PART 2 Basic Science and Translation to Therapy

SECTION 1 Anatomy and Physiology

22 Structure, Function, and Pathology of Bruch's Membrane

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INTRODUCTION, HISTORY, EMBRYOLOGY

Bruch's membrane is a thin (2–4 μm), acellular, five-layered extracellular matrix located between the retina and choroid.[1,2] It extends anteriorly to the ora serrata, interrupted only by the optic nerve. Tissue resembling Bruch's membrane is visible anterior to the ora serrata extending forward to the pigmented epithelium of the ciliary body. Bruch's membrane lies between the metabolically active retinal pigment epithelium (RPE) and a capillary bed (choriocapillaris) and thus serves two major functions as the substratum of the RPE and a vessel wall. It has major clinical significance because of its involvement in age-related macular degeneration (AMD) and other chorioretinal diseases.

Early History

Carl Ludwig Wilhelm Bruch (1819–1884) (Fig. 22.1 online) first isolated the "lamina vitrea" that we now know as Bruch's membrane and described it in his 1844 doctoral thesis[3,4] where he also first described the tapetum found in many mammals. By light microscopy, Bruch's membrane appeared transparent, with little internal structure. Later studies by A.E. Smirnow[5] divided this membrane into an outer elastic layer (first described by Sattler in 1877) and an inner cuticular layer, separated by a dense plexus of very fine elastic fibers.[6,7]

Development of Bruch's Membrane

The bipartite character of Bruch's membrane arises from the embryology of its tissue. When the optic cup invaginates and folds, its inner layer forms the neural retina, and its outer layer, the RPE. The RPE lies in contact with mesenchyme. At this apposition, Bruch's membrane forms by 6–7 weeks' gestation. Thus, its inner layer is composed of ectodermal tissue and its outer, mesodermal. At the border of two layers, the elastic layer forms last, becoming histologically visible by 11–12 weeks.[8–10]

The collagen that fills the extracellular space, and the later appearing elastin, appear to be made by invading fibroblasts and the filopodia of endothelial cells lining the adjacent choriocapillaris. The two basal laminas are produced by their associated cell layers.[11] In addition to collagen IV subunits specific to specialized basal lamina, RPE expresses genes for

structural collagen III and angiostatic collagen XVIII in a developmentally regulated manner linked to photoreceptor maturation.[12]

By week 13, fenestrations are apparent in the endothelium facing Bruch's membrane,[10] indicating that at this stage, transport across this tissue may be functional. Choroidal endothelial cells originate from paraocular mesenchyme. Development of the choroidal vasculature, and Bruch's as part of it, depends on differentiated RPE and its production of inductive signals, including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).[13]

STRUCTURE OF BRUCH'S MEMBRANE IN THE YOUNG ADULT EYE

Hogan's five-layer nomenclature for Bruch's membrane[14,15] is commonly used. Gass proposed a three-layer system that did not include the cellular basal laminas as part of Bruch's proper.[16] These layers are shown in Fig. 22.2 and their constituents in Table 22.1. Important components in specific layers are structural collagens, elastin, and proteoglycans with negatively charged glycosaminoglycan side-chains.

RPE Basal Lamina (RPE-BL)

This ~0.15-μm thick layer is a meshwork of fine fibers like other basal laminas in the body.[17,18] The RPE-BL resembles that of the choriocapillaris endothelium (ChC-BL) in containing heparan sulfate proteoglycans with several sulfation motifs.[19–21] Unlike ChC-BL, RPE-BL does not contain collagen VI. The RPE-BL contains collagen IV α3-5,[22] like that of kidney glomerulus, another organ with specialized filtration and transport functions. The RPE synthesizes specific laminins that preferentially adhere Bruch's membrane to the RPE through interaction with integrins.[23]

Inner Collagenous Layer (ICL)

The ICL is ~1.4 μm thick and contains 70-nm-diameter fibers of collagens I, III, and V in a multilayered crisscross, parallel to the plane of Bruch's membrane.[1] The collagen grid is associated with interacting molecules, particularly chondroitin sulfate and dermatan sulfate proteoglycans.[19,21,24]

Elastic Layer (EL)

The EL consists of stacked layers of linear elastin fibers, crisscrossing to form a 0.8-μm thick sheet with interfibrillary spaces of ~1 μm. This sheet extends from the edge of the optic nerve to the ciliary body pars plana.[1] In addition to elastin fibers, the EL contains collagen VI, fibronectin, and other proteins, and collagen fibers from the two collagenous layers can cross the EL. Some EL elastin fibers cross the tissue space between the choriocapillaris and join bundles of choroidal elastic tissue.[25] The EL confers biomechanical properties, vascular compliance, and antiangiogenic barrier functions. It is discontinuous in the macula, perhaps explaining why choroidal neovascularization is more prominent there.[26] This concept is supported by the extensive laser-induced neovascularization in mice deficient in lysyl oxidase-like 1, an enzyme required for elastin polymerization.[27]

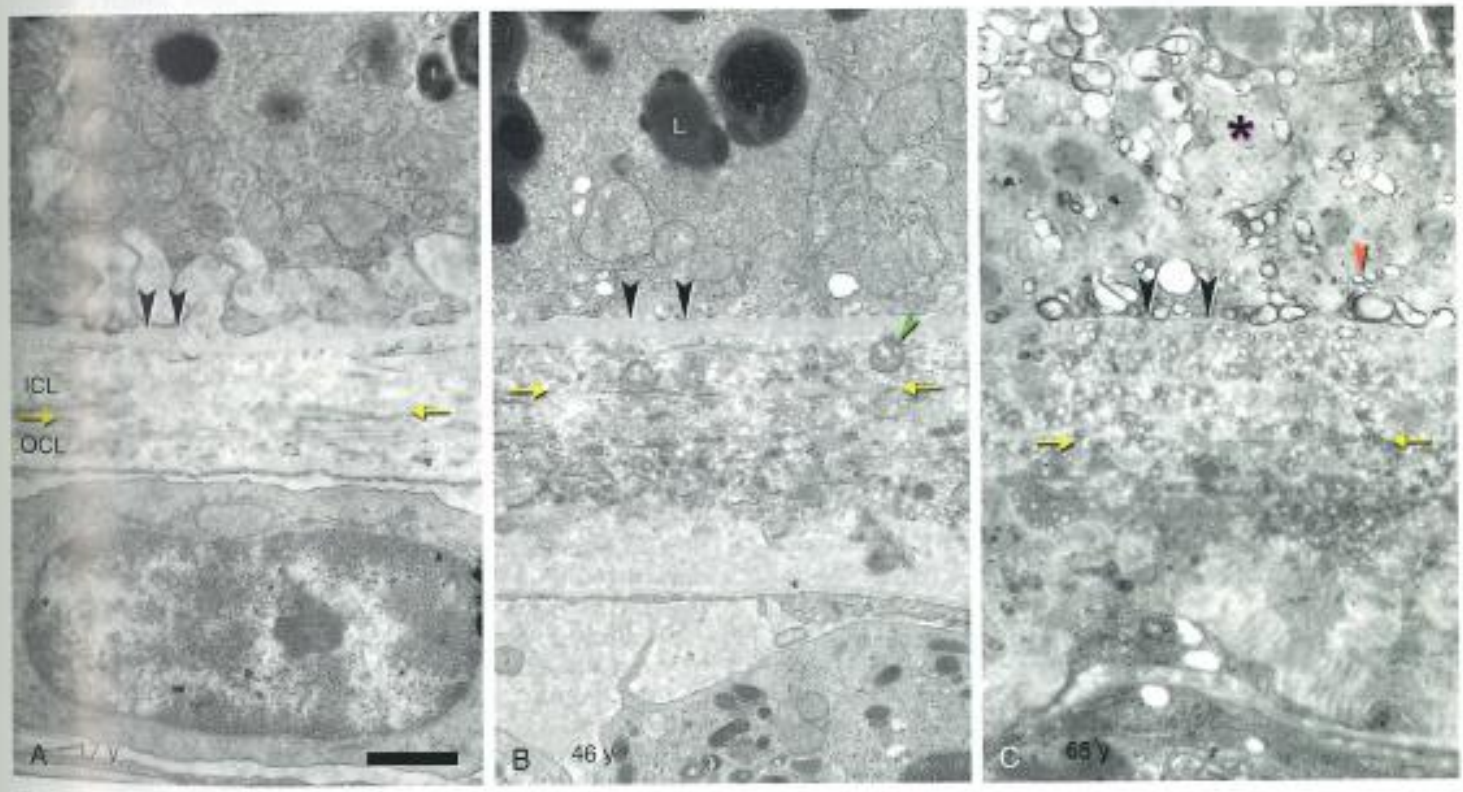

Fig. 22.2 Macular Bruch's membrane throughout the lifespan. Retinal pigment epithelium (RPE) is at the top of all panels. RPE basal lamina (*arrowheads*) and elastic layer (EL, *yellow arrows*, discontinuous in macula) are shown. (A) 17 years: Electron-dense amorphous debris and lipoproteins are absent. Scale bar: 1 μm. (B) 46 years: Electron-dense amorphous debris and lipoproteins are present. A coated membrane bounded body (*green arrow*) contains lipoproteins. L, lipofuscin. (C) 65 years: Electron-dense amorphous debris and lipoproteins are abundant. Membranous debris, also called lipoprotein-derived debris (*red arrow*) has electron-dense exteriors within BLamD (*). Within OCL, banded material is type VI collagen, often found in BLamD.

TABLE 22.1 Structural and Molecular Components of Bruch's Membrane

Layer (Common Abbreviation)	Component; **Age Change**	References
Basal laminar deposit (BlamD)	+ **Fibronectin, laminin, IV α4-5, VI, endostatin, EFEMP1**	220, 223, 277–280
RPE–Basal lamina (RPE–BL)	IV α1–5, V, laminins 1, 5, 10, and 11, nidogen-1, heparan sulfate, chondroitin sulfate	19, 22, 23, 78, 281,282
Lipid Wall/ Basal linear deposit (BlinD)	+ **Lipoproteins**	52, 53, 283
Inner collagenous layer (ICL)	I, III, V, fibronectin, chondroitin sulfate, dermatan sulfate, **lipoproteins↑**, apoE, heme, clusterin, vitronectin	44, 47, 52, 53, 78, 189, 197, 281, 284–287
Elastic layer (EL)	**Elastin↑, calcium phosphate↑**	78–81, 281, 288
Outer collagenous layer (OCL)	I, III, V, fibulin-5, fibronectin, chondroitin sulfate, dermatan sulfate, **lipoproteins↑**, apoE, clusterin	19, 53, 189, 281, 285, 289
ChC–Basal lamina	IV α1,2, V, VI, laminin, heparan sulfate, chondroitin sulfate, endostatin	22, 279, 281, 292, 290
Bruch's, throughout or layer not specified	**I↑, collagen solubility↓**, perlecan, **MMP-2↑, MMP-9↑, TIMP-2↓ TIMP-3↑, pentosidine↑, CML↑, GA-AGE↑**, RGR-d, apoB, oxidized apoB-100, 7-KCh, MDA, LHP, **HHE↑, DHP-lys↑, FHL-1, C3d↑, C5b-9↑, pentraxin-3↑**, thrombospondin-1, zinc	74, 78, 85, 174, 183, 198, 290–302

Table shows definitely localized components. Most determinations were made in macula. Studies showing histochemical/ immunohistochemical verification of biochemistry and ultrastructural validation of structures identified by light microscopy techniques were given greater weight. Localizations were assigned to specific layers if immunogold-electron microscopy or high magnification confocal microscopy images were available. Roman numerals denote collagens. Components are ordered within each layer: structural components, lipoproteins, extracellular matrix and its regulation, modified lipids and proteins, complement/immunity, cellular response/activity, metals. Known changes with advancing age are **bold** with an arrow indicating direction of change. New additions with age are shown with a plus (+). Plain text means no change or not tested.

