CLEAR - Anatomy and Physiology of the Anterior Eye

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Abbreviations

CALT	Conjunctival-associated lymphoid tissue
FCAT	Federative committee on anatomical terminology
IVCM	*In vivo* confocal microscopy
MHC	Major histocompatibility complex
OCT	Optical coherence tomography
TRP	Transient receptor potential

ABSTRACT

A key element of contact lens practice involves clinical evaluation of anterior eye health, including the cornea and limbus, conjunctiva and sclera, eyelids and eyelashes, lacrimal system and tear film. This report reviews the fundamental anatomy and physiology of these structures, including the vascular supply, venous drainage, lymphatic drainage, sensory innervation, physiology and function. This is the foundation for considering the potential interactions with, and effects of, contact lens wear on the anterior eye. This information is not consistently published as academic research and this report provides a synthesis from all available sources. With respect to terminology, the report aims to promote the consistent use of nomenclature in the field, and generally adopts anatomical terms recommended by the Federative Committee for Anatomical Terminology. Advanced techniques for the examination of the ocular surface are also discussed.

1. Introduction

A key element of contact lens practice involves clinical evaluation of anterior eye health, including the cornea and limbus, conjunctiva and sclera, eyelids and eyelashes, lacrimal system and tear film. This report reviews the fundamental anatomy and physiology of these structures, as a foundation for considering the potential interactions with, and effects of, contact lens wear on the anterior eye, in the companion Contact Lens Evidence-based Academic Report papers. This information is often not published as academic research and hence when this is the case, has been drawn from consensus around other sources.

To promote use of standardised, descriptive nomenclature within contact lens practice, this report generally uses terminology recommended by the Federative Committee on Anatomical Terminology (FCAT), and published in *Terminologia Anatomica* [1] (Table 1). An exception to this approach is use of the eponymous term 'meibomian gland', rather than the FCAT term of 'tarsal gland' (see Table 1); this decision was based on consenus at the CLEAR harmonisation stage, given that the 'meibomian gland' is relatively entrenched in the field, its output is termed 'meibum' and the 'lacrimal gland' is not named after its structural location. In addition, as tarsal only describes a portion of the eyelid, the conjunctiva covering the eyelid has been termed the 'palpebral conjunctiva' throughout this report. The eponymous terms for common anterior eye structures are summarised in Table 1; where judged useful for interpretation, the associated eponym has been provided in square parentheses in this report.

Table 1 - Summary of the Federative Committee on Anatomical Terminology (FCAT) descriptive anatomical nomenclature [1] and the relevant eponymous term for anterior eye structures used in this report

FCAT nomenclature	Eponym
Anterior limiting lamina	Bowman's layer / Bowman's membrane
Canal of Schlemm	Scleral venous sinus
Ciliary portion of orbicularis oculi*	Muscle of Riolan
Fascial sheath of the eyeball	Tenon's capsule

Fornix accessory lacrimal gland^	Accessory lacrimal gland of Krause
Gland of Moll	Ciliary gland
Gland of Zeis	Sebaceous gland
Palpebral accessory lacrimal gland^	Accessory lacrimal gland of Wolfring
Posterior limiting lamina	Descemet's membrane
Tarsal gland – *note, based upon consensus, CLEAR terminology remains Meibomian gland*	Meibomian gland
Tarsal muscle	Müller's muscle
Not listed	Palisades of Vogt

*Name proposed in Bergmanson (2020) [2]; this structure is not listed by FCAT terminology [1]; ^Only described as 'accessory lacrimal gland'; 'Lacrimal glands' and not listed in FCAT.

2. Cornea and limbus

2.1. Anatomy

2.1.1. Gross anatomy

The cornea forms the anterior aspect of the outer coat of the eye and is highly transparent. It is continuous with the opaque sclera, the other component of the outer coat. The transitional zone, where the cornea becomes sclera, is known as the limbus or corneoscleral junction.

The human cornea has the shape of a negative meniscus ophthalmic lens, with an average central thickness of 535 μm; it is approximately 100 μm thicker at its periphery [3]. The cornea appears slightly oval in shape when viewed at the slit lamp biomicroscope. A commonly quoted dimension is 11.7 mm horizontally x 10.6 mm vertically [4], although these measurements are based on work

not explicitly defining the anatomical criteria used [5]. A more recent *in vivo* study reported a larger average corneal size (12.9 x 11.6 mm), but again anatomical landmarks were not defined [6]. Clinically, the approach of measuring horizontal visible iris diameter, albeit imprecise for similar reasons, is widely used [7].

The cornea is traditionally considered to comprise five layers (Figure 1): (i) epithelium (approximately 50 µm); (ii) anterior limiting lamina [Bowman's membrane] (approximately 8 µm); (iii) stroma (approximately 470 µm); (iv) posterior limiting lamina [Descemet's membrane] (3 to 20 µm); and (vi) endothelium (3 to 5 µm, based on typical measures [2]). There remains contention [8,9] surrounding the potential existence of a postulated sixth corneal layer, Dua's layer [10], located between the stroma and posterior limiting lamina. The two posterior corneal layers are customarily presented as single values, although ranges are more accurate since their thickness changes throughout life. With age, the posterior limiting lamina becomes thicker and the endothelium thins [2].

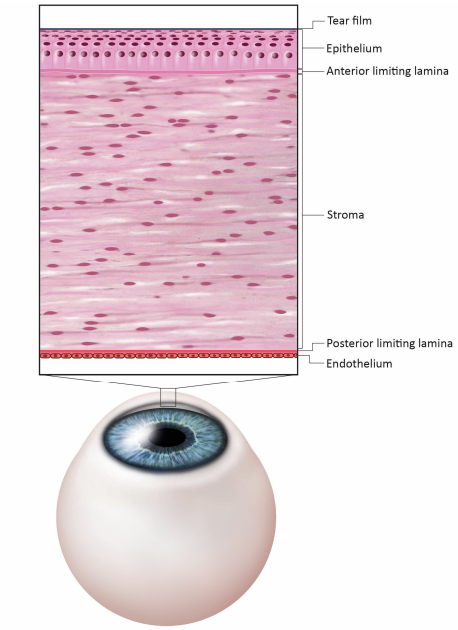

Figure 1 - Diagram of the corneal structure in transverse section. Copyright BCLA 2021.

The limbus measures about 1.5 to 2.0 mm in width, and is anatomically complex [11]. Anteriorly, it is the zone over which the corneal epithelium transitions into the bulbar conjunctival epithelium.

The limbus is defined by fibrovascular ridges that exist in a radial orientation, known as the Palisades of Vogt [12], interspaced by the limbal epithelial crypts that house the stem cells responsible for corneal epithelial regeneration [13,14].

2.1.2. Microscopic anatomy

2.1.2.1. Corneal epithelium

The corneal epithelium, derived from the surface ectoderm, is stratified with five to seven cellular layers, formed by basal, wing and squamous cells. These are not different categories of cells, but are the same type of epithelial cell captured in different life cycle phases. The cells are in a constant motion towards the surface and thus, older cells are located closest to the corneal surface. As the cells migrate towards the surface, they flatten and widen; the basal cells are tall and columnar (approximately 20x40 µm), whilst the thin surface squamous cells are much flatter (approximately 50x2 µm).

The corneal epithelium is non-keratinised and is internally limited by a basement membrane that is synthesised by the epithelium. Internally, the epithelium, with its basement membrane, faces the anterior limiting lamina [Bowman's membrane], while externally it forms part of the ocular surface and is bathed in tears. Peripherally, the corneal epithelium is continuous with the conjunctival epithelium via the limbal epithelium.

Key functions of the corneal epithelium are: (i) physical protection, as a resilient renewable surface that protects the deeper layers from insult; (ii) refraction, with its uniform structure supporting optical function and corneal transparency; (iii) radiation protection, providing ocular protection in the short wavelength band of the ultraviolet spectrum; (iv) tear stabilisation, with microvilli and plicae that help stabilise tears on the ocular surface; (v) barrier protection, via *zonula occludentes* (tight junctions) that prevent ocular entry of fluid and microorganisms; (vi) mucous production, in the form of glycocalyx (see Section 2.3.1.1).

The corneal epithelium is a compact structure with no intercellular spaces and interdigitations between cells. Along their circumference, the cellular surfaces are dotted with desmosomal cell junctions that function as 'spot weldings'. Basal cells, which internally have no neighbouring cells, adhere to the basement membrane through hemidesmosomes. The basement membrane (120 to 200 nm) is firmly attached to the underlying anterior limiting lamina primarily through Type VII collagen fibres [2]; this structural arrangement imparts substantial strength.

The epithelium undergoes constant renewal through mitosis, which it cannot accomplish by itself, but needs a peripheral influx of cells from the limbus and conjunctiva [15]. This steady flow of cells derives from stem cells in the limbal basal epithelium and Palisades of Vogt [15]. Stem cells are believed to be epithelial residents for life; if they are lost from trauma or disease, they are not replaced. The stem cells produce daughter cells or transient amplifying cells, which migrate into the cornea along the basement membrane [16]. The transient amplifying cells undergo mitosis a limited number of times, unlike the stem cells that have a lifelong capacity to reproduce, along their travel within the cornea. Mitosis is primarily observed in the basal layer, but may also occur in the wing cell layers [2]. Constant shedding of the surface squamous cells must be balanced by a supply of new cells. The concept of epithelial homeostasis is expressed by the X + Y = Z theory, which states that the mitosis of basal cells (X) together with a centripetal movement of cells (Y), equals the loss of cells from the surface (Z) [15].

The corneal epithelium is completely replenished approximately every 10 days [17]. At any time in the healthy corneal epithelium, about 4% of cells are mitotic [18], with approximately 1% of surface cells undergoing apoptosis [19]. Both cell renewal and programmed loss processes are complex physiological events that can easily be disturbed. For instance, contact lens wear can slow cell movement towards the surface and inhibit the number of cells desquamating [20,21] (see CLEAR Materials Report [22]). Furthermore, infected cells in eyes wearing contact lenses have a reduced rate of desquamation [23].

A variety of non-epithelial cells can also exist in the corneal epithelium, including:

(i) Putative dendritic cells (sometimes termed Langerhans cells in clinical studies), which have a dendritiform morphology and exist in the basal epithelium. Dendritic cells, also described in the context of the conjunctival epithelium in Section 3.1.1.2.1, are antigen-presenting cells that typically express Class II major histocompatibility complex (MHC II) antigens [24,25]. Dendritic cells occupy both the central and peripheral cornea, with a centripetally decreasing density under physiological conditions [26]. Changes to corneal dendritic cells, in particular density, have been described in a range of conditions, including dry eye disease [27], small fibre neuropathy [28] and herpetic keratitis [29].

(ii) Sensory nerves (Figure 2) - see Section 2.1.4. Nerve axons enter the corneal epithelium, shed their Schwann cells and reside as naked axons in the corneal epithelium [30]. Most epithelial axons travel in basal cell plasmalemmal infoldings, between the basal cell nuclei and basement

membrane [30]. Vertical projections from this neural plexus extend towards the ocular surface and, in these instances, the axons advance between the epithelial cells.

(iii) Leucocytes, which are not normally resident in the healthy corneal epithelium, reflect a response to corneal challenge.

2.1.2.2. Anterior limiting lamina [Bowman's membrane]

The anterior limiting lamina is modified stroma and is acellular except at locations where stromal nerve fibres penetrate the epithelium. The anterior limiting lamina is mesodermal in origin and formed by a dense, randomly oriented network of collagen Type I fibres. Delicate, Type VII collagen fibres, at the external aspect of the anterior limiting lamina attach the epithelial basement membrane to the underlying cornea [31,32]. At its external limit, the Type VII fibres fuse with the basement membrane, which itself is formed of Type IV collagen, and at the opposite extreme, the Type VII fibres are embedded in anchoring plaques [31,32].

The anterior limiting lamina terminates at the peripheral extreme of the cornea and is, for this reason, an anatomical landmark demarcating the corneal peripheral limit. Anteriorly, the anterior limiting lamina borders the epithelial basement membrane, and this interface is well defined. Posteriorly, the anterior limiting lamina faces the stroma, but due to shallow overlapping, up to 1 µm in each direction [33], the interface between these two layers is less distinct. *In vivo* confocal microscopy (IVCM) indicates that the anterior limiting lamina has a thickness of approximately 10.7 µm [34]. The anterior limiting lamina has no regenerative capacity, perhaps because of its acellularity. In radial keratotomy, the corneal incisions penetrate through the full thickness of the anterior limiting lamina and it has been noted that this layer remains severed several years after the surgery [35].

2.1.2.3. Corneal stroma

The stroma, sometimes called the *substantia propria*, forms the corneal bulk and imparts rigidity [36]. Peripherally, it is continuous across the limbus and into the sclera. Anteriorly, it borders the anterior limiting lamina, and posteriorly it interfaces with the posterior limiting lamina. The stroma has three components: (i) collagen Type I fibres; (ii) keratocytes, a connective tissue cell like fibrocytes; and (iii) matrix, a substance found around and between collagen fibres and keratocytes [37]. The anterior stroma also houses sensory nerve fibres.

2.1.2.3.1 Collagenous lamellae

The building blocks of the corneal stroma are collagenous lamellae, formed by bundles of Type I collagen fibres that run in parallel to one another and to the corneal surface, a feature essential for corneal transparency. The thickness of the lamellae typically varies from 2 to 3 µm, with thinner lamellae close to the anterior limiting lamina and thicker lamellae closer to the posterior limiting lamina [38]. Keratocytes are distributed primarily between the lamellae and are believed to have a tethering function, keeping the lamellae in their intended position [2].

Surprisingly little is known about the overall architecture of the lamellar layout. Anatomical literature generally describes lamellae as stretching from limbus to limbus [4,39]. In transverse section, the stroma has been reported to contain 242 ± 4 lamellae, but the distribution of lamellae in an anterior-posterior direction is not uniform [40]. As such, counts over a portion of the corneal thickness that are extrapolated for the whole cornea do not yield accurate estimates. The anterior 100 µm of the stroma has 50% more lamellae than the posterior 100 µm of the tissue [40]. Anteriorly, lamellae are thinner and intertwined, while posterior lamellae are thicker and laid down upon one another without distinct intertwining [9,40]. At this corneal depth, lamellae can be separated by blunt dissection, which underlies the surgical capacity for deep lamellar keratectomy.

