[38,39] Similarly, advanced glycation end-products, which are harmful byproducts of hyperglycemia, also promote increased caveolar transport with associated BRB permeability *in vivo*.[40]

More recent evidence has implicated plasmalemma vesicle-associated protein (PLVAP), a structural component of caveolae, in VEGF-induced permeability. Under physiological conditions, PLVAP is absent from the endothelium of the retina; however, it was shown to be induced by VEGF and required for VEGF-mediated increase in caveolar transport. PLVAP expression co-localizes with barrier loss, making it a good marker and/or therapeutic target for increased transcytosis.[41] The relative contribution of transcellular and paracellular flux is only beginning to be explored and will require genetic manipulation to fully elucidate.

The mechanisms that lead to oBRB breakdown have not been studied as extensively as for the iBRB, yet RPE barrier dysfunction may contribute to AMD and diabetic retinopathy pathology.[15] The disruption of RPE intercellular junctions favors the entry of fluid into the sub-retinal space; and similarly to vascular barrier dysfunction, proinflammatory mediators and VEGF seem to contribute predominantly. Both proinflammatory cytokines and VEGF mediate direct effects on TJ proteins, altering their expression and/or subcellular localization.[42–45]

Furthermore, upregulated basolateral VEGF secretion by RPE in hypoxia or loss of polarity of VEGF production not only induce barrier breakdown but are also implicated in the pathogenesis of choroidal neovascularization (CNV) in AMD.[46] CNV can further exacerbate RPE breakdown by physically separating cells and contribute to edema formation as the choroidal vessels are fenestrated and leak into the retina.

Vision threatening edema formation happens when fluid accumulates at the macula region.[47] Although the mechanisms involved are not yet fully understood, it is believed that increased barrier permeability is a key contributor.[48,49] Extravasation and accumulation of albumin following BRB loss promote a switch in the oncotic pressure leading to water entry into the retina.[49,50] Additionally, compromised active transport of water out of the retina by the RPE can also contribute to fluid accumulation in the subretinal space.[48] Therefore, targeting BRB permeability is one of the major therapeutic concerns, and developing appropriate representative experimental models that allow to rapidly test potential candidates is of great importance.

Modeling the BRB *In Vitro*

As new therapies are under development to prevent vascular leakage or restore vascular barrier function in ocular diseases, it is critical to develop *in vitro* models that recapitulate important physiological aspects of barrier biology. Ideally, suitable *in vitro* models would be relevant to human diseases by integrating human cells, provide a more robust understanding of disease biology, mitigate the use of cost and time-intensive animal studies, and enable improved throughput of early-stage compound screening. To achieve this end, engineered models are needed that recapitulate critical features of BRB biology and that can be designed for a broader use in standard biomedical laboratories. To date, this has focused on the design of static and planar models; however, new directions in organ-on-a-chip technology are enabling more sophisticated 3D models with flow, multicellularity, and control over microenvironmental properties.

Conventional in vitro *models of the BRB*

The BRB is a tightly regulated interface that isolates the retinal tissue from the circulation (Fig. 1).[5,6,51] As they provide a physical separation between 2 compartments that mimics this interface, transwell inserts—that is, permeable supports on which cells are seeded and that comprise an apical and basal chamber—have been used widely as *in vitro* systems to model the BRB. Further, these planar models enable facile quantification of barrier properties by transepithelial/transendothelial electrical resistance (TEER) and/or permeability to fluorescent tracers.[52] In the standard use of transwell inserts to model the BRB, retinal endothelial cells for the iBRB or retinal pigmented epithelial cells for the oBRB were seeded on the upper compartment of the transwell to generate monoculture devices or integrated as co- or tri-culture devices where the other cell types are seeded on