Abbreviations: 7-KCh, 7 keto-cholesterol;[303] CML, carboxymethyl-lysine;[298] DHP-Lys, dihydropyridine lysine;[78] GA-AGE, glycolaldehyde derived AGE;[299] HHE, 4-hydroxyhexenal;[78] MDA, malondialdehyde.[78,299]

Outer Collagenous Layer (OCL)

The OCL contains many of the same molecular components as the ICL, and the collagen fibrils running parallel to the choriocapillaris additionally form prominent bundles. This layer, unlike the ICL, has periodic outward extensions between individual choriocapillary lumens called intercapillary pillars, where thickness cannot be determined due to the lack of a boundary. Between pillars, OCL thickness can range from 1 to 5 μm.[28]

Choriocapillaris Basal Lamina (ChC-BL)

This 0.07-μm-thick layer is discontinuous with respect to Bruch's membrane due to the interruptions of the intercapillary pillars of the choroid. It is continuous with respect to the complex network of spaces defined by the choriocapillary lumens because the basal lamina envelops the complete circumference of the endothelium. A remarkable structural feature of the adjacent choriocapillary endothelium is fenestrations that are permeable to macromolecules (Fig. 22.3).[29] This basal lamina may inhibit endothelial cell migration into Bruch's membrane, as do basal laminas associated with retinal capillaries.[30]

BRUCH'S MEMBRANE IN AN AGED EYE

Aging is the largest risk factor for developing AMD,[31] and Bruch's membrane undergoes significant age-related changes. Identification of factors predisposing to disease progression is a priority. This task has been challenged by difficulty imposed by the thinness of the tissue, and the closely integrated functions of RPE, Bruch's membrane, and choriocapillaris. Current opinion holds that RPE and Bruch's membrane age in concert, and normal Bruch's membrane aging transforms insidiously into AMD pathology.[1,17,18,32] This section covers aging, to inform the following section on function.

Lipid Accumulation: Bruch's Membrane Lipoproteins

Early electron microscopists described aged Bruch's membrane as being filled with debris, including amorphous electron dense material, membrane fragments, vesicles, and calcification.[33,34] Debris deposition in ICL and OCL begins in the second decade of life in the macula and is delayed in equatorial regions.[35] Verhoeff speculated that calcification of aging Bruch's membrane might follow lipoidal deposition[36] as it does in atherosclerosis. Later investigators described aged Bruch's membrane as sudanophilic (i.e., histochemically detectable lipid).[37,38] Histochemical, ultrastructural, biochemical, gene expression, cell biologic, and epidemiologic evidence have converged to indicate that the lipid-rich material accumulating with age in Bruch's membrane is cholesterol-rich lipoprotein particles containing apolipoproteins B and E that are assembled and secreted by the RPE.[39] This process, ongoing throughout life yet first revealed by aging, has implications for formation of AMD-specific lesions, RPE physiology, nutrient and waste product transport to and from the outer retina, and maintenance of photoreceptor health. The physicochemical properties, physiologic roles, and distribution of cholesterol in relation to AMD pathology has been reviewed.[40]

Clinical observations on fluid-filled RPE detachments in older adults led to Bird and Marshall's hypothesis that a lipophilic barrier in Bruch's membrane blocked a normal, outwardly directed fluid efflux from the RPE[41] (as opposed to leakage from neovascularization). This hypothesis motivated a seminal histochemical study by Pauliekhoff[42] that demonstrated oil red O-binding material (EC, esterified cholesterol; TG, triglyceride; FA, fatty acid) localized exclusively to Bruch's membrane, unlike other stains. This lipid was absent <30 years, variably present at 31–60 years, and abundant at ≥61 years.[43,44] Biochemical studies confirmed the strongly age-related nature of the deposition.[45,46]

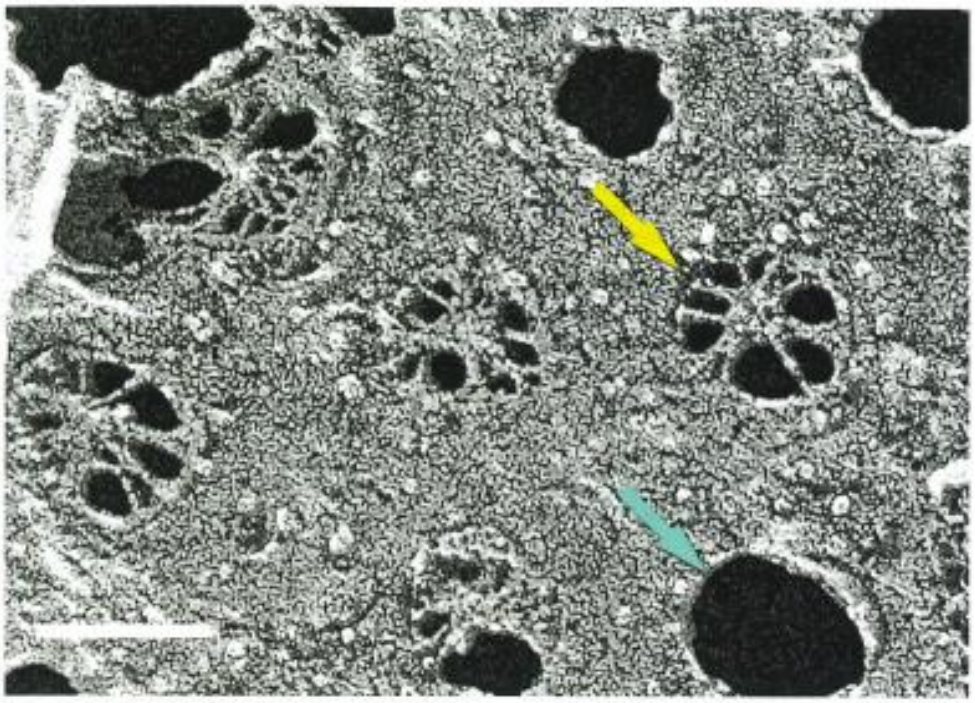

Fig. 22.3 Surface of the endothelium of the choriocapillaris showing fenestrations with a bicycle-spoke pattern (*yellow arrow*) and presumed artifactual openings arising from tissue preparation (*cyan arrow*); quick-freeze/deep-etch, 64-year-old eye, macula. Scale bar: 100 nm. *(Reproduced with permission from Johnson M, Huang J-D, Presley JB, et al. Comparison of morphology of human macular and peripheral Bruch's membrane in older eyes. Curr Eye Res 2007;32:791-9.)*

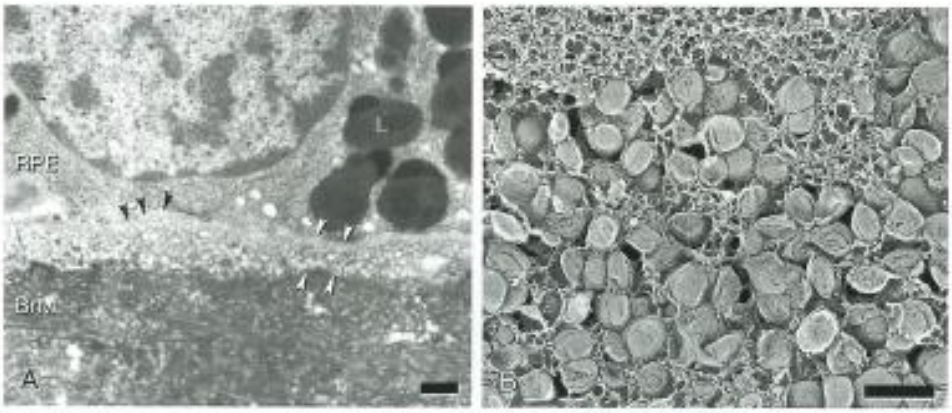

Fig. 22.4 Lipid Wall, a layer of lipoproteins on the inner surface of Bruch's membrane. (A) Lipoproteins (spherical vesicles of uniform diameter) accumulate 3–4 deep between the RPE basal lamina (*black arrowheads*) and Bruch's membrane ICL (*white arrowheads*). Thin section transmission electron micrograph following osmium postfixation. *RPE*, retinal pigment epithelium; *BrM*, Bruch's membrane; *L*, lipofuscin. Sectioning plane is vertical; scale bar: 1 µm. (B) Quick-freeze deep-etch shows tightly packed Bruch's membrane lipoproteins in the Lipid Wall, and that lipoproteins have classic core and surface morphology.[53] Fracture plane is oblique; scale bar: 200 nm.

The oil red O-binding material proved to be EC, which with unesterified cholesterol (UC) accumulates markedly in Bruch's membrane, in sevenfold higher quantities in macula than periphery.[44,47] Key techniques were use of the fluorescent marker filipin, which binds the 3-β-hydroxy group of sterols to reveal unesterified (free) cholesterol (UC) or EC depending on tissue pretreatment[47] and hot stage polarizing microscopy,[44] which showed very few birefringent crystals signifying the neutral lipid TG. Among lipids, EC is confined exclusively to Bruch's membrane,[48] focusing attention on lipoproteins, the only means by which EC is released by cells. Human RPE expresses apoB gene and protein, along with microsomal triglyceride transfer protein, required for apoB lipidation and secretion.[49] This suggests that RPE is a constitutive lipoprotein secretor. Indeed, human- and rat-derived RPE cell lines secrete full-length apoB.[50,51]

Lipid-preserving ultrastructure and analytic biochemistry support this concept. Ultrastructural studies described, in Bruch's membrane of older eyes,[35] numerous small (<100 nm), round, electron-lucent vesicular profiles, implying aqueous interiors. These so-called vesicles are actually solid, lipid-containing particles (Fig. 22.4B) when prepared by lipid-preserving methods including postfixation in osmium paraphenylenediamine (OTAP)[47] and, strikingly, quick-freeze/deep-etch (QFDE), a freeze fracture method with etching to remove frozen water.[52–54] Particles vary in size from 60 to 100 nm and occasionally appeared to coalesce (Fig. 22.4). Particles of comparable diameter with lipoprotein-like flotation

properties and spherical shapes indicating neutral lipid cores are isolable from normal human Bruch's membrane[50,55] (Fig. 22.5). These fractions include apolipoproteins B, A-I, and E. Bruch's membrane cholesterol is EC-enriched (EC/total cholesterol = 0.56)[47,50,55,56] and there is little triglyceride (EC/TG = 4-11), unlike hepatic very-low-density lipoprotein (VLDL), of similar diameter. An early report of TG-enriched Bruch's membrane[46] was not replicated. Thus, Bruch's membrane apoB-lipoproteins are unusual because they are large like VLDL yet EC-rich like atherogenic LDL (Fig. 22.5).

