CHAPTER 2

ANATOMY AND PHYSIOLOGY OF THE EYELID, CONJUNCTIVA, LIMBUS, CORNEA AND TEAR FILM

Any book on contact lens practice should include an overview of the anatomy and physiology of the cornea, limbus, tear film, conjunctiva, anterior sclera and the eyelids protecting the delicate surface of the eye from the environment. As students of the ophthalmic sciences, you will have encountered and covered the topics of this chapter in some detail from many excellent textbooks on the respective subject matter. This overview of the anatomy and physiology of the anterior structures of the eye, deemed important to contact lens practice, will add to your knowledge, hopefully fill some gaps, or at the very least, refresh your memory.

COLLAGEN

Collagen is an important component of the body in general, and the eye in particular. It is the major protein of the extracellular matrix. Collagen is classified into different types, with different functions, many being found in the eye [4]. The following table will highlight the different types and where they are found.

Table 2: General distribution of the different types collagen in the human body

Type	General Distribution
1	Most common type. Found in bone, tendons, dentine and adult skin
2	Defining characteristic of hyaline cartilage, also found in vitreous, embryonic cornea and neural retina
3	Important for wound healing. Found in tissues with major smooth muscle component
4	Found in basement membranes
5	Second most common collagen. Especially dense in muscle and tendon sheaths
6	Widely distributed throughout the body
7	Fibrils anchoring basement membrane to the underlying stroma
8	Secreted by endothelia of the vasculature and cornea
9	Found in cartilaginous tissues
10	Found in cartilage with growth plate
11	Minor type of collagen found in cartilage

EYELIDS

The structure of the eyelid is illustrated in Figure 1, and from an anterior to posterior direction can be divided into:

1. Skin
2. Subcutaneous layer
3. Muscles
4. Sub-muscular layer

5. Fibrous layer
6. Palpebral conjunctiva

Functions of the eyelids include: protection of the eye, contributes to tear production, spreads tears across the corneal and bulbar conjunctiva and drainage of tears.

Skin

The eyelid skin is very thin, elastic, and fat free. It is composed of the epidermis, dermis and sub-cutaneous tissue. The outermost layer is the epidermis, which is composed of keratinised, and several layers of non-keratinised epithelial cells. Five other cell types can also be found in the epidermis and they include: keratinocytes, melanocytes, Langerhans cells, Merkel cells and neurons. Of these the keratinocytes are most numerous and are responsible for producing keratin. The dermis is found internal and adjacent to the epidermis and is composed of dense irregular collagenous tissue. The sub-cutaneous tissue is found deeper to the dermis. Sensory innervation of the upper lid is via the ophthalmic division and the lower lid via the maxillary division of the 5th cranial nerve [5].

Appendages such as fine hairs, sweat glands and pigment cells are located on the surface of the eyelids and along the lid margin, two rows of lashes along with two associated rows of glands can be found. The skin meets the conjunctiva at the muco-cutaneous junction (also known as Marx's line) which can be visualised along the openings of the tarsal or Meibomian glands [6]. Keratinisation of the skin surface epithelium occurs all the way to the Marx's line and includes the epithelium lining the Meibomian gland orifice. The lid wiper surface starts at Marx's line, or at the transitions from keratinised squamous epithelium to cuboidal cells and occasionally columnar cells. The initial portion is elevated due to multiple cell layers (typically eight to 12), and the thickness gradually decreases toward the sub-tarsal fold over a distance of 0.3 mm to 1.5 mm. The width of the wiper region is about 1 mm; however, it is wider in the nasal and temporal regions compared to the centre of the eyelid (Figure 1). The epithelium of the lid wiper conjunctiva is non-keratinised and contain goblet cells, either singly or in clusters located in the superficial epithelial layers. The cells secrete soluble mucus onto the lid wiper surface [5–7]. Along with the tear film, these mucins form a hydrated gel between the lid wiper and the ocular surface to provide lubrication, so that the lid and the ocular surface are completely separated by a fluid film consisting of mucin and aqueous components.

Because the skin of the eyelid is so thin and delicate it is susceptible to much pathology. These pathologies include; malignant, as well as benign skin cancers such as melanoma, squamous cell and basal cell carcinoma. Evidence suggest that basal cell carcinoma is related to ultraviolet (UV) radiation and although benign growths are distributed evenly between the upper and lower lids, malignant tumours are 4x more common in the lower lid. This is probably due to the natural protection provided by the upper orbital rim from direct exposure to the sun [7].

Lid wiper epitheliopathy (LWE), is a clinical condition observed as vital staining of the upper and lower lid margin regions that are in contact with the globe or a contact lens. It is believed to be a result from an increase in friction between the palpebral lid and the opposing bulbar conjunctiva, cornea, or contact lens. Although the cause of LWE is not known, it is postulated that inadequate lubrication results in frictional damage and inflammation of the marginal conjunctiva of the lid wiper region, resulting in an epitheliopathy that is clinically observed as lid margin staining [8].

Muscles

Orbicularis Oculi

This striated, voluntary muscle is the most prominent muscle and is organised in bundles of muscle fibres. It has orbital, palpebral and ciliary portions, which are concentrically orientated, allowing a sphincter action. It helps to hold the lid tightly against the eye, assists in spreading tears and flushing away debris and waste products. The palpebral portion arises from the medial palpebral ligament, is confined to the lids, and passes across the lids in a series of half ellipses, which meet outside the lateral cantus in the lateral palpebral raphe (Figure 1). The ciliary portion, also called the Muscle of Riolan, keeps the lid margin tight against the eye. The orbital portion has a curved origin from the medial side of the orbit. From these origins, the fibres sweep across the orbital margin in a series of continuous loops, while the more central fibres form nearly complete rings. The orbicularis oculi is innervated by the 7th cranial nerve and paralysis leads to lagophthalmos, and dry eye due to incomplete closure of the eyelids [5, 6].

Levator Palpebrae Superioris

This striated, voluntary muscle originates from the inferior surface of the lesser wing of the sphenoid above and anterior to the optic canal. It is only found in the upper lid and is responsible for raising the lid (antagonistic to the orbicularis oculi). The muscle becomes tendinous as it enters the lid and at this point is called the, "aponeurosis of the levator" (Figure 1). The tendon spreads out and moves between the bundles of the orbicularis oculi and inserts in the skin. The levator muscle is innervated by the 3rd cranial nerve and paralysis leads to ptosis of the lid [5, 6].

Müllers or Tarsal Muscle

This smooth muscle has primary (excitatory) sympathetic innervation (superior cervical sympathetic ganglion) and parasympathetic (inhibitory) innervation (7th cranial nerve). Müller muscle is found in the upper and lower lids. In the upper lid it lines the levator internally and contraction aids in the action of the levator (Figure 1). In the lower lid it is associated with the facial sheath of the inferior rectus and lowers the lid. Müllers muscle maintains the "open" position of the lids in the waking state and its inhibition closes them during sleep. It is also associated with the wide-eyed expression of fear. Damage to the sympathetic innervation of the superior tarsal muscle causes ptosis [5, 6].

Sub-Muscular Areolar Tissue

This is a fat-free loose connective tissue between the muscular tissue, skin and the tarsal plate. The skin is loosely attached to the underlying muscle by this connective tissue and is easily pulled or pushed away. Therefore, immediately beneath the skin, a potential cavity can be readily filled with blood from a haemorrhage (black eye), or an escape of lymph (oedema of the lid), or air from a fractured sinus wall [6].

Orbital Septum and Tarsal Plates

The tarsal plate consists of dense fibrous tissue, which gives firmness and shape to the eyelids. The tarsal plates collagen is more developed in the upper lid than in the lower lid, which allows the upper lid to be everted. The upper tarsal plate measures about 10 mm in height at the centre and gradually narrows to the ends. Attached to its upper edge are the orbital septum and the smooth muscle fibres of the levator muscle (Figure 1). The lower tarsal plate is 5 mm in height in the centre gradually narrowing to the ends. The orbital septum is attached to its lower edge. The orbital septum forms the fibrous framework of the eyelids and is attached to the orbital margin, where it is continuous with the periosteum. The orbital septum separates the eyelids from the contents of the orbital cavity [6, 7].

Palpebral Conjunctiva

The conjunctiva is a thin mucous membrane that lines the eyelids and is reflected at the superior and inferior fornixes onto the anterior or bulbar surface of the eyeball. It covers part of the sclera, and its epithelium is continuous with that of the cornea. As mentioned earlier, the conjunctiva continues into the skin at the lid margin along the openings of the Meibomian glands. A shallow groove on the back of the lid, called the sub-tarsal sulcus, lies about 2 mm from the posterior edge of the lid margin. It tends to trap debris and foreign particles introduced into the conjunctival sack and is therefore clinically important. The palpebral conjunctiva is richly vascularised, giving the back of the lid its pinkish colour. It is firmly attached to the entire extent of the upper tarsal plate, but on only on the upper half of the lower tarsal plate [6, 7].

Glands of the Eyelids and Conjunctiva

Tarsal or Meibomian Glands

The Meibomian glands develop at two and a half month's gestation. The glands are modified sebaceous glands, secreting an oily secretion which forms the surface of the tears slowing evaporation. A single Meibomian gland is composed of clusters of secretory acini (special type of sebaceous gland) that are arranged circularly around a long central duct and connected to it by short ductules. This arrangement has been compared with onions on a string (Figure 1). One end of the central duct is blind, and the other end opens close to the posterior lid border, just anterior to the muco-cutaneous junction at the lid margin, where the oily secretion is delivered onto the tear meniscus. These separate glands are arranged in parallel in a single row throughout the length of the tarsal plates in the upper and lower lids. They presumably act in a coordinated fashion that is influenced by hormonal and neural regulation and by the mechanical forces of muscle contraction during an eye blink. The glands are richly innervated by parasympathetic, sympathetic and sensory fibres. Parasympathetic fibres are derived from the 7th cranial nerve and form the majority of the neural innervation [5].

The extent of the Meibomian gland roughly corresponds to the dimensions of the tarsal plates in the upper and lower eyelids and hence differs between them. In the upper lid the tarsal plate has the shape of a half circle that extends upward and centrally, for approximately 1 cm and narrows on the temporal and nasal sides. Whereas, the tarsal plate in the lower lids is smaller and forms a strip of rather equal length from the nasal to the temporal side. Approximately 30–40 and 20–30 glands can be seen in the upper and lower lid respectively [6].

The continuous production of lipid, is not only supported by the observed generation time of new meibocytes, but also by the finding that in the morning after sleep, during which the lids are closed, an increased amount of lipid that has apparently accumulated within the ductal system is then delivered in increased amounts onto the lid margin [6, 7].

Infections of the Meibomian glands are known as *internal hordeolums* and sterile chronic inflammation of the glands as *chalazia* which may sometimes manifest *de novo* or independent of chronic inflammation [9].

Exocrine Glands Associated with Cilia Anterior to the Tarsal Plate

Ciliary Glands of Moll

These are primitive sweat glands, observed around the eyelash follicles which have a spiral shape. The glands of Moll are more common in the lower lid, but usually not found on every cilium. They are thought to be sweat glands which have become arrested in their development. The secretions contain enzymes and lipid [5, 6].

Sebaceous Glands of Zeiss

The morphology of the glands of Zeiss, are similar to the Meibomian glands and are more common than the glands of Moll. Usually, two glands per cilium are present and their oily secretion prevents the eyelash from becoming dry and brittle [5, 6].

Infections of the ciliary glands are termed, *external hordeolums* or *styes*. Although they are painful, they are self-limiting an easily treatable. *Chalazions* may also develop in the sebaceous gland of Zeiss [9].

The Conjunctival Glands

Associated with the conjunctiva are a number of small glands, differentiated both histologically and topographically into different types.

The Glands of Krause

The glands of Krause are accessory lacrimal glands having the same structure as the lacrimal gland. They occur deeply in the subconjunctival tissue, mainly in the upper fornix, between the tarsus and the lacrimal gland of which they are offshoots. They number 42 in the upper and 6–8 in the lower fornix and occur largely on the lateral side. The glands produce a serous secretion [6].

The Glands of Wolfring

The glands of Wolfring are also accessory lacrimal glands but larger than the glands of Krause. 2–5 glands are situated in the upper border of the tarsal plate of the upper lid. The glands produce a serous secretion [6].

Crypts of Henle

The crypts of Henle are glands that occur in the palpebral conjunctiva, between the tarsal plates and fornixes. They are probably not true glands, but folds of mucous membranes [6].

Goblet Cells

These cells occur in all regions of the conjunctiva. They are large, oval, or round cells which look like fat cells. The goblet cells form in the deepest layer of the conjunctiva, pass toward the surface, but remain attached to the basement membrane by a pointed process (Figure 2). The goblet cells are true, unicellular holocrine mucus glands, moistening and protecting the conjunctiva and cornea. The glands produce mucin (MUC 5AC), which makes up the inner layer of the tears. Mucin combined with water forms mucus, which acts as a wetting agent and helps spread the tears across the cornea. Goblet cells are denser nasally, least dense in the upper temporal fornix, and absent from the bulbar conjunctiva to the nasal and temporal sides of the limbus. Although goblet cells occur normally in the conjunctiva, they are greatly increased in inflammatory conditions [5, 6].

Blood Supply and Lymphatics

Arteries in the lid anastomose to form two transverse arcades per eyelid – the marginal and peripheral arcades. Both these arcades are fed by branches of the facial artery, derived from the external carotid artery: and the orbital system derived from the internal carotid artery.

The eye itself does not contain lymph vessels, but both the conjunctiva and eyelids are provided with lymphatics. In the lid the lymphatics can be divided into a pre-tarsal plexus, draining the skin and orbicularis and a deep post-tarsus plexus, draining the tarsal plate and the conjunctiva. These lymphatic vessels drain into the superficial parotid nodes and the submandibular nodes [5].

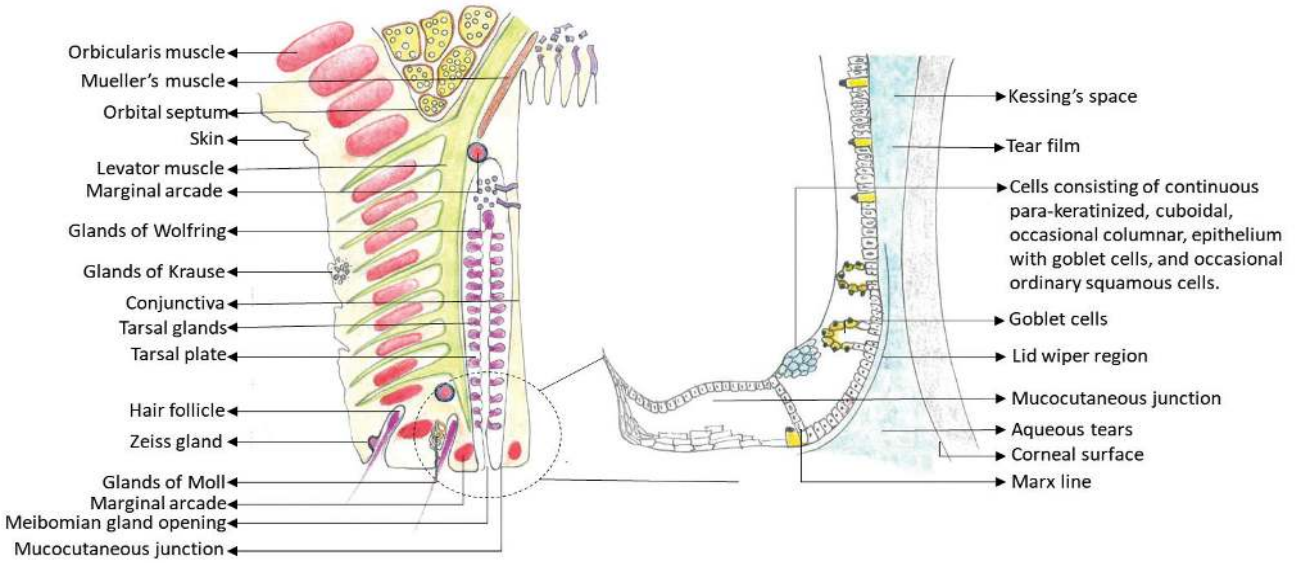

Figure 1: Anatomy of the eyelid highlighting the lid wiper region. Adapted from Bergmanson 2015 [5]

THE CONJUNCTIVA

The conjunctiva is a mucus producing tissue lining the ocular surface and inner surface of the lids, except for the cornea. Although it is described as a mucus membrane, its secretory output is more serous than mucus (more watery than slimy). The conjunctiva can be divided into the palpebral region lining the lids, bulbar region which lines the globe and merges with the limbus, and the fornix or cul-de-sac which connects the bulbar and palpebral regions. The depth of the conjunctival sac varies with the meridian, superior 14–15 mm, inferior 10–12 mm, lateral 5–8 mm, and medial it has no depth where the conjunctiva folds into the plica semilunaris. Therefore, if a contact lens has dislocated from the cornea, it is most probably located in the superior fornix, since it has the greatest depth [5, 6].

