The blood–retina barrier in health and disease

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The blood–retina barrier (BRB) is the term used to define the properties of the retinal capillaries and the retinal pigment epithelium (RPE), which separate the systemic circulation from the retina. More specifically, the inner blood–retina barrier (iBRB) is used to describe the properties of the endothelial cells that line the microvasculature of the inner retina, while the outer blood–retina barrier (oBRB) refers to the properties of the RPE cells that separate the fenestrated choriocapillaris from the retina. The BRB is not a fixed structure; rather, it is dynamic, with its components making unique contributions to its function and structural integrity, and therefore the retina. For example, while tight junction (TJ) proteins between retinal endothelial cells are the key molecular structures in the maintenance of the iBRB, other cell types surrounding endothelial cells are also important. In fact, this overall structure is termed the neurovascular unit (NVU). The integrity of the BRB is crucial in the maintenance of a ‘dry’, tightly regulated retinal microenvironment through the regulation of transcellular and paracellular transport. Specifically, breakdown of TJs can result in oedema formation, a hallmark feature of many retinal diseases. Here, we will describe the oBRB briefly, with a more in-depth focus on the structure and function of the iBRB in health and diseased states. Finally, the contribution of the BRB to the pathophysiology of age-related macular degeneration (AMD), diabetic retinopathy (DR) and other rarer retinal diseases will be discussed.

Introduction

The retina, a portion of the central nervous system and in effect an extension of the brain, has the highest oxygen consumption per unit weight of any tissue in the body. This environment along with high metabolic demand makes the retina, and particularly the macula (the central area of the retina with a high density of cone photoreceptors), susceptible to oxidative stress causing damage to central vision [1]. Therefore, the microenvironment of the retina must be tightly regulated, and is separated from the systemic circulation by the blood–retina barrier (BRB).

The so-called outer blood–retina barrier (oBRB) consists of the choroid, Bruch’s membrane (BM) and retinal pigment epithelium (RPE). The choroid from outer to inner portions consists of the suprachoroid, large and medium blood vessel layer, and the choriocapillaris [2]. The choriocapillaris contains fenestrations and is actively involved in the supply of nutrients and removal of waste material from the outer retinal layers, including the RPE and photoreceptors (PRs) [3]. BM is located between the choriocapillaris basement membrane and the RPE basement membrane.

Abbreviations
AMD, age-related macular degeneration; BRB, blood–retina barrier; DR, diabetic retinopathy; JAM, junctional adhesion molecule; NVU, neurovascular unit; POS, photoreceptor outer segments; RP, retinitis pigmentosa; RPE, retinal pigment epithelium; TJ, tight junction; VEGF, vascular endothelial growth factor; ZO-1, zonula occludens.

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and consists of outer and inner collagenous layers separated by a central elastic layer [4]. The BM allows for the size selective passive diffusion of molecules through it, blocking large molecule diffusion [5]. Other functions include prevention of inflammatory cell migration, stabilisation of the RPE layer and absorption of physical stress forces [6].

Specifically related to the oBRB, the RPE is a modified neuroepithelial cell. The RPE is a single layer of hexagonal, polarised, pigment-containing cells which directly underlie the neural retina. The TJ's of the RPE are located at the apical surface and are mainly responsible for maintaining oBRB integrity [7]. Numerous microvilli extend from the apical surface of the RPE and surround the photoreceptor outer segments (POS) increasing the surface area in contact with POS approximately 30-fold, thereby promoting increased cellular contact and regulation [8]. Stimulation of PRs by light results in the accumulation of photo-damaged molecules and free radical production, the majority of which occur in the POS. The POS is shed daily and phagocytosed by RPE cells, with new POS forming at the ciliary at the base of outer segments [7,8]. Phagocytosis is controlled by the circadian rhythm, mostly occurring in the early morning [8]. The RPE also contributes to the recycling of essential digested POS molecules, such as vitamin A, back to PRs along with retinoid storage and conversion, crucial to the visual cycle [7,8].

The RPE secretes several molecules and growth factors, such as pigment epithelial cell derived factor and vascular endothelial growth factor (VEGF), which help maintain retinal and choriocapillaris structural integrity [9]. VEGF secreted at the basolateral surface of the RPE stabilises the choriocapillaris by preventing apoptosis and maintains fenestrations of endothelial cells [8,10]. Melanin granules are located mostly near the apical surface and absorb scattered and out of focus light, which helps maintain clear vision [8].