A natural history of Bruch's membrane lipid deposition obtained with quick-freeze deep etch showed lipoprotein particles first gathering among fibrils of the elastic layer in early adulthood.[57,58] This accumulation then extends toward RPE to fill the ICL by the seventh decade of life,[54,57] consistent with an RPE origin. In many older eyes, a new layer, the Lipid Wall,[52] forms between RPE basal lamina and OCL, and this is considered a precursor to basal linear deposits, a specific lesion of AMD (see below). With solid lipoprotein particles occupying nearly 100% of this sub-RPE space, the Lipid Wall displaces ICL collagen fibrils that anchor the RPE basal lamina (Fig. 22.4).

The fatty acid composition of Bruch's membrane lipids implicates diet as a driving force in RPE lipoprotein secretion. A longstanding hypothesis that debris in aging Bruch's membrane originates as outer segment membranes phagocytosed by RPE[59] was tested through fatty acid profiling of Bruch's membrane lipoproteins and lipid extracts.[50,56] Outer segment membranes have characteristically high concentrations of docosahexaenoate.[60] In contrast, all lipid classes in Bruch's membrane were dominated by linoleate, the most abundant fatty acid in plasma, with little (<2%) docosahexaenoate. Bruch's membrane lipid deposition is thus proposed as a recycling system whereby plasma lipoproteins delivering dietary essentials (vitamins A, E, lutein, UC) are taken up by RPE, stripped of cargo destined for photoreceptors, and excess fatty acids and UC repackaged as lipoproteins for basolateral secretion and choroidal clearance.[61]

If the proposed primacy of diet is true, then cells in culture medium should be able to create deposits without supplementation by outer segments. A landmark study by Johnson et al. confirmed these predictions,[62] demonstrating that highly differentiated and polarized human fetal RPE, supplemented only by culture medium, secreted apoE-immunoreactive particles resembling native Bruch's membrane lipoproteins. While the source of cholesterol in Bruch's membrane lipoproteins has not been determined to date, endogenous synthesis, taken-up plasma lipoproteins, and phagocytosed outer segments are obvious choices (see below).

It is informative to contrast Bruch's membrane lipid deposition with the systemic process whereby extracellular oil red O-binding lipids increase with age in normal human connective tissues. In arterial intima, tendons, sclera, and cornea, perifibrous lipid provides the background to atherosclerosis, xanthomas, and lipid keratopathy.[44,63–66] The source of extracellular EC in these locations is LDL translocated from plasma and trapped via binding to proteoglycans.[67,68] After apolipoproteins degrade, the remaining lipid components fuse.[67] The evidence that lipid deposition in aging Bruch's membrane is a distinct process dictated by photoreceptor physiology and not simply an ocular manifestation of this systemic process is compelling[40] (see below, AMD lesions). Indirect evidence also emerges from epidemiology. If EC deposition in Bruch's membrane and AMD-associated lesions were a manifestation of systemic perifibrous lipid and atherosclerosis, then a strong positive correlation between disease status and plasma lipoprotein levels, like that in cardiovascular disease,[69] might be expected. Such an association has not emerged despite many studies.[70] Nevertheless, the commonality of cholesterol-rich lipoproteins in a vessel wall is a rationale for seeking

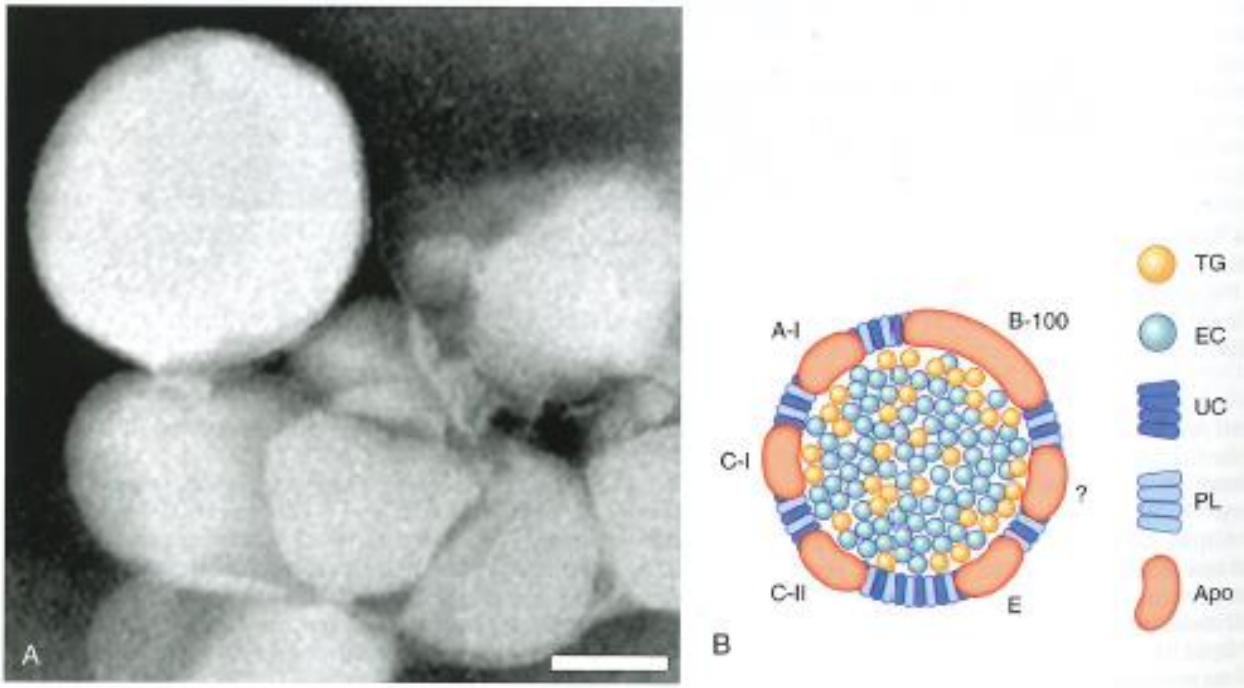

Fig. 22.5 Bruch's membrane lipoprotein composition. (A) Lipoprotein particles isolated from Bruch's membrane are large and spherical; negative stain.[190] Scale bar: 50 nm. (B) Bruch's membrane lipoprotein composition inferred from direct assay,[190,285] druse composition, and RPE gene expression.[174,191] *TG*, triglyceride; *EC*, esterified cholesterol; *UC*, unesterified cholesterol; *PL*, phospholipid; *Apo*, apolipoproteins. The question mark signifies that not all apolipoproteins are known.

guidance in cardiovascular disease for AMD pathogenesis and treatments.

Other Aging Changes

Bruch's membrane thickens throughout adulthood (20–100 years), two- to threefold under the macula and becoming more variable between individuals at older ages.[28,71,72] Equatorial Bruch's membrane changes little while Bruch's membrane near the ora serrata increases twofold during this time.[28] In the macula, the OCL thickens more prominently than the ICL.[73] A large ultrastructural study of 121 human donor eyes demonstrated that the macular EL is three to six times thinner than peripheral EL at all ages.[26]

Unbalanced regulation of extracellular matrix molecules and their modulators are thought to result in Bruch's membrane thickening. Increased histochemical reactivity for glycoconjugates, glycosaminoglycans, collagen, and elastin is seen in the macula relative to equator and near the ora serrata.[28] Collagen solubility declines with age.[74] Metalloproteinases MMP-2 and MMP-3 increase with age as does a potent inhibitor of metalloproteinases, TIMP-3. TIMP-3 immunoreactivity reaches mature levels at 30 years of age near vasculature in lung, kidney, and in Bruch's membrane, signifying the end of developmental organogenesis.[75] The reduction or absence of TIMP-3 is proangiogenic, as this protein not only regulates metalloproteinases during the normal turnover of Bruch's membrane matrix components but it also binds to VEGF.[76,77]

The EL thickens with age but decreases relative to overall thickening of Bruch's membrane.[26] Thus elastin referenced to other Bruch's constituents, as detected by Raman spectroscopy, decreases with age.[78] Similar arguments can be made for collagen III and IV. A prominent age-change,[79] noted early,[56] is calcification and ensuing brittleness. This process involves deposition of fine electron-dense particulate matter[80] confirmed as calcium phosphate[81] on individual elastin fibrils.

Long-lived proteins like collagens are modified *in vivo* by nonenzymatic Maillard and free radical reactions to yield advanced glycation end products (AGEs) and the formation of lipid-derived reactive carbonyl species like malondialdehyde (MDA), and 4-hydroxyhexenal (HHE), collectively called age-related lipoperoxidation end products (ALEs). Accumulation of AGEs and ALEs, characteristic of diabetes and atherosclerosis, also occurs in aging Bruch's membrane (Table 22.1). Finally, other components prominent in aged eyes include complement components C3d, C5b-9, and pentraxin-3, a homolog of the acute phase respondent C-reactive protein. Thus, at the molecular level, aging Bruch's membrane contains evidence of many biologic activities including remodeling, oxidative damage, and inflammation, in addition to lipoprotein accumulation.

New evidence from Clark, Bishop, Day, and associates indicates that variations in proteoglycan sulfation in Bruch's membrane have potential pathogenic significance for AMD.[21,82–86] Bruch's membrane has many heparan sulfate (HS) proteoglycan structures and motifs, as determined with antibodies to specific full-length and enzymatically truncated forms.[21] Sequence variants in the gene encoding the fluid phase regulator of complement factor H (CFH) are highly associated with risk for AMD.[87] Key in innate immunity, CFH can discriminate self from nonself for clearing by recognizing polyanionic structures such GAG chains of proteoglycans (e.g., HS and dermatan sulfate), thereby inhibiting complement activation on host cell surfaces. CFH of molecular weight 155 kD comprises 20 complement control protein (CCP) domains and contains main GAG-binding regions in CCP7 and CCP20. The Y402H polymorphism, in CCP7, alters CFH binding to sulfated GAGs. This polymorphism also affects factor H-like protein-1 (FHL-1), a 43 kD protein containing the CCP7 module that is generated by a splice variant of the CFH gene. The disease-associated 402H polymorphic variant of CFH was found to require 2-O- and/or 6-O-sulfation for binding to HS and dermatan sulfate in human Bruch's membrane tissue slices. In contrast, the non-disease-associated 402Y form binds to a broader range of proteoglycans, suggesting that it is overall more tightly bound and thus potentially more effective as an inhibitor. Importantly, in aged human Bruch's membrane, HS is reduced 50% overall and in the macula.[88] This change, attributed to either decreased production or increased turnover of HS core proteins, is one way in which disease-associated sequence variants could promote complement activation, lipoprotein binding to extracellular matrix,[88] and AMD progression in Bruch's membrane.

FUNCTION OF BRUCH'S MEMBRANE

As a vessel wall of the choroid, Bruch's membrane primary function is structural, like other vessel walls. Its architecture is similar to vascular intima, with a sub-endothelial extracellular matrix and elastic layer corresponding to the internal elastic lamina. The abluminal surface of Bruch's membrane differs from other vessel walls in that it abuts a basal lamina, that of the RPE. Essentially, Bruch's membrane is comprised of two epithelial membranes in apposition, consistent with its embryologic origin The luminal surface faces a fenestrated vascular endothelium and basal lamina, making Bruch's membrane structurally analogous to the renal glomerulus and providing a basis for commonality between retinal and kidney disease.[89–91] The importance of fluid and macromolecular transport across the renal glomerulus is well known.[92] Transport is a second important function of Bruch's membrane.