Histologically, a mucus membrane will generally compromise two layers: epithelium layer containing goblet cells straddling a basement membrane, and stroma or substantia propia containing connective tissue, nerve fibres and blood vessels [6].

Epithelium

Generally, the conjunctival epithelium is 2–4 layers thick and rests on a basement membrane. At the limbus where the conjunctival epithelium is continuous with the corneal epithelium (5–7 layers), a local thickening occurs, and the cells change to stratified squamous non-keratinised epithelium (Figure 2). At the palisades of Vogt close to the limbus, the organisation of the epithelium changes. It thickens 4–5x in an internal direction, due to the increased number of wing cells. Internally the conjunctival epithelium seems to undulate, but the surface remains smooth [5]. Stem cells are located in the palisades of Vogt and continually contribute cells to the cornea [10].

As previously discussed, goblet cells are formed in the deeper layers of the epithelium and then migrate to the surface, where they discharge their entire contents of secretory granules (Figure 2). On average a goblet cell is 11 μm wide and its secretory granules are just less than 1 μm in diameter. After the contents are expelled, a crater is left in the surface of the epithelium. Goblet cells are sparse in the bulbar conjunctiva, but frequently occur in the fornix and palpebral conjunctiva. Evidence suggest that the epithelial cells may also produce mucin (MUC 1 & 16), which functions to anchor the mucus to the epithelium [5]. Hypertrophy of goblet and epithelial mucus producing cells may account for the mucoid characteristics of ocular inflammatory conditions (Figure 2).

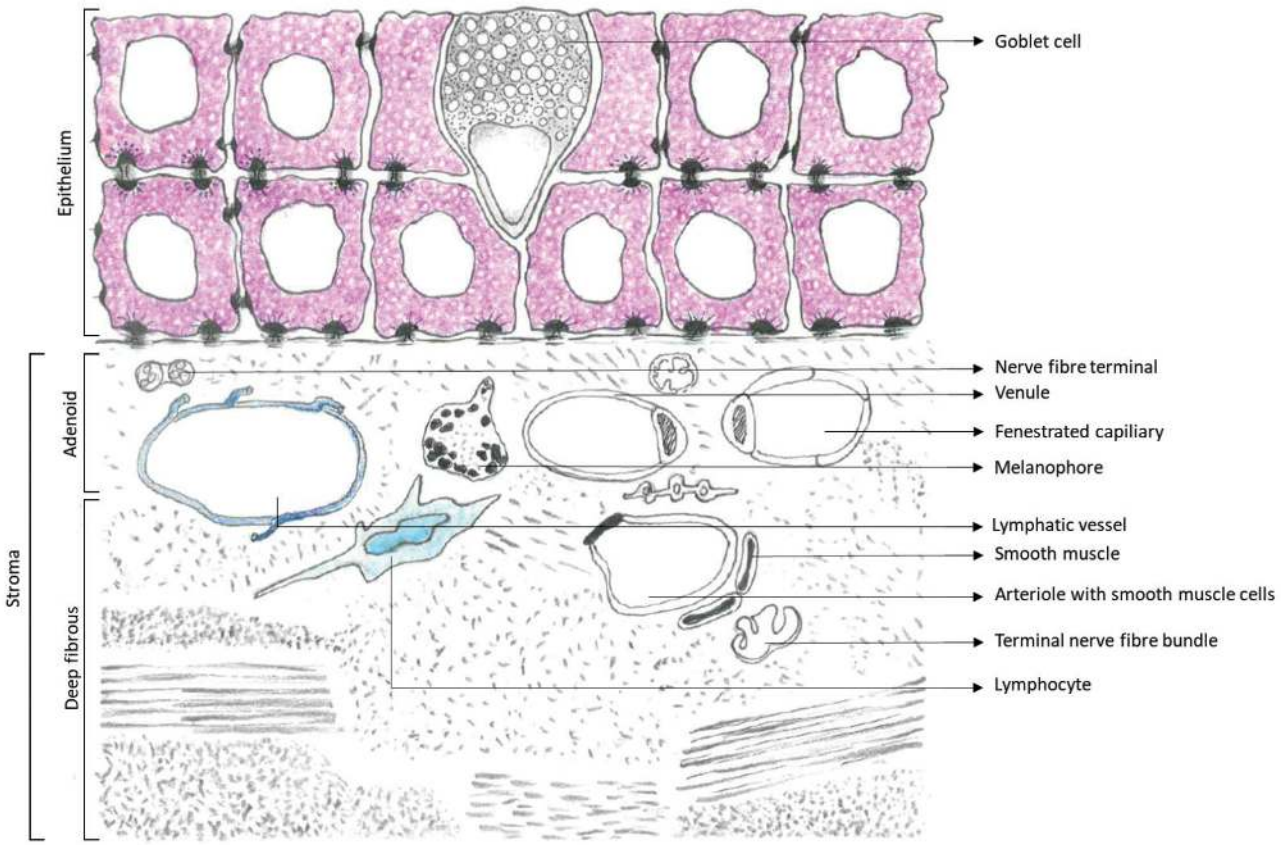

Figure 2: Cellular organisation of the bulbar conjunctiva. Adapted from Bergmanson 2015 [5]

Stroma

The stroma lies below the epithelium and consists of the adenoid layer and the deep fibrous layer. The adenoid layer is a superficial open structure of fibrous tissue containing lymph nodules, lymphocytes, blood capillaries, and pigment granules or melanophores, which is more common in the conjunctivas of darker skinned races. The capillaries are fenestrated, providing nutrition and oxygen to the conjunctiva for regeneration of the epithelium and formation of new goblet cells. Lymphoid tissue is more numerous in the region of the tarsal conjunctiva. Beneath the adenoid layer occur the deep fibrous layer, which harbours the larger blood vessels and nerves. In the palpebral conjunctiva, the deep fibrous layer is replaced by the tarsal plate of the lid [5]. The fibrous layer is reinforced by the muscle facia and Tenons capsule (Figure 2).

The conjunctiva is the most immunologically active tissue of the external eye. Normal conjunctival epithelium contains 6000 neutrophils and 14000 lymphocytes per mm^3. Eosinophils and basophils are not found in the normal conjunctival epithelium, but they occur in only two diseases: vernal conjunctivitis and giant papillary conjunctivitis. A variety of conjunctival reactions can be provoked by infections, allergy and inflammation. These reactions are characterised by two structural changes accompanied by inflammation: the formation of papillae and follicles [5].

Papillae are areas of conjunctival hypertrophy, which contain eosinophils and neutrophils. Papillae have a blood vessel in their centres and are most commonly found in the upper lid palpebral conjunctiva in response to allergy. Follicles are clear fluid-filled pockets containing lymphocytes and macrophages, with blood vessels passing either above or below but never within. Follicles are found in both the upper and lower fornix and commonly occur in response to viral infections [11] (Figure 3).

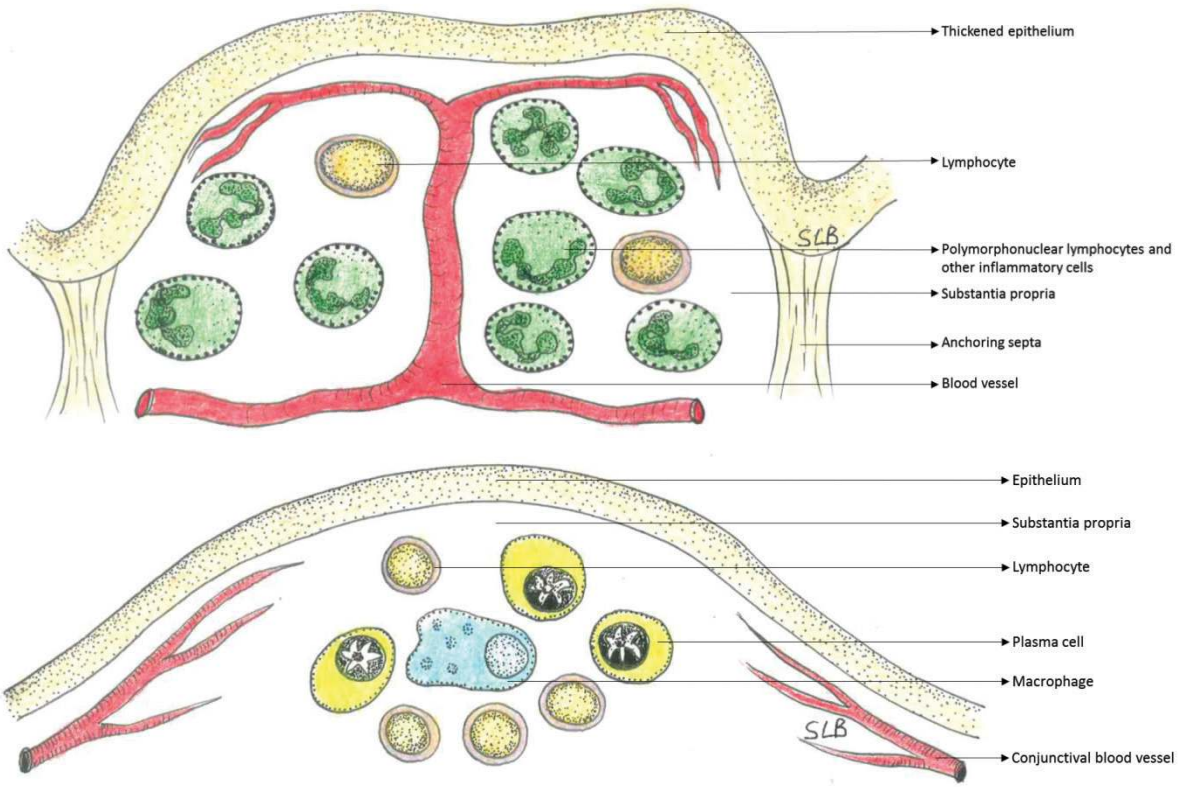

Figure 3: Comparative anatomy of a papillae (top) and a follicle (bottom)

Table 3: Differences between a papillae and follicle [11]

Papilla	Follicle
Elevated polygonal areas separated by paler areas	Discrete, round elevated lesion of conjunctiva
0.30–2.0 mm (giant > 1.0 mm)	0.5–5.0 mm
Anywhere on conjunctiva to limbus but usually on tarsal conjunctiva	Usually inferior conjunctiva
Central fibrovascular core Central vessel (vascular tuft)	Vascular network around follicle Vessels disappear to centre
PMNL's (polymorphonuclear leukocytes) and other acute inflammatory cells; epithelial hypertrophy	Lymphoid tissue, lymphocytes, macrophages and plasma cells
Connective tissue septum anchored into deeper tissue resulting in polygonal outline. Giant papilla occurs when the septums rupture	

Mast Cells

Mast cells are usually found in the adenoid layer of the conjunctival stroma, just below the junction with the epithelium. They are extremely numerous (>6000 mm^3) and up to 50 million per eye can be found. The mast cell is 20 μm in diameter and its cytoplasm is filled with preformed chemical mediators of inflammation, such as histamine and heparin, which are released when the cells are activated by neurogenic or antigenic stimulation [5].

Nerve Supply

Sensory innervation of the epithelium and stroma arise from the 5th cranial nerve, superior conjunctiva – ophthalmic division and inferior conjunctiva – maxillary division. The conjunctiva has both sympathetic, from superior cervical ganglion, and parasympathetic, from the 7th cranial nerve, innervation. Conjunctival blood vessels receive dual autonomic innervation. Sympathetic and parasympathetic terminals are found in the walls of arterioles, but also adjacent to the endothelial lumen of the fenestrated and non-fenestrated capillaries. This dense vascular innervation of the conjunctiva explains the remarkable local control of blood flow seen in the inflamed eye. The epithelium also receives dual autonomic innervation, which provides the neural control of goblet cell secretion [5–7].

Blood Supply and Lymphatics

The blood supply to the conjunctiva, arise from the palpebral branches of the dorsonasal and lacrimal arteries – supply palpebral region, as well as the anterior ciliary arteries that supply the bulbar region. The superficial lymphatic plexus drains the adenoid layer into the parotid node and the deep plexus drains the deep fibrous layer into the submandibular node. The flow of lymph is generally in a temporal direction toward the lateral extremes of the lid, eventually connecting with the parotid node. A medial route drains into the submandibular nodes [5–7].

