**Title:** Biomechanical properties of retina and choroid: a comprehensive review of techniques and translational relevance.

**Running Head:** Update on retinal and choroidal biomechanics.

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Authors have no conflict of interest.

No funding has been received for this work.

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**Abstract**

Studying the biomechanical properties of biological tissue is crucial to improve our understanding of disease pathogenesis. The biomechanical characteristics of the cornea, sclera and the optic nerve head have been well addressed with an extensive literature and an in depth understanding of their significance whilst, in comparison, knowledge of the retina and choroid in relatively limited. Knowledge of these tissues is important not only to clarify the underlying pathogenesis of a wide variety of retinal and vitreoretinal diseases, including age-related macular degeneration, hereditary retinal dystrophies and vitreoretinal interface diseases, but also to optimize the surgical handling of retinal tissues and, potentially, the design and properties of implantable retinal prostheses and subretinal therapies. Our aim with this article is to comprehensively review existing knowledge of the biomechanical properties of retina, internal limiting membrane (ILM) and the Bruch’s membrane – choroidal complex (BMCC), highlighting the potential implications for clinical and surgical practice. Prior to this we review the testing methodologies that have been used both *in vitro,* and those starting to be used *in vivo* to aid understanding of their results and significance.

# Introduction

Diseases affecting the retina, including age-related macular degeneration (AMD) and hereditary retinal dystrophies, comprise a large proportion of untreatable blindness globally. Considerable efforts have been made into unravelling their underlying pathogenesis, but there has been relatively little study into the biomechanical predispositions of, and consequences to the affected tissues, nor indeed of the biomechanical properties of the ocular tissues affected during normal human development and ageing.

Biomechanics aims to characterise the origin and effects of mechanical forces involved in biological processes at different levels, from whole body/organ down to the subcellular level.1 Soft biological tissues, including retina and choroid, can be regarded as hierarchical, collagenous structures that exhibit complex biomechanical behaviour which is directly related to the composition and organisation of their microstructural constituents. The mechanical behaviour of these tissues can be described in terms of their age-dependent, anisotropic, nonlinear (hyperelastic) and viscoelastic[[1]](#footnote-1)\* properties. Indeed, age-related changes in elastic and collagenous fibres result in variations of biomechanical properties, including reduced elasticity of the retina-choroid complex, that may play a significant role in the pathophysiology of age-related ocular diseases, in particular AMD.2 Similarly, changes can be linked to other ocular features and it has also been highlighted that the stiffness of the retina-choroid-sclera complex, perhaps counter intuitively to clinicians, increases as axial length increases.3,4

A more in-depth knowledge of the biomechanical properties of these tissues could improve our understanding of the pathogenic mechanisms of disease. For instance, it is known that tangential and anterior-posterior forces related to vitreous ageing are involved in the origin of vitreoretinal interface diseases5; however, the exact effect of these processes and why in some people macular holes are formed, whilst others only experience vitreomacular traction, is not fully understood. Moreover, many vitreoretinal diseases, including retinal detachment and macular hole, are currently treated with pars plana vitrectomy that involve direct manipulation of retinal tissue. A better understanding of retinal biomechanics might help us to identify safe force thresholds and/or optimal angles of membrane peeling and, thereby, improved surgical approaches to minimise trauma.6,7 Similarly, a more detailed knowledge of retinal anisotropic behaviour is key to improving specific procedures whilst also adding insight into the optimum properties of implantable retinal prostheses and advanced therapeutics e.g. subretinal therapies.7 In particular, the compressive moduli of retina and choroid are important parameters for these therapies, as a substantial mismatch between them and those of a polymer scaffold for example may result in reduced biocompatibility in vivo.7

There have recently been comprehensive reviews of the biomechanical properties of the sclera and optic nerve head in the context of glaucoma and myopia in particular.6,8-13 This manuscript aims to review existing knowledge of the biomechanical properties of retina and the choroid, with the associated Bruch’s membrane. We firstly review techniques used in their investigation, before providing a brief overview of relevant anatomy and summary of biomechanical data specific to each tissue. We have included a section specific to the internal limiting membrane of the retina because of its surgical relevance. Bruch’s membrane and the choroid are considered together because of their close relationship.

**Methods**

The studies for this review were identified using Medline and Embase to December 2019, searching also the reference lists of the studies selected. The MeSH terms used were: ocular biomechanics; eye biomechanics; elasticity; stiffness; anisotropy; thickness; retina; choroid; Bruch’s membrane; internal limiting membrane; Young’s modulus; elasticity modulus; biomechanical tests. We have tabulated and summarised the most relevant publications in table 1.

**Techniques used to assess retinal and choroidal biomechanics**

Biomechanical properties of tissues are assessed by measuring their deformation in response to an applied force. Several methods of inducing deformation of ocular tissues have been utilised for both *in vitro* and *in vivo* biomechanical characterisation. The following subsections will summarise the findings of these methods, as well as their advantages and disadvantages.