Structural Role of Bruch's Membrane

Bruch's membrane encircles more than half the eye and stretches with the corneoscleral envelope as intraocular pressure (IOP) increases. It withstands this stretch and return to its original shape when IOP decreases. This tissue also stretches to accommodate changes in choroidal blood volume. Finally, the choroid (and Bruch's membrane with it) may act as a spring that pulls the lens during accommodation.[93,94] For these reasons, then, Bruch's membrane requires elasticity.

Marshall and Hussain's group estimated the modulus of elasticity in Bruch's membrane-choroid preparations to be 7–19 MPa.[95] These values are similar to those of sclera (although sclera is much thicker and thus can support more load) consistent with the notion that Bruch's membrane contributes to load bearing. After early adulthood, the modulus of elasticity of human Bruch's membrane-choroid complex increases ($p<.001$) at a rate of ~1% per year. Bruch's membrane stiffness in AMD eyes does not differ from age-matched normals.[96]

Transport Role of Bruch's Membrane

The choroid services the metabolic needs of the outer retina, facilitated in part by fenestrated endothelium (Fig. 22.3). Oxygen, electrolytes, nutrients, and cytokines destined for the RPE and photoreceptors pass from the choriocapillaris and through Bruch's membrane, and waste products travel back in the opposite direction for elimination. Vitamins, signaling molecules, and other factors needed for photoreceptor function are carried to the RPE by lipoprotein particles passing through Bruch's membrane, as do the RPE-produced lipoproteins that are eliminated in the opposite direction. The RPE pumps water from the subretinal space to counter the swelling of the interphotoreceptor matrix glycosaminoglycans (GAGs).

This fluid flows across Bruch's membrane to reach the circulation. Thus, many transport processes involve Bruch's membrane, as reviewed here.

Hydraulic Conductivity of Bruch's Membrane

GAGs are concentrated in the interphotoreceptor matrix[97,98] and corneal stroma.[99] In both locations, these highly charged macromolecules maintain geometric fidelity essential for vision (periodic collagen spacing for corneal transparency, orderly photoreceptor spacing for visual sampling).[36,100,101] GAGs generate significant swelling pressure (up to 50 mm Hg in cornea).[102,103] Without a mechanism to maintain tissue deturgescence, GAGs would imbibe fluid, swell, destroy tissue geometry, and interfere with visual function. Corneal endothelium forestalls swelling by continuously pumping fluid out. This function is accomplished for retina by the RPE, and its failure can lead to retinal detachment.

The fluid pumped by the RPE must then flow from the basal surface of the RPE, across Bruch's membrane, and through the endothelial lining of the choriocapillaris to be adsorbed by the vasculature. A driving force adequate to overcome the collective flow resistance of these tissues is provided by a gradient in fluid pressure and oncotic pressure (the osmotic pressure generated by plasma proteins). This balance is embodied by Starling's Law that characterizes the relationship between fluid flux (q: flow per unit area; positive when flow is out of the blood vessel) across a capillary vessel wall and the forces driving this flow:

$$q = L_p * (\Delta P - \sigma \Delta \Pi) \qquad [22.1]$$

L_p is hydraulic conductivity, which characterizes the ease with which fluids flow cross the vessel wall. If the surface area of the blood vessel is A, then $1/(L_p\ A)$ is the flow resistance of the vessel wall. ΔP is the difference between the fluid pressure within the blood vessel (P_α) and the pressure at the basal surface of the RPE (P_{RPE}). $\Delta \Pi$ is the difference between the oncotic pressure within the blood vessel (Π_α) and that at the basal surface of the RPE (Π_{RPE}). σ is the reflection coefficient that characterizes the extent to which the vessel wall rejects the plasma proteins species generating $\Delta \Pi$. σ ranges from 0 for a freely permeable species to 1 when a species is completely rejected by the membrane.

We can estimate the magnitude of $\Delta P - \sigma \Delta \Pi$ using measured value of q and L_p. The fluid pumping rate by human RPE has been measured as q=11 μL h^{-1} cm^{-2}, similar to that in other animals (Table 22.2). The hydraulic conductivity of macular Bruch's membrane/choroid of healthy young humans ranges from 20 to 100×10^{-10} m s^{-1} Pa^{-1}.[104] Then, using q=11 μL h^{-1} cm^{-2} and L_p=50×10^{-10} m s^{-1} Pa^{-1},[a] we can calculate that the magnitude of $(\Delta P - \sigma\ \Delta \Pi)$ necessary to drive this flow through Bruch's membrane is roughly 0.05 mm Hg.

σ can be roughly estimated by assuming that the fluid in the suprachoroidal space is in equilibrium with blood in the choroid. Using measurements in monkeys of plasma protein concentration inside and outside of choriocapillaries (82.1 and 23.7 mg/ml, respectively),[107] and the Landis Pappenheimer equation[108] for osmotic pressure, the osmotic pressure difference, in this species, across the choroid can be estimated to be 33 mmHg − 6 mmHg=27 mmHg. The pressure in suprachoroidal fluid was measured by Emi[107] to be 4.7 mmHg below IOP while that in the choriocapillaris was measured at 8 mmHg higher than IOP[109] giving a pressure difference across this wall of 12.7 mmHg. Then, Eq. 22.1 is used to estimate that σ=0.5, assuming equilibrium conditions.

Using Π_α=33 mmHg, P_α=IOP+8 mmHg, Π_{RPE}=0 mmHg (fluid pumped by the RPE is assumed protein-free), and we take P_{RPE}=IOP (assuming no pressure is generated by the RPE above that necessary for crossing Bruch's), we find that $\Delta P - \sigma\ \Delta \Pi$ is approximately 8.5 mmHg pulling fluid into the choroid. Thus, in normal young adults, oncotic pressure within the choroid is more than sufficient to adsorb all the fluid pumped by the RPE. We can also use Eq. 22.1 to calculate that the lowest value of L_p that still adsorbs fluid pumped by the RPE without generating an elevated pressure at the RPE basal surface is $L_p > 0.3\times10^{-10}$ m s^{-1} Pa^{-1}.

Experiments using laser ablation of Bruch's membrane/choroid explants allowed Starita et al.[110] to conclude that the ICL was responsible for most of the flow resistance in Bruch's membrane. Attempts to further localize the flow resistance using morphometric methods are complicated by (i) stereologic issues[111] and (ii) the loss of ultrastructural fidelity from connective tissue conventionally processed for electron microscopy.[52] Failure to appreciate the former difficulty can lead to unphysiologically low estimates for tissue porosity and thereby hydraulic conductivity.[1]

TABLE 22.2 Retinal Pigment Epithelium Fluid Pumping Rates

Species	Fluid Transport Rate Across RPE (μL h^{-1} cm^{-2})	References
Frog	4.8-7.6	303, 304
Rabbit	12±4	305, 306
Canine	6.4	307
Primate[a]	14±3	308, 309
Human	11	310

Retinal pigment epithelium (RPE) pumping rates were measured by reabsorption of subretinal fluid or by direct measurement in culture.
[a]Cantrell and Pederson measured a much higher transport rate than that reported here,[308] but used fluorescein as a tracer which likely does not track fluid flow due to its high diffusion coefficient.

Age-Related Changes in Hydraulic Conductivity and Disease

Fisher was the first to measure L_p of human Bruch's membrane,[112] finding that L_p decreased significantly with age. However, his values for L_p of Bruch's membrane and other tissues are much lower than those found by later investigators.[106,113,114] Marshall and Hussain carefully revisited these measurements using Bruch's membrane/choroid with RPE removed, a preparation that was simpler to create. They showed using laser ablation that the flow resistance of these preparations was entirely due to Bruch's membrane.[110] They also found that flow rate increased linearly with driving pressure, indicating that L_p of Bruch's membrane is relatively insensitive to pressure up to 25 mmHg.

They reported that L_p of macular Bruch's membrane exhibited a dramatic, exponential decline throughout life (Fig. 22.6), dropping from 130×10^{-10} m s^{-1} Pa^{-1} in young children to 0.52×10^{-10} m s^{-1} Pa^{-1} in old age. L_p of macular Bruch's membrane dropped more rapidly with age than did that of the periphery, consistent with an accelerated process occurring in the macula.[1,104,105,115,116] Note that the lowest value measured for L_p of Bruch's membrane in normal eyes is similar to the

[a]This does not include the flow resistance of the choriocapillaris endothelium, which is not measured when L_p of a Bruch's membrane/choroidal preparation is determined. For this highly fenestrated endothelium with fenestra taking up roughly 80% of luminal surface area,[105] L_p can be estimated as roughly 25×10^{-10} m s^{-1} Pa^{-1},[106] which does not affect our conclusions.

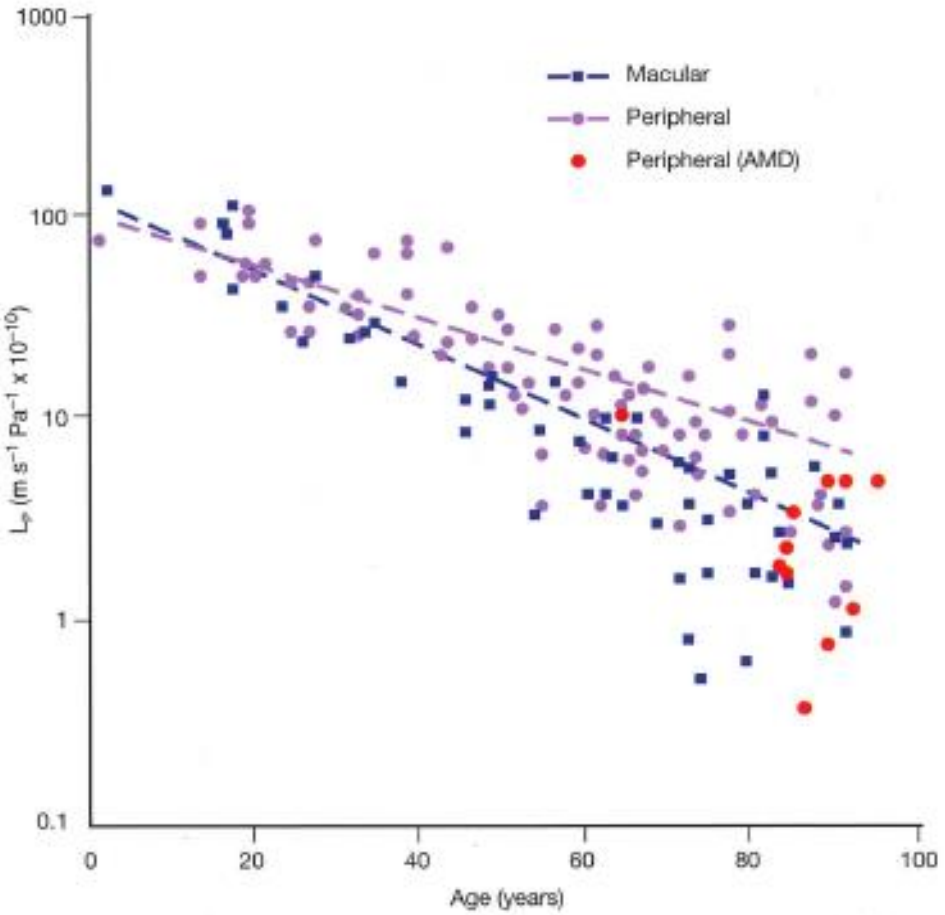

Fig. 22.6 Hydraulic conductivity (L_p) of Bruch's membrane as a function of age. Dotted lines are exponential fits to data from macular and peripheral regions, respectively. Note that all of the data from eyes with AMD (taken only in peripheral region) have lower values of L_p than the best fit to data taken from peripheral Bruch's membrane of nondiseased eyes.[115]

calculated minimum value of L_p (0.4×10^{-10} m s^{-1} Pa^{-1}, see above) that allows complete resorption of fluid pumped by the RPE without need of an elevated pressure at the basal surface of the RPE. Marshall and Hussain reached similar conclusions regarding this process.[115]