The second barrier system of the retina is the inner blood–retina barrier (iBRB). This barrier is composed of endothelial cells that line the retinal vasculature which originates from the central retinal artery and supplies the inner retinal layers. The vasculature penetrates the retina at three main plexuses: the superficial, intermediate and deep layers which correspond to the nerve fibre layer, inner plexiform layer and outer plexiform layer respectively [11]. The PR layer of the retina is avascular [12]. Figure 1 for comparison of the iBRB/oBRB.

While the iBRB refers to the unique properties of the retinal endothelial cells, the general make-up of the iBRB consists of the neurovascular unit (NVU), which is similar in structure and function to the blood–brain barrier (BBB) [13]. The retinal NVU comprises of retinal vascular endothelial cells with their dual basement membrane, surrounded by pericytes and glial cells including astrocytes, muller cells and microglia [13,14].

![Anatomical localisation of the inner and outer blood-retina barriers](image-url)
The NVU contributes to the overall integrity of the iBRB. Retinal pericytes, which contribute heavily to this integrity, are contractile, phagocytic cells embedded in the capillary basement membrane with a higher ratio in the retina compared to any other tissue in the body [15]. They stabilise the microvasculature, regulate blood flow and can control endothelial cell proliferation, and, thus, have a role in angiogenesis [14]. They can further regulate the retinal microenvironment by secretion of extracellular matrix components such as fibronectin [14,16].

Astrocytes, which are predominately located in the nerve fibre and inner nuclear layers, can modulate the BRB by releasing trophic factors, antioxidants and both pro- and anti-inflammatory cytokines to the NVU microenvironment [17,18]. Their processes surround retinal vascular endothelial cells which leads to a more intact TJ barrier [17,19]. High glucose disrupts astrocyte function, morphology and TJ integrity [19].

Muller cells located throughout all layers of the retina, interact via their foot processes at synapses with other neuronal cells in the retina (including ganglion, bipolar and amacrine cells) [17,20]. Their footplates form the internal limiting membrane and surround blood vessels providing a further support function [17,20]. Through these interactions they have been shown to release vasoactive substances, so called ‘gliotransmitters’ and, thus, modulate neuron transmission and endothelial cell permeability [17,20].

Microglia are resident glial macrophages which are predominately located close to the retinal vasculature where they can clear cellular and metabolic debris [13]. Despite the relative immune privilege of the eye, microglia become activated in an inflammatory environment, release proinflammatory molecules and undertake phagocytosis [13]. In addition to their proinflammatory immune function, they also interact and secrete biochemical factors which influence neuronal transmission, synaptic plasticity and other cells in the NVU [17,20].

**Molecular structure of tight junctions at the BRB**

Having discussed the important cellular structures and their functions at both the inner and outer BRB, our focus can now shift to the molecular organisation of TJs, which serve as the key barrier components in both the iBRB and oBRB. TJ proteins project inwards and interact in the paracellular space creating a ‘barrier’ effect. In the oBRB, they are concentrated at the apical surface of RPE cells helping to separate the choriocapillaris and sub-retinal space [21]. They consist predominantly of the claudin family proteins, MARVEL family transmembrane proteins and junctional adhesion molecules (JAMs).

In contrast to the epithelium of the RPE with TJs at the apical surface, TJs of endothelial cells are more structurally complex, dispersed between and frequently interacting with gap and adherens junctions, as tight regulation of vascular permeability is required [22]. Retinal endothelial cells have the smallest intercellular space and the highest number of TJ strands in comparison to any other tissue containing endothelial cells [23].