## *In vitro testing*

Biomechanical tests to determine the mechanical properties of individual ocular tissues have traditionally been performed using *ex vivo* tissue post mortem, due to the difficulties in assessing them *in vivo*.6 Moreover, since access to human ocular tissue for experimental purposes is limited, eyes from various animal species including pigs, rabbits, primates and mice have all been used in biomechanical studies, although significant differences can exist in their properties.14-18 Porcine tissue in particular has frequently been used as a substitute related to its widespread availability, similarities in size, absence of a tapetum and holangiotic vascular pattern similar to primates. Indeed, Chen et al. evaluated the elastic properties of both porcine and human posterior eyewall, observing that each porcine tissue layer had elastic moduli within an order of magnitude of the values obtained from human tissues, and could be used as an eqivalent.7

Variations in experimental setup can have a significant impact on the results obtained, meaning that straightforward comparison of results from different studies is rarely possible. Since the ocular tissues exhibit anisotropic and viscoelastic characteristics7,14,19-28, the direction and rate of stretch during an experiment can have a substantial effect on the results obtained. Another concern with testing *ex vivo* tissue is post-mortem changes, which can be significant in tissues characterised by high cell content, such as retina and choroid.6 The processing of tissue samples is not standardised and several factors, such as the post-mortem processing time, as well as storage and transport conditions, can influence the quality and measured properties, limiting the reproducibility of the experiments.29 Indeed, environmental factors, such as calcium concentration, pH, and temperature strongly affect the adhesion strength between the neurosensory retina and the retinal pigment epithelium (RPE) in the post-mortem period.30 Chen et al. highlighted that *in vitro* conditions should match as closely as possible *in vivo* conditions, to reduce the impact of these environmental confounders.14,25,31 For instance, it has been reported that choroidal stiffness decreased as temperature increased from 26°C to 33°C in a single cadaveric human eye19, whilst retinal stiffness increased with a temperature rise from 26°C to 37°C in 6 month-old porcine eyes.14 Based on these findings, a 37°C saline solution has been proposed as optimum for *in vitro* biomechanical testing to simulate physiologic conditions.7 However, to date, the vast majority of *in vitro* biomechanical characterisation of ocular issues has been performed at room temperature. In contrast, it has been demonstrated that the freeze-thawing of BMCC does not apparently alter the biomechanical properties of this tissue in terms of elasticity and stress-strain relation.6 Finally, it should also be observed that tissue may behave differently when in its normal anatomical relationship with other tissue, although there has been no quantification of this effect.

### Uniaxial Testing

Uniaxial tensile testing is currently the most common method used to determine retinal and choroidal biomechanics of both animal and human eyes.7,14,18-25,27,31-37 For tensile testing, the targeted tissue (e.g. BMCC, or retina with or without ILM) is dissected from enucleated eyes and strips of uniform width and desired length are excised. Each strip is then mounted between two grips/clamps, on a manual or computer-controlled tester, and stretched in one direction (i.e. uniaxially) while recording the resulting load and elongation. The strip width and gauge length (i.e. grip-to-grip distance) is measured using a Vernier calliper. While strip thickness has been measured using an ultrasound pachymeter38 or micrometer21 for cornea and sclera uniaxial experiments, optical coherence tomography (OCT)18 and histological methods7,14,25,27,31,33,34,36 have been used for the retina and choroid. Friberg and Lace21 noted that, in the case of the choroid, it was necessary to use histology to measure thickness as the pressure exerted using a micrometer damaged the tissue.

When the strip dimensions are considered, the stress and strain can be determined and, from this, further parameters that describe the material behaviour characteristics can be ascertained. The strain, *ϵ*, is the response of the material to an applied force and is defined as:

|  |  |  |
| --- | --- | --- |
|  |  | Equation 1 |

where *ΔL* is the change in length and *L0*is the original length of the strip.

The stress, *σ*, is the force per unit area generated within the cross-section of the material due to the strain and is defined as:

|  |  |  |
| --- | --- | --- |
|  |  | Equation 2 |

where *F* is the applied force and the *A* is the cross-sectional area of the strip.

After plotting stress against strain, Graebel and van Alphen35 reported a power law response for the behaviour of choroidal specimens. However, the stress-strain behaviour of soft biological tissues typically follows a J-shaped exponential curve, as is reported in the majority of studies for both choroid and retina tissue. In such a curve, three regions can be identified which have been attributed to the degree of collagen recruitment as the tissue is stretched. First, a plateau region in which the sample is not yet fully straightened. Second, the elastic region of interest, characterized by an increase in mechanical resistance due to progressive recruitment of collagen fibres.39 Third, a region of “rupture” in which the mechanical resistance decreases and the sample starts to tear.14 Upon unloading, energy dissipation within the tissue results in a lag behaviour which is evident in the difference between the loading and unloading stress-strain curves. When loaded to failure, the yield strength and ultimate yield strength can also be quantified from the stress-strain graph as shown in Figure 1.

The modulus of elasticity is a measure of a materials resistance to deformation within its elastic limit. For a linear elastic material, the change in stress is directly proportional to the change in strain and the modulus of elasticity remains constant. In this situation the parameter is referred to as the Young’s modulus, *E*, where:

|  |  |  |
| --- | --- | --- |
|  |  | Equation 3 |

However, the behaviour of soft biological tissues such as the retina and choroid is non-linear, and the stress typically increases exponentially in relation to strain. Therefore, it is more appropriate to use the term tangent modulus, *E-tan*, as the value will be determined as the slope of a tangent line at a chosen point on the stress-strain curve. Tangent moduli are typically compared at specified physiologically relevant levels of stress35 or strain.18 Another method is to present the toe and heel modulus which corresponds to *E-tan* measured in the lower and upper slopes of the stress-strain curve, respectively, within the elastic limit.7,34 Higher values correspond to stiffer, less extensible material responses to stretching. Contrarily, lower values characterise more compliant tissues.