THE LIMBUS

The area where the cornea meets the sclera and conjunctiva is known as the limbus. It is ±1 mm wide and its most important functions include:

- To nourish the peripheral cornea
- To provide an outflow for the aqueous humour, via the trabecular meshwork and Schlemm's canal
- To assist the corneal epithelial regeneration and maintenance of transparency via the stem cells and stockpiles of epithelial cells located in the limbus

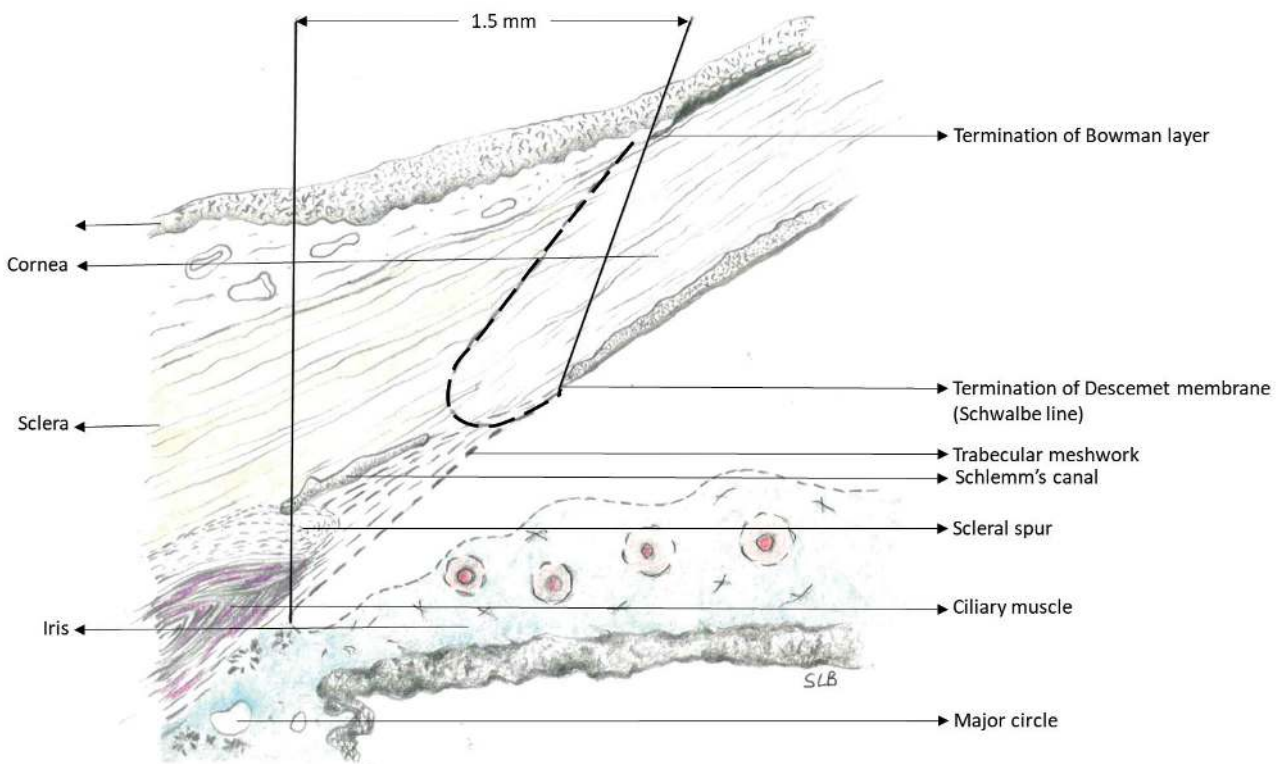

Figure 4: Anatomy of the limbus

Epithelium

The corneal epithelium continues into the limbus, where it becomes thicker and less uniform. It contains 10 or more layers due to greater numbers of wing cells. The single row of basal cells is smaller and more densely packed. The corneal epithelial basement membrane becomes somewhat undulating before continuing into the conjunctiva (Figure 4). On the conjunctival side, the epithelium tapers down to 3–4 layers, except at the palisades of Vogt, which form epithelial ridges with huge stockpiles of newly formed cells. Stem cells are found in the horn-like extensions (limbal epithelial crypts) of the thickened limbal epithelium [10]. Melanin granules, from the conjunctival melanocytes, can sometimes be found in the basal cells, but no goblet cells are present in the limbal epithelium [5].

Bowman's Membrane

Bowman's membrane stops at the peripheral cornea and demarcates the histological limit of the cornea [5].

Stroma

The corneal stroma is continuous with the scleral stroma, but at the limbus the stroma loses the regularity of the lamellae found in the cornea. Due to the increase in diameter, as well as, less uniform spacing of the collagen fibres, some of the corneal transparency is lost at the limbal region. Blood vessels (limbal loops) are found among the anterior bundles of collagen fibres and extend no more than 1 mm into the cornea (Figure 4). The sensory corneal nerves lose their medullary sheaths and enter the cornea radially, forming the neural net of the cornea [5].

Descemet's Membrane

Descemet's membrane forms a circum-corneal ring, demarcating the peripheral limit of the cornea posteriorly (Figure 4). This can be viewed using a gonio lens and is known as Schwalbe's ring/line. However, the endothelial cells' basement membrane is continuous into the trabeculum, forming the trabecular meshwork [5].

Endothelium

The endothelium is continuous from the cornea, but some drastic modifications occur at the limbus, where it forms the trabecular meshwork and canal of Schlemm is formed [5].

THE SCLERA

The sclera is the largest component of the fibrous tunic of the eye. Estimates of the area of the sclera range from, 5/6 to 9/10's of the total outer surface of the eyeball. The main function of the sclera is to protect the intraocular contents and to maintain the overall shape of the eyeball. The thickness of the sclera shows some variation, it is thickest at the posterior pole of the glode (1–1.35 mm) and decreases toward the equator to reach a minimum thickness under the tendons of the rectus muscles (± 0.3 mm). The combined thickness of the tendons and sclera is 0.6 mm and from this point it thickens toward the limbus, where it is ±0.83 mm thick. The diameter of the scleral coat varies from, 23 to 25 mm and it is wider horizontally than it is vertically. The male sclera is also thicker than the female sclera [5, 6].

Of importance in scleral contact lens fitting, is the anterior scleral curvature. The measurement of the scleral radius of curvature using AS-OCT, has shown that the mean radius of the anterior sclera was 13.12 ± 0.80 mm and that the nasal scleral curvature (13.33 ± 1.12 mm) was significantly greater (flatter) than that of the temporal scleral curvature (12.32 ± 0.77 mm) [12]. Newer technology such as, the sMap3D topographer (Precision Ocular Metrology) and the Eye Surface Profiler (Eaglet Eye, Netherlands) are being introduced, which will hopefully make the measurement of the anterior scleral radius simpler and therefore improve our ability to fit scleral lenses.

The corneo-scleral profile was defined by Meier, 1992 based on observations of the limbal zone, using the naked eye or slit lamp. He described five transition profiles, where profile 1 exhibits the greatest sagittal depth [13] (Figure 5). However, more recent study by Hall, 2013 found that 77% of all transition angles demonstrated a tangential profile 2 [14]. The work of van der Worp, Caroline and André, 2015 using AS- OCT, demonstrated that the central and mid-peripheral cornea can be accurately described by radii, due to the fact that the corneal surface is curved [15]. However, beyond a chord of 10.0 mm, the peripheral cornea, limbus and sclera generally form a straight line and therefore are more appropriately described as a tangent angle. In most eyes evaluated, there was not a discernible junction between the cornea and sclera. Instead, the limbal area is simply part of the tangent continuum between the 10.0 mm and 20.0 mm chord [15]. Ritzmann et al., 2017 measured sagittal depth with the "Eye Surface Profiler (Eaglet Eye, Netherlands)". The measurements were referenced from the corneal apex and taken in primary and oblique positions of gaze and showed marked asymmetry between horizontal and vertical meridians [16]. Findings of this study indicate that:

- At a 10 mm chord, sagittal height values reflect with-the-rule corneal astigmatism
- The ocular surface is relatively more symmetric at a 12.8 mm chord
- Sagittal height measurements at a 15 mm chord reveal a rotationally asymmetric shape
- Scleral sagittal height at a 15 mm chord was found lowest nasal and highest temporal
- The Sclera is steepest in the superior nasal quadrant
- Corneo-scleral transition angles were more concave nasally and tangent temporally

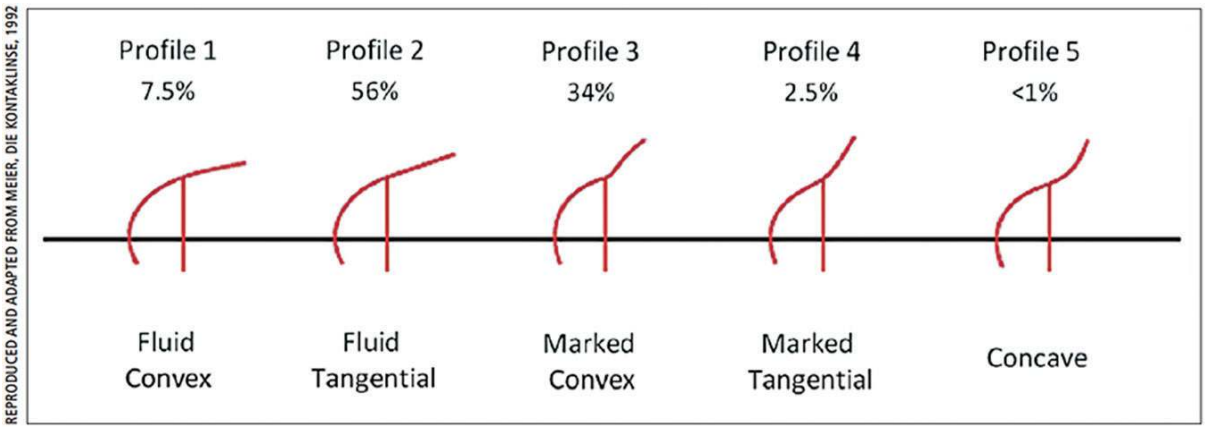

Figure 5: Meier's anterior scleral profiles

Although the sclera is not a truly layered structure, it is traditionally divided into three regions. All three regions are composed of the connective tissues collagen and elastin.

Episclera

The episcleral connective tissue is anterior to the stroma of the sclera and firmly attached to the overlying conjunctiva. It is generally less compact and more vascular than the stroma and Tenons capsule (lying on its outside). The episclera is thicker anteriorly, and thinner posterior to the rectus muscle insertions. The bulk of the episclera, consist of medium diameter collagen fibres (50–60 nm), which is uniformly distributed. Additionally, some elastin fibres are also present. The blood vessels in the episclera are derived from the anterior and posterior ciliary arteries [5].

Scleral Stroma

The stroma is composed of dense bundles of collagen, some sclerocytes (similar to the corneal keratocytes). The bundles of collagen fibres are not organised into uniform flat sheets or lamellae, neither are they regular and often have an undulating pattern. The diameter of the fibres varies depending on the depth in the stroma, ranging from as small as 50 nm, to as large as 400 nm. The large diameter of the fibres scatters light and is the main reason for the opaque nature of the scleral tissue. Elastin fibres can be found weaving between the bundles of collagen fibres, especially in the region of the rectus muscle insertions [5].

Lamina Fusca

This is a modified part of the most internal aspect of the scleral stroma. Melanocytes from the choroid are present. The fibrous tissue of the lamina fusca is more loosely packed than the scleral stroma, and due to the presence of the melanocytes, it appears similar to the suprachoroid [5].

Penetrations of the Sclera

The most prominent penetration is the optic nerve, just nasal and superior to the posterior pole of the eye ball. Here the sclera becomes the lamina cribrosa, allowing the axons of the optic nerve to exit the eye. Upon leaving the eye, the axons become myelinated resulting in an increase in the overall diameter of the optic nerve. At the lamina the diameter is 1.5–2.0 mm and the external diameter 3–3.5 mm. 8–20 short posterior ciliary arteries pierce the sclera in a ring (circle of Zinn) around the optic nerve. The arteries are accompanied by the short posterior ciliary nerves. More anteriorly, the long posterior arteries and nerves (2 pairs) penetrate the sclera, mostly along the horizontal meridians. About 4 mm posterior to the equator of the eye, 4 or occasionally more vortex veins can be found. The vortex veins originate from the choroid and drain the uvea. Anteriorly, about 3 mm from the limbal region 7 anterior ciliary arteries penetrate the sclera to supply the anterior uvea and episclera. Finally, the canal of Schlemm follows an annular course within the limbal region of the outer wall, while communicating internally with the anterior chamber via the trabeculum and externally with the anterior veins, which drain the eye via the collector channels [5, 6].

ANATOMY OF THE CORNEA

The following two quotations give some perspective on the cornea and its function.

"The front chamber is completed by a layer of skin specialised to be glass-clear and free from blood vessels, which if present, with their blood, would throw shadows within the eye. This living glass-clear sheet is covered with a layer of tear-water which is constantly renewed. This tear-water has the special power of killing germs which might inflame the eye". Sir Charles Sherrington (1857–1952).

"The glass-clear bit of skin has only one of the fourfold set of skin senses; its touch is always "pain", for it should not be touched". Man, on his nature. Cambridge 1940.

The cornea is part of the outer coat and is embryologically related to the skin. However, its physiology is uniquely adapted to its main function, transparency as it has an important optical function. It is responsible for 2/3rd of the refraction taking place in the eye, as well as protection through its renewable epithelial surface. The cornea covers 1/6th of the circumference of the eyeball.

The layers of the cornea include: Epithelium, Bowman's membrane, stroma, Dua's layer, Descemet's membrane, and endothelium.

Table 4: Corneal characteristics

Diameter	Adult size is 12.5 horizontally x 11.5 vertically in mm New born cornea is relatively large, 10 mm vertically Premature infants at 34 weeks has 8.2 mm Adult size at age 2–3 years
Central thickness	520–535 µm
Peripheral thickness	650 µm
Radius of curvature	Adult anterior curvature: 7.8 mm (43.25 D) New born steeper: 6.62 mm (51.00 D) Premature infants at 34 weeks: 6.49–6.37 mm (52–53.00 D) Adult posterior curvature: 6.42–6.8 mm (52.5–49.62 D)
Refractive power of front surface	+48.8 D
Refractive power of the posterior surface of the cornea	-5.8 D
Net refractive power of the cornea	+43 D or 70% of the total refractive power of the eye
Refractive index	1.376
Water content	78%
Collagen content	15%
Other proteins	5%

Epithelium

The corneal epithelium makes up about 10% of the corneal thickness (50.6 ± 3.9 µm). It is stratified with 5–7 layers of tightly packed cells. Three types of cells are found: basal or tall columnar cells, wing or umbrella cells, and squamous or flattened cells. The basal cells are firmly attached to the basement membrane and the underlying Bowman's membrane by hemidesmosomes (half desmosomes) and are mitotically active. The basement membrane is normally between 120–200 nm thick and it increases in thickness by 3 nm per year in young patients. Along the lateral and anterior walls of the basal cells, numerous desmosomal contacts with neighbouring cells are found (Figures 7 & 8). These desmosomes and interdigitations make the epithelium mechanically strong and capable of significant mechanical shearing forces (such as rubbing the eye, or in contact lens wear) [5, 6]. The tight junctions serve as an anatomical barrier to the passage of substances into the intercellular space. The weakest part of the epithelium is the tall (18 µm) columnar basal cells, which will typically rupture just internal to the nuclei, leaving cytoplasm and other cell parts still attached to the basement membrane (Figure 6). As basal cells mature they migrate to the anterior surface. Once the basal cells have lost contact with the basement membrane, it becomes post-mitotic and therefore loses its ability to divide. The columnar shape transforms to flattened wing cells (three layers thick) and later to very flat squamous cells. Extensive interdigitation and desmosomal attachments are found which maintain cell shape. On reaching the surface the squamous cells are sloughed off into the tear film. This exfoliation process is driven by an apoptotic mechanism. It is estimated that the total epithelium renewal rate is ±7 days [5, 6].

The surface cells exhibit numerous microprojections (microvilli and microplicae) and have extensive fibrillar glycocalyx (glycocalyx is a glycoprotein secreted by the epithelial cells) facilitating the adherence of the tear film to the cells surface membranes. Squamous cells are the only corneal epithelial cells, expressing zonula occludens, preventing fluid from leaking into the cornea. Basal cells have higher levels of mitotic activity than the wing and squamous cells and therefore have large reserves of glycogen [5].

The corneal epithelial stem cells are located, as previously mentioned, between the palisades of Vogt at the limbus [10]. Limbal stem cells are slow-cycling (they don't divide frequently) but have tremendous potential to divide (up to the life of the host). These cells can be easily stimulated, to temporarily up-regulate mitosis in severe corneal injury or stress. The stem cells give rise to daughter cells, which move centripetally to form the basal epithelial cells of the cornea. The daughter cells divide more rapidly but are limited in the number of divisions before becoming terminally differentiated.

As previously mentioned the normal renewal rate of the epithelium is ±7 days. However, during contact lens wear this rate is altered due to: basal cells dividing at a slower rate, epithelium cells migrating at a slower rate, and slower apoptotic surface cell death of squamous cells. This is probably due to the reduction in oxygen caused by the lower oxygen transmissibility of the contact lens material. Keep in mind that a cornea with a stagnant epithelium is more prone to infection [5].

