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# Mechanobiological implications of age-related remodelling in the outer retina

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**Abstract**

The outer retina consists of the light-sensitive photoreceptors, the pigmented epithelium, and the choroid, which interact in a complex manner to sustain homeostasis. The organisation and function of these cellular layers are mediated by the extracellular matrix compartment named Bruch's membrane, situated between the retinal epithelium and the choroid. Like many tissues, the retina experiences age-related structural and metabolic changes, which are relevant for understanding major blinding diseases of the elderly, such as age-related macular degeneration. Compared with other tissues, the retina mainly comprises postmitotic cells, making it less able to maintain its mechanical homeostasis over the years functionally. Aspects of retinal ageing, like the structural and morphometric changes of the pigment epithelium and the heterogenous remodelling of the Bruch's membrane, imply changes in tissue mechanics and may affect functional integrity. In recent years, findings in the field of mechanobiology and bioengineering highlighted the importance of mechanical changes in tissues for understanding physiological and pathological processes. Here, we review the current knowledge of age-related changes in the outer retina from a mechanobiological perspective, aiming to generate food for thought for future mechanobiology studies in the outer retina.

**Keywords:** outer retina, ageing, retinal pigment epithelium, mechanobiology, extracellular matrix, age-related macular degeneration;

In the last decades, mechanical forces at the cellular and molecular level have been recognised as critical components in controlling physiological and pathological processes [1]. Mechanobiology gains increasing attention for understanding ageing and age-related diseases, where cellular senescence, in combination with the mechanical changes of the surrounding tissue, such as extracellular matrix (ECM) stiffening, may generate conditions for the development of age-related pathologies [2,3]. During ageing, mammalian biological systems experience different cellular mechanics, cytoskeletal re-organisation, altered mechanotransduction and changes in the ECM composition [2,3]. Despite this growing understanding of mechanobiology, until now, very little is known about the role of mechanics in retinal physiology and its possible impact on the age-related decline of functionality. In the following chapters, we aim to summarise the current knowledge of age-related changes in the outer retina with a particular focus on retinal pigment epithelium as a key layer for retinal organisation and function. Furthermore, we will focus on the possible implications of these changes for retinal mechanobiology.

**Age-related structural changes of retinal pigment epithelial cells**

Cellular mechanics is significantly influenced by the arrangement of the cytoskeleton elements and the regulation of their activity [4]. The cytoskeleton of cells encompasses a filamentous network of polymers and regulatory proteins that controls cell shape and adhesion, migration, division, directed transport of organelles, targeting and sorting of membrane proteins and membrane fusion events [5]. It consists of actin filaments, microtubules and intermediate filaments [6]. In the retinal pigment epithelium (RPE), actin filaments organise as a circumferential actin belt around each cell, forming a contractile structure that associates with the adherens junctions [7]. Branched actin filaments localise abundantly at the apical side of cells, where they ensure the proper structure and orientation of the microvilli, maintain contact with the photoceptor outer segments (POS) and facilitate the internalisation of shed POS [8,9]. Microtubules emerge from a microtubule organising centre close to the nucleus and reach out in parallel arrays to form the cores of the apical processes [10]. These hollow tubes are crucial for mechanical stability [11], proper phagosome translocation [12,13], pigment granule transport and aggregation [10,14], and microvilli integrity [15]. Healthy RPE cells also present an intermediate filament network composed of keratin-8 and keratin-18 [16,17], which provide structural stability and absorb mechanical stresses [18,19]. The keratin network in the native murine RPE is prominent at the side facing the photoreceptors and in the perinuclear cage with thicker filaments apically, while the basal cytoplasmic domain is devoid of intermediate filaments [16].