Uniaxial tests have been used to assess the effect of various factors on the biomechanical properties of the retina and choroid, namely ageing7,21,23,35, disease34, strain rate20,27 and temperature.14,19,31 Although restricted to assessing the localised uniaxial behaviour of the excised tissue strip, this method has also been used to investigate anisotropy, that is directionally-dependent mechanical properties, which are directly related to the orientation of collagen fibres within the tissue.40 In the posterior eye, the anatomical landmark for sample harvesting is usually the optic nerve head or the foveal centre, with strips excised in the horizontal or vertical direction.7,14 However, strips have also been excised with circumferential (equatorial) or meridional (anterior to posterior) orientations for comparisons between the anterior21, equatorial23 and posterior regions, as well as regions with and without blood vessels.20,22,24,25 Since retina and choroid collagen fibres are mainly aligned tangentially to the ocular surface, both tissues exhibit their maximum strength along surface directions.14 It has to be noted that a surface anisotropy may also be present.14,22 In this regard, biaxial tensile testing can be used to simultaneously extend the tissue equally along two axes. However, so far, biaxial testing has been used only for the sclera.41

Due to its simple setup and post-test analysis, uniaxial tensile testing has been used extensively to characterise biomechanical properties of ocular tissues, however, the technique has a number of limitations. Firstly, excising the tissue strip from its native curved environment results in severing of load bearing collagen fibres on both sides along the length of the strip. Secondly, flattening of the strip can generate compression towards the outer surface and tension towards the inner surface, altering the measured biomechanical properties. Thirdly, applying tension along one axis only loads the tissue in a manner which is not representative of that which is experienced *in vivo*. Finally, cyclic preconditioning of the tissue is required to obtain repeatable behaviour from the tissue sample; that is repeated cyclical straining from 0% to a pre-set maximum value of stress or strain at a predefined rate to induce reorientation of elastin and collagen fibres toward the loading axis.6,18,42 In biomechanical tests performed on sclera and cornea, a stiffening effect has been related to the preconditioning. Nevertheless, this test method is regularly used to obtain meaningful results in comparative biomechanical studies of ocular tissues.

### Inflation Testing

Inflation testing has primarily been used for the biomechanical evaluation of cornea and scleral tissue6,42,43, but also of the choroid4,18 and retina.44,45 The technique is widely regarded at the most desirable *ex vivo* method of assessing ocular biomechanics, related to its similarities to the *in vivo* situation. Enucleated eyes, typically transected or whole globes, are inflated using phosphate buffered saline (PBS) to vary intraocular pressure (IOP) while monitoring the pressure change and tissue deformation. Although this removes the need to align collagens to a single axis of loading as is required in uniaxial testing, preconditioning cycles are normally required to obtain repeatable behaviour from the tissue. Moreover, due to the anatomical location of the choroid and retina within the eye, standard techniques such as laser displacement sensing and digital image correlation are not suitable for monitoring displacement *in situ*, since direct measurements are obscured by the enveloping sclera. In order to overcome these obstacles, alternative methods have been employed. For instance, Ugarte et al. used OCT to monitor the deformation of human BMCC during inflation testing.4 They removed the BMCC from the sclera and retina and mounted 4-mm diameter sections of the isolated membrane in a pressurisation chamber applying a positive pressure to the choroidal surface.4 Cross-sectional OCT images were then used to calculate the arc length of the inflated membrane and from this the change in arc length due to applied pressure was used to quantify the strain, *ϵ*, using Equation 1. Rather than calculating cross-sectional area of the tissue to determine stress, *σ*, the applied pressure within the inflated membrane was considered to be the induced stress within the sample. Contrary to Equation 3, the authors stated that the elastic modulus, *E*, was calculated as the ratio of strain to stress rather than stress to strain. Nevertheless, the determined values were used to assess the effects of freeze-thawing on the biomechanical properties of BMCC as well as age- and AMD-dependent variations. More recently, Wang et al.18 used inflation testing to assess porcine BMCC rupture in conditions of elevated IOP. After removing a 7 × 7 mm square section of the overlying sclera, whole eye globes were progressively inflated using PBS until vitreous leakage through the exposed BMCC was evident. The pressure at this point was then registered as the value required to rupture the BMCC.

However, although inflation test allows the measurement of biomechanical properties in a more physiological setting compared with uniaxial testing, determining accurate material properties is demanding due to the varying thickness and anisotropic behaviour of ocular tissues. The recent application of elastography methods to ocular tissue, as discussed later, has given researchers the ability to assess ocular biomechanics within their native environment. This technique has been applied to both pressurised cornea and sclera and, more recently, to pressurised enucleated whole eyes.44,45

### Finite and Inverse Finite Element Modelling

Finite element (FE) modelling is a computer-aided numerical technique originally developed to solve complex structural analysis problems in aeronautical and civil engineering but that has also been applied to ocular biomechanics. The geometry of the structure, in this case the eye, is discretised into a mesh of individual or “finite” elements. By assigning representative boundary conditions and material behaviour characteristics to the elements, and incrementally simulating an applied load (e.g. IOP), the equations required to determine the resulting deformation of each element are solved computationally to obtain global deformation of the structure. Using this technique, valuable insights have been gained into pathologies of the posterior eye such as trauma-induced retinal haemorrhage46 and macular hole formation47, surgical considerations such as vitrectomy-induced retinal shear stress48 and optimal intravitreal injection angles49 as well as elucidating important factors that influence optic nerve head deformations.18

In order to obtain meaningful results from FE modelling, it is important to accurately define the geometry and material properties of the structure. However, due to its anisotropy, complex geometry and varying thickness, inverse FE analysis is often used to determine material properties of the eye. Inverse FE analysis is a combined experimental and computational process where experimental deformations are first monitored while the tissue is stretched due to an applied force, such as during an inflation test. An FE model of the test specimen is then constructed and the material properties of its elements are optimised using an iterative process until the displacements observed during the experiment match those produced by the FE model.