Apart from the epithelial cells, the corneal epithelium also harbours neurons, Langerhans cells and lymphocytes. The Langerhans cells are a peripheral component of the immune system and are mostly located peripherally and always in the basal cell layer. UV radiation causes loss of the Langerhans cells and contact lens wear increases the total number of cells in the corneal epithelium [5].

Functions of the corneal epithelium include: physical protection by forming a renewable layer of the corneal surface, refraction, UV radiation filter, tear stabilisation due to microprojections and glycocalyx, fluid barrier and microorganism shield due to zonula occludens [5].

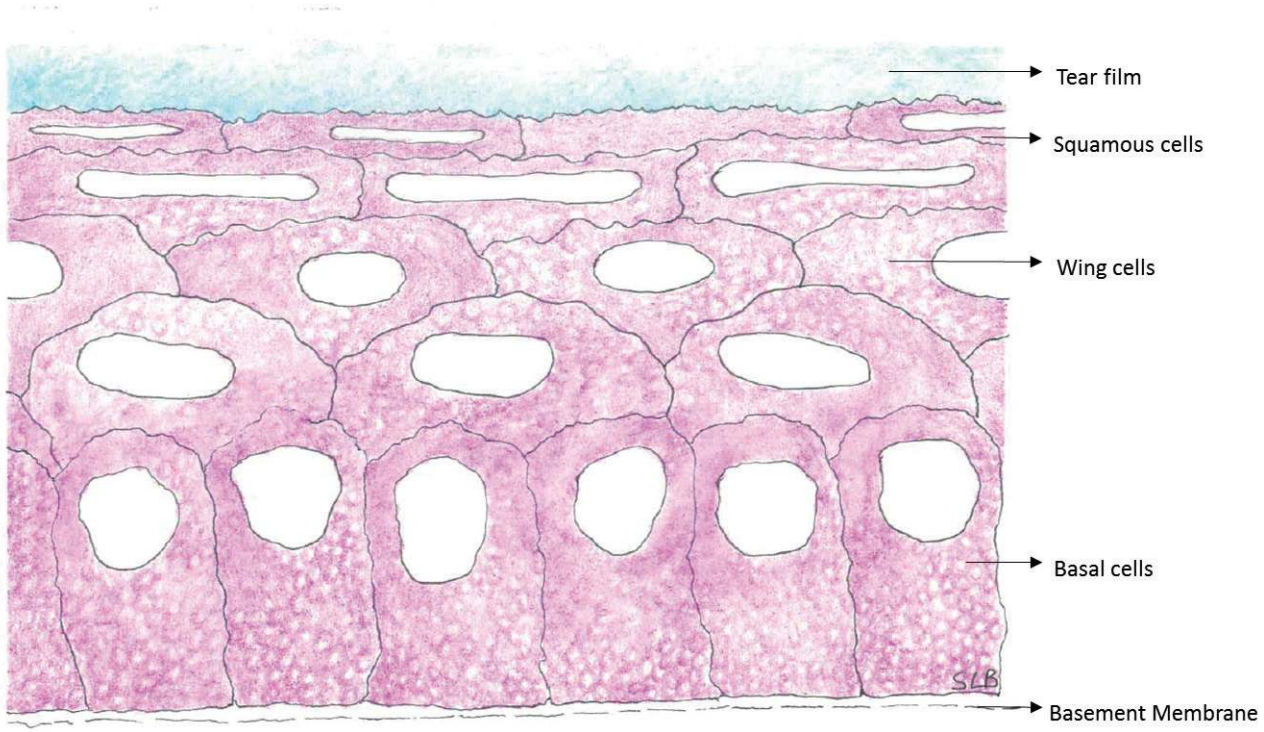

Figure 6: Cells of the corneal epithelium

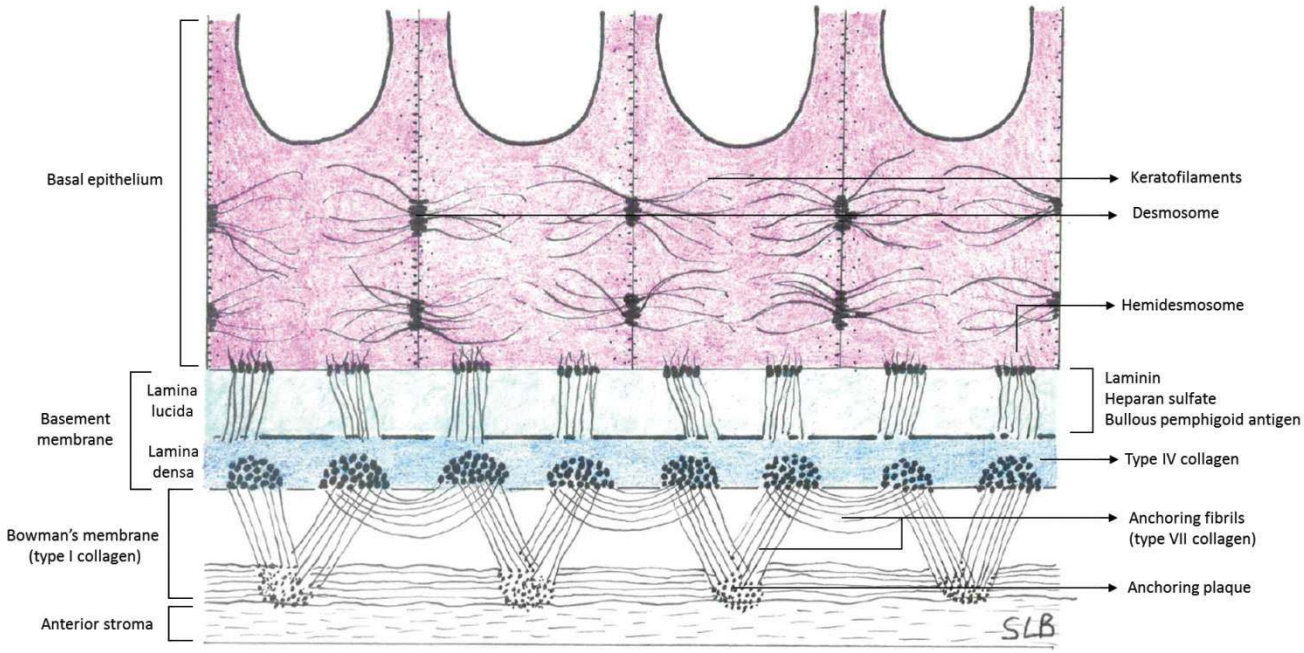

Figure 7: Anatomy of the corneal epithelium showing cell layers and the collagen of the anterior limiting membrane

Bowman's Membrane

Bowman' membrane consists of modified stromal tissue, is ±8.2 µm thick, and stops at the limbus. It is acellular and consists mainly of type 1 collagen fibres (smaller in diameter than stromal fibres). It is strongly attached to the epithelial basement membrane by type 7 collagen fibres, and the underlying stroma by fibrillar projections, as well as, stromal lamellae insertions (Figure 7). Bowman's layer has no regenerative capabilities [5, 6].

Stroma

The stroma makes up the bulk of the corneal thickness and is formed by collagen, ground substance and keratocytes. It is ±500 µm thick and peripherally continuous with the sclera [5].

Collagen

The bulk of the stroma is made up of type 1 collagen, type 3 collagen appears during stroma repair and type 4 makes up the basement membranes, as well as, the anterior and posterior limits of the stroma. Type 5 and 6 collagen fibres can also be found in the stroma. The collagen fibrils are arranged in into 200–300 (242 ± 4) lamellae parallel to the tear surface [17]. Interlacing lamellae cross each other in a highly regular fashion, at less than 90° in the anterior stroma, and are at nearly right angles, in the posterior stroma. The anterior lamellae tend to be thinner and therefore, ±50% of the lamellae are found in the anterior stroma than the posterior stroma which tend to have thicker lamellae [17]. The average thickness of stromal lamellae is 1.2 µm [17].

In the past, it was thought that the lamellae run the full width of the cornea. However, recent studies have shown that although this may be the case for posterior lamellae, some anterior lamellae end centrally in the cornea, as well as, below Bowman's membrane where they then run parallel to it. A cross section of the stroma will show some fibrils running parallel to the section and others nearly perpendicular. This layered arrangement of the fibrils facilitates lamellar dissection of the cornea. The small diameter of individual fibrils (22.5–35 nm) and the highly uniform arrangement of the lamellae contribute to the transparency of the cornea [5].

Ground Substance

The ground substance surrounding the collagen fibrils is rich in glycosaminoglycans (GAGs). Keratin sulphate and chondroitin sulphate are the primary GAGs of the stroma and occur in a 3:1 ratio. GAGs have a negative charge and high affinity for sodium creating a high imbibition pressure. GAGs attach to protein and form long cable like proteoglycans. Proteoglycans coat each individual collagen fibril and each fibril is connected along its length by strand of proteoglycans to other fibrils (crosslinks). Proteoglycans, therefore, maintain the regular array of collagen fibrils. With corneal oedema the collagen fibril size does not change, but the volume of the ground substance increase, and therefore the spacing between the fibrils also increase which leads to loss of transparency of the cornea [5, 6].

Keratocytes

Although keratocytes are the principal cells of the corneal stroma, small numbers of neutrophils, lymphocytes, plasma cells, and histocytes may also be present in the normal stroma. Around 2.4 million keratoocytes are present in the normal adult cornea. The keratocytes are large flat cells with a number of processes that extend out from the cell body in a stellate fashion. The cell bodies lie between packed collagen lamellae, and their processes extend within or between the same lamellar plane, although "trans-lamellar keratocytes" have been reported. Adjacent cells are linked via gap-junctions and communicate with one another. The distribution of the keratocytes is non-uniform throughout the cornea with larger numbers of cells occurring anterior adjacent to Bowman's membrane and decreasing by, as much as 30%, posteriorly through the depth of the cornea [5, 6]. Keratocytes are responsible for inter-corneal communication, wound healing, storing glycogen, and the production and maintenance of collagen and the proteoglycan ground substance. In response to stromal injury, the keratocytes migrate into the wound area and undergo transformation to fibroblasts. These transformed cells have increased rough endoplasmic reticula, Golgi complexes, and reduced cytoplasmic processes. They contribute to the scar formation by proliferation and collagen production [5].

Dua's Layer

This is a well-defined, acellular layer of 5–8 lamellae (10 μm thick), of predominantly type 1 collagen bundles arranged in transverse, longitudinal and oblique directions. It is located between the most posterior row of stromal keratocytes and Descemet's membrane [18].

Descemet's Membrane

Descemet's membrane is the basement membrane of the corneal endothelium and contains type 4 collagen and fibronectin. It is the thickest basement membrane in the body and is synthesised at a high rate by the endothelium throughout life. It is 3–20 μm thick and Schwalbe's line marks the termination of Descemet's membrane peripherally. The anterior portion is the oldest and least uniform. Peripherally, localised thickenings of Descemet's membrane are known as Hassall-Henle bodies. When these wart-like thickenings occur centrally, the condition is known as "guttata". In contrast to Bowman's layer, Descemet's membrane is easily separated from the stroma and after injury regenerates readily. Descemet's membrane is very resistant to pathological processes [5]. Fuchs describes this resistance as follows: "when the entire cornea has broken down into pus, we often see the thin Descemet's membrane offering resistance remaining unimpaired for days"[6].

Endothelium

The corneal endothelium consists of a single layer of flat squamous, predominantly hexagonal cells that cannot reproduce. At the corneal periphery (limbus), the endothelium terminates as a monolayer and then continues as a modified form to become the trabecular meshwork. The endothelial cell density naturally declines with age (average of 0.6% per year) [19], but surgery, trauma, and disease can greatly accelerate this decline in cell density [5, 6].

Table 5: Endothelial cell density throughout life [5]

Age	Endothelial cells/mm^2
Birth	2987–5632 (Average 4252–4425)
20–30 years	3000–3500
40–50 years	2500–3000
80 years	2000–2500
Functional limit	700–1000

Complete coverage of the posterior surface of the cornea by the endothelium is essential for corneal function. Without coverage, corneal oedema occurs. The endothelial cells are densely packed with mitochondria, as well as endoplasmic reticulum, and the cells have numerous interdigitations along their lateral walls. Cell junctions include: zonula occludens, zonula adherens, and gap junctions [5]. The function of the zonula occludens is to limit the movement of fluid between the cells. The gap junctions function is to facilitate extracellular exchange and communication between the cells. Zonula adherens are weaker and support cell-to-cell adherence (Figure 8). The endothelium controls the movement of fluid between the cornea and aqueous by actively pumping water from the cornea to maintain corneal transparency. Glucose travels from the aqueous to the cornea and lactic acid from the cornea to the aqueous via the endothelium [5, 20]. Endothelial cells react to chronic anterior hypoxia, often caused by contact lenses, by varying the size of its posterior apical side known as polymegethism. As the result of age, trauma, and UV radiation some cells may become larger than others. This reflects loss of cell density, with neighbouring cells spreading to fill the gap explaining polymegethism, pleomorphism (cells of varying shape) and polygonality (cells with varying numbers of sides) [5, 21]. These conditions are discussed in more detail in chapter 16.

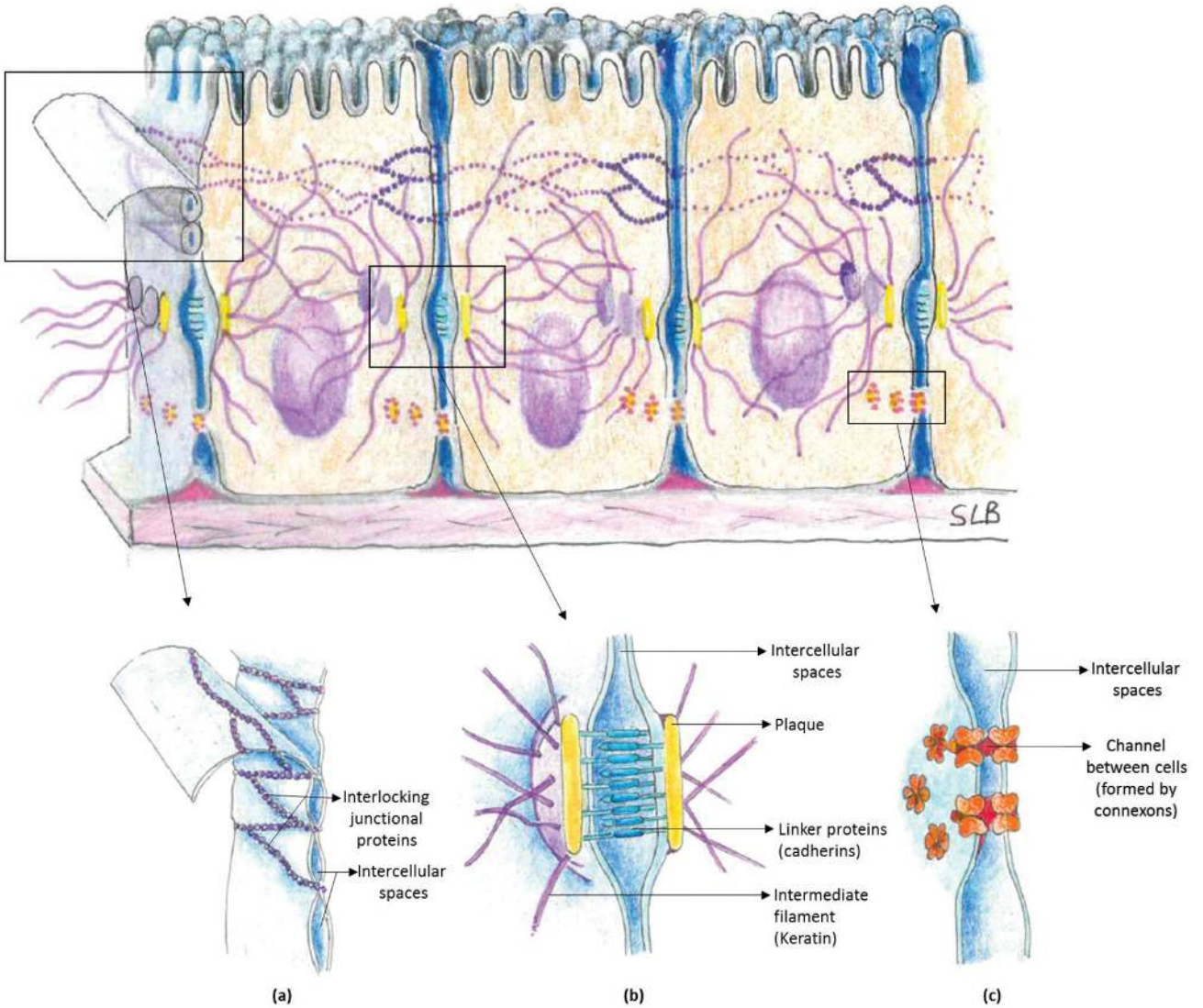

Figure 8: Cell junctions in the corneal epithelium and endothelium.