**Claudins**

The claudin family of proteins have at least 24 known members [21]. In general, claudins are 18–27 kDa in size [24]. They consist of four transmembrane regions with two extracellular loops and two C terminal intracellular domains containing the highly conserved PDZ-binding motifs which interact directly with the linker proteins zonula occludens (ZO), specifically ZO 1-3 [21,24]. The first extracellular loop domain is crucial in maintaining paracellular ion permeability and, therefore, is the major contributor to transepithelial electrical resistance (TEER) which measures the integrity of TJs [25] (Fig. 2). Claudins are likely the most important proteins in TJ formation, cell-cell adhesion and regulation of TJ permeability as they facilitate passive diffusion by forming pores in the paracellular space [26,27]. In the RPE, claudin-19 is the most important and predominant in maintaining TJ function [28]. Claudin-3 and -10 are also expressed to a detectable level but studies involving knock down of claudin-3 had no material effect on TJ formation and function in contrast to claudin-19 knockdown where TJ formation was impaired [28]. At the iBRB, claudin-5 is the most highly expressed claudin protein and indeed the highest expressed TJ component [29]. It is likely that it is the dominating claudin at the iBRB and its levels are critical in maintaining retinal homeostasis, as blood vessels of claudin-5 deficient mice are more permeable to molecules less than 800 Da [29].

**Associated TJ proteins**

The MARVEL proteins include occludin, tricellulin and marvelD3. Occludin has been shown to directly bind ZO1-3, but their role in TJ barrier function has yet to be fully characterised with contrasting in vitro and in vivo findings [30,31]. For example, the discovery that occludin deficient cells and knockout mice can form tight barriers that are viable, indicates that other
TJ proteins are more important for TJ formation and function [30,31].

JAMs are members of the immunoglobulin superfamily and are transmembrane proteins located at TJs, with one transmembrane domain, an extracellular domain including two Ig-like motifs, and a C terminus domain containing a PDZ-binding motif which can bind TJ scaffolding proteins such as ZO-1, occludin and cingulin [32,33]. Through this, they contribute to TJ formation and function [32,33].

ZO os are cytoplasmic scaffolding proteins which attach to transmembrane proteins and anchor them to the cytoskeleton [32]. This interaction facilitates correct transmembrane protein organisation (with claudins) in the cell membrane allowing multi protein cell signalling cascades [32,34]. The core structure of ZO consists of PDZ, Src homology 3 and guanylate kinase domains [21,32]. Through its first PDZ domain, ZO can bind the C terminal end of the actin cytoskeleton facilitating β actin binding [32,34]. Its second PDZ domain enables ZO-2 and 3 binding [21,32].

**Intraocular forces at the BRB**

Cellular structures and TJ proteins are not the only factors influencing the BRB and control of the retinal microenvironment. Forces involved in the transport of fluid and ions across the retina can help explain the events that occur in many retinal diseases during the breakdown of the BRB and subsequent oedema [35]. In the normal eye, intraocular pressure causes water to move into the extracellular space of the retinal layers, which is then counteracted by the osmotic gradient of the choroidal circulation pulling water into the vasculature and, thus, the retina remains ‘dry’ and in place [35,36]. Water and solute likely move freely across the internal limiting membrane of the retina – which is composed of collagen and matrix material – and intercellularly until they reach the external limiting membrane (ELM), sometimes referred to as the outer limiting membrane [36,37]. The ELM contains a band of adherens junctions which are less effective than TJs of endothelial cells but do prevent the movement of large proteins such as albumin across it [37,38]. Thus, to counteract this osmotic pressure within the retina, active transport of water across the RPE takes place from the apical to basolateral direction [8,35].

Most evidence suggests that it is the breakdown of the tight junctional complexes between endothelial cells which contributes to iBRB breakdown and subsequent retinal oedema that is observed as a co-morbidity in numerous retinal conditions [14,22,26]. Increased iBRB permeability results in increased capillary hydrostatic pressure, as TJ protein regulation is lost, and the
retina is exposed to systemic blood pressure [35,39]. As more extracellular fluid accumulates and retinal thickness increases, tissue compliance in the retina is decreased resulting in lower resistance to fluid accumulation [39].

Osmotic pressure in the retina is approximately zero, but with increased iBRB permeability, proteins can accumulate in the retina behind the ELM, increasing osmotic pressure which contributes to oedema formation [39]. Compensatory mechanisms such as the reabsorption of fluid by Muller cells and aquaporin-4 channels can resolve fluid accumulation, however, when these are overwhelmed, it will ultimately lead to RPE dysfunction and decreased RPE active transport which will result in retinal oedema [35,36,39].

