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Front Matter

Cornea

THIRD EDITION

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In the age of instantaneous electronic information, a bound textbook may seem to some, perhaps, anachronistic. We, and even more so, our residents and fellows are accustomed to finding quick facts and lengthy lists of citations with but a few key strokes at the computer. The time spent rifling through the pages of journals in a library or working one's way through a hard copy text is, for better or worse, diminishing in favor of the agility of the computer – ever faster, ever smaller, and ever more convenient. Indeed, there are some advantages to modern day electronic texts – universal access in spite of time of day or location, portability, rapidity of information access, and the ability to do complex Boolean searches in moments. Such features save hours of time. For these very reasons, this edition is made available in an electronic format as well as the print version.

At the same time, a multi-authored text which has been forged through the process of gathering the best minds in the field, written and rewritten through a laborious and meticulous editing process, and presented as a comprehensive and authoritative source that can be turned to repeatedly is highly desirable as a bound document – codifying the current state of our knowledge in one place.

The practice of our subspecialty in ophthalmology is more diversified than ever before. The proliferation of new surgical procedures targeted to specific corneal abnormalities, a variety of new diagnostic testing capabilities, and a dramatic broadening of our understanding of the pathophysiology of the cornea and ocular surface have revolutionized what we know about the remarkable structure through which we view the world. We hope that this book will continue to serve as a useful tool to all students and practitioners in our field, both in print and electronic forms.

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Dedication

To

my wife, Kathryn

our children, Edward, Kara, and Jill

our parents, Paul and Rebecca Krachmer

and Louis and Gertrude Maraist

with great love and appreciation

Jay H Krachmer

To

my sister, Libby, and in memory of my brother, Norman

Mark J Mannis

To

my wife, Lynette who is a great partner and always supportive

and our children, Colson, Kelsey and Natalie who keep me entertained, challenged and grounded

Edward J Holland

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Introduction

The avascular cornea is not an isolated tissue. It forms, together with the sclera, the outer shell of the eyeball, occupying one-third of the ocular tunic. Although most of both the cornea and sclera consist of dense connective tissue, the physiological roles of these two components of the eye shell differ. The cornea serves as the transparent ‘window’ of the eye that allows the entry of light, whereas the sclera provides a ‘darkbox’ that allows the formation of an image on the retina. The cornea is exposed to the outer environment, whereas the opaque sclera is covered with the semitransparent conjunctiva and has no direct exposure to the outside. The differences in the functions of the cornea and sclera reflect those in their microscopic structures and biochemical components.

Interwoven fibrous collagen is responsible for the mechanical strength of both the cornea and sclera, protecting the inner components of the eye from physical injury and maintaining the ocular contour.[1] The corneal epithelium forms an effective mechanical barrier as a result of interdigitation of cell membranes and the formation of junctional complexes such as tight junctions and desmosomes between adjacent cells. Together with the cellular and chemical components of the conjunctiva and tear film, the corneal surface protects against potential pathological agents and microorganisms.
The smooth surface of the cornea contributes to visual clarity. The regular arrangement of collagen fibers in the corneal stroma accounts for the transparency of this tissue.[2] Maintenance of corneal shape and transparency is critical for light refraction, with the cornea accounting for more than two-thirds of the total refractive power of the eye. A functionally intact corneal endothelium is important for maintenance of stromal transparency as a result of regulation by the endothelium of corneal hydration.
Anatomy and Physiology

Structure of the cornea and sclera

The anterior corneal surface is covered by the tear film, whereas the posterior surface is bathed directly by the aqueous humor. The highly vascularized limbus, which is thought to contain a reservoir of pluripotent stem cells, constitutes the transition zone between the cornea and sclera. The anterior corneal surface is convex and aspheric (Fig. 1.1), and it is transversely oval as a result of scleralization superiorly and inferiorly.
The adult human cornea measures 11 to 12 mm horizontally and 9 to 11 mm vertically. It is approximately 0.5 mm thick at the center, with the thickness increasing gradually toward the periphery, where it is about 0.7 mm thick.\[3\] The curvature of the corneal surface is not constant, being greatest at the center and smallest at the periphery. The radius of curvature is between 7.5 and 8.0 mm at the 3-mm central optical zone of the cornea, where the surface is almost spherical. The refractive power of the cornea is 40 to 44 diopters, constituting about two-thirds of the total refractive power of the eye.

The sclera, a tough and nontransparent tissue, shapes the eye shell, which is approximately 24 mm in diameter in the emmetropic eye. The anterior part of the sclera is covered with the bulbar conjunctiva and Tenon's capsule, which consists of loose connective tissue and is located beneath the conjunctiva (Fig. 1.2). The nontransparency of the sclera prevents light from reaching the retina other than through the cornea, and, together with the pigmentation of the choroid and retinal pigment epithelium, the sclera provides a dark box for image formation. The scleral spur is a projection of the anterior scleral stroma toward the angle of the anterior chamber and is the site of insertion for the anterior ciliary muscle. Contraction of this muscle thus opens the trabecular meshwork. At the posterior pole of the eyeball, where the optic nerve fibers enter the eye, the scleral stroma is separated into outer and inner layers. The outer layer fuses with the sheath of the optic nerve, dura, and arachnoid, whereas the inner layer contains the sieve-like structure of the lamina cribrosa. The rigidity of the lamina cribrosa accounts for the susceptibility of retinal nerve fibers to damage during the development of chronic open-angle glaucoma. The sclera contains six insertion sites of the extracocular muscles as well as the inputs of arteries (anterior and posterior ciliary arteries) and outputs of veins (vortex veins) that circulate blood through the uveal tissues.

**Optical properties of the cornea**

The optical properties of the cornea are determined by its transparency, surface smoothness, contour, and refractive index of the tissue.\[4\] If the diameter of (or the distance between) collagen fibers in the corneal stroma becomes heterogeneous (as occurs in fibrosis or edema), incident light rays are scattered randomly and the cornea loses its transparency. Given that the spherocylindrical surface of the cornea has both minor and major axes, changes in corneal contour caused either by pathological conditions such as scarring, thinning, or keratoconus or by refractive surgery render the surface regularly or irregularly astigmatic.
The total refractive index of the cornea is determined by the sum of refraction at the anterior and posterior interfaces as well as by the transmission properties of the tissue. The refractive indexes of air, tear fluid, corneal tissue, and aqueous humor are 1.000, 1.336, 1.376, and 1.336, respectively. The refractive power of a curved surface is determined by the refractive index and the radius of curvature. The refractive power at the central cornea is about +43 diopters, being the sum of that at the air–tear fluid (+44 diopters), tear fluid–cornea (+5 diopters), and cornea–aqueous humor (−6 diopters) interfaces. Most keratometry and topography measurements assume a standard refractive index of 1.3375.