2.1.2.3.2 Keratocytes

There are approximately 2.4 million keratocytes in the human cornea and these have a non-uniform distribution [41–43], with greatest density adjacent to the anterior limiting lamina [42]. Keratocytes are of mesodermal origin and are responsible for producing and organising the collagen fibres in lamellae during corneal development. They were generally believed to be quiet residents after accomplishing this task, but recent research has shown them to be rather active cells, structurally and physiologically, with many functional responsibilities; these include mediating intra-corneal communications, acting as an energy resource, contributing to matrix turnover, facilitating lamellar tethering, phagocytosis, as well as playing a role in wound healing [2,44–47].

Keratocytes are thin, flattened cells that form an intracorneal communicating network via gap junctions [45–47]. They store glycogen, which can be converted into glucose and used for energy. Keratocytes possess organelles, such as mitochondria, rough endoplasmic reticulum, centrioles and various vesicular formations, which support their cellular activities [45,47]. They are

considered important in matrix turnover and are active in wound healing and scar formation. In excimer laser procedures, the superficial and exposed cells are lost, but are gradually replaced by peripheral, activated cells.

Other cells, such as lymphocytes and neutrophils, may, at least transiently, be present in the corneal stroma [48].

2.1.2.4. Posterior limiting lamina [Descemet's membrane]

The posterior limiting lamina is the basement membrane (Type IV collagen) of the corneal endothelium and is the thickest such membrane in the body; its thickness increases throughout life by approximately 1 µm/year [49]. The thickness and elasticity of the posterior limiting lamina makes it relatively resilient to trauma and disease. In some individuals, the endothelium synthesises an excessive amount of basement membrane, which may be seen clinically as guttae [50]. This process is harmful to the endothelium and leads to endothelial cell loss, which can ultimately interfere with the cornea's ability to maintain normal hydration, resulting in corneal oedema; these are the features typical of Fuchs' endothelial dystrophy [51,52]. When this same endothelial excessive basement membrane synthesis occurs in the peripheral cornea, it is considered a normal age-related change, termed Hassall-Henle bodies [2].

2.1.2.5. Corneal endothelium

The corneal endothelium forms a single layer of squamous cells that have a mesenchymal origin. The full corneal span is covered by this layer. The point where the single layered endothelium begins to transform into the multi-layered trabecular meshwork marks the peripheral limit of the posterior cornea and the beginning of the limbus. Anteriorly, the endothelium borders the posterior limiting lamina, which it produces. In the healthy eye, the endothelium is strongly adherent to the posterior limiting lamina, but unlike the epithelium, it lacks hemidesmosomes to anchor it onto its basement membrane. Instead, it is believed that the continuous, healthy flow of basement membrane material from the cell to the posterior limiting lamina provides the necessary adhesion [2]. Posteriorly, the endothelium faces the anterior chamber.

In coronal section, or viewed using specular microscopy, corneal endothelial cells have a predominantly hexagonal shape [53]. The lateral walls of these cells show deep interdigitations, perhaps to increase the cell surface. In contrast, the anterior and posterior sides are strictly flat and parallel to each other and follow the plane of the cornea. The posterior side of the endothelium has microvilli, but these are not as plentiful as on the corneal surface epithelial cells. Along their

lateral sides, and towards their apical sides, the endothelium has junctional complexes, consisting of gap junctions, tight junctions and intermediate junctions. The tight junctions, or *zonula occludentes*, do not completely surround the cell and thereby create a so-called 'leaky membrane' [54]. The gap junctions facilitate intercellular communications, while the intermediate junctions (or the *zonula adherents*) function to hold the endothelial cells together [2]. Corneal endothelial cells are packed with mitochondria and rough endoplasmic reticulum, indicative of their metabolic activity to maintain corneal hydration.

Corneal endothelial cells cannot regenerate. Fortunately, the cornea is endowed with a healthy supply of cells at birth that permits continued corneal functionality despite progressive cell loss throughout life (Table 2). The natural, age-related endothelial cell decline is, on average, 0.6% per year [55], but can be accelerated by exposure to various factors, including trauma, surgery and ultraviolet radiation [53].

Table 2: Corneal endothelial cell density with age, from Bergmanson et al., (2020) [2].

Age / functional time point	**Corneal endothelial cells per mm^2**
Birth	5632 to 2987 (average: 4300)
20-30 years	3000 to 3500
40-50 years	2500 to 3000
80 years	2000 to 2500
Functional limit	500 to 1000

The human cornea is generally accepted to function sufficiently well with an endothelial cell density >1000 cells/mm^2; the indication for surgical intervention is the development of bullous oedema, which typically occurs at a level of 500 to 700 cells/mm^2 [56].

2.1.3. Vascular supply and lymphatic drainage

The cornea is avascular and does not possess lymphatic drainage, although this is subject to change due to disease and/or trauma.

2.1.4. Innervation

2.1.4.1. Source and distribution of the corneal nerves

The cornea is one of the most densely innervated tissues in the body, with an estimated 7000 nociceptors per square millimetre [57]. Its innervation derives primarily from the ophthalmic division of the trigeminal nerve, via the long and short ciliary nerves. There may also be a small degree of sympathetic corneal innervation, although this is less well established in the human cornea than in some animal species [58].

Approximately 60 sensory nerves arrive at the limbus and enter the cornea in the anterior half of the stroma, in a radial manner [59]. Many, if not most, fibres at this point are myelinated; within 1.5 mm of corneal penetration the myelin sheath is shed [30,60]. The nerve fibres progress into the cornea, while dividing and branching to form stromal and epithelial plexi (Figure 2). The epithelial plexus is formed by stromal fibres that cross the anterior limiting lamina. These anterior limiting lamina *rami perforans*, also termed corneal stromal-epithelial nerve penetration sites [61], occur predominantly peripherally, with approximately 185 such sites in the human cornea [62]. Once in the epithelium, the nerve axons shed their Schwann cells and turn anteriorly to innervate the apical aspect of the ocular surface [57]. Schwann cell function is supported by the epithelial cell [63]. This is considered to be the reason why, when epithelial metabolism is suppressed, as in the wear of low oxygen permeable or impermeable contact lenses, nerve function decreases [64].

Corneal epithelial nerve fibres travel primarily in infoldings of the basal cell basal plasmalemma [62,65]; this is also where most epithelial nerve endings are located. At intervals, the epithelial nerve fibres ascend between cells to the squamous cell layers, where they terminate at multiple levels within the corneal epithelium (Figure 2) [59,66]. The relative infrequency of these terminations underlies why manifest corneal staining or surface loss of cells, may not lead to symptoms. Epithelial nerve terminals undergo frequent remodelling, consistent with the continuous physiological renewal of epithelial cells [67]. The epithelial and stromal plexi respond to the same anteriorly presenting stimulus, with the stromal plexus serving as a second line of

defense [2]. If the cornea is denuded, the stromal plexus provides some somatosensory function, otherwise the cornea would become anaesthetised.

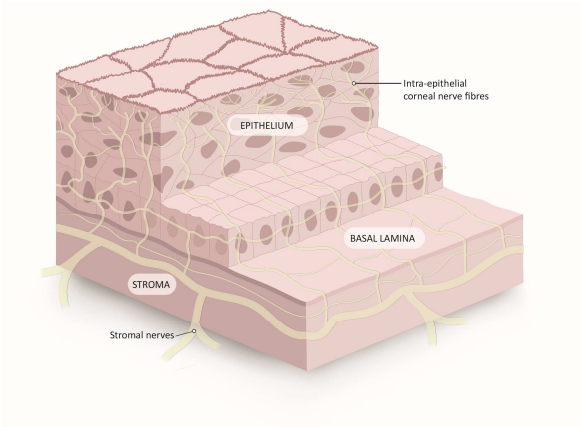

Figure 2 - Sensory innervation to the cornea. Stromal nerves penetrate the basal lamina and branch into leash-like assemblages of horizontally oriented fibres, called the intra-epithelial corneal nerve fibres [sometimes clinically defined as the sub-basal nerve plexus]. Adapted from Bergmanson (2020) [2]. Copyright BCLA 2021.

The corneal epithelial nerve plexus is often described in the clinical literature as the 'sub-basal nerve plexus' [68–70]. This is an unfortunate misnomer given the word 'sub' implies a structure beneath, or internal to, the basal cells, which is not anatomically accurate. Although in IVCM imaging, the corneal nerve fibres are observed between the keratocyte and basal cell nuclei (Figure 3), the acellular anterior limiting lamina, with the exception of an occasional *ramus perforans*, separates the two plexi into epithelial and stromal components; thus an accurate anatomical description for the nerves located within this epithelial plexus is 'intra-epithelial corneal basal nerves' [61].

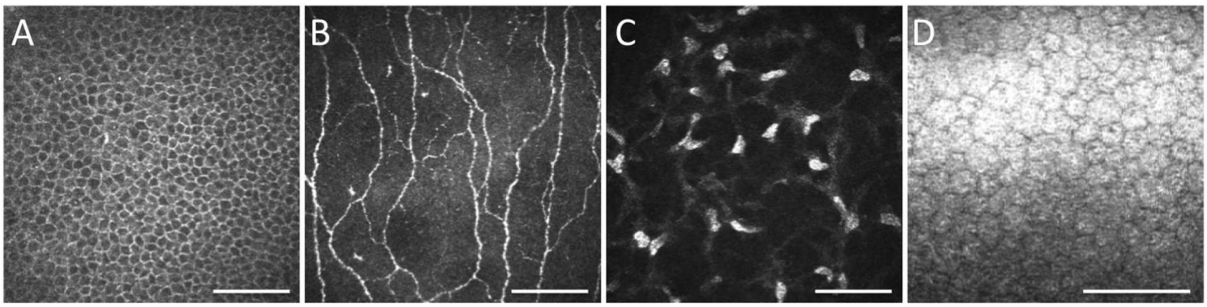

Figure 3 - Appearance of the central corneal layers using *in vivo* confocal microscopy (IVCM), showing cross-sectional images of the (A) epithelium, (B) intra-epithelial corneal basal nerves (also termed the 'sub-

basal nerve plexus' in the clinical literature), (C) stroma and (D) endothelium. Scale bars are 100 µm. Images for this figure were kindly supplied by Dr Stuti Misra, The University of Auckland, New Zealand.

The corneal 'inferior whorl' is an anatomical landmark, readily visualised using IVCM. Located approximately 2.5 mm infero-nasal to the corneal apex [59], it is identified by a clockwise spiralling of the intraepithelial corneal basal nerves [71] (Figure 3). It has been proposed to be a useful landmark for longitudinal corneal nerve evaluation [72], and may be a sensitive region for detecting early stages of corneal neuropathy [73].

2.1.4.2. Functions of the corneal nerves

Corneal sensory nerves have a major somatosensory role, but also mediate a range of physiological responses, including blinking, tear production, and maintenance of corneal health [74]. With respect to sensory function, based primarily on animal studies, three broad classes of corneal sensory fibres are recognised (Table 3): (i) polymodal nociceptors; (ii) cold thermoreceptors; and (iii) mechano-nociceptors [75,76]. Polymodal nociceptors are the most abundant. Comprising mostly unmyelinated C type fibres, polymodal nociceptors show a spectrum of responsiveness, including activity to mechanical, thermal and chemical stimuli, over a large range of intensities. The ion-channel transient receptor potential (TRP) sub-family V member 1 (TRPV1) is a molecular marker for polymodal nociceptors. Cold thermoreceptors account for 10 to 15% of corneal sensory nerves, and consist of A delta (Aδ) and C fibres [75]. These nociceptors detect changes in ocular surface temperature and tear osmolarity. Cold thermoreceptor responses are mediated by TRPM8 cation channel activity, which increase activity under conditions of tear hyperosmolarity [77]; these responses may underlie ocular discomfort responses in conditions characterised by elevated tear osmolarity, such as dry eye disease [78,79]. Mechano-nociceptors account for about 20 to 30% of the sensory nerves, and are responsive to mechanical forces applied to the corneal surface [80].

Table 3 - Summary of corneal sensory nerve sub-types, based on Belmonte et al. 2017 [75]

Receptor subtype	Main type of nerve fibre	Main ion channel type	Responsivity	Proportion of corneal nerves

Polymodal nociceptors	C fibres	TRPV1	- Heat - Chemical (e.g., low pH, carbon dioxide) - Mechanical	70%
Mechano-nociceptors	Aδ fibres	Piezo2	- Mechanical	20 to 30%
Cold thermoreceptors	Aδ and C fibres	TRPM8	- Small changes in ocular surface temperature - Tear osmolarity	10 to 15%

Corneal nerves have complex neurochemistry. They release multiple neuroactive agents, including substance P, neuropeptide Y, vasoactive intestinal peptide, calcitonin gene-related peptide and acetylcholine. Many of these neuromediators are trophic factors, involved with corneal healing and maintaining ocular surface health. Substance P is one of the key corneal neuropeptides; it is constitutively expressed in the tear film [81], and has an important role in corneal nerve regeneration and epithelial healing [82]. Substance P has also been implicated in mediating ocular surface pain [83]. Reduced substance P levels have been reported in the cornea and tears of older adults [84]. In addition to its expression on corneal nerves, the substance P receptor is expressed on some corneal immune cells, suggesting potential dual neuro- and immune-mediated involvement [85].

When corneal sensory nerves are stimulated, tear production is instigated by the trigeminal-parasympathetic reflex (see Section 6.2.2). Under physiological conditions, ocular surface sensory nerves provide basal input to the lacrimal nucleus. This input stimulates an efferent pathway, comprising sympathetic and parasympathetic fibres that innervate the lacrimal gland, resulting in basal tear secretion. Vigorous stimulation of the corneal sensory afferents (for example, due to foreign matter entering the eye) can lead to an up to 100-fold increase in tear flow [86]. Corneal sensory nerve endings thus rapidly respond to environmental perturbations to modulate tear secretion. Disruptions to these neural pathways can lead to a variety of ocular surface complications, including dry eye disease, neuropathic pain and neurotrophic keratitis [75].