Determining L_p of Bruch's membrane in isolated macular samples of AMD eyes is difficult due to scar formation and other changes.[115] However, Marshall and Hussein's group showed that in the periphery, L_p of Bruch's membrane is decreased in AMD eyes as compared to age-matched normal eyes (Fig. 22.6).[115] Assuming that similar processes occur in macular Bruch's membrane due to the profound lipid accumulation in this region, then in diseased eyes, the RPE must generate higher pressures at its basal surface to drive fluid into the choriocapillaris, with further pathologic consequences.[41] Above an unknown threshold level, higher pressure will cause the RPE-EL to separate from the ICL, leading to RPE detachment and fluid accumulation, as seen in 12–20% of AMD patients.[115]

What causes the dramatic age-related decrease in L_p of Bruch's membrane? It is natural to suspect the age-related lipid accumulation. In fact, McCarty et al.[117] showed that lipid particles trapped in an extracellular matrix can generate very significant flow resistance, more than would be expected based simply on their size and number. However, Marshall and Hussain observed that most of the marked change in L_p occurred before age 40 (Fig. 22.7A) while the increase in Bruch's membrane lipid content occurred largely after this age. They thus concluded that other age-related changes must be responsible for changes in L_p.[1,104]

A different conclusion can be reached from examining age-effects on flow *resistivity*, the *inverse* of L_p. Resistivity increases from a low of roughly $R=10^8$ Pa m^{-1} s^{-1} for young individuals to $R=10^{10}$ Pa m^{-1} s^{-1} for aged persons. Thus, when hydraulic conductivity L_p drops from roughly 100×10^{-10} m s^{-1} Pa^{-1} to 25×10^{-10} m s^{-1} Pa^{-1} between the ages of birth and 40 years of age, 75% of its total possible decrease, resistivity R increases from 1×10^8 Pa m^{-1} s^{-1} to 4×10^8 Pa m^{-1} s^{-1}, only 4% of the ultimate increase. Simply put, hydraulic conductivity drops more rapidly with age at young ages because its value is high to start with. Fig. 22.7B plots resistivity and histochemically detected EC against age for Bruch's membrane.[118] The agreement between the trends and the fits to the data are striking. This is strong evidence that the increasing lipid content and progressively hydrophobic character of Bruch's membrane are responsible for impairing fluid transfer with age, as postulated.[41] The strong correlation between flow *resistivity* of Bruch's membrane and lipid content was likewise found by Marshall and Hussain.[1,104,116] Laser ablation studies localizing flow resistance to the ICL[120] further supports this conclusion, because lipids accumulate prominently in the ICL with aging.[33] Further, more laser pulses were required to abolish flow resistance in the oldest eyes, consistent with presence of a Lipid Wall, requiring prior removal.

Thus, it appears that decreased L_p and increased resistivity of Bruch's membrane with aging is closely related to the age-related accumulation of lipids, primarily EC. Lipids accumulate more rapidly in the macular Bruch's membrane than in the periphery.[47,119] Thus, L_p of the macula decreases more rapidly with age than it does in the periphery.

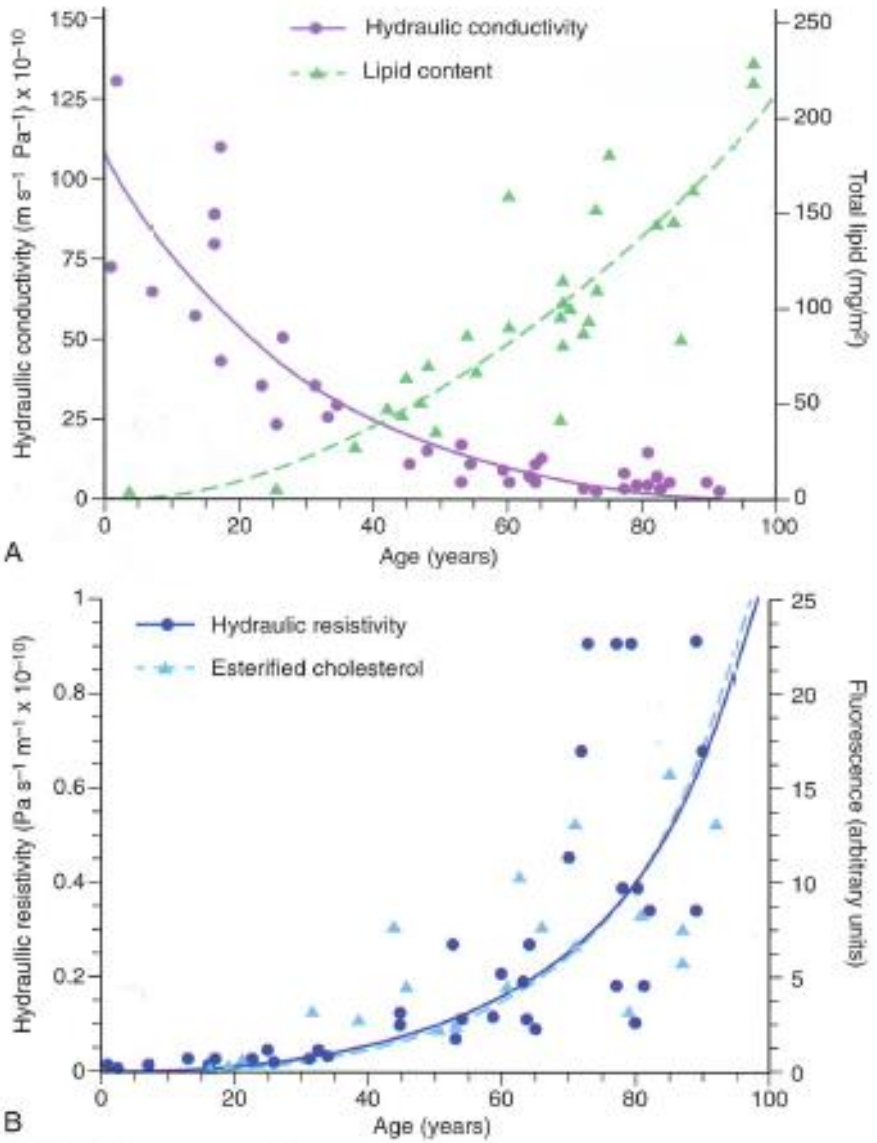

Fig. 22.7 (A) Hydraulic conductivity of human macular Bruch's membrane/choroidal preparations as a function of age, as compared to lipid accumulation in human macular Bruch's membrane; lines as exponential fits to the data. (B) Hydraulic resistivity of human macular Bruch's membrane/choroidal preparations as a function of age,[1] as compared to esterified cholesterol accumulation in human macular Bruch's membrane;[47] lines are exponential fits to the data (the fits nearly overlie one another). *(Panel A modified from Marshall J, Hussain AA, Starita C, et al. Aging and Bruch's membrane. In: Marmor MF, Wolfensberger TJ, editors. The retinal pigment epithelium: function and disease. New York: Oxford University Press; 1998. p. 669-92.)*

L_p in Other Species

L_p of dog Bruch's membrane was measured as 3.7×10^{-10} m s^{-1} Pa^{-1},[120,121] similar to the value found in an older human. Hillenkamp et al.[122] reported a very low value of L_p=0.345×10^{-10} m s^{-1} Pa^{-1}in cow eyes, lower than that found in any human eyes. Cankova et al.[123] examined calf eyes and found a much higher value of L_p (14.2×10^{-10} m s^{-1} Pa^{-1}) that was threefold lower in the cow eyes that they examined (4.9×10^{-10} m s^{-1} Pa^{-1}), although not as low as measured by Hillenkamp et al. Cankova et al.[123] concluded that, as in humans, L_p decreases with age.

Permeability of Bruch's Membrane to Solute Transport

Along with bulk fluid flow, there is significant transport of individual molecular species across Bruch's membrane, including dissolved gases, nutrients, cytokines, and waste products driven by passive diffusion. Flow crossing Bruch's membrane is too slow to influence this process. This can be seen through calculation of the Peclet number, the relative magnitude of convection of a species due to bulk flow to that of diffusion:[124]

$$\frac{VL}{D_0} \qquad [22.2]$$

where V is the velocity of the flow, L is the transport path length, and D_0 the free diffusion coefficient of the species being transported. (The free diffusion coefficient in saline is used rather than its value in tissue, since the species carried by flow is constrained to the same extent by the tissue as is its diffusion). Using the RPE pumping rate (Table 22.2) for V, Bruch's membrane thickness (average of 3 μm[71]) for L, and a range of diffusion coefficients of species crossing Bruch's

membrane (2×10^{-7} cm^2/sec for LDL to 2×10^{-5} cm^2/sec for oxygen)[124,125] we find that the Peclet number ranges in value from 5×10^{-5} to 5×10^{-3}. Thus, convection is negligible in transporting species across Bruch's membrane under physiologic conditions.

Diffusion follows Fick's law whereby the diffusive flux per unit area (j) is proportional to the diffusion coefficient (D) of that species in the medium through which it passes and to the concentration difference across the medium (ΔC), and inversely proportional to the diffusion length:

$$j = D\,\Delta C/L \qquad [22.3]$$

The *permeability* of a tissue to a given species is defined as $P = j/\Delta C$. We see then that $P=D/L$. For example, the permeability of Bruch's membrane to oxygen is ~0.067 cm/sec. Note that since diffusion moves down a concentration gradient, one species might be diffusing across Bruch's membrane toward the RPE (e.g., oxygen) while another species (e.g., carbon dioxide) diffuses simultaneously in the other direction.

With high diffusion coefficient and little interaction with extracellular matrix, small molecules (e.g., oxygen, cytosine, RNAaseA) diffuse quickly across Bruch's membrane with diffusion coefficients nearly the same as in free solution.[126] However, macromolecules have much smaller free solution diffusion coefficients due to their larger size. Their diffusion coefficients are further reduced by interactions with extracellular matrix and/or lipoproteins that accumulate with age. For example, the diffusion coefficients of albumin and ferritin are an order of magnitude smaller in Bruch's membrane than their values in free solution.[126]

The transport of amino acids,[127] serum proteins,[128] drugs,[129] and LDL[123,130] across Bruch's membrane has been examined. Transport experiments by Clark and associates[85] suggest that FHL-1 (43 kDa) crosses Bruch's membrane more readily than CFH (155 kDa) and is in fact the major CHF form in native aged Bruch's membrane.

There are technical challenges to these transport experiments. First, as indicated in Eq. 22.3, diffusional flux depends on the length of the tissue. Since the diffusion coefficient of the transported species is likely different in Bruch's membrane than in the choroid in a combined preparation, but the path lengths of both tissue components are usually not determined, it is difficult to use the measured values to determine absolute values of permeability. Instead the more easily measured flux rate (j; see Eq. 22.3) is usually presented. Second, an unstirred layer can develop near the membrane thereby complicating the results, and this can occur even in cases when the solutions are stirred.[131] Nonetheless, useful comparative results can be generated.