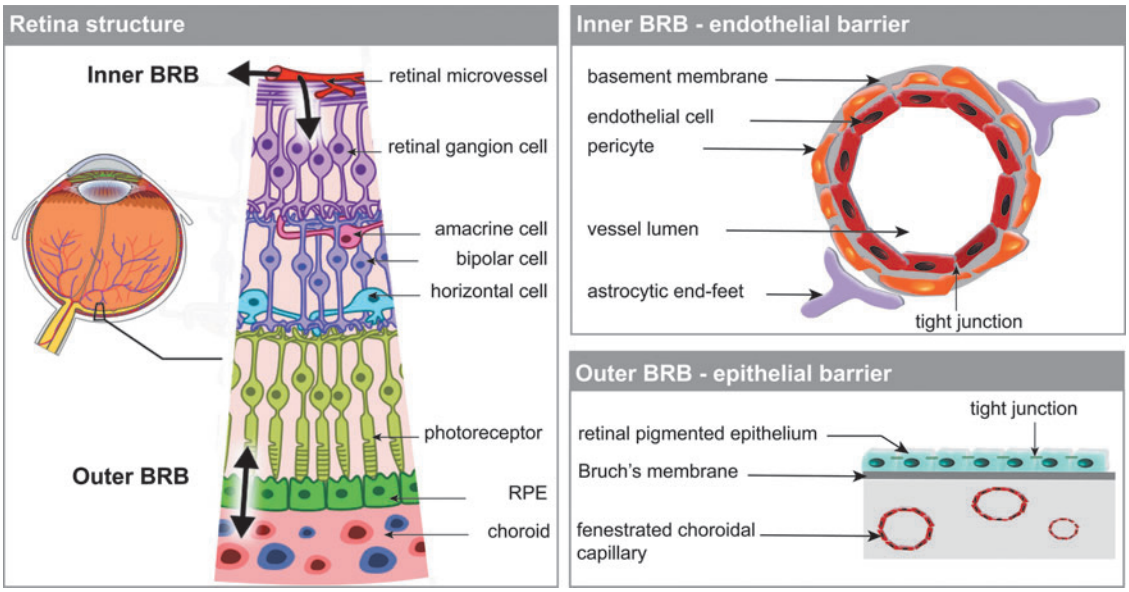

FIG. 1. BRB. The BRB ensures protection of neuronal layers of the retina and is composed of two tight barriers: an endothelial barrier called the inner BRB, and an epithelial barrier called the outer BRB (parts of this figure are adapted from Yeste et al.[115] with permission of the Royal Society of Chemistry). BRB, blood–retinal barrier.

the opposite side of the insert and/or at the bottom of the well.[53–55] The inclusion of multiple cell types enhanced physiological relevance by enabling crosstalk between neighboring cell populations.[54] Further, investigating cell–cell communication enabled mechanistic studies of developmental, functional, and pathological processes of the retina, as support cells are known to influence BRB permeability and endothelial cell functions.[56–58] For example, integrating pericytes, astrocytes, and/or astrocyte-conditioned medium with endothelial cells in an iBRB transwell model enhanced TJ protein content and TEER values compared with monoculture and provided a more relevant frame to investigate VEGF-mediated permeability.[59,60] In an oBRB model, a coculture of primary RPE and primary endothelial cells revealed specific cell–cell mechanisms that induce CNV as observed in AMD.[55]

Most BRB *in vitro* models have been established using primary cells isolated from animal sources or from human patient samples to increase model relevance to human diseases.[53–55,59] Human immortalized cell lines, such as the RPE cell line ARPE-19, have been developed in an attempt to improve robustness and availability.[61,62] Recently, induced pluripotent stem cells (iPSCs), which possess self-renewal capability and potential to differentiate into various lineages, have emerged as a source to generate retinal cells[63,64] and have been used to generate vascular progenitors and endothelial cells that express TJ and endothelial cell markers.[65–68] The generation of different retinal cell types derived from iPSC will advance the relevance and translation of BRB models.