2.2. Physiology and function

The cornea fulfills several important functions, including: (i) optical: transparency and a major refractive component, responsible for two-thirds of the approximately 60 D total ocular refraction (Table 4); and (ii) protection: physical protection to the intraocular components and filtering of damaging ultraviolet radiation.

Table 4 - Summary of typical corneal dimensions and measurements

Parameter	**Details**
Diameter	11.7 x 10.6 mm (horizontal x vertical) [4], reaching adult size by three years of age
Central thickness	Approximately 535 μm [87]
Radius of curvature	Average values [88]: (a) Anterior: 7.80 mm (43.25 D) (b) Posterior: 6.42 mm (52.50 D)
Eccentricity (E-value)	0.4 to 0.6 [89,90]
Front surface refractive power	Approximately 40 D
Refractive index	1.376, although this varies through the corneal depth: 1.400 at the epithelium, 1.380 at the anterior limiting lamina, 1.369 in the mid-stroma, and 1.373 at the endothelium [91]
Structural components	Water: 78% (bulk of the cornea) Collagen: 15% Other proteins: 5% Other: 2%

Attenuation of ultraviolet radiation	At 290 nm: approximately 100% absorption At 310 nm: approximately 50% absorption [92]

Housing the corneal epithelial stem cells, the limbus mediates physiological replacement of the corneal epithelial cells, and superficial wound healing. The limbus is also a physical barrier that ensures conjunctival cells do not proliferate into the corneal epithelium [13]. A rich vascular supply exists in the scleral portion of the limbus, transferring oxygen and nutrients to the peripheral cornea [93]. This region also houses components of the aqueous outflow system (i.e., the trabecular meshwork and scleral venous sinus [Canal of Schlemm]), essential to aqueous humour flow and debris removal.

2.2.1. Source of oxygen and nutrients

As an avascular tissue, the cornea derives its oxygen and nutrient supply from three main sources, the: (i) tear film; (ii) aqueous humour; and (iii) peri-limbal vasculature (as previously described). Oxygen consumption across the corneal sub-layers is approximately divided 2:2:1 over the epithelium, stroma and endothelium, respectively [94]. In open eye conditions, most of the corneal oxygen supply is from the atmosphere, accessed by diffusion through the tears. The partial pressure of oxygen, a measure of dissolved oxygen, is estimated to be 155 mmHg (or 21% volume per volume) in the tear film, and 20 to 30 mmHg (or 3 to 4% volume per volume) in the aqueous humour [2]. With eyelid closure, the partial pressure of oxygen in the tears reaches equilibrium with the palpebral vasculature, at approximately 55 mmHg [95]. A more dramatic reduction in this tear-derived oxygen supply, as may occur during contact lens wear, can result in corneal oedema [96] (see CLEAR Materials Report [22]). The aqueous humour supplies most of the glucose and essential amino acids required by the cornea [93].

2.2.2. Metabolism

Glucose, the main substrate for generating adenosine triphosphate (ATP), enters the corneal endothelium from the aqueous and is catabolised via three main pathways [97]: (i) the tricarboxylic acid (TCA), or Kreb's, cycle; (ii) glycolysis; and (iii) pentose phosphate shunt (also known as the hexose monophosphate shunt) (Figure 4). The TCA cycle is most active in the corneal endothelium, which possesses a large number of mitochondria, and relies on the availability of

oxygen to yield 36 molecules of ATP. Overall, anaerobic glycolysis is the major pathway for corneal glucose metabolism, accounting for 85% of its glucose consumption [97]. Once glucose enters the cornea posteriorly, it diffuses through the stroma; some is consumed by keratocytes, but most is used by the epithelium and undergoes glycolysis [97]. In the anaerobic glycolytic pathway, glucose is subsequently converted into lactate, with a net yield of two molecules of ATP. The pentose phosphate shunt is active in the corneal epithelium, producing intermediaries responsible for nucleic acid synthesis and preventing damage from oxygen free radicals [98].

In terms of metabolic waste, carbon dioxide readily diffuses out of the cornea [99]. Lactate diffuses through the endothelium into the anterior chamber under normoxic conditions [99]. Hypoxia results in an increase in anaerobic metabolism and the potential for lactic acidosis (lactic acid accumulation) [100], which causes movement of fluid into the cornea, seen clinically as corneal oedema [101].

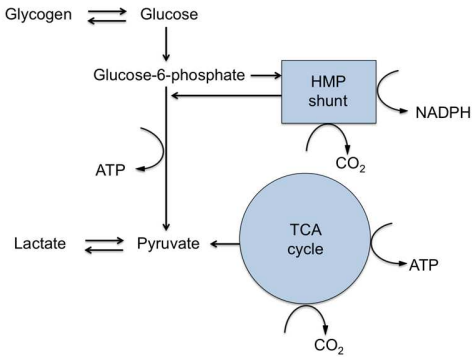

Figure 4 - Corneal metabolic pathways. HMP = hexose monophosphate shunt (also known as the pentose phosphate shunt); TCA, tricarboxylic acid (TCA) cycle; ATP = adenosine triphosphate; NADPH = nicotinamide adenine dinucleotide phosphate (reduced form). Based upon a figure from Lawrenson, 2010 [99].

2.2.3. Hydration

Maintenance of physiological hydration is essential to corneal transparency. With the cornea being 78% water by weight, the cornea is relatively dehydrated and inclined to take up water, causing a swelling pressure of approximately 60 mmHg in a healthy cornea [2]. Corneal tissue

will swell to many times its thickness, but only in an axial direction since collagen fibres are unable to stretch [102]. The negative imbibition pressure is the difference between the intraocular pressure and the swelling pressure, which promotes aqueous humour flow into the cornea. Both the epithelium and endothelium contribute to the maintenance of corneal hydration. Tight junctions between the superficial epithelial cells create a permeability barrier to ions and polar solutes [103]. Active ion transport systems for Na+ and Cl- also contribute to the tonicity of the tear film [104,105]. Since the corneal endothelial *zonula occludentes* do not encircle the entire cell, there is a steady influx of water (aqueous) into the cornea [54]. As the cornea is avascular, this aqueous flow into the cornea is essential for nutrition, but fluid is constantly removed simultaneously. This physiological process is driven by a metabolically active ion pump (Figure 5). As bicarbonate is formed within the corneal endothelial cell and released into the anterior chamber, it promotes the movement of water. Metabolic inhibitors and low temperatures will suppress this process, leading to excess fluid accumulation in the cornea, clinically observed as corneal stromal oedema [101]. Contact lenses with low oxygen permeability may also inhibit these corneal metabolic processes, leading to corneal oedema [106] (see CLEAR Complications Report [107]).

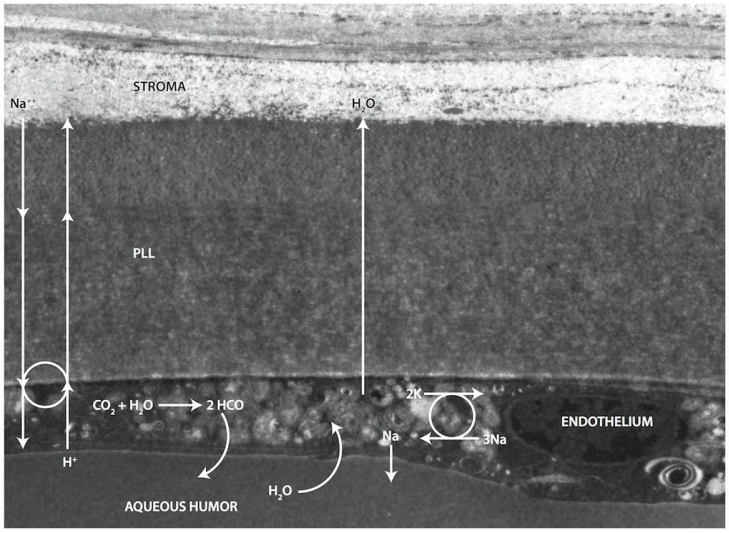

Figure 5 - Model of the key biochemical interactions between the corneal endothelium and stroma, and anterior chamber. Reproduced with permission from Bergmanson, 2020 [2]. Abbreviations: CO_2, carbon dioxide; H_2O, water; HCO, bicarbonate; K, potassium; NA, sodium; PLL, posterior limiting lamina.

With eyelid closure during sleep, access to atmospheric oxygen is interrupted, but a fraction of the open- eye oxygen supply is provided by the vascular palpebral conjunctival circulation. However, approximately 4% corneal swelling exists immediately upon waking; in healthy eyes, corneal thickness will return to baseline levels within about one hour [108].

2.2.4. Response to injury

The corneal response to injury varies by layer. The epithelium, a constantly renewing layer, is most able to achieve full recovery. If the epithelium is abraded by a blunt injury or over-wear of a low oxygen permeable contact lens, the injury may not involve the basement membrane. The epithelium is tightly packed and held together by desmosomes (see Section 2.1.2.1); the weakest point is the tall columnar basal cells, which rupture internally to their nuclei leaving cytoplasmic fragments attached to the basement membrane [109]. Immediately after injury, neutrophils are recruited from the tears [110]. The repair phase involves an inhibition of mitosis and rapid migration of epithelial cells to cover the affected area. If the basement membrane is unharmed, cells can form hemidesmosomes within two days but trauma to this membrane delays healing by several days [110].

The stroma has some capacity to heal, primarily by forming scar tissue, which can adversely affect vision. Stromal scarring involves keratocytes and activated cells recruited to the affected area [111]. Observations of radial keratotomy corneas, years after surgery, reveal that severed lamellae never heal or re-attach, meaning the cornea is biomechanically weakened [35].

Neither the anterior nor posterior limiting lamina have regenerative capacity. The mesenchymal endothelial cells also do not multiply after birth. However, in response to the steady, age-related decline in cell density, and also to injury, endothelial cells can stretch to occupy areas previously covered by missing cells; this explains the thinning of the endothelium with age [2].

2.3. Clinical assessment of the cornea and limbus

A range of clinical instruments are available to assess corneal and limbal structure and function (see supplementary appendix and CLEAR Evidence-Based Practice Report [7]).

3. Conjunctiva and Sclera

3.1 Conjunctiva

The conjunctiva is a thin, translucent mucous membrane, consisting of a superficial epithelium overlying a loose connective tissue called the *lamina propria* or stroma [112–114]. The conjunctiva develops from the ocular surface ectoderm [115]. It covers the anterior region of the eyeball, extending from the limbus to the eyelid fornices and then back to the mucocutaneous junction. Key functions of the conjunctiva include immune surveillance [116], production of tear constituents [117], and acting as a physical barrier to limit entry of foreign bodies and pathogens to deeper ocular tissues.

3.1.1. Anatomy

3.1.1.1. Gross anatomy

The conjunctival epithelium is continuous with the corneal epithelium at the limbus, and with the skin at the mucocutaneous junction of the eyelid margin [112–114]. The tissue reflects from the anterior portion of the sclera, at the superior and inferior fornices, onto the tarsal surface of the eyelids, forming the conjunctival sac. The conjunctiva is traditionally considered to have three main regions: bulbar, forniceal and palpebral (Figure 6); the marginal conjunctiva is a fourth region of clinical relevance to contact lens wear [118,119] (see Section 3.1.1.2.3).

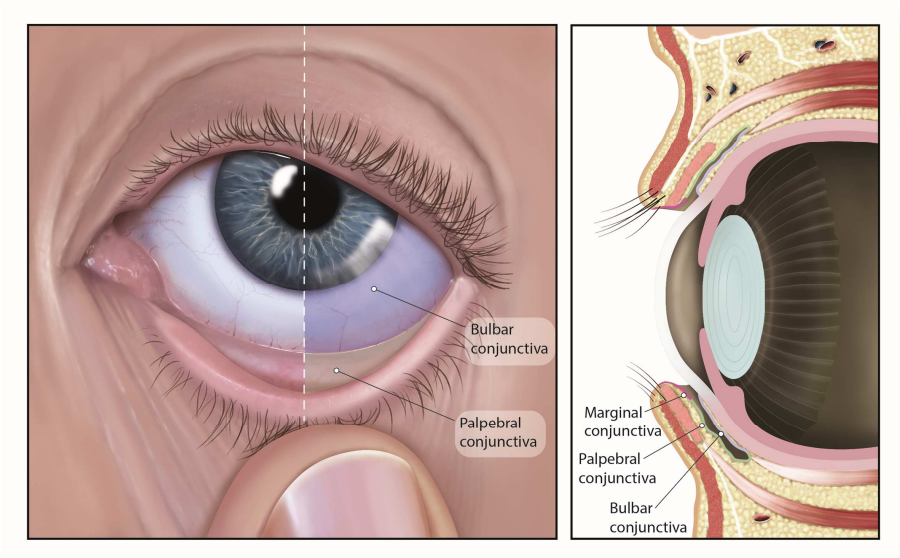

Figure 6 - Conjunctival regional anatomy, based upon a figure in Azari and Barney, 2013 [120]. Copyright BCLA 2021.

The bulbar conjunctiva covers the anterior sclera and the extraocular muscle insertions. The conjunctiva is tightly bound to the globe near the limbus, but further from the limbus there is a loose episcleral tissue layer within which the pericorneal vascular plexus lies [11]. As the bulbar conjunctiva is transparent, the underlying conjunctival and episcleral vascular supplies are visible. The forniceal conjunctiva is subdivided into superior and inferior regions, which are continuous at the medial and lateral canthi, forming a circular *cul de sac* [121]. The forniceal conjunctiva loosely attaches to the eyelid levator palpebrae superioris and rectus muscles. Within the medial fornix, there is a specialised region known as the semilunar fold (or *plica semilunaris*), which has a presumed role in allowing unrestricted lateral eye movements; this structure is also rich in goblet cells and interstitial immunocompetent cells [122]. The caruncle (or *caruncula lacrimalis*) is located medially; it consists of modified skin with a highly vascular node with accessory lacrimal and sebaceous gland tissues [123]. The palpebral conjunctiva lines the inner surface of the eyelids. It is subdivided into marginal, tarsal and orbital regions. A subepithelial connective tissue tightly binds the palpebral conjunctiva to the tarsal plate. The lacrimal punta comprise small openings in the palpebral conjunctiva, at the medial corners of the upper and lower eyelid margins; these structures support tear drainage [124]. A small subtarsal sulcus, positioned in proximity to the eyelid margin assists with trapping and clearing foreign matter.