The transport rate across human Bruch's membrane declines linearly with age for all molecules measured. Amino acids exhibited permeabilities of 0.6×10^{-4} cm/sec (phenylalanine) to 1.2×10^{-4} cm/sec (glycine) for young Bruch's membrane and exhibited a modest decline (twofold or less) with aging.[127] Serum proteins decrease more markedly, dropping from 3.5×10^{-6} cm/sec in the first decade to 0.2×10^{-6} cm/sec in the ninth decade, a >10-fold decrease.[128] In particular, proteins larger than 100 kDa have significantly decreased flux through Bruch's membrane of older individuals. Macular Bruch's membrane showed a steeper decrease with age than did the periphery.[132] Permeability was reduced in eyes with AMD relative to age-matched normal eyes.[132]

Decreased permeability of Bruch's membrane to transport is likely due to a decrease in diffusion coefficients, especially for the larger species affected by interaction with extracellular matrix and lipoproteins. As indicated in Eq. 22.3, increased path length due to age-related thickening of Bruch's membrane[71] could also have a significant effect.

An original proposal of a molecular weight (MW) exclusion limit to Bruch's membrane macromolecule transport of 66–200 kD[127,128] has been questioned by more recent work suggesting that if such a limits exists, it is much higher.[132] Because of the importance of lipoproteins in transporting lipophilic nutrients to the RPE for ultimate use by the photoreceptors, and also because lipoproteins accumulate with age in Bruch's membrane, Cankova et al.[123] specifically examined the reflection coefficient of bovine Bruch's membrane to plasma LDL. They measured a reflection coefficient of 0.62 (compared to a reflection coefficient of arterial endothelium to LDL of 0.998 and arterial intima to LDL of 0.827[131]). Thus, while LDL did not pass freely through Bruch's membrane, it could nonetheless pass. Hussain et al.[132] also concluded that particles as large as LDL could cross Bruch's membrane. Accordingly, RPE cells have been shown to internalize plasma LDL from the choroid.[134–136]

These considerations are relevant not only to understanding mass transfer between the choriocapillaris and the RPE, but also for transcleral drug delivery strategies including antiangiogenic agents for treating AMD and steroids for treating diabetic retinopathy.[137,138] Cheruvu and Kompella[129] reported that the choroid–Bruch's layer is a more significant barrier to drug transport than is sclera. It hindered the transport of lipophilic solutes more than hydrophilic solutes and in a more dramatic way than does sclera. Importantly, the reduction in transport across this layer directly correlated with solute binding to the tissue. Pitakänen et al. found significant lag times associated with transport of lipophilic beta blockers across the RPE-choroid, consistent with binding of these drug to the tissues; however, they found that the permeability of the lipophilic drugs across this tissue was greater than that of hydrophilic compounds or macromolecules.[139] Lipophilic substances are known to have both different transport characteristics in connective tissues and also bind to extracelluar matrix.[140]

Summary and Implications

Bruch's membrane's physiologic roles are structural and facilitating transport. Transport across Bruch's membrane is increasingly hindered with age, due at least partly to the marked age-related accumulation of EC-rich lipoproteins in this tissue, impeding pumping of fluid from RPE.[115] A ≥90% decrease in transport of some species from the choroid[126,132] may include lipophilic essentials delivered by lipoproteins. This decline in transport capability is thought to have functional consequences for photoreceptors.[141] A well-characterized change occurring through the lifespan of individuals with healthy maculas is slowed dark adaptation,[142] attributed to impaired translocation of retinoids across the RPE-Bruch's interface. This slowing, worse in AMD patients,[143,144] can be partly ameliorated by short-term administration of high-dose vitamin A,[145] presumably overcoming the translocation deficit via mass action.

However, it is important to recognize that except for the studies on tissue from individuals with AMD, all of the results in human summarized here were from eyes that did not have retinal disease. As such, while the age-related decline in the transport capability of Bruch's membrane has functional consequences on photoreceptor function it is not likely that this represents disease, but instead, is part of the aging process. Accordingly, a prospective study has shown that slowed dark adaption is detectable in 22% of older adults with maculas considered normal by color fundus photography and that these persons are two times more likely to have incident early

AMD 3 years later.[146,147] Thus the overall model of photoreceptor nutritional insufficiency, due to impaired transport, at the interface of aging and disease has strong *in vivo* validation.

PATHOLOGY OF BRUCH'S MEMBRANE

AMD Lesions

In aging and AMD, characteristic extracellular lesions accumulate in tissue compartments between the RPE-BL and ICL (Fig. 22.8). Known as drusen and basal linear deposits (BLinD),[32,148] these lipid-containing aggregations ultimately impact RPE and photoreceptor health by impairing transport, causing inflammation, and predisposing to choroidal neovascularization. Basal laminar deposit (BLamD), a stereotypic thickening of RPE basal lamina, forms in parallel with lipid deposition in Bruch's and may indicate RPE stressed by it. Since 2010, clinical optical coherence tomography (OCT) has revealed that AMD lesions include a major new component, called subretinal drusenoid deposits (SDD) by cross-sectional OCT and histology[149] and reticular pseudodrusen by en face imaging such as color fundus photography.[150] These solid space-filling lesions located between the photoreceptors and RPE (Fig. 22.8) are common in AMD but are not unique to AMD. In the section below, we refer to retinal regions as foveal (central 1 mm), perifoveal (0.5–3 mm from the foveal center), and extramacular (>3 mm).

Drusen

In a fundus view, drusen are 30–300 µm-diameter yellow–white deposits posterior to the RPE. By OCT, they appear as variably hyporeflective spaces in the same location.[151–153] Found in most older adults,[79,154] drusen are more numerous in extramacular retina than in macula.[155–157] Drusen are typically classified as "hard" and "soft" by the appearance of their borders. Other rare druse types exist and are less well characterized.[158] Soft drusen confer high risk of advanced disease.[159–162] Histologically, drusen are focal, domed lesions between the RPE basal lamina and the ICL (Fig. 22.8), as illustrated[163] and established[148,164] using transmission electron microscopy.

In separate 1854 publications, Donders (a Dutch ophthalmologist) and Wedl (an Austrian pathologist) described "colloid bodies" (*Colloidkugeln*) or "hyaline deposits" on the inner surface of the choroid in older or diseased human eyes[3,165,166] (translated by Busk). Both authors interpreted droplets filling these deposits as "fat-globules." The term drusen originated with Müller in 1856, from the German word for geode (not to be confused with *drüse*, meaning gland).[167] The name drusen was adopted by English writers early in the 20th century[168] yet "colloid body" was used by Verhoeff into the 1920s.[169] Lauber[170] (cited in reference 171) noted that deposits between the lamina vitrea and the RPE were sudanophilic in 1924. Wolter and Falls[38] stated that "hyaline bodies [drusen] ... stain reddish with ... oil red O" in 1962. Soft drusen were termed serogranular and hard druse hyaline (glassy) by S. Sarks,[159] implying different composition. Soft drusen are oily, difficult to isolate individually, biomechanically more fragile than hard drusen, and found only in the macula.[157] Friability upon processing for conventional paraffin histology was noted early.[172] Due to this and other technical challenges, few recent drusen compositional studies (Table 22.3 online) included true macular soft drusen.[173–177] Many studies analyzed peripheral drusen, combined macular and peripheral drusen, or did not specify location.

Extant theories for druse formation, extending back to their discovery,[167] fit into two general categories: transformation of the overlying RPE and deposition of materials onto Bruch's membrane. The latter is now accepted.[166] The RPE has been implicated as a source of many druse components, via budding of membrane-bound packets of cytoplasm or secretion of lipoproteins with retention by aged Bruch's membrane,[35] as experimentally confirmed.[178] BLinD and soft drusen are two physical forms (layer and lump) of the same AMD-specific lesion, located only in the macula. BLinD forms consequent to lipoprotein accumulation in Bruch's membrane and formation of the Lipid Wall, likely involving lipoprotein aggregation, oxidation of individual lipid classes, and local inflammation. Soft drusen involves these and other processes that cause the distinctive dome shape of these lesions. RPE expresses genes for many druse components, including lipoproteins.[18,179,180] The contribution of plasma-derived components, in contrast, has not been well characterized. The existence of druse subregions additionally suggest remodeling in the extracellular compartment, such as cellular invasion and enzymatic activity[181–183] and uplifting of the Lipid Wall.[174]

Most prominent among druse constituents are lipids (Table 22.3 online), as noted early. All drusen contain EC and UC, in addition to phosphatidylcholine, other phospholipids,

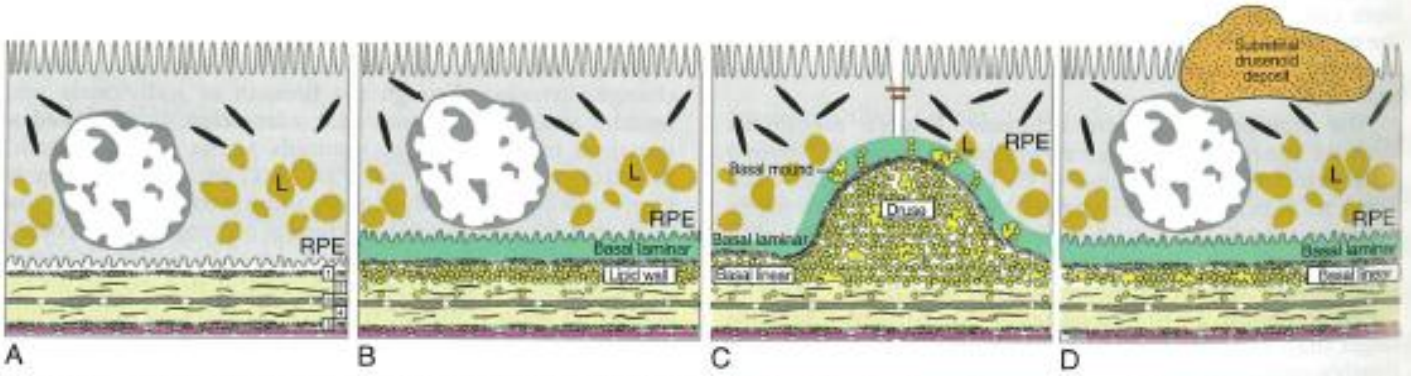

Fig. 22.8 Bruch's membrane and characteristic AMD lesions. (A) Bruch's membrane has five layers in a normal eye: 1, basal lamina of the retinal pigment epithelium (RPE); 2, inner collagenous layer; 3, elastic layer; 4, outer collagenous layer; 5, basal lamina of the choriocapillary endothelium (fenestrated cells, pink). *L*, lipofuscin. (B) Older eyes have basal laminar deposit (BlamD) and the Lipid Wall, precursor to basal linear deposit and soft drusen. (C) Drusen, basal linear deposit, and the Lipid Wall occupy the same tissue compartment. Basal mounds are soft druse material within BlamD. (D) Subretinal drusenoid deposit is an extracellular lesion compositionally distinct from drusen, located between the photoreceptors (not shown) and the RPE. *(Modified from Curcio CA, Johnson M, Huang J-D, et al. Apolipoprotein B-containing lipoproteins in retinal aging and age-related maculopathy. J Lipid Res. 2010;51(3):451-67.)*

ceramides, and 7-ketocholesterol, an oxidation product of UC that is angiogenic and proinflammatory.[44,47,163,174,182,184,185] Extractable lipids account for ≥40% of hard druse volume[186] and likely more for macular soft drusen.[174] This includes large EC-rich lakes in soft drusen (Figs. 22.9A–B), as in atherosclerotic plaques.[187] Apolipoprotein immunoreactivity appears in drusen with high frequency (100%, apoE; >80% apoB; 60%, A-I).[174,188–191] Importantly, hard drusen contain many solid, Folch-extractable electron-dense particles of the same diameter as the lipoproteins in Bruch's membrane. These observations together with the appearance of membranous debris in soft drusen (below) make an RPE-secreted apoB,E-containing lipoprotein particle an efficient mechanism to place multiple lipids and apolipoproteins within lesion compartments. Only half of macular drusen take up hydrophilic fluorescein in angiography,[192] possibly reflecting differing proportions of lipid classes in individual lesions.[184]

Discrete nonlipid components in some drusen granules of lipofuscin or melanin indicate cellular origin (Table 22.3 online). Spherical β-amyloid assemblies[175,193,194] now appear to be due to nonspecific binding of proteins to hydroxyapatite spherules (see below), consistent with immunohistochemistry.[195,196] Other constituents present in all drusen include vitronectin, TIMP-3, complement factor H, complement components C3 and C8, crystallins, ubiquitin, and zinc.[26,183,193,197–200] Many druse components are found also in retina IL-1[177] and/or choroid (carboxypyrrhole adducts[202]) of the same eyes and thus are less specific for these lesions than other components.