In addition to the importance of cell–cell communication, recent evidence indicates that cell–matrix interactions are critical to BRB function, as the extracellular matrix (ECM) provides cells with biochemical, mechanical, and molecular signals that contribute to the regulation of cell function, via reciprocal interactions between cells and components of the ECM.[69,70] Synthetic transwell membranes limit cell–cell contacts to within the plane, are made of polycarbonate that is stiffer than physiologic tissues, and rely on surface coating with protein solutions that often fail to recapitulate the complex composition of the ECM.[71] Therefore, recent efforts have focused on engineering a more physiologic 3D microenvironment for *in vitro* BRB models. For example, alginate-based hydrogels, basement membrane matrix, and silk fibroin membranes have been explored as RPE and retinal endothelial cell substrates.[72,73] To recapitulate the trilayered structure of the oBRB, RPE and endothelial cells were seeded on both sides of an amniotic membrane, which induced phenotypic changes in the endothelium that were not observed on synthetic transwell membranes.[74,75]

While these centimeter-scale systems have been useful for evaluating the efficacy of exogenous molecules on barrier breakdown, they remain quite simple, are static, and provide a limited toolbox to investigate complex biological questions and/or mechanisms of action of disease mediators on barrier properties. Cells cultivated in 2D are not able to recapitulate the characteristics found in 3D, such as polarized expression of specific receptors or proteins; they present altered cell signaling pathways and aberrant gene expression profiles.[76–78] These differences between 2D and 3D likely contribute to the observed discrepancies between *in vitro* and *in vivo* data and may impact the drug discovery process.[79,80] In addition, implementing physiological flow in a macroscale system is not trivial but is critical as shear stress regulates barrier properties, induces changes in gene expression, and glycocalyx maturity.[81–85] Finally, macroscale systems often lack multiscale architecture and tissue–tissue interfaces; nutrient and metabolite transport is limited by diffusion; growth factor gradients that regulate microvascular growth are absent; and the media-to-cell ratio is quite high leading to the dilution of paracrine signaling molecules.[86–88]

Therefore, advanced models are needed to better mimic *in vivo* physiology by allowing perfused culture, 3D multicellular organization, and controlled microenvironment.

Advanced *in vitro* systems such as organs-on-chip are engineered to integrate additional biological complexity while remaining easy to handle. Microscale organs-on-chip have proven to be particularly useful for vascular models and, therefore, represent a powerful tool to emulate the BRB. The following section explains general concepts for chip engineering and describes recent advances in BRB-on-a-chip development.

Improving in vitro *models through organ-on-a-chip microengineering*

Organ-on-a-chip systems are micrometer-sized devices that allow the culture of cells under perfusion and in a spatially controlled microenvironment in an attempt to better recapitulate tissue or organ physiology. It is believed that tissue organization and function are determined by the spatial arrangement of cells combined with specific cell–cell interactions and ECM cues and that the physical and geometrical constraints that regulate tissue expansion and decipher tissue organization also affect phenotype.[10,76,89] By providing a 3D scaffold for cells to grow and capturing the optimum biochemical and structural microenvironment, organs-on-chip can help approach actual tissue organization and function.

These devices are manufactured using standardized microfabrication techniques that enable a high-resolution and precise control over geometry and surface architecture at the micron scale, similar to the cellular length scale. Organs-on-chip generally consist of one or multiple microchannels (10–500 μm in height), made of glass, plastic and/or polydimethylsiloxane (PDMS), seeded with organotypic cells, and perfused using microfluidic pumps.[9,90,91]

The fact that these systems are fully microengineered provides them with several advantages such as flexibility of design features; the possibility of integrating analytics directly within the chips, including microsensors or microelectrodes for TEER measurements; and *in operando* high-resolution imaging. In addition, the microscale minimizes the use of reagents and cells, allows media-to-cell ratio closer to physiological values, enables analytical sampling on small volumes, and favors high-throughput experimentation.[9,92,93]