The majority of inverse FE studies on ocular biomechanics have focused on the corneoscleral tunic in *ex-vivo* models.50-52 Qian et al.53 applied inverse FE analysis to assess biomechanical properties of the choroid and retina *in vivo*. The authors injected saline into the anterior chamber of a cat eye while monitoring pressure using a pressure transducer. During the experiment, the choroid and retina was imaged using OCT at increasing levels of IOP, and the distance between locations in and around the optic nerve head were used as control points to monitor deformations for use in the material parameter optimisation process. However, the invasiveness of this approach would preclude its use in human participants.

### Atomic Force Microscopy

Atomic Force Microscopy (AFM) is a nanoscale surface imaging technique that is also capable of assessing mechanical properties of biological materials. Small sections of tissue, typically a few millimetres squared in size, are removed from their native environment and adhered to glass slides before being mounted in the AFM. The AFM has a cantilever with a tip, also known as a probe, which scans the sample surface up/down and side/side. In order to register the vertical and lateral motion of the probe during scanning, a laser beam is reflected off the cantilever and tracked through a deflection sensitive photo-detector. Knowing the cantilever stiffness, the Young’s modulus of the samples can be calculated from the deflection of the cantilever as it indents points on the sample surface using a range of equations that account for the changing force generated as a result of the probe shape contacting the underlying material. AFM has been used to assess thickness54,55 and a range of ILM biomechanical parameters, namely anisotropy28,55-58, indentation rate sensitivity and hysteresis28, developmental and age dependent stiffness variations54,59, as well as stiffness changes due to disease28,60 and treatments such as tissue staining57,58 and glycosaminoglycan removal.54 While AFM is restricted to scanning small areas in the region of 150 × 150 × 20µm, the primary advantage of this technique from a mechanical characterisation perspective is its ability to measure properties of nanoscale structures such as cells, collagen and nerve fibres. However, there are several variables that must be carefully considered to ensure reliable results. Firstly, the cantilever stiffness should be similar to the stiffness of the sample being tested. Secondly, the substrate on which the sample is mounted can influence the results; therefore, the indentation depth should be optimised. Thirdly, the most commonly used mathematical model (i.e. the Hertz model61) assumes linear elastic behaviour and homogeneity of the sample, and so it is important to focus on the region of the probe force-deflection curve which represents the structure that is being investigated.

Schematic illustrations of the tissue deformation modes experienced commonly used *ex vivo* test methods are presented in Figure 2.

***In vivo testing***

*In vivo* measurements of biomechanical properties of ocular tissues by non-invasive techniques may lead to a patient-specific assessment of both risk factors for the development and progression of ocular diseases and prognostic factors related to surgical treatment. Limited data are currently available regarding the use of high-resolution magnetic resonance imaging (MRI), ultrasound (US) biomicroscopy and OCT. MRI has been used to assess sclera and for the evaluation of IOP-induced changes of the whole globe with particular regard to the posterior sclera displacement.17 However, random eye movements in living or non-paralysed subjects can lead to blur artefacts and MRI has never been used to assess thinner tissue, such as retina and choroid.17 On the other hand, elastography techniques are being increasingly used for *in vivo* assessment of ocular biomechanics.

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### Ultrasound Elastography

Ultrasound elastography has been proposed as a reproducible method to assess the *in vivo* elasticity of the retina-choroid-sclera complex in several pathologic conditions. An ultrasonic transducer is used to transmit inaudible, high frequency soundwaves into the eye while the response is sensed by a second transducer which can, in some cases, be the sending transducer. Pekel et al. used US elastography to assess elasticity in myopic62 and diabetic eyes treated with argon laser panretinal photocoagulation.63 After applying gel, the ultrasound probe was placed in contact with the closed eyelid and small rhythmic compressions were manually applied by the operator. A similar approach was used by Agladioglu et al.64 to assess ocular elasticity patients with primary open angle glaucoma. Tissue elasticity in these studies was determined by the ultrasound system which provided unitless values that can be used to distinguish between areas of high and low stiffness. However, Shahbazi et al.2 used US to determine quantitative biomechanical parameters of the retina-choroid complex in healthy and AMD patients. In this study, the US probe monitored static pressure-induced changes in axial length and tissue thickness from which strain and elastic modulus values were then estimated.2 While the aforementioned studies have proven the ability of US to obtain semi-qualitative and qualitative biomechanical data from the posterior eye *in vivo*, the technique has been not yet been used to individually characterise properties of the retina and choroid.