Innervation

Innervation of the cornea is required for pain sensation as well as for tissue repair. In addition, autonomic innervation of the scleral spur and of blood vessels in the episclera, the surface of the sclera immediately beneath the subconjunctival connective tissue, plays an important role in the regulation of intraocular pressure.

The cornea is one of the most heavily innervated and most sensitive tissues in the body. The density of nerve endings in the cornea is thus about 300 to 400 times greater than that in the skin.[5],[6] Most of the sensory nerves in the cornea are derived from the ciliary nerves of the ophthalmic branch of the trigeminal nerve. The long ciliary nerves provide the perilimbal nerve ring. Nerve fibers penetrate the cornea in the deep peripheral stroma radially and then course anteriorly, forming a terminal subepithelial plexus.[7] The nerve fibers lose their myelination within a short distance of their point of entry into the cornea, penetrate Bowman’s layer, and terminate at the wing cell level of the epithelium. Loss of the superficial corneal epithelium results in exposure of the nerve endings and consequent severe ocular pain.

Slit lamp microscopy allows observation of nerve fibers in the corneal stroma. The fibers are especially prominent at the corneal periphery, where their diameter is relatively large. Laser-scanning confocal microscopy has revealed networks of fine nerve fibers (subepithelial nerve plexuses) in or below the basal cell layer of the corneal epithelium.[6,8,9] The diameter of these nerve fibers increases with distance from the anterior corneal surface (Fig. 1.3).

![Fig. 1.3](image-url) Confocal biomicroscopy of the human cornea. (A–C) Superficial, wing, and basal cell layers of the corneal epithelium. (D) Subepithelial nerve plexus. (E) Shallow layer of the stroma, containing a high density of polygonal keratocytes. (F) Mid layer of the stroma, containing thick nonbranching nerve fibers. (G) Deep layer of the stroma, containing keratocytes. (H) Amorphous appearance of Descemet’s membrane. (I) Endothelium, comprising hexagonal endothelial cells of uniform size.

Histochemical studies have revealed the presence of various neurotransmitters, including substance P, calcitonin gene-related peptide, neuropeptide Y, vasoactive intestinal peptide, galanin, methionine-enkephalin, catecholamines, and acetylcholine, in the cornea.[6,10–21] The cornea thus contains peptidergic, sympathetic, and parasympathetic nerve fibers. Degeneration or dysfunction of sensory nerves (trigeminal nerve branches) in the cornea can result in delayed healing of corneal injuries and the development of neurotrophic ulcer.

The short and long posterior ciliary nerves, which are branches of the trigeminal nerve, penetrate the sclera and provide fine sensory branches to the scleral stroma. In addition, nerve fibers are also present in the episclera. These fibers include those of vasodilative and vasoconstrictive nerves and are thought to regulate blood flow and volume in the episcleral vessels for modulation of episcleral venous pressure and outflow facility.[22] Cells in the scleral spur are also thought to contribute to the regulation of intraocular pressure. Axons of presumably parasympathetic origin are present in the scleral spur of humans. On the other hand, cholinergic innervation of scleral spur cells appears to be rare or absent.[23]

Vascular system
The cornea is one of the few avascular tissues in the body. Although the normal cornea does not contain blood vessels, factors derived from the blood play important roles in corneal metabolism and wound healing. The anterior ciliary artery, which is derived from the ophthalmic artery, forms a vascular arcade in the limbal region that anastomoses with vessels derived from the facial branch of the external carotid artery. The cornea is thus supplied with blood components by both internal and external carotid arteries. In certain pathological conditions, new vessels enter the transparent corneal stroma from the limbus and result in a loss of corneal transparency.

In contrast to the cornea, the episclera is highly vascularized. The episcleral vasculature shows a specialized morphology characterized by the absence of capillaries, numerous arteriovenous anastomoses, and a muscle-rich venous network, which is thought to play an important role in the regulation of intraocular pressure. Such vascularization is also apparent in the loose connective tissue of Tenon's capsule. The scleral stroma contains few blood vessels with the exception of the input and output of the vessels of the choroidal circulation.

**Oxygen and nutrient supply**

Corneal epithelial and endothelial cells are metabolically active. Cellular activities require adenosine triphosphate (ATP) as an energy source, with catabolism of glucose by glycolysis and the citric acid cycle generating ATP under aerobic conditions. A supply of glucose and oxygen is thus essential to maintain the normal metabolic functions of the cornea.[24–27] The cornea is supplied with glucose by diffusion from the aqueous humor. In contrast, oxygen is supplied to the cornea primarily by diffusion from tear fluid, which absorbs oxygen from the air. Direct exposure of tear fluid to the atmosphere is thus essential for oxygenation of the cornea. Disruption of the oxygen supply to the cornea, such as that resulting from the wearing of contact lenses with less gas permeability, can lead to corneal hypoxia and consequent stromal edema.[28–31] Closure of the eyelids during sleep also reduces the amount of oxygen that reaches the cornea. Corneal metabolism therefore changes from aerobic to anaerobic (with consequent accumulation of lactate) during sleep.[32][33]

**Tear fluid**

The corneal surface is covered by tear fluid, which protects the cornea from dehydration and helps to maintain the smooth epithelial surface. The thickness and volume of the tear film are about 7 µm and 6.5 µL, respectively.[34][35] The tear film consists of three layers: a superficial lipid layer (~0.1 µm), an aqueous layer (~7 µm), and a mucinous layer (~0.02–0.05 µm).[36] More than 98% of the total volume of the tear film is water. However, tear fluid also contains many biologically important ions and molecules, including electrolytes, glucose, immunoglobulins, lactoferrin, lysozyme, albumin, and oxygen. Moreover, it contains a wide range of biologically active substances such as histamine, prostaglandins, growth factors, and cytokines (Table 1.1). The tear film thus serves not only as a lubricant and source of nutrients for the corneal epithelium but also as a source of regulatory factors required for epithelial maintenance and repair.[37–54]