Organ-on-a-chip systems offer flexibility and control over the spatial arrangement of cells and the microenvironment. The compartmentalization facilitates the integration of multiple cell types, and different configurations can be achieved depending on the tissue architecture one wants to recapitulate.[94] Cells can be placed as a monolayer at the interface between two chambers or encapsulated in a 3D matrix. Some systems allow direct cell–ECM contacts without the use of any artificial membrane, which is particularly useful for investigating the influence of matrix properties on cell behavior. In these systems, channels are filled with hydrogels, which mimic aspects of the ECM, in a controlled manner via the inclusion of microstructures in the chip that guide the air–liquid interface and enable spatial definition of gel formation.[95,96] Microfluidic channels are usually amenable to different matrix compositions, and collagen I, fibrin, and matrigel-based matrices have been investigated along with tunable, synthetic hydrogels.[97,98] For example, mature pluristratified keratinized epidermis has been developed by seeding keratinocytes on a fibrin-based dermal matrix and air–liquid interface. This human skin-on-a-chip showed improved barrier function, epidermal differentiation, and basement membrane protein deposition compared with static skin equivalent and constitutes a relevant platform to test percutaneous permeability and toxicity.[99]

Because they provide easy control over flow and enhanced throughput, organs-on-chip can serve as a platform to investigate the effect of flow and shear stress on cell form and function and on biological phenomena (eg, flow-induced endothelial cell migration, proangiogenic factor secretion, and mechanotransduction), as well as to study interactions between circulating cells and endothelium (eg, immune adhesion).[100–102] Flow is critical while modeling the BRB as it improves barrier properties. In some devices, perfusion is induced by gravity, meaning that flow is created by the movement of the chip on an inclination rocker. These devices do not need microfluidic tubing and induce an intermittent bidirectional[103,104] or unidirectional flow.[105] In other approaches, unidirectional perfusion is directed via pumps and valves enabling control over flow rate and pattern (ie, steady, pulsatile), which is useful as blood flow is extremely heterogeneous in the vascular system. The full physiological range of shear stresses (τ) can be accessed on microchips by modifying the volumetric flow rate (Q) and/or channel radius (r). The shear stress is calculated as $\tau = Q/r^3$ for Poiseuille flow, often observed in microfluidic culture devices, and can easily span from 0.1 to 10 Pa.[106,107] Beyond flow, chips can also integrate other mechanical forces to which cells are subjected *in vivo* such as cyclic strain to mimic breathing movements or peristaltic motion.[101,108] A lung-on-a-chip model integrating breathing motion was able to provide new insights in the contribution of lung mechanical activity to cell–pathogen interactions and nanoparticle transport.[101]

Microchips allow biochemical gradients to be generated, enabling the study of cellular response to controlled spatial and temporal distribution of molecular signals, such as endothelial cell migration under gradients of proangiogenic factors.[106] For example, diffusion-based concentration gradients can be created across a cell-containing channel that connects two compartments with differential concentrations of a signaling molecule.[109,110] As flow is laminar in microfluidic systems, no turbulent mixing will occur. Therefore, molecules present in fluid streams flowing side-by-side will mix via diffusion at the interface, and stable gradients can be established based on molecular diffusion at fluid–fluid interfaces. By merging multiple microchannels containing different concentrations and/or molecules, one can generate flow-based gradients of signaling molecules or therapeutics and test their effects on cell-laden compartments.[111,112]

The current chips available offer different options in terms of designs and features from which one can choose depending on the tissue of interest and the biological application. The goal is not to capture the full organ function but rather to engineer a minimal functional unit that is the simplest model possible that recapitulates the physiological response(s) of interest[9,92] The following section describes BRB-on-a-chip models that have been developed.

Physiological factors and design considerations for BRB-on-a-chip models

The oBRB is composed of a fenestrated choroidal vascular network adjacent to a tight RPE monolayer (Fig. 1).