### Optical Coherence Elastography

Optical coherence elastography (OCE) combining structural OCT imaging with elasticity measurement principles is able to provide tissue elasticity mapping with high sensitivity and high spatial resolution (about 10 µm).65-69 In this technique, the OCT detects the deformation or vibration of the sample induced by an external force that can be generated by different techniques, such as air-puff pulse65, acoustic radiation force (ARF)66, needle probe67, piezoelectric transducer68 and laser pulse.69 The excitation of the sample generates elastic waves propagating to the surrounding tissues. The ability to measure wave velocity and track wave propagation by the OCT provides a means of quantifying and mapping the elastic properties. Bulk moduli for longitudinal wave propagation are based mainly on water, and is dependent on short-range molecular interactions, while shear moduli are more related to tissue structure.70 Consequently, bulk moduli values for biological tissues fall within approximately one order of magnitude, whereas shear moduli range over several orders of magnitude. Therefore, monitoring shear waves provides greater contrast between different tissue types and increases the potential for distinguishing between healthy and diseased tissues.70

ARF is able to excite both superficial ocular tissue, such as cornea, and deeper tissue, such as retina and choroid. Although largely applied to the study of anterior segment structures, the use of elastography methods to assess retinal and choroidal biomechanics has been limited due to the inaccessibility of such tissues and unsatisfactory resolution.44 However, ARF-OCE has recently been used to evaluate the *in vivo* elastic modulus of the choroid and individual retinal layers in rabbit eyes.45,71 While Qu et al.71 acknowledged that the acoustic intensity used to excite the ocular tissue in their study exceeded FDA limits for diagnostic ultrasonography of the human eye, the required intensity reduction to satisfy the guidelines would still induce tissue displacements that could be detected by their imaging system. However, the current methodology required proptosis of the rabbit eye to expose the sclera during measurements and further refinement is required to translate the technology to a clinical setting.

**Retina**

The retina, the innermost tissue of the eye, is composed of neuronal and non-neuronal cells organized in laminated structure with a thickness in humans of around 250 µm.3 In particular, the neurosensory retina is composed of the 9 distinct layers from the internal limiting membrane on the vitreous side to the photoreceptor layer (Figure 3). The neurosensory retina, hereon called the retina, rests upon a specialised monolayer of hexagonal cells joined by tight junctions and forming the outer blood retinal barrier, the RPE, These cells, whose apical microvilli enshroud the outer segments of the photoreceptor, have multiple crucial functions for the maintenance of retinal health.72 The mechanical inter-digitation of the RPE microvilli to the outer segments of the photoreceptors, the maintenance of subretinal ions concentrations and the transport of subretinal fluid towards the choriocapillaris are all mechanisms that actively contribute to the adhesion between the RPE and neurosensory retina.

The role of Müller cells for the homeostasis and the structural integrity of the neurosensory retina is crucial.73 These specialized radial glial cells have two main stem processes radiating from the body in two opposite directions, one towards the vitreal surface and the other towards the photoreceptors, and ending in the ILM and ELM, respectively.73 Müller cells are involved in water and ion homeostasis, modulation of neuronal activity, but also structural support of the retina through intermediate filaments, such as the glial fibrillary acidic protein (GFAP).73 Under pathologic stimuli, these cells react with the upregulation of several proteins, amongst which GFAP, is considered a marker of retinal stress.73

The distribution, density and morphology of the above cells vary across the retina topographically but also with age or disease. The density of the 91 million rod photoreceptors progressively decreases centripetally and inversely to that of the 4-5 million cone photoreceptors, that represent the only cellular type in the foveola.74 From the fovea to the periphery, RPE cells progressively increase in diameter and decrease in height and density. Moreover, the peripheral density of RPE cells further declines with aging.74 Two different types of Müller cells have been described in the fovea: the Müller cells of the foveal centre, smaller with densely packed cytoplasm and straight inner processes, contrasting to the Müller cells of the foveal walls, whose outer processes have a characteristic “z-shape” running obliquely through the inner portion of the OPL (called Henle’s fibre layer) around the foveal centre.73 Spatial variation in retinal cells distribution and arrangement have been associated with topographical variations in retinal stiffness.73 Indeed, using AFM in guinea pig eyes, Franze et al. demonstrated that mean retinal stiffness increased moving from the optic nerve head (ONH) to a distance of 2.5mm, remained stable in the midperiphery (2.5-4.5 mm from ONH) and, then decreased in the far periphery (7 mm from ONH).56 Moreover, the stiffness of temporal and nasal retinal quadrants was significantly higher than that of the inferior and superior quadrants.56

In the retina, as well as in the choroid, elastin and the collagen are mainly responsible for the tissue’s stiffness, whilst proteoglycan molecules, mainly heparan and chondroitin sulfate proteoglycans, contribute to its incompressibility.75 A reduction of elastin in retinal vessels and, consequently, increased retinal stiffness has been demonstrated in patients affected by moderate-to-severe AMD and atherosclerotic vascular disease.75,76 It is known that a variety of tissue insults result in an increased production of proteoglycans.75,76 Consistently, a significantly higher content of proteoglycans has been reported in maculae with early AMD. Moreover, the heparan sulfate proteoglycans (HSPGs) also appear to be involved in the pathogenesis of AMD, due to their reduced ability to bind complement factor H (CFH) and, as a result, regulate the alternate pathway of complement, whose inappropriate activation plays a critical role in the progression of AMD.75