<table>
<thead>
<tr>
<th>Tear layer</th>
<th>Origin</th>
<th>Components</th>
<th>Physiological functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid layer</td>
<td>Meibomian glands, accessory lacrimal glands</td>
<td>Wax, cholesterol, fatty acid esters</td>
<td>Lubrication, prevention of evaporation, stabilization</td>
</tr>
<tr>
<td>Aqueous layer</td>
<td>Lacrimal gland, accessory lacrimal glands</td>
<td>Water, electrolytes (Na+, K+, Cl−, HCO3−, Mg2+), proteins (albumin, lysozyme, lactoferrin), transferrin, ceruloplasmin, immunoglobulins (IgA, IgG, IgE, IgM), cytokines, growth factors (EGF, TGF-α, TGF-β, TGF-β2, bFGF, HGF, VEGF, substance P), others (glucose, vitamins)</td>
<td>Lubrication, antimicrobial, bacteriostasis, supply of oxygen and nutrients, mechanical clearance, regulation of cellular functions</td>
</tr>
<tr>
<td>Mucinous layer</td>
<td>Conjunctival goblet cells, conjunctival epithelial cells, corneal epithelial cells</td>
<td>Sulfomucin, cylalomucin, MUC1, MUC4, MUC5AC</td>
<td>Lowering of surface tension, stabilization of aqueous layer</td>
</tr>
</tbody>
</table>

The components of the superficial lipid layer of the tear film are supplied by meibomian glands and other secretory glands of the eyelid. The aqueous layer is derived from the lacrimal gland and accessory lacrimal glands, and the mucinous layer is produced largely by goblet cells in the conjunctival epithelium. Hypolacrimation (dry eye syndrome) can thus be classified basically into three categories attributable to lipid, aqueous, or mucin deficiency.
Histology and Biochemistry

The cornea consists of three different cellular layers and two interfaces: the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium (see Fig. 1.1). The cell types that constitute the cornea thus include epithelial cells, keratocytes (corneal fibroblasts), and endothelial cells. Components of the cornea interact with each other to maintain the integrity and function of the tissue, with the precise arrangement of the various components contributing to its transparency and strength.

Corneal epithelium

The corneal and conjunctival epithelia are continuous and together form the ocular surface. They are both composed of nonkeratinized, stratified, squamous epithelial cells. Although their characteristics differ, both corneal and conjunctival epithelia cooperate to provide the biodefense system of the anterior surface of the eye. The thickness of the corneal epithelium is approximately 50 µm, which is about 10% of the total thickness of the cornea (see Fig. 1.1), and it is constant over the entire corneal surface.

The corneal epithelium consists of five or six layers of three different types of epithelial cells: superficial cells, wing cells, and columnar basal cells, the latter of which adhere to the basement membrane adjacent to Bowman's layer (Fig. 1.4). Only the basal cells of the corneal epithelium proliferate. The daughter cells differentiate into wing cells and subsequently into superficial cells, gradually emerging at the corneal surface. The differentiation process requires about 7 to 14 days, after which the superficial cells are desquamated into the tear film. Ultraviolet radiation, hypoxia, or mechanical stress induces apoptosis (programmed cell death) and desquamation of corneal epithelial cells.

Fig. 1.4 Transmission electron microscopy of the human corneal epithelium. (A) The epithelium comprises five or six layers of epithelial cells. The electron-dense cell is about to undergo desquamation. (B) Basal cells. Note the numerous junctional complexes. (C) Basement membrane and anterior portion of Bowman's layer. Note hemidesmosomes at the basal surface of the epithelial cells. (D) Intergilitation and junctional complexes at the lateral surface of basal epithelial cells. (E) Gap junction at the lateral surface of basal cells.

An important physiological role of the corneal epithelium is to provide a barrier to external stimuli. The presence of junctional complexes between adjacent corneal epithelial cells prevents the passage of such agents into the deeper layers of the cornea. Both cell–cell and cell–matrix interactions are important for maintenance of the normal stratified structure and physiological functions of the corneal epithelium. The characteristics of the different types of intercellular junctional complexes present in the corneal epithelium are summarized in Table 1.2 and in Figures 1.4, 1.5, and 1.6. Tight junctions...
(zonula occludens) are present mostly between cells of the superficial cell layers and provide a highly effective barrier to prevent the penetration of tear fluid and its chemical constituents. Hemidesmosomes (zonula adherens) and desmosomes are present in all layers of the corneal epithelium, whereas gap junctions, which allow the passage of small molecules between cells, are present in the wing cells and basal cells. After damage to the corneal epithelium, actively migrating epithelial cells no longer manifest gap junctions or desmosomes in the wounded region lacking a basement membrane. Reestablishment of the continuity of the corneal epithelium is accompanied by the synthesis and deposition of basement membrane proteins and by the reassembly of the various types of junctional apparatus, suggesting that the presence of the basement membrane may be required for re-formation of cell–cell junctions in the corneal epithelium (Fig. 1.6).[63]

Table 1.2 -- Characteristics of the various types of corneal epithelial cells

<table>
<thead>
<tr>
<th></th>
<th>Shape</th>
<th>Layers</th>
<th>Size</th>
<th>Mitotic activity</th>
<th>Junctional complexes</th>
<th>Cytoplasmic organelles</th>
<th>Keratin Microfilaments(α-actin)/Microtubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial cells</td>
<td>Flat Microvilli Microplicae</td>
<td>2–4</td>
<td>40–60 µm in diameter</td>
<td>–</td>
<td>Desmosomes Tight junctional complexes (zonula occludens)</td>
<td>Sparse</td>
<td>+</td>
</tr>
<tr>
<td>Wing cells</td>
<td>Winglike processes</td>
<td>2–3</td>
<td>–</td>
<td>Entire surface</td>
<td>Desmosomes Gap junctions</td>
<td>Sparse</td>
<td>+++</td>
</tr>
<tr>
<td>Basal cells</td>
<td>Columnar Mono layer</td>
<td>18–20 µm high 8–10 µm in diameter Flat at posterior surface</td>
<td>+</td>
<td>Apical surface</td>
<td>Desmosomes Gap junctions Hemidesmosomes</td>
<td>More than superficial cells Large numbers of glycogen granules Prominent mitochondria and Golgi apparatus</td>
<td>+++</td>
</tr>
</tbody>
</table>
In corneal epithelial cells, intermediate filaments of the cytoskeleton are formed by specific types of acidic (type I) and basic (type II) keratin molecules. Basal cells of the corneal epithelium express keratin 5/14, like basal epidermal cells of the skin. However, keratin 3/12 (64-kDa keratin) is specifically expressed in the epithelium of the cornea, not being found in that of the conjunctiva or in the epidermis.[64][65] Genetic mutation of the keratin 12 gene is responsible for Meesmann’s dystrophy of the corneal epithelium.[66]

Replacement of most organs or tissues by transplantation from a genetically nonidentical individual is associated with an immune response that may lead to rejection. In contrast, the cornea is ‘immune privileged,’ a characteristic that is critical for the success of corneal transplantation. Dendritic Langerhans cells, specialized macrophages derived from the bone marrow that are implicated in antigen processing, are abundant at the periphery of the corneal epithelium but are not present in the central region of the normal cornea.[67][68] These cells express human leukocyte antigen (HLA) class II molecules and are thought to function in the afferent arm of the ocular immune response by presenting antigens to T lymphocytes.[69][70] Injury to the central cornea results in the rapid migration of peripheral Langerhans cells to the damaged area.