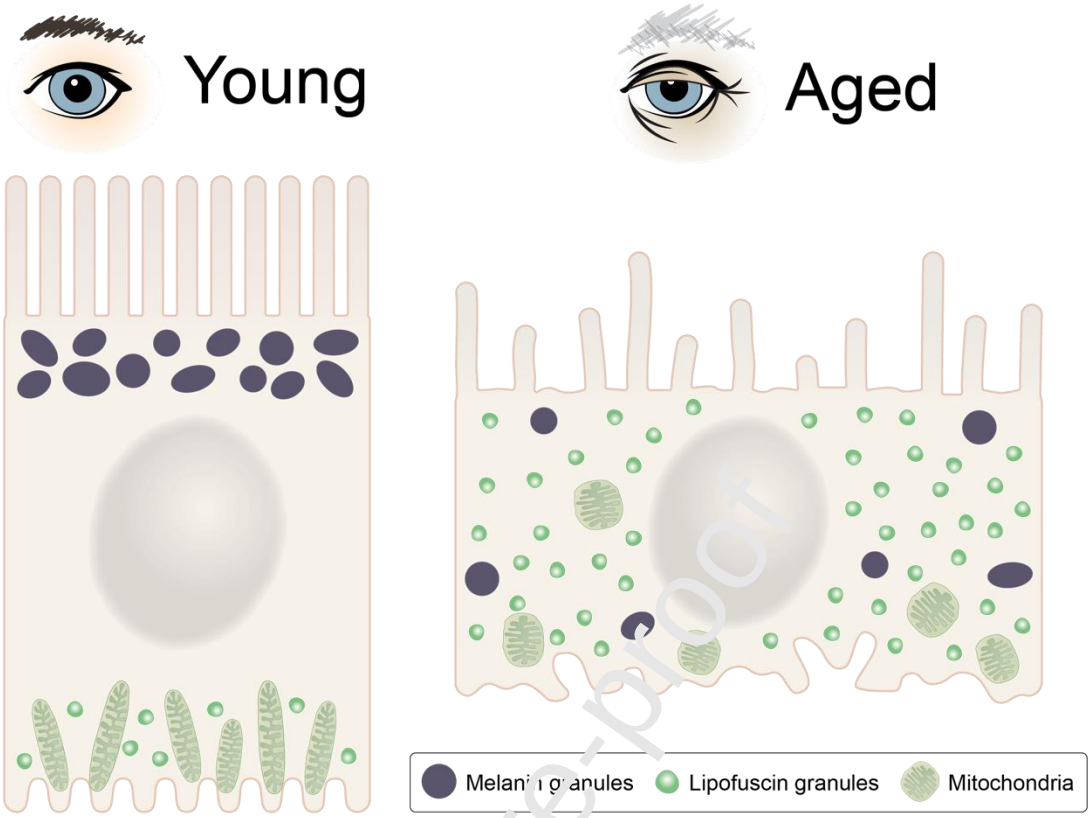


Figure 1: Structural differences between RPE cells in young (left) vs aged (right) retina. RPE cells in young retinas are polarized along the apicobasal axis, with dense microvilli at the top. In the apical portion below the villi, melanin pigments are localised. The basal domain contains other organelles like bacillus-like mitochondria and is rich in membrane infoldings. In ageing, RPE cells become larger and thinner with apical microvilli getting shorter, more disorganised, and sparse. The cellular cytoplasm becomes clogged with aberrant pigments, while mitochondria change morphology and size.

With age, RPE cell morphology undergoes significant alterations with an increase in average cell size in the macula and in the retinal far periphery [20] (Fig. 1) and has been reported to become thinner in the macular region [21] (Fig. 1). Since cell mechanics and cell shape within a tissue is defined by its cytoskeleton [4,5,22], these age-related changes in cell geometry suggest a gradual cellular “stretching” in time that implies rearrangement of the cytoskeleton elements [4,23] (Fig. 2). Interestingly, studies on cultured kidney epithelium (MDCK), which for many structural aspects can be compared to RPE [16], demonstrated that cellular enlargement within monolayers coincides with cellular stiffening [24]. Thus, the increased size of aged RPE cells most probably correlates with altered mechanical properties of cells. Another explanation for the morphological changes of RPE cells in ageing, is an alteration in cellular contractility. Actomyosin contractility shapes cellular and tissue morphology in

morphogenesis[25,26], however, less is known about the homeostatic case. Furthermore, the principle of force balance between cell-ECM and cell-cell adhesion[27] as well as the changes in the height and area of RPE cells during ageing[20,21] suggest changes in overall cell contractility, the extent of which is yet to be investigated.

Cytoskeletal remodelling affects basolateral polarity, which is crucial to preserve epithelial functionality [28]. RPE basolateral organisation is also a prerequisite for the retina organisation and enables the epithelium to fulfil barrier function, perform directed transport, secrete growth factors and daily phagocyte POS [29]. Structurally, the RPE possesses dense apical processes facing the interphotoreceptor matrix and attached to the photoreceptors, and a basal side enriched in membrane infoldings [29]. Two distinct types of microvilli exist in the adult RPE: long and thin microvilli (5-7 µm) to maximise plasma membrane area for transport and specialised shorter microvilli that enwrap the POS [30]. Studies on primates demonstrated that RPE villi become sparse and thinner while ageing, correlating with an increased number of displaced photoreceptors [31] (Fig. 1). Furthermore, studies in rats reported that while RPE height decreases, the apical processes shorten with ageing [32] (Fig. 1). Reduced villi size and number signify a decreased interface area that interacts with the POS. This may have implications for the attachment strength of the neural retina to the RPE-choroid complex and the efficiency of POS recycling (Fig. 2).

Cytoskeletal remodelling is facilitated by associated proteins controlling filament polymerisation, interaction, or degradation [5]. A recent single-cell transcriptomic study in monkey RPE identified age-related downregulation of genes linked to the regulation of cytoskeletal organisation and actin filament-based processes [33]. Expression of microtubule-associated protein MAP1B, actin filament-binding protein beta-tropomyosin (TPM2), and the mechanoresponsive nuclear intermediate filament protein Lamin-A/C (LMNA) were down-regulated in RPE with age [33]. These alterations may contribute to an aberrant age-related cytoskeleton organisation and changes in the mechanical properties and mechanotransduction of aged RPE cells and tissue (Fig. 2).