3.1.1.2 Microscopic anatomy

Histologically, the conjunctiva comprises a surface layer of non-keratinised stratified squamous epithelium overlying vascular stroma composed of loose connective tissue [122].

3.1.1.2.1 Conjunctival epithelium

Conjunctival epithelial structure varies according to anatomical location. Closer to the eyelid margin, the conjunctiva is composed of a stratified, non-keratinised epithelium. The bulbar conjunctiva consists of stratified columnar epithelium. Two main cell types exist in the conjunctival epithelium: epithelial cells and goblet cells [125]. The epithelial cells arrange in several layers, organised into basal, intermediate and superficial cells. These epithelial layers are interspersed with goblet cells in non-keratinised regions that vary in density according to location (see Section

3.1.1.2.3 and Figure 7), along with blood vessels, fibrous tissue, lymphatic channels, melanocytes, resident immune cells and accessory lacrimal glands [126,127].

The conjunctival epithelium is self-renewing and has rapid turnover. The location of the conjunctival stem cell population is controversial, but there is evidence this population resides in the forniceal [128,129] and/or bulbar conjunctiva [125] at both the limbus and the mucocutaneous junction [130],[131]. Stem cell markers are expressed throughout the conjunctiva, with highest levels in the medial canthal and inferior forniceal areas [132].

Conjunctival epithelial cells are joined mechanically by desmosomes and are interconnected by intercellular junctions at their apical aspect. These features act against ocular surface shear stresses and as a barrier to the external environment [133]. Alterations to these intercellular junctions compromise conjunctival integrity and are associated with ocular surface disease [134]. Conjunctival epithelial cells have transmembrane water channels, called aquaporins, which mediate water transport between the conjunctiva and aqueous phase of the tear film [135]. Intercellular spaces between conjunctival epithelial cells have a role in the maintenance of water transport across the tissue [136].

Superficial membrane-spanning mucins, principal components of the glycocalyx, are an integral component of the membrane mucins produced by conjunctival epithelial cells [137]. They have a prominent role in defining interactions between the surface epithelium and overlying tear film and also form a barrier to prevent adhesion of pathogens, debris and other cells to the epithelium [138]. The three major membrane-associated mucins expressed in human conjunctival epithelia are MUC1, MUC4, and MUC16 [139]; these expressions have been verified at mRNA and protein levels [137,140–143]. Conjunctival epithelial cells also produce functional proteins, such as lubricin [144], keratin 7 [145], claudin 10 [146] and trefoil factor family peptides [147]. Secretory mucins are primarily produced by goblet cells (see Section 3.1.1.2.3 and Section 6.1.2.2).

3.1.1.2.2. Conjunctival stroma

The conjunctival stroma, also known as the *substantia propria*, consists of loose connective tissue that resembles the *lamina propria* of other mucous membranes. The stroma has two principal layers: an outer adenoid layer and inner fibrous layer [148]. The adenoid layer contains lymphocytes, predominantly B cells, which exist in aggregations as lymphoid follicles and are a key component of the local mucosal lymphoid tissue, known as conjunctiva-associated lymphoid

tissue (CALT) [149]. CALT has a pivotal role in initiating and regulating ocular surface immune responses, including as a primary defense against microbes and mediating immune tolerance [149]. MHC Class II positive dendritic cells have been detected in CALT follicles and are distributed throughout the conjunctiva, including the epithelium. The adenoid layer also houses a large population of mast cells, involved in ocular allergic responses [148]. The deeper fibrous layer, which is thicker than the adenoid layer, contains blood vessels, nerves and the forniceal accessory lacrimal glands [126,127]. The rich vascular network within this layer receives blood from the anterior ciliary arteries [126,127].

3.1.1.2.3. Goblet cells

Goblet cells are specialised apocrine cells in the conjunctival epithelium; their density increases from the superior temporal to the inferior nasal region of the conjunctival sac [150] (Figure 7). Goblet cells are larger than conjunctival epithelial cells. Their cytoplasm is densely packed with membrane-bound secretory granules containing MUC5AC that are discharged from the apical surface in an apocrine manner [151]. The lid wiper region of the eyelid margin, which interacts with the ocular surface during blinking, has goblet cells within mucus crypts that are similar to those found in the palpebral conjunctiva [152]. The anatomy of the lid wiper region has been thoroughly characterised [112–114] (see Section 4.1.2.2).

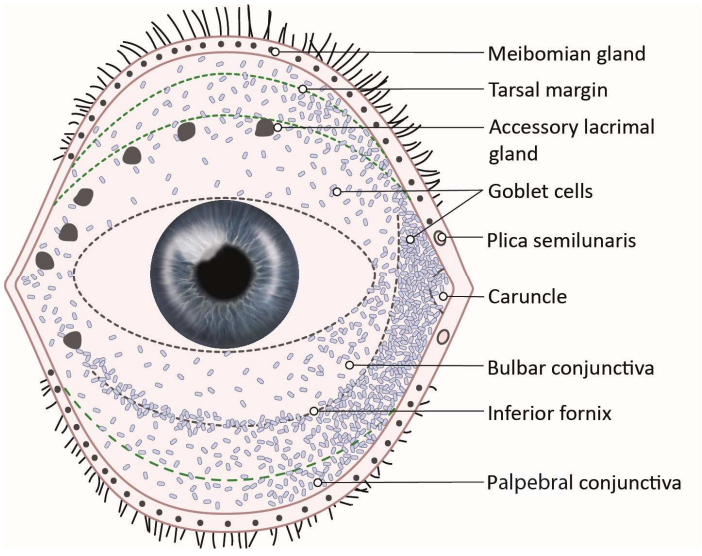

Figure 7 - Regional variation in human goblet cell density, based upon Kessing, 1968 [150], from Gipson, 2016 [153]. Copyright BCLA 2021.

3.1.1.3. Conjunctival vascular supply

The conjunctiva is richly vascularised. The bulbar conjunctiva obtains its blood supply primarily from the anterior ciliary arteries and peripheral tarsal arcades of the eyelids [154]. Vascular supply to the forniceal conjunctiva mirrors that of the bulbar tissue [154]. The palpebral conjunctiva has a dual blood supply, with the main source from the dorsal, nasal, frontal, supraorbital, and lacrimal arteries (terminal branches of the ophthalmic artery) and a supplementary supply from the facial, superficial, temporal, and infraorbital branches of the facial artery [155].

3.1.1.4. Conjunctival venous drainage

The anterior ciliary veins and peripheral conjunctival veins form the conjunctival venous drainage system that leads to the venous plexus of the eyelids, and then later connects to the superior and inferior ophthalmic veins [154]. Venous drainage of the palpebral conjunctiva occurs through post-tarsal veins of the eyelids, deep facial branches of the anterior facial vein, and pterygoid plexus.

3.1.1.5. Conjunctival lymphatic drainage

The conjunctival tissue possesses an extensive lymphatic network [156]. Lymphatic drainage from the nasal bulbar conjunctiva joins the submandibular nodes, while lymphatics from the temporal bulbar conjunctiva drain into preauricular nodes [154]. The medial lymphatics of the palpebral conjunctiva drain into the submandibular lymph nodes via eyelid lymphatics, while the lateral lymphatics drain into the preauricular nodes. The lymphatics of the forniceal conjunctiva are similar to those of the bulbar conjunctiva [154].

3.1.1.6. Conjunctival innervation

The conjunctiva has both sensory and autonomic neural innervation (Figure 8). Sensory innervation to the conjunctiva derives from the first or ophthalmic branch of the trigeminal nerve. The superior conjunctiva is supplied by the supraorbital nerve, supratrochlear nerve, infratrochlear nerve, whereas the inferior conjunctiva is innervated by the infraorbital nerve [157]. The lacrimal nerve, with contribution from the zygomaticofacial nerve, supplies the lateral part of the conjunctiva. In addition, parasympathetic and sympathetic nerve fibres supply the conjunctival tissue [158], which project free nerve endings into blood vessels, the epithelium surrounding goblet cells and into the squamous epithelial cells. Parasympathetic and sympathetic nerves,

along with muscarinic α_{1A}- and β -adrenergic receptors, have been reported to exist on mouse and human conjunctival goblet cells, suggesting these nerves have a role in regulating goblet cell secretion [159].

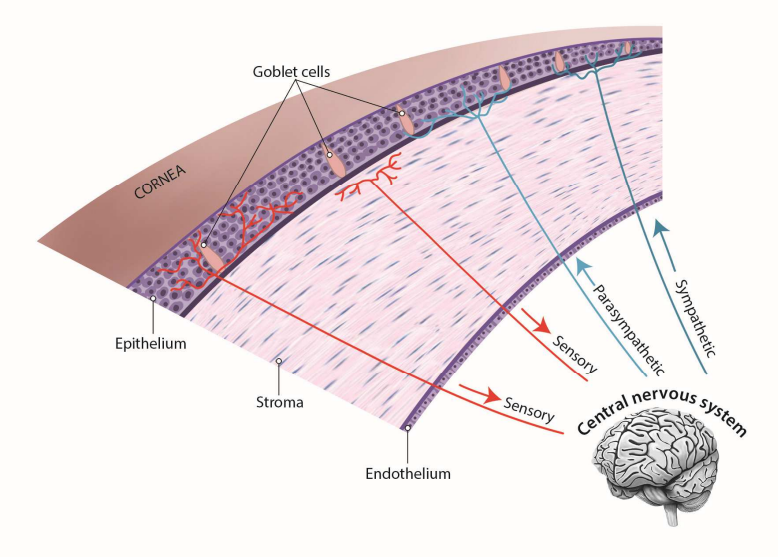

Figure 8 - Schematic representation of conjunctival neural innervation. Goblet cells are surrounded by autonomic nerves, whereas sensory nerves only innervate the stratified squamous cells. The cornea is innervated with sensory nerves, which can activate a neural reflex to stimulate conjunctival goblet cell secretion via parasympathetic nerves releasing acetylcholine (Ach) and vasoactive intestinal peptide (VIP). Copyright BCLA 2021.

3.1.2. Conjunctival physiology and function

The principal functions of the conjunctiva include acting as a physical barrier to pathogens and facilitating mucin secretion [150,160] (see Section 6.1.2.2). Conjunctival tissue is immunologically active, equipped with immune components such as toll-like receptors that recognise pathogens through pathogen associated molecular patterns. Dendritic cells can initiate and modulate ocular surface immune responses. Often described as 'immune sentinels', dendritic cells present antigen to adaptive immune cells through pathways involving lymph nodes and/or the CALT (see Section

3.1.1.2.2). The main humoural mediator of the secretory immune response is secretory IgA, produced by transformed B cells (plasma cells); secretory IgA protects the mucosa through multiple actions, including preventing microbial binding [161,162]. Endothelial venules, specialised vessels that facilitate lymphoid cell migration, along with lymphocytes, lymphoid follicles, IgA-positive plasma cells and their associated transporter molecules, further support the functions of the CALT [149,162,163].

The conjunctiva, in particular the bulbar region, harbours a large population of resident lymphocytes [164]. The most common cell type is CD3+ T cells, of which the majority are CD45Ro+ (memory) T cells [164]. Additional conjunctival T-lymphocyte populations include CD8+ and CD4+ cells [164–166]. B cells, macrophages, plasma cells, natural killer cells and mast cells have been described in the healthy conjunctival epithelium and/or stroma [149]. Melanocytes and stem cells also contribute to conjunctival immunological defense [149].

Consistent with its denser sensory innervation [30,66,167,168], the cornea has higher sensitivity than the conjunctiva [169–172]. Nevertheless, a positive correlation has been described between corneal and conjunctival sensitivities [173]. The marginal conjunctiva has been reported to have greater mechanical sensitivity than other regions, such as the palpebral conjunctiva [174,175] and bulbar conjunctiva [170,175]. No difference has been observed in the sensitivity of the marginal conjunctiva between the superior and inferior eyelids [175]. This increased sensitivity of the marginal conjunctiva may be a factor involved in ocular comfort, especially during contact lens wear [175]. Although regional variations in sensory innervation have not been comprehensively investigated, light and electron microscopy have shown free, non-specialised nerve endings in the eyelid margin area [176], which may explain greater sensitivity in this region. Soft contact lens wear has been shown to have the capacity to affect inferior conjunctival sensitivity to air [169] and chemical [177] stimuli; these effects are influenced by the contact lens material (see CLEAR Materials Report [22] and CLEAR Complications Report [107]).

3.2 Sclera

3.2 Anatomy

3.2.1 Gross and microscopy anatomy

The opaque sclera covers most of the surface of the globe, ranging in thickness from 0.3 mm immediately posterior to the insertion of the rectus muscles, up to 1.0 mm near the optic nerve [178]. From the limited literature describing scleral anatomy, it has been reported that the sclera is composed of collagen bundles that are approximately 64 nm in length, and branched fibrils that are randomly arranged and vary in diameter from 20 to 30 nm [178]. The sclera has three main layers, which from outermost to innermost, are the: (i) episclera, consisting of fibroblasts, proteoglycans, melanocytes and glycoproteins, interlaced within the loosely arranged thin collagen bundles; (ii) stroma, with elastic fibres and occasional melanocytes with relatively thicker collagen bundles; and (iii) lamina fusca, made of shorter collagen bundles and an increased number of elastic fibres, situated adjacent to the uvea [178]. This latter region appears brownish in colour due to large numbers of melanocytes. The lamina fusca also has a groove to allow the passage of ciliary vessels and nerves. The space between the lamina fusca and choroid (perichoroidal space) contains thin collagen fibres that provide weak attachment to the sclera. The scleral and episcleral layers connect to the fascial sheath of the eyeball [Tenon's capsule], between the conjunctival and episcleral plexi [179]. The scleral shell has two openings, the anterior scleral foramen that surrounds the cornea, and the posterior scleral foramen that surrounds the optic nerve.

3.2.2 Scleral vascular supply

The episclera has a rich vascular supply from the anterior ciliary arteries at its connection to the fascial sheath of the eyeball; the posterior ciliary arteries supply blood to the equator and posterior episclera [179]. These vascular networks, along with the choroidal blood vessels, supply capillary beds in the scleral stroma. The venous system in the limbus collects blood from the conjunctival and limbal venous arcades and drains into the episcleral-collecting veins that collect blood from the anterior episcleral veins and scleral veins. These veins run posteriorly to form the anterior ciliary veins to exit the anterior surface of the eye [179].