Note that the junctions are not all present in both types of cells. Tight junction (a) are impermeable preventing molecules from passing through the intercellular space. Desmosome (b) are anchoring junctions which hold adjacent cells together like molecular "Velcro". They help form an internal tension reducing network of fibres. Gap junctions (c) are communicating junctions which allow small molecules to pass between cells for intercellular communication.

Corneal Sensory Innervation

The bulk of the corneal sensory fibres arise from the ophthalmic division of the 5th cranial nerve and some from the maxillary division. The fibres enter the eye posteriorly through the short ciliary nerves, which is close to the optic nerve, or through the long ciliary nerves more anteriorly. The fibres enter the cornea radially and lose their myelination after ±1.5 mm. The fibres branch toward the apex and traverse approximately 2/3 of the cornea. The normal corneal touch threshold is 40 mgm/mm^2. In contact lens wear, this threshold often reduces to 80 mgm/mm^2 due to hypoxia [22]. Some authors report a variation in touch threshold according to ocular pigmentation, race and age. Blue eyes are the most sensitive, followed by, hazel eyes, green eyes, brown eyes and dark brown eyes [23, 24]. However, the literature is ambiguous with more recent research confirming that corneal sensitivity is affected by iris colour and ethnicity; and that whites have a progressive decrease in sensitivity with increasing iris pigmentation [25]. Others report no relationship between eye colour and corneal sensitivity [26].

It is also interesting to note that the lid margin touch threshold is similar to that of the corneal apex (20 mgm/mm^2) but the conjunctiva is much less sensitive (70–200 mgm/mm^2) [20].

Table 6: Corneal touch thresholds at different ages [5]

Age	Touch threshold (mgm/mm^2)
7–10	18.3
11–20	20.9
21–30	21.3
31–40	19.7
41–50	25
51–60	26.7
61–70	33.5
71–80	36

Two plexi are formed, one in the epithelium, which is most dense and one in the stroma, which forms a second line of defence. The epithelial nerve fibres are usually found deep in the basal cells, but small branches move anteriorly between the cells to just beneath the surface squamous layer of the cells. The fibres in the corneal stroma terminate very close to the epithelium, but there is very little communication between the stromal and epithelial plexi in the central cornea. As previously mentioned, the nerve fibres of the stroma are unmyelinated with only Schwann cell wrappings. The nerve fibres of the epithelium are naked without Schwann cell or myelin wrappings. The corneal nerve fibres convey more than one type of stimuli. Touch and pain are the primary stimuli carried by the corneal nerves. Temperature is sensed less than these two main stimuli [5, 20].

PHYSIOLOGY OF CORNEAL HYDRATION

Corneal Transparency

The normal cornea transmits > 90% of light at 400 nm, its refractive index is n = 1.376 and is made up of a collagen and ground substance component (collagen n = 1.345 + ground substance n = 1.550 = 1.376). Collagen fibrils are small, 20–40 nm in diameter, and uniformly separated by distances of ±60 nm [5, 20].

Maurice, 1957 originally proposed that the collagen fibrils of the stroma were organised into a "hexagonal" or "lattice" array that would act like a diffraction grating, eliminating scattered light through destructive interference, and thereby promoting light transmission through the tissue [27]. Other theories suggested that the closely spaced fibrils were simply too small to scatter light. More recent studies using the electron microscope show little evidence of the "hexagonal" order suggested by Maurice. It has been concluded that although order is important, the corneal stroma is transparent because the fibrils are so small and the fact that the balance between the refractive index of the fibrils and ground substance is just right to promote transparency. Keratocytes and other cellular components are too few in number and too small to hinder light transmission [20]. The balance of refractive index distribution is partly determined by the hydration of the corneal tissue, which also affects the fibril organisation and keratocyte light scattering properties, and therefore corneal transparency [5].

Oxygen, Glucose and the Biochemistry of the Corneal and Pre-Corneal Fluids

The biochemical properties of the fluid within the corneal stroma (interstitial fluid), fluid between the cell layers (para-cellular fluid), and the aqueous and tear film are important when considering the overall hydration of the corneal tissue.

Oxygen Availability and Solubility

In the open eye, oxygen is obtained from the atmosphere and dissolves rapidly into the tear film. The partial pressure of oxygen in the tear film is 155 mmHg (21%) at sea level, and 35–50 mmHg (5–7%) for the aqueous humour.

This creates a steep gradient of oxygen from the anterior to posterior cornea. Oxygen supply to the cornea can be altered by sleep (closed eye environment) and the presence of a contact lens on the eye [5]. The partial pressure of oxygen in the tear film is affected by altitude. The higher the altitude, the lower the partial pressure of oxygen. At an altitude above sea level of 5500 feet (as in Johannesburg South Africa), the partial pressure of oxygen is around 129 mmHg (17%), which is significantly lower than at sea level.

Glucose Supply and Utilisation

The aqueous humour contains 5 mM of secreted glucose from the blood supply. This glucose can diffuse across the corneal endothelium into the corneal tissues. In contrast, the normal tear film, normally only contains trace elements of glucose (<0.05 mM). This concentration can increase significantly with uncontrolled diabetes causing vascular stress in the conjunctiva. Other sources of glucose are from the limbal portions of the stroma. This creates a steep gradient of glucose from posterior to anterior cornea, with the endothelium and stromal keratocytes readily obtaining glucose from the aqueous. The epithelium is impermeable to glucose and the aqueous is therefore the main glucose source, with the limbus the secondary source. The endothelium and keratocyes use only small amounts of glucose with the remainder (±60%) diffusing anteriorly to be used by the epithelium. However, some glucose is stored as glycogen in the corneal epithelial cells, which can be converted back to glucose for use when needed [5, 20].

High levels of glucose in poorly controlled diabetes leads to an excess production of sorbitol within the cornea that affects both the corneal epithelium and endothelium. This leads to delayed wound healing, as well as, corneal swelling.

Oxygen and glucose is essential for metabolism, which provides energy for cell function. Epithelial metabolism is *normoxic* in the open eye, becoming *hypoxic* in the closed eye or in the presence of a contact lens. In contrast, the endothelial metabolism is always *hypoxic* [5, 20]. The Embden-Myerhoff pathway describes the metabolic process by which, corneal cells convert glucose into pyruvate that enters the mitochondria to be oxidised, to produce large quantities of ATP in a *normoxic* environment. Tissue levels of at least 50 mmHg (7%) oxygen is probably required for this metabolism to occur and cell cytoplasm, pH is probably around 7.3. In a *hypoxic* environment epithelial cells can convert glucose to pyruvate (called glycolysis), by producing a small amount of adenosine triphosphate (ATP) along with the metabolic by product lactate. Cellular cytoplasm pH is more acidic around 6.75, due to increase tissue levels of CO_2 and lactate. Deeper corneal tissues never achieve *normoxic* conditions and therefore, some lactate is always present in the cells (5–10 mM). In a healthy cornea, lactate is actively transported out of the tissue, normally into the aqueous humour. Sustained lactate levels > 10 mM in the cells and para-cellular spaces between the cells, exerts a hypertonic effect, resulting in corneal swelling [20].

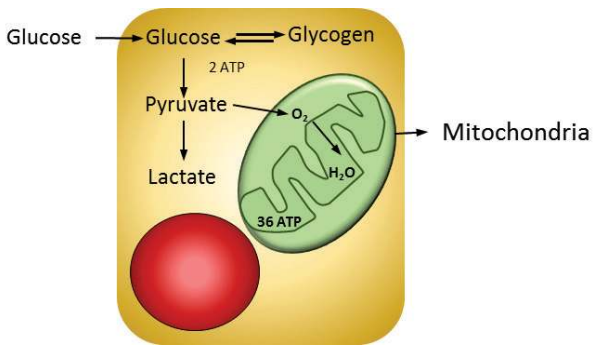

Figure 9: Metabolism in the corneal epithelium

This diagram shows the use of glucose by the cells for energy production. Glucose enters the cell by diffusion and is stored as glycogen. Glycogen can be metabolised by glycolysis to pyruvate and 2ATP molecules. In the presence of oxygen some pyruvate can be further metabolised in the mitochondria to yield a further 36ATP. If oxygen is not present, the pyruvate is metabolised to lactate, which must be excreted from the cell.

Buffering and Osmolarity

The pH buffering capacity of the corneal fluids is primarily determined by the concentration of bicarbonate that is in equilibrium with dissolved CO_2 levels. There is an iron clad relationship between the bicarbonate concentration, pH, and CO_2 concentration defined by the Henderson-Hasselbach equation. It is important to understand that if one of the variables is changed, the others will have to change too, and these changes are very predictable [5, 20]. In the open eye, the tear bicarbonate varies between 5–25 mM, depending on the lacrimal flow rate. The pCO_2 is low at < 5 mmHg and the pH therefore is 7.5–7.8. In contrast, the aqueous bicarbonate is 30 mM, at 55 mmHg pCO_2 and it pH is 7.4 [5].

In the closed eye, this changes with the tear bicarbonate <5 mM and the pCO_2 rising to be close to that of the blood vessels of the lid (55 mmHg), resulting in a condition of hypercapnia for the corneal epithelium and acidic tear pH 6.95. The aqueous also changes with bicarbonate levels 25 mM at pCO_2 of 55 mmHg with pH 7.10 [5].

The corneal tissue being between the tear film and aqueous will adopt pH, pCO_2, bicarbonate and pO_2 values between these two fluid layers. The osmolality of the tear film is influenced by its ability to evaporate (usually 305 mOsm/kg). The aqueous humour osmolality is in equilibrium, and maintained by, the vasculature of the anterior segment of the eye (usually 295 mOsm/kg). Creating a hyperosmotic force, by using hypertonic saline, can cause fluid to move from the anterior stroma and epithelium. This is a commonly used clinical therapy for instances of superficial corneal oedema [5].

Hydration of the Corneal Stroma

The corneal stroma has an enormous capacity to increase its hydration, but these changes are never realised *in situ.* Corneal tissue normally has a hydration equivalent to 3.55 mg H_2O/mg of dry tissue or the equivalent of the tissue being 78% water. A change to 80% water or 4.55 mg H_2O/mg of dry tissue indicates slight oedema and 83% water or 6 mg H_2O/mg of dry tissue, severe oedema. Values higher than 83% water are seldom found in clinical practice [5]. When the cornea swells, its thickness increases, and this relationship is linear. However, the cornea swells more centrally than peripherally with the endothelium moving into the anterior chamber, this results in a well-known phenomenon called posterior striae. The fact that the peripheral cornea swells less than the central cornea is due to "scleral clamping", indicating the surrounding sclera physically stops the peripheral stroma from swelling. The normal imbibition (suction) pressure of the stroma in the living eye is between 40–60 mmHg and to counter this, *in vivo,* requires intact barrier functions of both the endothelial and epithelial cell layers of the cornea [5].

The Role of the Corneal Epithelium in Maintaining Corneal Hydration

Normal corneal transparency, thickness and hydration are maintained within narrow limits by healthy cells. The epithelium is a "tight" barrier, which limits fluid flow in and out of the tissue. Although the epitheliums role is relatively passive in the maintenance of corneal hydration, a number of unique properties determine this passive barrier function. Damage to the epithelium can result in either dehydration or an increase in hydration of the cornea, depending on the exact nature of the epithelial damage and changes. The epithelium supports a stable tear film. However, if the epithelium is compromised the disruption of the tear film, can lead to increased tear evaporation and dry eye problems, which is associated with dehydration of the corneal tissues. Another important property of the epithelium is the "tight" barrier function limiting permeability to water, cations and anions. This effectively stops the ingress of water into the cornea from the tear film. This barrier function is achieved due to the fact that the epithelium is a high resistance, ion-transporting cell layer [5–7]. An active pump (Na/K ATPase) mechanism uses energy from metabolism and ions are pumped from the tissues so that water will follow by osmosis. Sodium is pumped to the corneal stroma and potassium to the tear film. A trans-epithelial potential difference

(15–20 mV) and high electrical resistance, result due to the efflux of chloride ions from the epithelial cells to the tear film. In the *normoxic* cornea, 1 glucose molecule is the fuel for glycolysis and is broken down into 2 pyruvate molecules and lactate producing 2 ATP molecules. Furthermore, pyruvate is burned by the citric acid cycle (oxidative phosphorylation) in the cells mitochondria, producing CO_2 and 36 ATP molecules (Figure 9). Therefore, 1 Glucose molecule = 2 ATP (from glycolysis) + 36 ATP (citric acid cycle & oxidative phosphorylation). In the *hypoxic,* cornea stored glycogen undergoes glycolysis, which is very inefficient at producing ATP [20]. Extended hypoxia can result in substantial depletion of the epithelial glycogen reserves. Glucose metabolism slows down and a disproportional increase in the production of lactate in the cells result. Intracellular pH is lowered slowing glycolytic activity. Although lactate can be converted back to pyruvate, this only occurs only if the citric acid cycle is active which requires oxygen. The superficial epithelium is impermeable to lactate, which means it must couple with a proton to move into extracellular space with help of a co- transporter (Figure 10). It can then move into the stroma, endothelium and eventually aqueous humour. Increased lactate leads to cellular acidification, cell glycogen depletion, reduction in ATP levels, corneal swelling and loss of barrier function [5].