Mechanosensing signalling in ageing RPE may be affected by the cytoskeletal remodelling. A prominent mechanosensitive pathway is the YAP/TAZ pathway. YAP/TAZ nuclear translocation responds to mechanical cues such as substrate stiffness[34] and its activity declines during ageing in other systems like stromal cells[35]. In the RPE, YAP/TAZ plays an important function during embryonal development[36–38], and one study reported that the lack of YAP-1 causes alteration of RPE phenotype including depolarisation, tight-junction disruption, reduced RPE65 expression and diminished pigmentation[39]. This suggest that YAP-1 signalling is required to maintain the RPE differentiation[39] .[39] Since YAP/TAZ signalling is controlled by cell density with increased translocation to the nucleus at low cell

numbers[40] and monolayer strain[41], the age-related RPE cell loss may alter YAP/TAZ regulation of specific genes involved in outer retina ageing including RPE polarity.

At the basal side, RPE architecture enables the secretion of components of the Bruch's membrane and choroidal trophic factors and the exchange of nutrients between the general circulation and the retina [29]. Ageing causes disorganisation of RPE basal infoldings [42] (Fig.1). For example, basal infoldings in old rats become enlarged and heterogeneous in size, together with thickening of the basement membrane [43]. Altogether this evidence suggests that as RPE cells age, they may change their apical-to-basal membrane ratio, which seems to be constant in foetal and young RPE cells (three to one) and may be essential for proper polarisation and transport [44]. If this ratio shifts in ageing, different demands for transport-linked membrane remodelling may locally influence cytoskeletal dynamics at the apical and basal sides (Fig. 2). Lastly, since cell polarity is shaped and sustained by biochemical and mechanical cues from the environment [45], age-related alterations in RPE polarisation may reflect changes in the environment of the outer retina - altered matrix composition, different tissue mechanics and, overall, an increased mechanical heterogeneity of the tissue.

In the outer retina, the RPE faces constant light exposure – a condition that favours oxidative damage [46,47]. Electron-dense, cigar-shaped melanin granules are organised underneath the apical processes of RPE to mask reactive DNA-damaging blue light entering the cell [30]. With increasing age, melanin granules decrease in number and become amorphic [30], while complex granules like melanolysosomes and melanolipofucsin build up in the cytoplasm [42] (Fig.1). By the age of 80, up to 19% of the cytoplasmic volume is occupied by the photoreactive pigment lipofuscin [48]. Lipofuscin granules first appear only basally in young RPE, while, in older eyes, granules form clumps and fill the entire cytoplasm, possibly causing mechanical disruption in the cellular organisation [49]. A further age-related phenomenon in the RPE is the increased density of residual bodies [49]. Accumulation of residual bodies was observed in other post-mitotic cells like cardiomyocytes and hepatocytes and indicates an age-related reduction of lysosome activity [50]. Altogether, the elevated number of residual bodies and aberrant pigments could hinder cytoplasmic activity and enhance macromolecular crowding phenomena that affect reaction rates, such as protein folding, oligomerisation and micro-compartmentalization [51]. Interestingly, different regulations of actomyosin contraction via Rho GTPases were shown to influence RPE functions, both as a barrier [52] and the phagocytosis of POS [53]. Thus, macromolecular crowding could significantly affect the kinetics of actin polymerisation [54], which in turn may influence cellular function (Fig. 2).