The principal lipid-containing component of soft drusen and BLinD was called "membranous debris" by the Sarks[155,203,204] and "lipoprotein-derived debris" was suggested as an alternative, for two reasons.[39] First, these lesions are richer in histochemically detectable UC than surrounding cellular membranes.[184,205] By transmission electron microscopy following osmium tetroxide postfixation, membranous debris appears as variably sized, contiguous coils of uncoated membranes consisting of uni- or multilamellar electron dense lines, that are denser than cellular membranes and surround an electron-lucent center (Fig. 22.2). Since conventional ultrastructural preparation methods can remove lipids, the building blocks of membranous debris are likely the UC-rich exteriors of lipoproteins (native and fused) whose neutral lipid interiors are not well preserved in postmortem tissue.[53,190,206] Second, all drusen have abundant EC that can only be explained by lipoprotein particles in addition to cellular membranes.

By ophthalmoscopy, refractile or glistening deposits thought to be calcified drusen[172] regularly appear in areas where drusen regress, before the onset of geographic atrophy.[207,208] Recent investigation has confirmed that glistening drusen in fact contain calcium phosphate in the form of hydroxyapatite spherules, culminating a five-decade quest. Studies included detection of refractile nodules by light microscopy,[32,209–212] phosphate detection via von Kossa histochemistry,[172,208] confirmation of calcium and phosphate signals in nodules by energy dispersive X-ray analysis,[213,214] and identification of hydroxyapatite mineral by synchrotron micro-X-ray diffraction.[215] Most drusen contain 0.5–20 μm diameter spherules that become clinically visible when the RPE degenerates.[208] Concentric shells within the spherules account for the refractility.[208] Spherules also have UC within them.[215] As hydroxyapatite binds proteins well and is widely used as a stationary phase for chromatography, these nodules are strong candidates for nucleation sites for further protein deposition and druse enlargement.[215] Calcification in drusen differs from that within Bruch's membrane itself by not requiring the presence of elastin fibers. The source and mechanism of high calcium and phosphate concentration in the sub-RPE space, likely reflecting RPE physiology, are important areas for future research.

Basal Linear Deposit

BLinD is a thin (0.4–2 μm) layer located in the same sub-RPE compartment as soft drusen (Fig. 22.8). BLinD is not visible clinically except as associated with other pathology. By lipid-preserving ultrastructural techniques, BLinD is rich in solid lipoprotein particles and lipid pools (Figs. 22.10A,C) and can contain hydroxyapatite.[203] BLinD and soft drusen are considered alternate forms of the same entity.[216] ApoE and apoB are present in BLinD and its precursor, the Lipid Wall.[174,188,189] Transitional morphologies between Lipid Wall and BLinD have been reported.[217] BLinD is thicker in the fovea than in the

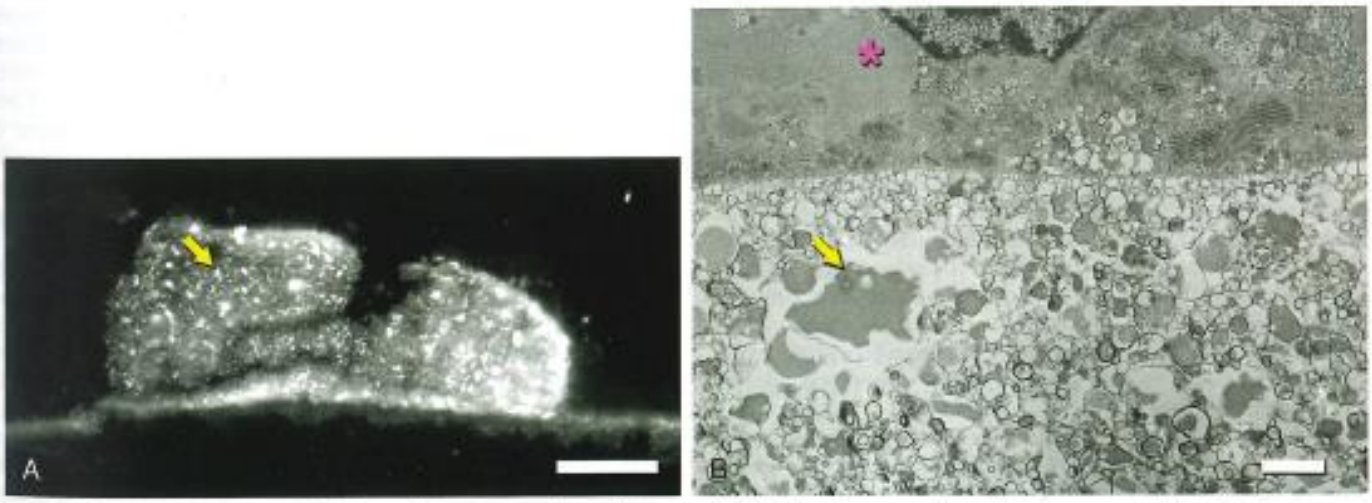

Fig. 22.9 Esterified cholesterol (EC) forms lakes in macular soft drusen. (A) EC lakes in a macular soft druse revealed by filipin fluorescence (*arrow*). Scale bar: 25 μm. (B) Macular soft druse from an AMD eye has lakes of homogeneous electron-dense lipid (*arrow*) among partially preserved lipoprotein-like material. Basal laminar deposit (*asterisk*) overlying the druse has similar material, called membranous- or lipoprotein-derived debris (to the right of the asterisk). Scale bar: 1 μm. *(Panel A modified from Malek G, Li C-M, Guidry C, et al. Apolipoprotein B in cholesterol-containing drusen and basal deposits in eyes with age-related maculopathy. Am J Pathol. 2003;162(2):413-25.)*

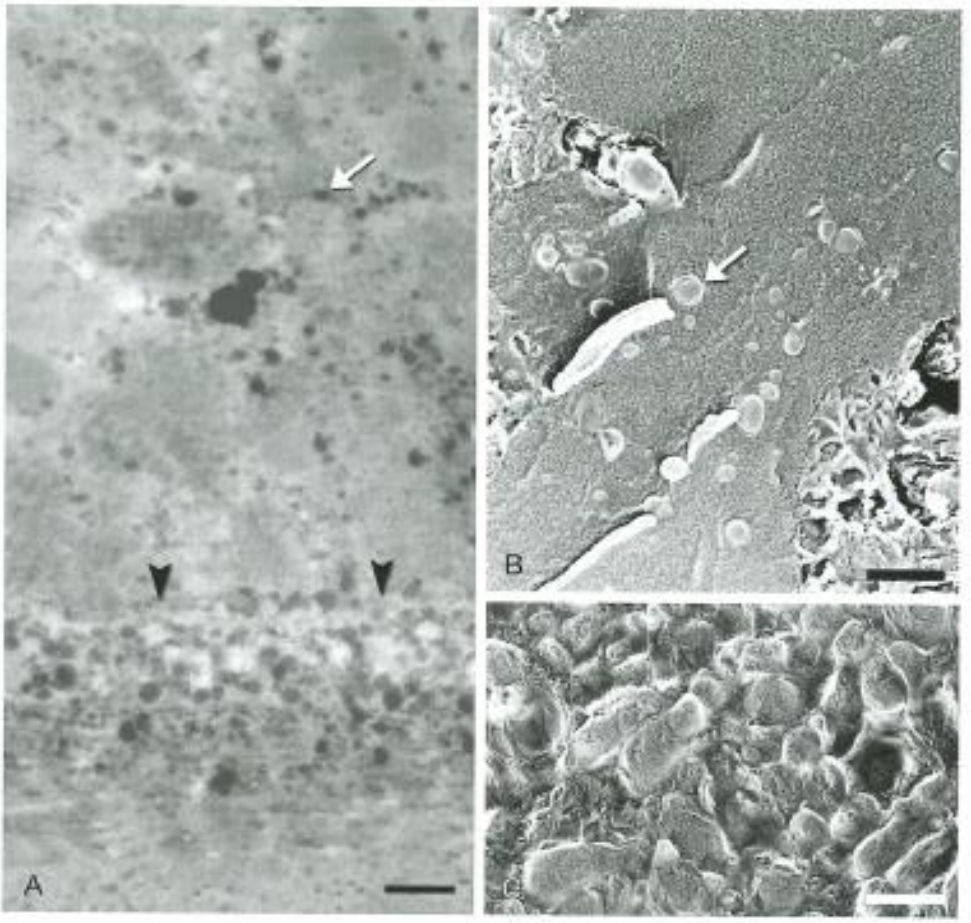

Fig. 22.10 Lipoprotein-derived debris and lipid pools in AMD lesions are solid rather than vesicular material. (A) Above retinal pigment epithelium (RPE) basal lamina (*arrowheads*) is basal laminar deposit (BlamD) with individual particles indicated (*arrow*). Below RPE basal lamina are numerous solid particles in basal linear deposit (BlinD). Transmission electron microscopy, OTAP fixation; scale bar: 500 nm. (B) BlamD appears as a solid column of basal lamina-like material, with solid particles embedded within (*arrow*). Scale bar = 500 nm. (C) BlinD has lipoproteins of heterogeneous sizes and shapes as well as pooled lipid, consistent with a model of surface degradation and particle fusion. Scale bar = 200 nm. *(Panel A Curcio CA, Presley JB, Millican CL, Medeiros NE. Basal deposits and drusen in eyes with age-related maculopathy: evidence for solid lipid particles. Exp Eye Res 2005;80(6):761–75. Panel B image courtesy of J.-D. Huang, PhD. Panel C Curcio CA, Johnson M, Rudolf M, Huang J-D. The oil spill in ageing Bruch's membrane. Br J Ophthalmol 2011;95(12):1638–45.)*

perifovea, unlike SDD, consistent with these lesions reflecting differential aspects of cone and rod physiology[61] (see below).