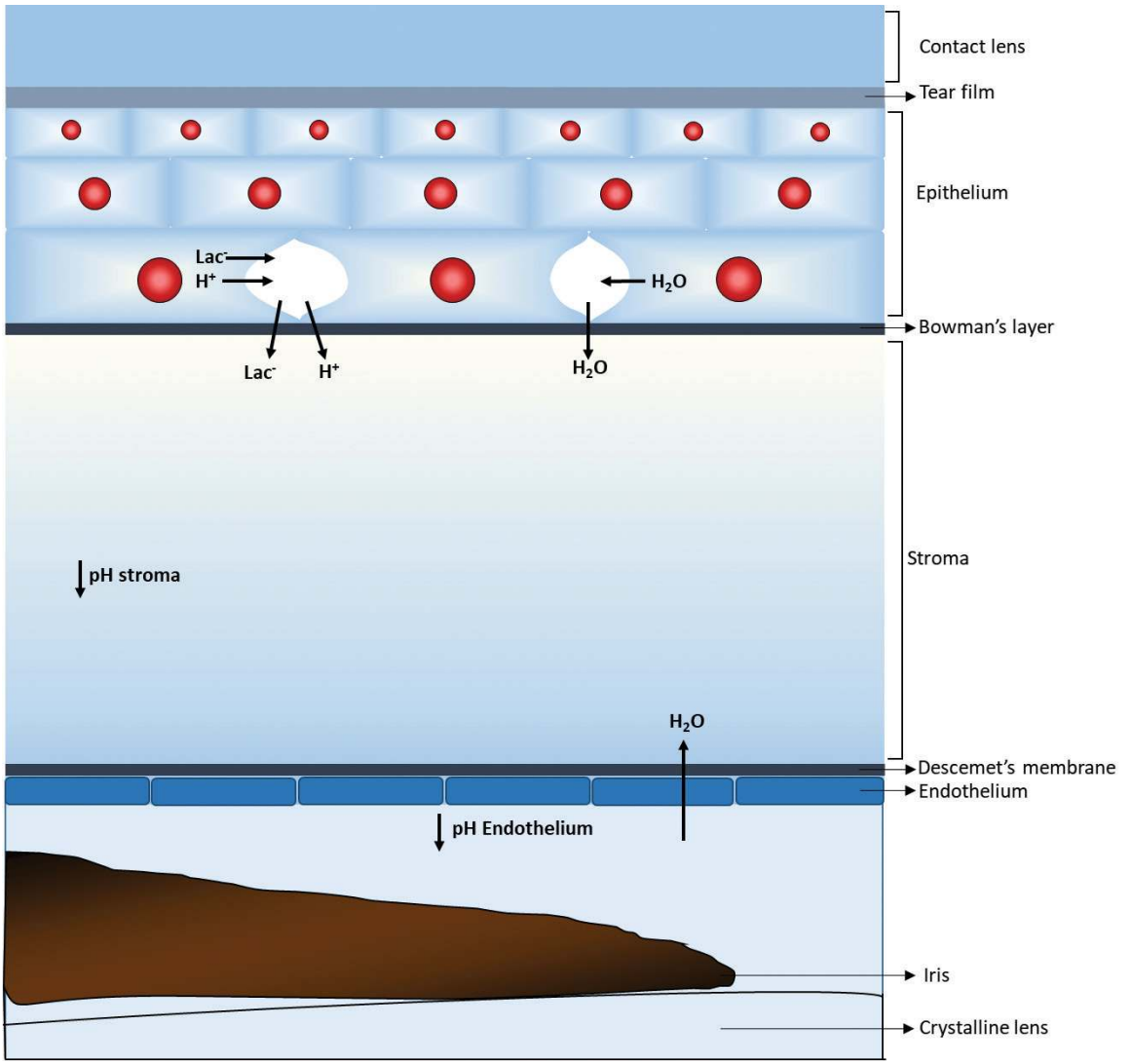

Figure 10: Diagram showing the fate of lactate and H+ produced by the epithelium

Both leave the epithelium via lactate H^+ cotransporters and diffuse into the stroma to eventually end in the aqueous. The accumulation of lactate in the stroma in between the epithelial cells osmotically draw fluid into these spaces. In the epithelium this leads to increased light scatter and in the stroma swelling (oedema). Stroma and endothelial pH drops which may affect the fluid pump leading to more swelling.

The Role of the Corneal Endothelium in Maintaining Corneal Hydration

Many texts consider that the endothelium is the site of a fluid pump that removes fluid from the corneal stroma and that this pump is totally responsible for maintaining corneal hydration and thickness. The *pump-leak hypothesis* simply states that the passive natural leakage of fluid into the stroma driven by imbibition pressure is counteracted by an active bicarbonate-and CO_2-dependent endothelial pump. This pump works by transporting bicarbonate in the stroma toward the aqueous, both across the apical membrane of the endothelial cells and into the para-cellular spaces (Figure 10). Water passively follows the bicarbonate transport to maintain corneal hydration. Experiments have shown that the net fluid pump rate is about 5 µl/h/cm^2, which thins the corneal stroma by 50 µm/h. Evidence also shows that Na^+/K^+ is actively pumped from the corneal stroma across the endothelium into the aqueous against a concentration gradient for Na^+. The normal concentration of Na^+ within the aqueous is higher (143 mEq/L) than that of the stroma (134 mEq/L). The fact that the epithelium and stroma consumes about 40% and 39% oxygen respectively, the endothelium consumes 21% oxygen but this consumption is 6x faster than that of the epithelium and endothelium supports this hypothesis [20]. Most recently, it has been proposed that the endothelium is not as tight a barrier as the epithelium, but it has a finite permeability to cations and anions. It is thought that in addition to the active pump function, permeability is regulated by cell metabolism and its functions. Changes in cell volume alter the para-cellular space pathway and thus change the net ionic permeability and fluid flow across the endothelial cell layer [5].

Table 7: Summary of corneal physiology [5]

Biological chemistry of the corneal and pre-corneal fluids	
Physiological range of pH	6.5 to 8.0
PCO_2 of aqueous	55 mmHg
PCO_2 of the tear film	<5 mmHg
Bicarbonate concentration of the aqueous	30 mM
Bicarbonate concentration of the tear film	12 to 25 mM
pH of tear film in the open eye	7.5 to 7.8
pH of aqueous in the open eye	7.4
pH of the tear film in the closed eye	6.95
pH of the aqueous in the closed eye	7.1
PO_2 of the aqueous	35 to 50 mmHg
PO_2 of the tear film in the open eye	155 mmHg
Osmolality of the tear film	305 mOsm/kg
Osmolality of the aqueous	295 mOmsm/kg
Donnan osmotic pressure in the stromal tissue	40 to 60 mOmsm/kg
Glucose metabolism by the corneal cells	
Glucose concentration in the aqueous	5 mM
Glucose concentration in the tears	<0.5 mM
Sea level oxygen tension	155 mmHg
Blood stream oxygen tension	55 mmHg
pH at which lactate production is favoured	6.75
Normal cellular lactate concentration	5 to 10 mM

Normal vs stressed cornea – characteristics	
Average corneal thickness (CCT)	535 µm (95% within range 445 to 600 µm)
Average corneal thickness - periphery	700 µm or 23% thicker than CCT
Corneal thickening overnight	3%
CCT in the new born	650 µm
CCT increase in sustained hypoxia	Up to 15%
CCT increase with gross epithelial damage	Up to 150%
CCT increase with gross endothelial damage	300%
Average corneal stromal thickness in keratoconus	350 µm

How Much Oxygen Does the Cornea Require to Maintain Normal Physiology?

Using evidence of corneal oedema, problems with mitosis and cell metabolism, lactate accumulation, and loss of corneal sensitivity, the following criteria were established by different researchers (Table 8).

Table 8: Oxygen requirements of the human cornea [22, 28–31]

Oxygen Level	Physiological Response
8%	Avoids loss of corneal sensitivity (Millodot & O'leary, 1980)
9%	Minimum safe daily wear
10%	Avoids corneal oedema in open eye wear (Holden, Sweeney & Sanderson, 1984)
12%	Minimum safe extended wear
13%	Maintains epithelial mitosis and avoids lactate accumulation in anterior chamber (Hamano et. al., 1983)
15%	Avoids microcysts and polymegethism in extended wear (Holden, 1988)
18%	Avoids swelling beyond physiological amount during normal eye closure of 4% (Holden & Mertz, 1984)

Permeability (Dk), Transmissibility (Dk/T Or Dk/L), EOP, Oxygen Flux and Consumption [32]

Permeability (Dk) is a property of the lens material and is the product of diffusivity and solubility of a gas. Water content governs permeability in hydrogel lenses and silicone content in silicone hydrogel lenses.

Transmissibility (Dk/t or Dk/L) is the function of the permeability related to the thickness of the lens. This gives a measure of oxygen transmissibility that shows the amount of oxygen that can pass through a contact lens in air. The measure of Dk/t has been used as an industry standard for hydrogel contact lenses for more than 30 years. In this text Dk/t will be used to refer to transmissibility. However, originally Fatt and Hill used Dk/L where L refers to thickness. As Dick Hill and Alan Saks state: "it makes an L of a difference". Although this measure is valid for hydrogel lenses, it is arguably no longer relevant where silicone hydrogels are concerned. Transmissibility in hydrogel materials is influenced by:

- Temperature
- pH
- On eye hydration (up to 6% less than absolute water content)
- Lens prescription
- Edge thickness

Equivalent oxygen percentage (EOP) is described by Hill as the oxygen availability beneath the lens, in other words EOP predicts the partial pressure of oxygen at the front surface of the cornea [33]. EOP has a high correlation (95%) with Dk/t. Dk/t only describes the amount of oxygen passing through a contact lens in laboratory conditions [33]. It does not describe the on-eye situation. The measurement of oxygen flux takes a step further than Dk/t by calculating the amount of oxygen that passes through a lens on the eye and enters the cornea. As such it attempts to better describe the on-eye situation of a contact lens [34]. An eye wearing no contact lens would have an oxygen flux of 100% with a normal amount of atmospheric oxygen being able to enter the eye. Brien Holden aptly stated that: "flux sucks". The amount of oxygen that can enter the eye has a natural upper limit due to the partial pressure of oxygen in the atmosphere. This limit is 159 mmHg at sea level. It is because of this that beyond a certain level doubling the Dk/t does not lead to the doubling of oxygen entering the eye. This is called the law of diminishing returns and is illustrated in the graph [35] (Figure 11). In fact, moving from a Dk/t of 60 to one nearly three times greater of 175 only results in an extra 3% of oxygen entering the central cornea (Table 9) [32].

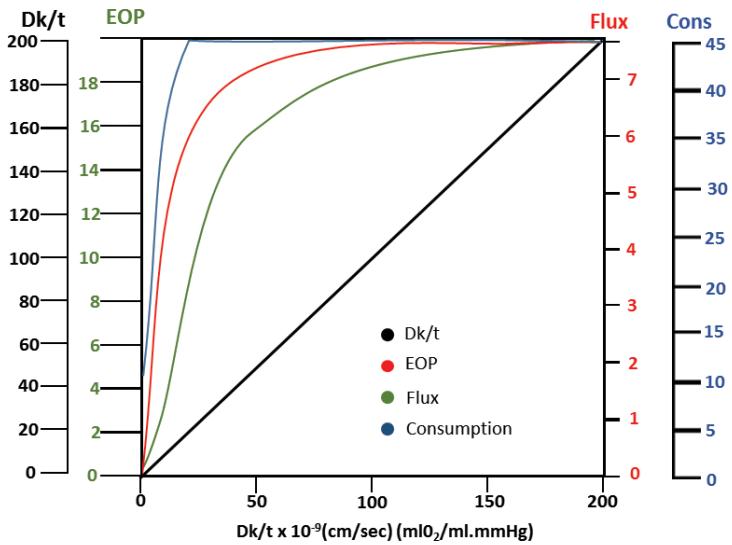

Figure 11: Combined graph showing DK/t, EOP, anterior corneal oxygen flux (µl/cm2hr), and consumption (nl/cm3sec) as a function of DK/t for the open eye

This graph adapted from Brennan and Morgan, 2009 clearly illustrates the "law of diminishing returns" [32, 35]

Fick's law: oxygen flux = Dk/tΔP where ΔP represents the oxygen pressure gradient between the front and back of the lens. When the pressure gradient is high the oxygen flux is high.

While oxygen flux does a better job of illustrating the amount of oxygen entering the anterior cornea, it still falls short as a measure of corneal metabolism because oxygen can also enter through the posterior cornea and limbal vessels. It is important to be able to model corneal metabolism to understand the amount of energy that the cornea is generating to carry out its normal functions. The amount of oxygen available for these processes is the rate limiting step. And so, by understanding the oxygen consumption needs of the cornea we can in turn understand if a contact lens placed on its surface is compromising that process or not. Finally, the point to remember when selecting a lens is that all currently available silicone hydrogel contact lenses provide adequate oxygen to the eye for daily wear irrespective of their Dk/t.

The following table illustrates the transmissibility and oxygen flux of commonly used hydrogel and silicone hydrogel lenses.

Table 9: Oxygen flux (µl/cm2hr) comppared to Dk/t x10–9(cm/sec)(mlO_2/ml.mmHg) of some commonly used disposable contact lenses

Contact lens	Dk/t	Open eye flux	Closed eye flux
Hema 0.1 mm thick	7.5	52%	25%
Acuvue 2 Etafilcon A	26	88%	68%
Acuvue Advance Galyfilcon A	86	97%	92%
Acuvue Oasys Senofilcon A	147	98%	96%
Pure Vision Balafilcon A	110	98%	94%
Night and Day Lotrafilcon A	175	99%	97%
No lens	∞	100%	97%

The following table illustrates the new minimum oxygen thresholds for extended wear contact lenses including the rational used for each threshold.

Table 10: Minimum oxygen thresholds for extended wear contact lenses [29, 36–39]

Author	Rational	Dk/t $x10^{-9}$(cm/sec) (mlO_2/ml.mmHg)	Oxygen Flux µl/cm^2hr
Papas, 1998	**Absence of induced limbal hyperaemia**	**56**	**87%**
Holden & Mertz, 1984	**Corneal swelling with lens wear of 4%**	**87**	**92%**
Harvitt & Bonanno, 1999	**Absence of epithelial anoxia**	**89**	**92%**
Sweeney, 2004	**Absence of epithelial anoxia**	**125**	**95%**
Papas, 1998	**Absence of induced limbal hyperaemia, mean result**	**125**	**95%**
Papas, 1998	**Absence of induced limbal hyperaemia, 95% confidence level**	**274**	**98%**
Giasson & Bonanno, 1994	**Absence of epithelial pH change**	**300**	**98%**

PHYSIOLOGY OF CORNEAL DEFENCE MECHANISMS [40, 41]

We are constantly exposed to a variety of microorganisms, which includes bacteria, viruses, and fungi. In most cases these microorganisms do not produce infection because the skin and mucous membrane surfaces provide effective barriers against invasion. Some organisms can invade directly through these barriers or can be introduced into the body through lesions from trauma or surgery. The immune system usually deals with them quite effectively. Conversely, some organisms possess special properties that allow them to overcome the immune system or the patient's immune system does not always function optimally. This may allow microorganisms that would not normally

pose a problem to cause an infectious disease. The normal conjunctiva as with other mucus membranes sustain a permanent flora of indigenous bacteria. These organisms constitute a protective host defence mechanism that helps to prevent pathogens from multiplying efficiently. Normal flora can become pathogens in immune-compromised or debilitated patients. Viruses and parasites often present in asymptomatic individuals are not considered a part of the normal flora. Several studies have documented that the normal flora resembles that of the upper respiratory tract and eyelid skin. The normal flora of the conjunctiva and anterior eye include the following organisms [42]:

Gram-Positive Organisms

- Staphylococcus aureus
- Staphylococcus epidermidis (most common organism)
- Streptococcus pneumoniae occurs occasionally
- Corynebacterium diphtheria is more common in over 20-year age group
- Propionibacterium acnes are an anaerobe from the skin also commonly found on the conjunctiva

Gram-Negative Organisms

- Haemophilus influenza
- Escherichia coli
- Pseudomonas aeruginosa

Although these organisms are everywhere they rarely cause infections. As mentioned previously, the immunological status of the individual is important. However, the ocular defence mechanisms also play a vital role.