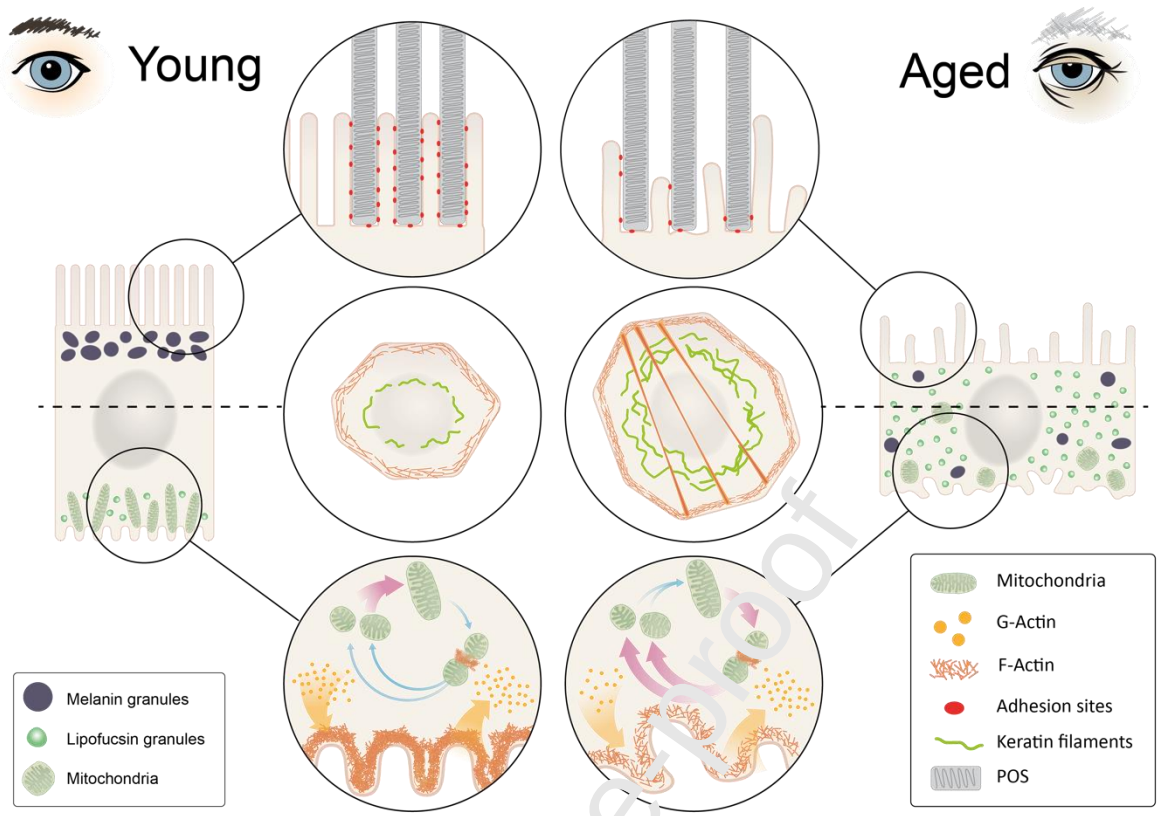


Figure 2: Hypothetical consequences of age-related structural changes for RPE mechanics and function. The altered structure of apical microvilli may influence the adhesion of the photoreceptors to the RPE – fewer microvilli present less contact area for the neural retina to adhere to the RPE (top). Altered cell size and morphology, may imply altered cytoskeletal organisation and mechanical properties (middle), and, thus, influence cytoskeletal dynamics, which is a prerequisite for an array of cellular functions, such as phagocytosis and transport. Changes in basal membrane organisation and mitochondria size and morphology may be linked to different turnovers of the cytoskeleton (down).

Age-related pigmental changes in the RPE have been reported in the context of oxidative stress and increased reactive oxygen species [55–57]. The major sites of reactive oxygen species (ROS) production are the mitochondria, which are particularly important for the highly metabolically active RPE cells [58]. Mitochondria in young RPE cells are abundantly located in the basal site with their elongated morphology and the presence of numerous cristae [59]. A recently published review summarises mitochondrial changes in RPE ageing[60]. Briefly, the number of mitochondria per cell decreases with time, and they become roundish and disorganised with partial loss of cristae [61] (Fig. 1). These changes may be linked to different dynamics of fission and fusion and cellular mechanosensitivity, both mechanisms that depend on the actin cytoskeleton [62,63]. Steady-state mitochondrial network morphology is regulated by cyclic actin assembly and disassembly[64], whereas mitochondria-associated

actin filaments promote the activity of GTPases like Drp1 to facilitate mitochondrial fission[65]. Furthermore, mitochondrial fission is mechanoresponsive and can be triggered by biomechanical stimuli such as force application by atomic force microscopy[66]. Overall, these reports suggest that the observed structural changes in RPE mitochondria result from age-related mechanical changes of the RPE and may affect its mechanobiology.

**Age-related alterations of RPE collective organisation**

The RPE is a simple cuboidal epithelium [67], where each cell is connected to its neighbours, forming a continuous cell sheet. At this level of multicellular systems, cell organisation, adhesion, and mobility give rise to mesoscale physical phenomena that govern biological functions [68]. The RPE monolayer extends from the highly illuminated fovea and macula to the lower light-exposed retinal periphery. Thus, cells face a gradient of functional demand, pre-determined by the heterogenous photoreceptor distribution along the visual axis [69,70]. Photoreceptors have the highest density in the macula, with a ratio of photoreceptors to RPE cells of 32 to 1. Proceeding towards the periphery, the ratio drops to 13 to 1 due to reduced photoreceptor numbers [69,70]. RPE cell density similarly changes along the visual axis - it is highest in the fovea and macula and linearly decreases in the mid- and far-periphery [20,71–73]. During ageing, the outer retina experiences an overall loss of photoreceptors [74]. Particularly in the periphery, both rod and cone photoreceptor numbers undergo age-related reduction, while in the more central region (fovea), cone densities remain constant [74].