Both are separated by a thin ECM layer called the Bruch's membrane. In pathologic conditions, aberrant vascular growth from the choroid invades the Bruch's membrane and induces RPE monolayer breakdown. The objective of most oBRB-on-a-chip devices that have been developed is to model epithelium phenotype and barrier function, mimic pathological CNV and angiogenesis, and investigate the effect of antiangiogenic compounds on vascular growth. As cell–cell interactions are critical in this process, oBRB-on-a-chip devices are usually a co-culture of RPE and endothelial cells.[1,96,113]

One of the simplest oBRB model is a microfluidic device made of 2 parallel channels, one containing RPE (ARPE-19) and the other one Human umbilical vein endothelial cells (HUVEC) and separated by a perforated PDMS membrane (Fig. 2a).[113] This co-culture model was used to investigate choroidal angiogenesis induced by hypoxia and/or low glucose. The authors show that increased VEGF secretion by RPE cells caused HUVEC migration and ultimately led to RPE monolayer destabilization.[113,114]

Instead of a synthetic membrane, another oBRB-on-a-chip includes a 3D fibrin matrix to separate the epithelium layer (monolayer of ARPE-19) from the choroidal vascular network (HUVEC embedded in a fibrin matrix) (Fig. 2b). Without this gap, the co-culture was not stable and choroidal vessel regression was observed. In addition, fibroblasts were seeded in lateral channels on both sides of the chip to support vessel formation and to stabilize epithelium monolayer. Indeed, asymmetric fibroblast patterning only on the vascular side of the chip led to a growth factor gradient that resulted in RPE breakdown. RPE polarization, expression of TJ proteins, and barrier formation as measured by