**Superficial cells**

The surface of the corneal epithelium contains two to three layers of terminally differentiated superficial cells. In contrast to the epidermis of the skin, the corneal epithelium is not normally keratinized, although it may become so under pathological conditions such as vitamin A deficiency. These cells are flat and polygonal with a diameter of 40–60 µm and a thickness of 2–6 µm (see Table 1.2). Their surface is covered with microvilli.[71] Given that superficial cells are well differentiated, they do not proliferate.

Numerous glycoprotein and glycolipid molecules are embedded in the cell membrane of epithelial cells. These oligosaccharide-containing molecules form floating particles in the membrane that are collectively termed the glyocalyx and which confer hydrophilic properties on the anterior surface of the superficial epithelial cells. The glyocalyx interacts with the mucinous layer of the tear film and helps to maintain the trilayered structure of the latter.[72][73] Loss either of the glyocalyx of corneal epithelial cells or of goblet cells in the conjunctival epithelium results in tear film instability and the mucin-deficiency form of dry eye.

The superficial cells of the corneal epithelium are joined by desmosomes, adherens junctions, and tight junctions (see Figs 1.4, 1.5, and 1.6), which prevent the passage of substances through the intercellular space. Examination of fluorescein penetration into the corneal stroma with a fluorophotometer provides a measure of the barrier function of the corneal epithelium.[74]

**Wing cells**

Beneath the superficial cells lie two to three layers of wing cells, so called because of their characteristic winglike shape. Wing cells are in an intermediate state of differentiation between basal and superficial cells and are rich in intracellular tonofilaments composed of keratin (see Table 1.2). The cell membranes of adjacent wing cells are interdigitate. Numerous desmosomes, adherens junctions, and gap junctions are present between wing cells (see Figs 1.4, 1.5, and 1.6).

**Basal cells**

The single layer of columnar basal cells of the corneal epithelium rests on the basement membrane. Basal cells, unlike superficial and wing cells, possess mitotic activity, and they differentiate consecutively into wing and superficial cells (see Table 1.2). Neighboring basal cells interdigitate laterally and are joined by desmosomes, gap junctions, and adherens junctions. The posterior surface of basal cells is flat and abuts the basement membrane.

Basal cells adhere to the basement membrane via hemidesmosomes that are linked to anchoring fibrils of type VII collagen secreted by themselves (see Fig. 1.4).[75][76] The anchoring fibrils penetrate the basement membrane and course into the stroma, where they form anchoring plaques together with type I collagen, a major component of the stroma. The adherens junctions are present at the lateral surface of the basal cells of the corneal epithelium and are thought to mediate cell-cell interaction.[77]

Members of the integrin family of cell surface receptors for extracellular matrix components (ECM) proteins exist as heterodimers of α and β subunits.[78] The integrin α6β1 heterodimer, which is the major receptor for fibronectin, is present at the surface of basal cells in the normal corneal epithelium.[79][80] All epithelial cells undergoing active migration after debridement of the corneal epithelium express integrin α6β1.[81][82]
**Basement membrane**

As in epithelia in other parts of the body, basal cells of the corneal epithelium are anchored to a basement membrane. The presence of the basement membrane between the basal epithelium and the underlying stroma fixes the polarity of epithelial cells. Ultrastructurally, the basement membrane, which is 40–60 nm thick, is composed of a pale layer (the lamina lucida) immediately posterior to the cell membrane of the basal epithelial cells as well as an electron-dense layer (the lamina densa) (see Fig. 1.4). Type IV collagen and laminin are major components of the basement membrane (Fig. 1.7).

![Fig. 1.7](image1.png)

*Immunofluorescence analysis of the expression of matrix proteins in the rat corneal epithelium. (A) Fibronectin. (B) Type I collagen. (C) Type IV collagen. (D) Laminin.*

The basement membranes of the corneal and conjunctival epithelia contain different type IV collagen chains, although the functional relevance of this difference is unknown. Whereas collagen α5(IV) is present in the corneal basement membrane, collagen α2(IV) is present in the conjunctival basement membrane (as well as in the amniotic membrane) (Fig. 1.8).

![Fig. 1.8](image2.png)

*Differential expression of α2(IV) and α5(IV) isoforms of type IV collagen in the human cornea, conjunctiva, and amniotic membrane. The arrow shows the termination of Bowman's layer. (Reprinted with permission from: Fukuda K et al. Differential distribution of subchains of the basement membrane components type IV collagen and laminin among the amniotic membrane, cornea, and conjunctiva. Cornea 18:73-79, 1999.)*

**Bowman's layer**

An acellular, membrane-like zone known as Bowman's layer, or Bowman's membrane, is detectable by light microscopy at the interface between the corneal epithelium and stroma in humans and certain other mammals (but not in rodents). Given that this structure, which is 12 µm thick, is not a membrane but rather a random arrangement of collagen fibers and proteoglycans, the term Bowman's layer is preferable. The collagen fibers in Bowman's layer are primarily collagen types I and III. The diameter of these fibers is 20–30 nm, which is smaller than that of the collagen fibers present in the corneal stroma (22.5–35 nm) (see Fig. 1.4).

Bowman's layer is considered to be the anterior portion of the corneal stroma. The anterior surface of this layer, which faces the basement membrane, is smooth. Given that the collagen fibers in Bowman's layer are synthesized and secreted by stromal keratocytes, they appear continuous with those in the stroma.

Biological functions originally attributed to Bowman's layer are now thought to be mediated by the basement membrane. Bowman's layer does not regenerate after injury. Recent clinical experience with excimer laser photorefractive keratectomy demonstrates that a normal epithelium is formed and maintained even in the absence of Bowman's layer. Furthermore, many mammals do not have a Bowman's layer but still exhibit a well-organized epithelial structure. The physiological role of Bowman's layer therefore remains unclear.

**Stroma of the cornea and sclera**

**Overview**

The stroma, which constitutes the largest portion, more than 90%, of the thickness of the cornea. The peripheral portion of the cornea connects to the anterior sclera at the limbus, where the tissue loses its transparency. Many characteristics of the cornea, including its physical strength, stability of shape, and transparency, are largely attributable to the anatomic and biochemical properties of the stroma. The uniform arrangement and continuous slow turnover (production and degradation) of collagen fibers in the stroma are essential for corneal transparency.