The ratio of photoreceptors to RPE cells seems to be conserved during ageing [73,74], supporting the idea that also RPE have a differential cell density change over time. Nevertheless, the insights about RPE cell numbers with age are somewhat controversial, mostly due to the different and incomplete sampling. In most of the reports, there is an agreement that overall RPE cell density decreases yearly by around 0.23% - 0.3% [72,73]. Different regions of the retina experience distinct frequencies of cell density changes. RPE cell numbers in the macula have been reported to either decrease in an age-dependent manner [20,73] or remain constant [71,72,75,76]. The equatorial region or mid-periphery mostly remains unchanged in terms of RPE density [20,72,75], while the periphery experiences a well-documented age-related cell loss[20,72,74]. Furthermore, Harman et al. observed a band of larger RPE cells at the extreme periphery close to the ora serrata, which by the age of 90 widens to occupy the outermost 30% of the retinal area [75].

The cellular arrangement within an epithelium reflects its physical properties and the mechanics of cellular interactions [77]. Hexagonal packing represents the most stable configuration that tiles a flat surface, ensuring efficient coverage without cell overlap or empty areas [78]. This type of arrangement arguably results naturally from cell proliferation during development and is not a consequence of active cell sorting into an energetically

optimal state [79]. This is the case for RPE cells that organise themselves in a honeycomb-like structure [80]. In the macula, healthy RPE cells have mostly hexagonal shapes with five to seven neighbours [59,81] (Fig. 3). This organisation changes with increasing distance from the macula, with a reduction of hexagonal cells [20]. Due to the postmitotic nature of RPE cells and the natural cell loss in ageing [72], the epithelium needs to compensate for cell density changes in time by cellular enlargement and geometrical remodelling to preserve the overall integrity of the sheet [20]. Manifestations of this include cellular rosettes and the overall disorganisation of the monolayer [20,81,82]. Evident age-related alterations in cell morphology include an increased number of cell sides (pleomorphism) and increased variability of cell area (polymegmetism) [71]. In particular, the number of hexagonal cells decreases in the ageing fovea, while the density of pentagonal cells increases [83]. Furthermore, in advanced age, cell geometry becomes increasingly irregular [71,81] (Fig.3). Cells adopt a more elongated and less symmetric shape (polymorphic) in the macula [84] and in the far periphery [20], while cell geometry in the mid-periphery seems to be rather stable [20]. This morphological adaptation could signify altered cell-cell adhesion – a phenomenon not yet investigated in RPE, but demonstrated in different systems during ageing. For example, levels of cell-cell adhesion molecule E-cadherin in aged skin become reduced[85], while airway epithelial cells experience a general reduction of the expression of epithelial cell adhesion molecule, EPCAM[86].

The initial morphometric gradient in the retina may prime RPE cells to experience shape-related behaviours, including shape driven movement or morphotaxis [87]. If repeated cell divisions are normally the driver of hexagonal arrangement, the post-mitotic nature of RPE, thus the lack of proliferation, will account for the increasing dysmorphia of cells and disorganisation of the sheet (Fig. 3). Patterning of epithelial tissues is responsive to mechanical cues and may reflect stiffness gradients within the tissue [87]. The broadening of the neighbour distribution, manifesting in the appearance of large or small cells, shows increasing disorder and could reflect increasing heterogeneity of surface tension, as seen in foams [88].