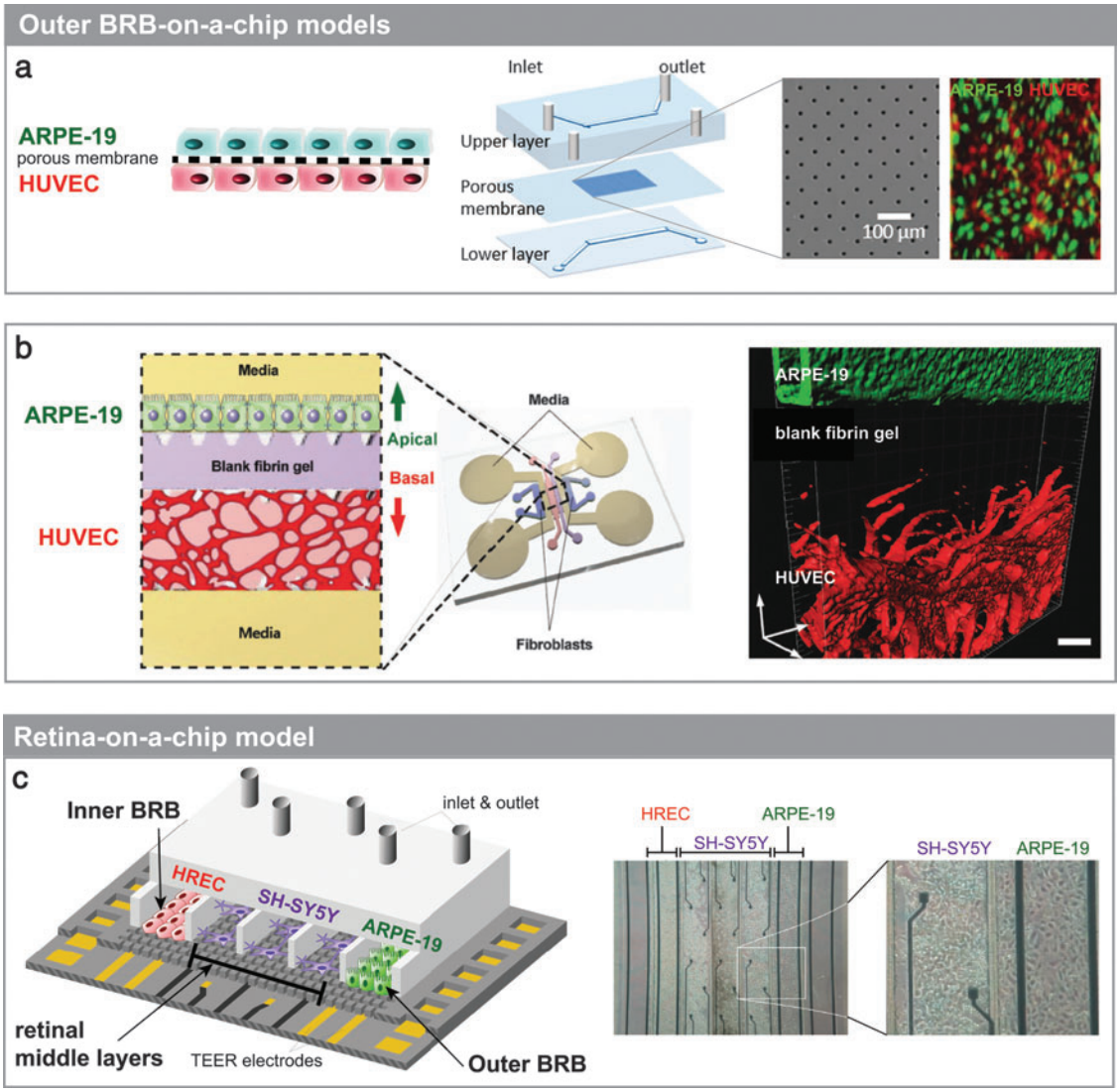

FIG. 2. Organ-on-a-chip models of the BRB. **(a)** Outer BRB-on-a-chip composed of monolayers of ARPE-19 and human umbilical vein endothelial cells (HUVEC) separated by a synthetic membrane (reproduced from Chen et al.[113]; https://creativecommons.org/licenses/by/4.0/); **(b)** outer BRB-on-a-chip integrating a fibrin matrix: the blank matrix serves as a support for the ARPE-19 monolayer and is used as scaffold for the 3D encapsulation of HUVEC (reproduced and adapted from Chung et al.[96] with the permission of John Wiley and Sons); **(c)** retina-on-a-chip model that integrates a monolayer of HREC as the inner BRB, three channels seeded with neuroblastoma cell SH-SY5Y to mimic retinal neuronal layers, and a monolayer of ARPE-19 as the outer BRB (reproduced and adapted from Yeste et al.[115] with permission of the Royal Society of Chemistry).

permeability assay were observed in this model. This oBRB-on-a-chip was able to mimic the pathological morphology of CNV and showed use to test the effect of antiangiogenic molecules.[96]

To recreate the multiple tissue–tissue interfaces of the different layers of retina, iBRB (primary human retinal endothelial cells), neuronal middle layers (SH-SY5Y human neuroblastoma cells), and oBRB (ARPE-19) have been combined on a chip (Fig. 2c).[115] In this retina-on-a-chip system, cells are compartmentalized in parallel channels that are tightly separated from each other to maintain a microenvironment specific to each cell type and/or to expose cells to specific and independent stimuli (eg, flow to endothelial cells, electrical signal localized to the neuronal layer). A grid of microgrooves underneath the cells allows for paracrine signaling between cells from adjacent channels, but no cell–cell contact is *a priori* possible. The formation of endothelial and epithelial barriers was confirmed by the presence of TJ, tracer permeability assays, and TEER measurements as the device integrates electrodes.[115]

Although no iBRB-on-a-chip has been developed yet, numerous BBB models exist, and design specifications can be taken from these BBB models, as both barriers show similarities in structure. They both consist in a tight endothelium surrounded by pericytes embedded in a basement membrane and in contact with glial cells.[14] To date BRB-on-a-chip systems have been mostly developed using immortalized cell lines as this facilitates device validation, but the integration of iPSC-derived retinal progenitors or mature cells could further improve disease modeling, as it has been done on BBB models and vascular organoids.[65,66,68] Both the brain and the eye are soft tissues, and providing an ECM interface with relevant mechanical and biochemical properties is important, as ECM stiffness has an impact on endothelium tightness, and ECM composition imparts cells with critical signals for their function.[116–121] In addition, the effect of shear stress, which can regulate barrier properties, may be explored.[122]