Transcellular transport and caveolae

One of the main mechanisms of molecular transcytosis involves caveole formation. Caveolae are microscopic areas of invaginations in the plasma membrane which consist of lipid rafts and the important proteins caveolin-1 and cavin [43]. They can undergo endocytosis within retinal endothelial cells where caveolae vesicles within the cytoplasm can induce signal transduction, be recycled to the plasma membrane or deliver their contents to the opposite plasma membrane [43].

A low rate of vesicle transport is present in retinal endothelial cells, but several studies have shown that the rate of caveolae-mediated endocytosis increases and thus the permeability of the iBRB in an increased VEGF environment [44,45]. In addition, inhibition of PLVAP (a structural protein of caveolae that is expressed in the presence of VEGF) in vivo and in vitro resulted in inhibition of VEGF-induced caveolae formation and decreased BRB permeability [46]. Animal studies which demonstrated decreased caveolae and caveolin-1 expression showed decreased expression of some TJ proteins leading to an increase in iBRB and BBB permeability, suggesting caveolae may also have a role in regulating TJs and, thus, paracellular transport [47,48].

Caveolae also contribute to the establishment of the oncotic pressure gradient across the endothelial barrier through the transcytosis and delivery of albumin to the abluminal side of the endothelium [49]. Albumin binds glycoprotein 60 on the luminal side, forming caveolin –1 clusters thereby promoting transcytosis [22,49].

Mfsd2a

Major Facilitator Superfamily Domain Containing 2A (mfsd2a) is a transmembrane protein which transports lyso phosphatidylcholine to the brain and retinal PRs [50,51]. It is highly expressed in the BBB endothelium, BRB endothelium and in the RPE [50,51]. Docosahexaenoic acid (DHA), an omega-3 fatty acid synthesised from α-linolenic acid but transported in a lysophosphatidylcholine form, is essential for cognitive function, brain development, BBB establishment and PR OS formation [51,52]. Mfsd2a mutants develop BBB breakdown and lethal microcephaly [50].

The BBB, BRB and RPE exhibit a low rate of transcytosis compared to the periphery [51,53,54]. Mfsd2a transports lipids which create a specific lipid cellular microenvironment, with increased levels of phospholipids containing DHA isolated from brain compared...
to peripheral endothelial cells [50,53]. These lipid species are thought to disrupt caveolae-mediated vesicle formation, as caveolin-1 is displaced from the plasma membrane by DHA, thus, suppressing caveolae-mediated transcytosis [53,55].

Contrasting results show mfsd2a mutant mice have increased BBB permeability with increased levels of transcytosis [52], while others demonstrated no loss of BBB and BRB integrity [51,54]. All studies demonstrated preservation of TJs and vascular structure suggesting paracellular integrity remains [51,53,54]. Pericyte deficient mice and endothelial cell models demonstrate decreased mfsd2a expression, further indicating the role of NVU regulation of the BBB and BRB [52].

Mfsd2a deficient mice demonstrate rods that are DHA deficient, atrophied and with disorganised outer segments with rhodopsin reduction [51,54]. The RPE is thickened with loss of apical microvilli [51]. Interestingly, these mice have no significant loss of PRs and maintain phototransduction as measured through electoretinography [51,54]. DHA is thought to be transported to PRs by mfsd2a in the RPE and not the retinal vasculature, as mfsd2a deletion from vascular endothelium does not impact DHA accumulation in PRs or their morphology [50,54]. Overall, mfsd2a is a key transporter protein which influences cellular lipid constituents, limiting transcytosis and maintaining BRB integrity crucial to PR development.

**Paracellular transport and claudins**

TJs are the main regulators of paracellular transport at the BRB. By restricting the passage of molecules paracellularly between apical and basolateral membranes they maintain cell polarity [56]. While transcellular transport mechanisms are fundamental to maintain retinal homeostasis, it is the claudin proteins that prevent the flow of molecules paracellularly, forming ion selective pores [56]. Ion selectivity is determined by their first extracellular loop, whereas their second extracellular loop is crucial during cis (same cell) and trans (between cell) claudin–claudin interaction in a homotypic or heterotypic fashion [57]. The scaffolding protein ZO-1 links the transmembrane claudin with the actin cytoskeleton allowing precise membrane localisation and intracellular signalling, both important in paracellular regulation [57]. After birth, claudin-5 KO mice die within 10 h and display profound BBB leakiness to molecules < 800 Da [29]. Other studies modelling ischaemia and diabetes demonstrate that claudin-5 loss is the main driver of increased BBB and iBRB permeability, thus, further indicating claudin-5’s importance in supporting the BBB and iBRB [58,59]. Claudin-5 is not expressed in RPE cells of the oBRB, with claudin-19 being the dominant TJ component in RPE cells [28].