Basal Laminar Deposit

BLamD forms small pockets between the RPE and the RPE-BL in many older normal eyes or a continuous layer as thick as 15 μm in AMD eyes[204,205,218] (Fig. 22.8). The presence and abundance of BLamD has been used to stage AMD.[52,219] Ultrastructurally, BLamD resembles basement membrane material (Fig. 22.10B), containing laminin, fibronectin, type IV, and type VI collagen.[220–223] The latter is a distinctive banded material with 120 nm periodicity, called wide- or long-spacing collagen, which also appears in other ocular locations like epiretinal membranes. Thick BlamD, associated with advanced AMD risk,[204] contains histochemically detectable lipid including UC and EC[184,205] and is a classically described site for membranous debris (Fig. 22.2). By lipid-preserving methods, solid particles are seen in BLamD (Figs. 22.10A–B). Especially enriched in basal mounds[204] (Fig. 22.8C), lipoprotein-derived debris in BLamD may be considered as retained in transit from the RPE to BLinD and/or drusen.[174,184,205] Morphologically heterogeneous BLamD also contains vitronectin, MMP-7, TIMP-3, C3, and C5b-9,[218] EC, and UC.[203] Evoked in numerous mouse models of aging, stress, and genetic manipulation, BLamD is a reliable marker of RPE stress.[224,225]

Subretinal Drusenoid Deposit

Hypotheses of druse formation must eventually also account for SDD, the extracellular deposits between RPE and photoreceptors (Fig. 22.8). These lesions were first illustrated histologically in AMD by the Sarks[203] and independently described as drusen "visible en lumière bleu" by Mimoun et al.[226] Conferring risk for late AMD,[227] SDD appear in 60% or more of eyes with geographic atrophy,[227,228] 49% of early AMD eyes, and 23% of older eyes considered normal by color fundus photography.[229] SDD are also detectable in several Mendelian inherited disorders affecting the RPE–Bruch's membrane complex.[230–232] Comprehensive histology, clinicopathologic correlation, and adaptive optics scanning laser ophthalmoscopy[61,233–235] have definitively localized these lesions in the subretinal space, after some early debate. SDD lack markers for cells bounding this space (photoreceptors, Müller cells, RPE).[175,205] SDD share protein components with drusen,[175] and importantly, SDD lipid composition differs from drusen (UC only vs. UC and EC, respectively[236]). SDD are abundant in the perifovea and peripapillary area[149,150,223,237–239] and sparse in central macula, whereas BLinD is thickest under the fovea.[61] Cones are numerous in the fovea, and rods are numerous in the perifoveal region and just beyond.[240] Thus drusen and SDD have been linked in a system of outer retinal lipid recycling serving the differential lipid requirements of rod and

cone photoreceptor outer segment membranes.[61,241] The low content of EC relative to drusen has been explained by invoking a hypothetical high-density lipoprotein particle, rich in UC, that shuttles UC from and docosahexanoate to rod outer segments selectively.[61] If this hypothesis is true, SDD might be also rich in docosahexanoate and its derivatives, an important question to address experimentally.

Summary

Levels of significance ascribed to molecules sequestered in drusen (Table 22.3 online), and by inference, BLinD, include toxicity to the overlying RPE, stigmata of formative processes (extrusion of cellular materials, secretion, extracellular enzymatic processing, cellular activity), and markers of a diffusely distributed disease process affecting RPE and Bruch's membrane. Additional significance can be ascribed to these lesions as physical objects that increase path length between choriocapillaries and retina and provide a biomechanically unstable cleavage plane between RPE-BL and ICL. Many of these processes likely also occur in the subretinal space in relation to SDD.[149]

Response-to-Retention Hypothesis of AMD

The parallels between the pathology of arterial intima of large arteries in atherosclerosis and that of Bruch's membrane in AMD are striking. Both diseases feature cholesterol-rich lesions in subendothelial compartments within the systemic circulation, involving many of the same molecules and biologic processes at multiple steps, as long anticipated.[242,243] According to the Response-to-Retention theory of atherosclerosis, plasma lipoproteins cross the vascular endothelium of large arteries, and bind to extracellular matrix. By itself, this process is not pathologic. However, lipoprotein components become modified via oxidative and nonoxidative processes and launch numerous downstream deleterious events, including inflammation, macrophage recruitment, and neovascularization leading to disease.[244,245] Parallel with apoB-lipoprotein-instigated disease in arterial intima, an intraocular Response-to-Retention involving the RPE and Bruch's membrane in aging and AMD would begin with age-related accumulation of lipoproteins, but *of local origin*. Oxidation, perhaps driven by reactive oxygen species from adjacent RPE mitochondria, would then initiate a pathologic process resembling that in the vascular system with inflammation-driven downstream events including complement activation and structurally unstable lesions.[39] New model systems such as highly differentiated and polarized cultured RPE[178] and mice with genetically modified lipoprotein pathways[246] will allow rigorous experimental test of these concepts.

Neovascular AMD

Choroidal neovascularization (CNV), the major sight-threatening complication of AMD, involves angiogenesis along vertical and horizontal vectors: vertically across Bruch's membrane, and either laterally external to the RPE (type 1 CNV,[247]), laterally within the subretinal space (type 2 CNV), or further anteriorly into the retina (type 3 CNV).[247–249] Of 40+ conditions involving CNV, AMD is the most prevalent, followed by ocular histoplasmosis,[247] and including angioid streaks (below). CNV is a multifactorial nonspecific wound healing response to various specific stimuli, involving VEGF stimulation of choriocapillaris endothelium, compromise to Bruch's membrane, and participation of macrophages.[247] Impaired transport across Bruch's membrane in AMD increasingly isolates the RPE from its metabolic source in the choriocapillaries and enhances the challenge in waste product disposal. VEGF released by RPE as a stress signal initiates an angiogenic response by the endothelium. However, Bruch's membrane compromise is essential for CNV to proceed, as evidenced by intrachoroidal neovascularization without CNV in a mouse overexpressing VEGF in the setting of an intact Bruch's membrane.[250]

Bruch's membrane in a state of compromise can be breached easily by new vessels in AMD. It is notable that the EL is thinner and more interrupted in eyes with neovascular AMD.[26] The length of gaps in the EL is greater in eyes with early AMD and any CNV.[26] In paired donor eyes with and without CNV secondary to AMD, progressed eyes are distinguished by calcification and breaks in Bruch's membrane.[251] In contrast, calcification in a small number of geographic atrophy eyes is unremarkable.[252]

BLinD and soft drusen together further this process by presenting a horizontal cleavage plane for vessel formation to exploit. The lipid-rich composition, relative lack of structural elements like collagen fibrils, lesion biomechanical instability,[157] and proinflammatory, proangiogenic compounds like 7-ketocholesterol and linoleate hydroperoxide[247,253,254] likely promote vessel growth in this plane.[253] Interestingly, SDD is strongly associated with type 3 neovascularization,[255,256] suggesting that this lesion also plays a similarly important proangiogenic role.

Angioid Streaks (*ABCC6*, *MTP* Genes)

Angioid streaks are ruptures in Bruch's membrane associated with multiple disorders, caused by excess calcification of the elastic layer[257] and often accompanied by CNV. They are prominent ocular manifestation of pseudoxanthoma elasticum (PXE), a systemic connective tissue disorder. PXE patients harbor mutations of a hepatically expressed lipid transporter ABCC6.[258] Clinical presentation includes, in addition to streaks and CNV, peau d'orange (flat, yellow, drusen-like lesions), optic nerve head drusen, outer retinal tubulations, subretinal fluid, and pigmentary changes.[250] PXE clinical manifestations are believed related to ectopic mineralization of nonhepatic tissues, suggesting a defect in the transport of antimineralization agents.[259]

Angioid streaks are associated with abetalipoproteinemia,[260–263] an extremely rare disorder with low plasma apoB-containing lipoproteins, acanthocytosis of erythrocytes, neuropathy, and pigmentary retinopathy. It is historically attributed to lack of lipophilic vitamins delivered by plasma LDL.[264] The RPE expresses the abetalipoproteinemia gene (microsomal triglyceride transfer protein),[265] which cotranslationally lipidates apoB (see above). How this deficiency leads to angioid streaks is unknown. The finding, however, highlights that *lack* of apoB lipoproteins has negative consequences for Bruch's membrane health, likely by impacting RPE health, just as an *excess* of retained apoB lipoproteins has negative consequences via lesion formation and impaired transport (see above). Good chorioretinal function thus requires an optimal balance between these extremes.

Thick Basal Laminar Deposits (*TIMP-3*, *CTRP5*, *EFEMP1* Genes)

Three autosomal dominant-inherited disorders with adult onset – Sorsby fundus dystrophy, late-onset retinal degeneration (LORD) and Malattia Leventinese–Doyne honeycomb retinal dystrophy (ML–DH) – share phenotypic similarities with AMD and provide mechanistic support for many aspects of Bruch's membrane physiology and pathophysiology discussed above. All three conditions result from mutations in genes encoding extracellular matrix proteins or their

regulators (Sorsby – *TIMP3*[266], LORD – *CTRP5*[267], and ML–DH – *EFEMP1*[268]). All three can progress to CNV. All three have visual dysfunction, especially rods, attributed to a nutritional night blindness that is responsive to short-term administration of high-dose vitamin A.[269–271] Sorsby and LORD are notable for panretinal thick BLamD and areas of RPE atrophy,[272] while ML–DL is notable for radially distributed drusen and peripapillary deposits. In Sorsby mutant *TIMP-3* localizes to BLamD. In ML–DL, *EFEMP1* localizes to BLamD and not to the pathognomonic drusen themselves, suggesting an important role of BLamD in druse formation. Notably drusen in ML–DL are immunoreactive for fibulin-3 and collagen IV unlike drusen associated with aging and AMD.[273]

BLamD in Sorsby and LORD, like that in AMD, is notably rich in oil red O-binding lipid.[274–276] In LORD eyes[276] deposits contain EC, UC, and apoB, and lipid-preserving ultrastructural methods revealed solid electron-dense particles tracking in intersecting networks across the BLamD. Although not initially apparent, these may represent native lipoproteins in transit from RPE to the choriocapillaris. Lipid particle disposition within these thick deposits has been replicated in a mouse model expressing the R345W *EFEMP1* mutation.[224]

CONCLUSION

Bruch's membrane serves essential functions as substrate to the RPE and vessel wall of the outer retina. Its layers and constituent proteins collectively represent a barrier that keeps choroidal vessels at bay, provides a route for water, solutes, and macromolecules that transfer between RPE and choroid while supporting the structural integrity of both. It is unusual among human tissues in accumulating a high content of EC-rich neutral lipid over the lifespan. A natural history and biochemical model now suggests this lipid is due to apoB lipoprotein secretion by RPE, which may be part of an outer retinal nutrition system with an second component possibly also involving lipoproteins in the subretinal space. This deposition can account for the impaired outward movement of fluid from RPE, increasing risk for RPE detachments more common in older persons, and impaired macromolecular transport also leading to RPE stress. Oxidation of these lipid deposits in Bruch's membrane likely initiates an inflammatory process that leads to lesion formation and choroidal neovascularization in AMD.

Bonus material for this chapter can be found online at http://www.expertconsult.inkling.com

Fig. 22.1 Carl Bruch (1819–1884). (With permission from the Archives of the University of Heidelberg.)

Table 22.3 Localized components of drusen

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