3.2.3 Scleral sensory innervation

The short ciliary nerves supply the posterior region of the sclera, while long ciliary nerves supply the anterior portion [180]; the ciliary nerves perforate the sclera around the optic nerve. Due to the profuse sensory innervation of the sclera, inflammation of this region causes severe pain and may interfere with eye movements due to the location of the extraocular muscle insertions [180].

3.3 Scleral physiology and function

A major function of the sclera is to protect the intraocular contents from traumatic injury and mechanical displacement; the tissue also provides a rigid insertion point for the extraocular muscles. The sclera has poor distensibility and water binding capacity [181], as a result of the structural arrangement of collagen bundles that provide both a degree of rigidity and flexibility [182,183]. The posterior sclera is more distensible than the anterior sclera, owing to its higher water-holding capacity. Scleral distensibility decreases with age [184].

3.1.3. Clinical assessment of the conjunctiva and sclera

A range of clinical instruments are available to assess scleral structure and function (see supplementary appendix and CLEAR Evidence-Based Practice Report [7]).

4. Eyelids and eyelashes

4.1. Anatomy

4.1.1. Gross

Anatomically, the superior and inferior eyelids can be divided into two parts, or 'lamellae', separated by the anatomical grey line [185] (Figures 9 and 10); this distinction is also useful for describing eyelid disease. The relatively soft, anterior lamella includes the skin, eyelashes (cilia) and the orbicularis oculi muscle. The stiffer posterior lamella includes the fibrous tarsal plate (housing the meibomian glands) and the palpebral conjunctiva. The striated orbicularis oculi muscle surrounds each orbital orifice and the palpebral part of the muscle extends into each eyelid [186]. This muscle, together with the levator palpebrae superioris, is responsible for eyelid movements.

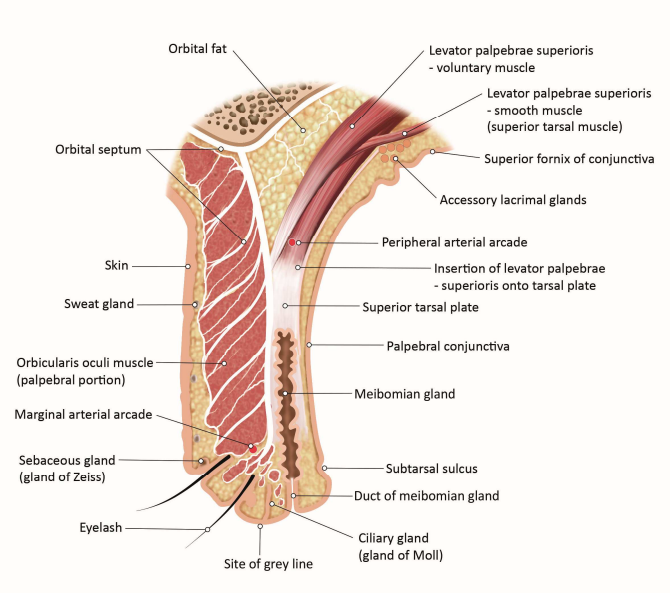

Figure 9 - Structure of the superior eyelid, as seen in cross-section, based on Snell and Lemp (2013) [187]. Copyright BCLA 2021.

The crescent-shaped superior tarsus measures approximately 21 mm x 8 mm [188] and is larger than its inferior counterpart. The lateral and medial extremities of the eyelids join together to form the inner and outer canthi. The inner canthus houses the caruncle, which contains both sebaceous and sweat glands, and is bordered on its lateral side by the *plica semilunaris*. The free portions of the eyelids are known as the 'occlusal' surfaces and extend horizontally between the canthi.

4.1.2. Microscopic anatomy of the eyelid margin

The eyelid margins comprise three zones: an anterior (outer) and posterior (inner) border, and the free eyelid margin area (in between) that extends from the cilia to the meibomian gland orifices [112]. The anterior cutaneous border is rounded, whilst the posterior conjunctival border is relatively sharper. The latter is responsible for maintaining lubrication of the anterior structures by continuous re-formation and distribution of the tear film with blinking [118,152].

4.1.2.1. Mucocutaneous junction [line of Marx]

The eyelid margin (Figure 10) transitions through different types of epithelia from its anterior to posterior zones. From the anterior border, the stratified squamous epidermis of the skin travels posteriorly past the cilia and the meibomian gland orifices, where it stops abruptly. It is then replaced by a zone of continuous, squamous parakeratinised epithelial cells that transition into a zone of discontinuous, squamous parakeratinised cells [112]. Parakeratinised cells are distinct from the anuclear (flattened) 'squames' at the surface of the skin (*stratum corneum*) in that they are partially keratinised and have a dense cytoplasm that still contains a nucleus. The continuous and discontinuous parakeratinised zones together make up the mucocutaneous junction (line of Marx), which is approximately 0.2 to 0.3 mm in width [112]. The entire mucocutaneous junction is kept moist by the presence of the tear film meniscus.

4.1.2.2 Lid wiper

The discontinuous, squamous zone of parakeratinised cells of the mucocutaneous junction transitions into a stratified cuboidal, and partly columnar, epithelium that contains interspersed squamous parakeratinised cells and goblet cells [112]. This epithelium represents the initial part of the conjunctiva, and is not part of the mucocutaneous junction. An epithelial 'lip' or elevation of approximately 100 µm in thickness is observed in this region; it has been reported to contain up to 15 cell layers and protrude towards the ocular surface [152]. This apposing surface is thought to 'wipe' the surface of the cornea and, for this reason, is termed the 'lid wiper' [189]. Further evidence for this function was provided by the finding that the area contains single, as well as clusters of, goblet cells both at the surface and deep within the epithelium [152]. These cells produce the gel-forming secretory mucin MUC5AC, effectively providing the lid wiper with its own in-built lubrication system that is integral to reducing frictional forces between the eyelid and ocular surface [152].

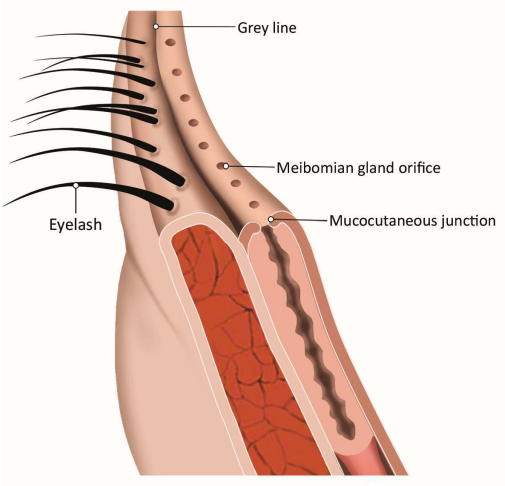

Figure 10 - Schematic of the eyelid margin. Copyright BCLA 2021.

From the thickened crest, the lid wiper tapers towards the conjunctiva at the sub-tarsal fold over a distance of 0.3 to 1.5 mm [112]. Both the mucocutaneous junction and the lid wiper extend from the medial to the lateral side of the eyelid margins, but the lid wiper is wider in the nasal and temporal regions compared to the centre [112]. The blood vessels underneath the superficial layers of the lid wiper region are more superficial than those at the mucocutaneous junction and immune cells have also been observed in this area [190,191].

4.1.2.3 Eyelid neuroanatomy

The human eyelid has a complex pattern of sensory innervation; the follicles surrounding the cilia in the anterior lamella are innervated by four types of nerve endings: lanceolate, circular Ruffini nerve terminals, free nerve endings and Merkel cells [176]. The free lid margin area contains dermal and epidermal free nerve endings and Meissner corpuscles. The posterior lamella, including the mucocutaneous junction and lid wiper areas, contain simple and Meissner corpuscles [176]. Mechano-nociceptors, such as Merkel's disks and Meissner corpuscles, respond to hair movement and light touch, respectively, but are rare in the eyelid, occurring around the cilia roots and being more prevalent in the central compared to the outer eyelid margin [192]. The eyelid margin is more sensitive than other conjunctival areas, but less sensitive than the cornea [175,193].

4.1.3. Eyelid muscles

The orbicularis oculi muscle (Figure 11) is divided into three sub-parts: orbital, palpebral and lacrimal [122]. The palpebral portion is further divided into the preseptal, pretarsal and ciliary (marginal) portions [186]. The striated fibres of the palpebral part of the orbicularis oculi muscle are arranged concentrically around the orbital orifice [194]. In the superior eyelid, the tendon of the levator palpebrae superioris passes through this muscle before inserting into the underside of the skin [195]. The levator palpebrae superioris joins to the superior tarsus by a smooth muscle called the superior tarsal muscle [Müller's muscle], which originates on the underside of the levator palpebrae superioris [196]. In the inferior eyelid, the lower tarsus is attached to a prolongation of the inferior rectus muscle, called the inferior tarsal muscle. The portions of the orbicularis oculi that extend almost to the occlusal surface and envelop the cilia and meibomian glands are given the eponymous term 'muscle of Riolan' [186].

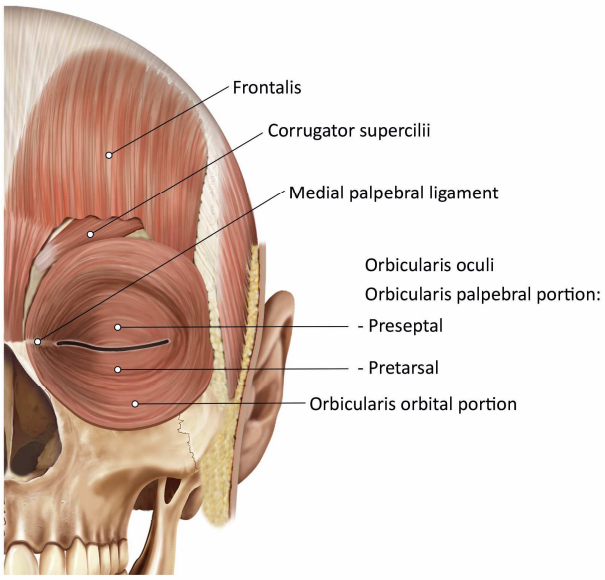

Figure 11: Different regions of the orbicularis oculi muscle. Copyright BCLA 2021.

4.1.4. Eyelid vascular supply

The vascular system of the superior eyelid has three arcades: the preseptal, supratarsal and marginal arcades [195,197–199]. The marginal arcade runs along the free eyelid margin, the

supratarsal arcade lies on the tarsal plate above the marginal arcade and the preseptal arcade runs along the orbital septum. This dense vascular network is formed by anastomoses arising from the internal and external carotid arteries. The internal carotid artery contributes via branches of the ophthalmic artery (supraorbital artery, supratrochlear artery, lacrimal artery, and the medial palpebral artery) and the external carotid artery contributes via branches of the facial (angular artery) and the superficial temporal artery [197]. The three arcades communicate via a network of vertical branches forming an anastomotic network [197,200].

Similar to the superior eyelid, the single inferior marginal arcade in the inferior eyelid is formed by anastomoses originating from the internal and external carotid arteries [200,201]. Arteries originating from the external carotid (superficial temporal, zygomatico-orbital, facial, and infraorbital arteries) anastomose with those from the internal carotid (lacrimal, dorsal nasal, and ophthalmic arteries).

4.1.5. Eyelid venous drainage

In contrast to veins elsewhere in the body, orbital veins, including those of the eyelids, do not generally accompany the arteries [202,203]. Venous drainage occurs via the pre- and post-tarsal veins. In front of the tarsus on the lateral side, blood drains into the superficial temporal vein and the lacrimal vein; medially, blood drains to the angular and ophthalmic veins [204]. Behind the tarsal plates, blood drains into the orbital veins and the deeper branches of the anterior facial vein and pterygoid plexus.

4.1.6. Eyelid lymphatic drainage

Eyelid lymphatics are arranged in pre- and post-tarsal plexi. Traditionally, lymphatics serving the lateral side of both eyelids were considered to drain into the superficial preauricular (parotid) nodes whilst those serving the medial side were thought to drain into the submandibular lymph nodes. However, more recently, studies have shown that the preauricular basin is likely to be the prime site for eyelid lymphatic drainage [205,206].

4.1.7. Eyelid innervation

The eyelids are innervated by both sensory and motor systems. The sensory nerves (Figure 12) derive from the trigeminal nerve, which has three main subdivisions: ophthalmic, maxillary and mandibular. The ophthalmic division conveys sensory information to different regions of the

superior eyelid via the supraorbital, supratrochlear, infratrochlear, and lacrimal branches [207]. The maxillary division supplies the inferior eyelid via the single infraorbital branch. The number of nerve projections reaching the eyelids is considerably less than the number supplied via the naso-ciliary branch of the ophthalmic nerve to the cornea and limbus [75].

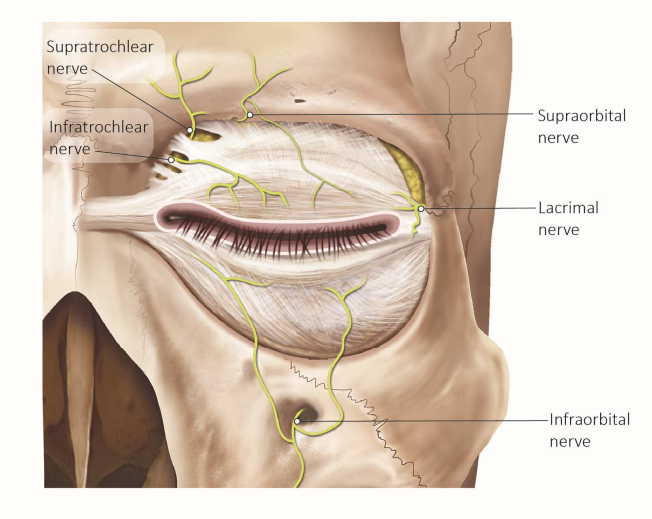

Figure 12 - Sensory innervation to the eyelids. Copyright BCLA 2021.