**Retinal diseases**

At this point, we have discussed the various elements of the BRB including the cellular, molecular, intracellular forces and transport mechanisms involved in its function. We have also touched on how their dysfunction can lead to the breakdown of the BRB, particularly manifesting in protein and fluid accumulation within the retina. In the following sections, we discuss some of the more common retinal diseases where BRB breakdown is the key element in disease origin and progression, while also briefly discussing other rare retinopathies and the role of the BRB in the disease process.

**Age-Related Macular Degeneration (AMD)**

AMD is the number one cause of retinal blindness worldwide with the incidence expected to continue to increase [60]. AMD results in irreversible loss of central vision due to retinal degeneration in the macula, the cone rich central portion of the retina [61]. Risk factors include cigarette smoke, alcohol, Western style diet, ageing and genetic polymorphisms (e.g. complement factor H and the ARMS2 variants) all of which contribute to increased oxidative stress thought to be the central mechanism leading to PR/RPE dysfunction and BRB breakdown [62]. AMD can be classified into two types ‘dry’ and ‘wet’. AMD in its early stages is generally asymptomatic and can be identified during clinical examination of the retina by yellowish deposits known as drusen in the subretinal or sub-RPE regions [61]. RPE dysfunction, loss and PR death can progress at the macula resulting in the end stage of ‘dry’ AMD termed geographic atrophy (GA) [60,61]. ‘Wet’ AMD has a faster progression, occurs in approximately 10% of cases and is characterised by choroidal neovascularisation (CNV), where the underlying choroid (underlying the RPE) neovascularises and can lead to haemorrhage, oedema and ultimately PR cell death [60,61]. Increased levels of VEGF are the main driver of CNV formation, therefore, anti-VEGF intravitreal injections are an effective treatment option for ‘wet’ AMD [63]. There are no treatments currently available for GA secondary to ‘dry’ AMD.
In early AMD, RPE function begins to decline due to oxidative stress and the accumulation of undigested material known as lipofuscin [64]. In later stages, RPE cells lose their unique hexagonal morphology and functions [65]. Recently, this loss of cell differentiation, known as epithelial–mesenchymal transition, has been shown to result in multinucleation, loss of TJ’s and loss of RPE morphology, all contributing to oBRB loss, dysregulated gas and molecule exchange and further RPE/PR damage [65].

The build-up of waste material and thickening with calcification of BM leads to impaired diffusion of nutrients and oxygen along with impaired removal of waste material between the RPE and choriocapillaris [66]. This can further damage the retina and RPE cells, leading to further oBRB integrity loss [66]. Decreased choroidal thickness and reduced choriocapillaris vascularity are also features of AMD. This likely leads to decreased oxygen and nutrient supply to the RPE, adding further metabolic stress to RPE cells and contributing to oBRB breakdown [67, 68].

Post-mortem analysis of human donor eyes with dry AMD revealed increased levels of plasma proteins such as fibrinogen, albumin, complement and immunoglobulins indicating iBRB breakdown may also be a key element of AMD progression [69]. This iBRB breakdown can result in loss of immune privilege and subsequent inflammatory cascade with neural retina injury [70]. Indeed, it has also been shown in mouse, non-human primates, and human subjects, that the iBRB is highly dynamic and appears to be regulated by the circadian clock. The key iBRB-associated TJ protein claudin-5 cycles in a circadian manner, and it is thought that its dysregulation may be a key driver of the early stages of dry AMD [71].

Diabetic retinopathy

A recent global estimate of diabetes burden in 2019 was 463 million people with an expected rise to 745 million people in 2045 [72]. In 2020, global diabetic retinopathy (DR) prevalence was estimated to be 103 million, 22.27% of the total diabetes burden, with this projecting to increase to 160.5 million by 2045 [72]. The detection of DR clinically is on average 10 years post diabetes diagnosis and is due to accumulation of retinal microvasculature biochemical dysfunction and subsequent damage [73]. Indeed, it is almost an inevitability that every patient with diabetes will develop some form of retinal disease if they live long enough.