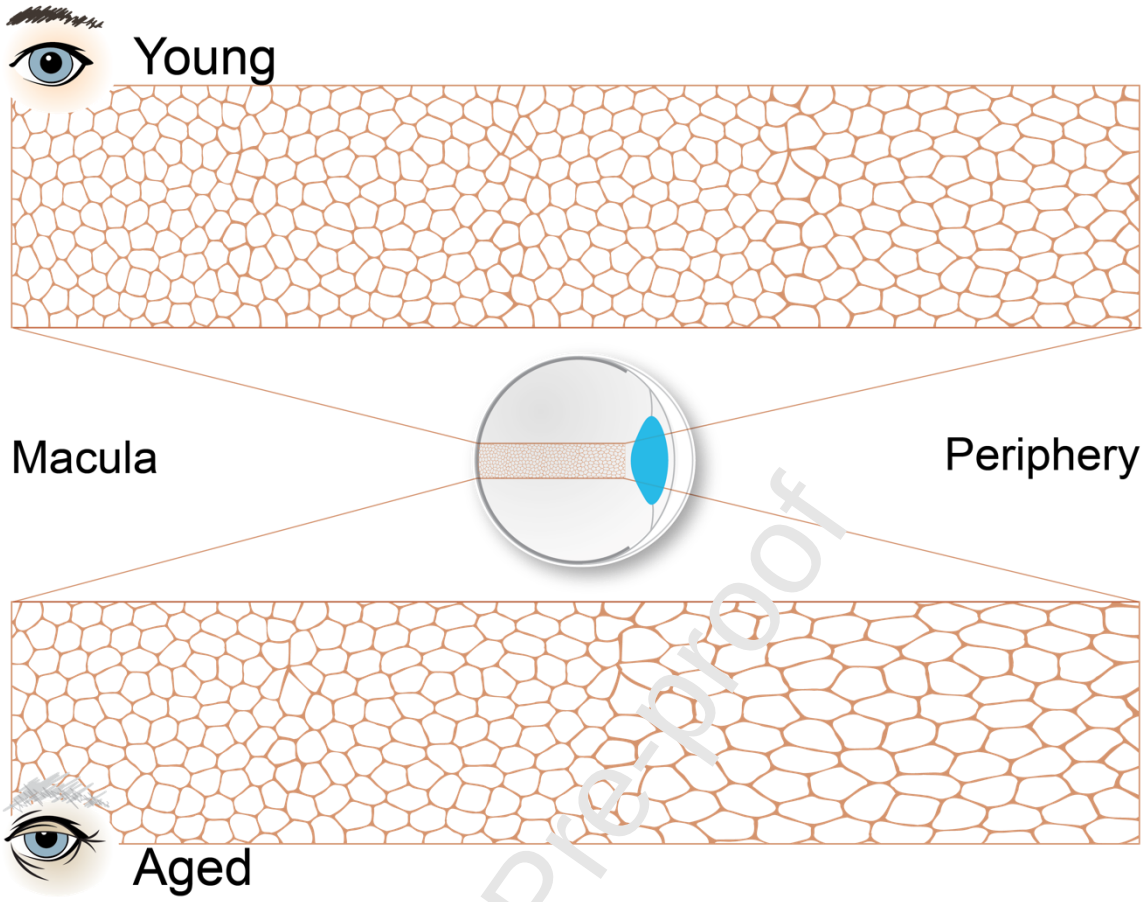


Figure 3: Collective morphological organisation of the RPE in young (top) vs aged (bottom) eyes. In young individuals, the RPE monolayer in the macula consists predominantly of hexagonal cells of uniform sizes, while cells become slightly larger and less regular towards the periphery (top). In old individuals, cells in the macula are bigger and of more variable sizes (down). Towards the periphery, cells become highly hypertrophic and irregularly shaped (down).

Overall, the heterogenous RPE cell organisation along the visual angle of the adult may reflect a gradient of mechanical homeostasis within the regions. Furthermore, age-related monolayer remodelling could directly affect changes in the intercellular stresses within the monolayer and directly modulate the ability of the RPE to support the visual cycle and photoreceptor health. Although speculative, these concepts need to be addressed in the future to better understand retinal physiology.

**Age-related RPE functional changes and mechanobiological implications.**

RPE functions are crucial for the physiology of the retina [89]. These include sustaining the outer blood-retinal barrier, facilitating transepithelial transport of nutrients and waste products, re-isomerizing the all-trans-retinal to 11-cis-retinal for the visual cycle,

phagocytosis and degradation of shed POS, protection against light and free radicals, and secretion of growth factors [30,90,91]. Some of these functions experience age-related changes, which may be conveyed by mechanobiological regulation.

One of the pivotal roles of the RPE is the daily phagocytosis of POS [92]. The phagocytosis of POS is a complex process that involves mechanobiologically regulated processes like binding, ensheathment, ingestion of POS and following phagosome maturation [8]. RPE cells recognise exposed phosphatidylserine phospholipids on the plasma membrane of the photoreceptors [93]. The ensheathment of the outer segment requires heavily branched actin networks, which create membrane 'ruffles' that envelop the photoreceptors [94–96]. Here, the activation of the tyrosine kinase Mer (MerTK) modulates Rac-GTPase activity via the guanine exchange factor Vav [97] or enhances its activity via focal adhesion kinase (FAK) mediated activation of $\alpha v\beta 5$ integrin [98]. Rac ultimately promotes Arp2/3-mediated F-Actin branching [99], while bundled submembrane actin is removed to relieve tension against the membrane ruffling and to ease the delivery of membrane from the endocytic pathway [100–102]. Each cell of macular RPE maintains roughly 32 photoreceptors with a turnover rate of 10 days for rod photoreceptors [69,103]. During ageing, the capacity for POS phagocytosis decreases [104], which may be influenced by altered mechanical homeostasis. In fact, the reported age-related stiffening of the Bruch's membrane [105,106] may affect cellular mechanics and cause reduced phagocytic activity in RPE [107]. Increased actomyosin contraction via the activation of RhoA reduces POS internalisation [53]. RhoA activity is responsive to externally generated forces such as compression [108] and tension [109,110], as well as to altered actomyosin contractility due to changes in substrate properties [111]. Rac1 activity is responsive to the remodelling of cell-cell adhesion [112]. These facts strongly suggest that age-related changes in the Bruch's membrane and RPE mechanics may indirectly impact RPE phagocytosis efficiency. The sealing of the nascent phagosome requires the activity of myosin IIA and myosin IIB [113], which may be affected by age-related changes in cell mechanics, cytoplasmic composition or altered expression of regulatory proteins such as tropomyosins [33]. Furthermore, the phagosome maturation involves apicobasal movement on both actin filaments [114] and microtubules [12], which may be affected by potentially altered macromolecular dynamics of the cytoplasm in older RPE cells.