Eyelid motor innervation stems from the facial nerve that innervates the striated orbicularis oculi muscle [208]. The superior division of the oculomotor nerve innervates the levator palpebrae superioris, whilst the nonstriated muscles of the superior and inferior eyelids (superior and inferior tarsal muscles) have both sympathetic and parasympathetic innervation [122]. Damage to the muscle or the sympathetic nerves that supply these nonstriated muscles can cause superior eyelid ptosis, as observed in Horner's syndrome.

4.1.7. Eyelid glands

The eyelids contain various secretory glands that each have specialised functions.

4.1.7.1. Meibomian glands

The exocrine eyelid glands (or 'tarsal glands' according to the FCAT terminology, see Table 1) are typically referred to as 'meibomian glands', after the 17th-century German anatomist Heinrich

Meibom who published the first detailed description and drawings of these glands. They are responsible for producing the lipid-rich, oily meibum of the tear film that helps retard evaporation [209]. There are approximately 25 to 40, and 20 to 30, individual meibomian glands in the superior and inferior eyelids, respectively, which span the vertical height of each tarsus (Figure 13) [210]. The glands in the superior eyelid consequently occupy a larger volume than those of the inferior eyelid (Figure 14) [211,212].

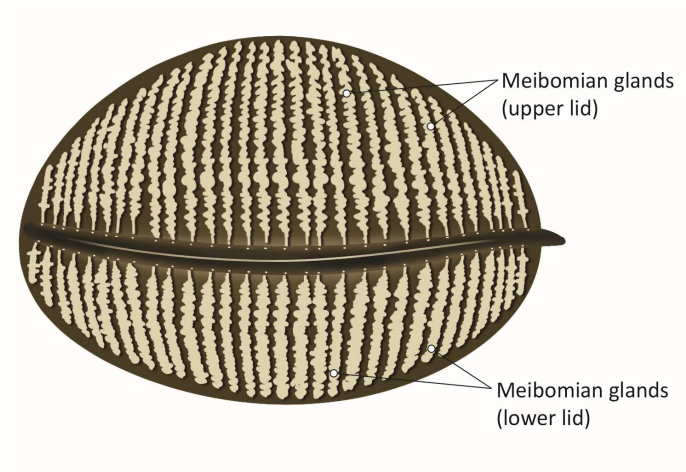

Figure 13: Configuration of the meibomian glands. Copyright BCLA 2021.

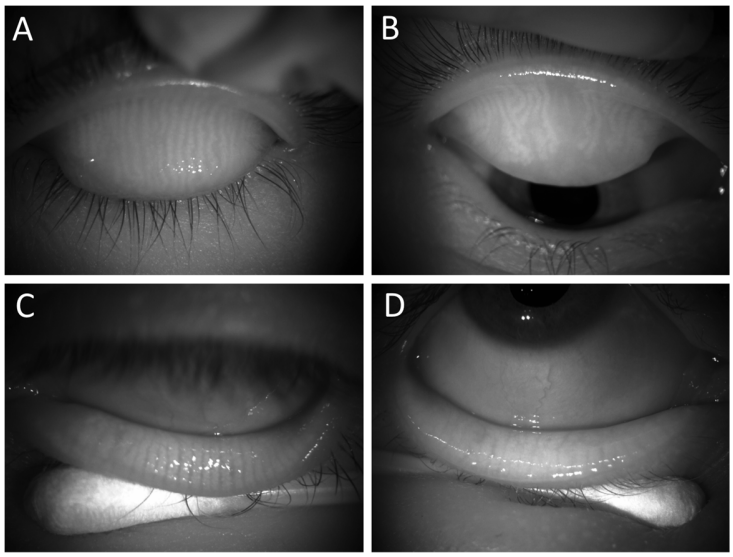

Figure 14: Infrared meibography of the superior (A&B) and inferior (C&D) eyelids, in an individual with minimal meibomian gland dropout (A&C), and in another individual with regional atrophy of the meibomian glands (B&D).

Each meibomian gland consists of a main central canal (or duct) that opens onto the free eyelid margin just anterior to the mucocutaneous junction. The main duct divides into a number of smaller 'ductules' that each terminates in one, or more, roundish acini (sacs). These acini are grouped together in clusters within an individual gland. The ductules and the central duct are lined by a four-layer stratified squamous epithelium [213], however only the epithelium lining the terminal part of the main central duct (close to the orifice) is fully keratinised, showing both a stratum corneum and stratum granulosum [214]. Each acinus is lined by a basement membrane and is filled with secretory meibocytes that move towards the ductule as they mature and eventually disintegrate, discharging their contents into it, in a 'holocrine' fashion. The area that encircles the gland orifices, the ciliary portion of the orbicularis oculi [muscle of Riolan] contains striated fibres [157,186].

4.1.7.2. Ciliary glands

Two types of glands exist in the ciliary region of both eyelids: the sebaceous gland [of Zeis] and ciliary gland [of Moll]. The sebaceous gland is an exocrine gland that, like the meibomian gland, has a holocrine secretion method. The gland consists of a few alveoli [215], which attaches directly to the eyelash follicle and produces an oily sebum whose exact function is not well defined [127]. The sebum may condition the eyelashes or maintain the hair follicles; it is unlikely to contribute significantly to tear composition.

The ciliary gland is a specialised apocrine gland (i.e., secretes its contents through membrane 'budding' or 'pinching-off') whose meandering ducts also empty into the eyelash follicle [215–217]. An individual gland is composed of two parts: a proximal coiled glandular (secretory) portion and the ductal portion. Its secretions contain antimicrobial peptides that may be important for defense against microorganisms at the eyelid margin [216,217].

4.1.7.3. Lacrimal accessory glands

The accessory lacrimal glands are divided into two anatomical groups: fornix [Krause] and palpebral [Wolfring]. These glands have similar morphologies, but differ in their eyelid locations [218]. These serous glands secrete a similar water component to the main lacrimal gland, containing electrolytes, immunoglobulins, and antimicrobial proteins [127]. The fornix accessory lacrimal glands [of Krause] are more numerous in the superior than inferior eyelid. The palpebral

accessory lacrimal glands [of Wolfring] are situated close to the superior border of the tarsal plate and are larger than the forniceal glands [127,219]. The accessory lacrimal glands lack true acini and have been categorised as tubular glands [219]. Each gland 'nodule' has its own excretory duct that opens onto the palpebral conjunctiva and is surrounded by myoepithelial cells and nerve fibres, mainly of parasympathetic origin [220].

4.2. Eyelid physiology

4.2.1. Functions of the eyelids

The eyelids: i) protect the anterior ocular surface from injury and other external factors (e.g., debris, pathogens); ii) distribute the tear film and provide the eye with an optically-smooth refractive surface; iii) provide a 'static-dam' for the tear film that prevents it from spilling onto the cheeks; and iv) assist with controlling the amount of light that enters the eye.

4.2.2. Eyelid movements

Three different types of blink are recognised: spontaneous, reflex, and voluntary. During blinking, the globe is displaced posteriorly by approximately 1.0 mm [221] and rotates upwards (Bell's phenomenon).

4.2.1.1 Blinking

A spontaneous blink is an unconscious blink in the absence of an evident stimulus, whilst a reflex blink is an involuntary protective mechanism that occurs in response to a stimulus [222]. The 200 to 300 ms rhythmic opening and closing of the eyelids during spontaneous blinking distributes the tear film over the ocular surface and provides a pumping mechanism to facilitate aqueous tear flow through the lacrimal drainage apparatus. The blink mechanism can be subdivided into a rapid closing phase and a slower opening phase. The palpebral portion of the orbicularis oculi muscle moves the eyelid down with concurrent inhibition of the activity of the levator palpebrae superioris and eyelid retractors. The levator palpebrae superioris, together with the tarsal muscle [Müller's muscle], returns the eyelid to an elevated position [223]. As the globe retracts during the closing phase, the lid wiper is thought to lift away from the ocular surface [224]. Whilst movement of the superior eyelid is predominantly vertical, the inferior eyelid (which moves lesser distance) has a mainly horizontal and inward movement [221,225].

Spontaneous blinking is thought to be triggered by ocular surface stimulation and particularly by enhanced corneal cold thermoreceptor activity that may produce a sensation akin to irritation during tear break-up or evaporation [226]. These cold thermoreceptors detect small changes in temperature and tear osmolarity.

4.2.1.2 Tear distribution and spreading of the lipid layer

Meibum migrates posteriorly from the meibomian gland orifices on the free eyelid margin, and is drawn into the inferior tear meniscus where it is swept across the ocular surface during the upstroke of each blink. The crest of the lid wiper is likely to present a softer 'rubbing' surface than that of the mucocutaneous junction as the cells are more loosely arranged compared with a squamous epithelium [112]. This arrangement, together with in-built lubrication from the gel-forming mucins, reduces the potential for friction between the two ocular surfaces [152]. The lid wiper action hypothesis is strengthened further by the observation that sub-dermal papillae, which 'reinforce' the tissue, are found only in the lid wiper region of the posterior margin [112]. The lid wiper forces the tear film downward during eyelid closure, causing the viscoelastic lipid film to compress [227]. On eye opening, the lipid layer undergoes expansion that in turn, draws the underlying tear film upwards by capillary action causing the superficial lipid layer to drift upwards over the aqueous phase and reform in between each blink [227]. The rate at which the lipid layer spreads is much slower than the speed of the blink.

4.2.1.3 Tear pump mechanism

The major forces involved in tear drainage are the blink and gravity [228,229]. When the eye is open, the puncta are open and covered by tear fluid. During the initial phase of eyelid closure, the puncta and the canaliculi become compressed through contact between the superior and inferior eyelid margins and also by the effects of contraction of the palpebral orbicularis oculi. As the eyelid reaches its maximum downward position, the preseptal orbicularis (which inserts into the lacrimal sac), pulls the lacrimal sac open and draws tears from the canaliculi into the sac. As the eyelid retracts upwards, the puncta open and the canaliculi refill. Simultaneously, the lacrimal sac collapses, moving tears into the nasolacrimal duct [230].

4.2.3. Eyelid gland physiology

4.2.3.1. Meibomian glands

The main function of the meibomian glands is to produce meibum, the lipid-rich sebum that has a key role in maintaining an optically smooth surface over the cornea and in retarding tear evaporation. Using advanced mass spectroscopy techniques, more than 17 lipid classes (polar and non-polar), with hundreds of individual molecular species, have been identified in the human tear film lipidome [231], which interact with contact lenses (see CLEAR Biochemistry Report [232]).

The meibocytes constantly produce meibum in the acini. This drives the meibum along the ductules towards the main central duct and ultimately to the orifice on the free eyelid margin. Compression of the meibomian glands during blinking, along with contribution from the ciliary portion of the orbicularis oculi [muscle of Riolan] around the orifices, results in a small amount of the lipid-containing meibum being squeezed onto the eyelid margin; this is then incorporated into the tear film lipid layer. In this way, a constant flow of these lipids enters the tears [210].

4.2.3.2. Lacrimal accessory glands

The accessory lacrimal glands can be thought of as ‘mini’ lacrimal glands, and may contribute a significant portion of the tear fluid [233]. This is further supported by estimates that the lacrimal accessory glands comprise about 10% of the total lacrimal tissue, and are innervated similarly to the main gland [215,220].

4.3. Clinical assessment of the eyelid and eyelashes

A range of clinical instruments are available to assess eyelid and eyelash structure and function (see supplementary appendix and CLEAR Evidence-Based Practice Report [7]).

5. Lacrimal system

The lacrimal system comprises three major divisions (Figure 15). The first is the secretory component, consisting of the exocrine tubuloalveolar serous lacrimal gland and two types of accessory lacrimal glands [Krause and Wolfring] (see Section 4.1.7), which contribute the aqueous components of the tear film. Distribution is the second component, consisting of the tear meniscus and blinking. The third component is the system that mediates the drainage of these secretions, which includes the puncta, lacrimal canaliculi, lacrimal sac, and nasolacrimal duct.

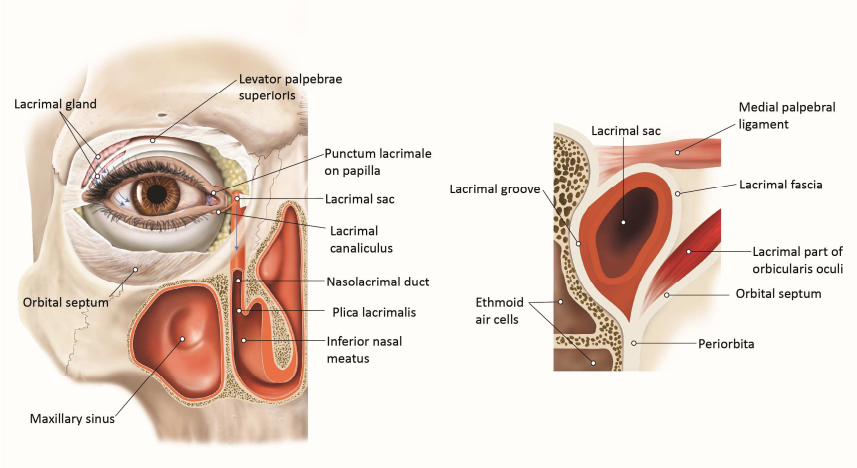

Figure 15 - A: Tear fluid secretion from the lacrimal gland and its distribution across the anterior surface of the eye before draining into the nose. B: Cross-section of the lacrimal sac and associated drainage structures, based on a figure by Snell and Lemp, 2013 [187]. Copyright BCLA 2021.

5.1. Lacrimal gland

5.1.1. Anatomy

5.1.1.1. Gross

The almond-shaped lacrimal gland is a bi-lobed exocrine gland of approximately 20 x 12 x 5 mm in size that is anatomically similar to mammary and salivary glands. The lacrimal gland is located in the lateral portion of the roof of the orbit, at the lacrimal gland fossa of the frontal bone. The lacrimal gland is often regarded as a continuation of the epithelial cells of the superior temporal conjunctiva that do not mature until about six weeks after birth [234]. The larger orbital and smaller palpebral lobes are separated anatomically by the lateral horn of the levator aponeurosis of the palpebral superioris muscle, with the orbital lobe situated superior to the palpebral lobe.