From a mechanical point of view, the first investigations of the retina with regard to its elastic behaviour estimated Young’s modulus to be approximately 20kPa77; this value was determined using uniaxial testing on isolated bovine retina. The retina however is an anisotropic and heterogeneous tissue, with its nonlinear elastic behaviour differing between the vertical and horizontal meridians.7 Indeed, Chen et al. using uniaxial testing on human retinal strips, showed that retinal transition stress, heel modulus and toe modulus all tended to be higher in the vertical, compared to the horizontal direction.7 The authors reported that the retinal heel modulus was approximately 19 kPa and 13 kPa in the vertical and horizontal meridians respectively, which is of translational relevance when peeling adherent membranes from the retinal surface to avoid retinal tearing. This study also confirmed the significantly lower stiffness of the retina when compared with the choroid (Heel modulus of 387 and 362 kPa in the vertical and horizontal meridians, respectively).7 Moreover, the retinal stress-strain curve showed that, above an inflection point, the stiffness decreased as the strain increased.7 Anisotropic differences in retinal tensile responses have also been described for the meridional (i.e. axially, anterior to posterior) and equatorial (circumferential) axes , as retinal strips in the former orientation endure higher stresses than the latter.24 In addition, blood vessels significantly contribute to retinal stiffness.25

The mechanical properties have been also studied in relation to ageing. Reinchebach et al. demonstrated that in adult rabbit eyes all retinal regions showed reduced tensility (i.e. reduced ability to extend) when compared with neonatal tissue, consistent with a progressive increase in retinal elasticity during development.78 However, the retinal compressive modulus appears to relatively stable with age.7 Retinal strain anisotropy appeared to decrease with age due to the decrease of the vertical transition strain and the increase of horizontal transition strain.7 However, the authors, constrained by tissue access, only analysed 24 eyes with a strong male predominance and a minimum age of 30 years. Moreover, one of the main limitation of *in vitro* retinal strip testing lies in the isolation of the retinal samples itself. *In vivo* the neurosensory retina is strongly adherent to the RPE, which adds to its biomechanical strength and properties. Such adhesive force has been studied in living eyes of different species and assessed at 0.001 N/cm on average in rabbits.79 Adhesion is stronger in cats and monkeys, which show mean values that are 180% (for cats) and 140% (for monkeys) of that in rabbits.79

Recently, Qu et al. used ARF-OCE to measure the elasticity of the retinal layers *in vivo* in porcine eyes.44 The authors found that the stiffness decreased from the top to the bottom layers, with Young’s modulus increasing from 1.3 kPa in the inner retinal layers to 26 kPa in the photoreceptor layer, consistent with the proximity of the photoreceptor layer to the choroid and sclera whose stiffness is much higher than the retina.44

**Internal limiting membrane**

The ILM is formed principally by the basement membrane of the Müller cells, forming the inner surface of the retina.80 Structurally, the main components are collagen type IV, fibronectin and laminin. The thickness varies from 0.01-0.10 µm at the optic disc and the fovea, to 0.5-3.2 µm at the posterior retina.80 The posterior vitreous cortex (PCV) is firmly and broadly adherent to the vitreal side of the ILM through adhesion molecules, including fibronectin, laminin, and heparan sulphate proteoglycans, forming the vitreoretinal interface (VRI).80 With age, vitreous liquefies and the gel structure collapses. Combined with this, there is a weakening of the VRI, and the vitreous separates from the ILM in a stereotypical way, peri-foveally initially, followed by the fovea and then optic disc. In vitreoretinal surgery, ILM removal is commonly peeled off for a variety of indications by using end-gripping forceps to remove tangential traction at the retinal surface. Since the ILM is thin and transparent, different dyes have been commonly used to improve ILM visualisation and minimize surgical trauma.81

The biomechanical properties of ILM have been investigated using the AFM.55,57,57,60 In most studies, after dissection of fragments of chick, mice or human cadaveric retina, the ILM has been isolated by incubation in 2% Triton-X-100, as basement membranes are detergent-insoluble.54,60 The ILM is mechanically stronger and stiffer than the cellular layers of the retina being 1000-fold stiffer, and accounting for approximately 50% of retinal tensile strength. This is comparable to articular cartilage, suggesting it has a major role in the structural integrity of the retina.55,59 Moreover, its retinal side, where there is a higher density of proteins typical of extracellular matrix55, is over 5 times more rigid than the vitreal side.55,57 Evaluating human ILM in 2-mm2 retinal segments taken from within the vascular arcades, Henrich et al.55 assessed the variation in ILM thickness and stiffness from the foveal centre. The ILM thickness and stiffness which were closely related, reached their maximum at about 1000 µm from the foveal centre, then decreased progressively towards the periphery, which can help guide surgical approaches for peeling.55 Candiello et al.54 demonstrated that the thickness and the stiffness of human ILM, outside the posterior pole, increase in an age-dependent manner. It has to be noted that, as the ILM acts as a barrier against the access of therapeutic antibodies, viruses or cDNAs injected intravitreally, differences in thickness are likely to have a role on the effect of these agents on retinal targets.82

As already observed, ILM plays an important role in several vitreoretinal diseases. Ciasca et al.28 used AFM to compare ILM specimens obtained from patients who had undergone PPV and ILM peeling due to macular hole (MH) and epiretinal membrane (ERM).They found that the ILM retinal side was significantly stiffer in cases with MH than in ERM, suggesting that, in the former, the higher ILM stiffness could stabilise and strengthen the adhesion between ILM and Müller cells.28