RPE cells are subjected to high oxidative stress from POS phagocytosis and lipid peroxidation [115–117], intense light illumination [118] and high oxygen pressure, especially in the macula [119]. With ageing, the oxidative capacity of the outer retina decreases with reduced catalase activity in both macular and peripheral RPE [120] and with increased damage to the mitochondrial DNA [121]. The RPE expresses a major transcriptional regulator of the antioxidative response – the transcription factor NRF2 [122]. Despite the elevated expression of NRF2 in aged mice, the response to oxidative insult has been shown to be impaired in

older animals [123]. The cytoplasmic protein Kelch-Like ECH-Associated Protein 1 (Keap-1) binds NRF2 and induces its degradation, thus reducing its activity as a transcription factor [124]. Keap-1 has been shown to directly bind actin filaments [125], supporting the idea that cellular mechanics and actin cytoskeleton may directly influence antioxidative response via the regulation of NRF2 degradation levels.

**RPE and age-related remodelling of Bruch's membrane**

One of the main factors that define epithelial mechanobiology is the nature of its ECM, both in composition and physical properties [126]. In the context of the outer retina, RPE cells are tightly connected to the underlying Bruch's membrane. Structurally Bruch's membrane is divided into five distinct functional layers: the RPE basement membrane, the inner collagenous layer, the elastic layer, the outer collagenous layer, and the basement membrane of the choroid capillaries [127] (Fig. 4). The basement membranes are thin and tight ECM networks (0.14–0.15 µm) composed of laminins, collagen type IV, heparan sulphate proteoglycans, nidogens and other minor glycoprotein components. On the other hand, the collagenous layers are thick and loose (0.7–1.4 µm) and consist of fibrillar collagen types I, III and V, embedded in glycosaminoglycans, fibronectin, and complement system proteins. The elastic layer is mainly formed by several layers of linear elastin fibres attached to each other. Additionally, it contains collagen type VI, fibronectin and other protein-associated substances [106,127]. Thanks to its multilayer organisation, the Bruch's membrane represents a complex structure that supports and controls the homeostasis of the outer retina layers with the primary function of regulating the diffusion and reciprocal exchange of ions, molecules and nutrients between RPE and choroid. Additionally, with its elasticity, the Bruch's membrane plays a crucial mechanical role in withstanding intraocular pressure and may have other biomechanical roles in accommodating eye changes during vision [128].

With age, the Bruch's membrane undergoes significant remodelling of its composition, altering the cell-ECM adhesion sites with subsequent putative changes in epithelial functionality [106]. Given its acellular nature, the exchange of biomolecules through the Bruch's membrane is primarily passive, with more trafficking in the macular region compared to the less functional demanding periphery [129]. Age-related Bruch's membrane remodelling mainly includes an increased crosslinking of collagen fibres, calcification of elastic fibres and turnover of glycosaminoglycans [130,131] (Fig. 4). In addition, advanced glycation end products (AGEs) and lipids accumulate with time in deeper layers of Bruch's membrane [132–135]. Finally, despite a large amount of literature on the remodelling of Bruch's membrane's interstitial matrix, little is known about changes occurring in the basement membranes.

The Bruch's membrane has a heterogeneous thickness along the visual angle, thicker in the periphery and thinner in the macula region. This is mainly caused by the different thickness

of both collagenous and elastin layers [136]. Due to this heterogeneous distribution, the extent of Bruch's membrane remodelling also appears to be heterogeneous, first thickening at the periphery and then gradually towards the macular region [137]. This increased thickness correlates with reduced elasticity, flexibility, and permeability [129]. Altogether these changes in the Bruch's membrane may alter its stiffness locally with a direct impact on epithelial polarity, function and organisation, as shown in different systems and thoroughly discussed in a previous review [126].

Bruch's membrane remodelling over time also includes the accumulation of several types of minerals, including calcium and zinc, within the interstitial matrix [131,138,139] (Fig. 4). At this level, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are active to ensure ECM protein turnover [140]. The activity of these enzymes is affected by the presence of minerals, therefore, accumulation of zinc and calcium may directly affect the fine equilibrium in the activity of both enzymes, leading to altered ECM turnover. One example is the calcification and fragmentation of the elastic layer, which makes the membrane less elastic and brittle, allowing for faster neovascularisation in late AMD or in Autosomal dominant neovascular inflammatory vitreoretinopathy (ADNTV) [136].

In addition to minerals, there is an increased accumulation of lipids in the Bruch's membrane with age, negatively influencing its permeability. In fact, this age-related reduced hydraulic conductivity is associated with the level of lipid deposits [141,142]. In AMD, the elderly macula's Bruch's membrane contains seven times more cholesterol esters in comparison with the peripherical region [134]. It has been proposed that the acquired hydrophobicity of the Bruch's membrane and its reduced conductivity is responsible for the sub-RPE fluid accumulation and RPE detachment that is often observed in AMD patients [143]. Furthermore, the highest water flow resistance, defined by Bruch's membrane hydrophobicity, is a prerequisite for consequent tears in the epithelium, implying that sufficient tangential stress is induced in the detached tissue to cause the rupture [143].