One of the challenges while building *in vitro* systems with cellular complexity is to maintain specific microenvironments for each cell type while enabling paracrine signaling and cell–cell contacts. While multicellular architecture is tightly regulated *in vivo*, it can be quite challenging to implement a stable co-culture *in vitro.* Cells need to be separated into physiologic compartments to maintain function of the multicellular tissue construct. For example, aphysiologic proximity between RPE cells and HUVECs, caused by a lack of Bruch's membrane substitute, induced RPE migration and vessel regression.[96] In another model, different cell types had to be separated by a thick ECM lane.[123] In addition, some growth factors in the cell culture medium of one cell type can activate the other cell types, or asymmetric cellular patterning can create gradients that result in cell migration.[96]

Toward the development of physiologically relevant models, recapitulating tissue-relevant ECM properties in the device is critical. In the case of ECM-based interfaces, there is no physical separation between the cellular compartments, as opposed to a porous synthetic membrane, and this makes co-culture optimization more challenging. Indeed, cell–ECM interactions are dynamic, and cells can modulate ECM properties, induce gel contraction or delamination, which can ultimately affect the other cell type seeded on the matrix.[102] Sometimes, physiological relevancy has to be compromised on account of technical limitations, and it is important to consider to what extent this compromise affects the validity of the final model. For example, RPE and choroid are separated by a much thinner matrix *in vivo* (4 vs. 300 μm in an oBRB model), and authors were still able to mimic pathological CNV.[96]

Developing a successful BRB-on-a-chip model requires a fundamental understanding of cell–cell and cell–matrix interactions in 3D, or at least the minimal required factors. Recapitulating in one device the required microenvironment for multiple cell types to develop and organize into a specific, functioning biological structure is a complex task. Beyond the potential of these systems to help drug discovery, they will also certainly contribute to our understanding of tissue organization and cell interactions as they are being developed.

Potential of BRB-on-a-Chip in Ophthalmic Drug Discovery and Development

Organs-on-chip represent a powerful *in vitro* platform to identify and validate the biological activity of potential targets as well as to assess toxicity and pharmacology profiles of therapeutics because they can better recapitulate clinically relevant disease phenotypes and enable better predictions on pharmacological responses than conventional *in vitro* systems.[124] A suitable BRB-on-a-chip should demonstrate good barrier properties with the formation of TJ, reproducible permeability to reference compounds, and medium-to-high throughput screening capacity. Further, it should integrate multiple cell types that are relatively robust and express relevant protein markers and transporters, and ideally should be relatively easy to use. No single system will be useful for assessing all the aspects of drug discovery, and some aspects might have to be compromised depending on the application, that is, target validation, toxicity, or drug disposition and metabolism.

Disease modeling for compound screening and elucidating molecular mechanisms

Ophthalmic drug development commonly focuses on targets that can restore or prevent BRB dysfunction, as vascular permeability is one of the hallmarks of the most prevalent ocular diseases, that is, diabetic retinopathy and wet AMD. The evaluation of potential targets is mainly performed using *in vitro* monoculture on transwell systems and *in vivo* models; therefore, a BRB-on-a-chip model would be beneficial to bridge the gap by maintaining physiological relevance while allowing increased throughput for efficacy and safety screening. Not only this would expand fundamental knowledge of barrier biology, but also enable screening of multiple molecules and/or molecule combinations and concentrations in parallel and limit animal use.