As ILM peeling can be a challenging technique, various vital dyes have been introduced as intraoperative tools to facilitate ILM visualization for peeling.57 It has been demonstrated that different dyes not only exhibit different interactions with surrogate ILM membrane models83 but also result in different histological planes of separation of the peeled ILM from the underlying retina84 and different immunohistochemical findings of the peeled ILM.85 To evaluate the potential influence of vital dyes on the biomechanical properties of human ILM, Haritoglou et al.57 collected unstained ILMs peeled from human eyes undergoing vitreoretinal surgery and analysed them with AFM. Samples were stained with brilliant blue (BBG) 0.025% or indocyanine green (ICG) 0.05% and an unstained fragment was used as a control.57 They also illuminated the stained fragments for 1 minute with a standard vitreoretinal light source to assess any variations related to intraoperative illumination.57 The authors reported that the rigidity of ILMs on both the retinal and vitreal sides significantly increased after staining with ICG and BBG.57 The increased stiffness was more pronounced on the vitreal side and with ICG.57 In addition, there was a further increase in stiffness, of around 1.2 fold, with ICG after illumination, but not with BBG.57

Diabetes has been associated with changes in the composition of ILM with resultant changes in its biomechanical properties.60 Long-standing diabetes is characterised by significant thickening of basement membranes with the formation of advanced glycation end products of their constituent proteins.86 Several extracellular matrix components have been shown to be increased in diabetic patients, namely fibronectin (on the retinal side), laminins, collagen types I, III, IV and V, and heparan sulphate proteoglycans.60,87 Using AFM on ILM obtained from cadaveric human eyes, samples from diabetic donors were stiffer than those from age-matched non-diabetic donors , with the differences ranging from 20 to 60%, and explaining perhaps the observed variability in ILM characteristics noted during surgical peeling in diabetic retinopathy.60 However, it is worth noting that AFM only has limited penetration of tissue samples and, therefore, provides measurements relative to the tissue surface. Moreover, changes in ILM in association with gender and other posterior segment diseases have not yet been studied.

**Bruch’s membrane/Choroid Complex (BMCC)**

Bruch’s membrane is a 2-4 μm-thick acellular sheet positioned between the RPE and the choroid. It is composed of five distinct layers that are (from the innermost to the outermost): the basement membrane of the RPE, the inner collagenous layer, the elastic layer, the outer collagenous layer, and the basement membrane of the choriocapillaris endothelial cells.88 The main components of BM are collagen types I, III, IV, V and VI, fibronectin, laminin, elastin and proteoglycans, in particular heparin and chondroitin sulfate proteoglycans.88 Bruch’s membrane acts as a physical and biochemical barrier for both molecules and cells between the retina/RPE and the choroid, as reservoir of anti-angiogenic factors and as scaffold for the adhesion, growth and support of the RPE cells.88,89

It has been suggested that changes in the biomechanical properties of BM are associated with age-related macular degeneration (AMD).90 It is known that BM undergoes several changes with age, including an increase in thickness with a blurring of the boundaries between the five layers, accumulation of lipids as well as advanced glycation end products (AGEs) and a decrease in both the amount and sulfation of heparin sulfate proteoglycans in the macular area.88,91 Moreover, a physical breakdown of BM has been associated with both aging and AMD.88 The involvement of BM has been also hypothesized in the physiological process of emmetropisation as well as pathological myopisation, through the generation of an active force in the midperiphery, influencing the axial elongation of the globe.92 Since it is difficult to isolate the BM, the biomechanical properties of this tissue have been commonly indirectly assessed through the biomechanical study of the BM-choroid complex (BMCC).4,18,21

The choroid is the 200 µm-thick vascular layer located between the sclera and the retina, responsible for supply of oxygen and nutrients to the outer retina.6,93 Going from the retinal to the scleral side, the choroid is structured in several layers, namely: the choriocapillaris, a capillary network with maximum thickness of 10 μm at the fovea, progressively decreasing to about 7 μm peripherally; Sattler's layer, composed of arterioles feeding the choriocapillaris and medium/small arteries and veins; Haller's layer, composed of large blood vessels; the Suprachoroid, a transitional zone containing elements of both choroid and sclera, such as collagen fibers, melanocytes and fibroblasts; and, finally, the lamina fusca, a 30μm-thick layer separating the suprachoroid from the sclera.6,93 In the choroid, the flow per perfused volume is the highest of any other human tissue, and the choroidal vessel and capillaries contain about 85% of overall ocular blood flow.93 Structurally, the main components of this tissue are heparan sulfate, laminins, collagens type IV, V, and VI, and a network of elastic fibrils.6,94 The elastic network is connected to both the posterior tendons of the ciliary muscle and a network of contractile cells extending from the optic nerve to the area of the vortex veins.94 Through this connection, the contractile cells has been hypothesised to counteract the variations in diameter and position of the choroidal vessels potentially induced by the pulling action of the ciliary muscle towards the elastic network during accommodation.94,95 Moreover, the ability of the choroid to modulate its thickness is also thought to be important for emmetropisation.96 It is also worth noting that changes in choroidal volume and, consequently, thickness result in changes of intraocular pressure.97

To date, the literature on BMCC biomechanics has been limited as its influence on the stiffness of the eye has been supposed to be negligible6; however, there is a growing interest in their mechanical properties as their role in ocular development and pathologic conditions has become clearer.