5.1.1.2. Microscopic

The lacrimal gland does not have an encapsulated structure, but is a collection of lobules of secretory tissue positioned in orbital fat [235]. The individual lobules are separated by loose connective tissue [235]. In cross-section, the smallest units of secretory lacrimal glands, known as acini, appear as ring-like structures. Acini comprise approximately 80% of the total mass of the lacrimal gland and are essentially composed of serous secretory cells that resemble grape-like structures on a platform of basal myoepithelial cells [236]. Acinar cells are polarised secretory cells that only secrete in one direction; this is achieved by a ring-like tight junction that surrounds the cells, with a lumen at the centre [237]. Acinar cells have prominent endoplasmic reticulum and Golgi apparatus, with the organelles and nucleus situated at the basal portion, and the major protein-containing secretory granules located towards the apical area [237].

Cuboidal cells are arranged in single- or dual-layers of the excretory lacrimal glands ducts. Similar to the acinar cells, these cells contain luminal tight junctions to create polarisation. These cells secrete primarily water, electrolytes and a limited number of proteins, which form the bulk of the tear film. The myoepithelial cells surround the acinar and ductal cells, to help maintain glandular structure and shape. They have multiple processes on their basal side with smooth muscle actin, which are involved in the contraction of acinar and ductal cells to facilitate the secretory process of the lacrimal gland [237]. Myoepithelial cells facilitate transport of secretions, via receptors for various neurotransmitters that stimulate protein secretions from acinar cells [238,239]. These cells

are connected by terminal interlobular ductules, leading to excretory ducts. Up to six secretory ductules join the ducts from the palpebral lobe to form six to 12 tubules that drain accumulated tear secretions into the superior fornix (Figure 16).

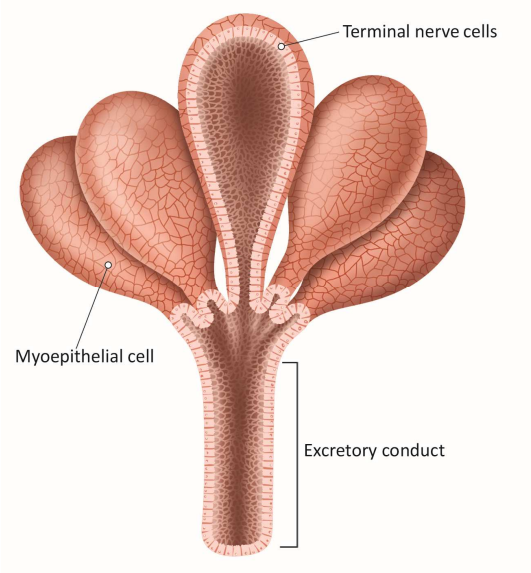

Figure 16: Microscopic structure of the lacrimal gland. Copyright BCLA 2021.

5.1.1.3. Lacrimal gland vascular supply and lymphatics

Blood supply to the lacrimal gland is provided by the lacrimal artery, a division of the ophthalmic artery, a branch of the infraorbital artery [240]. The lacrimal artery arises close to the optic foramen and is one of the longest branches derived from the ophthalmic artery. The lacrimal artery is accompanied by the lacrimal nerve along the upper border of the lateral rectus. Before supplying the lacrimal gland, the lacrimal artery gives off one or two zygomatic branches. After supplying the lacrimal gland, the lacrimal artery branches to supply the eyelids and conjunctiva; two of these branches are substantial and are called the lateral palpebral arteries. Venous drainage from the lacrimal gland follows a similar infraorbital course to that of the artery, and drains to the superior ophthalmic vein [241].

The lymphatic drainage of the lacrimal gland is believed to occur through the preauricular and submandibular lymph nodes [242]. However, this is still an area of ongoing research and debate [243].

5.1.1.4. Lacrimal gland innervation

Multiple highly coordinated neural mechanisms are involved in the secretion of components from the lacrimal gland. A small change in the relative proportions of the aqueous tear constituents has been correlated with dry eye disease [244] and contact lens discomfort [245] (see CLEAR Materials Report [22]). The lacrimal gland has both sensory and autonomic innervation [246]. Sensory nerves derive from the lacrimal nerve, a branch of the ophthalmic division of the maxillary nerve, which arises from the trigeminal nerve [246]. The facial nerve supplies parasympathetic secretory fibres to the lacrimal gland. The postganglionic cervical sympathetic fibres derive from the superior cervical ganglion to ultimately reach the lacrimal gland via the zygomaticotemporal nerve. A communicating branch to the lacrimal nerve from the zygomaticotemporal nerve contributes both sympathetic and parasympathetic innervation. Other sympathetic fibers may pass from the carotid plexus to the trigeminal nerve and to the lacrimal nerve.

5.1.2. Lacrimal gland physiology and function

The lacrimal gland synthesises and secretes tear components, including hundreds of proteins, peptides and electrolytes [247]. The lacrimal gland has a pivotal role in forming and maintaining the tear film [244] (see Section 6). Excretions from the lacrimal acinar glands can be influenced by various factors, including hormones [248]. This is particularly important for contact lens wearers as excessive protein content from acinar cells in the tears can increase tear debris, leading to decreased visual clarity and increased contact lens deposits [249] (see CLEAR Materials Report [22]). Various hormones are known to influence the secretion of acinar cells; a detailed summary has been previously published [250].

5.2. Lacrimal drainage apparatus

5.2.1. Anatomy

Tear fluid secreted by the lacrimal and accessory glands drains via the lacrimal drainage apparatus [213] (Figure 15). The lacrimal excretory pathway starts with an approximately 0.3 mm opening on the medial portion of each of the superior and inferior eyelids, called the punctum. The excretory apparatus comprises a membranous lacrimal drainage system that runs through a bony passage. The bony passage is formed by the lacrimal bone superiorly, the inferior turbinate

bone inferiorly and the maxillary bone temporally [213]. The lacrimal drainage system comprises three major parts: the lacrimal canaliculi, lacrimal sac and nasolacrimal duct.

5.2.1.1 Lacrimal canaliculi

The superior and inferior punctal openings each widen into a roughly 2 mm long channel, perpendicular to the eyelid margins which then makes a sharp turn, in a nasal direction, to form an 8 to 10 mm long canaliculus. The superior and inferior lacrimal canaliculi are essentially 0.5 to 1.0 mm wide tear drainage pipes, lined with stratified squamous epithelium. Both canaliculi then bend approximately 118° to form the common canaliculus. In more than 90% cases, the superior and the inferior canaliculi merge forming the common canaliculus, but for the rest they drain completely separately to the lacrimal sac [246]. The common canaliculus enters the sac at an angle of approximately 58°, often appearing as a valve-like structure that prevents back-flow of tears from the lacrimal sac [251].

5.2.1.2 Lacrimal sac

The lacrimal sac is the area where tears, drained from the lacrimal canaliculi, accumulate prior to drainage. The sac measures a length of 12 to 15 mm vertically and 4 to 8 mm antero-posteriorly, and rests in the lacrimal sac fossa, where the central portion adheres to the periosteal lining of the fossa. The lacrimal sac is essentially the upper part of the nasolacrimal duct, lined by nonciliated columnar epithelium [252].

5.2.1.3 Nasolacrimal duct

The nasolacrimal duct, which is about 18 mm in length, is a continuation of the lacrimal sac; it is lined with two columnar epithelial layers, including some ciliated cells. The nasolacrimal duct connects with the inferior meatus of the nose; this drainage point has a mucous membrane flap, the *plica lacrimalis* [Valve of Hasner], which acts as a valve to prevent air from entering the sac.

5.2.2. Lacrimal physiology and function

The mechanism of lacrimal drainage relies on combined functionality of the lacrimal canaliculus, lacrimal sac and nasolacrimal duct. The lateral four-fifths of both lacrimal canaliculi are encircled by the palpebral part of the orbicularis oculi muscle, which contracts during eye closure leading to closure of the associated portion of the canaliculi. This action triggers posterior traction on, and

opening of, the nasal one-fifth portion of the lacrimal canaliculi, drawing tears along each canaliculus, in a temporal to nasal direction, ultimately reaching the lacrimal sac [253]. This mechanism of aspiration is dependent on the capillary action and negative pressure built up within the canalicular lumen. The drainage mechanism in the lacrimal sac is similar to the canaliculi, where traction secondary to Horner's muscle contraction causes the sac to expand [254]. During the opening of an eye, the palpebral part of the orbicularis oculi muscle [Horner's muscle] relaxes and the lacrimal sac shrinks. This is a vital component of the pumping mechanism that is coordinated with blinking to ensure unidirectional tear drainage. Although the nasolacrimal duct does not perform active lacrimal drainage, it contributes by making the drainage process smoother, including facilitating tear resorption through the large area of stratified columnar epithelium and microvilli of the ductal surface. The remaining (unabsorbed) tears drain into the nasal cavity.

5.3. Clinical assessment of the lacrimal system

A range of clinical instruments are available to assess the lacrimal system (see supplementary appendix and CLEAR Evidence-Based Practice Report [7]).

6. Tear film

6.1. Anatomy

The tear film is a protective fluid layer, containing lipids, proteins, and electrolytes, which overlies the surface of the eye [247]. The ocular surface and tear film are intimately related; a loss of homeostasis in either component can trigger a vicious circle of dry eye disease, a self-perpetuating cycle of tear instability, tear hyperosmolarity, ocular surface inflammation and epithelial damage [255]. Contact lens wear is recognised as a cause of homeostatic imbalance, therefore optimising the tear film and ocular surface prior to, and during, contact lens wear is essential [256,257].

6.1.1. Macro-structure

The tear film is approximately 3 to 4 µm thick [258], and, facilitated by blinking, is replenished at a rate of around 16% per minute [259].

6.1.1.1. Models of tear film structure

The traditional, simplistic concept of a three-layered tear film model [260,261], has been superseded by contemporary appreciation of a more complex, ordered tear film structure comprising a superficial thin lipid layer overlying a thicker aqueous-mucin phase, which in turn overlies the glycocalyx (membrane bound mucins) that is tightly bound to the ocular surface epithelium [247,262] (Figure 17).

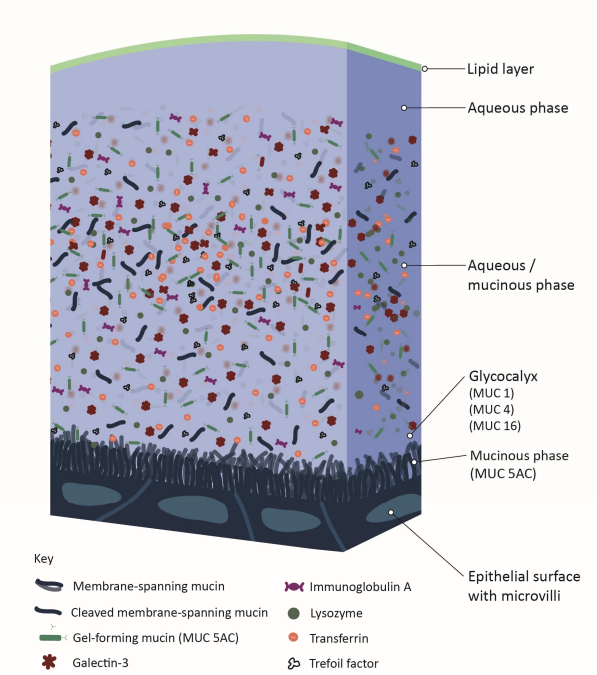

Figure 17: Current conceptualisation of tear film structure. Copyright BCLA 2021.

The lipid layer is approximately 40 nm thick, ranging from 15 to 157 nm [263], with a possible duplex structure [264]; a thin polar layer, interfacing with the hydrophilic aqueous-mucin phase, and a thicker, hydrophobic non-polar layer on the surface that forms the air-tear film boundary [265]. The lipid layer arises predominantly from the meibomian glands embedded in the superior

and inferior eyelids that release meibum from their orifices at the eyelid margin [210] (see Section 4.1.7).

The aqueous-mucin phase makes up the majority of the tear volume. The primary source of aqueous fluid is the main lacrimal gland [266,267] (see Section 5.1), located in the superotemporal quadrant of the orbit, supplemented by secretions from the accessory lacrimal glands (see Section 4.1.7) and ocular surface epithelial cells [268]. Stimulated (reflex) tearing can increase tear volume to around 100-fold that of unstimulated (basal) tears [269], with any volume exceeding the capacity of the lacrimal drainage system, spilling over the eyelid margins. Soluble, gel-forming mucins, produced by conjunctival goblet cells, also contribute to this phase (see Section 3.1.1.2.1). These mucins are found throughout this phase of the tears, increasing in concentration towards the ocular surface, where they interface with the thin layer of apical membrane-bound mucins that form the glycocalyx [270].

6.1.2. Components and biochemistry

Tear composition can be defined according to its proteome (proteins and protein fragments), lipidome (lipids), mucins (soluble and transmembrane), electrolytes, and other components.

6.1.2.1. Lipids

Meibum, which forms the bulk of the tear lipid layer, is a unique secretion delivered by meibocytes from within the meibomian glands [210] (see Section 4.2.3.1). Important functions of the lipid layer are to inhibit aqueous tear evaporation [209] and to reduce tear surface tension to encourage its retention and spread across the ocular surface [247]. Meibum contains a diverse range of long-chain lipids, made up largely of neutral lipids, including non-polar wax esters, cholesteryl esters, free cholesterol and triacylglycerols, and a lesser proportion of polar lipids [271]. Polar components, some of which may arise from sources other than the meibomian glands [272], include free fatty acids, phospholipids, ceramides, sphingomyelins, as well as long chain (O-acyl)-ω-hydroxy fatty acids, which are believed to play a stabilising role at the interface between the lipid and aqueous tear components [273].

6.1.2.2. Mucins

Two forms of mucins are present in the tear film; soluble, gel-forming mucins secreted by the conjunctival goblet cells (see Section 3.1.1.2.3) and transmembrane mucins produced by, and

anchored to (although can be released from), the apical surfaces of corneal and conjunctival epithelial cells [274] (see Section 3.1.1.2.1). Gel-forming mucins within the aqueous-mucin phase of the tears help lubricate the ocular surface by reducing friction during blinking, and protect the ocular surface cells by entrapping debris for safe passage to the caruncle for removal [138,275]. Heavy glycosylation of these high molecular weight proteins allows them to bind water, which serves to protect the ocular surface against desiccation. MUC5AC is the principal secreted mucin while MUC1, MUC4, and MUC16 are transmembrane mucins, which form the protective glycocalyx [274].