DR can be characterised by non-proliferative (NPDR) and proliferative (PDR) disease [73, 74]. Clinical features consistent with NPDR include microaneurysms and dot, blot, flame haemorrhages due to retinal vasculature dysfunction. Cotton wool spots are ischaemic retinal nerve fibre layers that occur secondary to capillary dropout [73, 74]. PDR is characterised by the growth of new blood vessels at the optic disc or elsewhere in the eye, secondary to the release of growth factors from the ischaemic retina, most notably VEGF [73, 74]. Fragile new blood vessels of the iBRB can leak fluid and protein resulting in macular oedema and haemorrhage. These dysregulated vessels can also fibrose and pull on the retina leading to tractional detachment [73, 74].

The main treatment option for PDR is pan retinal photocoagulation which destroys the peripheral RPE cells and PRs, which receive most of their oxygen from the choroidal circulation [75]. This reduces the oxygen consumption of the outer retinal layers allowing more oxygen to diffuse from the choriocapillaris towards the inner retinal layers, thus, reducing the stimulus of hypoxia-induced cytokine production (such as VEGF) which helps prevent further angiogenesis and thus maintains central vision [75].

For macular oedema (termed diabetic maculopathy) intravitreual anti-VEGF agents are generally the first line treatment as they decrease the stimulus which leads to the growth of 'leaky' blood vessels as discussed above [76]. Focal or grid laser photocoagulation, IVT steroids and slow-release steroid implants such as dexamethasone or fluocinolone implants (Ozurdex, Allergan) are also used to decrease exudate and oedema at the macula [75]. Steroid preparations are a good choice for chronic oedema, not responsive to other treatments [75, 76]. Vitreoretinal surgery is generally reserved for uncontrolled disease [75, 76].

Factors influencing iBRB breakdown in DR

The plasma kallikrein-kinin system (KKS) is a proteolytic pathway consisting of tissue kallikrein (TK) and plasma kallikrein (PK) [77]. PK is produced in the liver, complexes with other proteins and has a high affinity for endothelial cells where it binds and becomes activated [77]. PK participates in the breakdown of the iBRB in DR through inflammation and angiogenesis [77]. Activation leads to production of kinins, potent vasodilators, with bradykinin as the main mediator [77, 78]. Kinins exert their effects though the B1 and B2 G protein-coupled receptors on cell surfaces, where retinal B2 is constitutively expressed, but B1 is upregulated during ocular inflammation, injury and DR [79]. Several studies have shown the importance of the KKS in the promotion of
iBRB permeability and oedema, through the direct intravitreal administration of PK, B1 and B2 receptor agonists in animal models [80,81]. In addition, administration of B1 receptor antagonists can reduce retinal permeability and oedema [82].

TK has similar activity to PK but is virtually undetectable in the vitreous of PDR patients in contrast to PK which has high concentration levels, thereby indicating PK as the likely active mediator in DR [83]. Increased carbonic anhydrase levels in the vitreous of diabetic eyes was also postulated to increase the pH and activate the KKS system resulting in BRB breakdown [84].

VEGF, a well-characterised pro-angiogenic factor and a proven strong inducer of vascular permeability, is increased in the hypoxic retina of DR [73]. In hypoxia, the hypoxia-inducible factor complex forms, translocates to the nucleus and induces VEGF expression [85]. Increased VEGF leads to the decreased expression of ZO-1 and occludin resulting in increased paracellular permeability at the iBRB [86]. VEGF induces disruption of the iBRB through the phosphorylation, ubiquitination and subsequent inactivation of occludin in a PKCβ dependent manner [87]. VE-cadherin phosphorylation and inactivation also result in TJ disorganisation and increased vascular permeability [87,88].