The sclera is also composed mostly of collagen fibers and other matrix macromolecules, but nonuniformity in the arrangement of these fibers accounts
for its lack of transparency.\textsuperscript{[85]} The thickness of the scleral stroma ranges from approximately 0.5 mm to 1.0 mm depending on the area, with the exception of the sites of insertion for the rectus muscle, where the sclera is thinnest. The toughness of the scleral stroma is essential for its role as a container of the intraocular tissues. Scleral fibroblasts are embedded within the collagen lamellae.

Cells

Keratocytes are the predominant cellular components of the corneal stroma and are thought to turn over about every 2 to 3 years. The spindle-shaped keratocytes are scattered among the lamellae of the stroma (Fig. 1.9). These cells extend long processes, and the processes of neighboring cells are connected at their tips by gap junctions (Fig. 1.10).\textsuperscript{[86]} The three-dimensional network structure of keratocytes can be observed by light microscopy in flat preparations of the corneal stroma by confocal biomicroscopy, and, after digestion of stromal collagen, by scanning electron microscopy (Fig. 1.10).\textsuperscript{[87]}

Fig. 1.9 Transmission electron microscopy of the human corneal stroma. (A) A keratocyte localized between stromal lamellae, (B) A higher-magnification view showing a keratocyte
Keratocytes are similar to fibroblasts and possess an extensive intracellular cytoskeleton, including prominent actin filaments. Keratocytes are thus quiescent in the normal cornea but are readily activated and undergo transformation into myofibroblasts, that express α-smooth muscle actin, in response to various types of insult to the stroma.[88] Myofibroblasts produce ECM, collagen-degrading enzymes, matrix metalloproteinases (MMPs), and cytokines for stromal tissue repair, and their ability to contract contributes to wound closure.

Although scleral fibroblasts are not as well characterized as keratocytes, they are thought to be similar to fibroblasts in other parts of the body. As in the corneal stroma, a slow turnover of collagen fibers by scleral fibroblasts is required for connective tissue homeostasis. Matrix degradation by scleral fibroblasts is promoted by prostaglandin derivatives, which accounts in part for the increase in uveoscleral outflow of aqueous humor and the reduction in intraocular pressure induced by such drugs.[89] Activation of scleral fibroblasts by external stimuli, such as injury or surgery, also results in their transdifferentiation into myofibroblasts and consequent tissue fibrosis.

**Collagen**

The cellular components (mainly keratocytes) occupy only 2–3% of the total volume of the corneal stroma,[90] with the remaining portion comprising mostly the ECM components collagen and proteoglycans. Collagen constitutes more than 70% of the dry weight of the cornea. Collagen in the corneal stroma is mostly type I, with smaller amounts of types III, V, and VI also present.[91–100] Proteoglycans are distributed among the major collagen fibers.

Both the mean diameter of collagen fibers and the mean distance between such fibers in the corneal stroma are relatively homogeneous and are less than half of the wavelength of visible light (400–700 nm). This anatomic arrangement is thought to be responsible for the fact that scattering of an incident ray of light by each collagen fiber is canceled by interference from other scattered rays,[4] allowing light to pass through the cornea. If the diameter of or the distance between collagen fibers becomes heterogeneous (as occurs in fibrosis or edema), incident rays are scattered randomly and the cornea loses its transparency.

Procollagen molecules are secreted by keratocytes into the extracellular space, after which the propeptides at both ends are cleaved to yield the mature collagen molecules. The collagen molecules self-assemble into fibrils with a diameter of 10–300 nm, and these fibrils subsequently further assemble into collagen fibers. Individual collagen fibers in the corneal stroma can be observed by transmission electron microscopy (see Fig. 1.9). As mentioned above, both the diameter of (22.5–35 nm)[99],[101] and distance between (41.4 ± 0.5 nm)[102] collagen fibers in the corneal stroma are highly uniform, with this regular arrangement being a major determinant of corneal transparency. At high magnification, each collagen fiber exhibits a characteristic cross-striation pattern with a periodicity of 67 nm (see Fig. 1.9). In the corneal stroma, the collagen fibers form about 300 lamellae.[103] Each lamella courses parallel to the surface of the cornea from limbus to limbus. The turnover of collagen molecules in the cornea is slow, requiring 2 to 3 years.

The histological features of the scleral stroma are similar to those of the corneal stroma, with the scleral stroma also being composed largely of major collagen fibers and proteoglycans.[104] The collagen types detected in the scleral stroma are also similar to those in the corneal stroma. In contrast, the matrix components present in the spaces between the major collagen fibers in the scleral stroma differ from those in the corneal stroma. This difference in the noncollagenous matrix largely accounts for the difference in ultrastructure between the cornea and sclera. Whereas the collagen fibers in the corneal stroma are highly uniform in diameter, those in the scleral stroma range in diameter from 25 to 250 nm. Furthermore, whereas collagen fibers are arranged regularly with a relatively uniform interfiber distance in the corneal stroma, the distance between collagen fibers in the scleral stroma varies. The ECM of the scleral stroma, including both collagen and noncollagenous components, is produced by the stromal fibroblasts.
Proteoglycans

Proteoglycans, the major matrix components located in the spaces among major collagen fibers in the stroma of the cornea and sclera, are composed of a core protein and glycosaminoglycan chains and are thought to modulate collagen fibrillogenesis.[109] Glycosaminoglycans themselves also play important roles regardless of the core protein to which they are attached. The functions of proteoglycans can thus be considered from the points of view of both the core protein and glycosaminoglycans.

With the exception of hyaluronan (hyaluronic acid), the glycosaminoglycans of the corneal stroma are present in the form of proteoglycans. The most abundant glycosaminoglycan in the cornea is keratan sulfate,[106] constituting about 65% of the total glycosaminoglycan content. The remaining glycosaminoglycans include chondroitin sulfate and dermatan sulfate. Glycosaminoglycans have the ability to absorb and retain large amounts of water. Although corneal hydration is regulated predominantly by an endothelial pump, it is also influenced by the epithelial barrier, surface evaporation, intraocular pressure, and stromal swelling pressure. The tendency of the stroma to swell results from interstitial fluid and fluid repulsion between the fixed negative charges on keratan sulfate and chondroitin sulfate. This swelling tendency has been termed the swelling pressure (SP) and is approximately 50 mmHg in the excised cornea. The negative pressure that draws fluid into the cornea is termed the imbibition pressure (IP), which, in the excised cornea, is equal to the swelling pressure. In vivo, however, the imbibition pressure is lower than the swelling pressure because of the compressive effect of intraocular pressure (IOP). The relationship between these three parameters is described by the equation: $IP = |IOP - SP|$

An appreciation of the dynamics of corneal edema therefore requires an understanding of the role of the stromal ground substance (glycosaminoglycan chains of proteoglycans) in the hydration state (and hence the clarity) of the cornea. If the pump function of the corneal endothelium is lost, the corneal stroma swells, leading to a disturbance in the regular spacing between collagen fibers. The irregularity of the interfiber distance results in scattering of incident light and renders the cornea hazy.