Because the chip recapitulates several levels of biological complexity, one could deliver combinations of signals in specific amounts and intervals to modulate metabolic, phenotypic, and macroscopic tissue characteristics that are specific or relevant to a pathology or a specific pathological event. In the same way, it should be possible to simplify the disease pathology focusing, or modeling, specific events rather than examining the broad disease state. For example,

the specific effects of metabolic dysregulation, cytokine secretion, or pericyte loss on barrier breakdown may be investigated individually. An optimized organ-on-a-chip would provide multiple critical read-outs, simultaneously and overtime, as opposed to traditional *in vitro* assays that usually look at one single function at a given time point. Sensor integration and real-time imaging could provide dynamic functional read-outs of multiple parameters such as electrical resistance, inflammatory cytokine secretion, metabolic profiles, reactive oxygen species, allowing a direct visualization of disease evolution in various experimental conditions.[125–127] Control system and feedback loops could be integrated to test various treatment schemes with direct measurements of their cellular effects.[128]

One could then fabricate a stable cellular system with in-line sensors, monitor in real time specific relevant parameters in healthy state and after disease induction, as well as follow cell response to various treatment protocols. This should improve our understanding of a pathology and might bring new insights into the potential differential molecular mechanisms of disease mediators and drugs.

Potential applications for drug safety, disposition, and delivery

A major challenge in toxicology is to define models that predict potential liabilities in patients of clinical studies. This is mainly due to a lack of biological complexity of current *in vitro* models and species-to-species variability of preclinical animal models. Organ-on-a-chip models that include human cells could enhance toxicity prediction between *in vitro* and *in vivo* experiments, as well as elucidate critical interspecies differences, including pathways or biological feedback that are present in one species but absent in the other one, or differences in the expression of membrane transporters that affect drug absorption.[10,129,130]

In addition, because organs-on-chip facilitate the integration of multiple cell types, drug safety can be assessed on the full human tissue, and potential toxicity on adjacent cell layers such as pericytes, retinal glial cells, or neurons can be revealed. For example, a complete retina-on-a-chip that recapitulates different layers of the retina could be then helpful to investigate how BRB breakdown and subsequent events impact the functions of other retinal layers.[115] One could also model disease-induced immune infiltration. Similar to biological activity assessment, the integration of dynamic high-content read-outs and live cell imaging could help measure simultaneously different viability indicators and metabolic parameters (eg, apoptosis, proliferation, mitochondrial function, cell and nucleus morphology), and monitor the global health condition of the organ model following various treatment protocols. Gradient-enabling microfluidic systems could be implemented to help identify threshold concentrations of drugs and predict clinically relevant doses with fewer experiments.[112]

Another useful application is the investigation of transport mechanisms that are altered in diseased conditions. The BRB express numerous membrane transporters that regulate nutrient supply and metabolite efflux to meet the high energy demand of the neuronal retina, and changes in transporter expression during disease affect the uptake of endogenous substrates as well as xenobiotics.[131] In addition, inflammatory cytokines that contribute to BRB breakdown have an effect on both paracellular and transcellular flux, and monitoring in real time both transport routes could provide a mechanistic understanding of pathologically increased permeability as well as help pharmacokinetic predictions.[7,14] Recapitulating critical BRB transport features would be helpful for the development of drug delivery approaches and modeling of drug transport following intravitreal injection or systemic administration.[132]

Conclusions

New therapies in ophthalmology focus on the restoration or maintenance of barrier function, yet physiologically relevant *in vitro* models are lacking. By integrating biological complexity (eg, multiple cell types, ECM, and physiologic flow) within one device, organs-on-chip have the potential to improve our mechanistic knowledge of disease and drug mechanisms of action, facilitate target validation, as well as enhance drug toxicity predictions and pharmacokinetic/pharmacodynamic assessment. So far these systems have been useful to predict acute reactions; however, stable long-term culture over a month for modeling chronic disease is still difficult to achieve. Technical robustness is important for a successful implementation, and while several companies are developing organ-on-a-chip platforms, many of the devices described in the literature are custom-made for a specific application in academic laboratories. The lack of standardization of microfluidic chips makes comparison between different models difficult, and relevant biological criteria will have to be defined and validated for each application. Systems that are easy to handle and enable sufficient throughput with a minimal learning curve for use will facilitate the acceptance of microfluidic devices in the drug discovery process. As these systems and processes are being developed, they will also require fundamental knowledge on stable 3D co-culture of multiple cell types.

Author Disclosure Statement

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