Uniaxial tensile tests on choroidal strips have been used to investigate BMCC elasticity in relation to surgical procedures and trauma.7.21 The BMCC behaves as a nonlinear soft tissue as the stiffness increases with stretching.18 Unlike the retina, human choroid shows no significant difference in elastic behaviour between the vertical and horizontal meridians.7 However, it has been reported, similar to retina that meridional choroidal strips are stiffer than equatorial ones.7 Moreover, the elastic modulus of BMCC-RPE complex strips was significantly greater in samples taken posterior to the equator than those taken more anteriorly, whereas the elastic modulus of radial choroidal strips (straddling the equator) did not significantly change by location (superior, inferior, temporal or inferior).7

Compared with scleral tissue, human specimens of BMCC exhibit lower stiffness e.g. mean Heel modulus approximately 370kPa versus 4400kPa and a more linear stress-strain curve.7,21 On the contrary, testing BMCC specimens excised from porcine eyes, Wang et al.18 found that BMCC samples had elastic moduli (~1-2MPa) at least comparable or higher than those reported for sclera (~1-8Mpa)98, and far higher than the retina, cornea and iris (~0.01 MPa, 0.3 MPa and 0.004 MPa, respectively).98,99 The authors argued that their results could be due to the smaller amount of choroid included in the specimens compared with previous studies and, therefore, could be more representative of BM biomechanical properties rather than BMCC.18 Using inflation tests on completely excised BMCC specimens and assessing their deformation with OCT, Ugarte et al. reported that BMCC stiffness significantly increases with age, potentially leading to a reduction of choroidal blood flow with consequent alterations to the oxygen and nutrient supply to the retina.4 Finally, it has been reported that a substantially high IOP can be sustained by BMCC alone, that in burst tests exhibited a rupture pressure of about 80 mmHg, with significant deformation before reaching the point of mechanical failure.18

Graebel and van Alphen suggested a tendency for choroidal elasticity to decrease with age35 and an increase in the horizontal stiffness of human choroid with age has been recently demonstrated using uniaxial testing.7 Moreover, the decreased elasticity of BMCC with age does not appear to be exaggerated in AMD, as demonstrated by comparing BMCC samples taken from human donor eyes with and without signs of AMD, although further study is needed.7

**Conclusion and future perspectives**

The study of ocular biomechanics is a research area of growing interest due to its significant translational value with both diagnostic and therapeutic implications.10 This aspect has been clearly highlighted for ocular tissues such as cornea and sclera, whereas retina and choroid have not been investigated so extensively so far. With regard to diagnosis, alterations in mechanical properties at cellular and tissue level are thought to determine the onset or progression of detectable structural alterations. For instance, the decrease of retinal elastin and, consequently the increase in stiffness, have been detected in moderate-to-severe AMD but not in early disease.37 and there are other changes in the composition of Bruch’s membrane that are associated with both ageing and disease. Furthermore, although changes to the stroma will account for changes in the bulk mechanical properties of a tissue, the contribution of the direct contact point (the basement membrane for sheets of cells) should not be overlooked. Each basement membrane contains two linked supramolecular networks of type IV collagens, and of laminins. Crosslinking of type IV collagen is well known to occur with age and some diseases, leading to biomechanical changes that have been described above. Not all laminin isoforms are capable of network formation. A change in the ratio of polymerising to non-polymerising laminins will local soften or stiffen a basement membrane and, in turn, change the signalling from matrix-bound receptors. Modification to basement membrane structure need not only occur in terms of absolute abundance; matrix remodelling proteins including matrix metalloproteinases and post-translational modifications such as glycoslylation will change the structure, orientation, and hydration of these specialised extracellular matrix structures. All of these will influence the biomechanical properties, perhaps in a hyper-local manner.

Knowledge of biomechanical properties of ocular tissues is crucial for both the optimisation of pre-existing and novel surgical techniques and devices. Biomechanics can help predict the response of targeted tissues to surgery and the remodelling of such tissues after surgical manoeuvres or the implantation of various medical devices.10 One of the main aims therefore of the current research into biomechanics is the development and improvement of testing methodologies *in vivo*, for both the whole globe and the individual ocular layers. This could result in the use of biomechanical tests in clinical practice as well as allowing the optimization of FE models of the eye.10

In conclusion, the study of retinal and choroidal biomechanics is worthy of further investigation with potential to improve both the diagnosis and therapy of a variety of sight-threatening diseases.

**Acknowledgments**

None

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**Figure Legends**

Figure 1. Stress-stain curve for soft tissues. Schematic illustration of a typical nonlinear stress-strain curve for soft biological tissue showing the points at which the toe modulus, heel modulus, yield strength and ultimate yield strength are calculated.

Figure 2. Biomechanical ex-vivo test methods. Schematic illustration of deformation modes experienced in commonly used test methods to characterize mechanical properties of ocular tissues showing (a) uniaxial tension, (b) biaxial tension, (c) inflation and (d) AFM.

Figure 3. Retina and choroid. Schematic diagram of retinal layers, Bruch’s membrane and choroid.

1. \* Anisotropy: property of materials whose mechanical properties change in different directions (this is explained in the section “uniaxial testing”).

   Elasticity: property of materials able to resume their original shape and size after the removal of forces inducing their deformation.

   Hyperelasticity: property of materials for which the stress-strain relationship is not linear.

   Viscoelasticity: property of materials exhibiting both elastic and viscous behavior in response to deformation. [↑](#footnote-ref-1)