In terms of core proteins, the corneal stroma contains lumican, keratocan, and mimecan (osteoglycin) as keratan sulfate proteoglycans as well as decorin and biglycan as chondroitin sulfate or dermatan sulfate proteoglycans (Table 1.3).[107][108] These core proteins are members of the family of small leucine-rich proteoglycans (SLRPs), which contain a common central domain consisting of about 10 leucine-rich repeats.[109] They first accumulate as low-sulfate glycoproteins in the embryonic stroma and subsequently bind glycosaminoglycans to form proteoglycans typical of the adult cornea. Although the roles of specific proteoglycans in the maintenance of corneal transparency or shape under physiological conditions or in the development of corneal haziness under pathological conditions remain unclear, spontaneous mutation of a core protein gene has provided some insight. Mutation of the keratocan gene was recently shown to result in cornea plana, an anomaly characterized by abnormal corneal curvature, but it did not affect the transparency of the corneal stroma.[110][111]

Recent studies with transgenic or knockout mice have also provided insight into the roles of proteoglycan core proteins. Lumican-deficient mice have been shown to undergo age-dependent opacification of the corneal stroma.[112][113] Transmission electron microscopy revealed an irregular arrangement of collagen fibers in the posterior stroma of these animals. Humans with a mutated lumican gene have not yet been described, however. Keratocan-deficient mice show a change in the shape of the eye shell, but the transparency of the corneal stroma is not affected.[119] Mice lacking decorin exhibit abnormal collagen fibrillogenesis in the tail tendon but not in the corneal stroma,[114] indicating that decorin may not play an important role in maintenance of corneal stromal transparency, despite its abundance in the stroma. Such genetically modified mice not only shed light on the functions of specific molecules but also provide models of human genetic disorders of the cornea.

The main difference between the proteoglycan composition of the sclera and that of the cornea is the absence of keratocan, a specific marker of keratocyte differentiation,[108] in the sclera. However, this difference alone does not explain the lack of uniformity in the size and arrangement of collagen fibers in the sclera. The relative amounts of proteoglycan components in the sclera are changed in an animal model of myopia.[115] Recent studies suggest that matrix components of the cornea or sclera play specific roles in regulation of the shape or size of the eye shell. The eyeball of lumican-deficient mice is larger than that of wild-type animals, whereas that of keratocan-deficient mice is smaller.[110,112,113]

### Descemet's membrane

Descemet's membrane, the basement membrane of the corneal endothelium, gradually increases in thickness from birth (3 µm) to adulthood (8–10 µm) in humans. Histological analysis reveals it to be stratified into a thin (0.3 µm) nonbanded layer adjacent to the stroma, an anterior banded zone (2–4 µm), and a posterior amorphous, nonbanded zone (>4 µm), the latter of which can represent up to two-thirds of the thickness of the membrane and is laid down over time.[116]

Descemet's membrane is composed primarily of collagen types IV and VIII and laminin[117] but also contains fibronectin.[118][119] Type VIII collagen, which is produced by the corneal endothelium, forms a hexagonal lattice that is substantially different from the structure of type IV collagen in the basement membrane. Collagen fibrils in the stroma are continuous with those in Bowman's layer but not with those in Descemet's membrane.

Descemet's membrane adheres tightly to the posterior surface of the corneal stroma and reflects any change in the shape of the stroma. Rupture of Descemet's membrane by physical stress, such as compression birth injury, results in the penetration of aqueous humor into the corneal stroma and consequent stromal edema. Descemet's membrane does not regenerate after endothelial cells re-cover the ruptured area. Diseases such as Fuchs' dystrophy are associated with an atypical striated pattern of collagen deposition in Descemet's membrane.[120] A patient with early-onset Fuchs' dystrophy was found to harbor a mutation in COL8A2[121] which encodes the α2 chain of type VIII collagen.

### Table 1.3 - Glycosaminoglycans and proteoglycan core proteins in the cornea

<table>
<thead>
<tr>
<th>Glycosaminoglycan</th>
<th>Size (kDa)</th>
<th>Constituent disaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparan sulfate</td>
<td>5–12</td>
<td>N-acetylgalactosamine, glucuronic acid</td>
</tr>
<tr>
<td>Heparin</td>
<td>6–25</td>
<td>N-acetylgalactosamine, glucuronic acid</td>
</tr>
<tr>
<td>Dermatan sulfate</td>
<td>15–49</td>
<td>N-acetylgalactosamine, iduronic acid</td>
</tr>
<tr>
<td>Chondroitin 4,6-sulfate</td>
<td>5–50</td>
<td>N-acetylgalactosamine, glucuronic acid</td>
</tr>
<tr>
<td>Keratan sulfate</td>
<td>4–19</td>
<td>N-acetylgalactosamine, galactose</td>
</tr>
<tr>
<td>Hyaluronan</td>
<td>4–8000</td>
<td>N-acetylgalactosamine, glucuronic acid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Core protein</th>
<th>Glycosaminoglycan</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumican</td>
<td>Keratan sulfate</td>
<td>Interaction with corneal epithelial cells</td>
</tr>
<tr>
<td>Keratocan</td>
<td>Keratan sulfate</td>
<td>Mutation causes cornea plana</td>
</tr>
<tr>
<td>Mimecan</td>
<td>Keratan sulfate</td>
<td>Unknown</td>
</tr>
<tr>
<td>Decorin</td>
<td>Chondroitin sulfate or dermatan sulfate</td>
<td>Wound healing</td>
</tr>
</tbody>
</table>

In the normal cornea, proteoglycans are synthesized by stromal keratocytes. They are transiently synthesized by corneal epithelial cells during the early phase of wound healing.

![Image](449x621 to 507x634)
**Endothelium**

A single layer of corneal endothelial cells covers the posterior surface of Descemet's membrane in a well-arranged mosaic pattern (Fig. 1.11). These cells are uniformly 5 µm in thickness and 20 µm in width and are polygonal (mostly hexagonal) in shape. The uniformity of endothelial cell size has been evaluated by statistical analysis based on photographs taken by a wide-field specular microscope. In young adults, the cell density is about 3500 cells/mm². The coefficient of variation of mean cell area (standard deviation of mean cell area/mean cell area) is a clinically valuable marker and is about 0.25 in the normal cornea. An increase in the variability of cell area is termed polymegathism. Another morphometric parameter of the state of the endothelium is hexagonality. In the normal healthy cornea, about 70–80% of endothelial cells are hexagonal. However, endothelial damage can result in a decrease in the hexagonality value and an increase in the variability of cell area (Fig. 1.11). Deviation from hexagonality is referred to as pleomorphism.