The activity of proinflammatory cytokines and chemokines has also been shown to contribute to iBRB breakdown in DR [14,73]. Advanced glycation end products (AGEs), which are glycated lipids and proteins, and reactive oxygen species (ROS) form in the presence of hyperglycaemia leading to activation of NFκB which induces expression of these pro-inflammatory molecules [89]. Specifically, TNFα expression leads to PKCζ activation which down-regulates claudin-5 and ZO-1 levels at the iBRB [89]. Higher IL-1B levels are found in the diabetic rat retina, where upregulated expression of IL-1B and iBRB breakdown was mediated by recruitment of leukocytes and histamine release [90]. Chemokines, and specifically, CCL2 contributes to iBRB breakdown mostly indirectly through monocyte recruitment, which secrete further growth factors and pro-inflammatory mediators such as VEGF and TNF-α [91]. CCL2 levels were found to be higher in the vitreous of DR patients [92].

**Cellular and structural dysfunction of the iBRB during DR**

Pericyte dropout is another characteristic feature of retinal hyperglycaemia. These contractile cells surround retinal capillary cells in a near 1 : 1 ratio where they share a basement membrane, influence blood flow and contribute highly to the BRB [93,94]. They signal to endothelial cells through molecules such as TGF-β and PDGF-β [94]. Hyperglycaemia and AGEs cause apoptosis of pericytes, one of the earliest changes in DR [94,95]. Subsequent iBRB breakdown occurs in addition to dysregulated angiogenesis secondary to loss of control of endothelial cell proliferation [95].

The glycocalyx is a layer of soluble proteins, glycoproteins and proteoglycans such as heparan sulphate which covers the luminal surface of the vascular endothelium [96]. It functions as a barrier, prevents the adhesion of leukocytes and platelets and acts as a mechanotransducer (e.g. upon encountering a shear stress it stimulates nitric oxide (NO) production) [96,97]. Hyperglycaemia leads to endothelial dysfunction, leukocyte adhesion molecule upregulation (e.g. ICAM-1) and activation of matrix metalloproteinases (MMP-9 and −2) causing shedding and cleavage of the glycocalyx [98].

Decreased glycocalyx density has been observed in diabetic models in animals and in humans with elevated levels of heparinase and subsequent increased glycocalyx and TJ protein occludin shedding observed [99]. In *vitro*, high glucose cell cultures showed an inability of endothelial cells to position correctly when exposed to shear stress [100]. Decreased NO production secondary to decreased endothelial-derived nitric oxide synthase phosphorylation was proposed as a mechanism [100]. NO also contributes to MMP inhibition and decreased oxidative stress, therefore, decreased NO results in increased MMP-2 and −9 activity and ROS production leading to further decreased glycocalyx [100,101].

Finally, in a recent *in vitro* model of the human iBRB, a high glucose environment led to increased iBRB breakdown compared to a normal glucose environment [19]. This was demonstrated via increased paracellular permeability as measured by a reduced TEER and increased Na-F movement, a fluorescent inert tracer molecule [19]. Immunohistochemistry analysis revealed a discontinuous endothelial cell brush border with decreased levels of ZO-1 and VE-cadherin [19]. Astrocytes present in the high glucose environment demonstrated pro-inflammatory behaviour through increased IL-1B and IL-6 production [19].

**Retinitis Pigmentosa (RP)**

RP is a group of hereditary disorders resulting in first rod and then cone degeneration in the retina, with the usual onset in adolescence with characteristic symptoms including night blindness, loss of peripheral vision and
finally loss of central vision progressing to complete blindness [102]. Characteristic fundal findings include retinal vessel attenuation, optic disc pallor, RPE mottingling and bone spicule pigmentation in the periphery [102]. Inheritance patterns vary and include autosomal dominant, recessive and X linked, with over 50 genes reported to be associated [102]. The exact molecular pathogenesis is unclear but the presence of cystoid macular oedema (CMO) in many cases and evidence of increased permeability in inner retinal layers, points to iBRB breakdown [103]. Increased fluorescein leakage was demonstrated on fundus fluorescein angiography (FFA) in RP patients, with the most frequent site of leakage at the macula [104]. Animal studies have revealed more contrasting results regarding TJ integrity, with increased and decreased levels of ZO-1 demonstrated in different mouse models of RP [105,106].