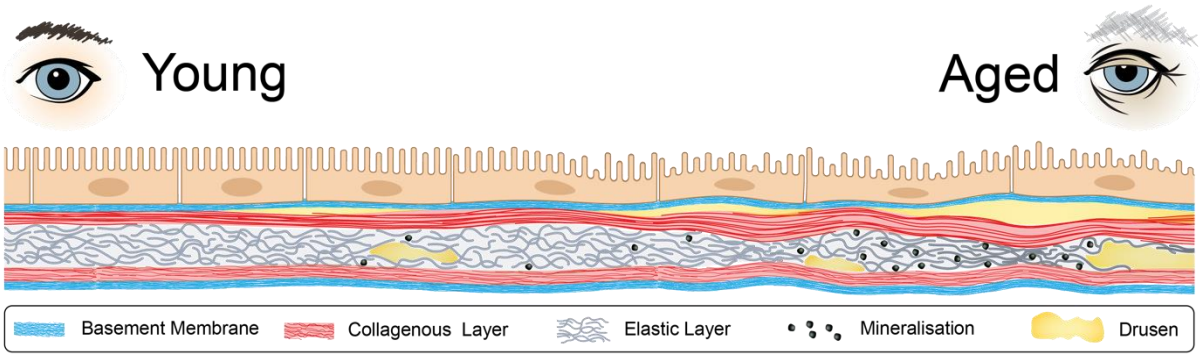


Figure 4: Bruch's membrane changes in ageing. The Bruch's membrane separates the RPE from the choroid capillaries (here not represented) and is composed of five distinct layers:

two basement membranes (blue), two collagenous layers (red) and an elastic layer in the middle. Over the years Bruch's membrane experiences numerous changes including mineralization and the formation of drusen. Drusen are localized accumulations of lipids and proteins mostly originating from blood serum and can vary in size and density between individuals and ages.

The main detectable change in Bruch's membrane ageing is the appearance of drusen [144]. Drusen are local deposits of amorphous extracellular material that accumulate within the Bruch's membrane (Fig. 4) and are formed by the aberrant accumulation of lipids and proteins, originating mainly from blood serum [145,146]. The morphology and composition of drusen vary between individuals, but epidemiological studies have shown that a high density of large drusen (63–125 μm in diameter and 17–30 μm in height) causes phenotypical switches and death in RPE cells, leading to detachment of photoreceptors in AMD [147,148]. From the mechanobiological perspective, the RPE monolayer is subjected to localized mechanical stress created by the drusen. This stress may be absorbed differently by an aged RPE monolayer, promoting phenotypical changes. This possibility is supported by the appearance of stress fibres in RPE cells on drusen [81], indicating a change in the equilibrium between cell-cell and cell-ECM adhesions.

**Conclusions**

Ageing is the main risk factor for many retinal pathologies, including AMD, diabetic retinopathy, and retinal detachment, whose prevalence significantly rises with increased life expectancy [149]. However, the differences between physiological and pathological ageing of the outer retina remain mostly unclear today. Understanding the unexplored mechanobiological features involved in the ageing process can give a novel perspective into the pathogenesis of retinal diseases. Morphological and organizational changes in the RPE may affect its mechanical features, forming local stress anisotropies. Intercellular stress may become higher and more heterogeneous, rendering the monolayer less flexible, thus losing plasticity to accommodate environmental stresses, like the accumulation of drusen, the stiffening of the Bruch's membrane and its increased hydrophobicity. Notable are the cell density changes that the RPE monolayer experiences over the years. As discussed, the reduction of cell density is particularly noticeable at the retinal periphery, whereases at the macular region, density remains constant. This suggests the necessity of RPE to preserve a defined mechanical equilibrium to best function. Finally, the overall mechanical and cytoskeleton shifts observed in RPE may directly lead to aberrant neural retina adhesion and local degeneration, as observed in age-related macular degeneration [150]. Certainly, future

research endeavours are still necessary to understand the mechanobiological processes of the outer retina physiology to reveal their role in different aspects of age-related pathologies.

**Author contributions**

TP, ANK and JDR wrote and proofread the manuscript and prepared the illustrations. All authors contributed to the article and approved the submitted version.

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**Declaration of competing interest**

The authors declare no conflict of interest.

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# Mechanobiological implications of age-related remodelling in the outer retina

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## Highlights

- Retinal epithelium undergoes structural and metabolic changes in ageing
- These changes are heterogeneous and may affect its mechanical homoeostasis
- Bruch's membrane remodelling suggests changes in epithelial mechanobiology
- Drusen may cause mechanical overload on a less flexible postmitotic epithelium
- Local mechanical anisotropies within the epithelium may cause retinal degeneration