Ocular Defence Mechanisms [11, 43]

- Lower ocular surface temperatures – most bacteria have very specific temperature requirements and therefore cannot thrive on the ocular surface
- Mechanical action of blinking sweeps the corneal surface at least three times per minute, effectively removing microorganisms from the ocular surface
- Irrigation by the tear layer and blink sweeps microbes into the nasolacrimal system and nasopharynx
- Soluble mucins and membrane bound mucins bind and prevent bacterial adherence to the corneal epithelium
- Tear lysozyme, lactoferrin, specific immunoglobulins, antimicrobial peptides as well as protein complement in tears inhibit bacterial growth and viability
- Intact epithelial surface of the cornea as well as its active and passive defence mechanisms inhibit bacterial penetration as well as growth and viability

Corneal Specific Defence Mechanisms [11, 21, 43–46]

- Passive or structural defence mechanisms are due to the tight epithelial cell junctions and cell orientation or polarity. The epithelial cells are only susceptible from underneath and the tight junctions maintain polarity preventing para-cellular bacterial penetration
- Active or biochemical defences involving β-defensin 2, surfactant protein D, cytokines, and pattern recognition receptors are produced by the cell nucleus, and then secreted by the cell when bacteria are detected. β-defensin 2 has a direct bactericidal activity
- Corneal epithelial cells can internalise bacteria and subsequently traffic them to perinuclear vacuoles within the cell. Acidification then reduces the viability of the intracellular bacteria

- Glycocalyx is negatively charged and repels bacteria while mucin binds bacteria and inhibits bacterial binding to epithelial cells. Bacteria does not particularly like the apical surfaces of corneal epithelial cells and remains a significant distance above the cell surfaces. Only occasionally will the bacteria home in on cells. Usually, this occurs only when the cells are dead or dying
- Tear fluid bathes the epithelial cells, increasing the epithelial cells barrier function, thereby regulating the immunity of the epithelial cells
- Exfoliation and normal cell sloughing removes infected cells
- The basal lamina separates the epithelium from the stroma and acts as a final barrier to infection by bacteria. It acts as a filter because its pores are smaller than the bacteria, preventing the bacteria from entering the corneal stroma

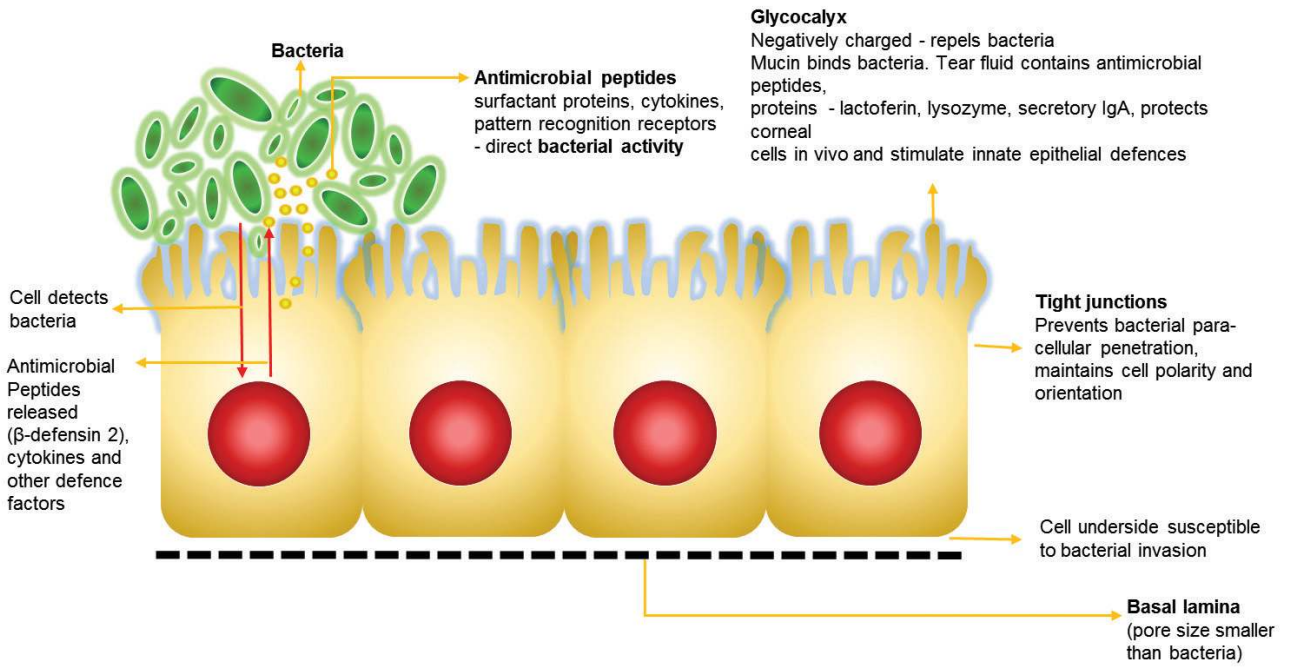

Figure 12: Corneal defence mechanisms

Why Does Contact Lens Wear Predispose the Cornea to Infection? [21, 45, 46]

Contact lens wear is a leading risk factor for the development of microbial keratitis (MK), especially *Pseudomonas aeruginosa* keratitis. The development of silicone hydrogel contact lens materials with vastly improved oxygen transmissibility has not reduced the incidence of MK suggesting that hypoxia is not critical to its pathogenesis. Based on current knowledge, and because extended wear is a risk factor, it is believed that bacterial adaptation coincident with changes to the biochemistry of the tear film, under the contact lens, is the most important contributors to the development of MK. When a contact lens is placed on the eye it interferes with the effective sweeping action of the lids as well as the normal tear flow and exchange. Furthermore, the contact lens provides a surface for the bacteria to stick to and form a biofilm on the lens surface, which protect the bacteria from the corneal and ocular defence mechanisms. Contact lens wear also hinder epithelial antimicrobial defences while still allowing pro-inflammatory mediators to compromise epithelial barrier function.

A common theme for the above mechanisms is the requirement for an extended exposure time. This entails the development contact lens biofilms, suppression of epithelial antimicrobial peptide expression, changes to tear film biochemistry and activation of immune responses. The need to wait for these events to unfold is likely

to explain the elevated risk of MK during extended wear and it provides us with an avenue to reduce the risk of MK.

Finally, the three critical defence layers include; tear layer, epithelium and the basal lamina. Redundancy suggests that all three may need to be compromised to get a corneal infection.

What are the Effects of Severe Hypoxia on Corneal Cell Function [11, 20]

- Stromal and aqueous pH is lowered and has a negative effect on the endothelium function
- When glucose consumption exceeds diffusion from aqueous, the stored glycogen is used until depleted, this takes about two hours in severe hypoxia
- Corneal swelling takes place due to the loss of the endothelial, epithelial and endothelial ion pumps
- Corneal transparency is lost
- Epithelial cell mitotic activity slows down as well as the formation of tight junctions and the active as well as passive defence mechanisms are lost
- Superficial epithelial cell desquamation (exfoliation) takes place making the epithelium more susceptible to microbial penetration and infection
- Finally, the cells die

THE TEAR FILM

Recent tomographic, interferometric and reflectance spectral techniques indicate central corneal tear film thickness values around 3 μm. The thickness of the thin outermost lipid layer of the tear film is around 50 to 100 nm in thickness and forms the barrier between the environment and the eye. The lipid secretions arise mainly from the Meibomian/tarsal glands, are combined with a lesser lipid contribution from the eyelid glands of Moll and Zeiss, and then spread across the tear film surface by blinking. The lipid layer comprises a thin inner polar layer overlaid by a thicker outer non-polar layer. In addition to preventing overspill of tear fluid onto the eyelids, prevention of contamination of the tear film by skin lipids, the most significant role of the lipid envelope is considered to be in retarding evaporation from the ocular surface [7, 20, 47–49].

The aqueous phase of the tear film forms the bulk of the tear film thickness. It arises primarily from the main lacrimal gland and accessory lacrimal glands of Krause and Wolfring. Additional fluid and electrolytes are secreted by the ocular surface epithelial cells. The tear flow rate varies according to the level of sensory stimulation in response to the demands of the external environment. The overnight tear production rate is significantly lower than production during the day. The role of the aqueous phase is to nurture and protect the epithelium by providing a medium for the transfer of oxygen and nutrients to the avascular corneal tissue, conveying signals between the structures bathed in aqueous and flushing away epithelial debris, toxins, and foreign bodies [7, 20, 47–49]. The electrolytes within the aqueous phase dictate the osmolarity of the tear fluid, play a role in regulating pH, and maintaining epithelial integrity. Hyperosmolarity, reflects an increased electrolyte concentration and is recognised to damage the ocular surface. Aqueous layer proteins contribute to ocular surface defence and maintenance of tear film stability [7, 20, 47–49]. As well as electrolytes and proteins, the tear film contains antioxidants to scavenge free radicals, and growth factors which are important in epithelial regeneration and wound healing. Inflammation causes changes in the tear film constituents with release of inflammatory markers precipitating an escalating cycle of inflammation and ocular surface irritation, tear film instability, epithelial cell dysfunction, and apoptosis. This ultimately affects the corneal epithelial barrier function [7, 20, 47–49].

Mucins are nutrient dependent glycoproteins expressed by epithelial tissues of mucosal surfaces. Mucins are classified as either secretory or membrane spanning and the tear film as well as ocular epithelium contain both

types. These mucins are important for removing pathogens and debris from the ocular surface, preventing bacterial adherence to the cornea, smoothing the ocular surface, and for protecting the surface through lubrication from the blink and environmental insult. A deficiency in mucin production can lead to decreased tear break-up time (TBUT), affecting ocular surface health. Currently, the proposed sources of ocular mucin include the conjunctival goblet cells, conjunctival, corneal epithelial cells, the lacrimal gland, as well as ocular surface wound healing growth factors that may stimulate goblet cell mucin secretion. Anchored to the apical plasma membrane of the corneal and conjunctival epithelial cells are the transmembrane/membrane-spanning mucins, which contribute to forming the glycocalyx. Eleven mucins (of the known 21) have been identified in the tear layer and they are listed in Table 11 with their functions [50].

In summary, mucins have: non-adhesive properties, which prevents inflammatory cells, debris, and pathogens from adhering to ocular surface; transmembrane/membrane-spanning properties, where the hydrophobic segment allows the mucin to be intimately associated with the epithelial cells, thereby providing lubrication and protection of the ocular surface; gel-forming properties, which stabilises tear layer, and due to their large structure they prevent foreign bodies and pathogens from binding to the ocular surface; small soluble properties, which provides mechanism for effectively mixing all the secretory mucins in the aqueous layer of the tear film [5, 50].

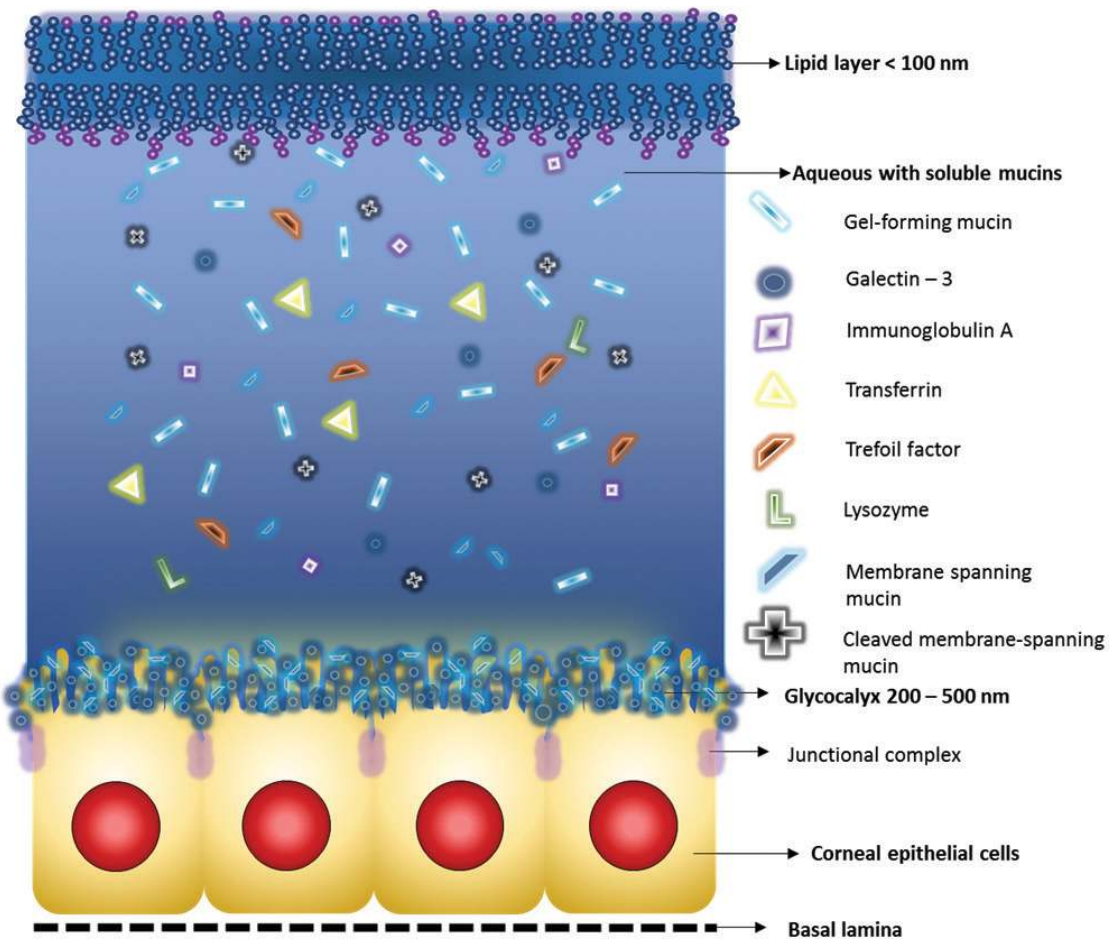

Figure 13: Structure of the tear film

Table 11: Function of mucins found on the ocular surface

Mucin	Secreted/produced by:	Non-adhesive:	Membrane-spanning	Gel-forming	Small Soluble
MUC1	Corneal and conjunctival epithelium	*	*		
MUC2	Corneal and conjunctival epithelium			*	
MUC4	Corneal and conjunctival epithelium	*	*		
MUC5AC	Conjunctival goblet cells			*	
MUC7	Lacrimal gland acinar cells				*
MUC13	Conjunctival epithelium		*		
MUC15	Conjunctival epithelium		*		
MUC16	Corneal and conjunctival epithelium	*	*		
MUC17	Conjunctival epithelium		*		
MUC19	Corneal and conjunctival epithelium, goblet cells			*	
MUC20	Conjunctival epithelium		*		

Non-adhesive – prevents inflammatory cells, debris, and pathogens from adhering to ocular surface. Membrane-spanning – hydrophobic [5, 50].