Current treatment options are limited but include gene therapy and retinal implant devices. Luxturna is used in patients who have a genetic mutation in the RPE65 gene [107]. An adeno-associated viral vector introduces a functional copy of the RPE65 gene to the RPE thereby restoring the production of 11-cis-retinal, a crucial step for rhodopsin and, thus, PR function [107]. CMO can be treated using oral and topical carbonic anhydrase inhibitors such as acetazolamide and dorzolamide respectively [108]. These agents decrease leakage from the retinal vasculature as demonstrated by FFA and may stimulate active transport across the BRB [108]. However, rebound CMO has been noted in up to 40% of cases [108]. Intravitreal anti-VEGF agents and corticosteroids have also been trialled with limited success [108].

Coats disease

Coats disease is a sporadic neovascular retinal disease with a male predominance, diagnosed in the first two decades of life, with most cases unilateral [109]. The most common finding is decreased visual acuity, while other findings include strabismus (eye squint), nystagmus (involuntary movement of the eye) and pain [110]. Retinal telangiectasia (broken, spider like vessels) is the characteristic feature with capillary dropout, vessel aneurysm, exudation and sub/intra-retinal fluid [109,110]. Retinal detachment, haemorrhage, fibrosis and macular oedema can also be present [109,110]. Treatment options include laser photocoagulation and cryotherapy to telangiectatic vessels and vitrectomy in cases of extensive retinal detachment [111]. Enucleation, while a last resort, is the best option for a blind painful eye. Emerging adjuvant therapies including intravitreal triamcinolone and anti-VEGF, which reduce retinal oedema, lead to vessel regression, and can improve visual acuity [111].

iBRB breakdown has long been implicated in the pathogenesis of Coats disease. The characteristic widening of retinal vessels and retinal exudate is due to plasma extravasation and accumulation in vessel walls and retinal layers [112]. Abnormal and reduced pericyte numbers have been observed leading to decreased vascular endothelium support contributing to abnormal ‘telangiectatic’ blood vessels and saccular/fusiform (spindle shaped) aneurysm formation [113,114].

Capillary non-perfusion is present in areas of the retina with telangiectasia, a further demonstration of retinal capillary dysfunction [109]. Decreased numbers of endothelial cells in vessel walls have been demonstrated using immunostaining and electron microscopy, with the underlying choroid normal and an intact BM [113,114]. The presence of lipid laden macrophages in retinal fluid and granulomatous immune reaction in retinal layers indicates a catastrophic loss of iBRB integrity to immune cell infiltration and ocular immune privilege [113,114].

**Fig. 3.** Schematic representation of blood–retina barrier breakdown. Ganglion cell layer (GCL), inner nuclear layer (INL), outer nuclear layer (ONL) and Outer plexiform layer (OPL). The oBRB is formed by the retinal pigment epithelial cells (RPE).
Conclusion

While the oBRB consists of the choroid, BM and most importantly the apical TJ of RPE cells, the iBRB consists of the junctional components located between endothelial cells of the retinal vasculature and the surrounding supporting cells, known as the NVU. The retina is highly metabolically active and, therefore, a fine balance of fluid, molecule and solute levels is critical to preserve cellular health and clear vision. Osmotic, oncotic, capillary hydrostatic and active transport mechanism are all centrally involved in maintaining this balance. TJ are the most important functional component of the iBRB. Transport of molecules to and from the retina is tightly controlled. Claudin family proteins are important both in maintaining the barrier function of TJ and in the regulation of paracellular transport. It is now clear that claudin-5 is the dominant member expressed in the iBRB with claudin-19 the dominant claudin in the oBRB. Transcellular transport in the retina is also tightly regulated, with caveolae centrally involved. Dysfunction and loss of iBRB integrity resulting in retinal ischaemia, cellular stress and oedema formation is central to the development of many retinal diseases including AMD and DR. iBRB loss is also present in rarer retinopathies including RP and Coats disease. A representation of various iBRB and oBRB breakdown scenarios is outlined in Fig. 3. Identifying the underlying causes of BRB dysfunction in retinal diseases may allow us to better tailor drugs of the future to restore BRB integrity and, therefore, treat disease.

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Conflict of interest

The authors declare no conflict of interest.

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References


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89 Aveleira CA, Lin CM, Abcouwer SF, Ambrosio AF, Antonetti DA. TNF-alpha signals through PKCzeta/NF-kappaB to alter the tight junction complex and increase retinal endothelial cell permeability. *Diabetes*. 2010;59:2872–82.
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