6.1.2.3. Proteins

The tear proteome describes the myriad of proteins and peptides found in tear fluid. The human tear film contains >2000 different proteins and peptides; up to 35% are shared with the proteome of other bodily fluids, but many are unique to tears [276]. Basal and reflex tears have comparable amounts of total protein (approximately 3.5 to 9.5 mg/ml); this concentration is increased in closed eye tears (following sleep) to approximately 16 to 18 mg/ml [256,277]. Individual tear protein concentrations are unequally affected by fluctuations in tear flow rate. For regulated proteins, such as lysozyme, lactoferrin, and lipocalin-1, the production rate alters with flow rate so that tear concentrations remain fairly constant across basal, reflex and closed eye tears [276]. In contrast, serum-derived proteins, such as albumin, and constitutive proteins, including IgA, have a more constant production rate, which results in the highest concentrations being present in the more stagnant (closed eye) tears, and relatively lower concentrations in reflex tears [278].

Tears also contain a wide variety of tightly regulated inflammatory mediators, such as complement, arachidonic acid metabolites and cytokines, which can be upregulated in ocular surface conditions [279–282]. Tear mediators are tightly regulated, courtesy of inhibitors that are also present within tears, facilitating modulation of inflammatory responses. Tears also contain a range of neuromediators, the concentration of which can be affected by impaired lacrimal gland function [283].

6.1.2.4. Electrolytes

Electrolytes within the tears contribute to ocular surface health and epithelial integrity and are the main drivers of tear osmolarity [284]. There are a number of electrolytes present in tears, the principal ones being sodium, potassium, chloride and bicarbonate [285]. Tear osmolarity is a key

indicator of tear homeostatic balance [286]. Tear hyperosmolarity, a core component of the pathophysiology of dry eye disease, reflects an increase in electrolyte concentration as a result of a loss of tear film integrity [255].

6.1.2.5. Other tear film constituents

The tears contain a number of other components, such as antioxidants that help protect the ocular surface from oxidative stress [287]. Closed eye tears, in particular, have been found to contain polymorphonuclear leukocytes, and this is thought to be mediated by increased tear cytokine levels overnight [288]. The tear film also contains debris that includes dead cells and cellular fragments from the epithelium, as well as non-organic components that migrate into the eye, such as cosmetics debris [256].

6.2. Tear film physiology

6.2.1. Function and properties

The tear film has a range of protective functions, including maintaining the health of the underlying ocular surface tissues and influencing vision quality [289,290]. Normal tear fluid has a refractive index of 1.337, a pH between 6.8 and 8.2 [291,292]. The extensional viscosity of the healthy tear film is >0.0082 Pa.s., which is reduced in dry eye disease [293]. Tear osmolarity, which describes the concentration of osmotically active particles in a solution, ranges from 270 to 315 mOsm/L in normal eyes [247]. Tear osmolarity is an overall indicator of the balance between tear production, evaporation, drainage and absorption [294]. Tear instability results in excessive evaporation of the aqueous tear component, resulting in tear hyperosmolarity.

6.2.2. Production and dynamics

Tear production is controlled by the ‘lacrimal functional unit’, comprising the cornea, conjunctiva, lacrimal gland and its accessory glands, meibomian glands, and their associated neural connections [295,296] (Figure 18). Basal, reflex, and emotional tears are produced mainly by the main lacrimal gland (see Section 5.1), with minor contributions from the accessory lacrimal glands, and epithelial cell secretions [75,268,297]. Basal tear production is between 0.19 and 1.2 µl/min [256,298]. Reflex tearing occurs in response to external stimulation, resulting in increased tear production of up to 100 µl/min [256,299]. Emotional tears arise from a complex secretomotor

response that occurs without ocular irritation and results in a rise in tear production, up to 400 µl/min [269,298,300].

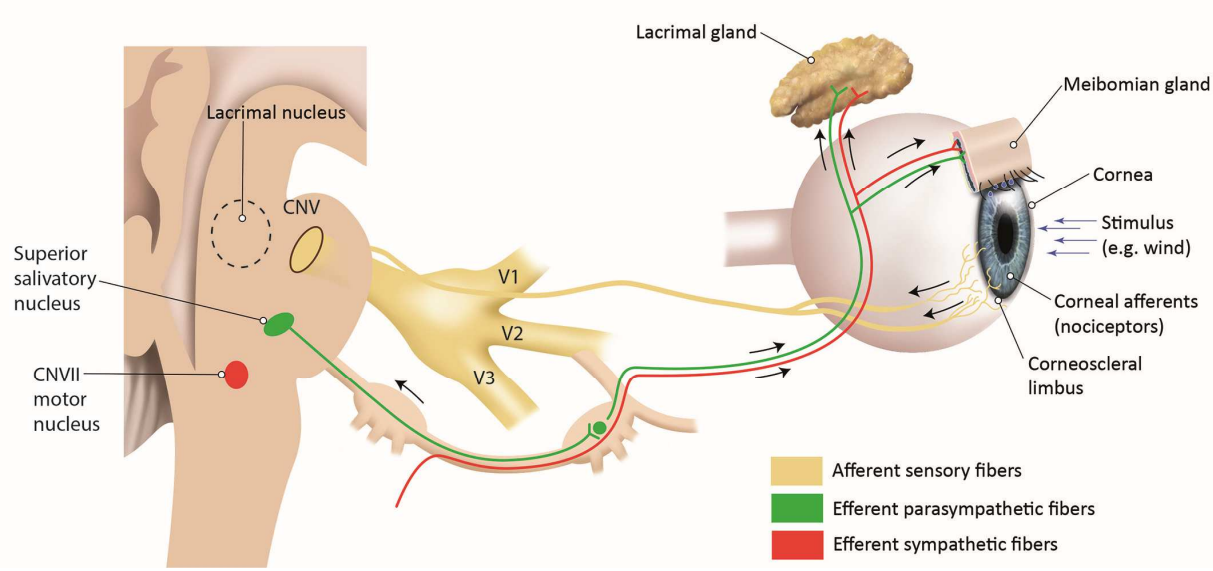

Figure 18 - Neural aspects of tear production, based on a figure in Labetoulle et al., 2019 [74]. Stimulation of corneal sensory nerves promotes lacrimal gland secretion via a trigeminal (cranial nerve V; CN V) - parasympathetic reflex. Facial nerve (CN VII) efferents promote the lacrimal gland, meibomian glands and goblet cells to secrete aqueous, lipids and mucin tear components, respectively. Copyright BCLA 2021.

Newly produced tears flow into the conjunctival sac and, due to negative hydrostatic pressure, are drawn into the superior and inferior menisci [301,302] (see Section 5.2.2). With each blink, lipids from the eyelid margins are compressed and spread over the ocular surface, causing a reduction in surface tension that encourages aqueous tear fluid from the menisci to follow the lipids [294,303]. In the interval between blinks, the low pressure in the menisci draws the tear film back into the mensici [301,304]. During the inter-blink period, a proportion of the tear fluid is lost by evaporation from the ocular surface [255,305].

6.2.3. Lipid-protein-mucin interactions

Tear lipids, proteins and mucins play roles in ocular surface wound healing, defense and tear stability [306]. The secreted gel-forming mucins (MUC5AC, MUC5D, MUC2, MUC19) promote

tear film spreading and act as a surface barrier to prevent contamination of the ocular surface by lipids [307]. Membrane-associated mucins (MUC1, MUC4, MUC16) ensure wettability of the ocular surface and bind galectin-3 to support ocular surface barrier function [307–309]. Phospholipid transfer proteins are believed to flush lipophilic substances from ocular mucins to maintain stability of the anterior tear lipid layer [310]. Other proteins, such as lipocalin, apolipoprotein D, lysozyme, keratins, secretoglobins, and protein B and C, interact with lipids, however there are no data that show a direct interaction between tear mucins and lipids [247].

6.2.4. Tear metabolome

Tear metabolites are small molecule intermediates and derivatives of cell metabolism. They range from highly water-soluble molecules through to those with an affinity for lipids and are generally <1 kDa in size. Using analytical techniques such as mass spectrometry or nuclear magnetic resonance, coupled with high performance liquid chromatography, gas chromatography or capillary electrophoresis, the profile of metabolites has been shown to change in dry eye disease, offering diagnostic and prognostic potential. The ability to alter the metabolomic profile of tears via oral supplementation has been demonstrated [311]. An extensive range of metabolites have been explored in dry eye disease, including steroids and sex hormones [312], antioxidants [313]) and pro-resolving mediators [314]. Vehof et al. evaluated associations of 222 serum metabolites [315,316], but it has been postulated that many more metabolites exist than have been reported to date [315,316].

6.3. Clinical assessment of the tear film

A range of clinical instruments are available to assess the tear film structure and function (see supplementary appendix and CLEAR Evidence-Based Practice Report [7]).

7. Advanced testing of the ocular surface

As well as the routine clinical assessment of ocular anatomy, described in the supplementary appendix and CLEAR Evidence- Based Practice Report [7], this section considers some specialist techniques that can be used to further understand ocular surface structure and function.

7.1 Pachymetry

Corneal pachymetry is used to measure corneal thickness. The procedure can be performed using optical or ultrasound methods. Monitoring corneal thickness is important in a variety of clinical scenarios, including for patients with keratoconus, corneal transplants, and corneal dystrophies, prior to corneal refractive surgery, as well as for glaucoma risk factor assessment.

7.2. Corneal sensitivity

Corneal sensitivity can be quantified using a variety of approaches. As a gross measure, the blink reflex can be tested with a wisp of cotton lightly touched to the edge of the cornea; a blink should be elicited in response to corneal stimulation. Different sectors of the cornea can be evaluated to determine potential regional sensitivity losses.

Cochet-Bonnet aesthesiometry measures ocular surface sensitivity to a mechanical stimulus, using nylon filaments of variable diameter and length [170,317–319]. This approach has several limitations, including being relatively invasive, the minimum stimulus possibly being suprathreshold, and the visibility of the stimulus potentially inducing patient apprehension and biased responses [320]. Accurate and repeatable positioning of the stimulus by the examiner is also a challenge [321]. Alternative methods to quantify ocular surface sensitivity include the delivery of stimuli using saline [322], a carbon dioxide laser [172], and air pulses [171,321,323]. A combined carbon dioxide/air aesthesiometer, developed by Belmonte and colleagues, further enables measurement of thresholds to mechanical, chemical and thermal stimuli [324].

Corneal sensitivity has been suggested to decrease with age [325,326, 327], although this has not been consistently demonstrated [328]. Corneal sensitivity may also be affected by a range of ocular conditions, including dry eye disease [329], atopic keratoconjunctivitis [330], Fuchs' endothelial dystrophy [331] and herpetic infection [332], and systemic disease, including diabetes [333]. Although contemporary soft contact lenses do not alter corneal mechanical sensitivity, other modalities (such as orthokeratology and hydrogel contact lens wear) can reduce corneal sensitivity [334,335]. Hypersensitivity to cooled air stimuli has been described in symptomatic soft contact lens wearers, relative to asymptomatic wearers [336] (see CLEAR Material Impact Report [22]).

Corneal sensory nerve dysfunction may manifest in the form of neuropathic disease, which involves abnormal corneal sensations (including stinging, burning, photoallodynia and pain) in the

absence of standard nociceptive signals or clinical findings of corneal disease [337]. Corneal neuropathic pain is typically diagnosed on the basis of corneal somatosensory symptoms in association with evidence of sensory nerve damage, from anatomical or functional tests. In corneal neuropathy, pain may be mediated by peripheral and/or central pathways; many patients have contributions from both mechanisms. A simple clinical method that can be useful for differentiating peripheral from central neuropathic pain is the topical anaesthetic challenge test [338]. This procedure involves assessing whether administration of topical anaesthetic eliminates, or reduces, the corneal pain response; the level of pain reduction is an indication of the relative contribution of peripheral nerve sensitisation to the patient's symptoms.

7.3 Conjunctival impression cytology

Conjunctival impression cytology, a technique first described by Egbert and colleagues [339], involves applying a specialised filter paper to the conjunctiva to collect a cell sample that can be subsequently used to evaluate the histological, immunohistological or molecular characteristics of collected cells [339,340]. The technique is not performed routinely, but used instead to evaluate the ocular surface in specific circumstances, such as dry eye disease, cicatricial pemphigoid, neoplasia and vitamin A deficiency; it has also been used to investigate the immune response in contact lens wear [190]. Understanding the aetiology and sequence of conjunctival changes over time and evaluating the effect of treatments are some of the specific applications of impression cytology [341]. This procedure can also be used as a diagnostic technique, in addition to immunostaining and DNA sequencing [341].

Impression cytology samples are typically acquired under topical anaesthesia. Paper, usually composed of cellulose acetate, is gently pressed onto the conjunctival surface for several seconds to allow adherence of epithelial and goblet cells. For goblet cell analysis, the sample can then be stained with solutions, such as Periodic acid-Schiff and Gill's haematoxylin. Impression cytology specimens can be graded based on the morphology of epithelial cells and goblet cell numbers [341]. A spectrum of goblet cell densities has been reported in the literature, ranging from 24 to 2226 goblet cells/mm^2 in nominally healthy eyes [342]. This wide range may be due to uneven distribution and/or clumped goblet cells [343] and/or variability in conjunctival histology across a given sample [344].

8. Conclusions and future directions

This report has reviewed current understanding of the anatomy and physiology of the anterior eye, as the basis for contact lens practice. In addition to foundational studies involving histological examination of the ocular anatomy, technological advancements (such as OCT and IVCM) have expanded knowledge of the dynamic, *in vivo* anatomy and physiology of the human eye, and enabled longitudinal evaluations in clinical populations to define the effect of exogenous factors, including contact lens wear. Evolving scientific understanding of these complex structure-function relationships provides a basis for ongoing advancements in contact lenses, and provides opportunities to further optimise on-eye contact lens comfort, biocompatibility and safety.

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