![Fig. 1.11](image)

**Fig. 1.11** Scanning electron microscopy of rabbit corneal endothelial cells in situ (A) and specular microscopy of the endothelium in both a normal human cornea (B) and the cornea endothelial cells associated with bullous keratopathy.

Endothelial cells contain a large nucleus and abundant cytoplasmic organelles, including mitochondria, endoplasmic reticulum, free ribosomes, and Golgi apparatus (Fig. 1.12), suggesting that they are metabolically active. The endothelial cells interdigitate and contain various junctional complexes, including zonula occludens, macula occludens, and macula adherens. In addition, gap junctions allow the transfer of small molecules and electrolytes between the endothelial cells. The interconnected endothelial cell layer provides a leaky barrier to aqueous humor.
Loss of or damage to corneal endothelial cells results in increased imbibition of water by the corneal stroma. The endothelial cells contain ion transport systems that counteract the imbibition of water into the stroma. An osmotic gradient of Na⁺ is present between the aqueous humor (143 mEq/L) and the stroma (134 mEq/L). This gradient results in the flow of Na⁺ from the aqueous humor to the stroma and in a flux of K⁺ in the opposite direction. The Na⁺- and K⁺-dependent ATPase and the Na+/H⁺ exchanger are expressed in the basolateral membrane of corneal endothelial cells. Carbon dioxide also diffuses into the cytoplasm of these cells and, together with water, generates bicarbonate ions (HCO₃⁻) in a reaction catalyzed by carbonic anhydrase. The HCO₃⁻ then diffuses or is transported into the aqueous humor. Coupled with this movement of HCO₃⁻ is a flux of water across endothelial cells into the aqueous humor. Given that this ion transport system is partially dependent on cellular energy, cooling of the cornea results in its thickening and in it becoming opaque. The return of the cornea to normal body temperature, however, results in restoration of its normal thickness and clarity in a phenomenon known as temperature reversal.

Corneal endothelial cells essentially do not proliferate in humans, monkeys, and cats, but they do divide in rabbits. Endothelial cell density in the normal healthy cornea decreases with age.[122] It is important that corneal endothelial cells are protected during surgery. The loss of endothelial cells for any reason results in enlargement of the remaining neighboring cells and their spreading to cover the defective area, without an increase in cell number. The indices based on specular microscopy fluctuate as endothelial damage is resurfaced by the migration and enlargement of the remaining endothelial cells. The coefficient of variation of mean cell area is the most sensitive index of corneal endothelial dysfunction, whereas hexagonality is a good index of the progress of endothelial wound healing.
Maintenance of Normal Corneal Integrity

Overview

Maintenance of corneal structure is crucial for the physiological roles of this tissue in refraction and biodefense. A smooth epithelium, a transparent stroma, and a functioning endothelium are all essential for clear vision. The cornea is vulnerable to various chemical or biological agents as well as physical events in the outside world. It is, therefore, equipped with an active maintenance system responsible for renewal of the corneal epithelium and wound healing.

The widespread application of corneal surgery, including keratoplasty and refractive surgery, has necessitated a more detailed understanding of recent advances in cellular and molecular biology of corneal wound healing. In most parts of the body, wound healing is initiated by the extravasation of blood constituents that accompanies disruption of blood vessels. The cornea, however, is an avascular tissue. The mechanism of wound healing in the cornea thus differs from that elsewhere in the body.

Epithelial maintenance

Role of limbal stem cells

Corneal epithelial cells renew continuously to maintain the normal layered structure of the epithelium. The centripetal movement of corneal epithelial cells has been well demonstrated, as has the fact that only the basal epithelial cells are capable of proliferation. Thoft and Friend proposed that an equilibrium exists between the centripetal movement of epithelial cells, the differentiation of basal cells into superficial cells, and the desquamation of epithelial cells from the corneal surface (X, Y, Z hypothesis). The existence of corneal epithelial stem cells at the limbus has also been postulated. Indeed, the limbal epithelium exhibits a higher proliferative activity and a lower differentiation capability than those of the corneal epithelium, and basal limbal epithelial cells are thought to be a type of undifferentiated stem cell because they do not express corneal epithelium-specific keratin (keratin 3/12).

Basal epithelial cells of the human limbus express various possible stem cell markers. The expression of p63, α-enolase, keratin 5/14, and the hepatocyte growth factor (HGF) receptor has also been shown to be higher in the limbal epithelium than in the corneal epithelium. Although no direct evidence for the existence of limbal stem cells has been obtained to date, ABCG2 appears to be the most promising surface marker for the identification of such cells. Deficiency of limbal stem cells has been suggested to result in impairment of corneal epithelial homeostasis in individuals with aniridia, inflammatory disorders of the ocular surface such as Stevens–Johnson syndrome, or severe alkali burn of the ocular surface.

Epithelial movement

Injury to the corneal surface is not uncommon and results in an epithelial defect, the rapid resurfacing of which is required for restoration of the continuity of the corneal epithelium. Repair of epithelial defects occurs in three distinct phases characterized by epithelial cell migration, proliferation, and differentiation,
Epithelial migration is thus the initial step in the resurfacing of epithelial defects. Trauma to the corneal epithelium induces the sliding and migration of the remaining epithelial cells adjacent to the injury site toward the defective area. Dynamic changes in cell–cell and cell–matrix (fibronectin–integrin system) interactions, up-regulation of hyaluronan, and modulation of the ECM by newly expressed proteolytic enzymes play important roles in these two types of epithelial cell movement in response to injury. Such changes are under the overall control of growth factors and cytokines.

**Fibronectin–integrin system**

Fibronectin provides a provisional matrix during the first phase of epithelial wound healing in many tissues. Fibronectin appears at the newly exposed corneal surface soon after epithelial or stromal injury, and epithelial cells then attach to and spread over the fibronectin matrix. Adhesion of migrating corneal epithelial cells to a fibronectin matrix is mediated by integrins, which constitute a family of cell surface receptor proteins. To date, this family has been shown to include 24 different α subunits and nine different β subunits, with the selective combination of these α and β subunits determining the specificity of binding to ECM proteins. The integrin subunits α2, α3, α5, α6, αv, β1, β4, and β5 have been detected in the human cornea. The binding of integrins α5β1, αvβ3, and αvβ5 to fibronectin is mediated by the RGD sequence. The appearance and disappearance of the integrin β1 chain and fibronectin during corneal epithelial wound healing are well coordinated (Fig. 1.13).