Table 12: Properties of the tear film [5]

Tear Film	Normal Range in Tear Film
Volume	2–4 µl
pH	6.5–7.8
Osmolarity	280–318 mOsm/kg
Thickness	1–7 µm
Turnover	10.9–22.2%/minute
Exchange	10–20% per blink
Evaporation	0.4–167g/m^2/h
Surface temperature	32–36°C

Functions of the Tear Film [5, 20]

- The tears act as a refractive surface, smoothing the surface of the corneal epithelium
- Tears also reduce friction during the blink, protecting the ocular surface
- Tears maintain ocular transparency by keeping the cornea wet
- Tears provide lysozyme (protein) in a high enough concentration to be bacteriolytic. Other antibacterial molecules are also present and includes: defensins, lactoferin, secretory IgA, and phospholipase A_2
- Tears protect the ocular surface by flushing away debris and other metabolic by products, which may be hazardous to the eye and adnexa
- The tear film also contains antioxidants, to scavenge free radicals and growth factors which are important in epithelial regeneration and wound healing
- Tears provide ions such as potassium, calcium, zinc, magnesium and oxygen to the avascular cornea. Tears contain no significant amounts of glucose and Vitamin A

Effect of a Contact Lens on The Tear Film [47, 51–55]

In situ, contact lenses (CLs) divide the tear film into pre-lens and post-lens films (Figure 13). This compartmentalisation impacts the tear film in a number of ways, affecting both its biophysical and biochemical properties [47, 56]. Contact lens wear can deplete the tear layer of specific components and stimulate the biochemical generation of additional components, such as albumin or inflammatory mediators [56]. The contact lens is approximately 10x thicker than the aqueous layer of the normal tear film and its interaction with the tear film is dependent on the lens material and the individual tear chemistry of the wearer [56]. The lens sits in the ocular environment and creates two active interfaces which result in interaction between the surface of the lens and the lid, leading to movement of the lens on the ocular surface. These forces are dynamic and involve both sliding and shearing forces rather than just simple compression [56]. It is therefore not surprising that contact lens wearers are recognised to exhibit significantly more ocular symptoms than non-wearers [47].

Biophysical Changes

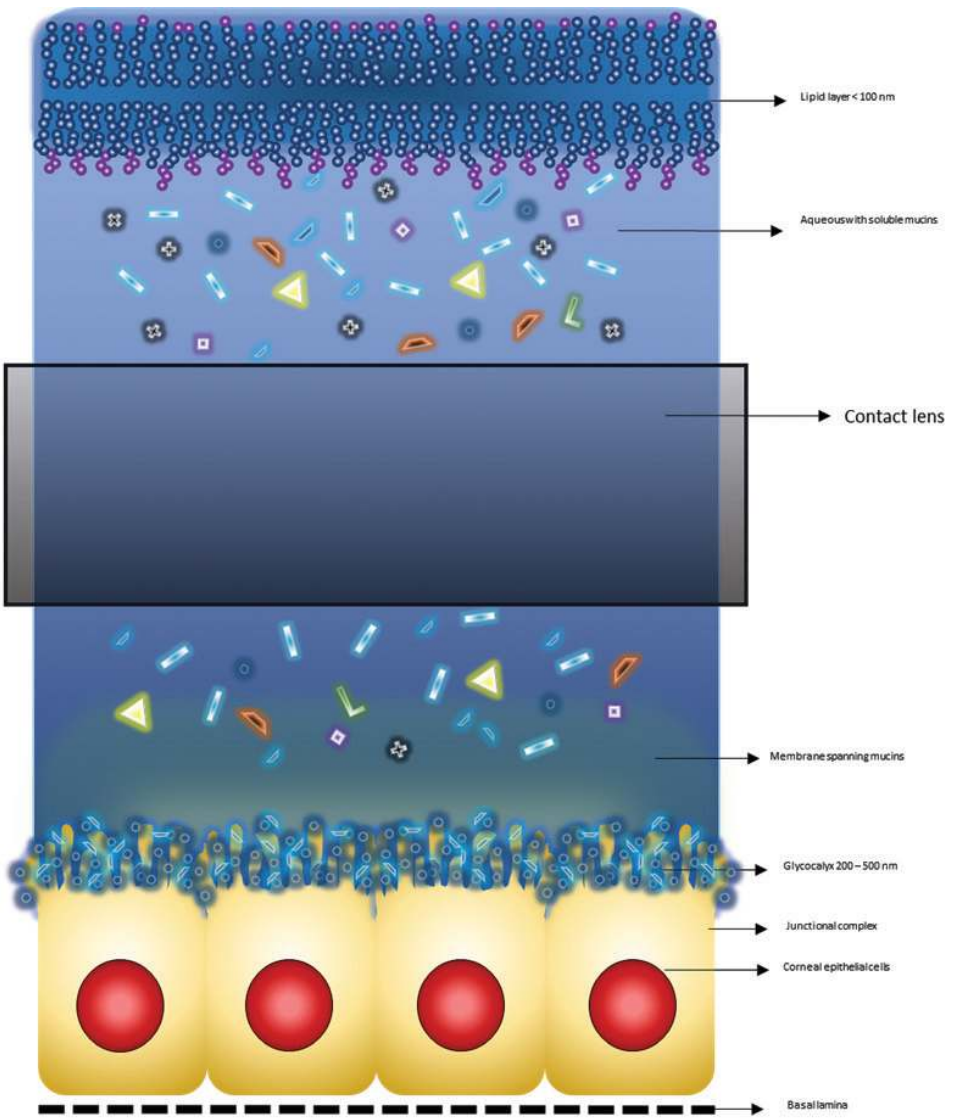

Figure 14: Effect of a contact lens on the tear film

Blink Impact on Pre-Corneal and Pre-Lens Tear Film Spread and Volume

To help maintain clear vision and ocular surface health, a blink occurs to distribute tears over the ocular surface and cornea. Two types of blink can be distinguished, complete and incomplete blinks. The eyelid covers less than 67% of the cornea [57]. The proportion of incomplete blinking in healthy subjects (without contact lenses) is ±20% [58]. The movement of the eyelid over the surface of a contact lens causes frictional stimulation which may lead to a biochemical response and the release of inflammatory mediators into the tear film [56]. With a contact lens *in situ* the following have been observed repeatedly:

- Higher percentage of incomplete blinks in RGP wearers [59]
- Soft lens wearers have a similar percentage of incomplete blinks to non-wearers. However, there is an association of incomplete blinks in soft contact lens wearers with corneal staining, discomfort, lens deposits and dryness [60]
- Inter-blink interval can be increased in both RGP and soft lens wearers to compensate for tear film instability and continuous friction between contact lens, palpebral conjunctiva and the cornea [47]

Lipid Layer

The two major functions of the lipid layer are to; lower the tear surface tension, thereby allowing the tear film to maintain a high area-to-volume ratio, and to inhibit the aqueous tear evaporation [47]. With a contact lens *in situ*, the aqueous layer is split in two and the thin pre-lens aqueous layer (2–6 μm) results in a deteriorating lipid layer [47]. This can result in patches of poor wettability and therefore, direct interaction between the lipid layer and the lens surface [61]. Lipid deposition is especially problematic in silicone hydrogel lenses, where after continuous wear hydrophobic lipid-attractive patches readily appear over the contact lens surface [56, 62]. Once formed, these deposits result in impaired vision and non-wetting lens surfaces with instantaneous TBUT's [56].

Clinically no pre-lens lipid layer is visible with RGP lenses [47].

Tear Film Stability

Contact lenses disrupt the tear film lipid layer and reduce tear film thickness [56]. This disruption is most marked with RGP lenses, where typically no pre-lens lipid layer is visible clinically and tear break up (TBU) occurs within 2–3 seconds, in contrast to 5–6 seconds with soft lenses [47]. Overall, tear film thinning has been shown to be significantly faster on the surface of a contact lens than on the corneal surface [63]. The location of the TBU is also different in the lens-wearing eye, occurring in the centre of the lens, compared to the non-lens-wearing eye, where the location is more peripheral [64]. Pre-ocular TBUT is initially significantly lower after lens removal. However, over the longer term it appears to be largely unaffected by contact lens wear [47]. Lower pre-ocular TBUT's are associated with increased symptoms of discomfort with both hydrogel and silicone hydrogel lens wearers [47]. Hom and Bruce, 2009 suggested a cut-off TBUT value of 3 seconds as a suitable criterion for identifying tear film dysfunction likely to cause symptoms of dryness in contact lens wearers [65]. Lower pre-lens TBUT can cause problems with visual acuity, which combined with changes in tear film quality and comfort may lead to increased intolerance of contact lens wear [66, 67].

Tear Film Evaporation

The normal tear film is lost from the ocular surface by evaporation, absorption, and drainage [47]. Evaporation is the main cause of tear film thinning [68, 69]. The rate of tear film evaporation increases with contact lenses *in situ* [51, 70]. This is due to the disruption of the aqueous and lipid layers caused by the lens [51, 70]. Contact lens wear

typically result in a 1.2–2.6x increase in the rate of tear evaporation independent of either lens type or water content [47]. Increased evaporation rates lead to discomfort and dryness in contact lens wearers [47].

Tear Film Temperature

Due to its exposed position the temperature of the normal ocular surface and tear film is lower than core body temperature (32–36°C) [71]. Pre-lens temperatures in soft lens wearers are cooler than that of the ocular surface without lenses, while the temperature of the post-lens tear film beneath the contact lens is higher [47] [72]. Higher water content lenses with corresponding rapid rate of water loss, show lower surface pre-lens temperatures [72, 73]. Silicone hydrogel lenses have higher post-lens tear temperatures than hydrogel lenses due to lower rate of water loss from these lenses [73].

Tear Film Thickness

The pre-lens tear film initially increases when the lens is placed on the eye. This is due to reflex tearing and excess wetting solution. However, this increase is transient and fairly rapidly settles down to about 2 μm thickness [74]. Post-lens tear film is thinner (1–3 μm) and the thinning rate is higher compared to the pre-corneal tear film [74]. Depletion of the post lens tear film may impact lens movement, cause lens adherence and surface staining, which can lead to contact lens complications and discomfort [47].

Tear Production/Turnover

An average tear turnover rate of 15.5%/minute is typical of normal young subjects without lenses [75, 76]. Tear turnover rate decreases significantly in contact lens wear, 12.4%/minute in hydrogel lens wear, and 13.2%/minute in silicone hydrogel wear [47, 76].

Tear Volume

In the normal tear system as little as 2–4 μl of tear volume is required to maintain a wet surface [47]. With each blink the tears are mixed and redistributed. The normal tear meniscus volume is 1.5 μl [77]. With a contact lens on the eye the tear meniscus volume is 1 μl and this further decreases over time during lens wear [78]. Lower tear meniscus volumes are related to increased ocular discomfort at the end of the day as well as corneal staining [47].

Tear Exchange

Tear exchange or mixing during lens wear may be regulated by the interrelationships between four variables: lens diameter, movement, the blink, and tear replenishment rate [79]. The tear exchange rate beneath a soft contact lens was about 9%/minute [80].

Osmolarity

During contact lens wear tear film osmolarity undergoes a series of changes. Initially, the insertion of a contact lens results in a reduction of osmolarity due to reflex tearing [47]. A subsequent increase in osmolarity is then observed [47]. Wearing soft lenses on an extended wear basis and RGP lenses on a daily wear basis significantly increased tear osmolarity. However, this did not occur while wearing soft lenses on a daily wear basis [47]. No differences have been observed between hydrogel and silicone hydrogel lenses [47]. The increase in tear osmolarity in contact lens wear have been attributed to two factors; reduced tear production due to reduced corneal sensitivity, and excessive evaporation due to disrupted tear film and reduced tear film stability [81].

Tear pH

The tear film pH varies throughout the day shifting from acid to alkaline (0.6 units) [47]. The tear film is more acidic in contact lens wear, decreasing between 0.27–0.53 units due to increased tearing and blinking. Whereas, eyelid opening leads to alkalisation due to equalisation of the partial pressure of CO_2 to that of the surrounding air [47]. This acidification (decrease in pH) has been observed in the post-lens tear film in both RGP and soft lenses and have been attributed to the lens preventing CO_2 loss from the ocular surface and tear fluid [82].

Viscosity

Normal human tears have viscosity rates of 1–10 mPa depending on the shear rate [47]. Although, it was initially thought that the main contributor to tear viscosity was mucin, recent studies have shown that proteins and lipids also play a role [83]. The effect of contact lens wear on viscosity is currently not known [47].

Surface Tension

Tear film surface tension is around 2/3rds of that of water or saline [84, 85]. The higher the surface tension the quicker the TBUT. Tear film lipids are probably the most important contributors to surface tension due to the amphipathic nature of their polar components. Not much information is available on the effect of a contact lens on the tear surface tension. However, the lipid layer is disturbed by the presence of a lens and one can therefore assume that the surface tension will also be influenced [47].

To summarise, the presence of a contact lens divides the tear film into a pre-lens and post-lens tear film and creates new interfaces with and within the ocular environment. The biophysical changes to the tear film properties introduced include; decreased tear film stability, decreased tear turnover rate, decreased pre-lens lipid layer thickness, decreased tear volume, and an increase in evaporation rate. Additionally, tear osmolarity increases, tear temperature decreases, and pH becomes more acidic.

Biochemical Changes

The lens is the guest and the ocular environment is the host. Interaction between the two will be affected by both the characteristics of the guest, such as material, modulus, permeabilities and coefficient of friction, as well as those of the host, such as individual tear chemistry, level of adaptation and duration of lens wear [56]. As soon as the lens is inserted it will be coated by components of the tear film including lipids, proteins and mucins. The effects of these lens deposits are dealt with in chapter 17. This section will deal with the effects of the interfacial interactions and ocular response to the lens on the eye.

The movement of the lens over the surface of the lens causes friction and mechanical stimulation which can upregulate the inflammatory response. The reduced tear flow between the posterior surface of the lens and cornea, due to a less dynamic interaction between the tears and the corneal epithelium, can lead to alterations in protein deposits on the posterior surface compared to the anterior surface of the lens and its sequelae [56].

Lipidome

As mentioned earlier, the pre-lens tear film lipid layer's thickness and stability is disrupted by the presence of a contact lens on the eye and silicone hydrogel lenses are particularly prone to lipid interaction and deposition [47, 56]. Exposure to oxygen and UV light leads to lipid oxidation, a process known as auto-oxidation, which in combination with enzymes break down the lipids from their native states to the end products of lipid oxidation – peroxide and hydroperoxide [56]. The presence of these end products and their effect on contact lens wear has

not been fully investigated. However, they may contribute to "end of day" discomfort in symptomatic contact lens patients [56].

Proteome

Protein denaturation, or a change in conformation of the protein, results in the protein losing its native biological function or properties. Protein denaturation on the lens surface (and in lens matrix) may be due to interaction with lens surface, material, tear chemistry changes, lens drying, and solution interactions [56]. Denatured protein is not recognised as "self" and has been linked to CLPC or GPC [56]. Additionally, plasma-derived proteins can be found in the tears of contact lens wearers. This indicates that the tear-blood barrier is compromised. This is probably due to some level of material-induced plasma leakage, increased vascular permeability, and/or changes in indigenous protein secretion such as IgA from the lacrimal gland [56]. Albumin is a plasma derived protein and is useful as a marker for vascular leakage into the ocular environment. Although contact lens wear leads to leakage of albumin into the tears, levels decrease rapidly upon lens removal, indicating that once the stimulus is removed the plasma leakage subsides [56]. The presence of albumin in tears suggest that other plasma derived components such as cytokines should also be present and indeed lens wear has been shown to have an influence on cytokine levels in the tear film [47, 56].

Mucin

Contact lens wear and cleaning solutions are associated with a decrease in the amount of secreted mucins at the ocular surface as well as damage to the glycocalyx formed by transmembrane mucins [47]. This is in part due to a decrease in the number of conjunctival goblet cells [56]. Mechanical interaction of the contact lens, the epithelial surface and the blinking forces of the lid are involved in the formation of mucin balls which are discussed in chapter 16. Mucin fragmentation and degradation was observed in asymptomatic contact lens wearers as a result of a new material leading to increased discomfort [47, 56].