Immediately after an incision to the rat cornea, fibronectin was detected on the surface of the V-shaped defect in the stroma. Epithelial cells expressing the integrin β1 subunit then began to migrate over and to fill in the defect. With the exception of that in basal cells, expression of the integrin β1 chain in epithelial cells was down-regulated coincident with the completion of wound healing. The abundance of fibronectin at the interface between the new epithelium and the stroma also markedly decreased at this time.

**Fig. 1.13** Changes in the localization of the integrin β1 chain, fibronectin, and laminin after a nonpenetrating incision in the cornea.
The integrin α6β4 heterodimer is a component of hemidesmosomes and is not related to fibronectin-mediated cell adhesion and migration. In response to wounding of the corneal epithelium, hemidesmosomes in the basal cell layer are disassembled. They eventually reappear after migration of the remaining epithelium has resulted in a recovering of the denuded area.[137]

**Hyaluronan**

Hyaluronan is also recognized as a biological signaling molecule and, like fibronectin, plays an important role in inflammation and wound healing.[138] Hyaluronan is not present in the normal cornea. Unlike other glycosaminoglycans, a core protein for hyaluronan binding has not yet been identified. Hyaluronan is transiently expressed in the rabbit cornea during wound healing.[139],[140] These observations suggest that hyaluronan plays a key role in the late stages of corneal wound healing.

Exogenous hyaluronan also increases the rate of corneal epithelial wound healing. The administration of hyaluronan eyedrops thus promotes corneal epithelial wound closure after epithelial debridement in rabbits[141] and in diabetic rats.[142],[143]

**Proteolytic enzymes**

Proteolytic enzymes also play an important role in wound healing. Cellular motility depends not only on the interaction of cells with the underlying ECM but also on the termination of such interaction by degradation of matrix proteins. Proteases, including plasminogen activator, have been detected in tear fluid.[143–146] MMPs are also up-regulated in the migrating corneal epithelium.[147]

**Cytokines and growth factors**

**Epidermal growth factor**

Epidermal growth factor (EGF) was first isolated from the mouse submaxillary gland as a factor that stimulates eye opening and incisory tooth eruption in newborn mice.[149] This 53-amino acid polypeptide is a potent stimulator of proliferation in a variety of cell types, including corneal epithelial cells.[150],[151] It was also found to promote corneal epithelial wound closure in animals.[152],[153]

The continuous exposure of the corneal epithelium to EGF present in tear fluid suggests that the stimulatory effect of this growth factor on epithelial cell proliferation must be counteracted if the normal thickness and function of the epithelium are to be maintained. In addition to its stimulatory effect on cell proliferation, EGF exerts a variety of other actions in corneal epithelial cells, including promotion of cell adhesion to a fibronectin matrix.[154],[155]

**Transforming growth factor-β**

Corneal epithelial cells express transforming growth factor (TGF)-β1.[156] The stimulatory effects of EGF on corneal epithelial cell proliferation, attachment to fibronectin, and migration are modulated by TGF-β.[157],[158] Although TGF-β alone inhibits corneal epithelial cell proliferation, it has no effect on cell attachment to a fibronectin matrix in the absence of EGF. Endogenous TGF-β also promotes corneal epithelial cell migration.

**Basic fibroblast growth factor and platelet-derived growth factor**

Basic fibroblast growth factor (bFGF) is another polypeptide growth factor that stimulates the proliferation of various cell types of mesodermal or neuroectodermal origin.[159] The application of recombinant human bFGF (200–500 ng, twice a day) was shown to accelerate corneal epithelial wound closure in
rabbits. Rabbit corneal epithelial cells also express both the β and α type of receptors for platelet-derived growth factor (PDGF) at a density of $4.3 \times 10^4$ and $2.8 \times 10^3$/cell, respectively. Consistent with this observation, the BB and AB isoforms of PDGF increased the cytosolic concentration of free Ca$^{2+}$ in these cells. Although Ca$^{2+}$ is implicated as a second messenger in regulation of a variety of functions in many cell types, a role for this ion in corneal epithelial wound healing remains to be determined.

**Interleukins**

Interleukins are cytokines that regulate the function of the immune system, inflammation, and other reactions of tissue to external stimuli. They modulate the activities of immune or inflammatory cells both locally in tissue as well as systemically in the circulation and in bone marrow. Although 35 members of the interleukin family (IL-1 to IL-35) have been identified to date, the roles of many of these proteins in corneal wound healing remain to be investigated. For example, the corneal epithelium expresses IL-1, and exogenous IL-1 promotes the healing of corneal epithelial wounds. Corneal epithelial cells also express IL-6. Exposure of rabbit corneal epithelial cells in culture to IL-6 resulted in a marked increase in the number of cells that attached to a fibronectin matrix. IL-6 stimulates the expression of integrin α5β1 in corneal epithelial cells, suggesting that this cytokine may regulate corneal epithelial migration through modulation of the fibronectin–integrin system.

**Neural regulation**

The physiological role of corneal innervation in corneal epithelial wound healing remains to be fully clarified. The loss of corneal sensation, however, often results in breakdown of the normal integrity of the cornea. Persistent corneal epithelial defects or delayed epithelial wound healing are frequently observed in individuals with a reduced corneal sensation, such as those infected with herpes simplex or herpes zoster virus as well as those with diabetes mellitus. Abuse of topical anesthetics also impairs corneal epithelial migration in an organ culture system. Furthermore, frank corneal ulceration has been shown to develop in anesthetized eyes. These various observations thus implicate neural regulation in maintenance and repair of the corneal epithelium.

As discussed earlier, the cornea is densely innervated by sensory nerve fibers of trigeminal nerve origin which contain the sensory neurotransmitter substance P. Substance P is thought to regulate various physiological processes, including plasma extravasation, vasodilatation, and the release of histamine from mast cells. Exposure of rabbit corneal epithelial cells in culture to the combination of substance P and insulin-like growth factor-1 (IGF-1) resulted in a marked increase in the number of cells that attached to a fibronectin matrix. Substance P has also been implicated in neuronal responses to various stimuli in the eye as well as in other tissues.

Trigeminal denervation correlates with a reduction in the abundance of substance P in the cornea. Sectioning of the trigeminal nerve results in trophic or degenerative changes in the cornea as well as in the depletion of substance P. Substance P may thus contribute to maintenance of the normal integrity of the corneal epithelium. The combination of substance P and IGF-1 synergistically promotes corneal epithelial migration, with neither agent alone having an effect on this process. Furthermore, the administration of eyedrops containing IGF-1 and either substance P or a tetrapeptide derived from its carboxyl terminus has been shown to be an effective treatment for persistent corneal epithelial defects in individuals with neurotrophic keratopathy or diabetic neuropathy.

Nerve growth factor (NGF), first discovered by Levi-Montalcini in the early 1950s, is a polypeptide that stimulates the regeneration of peripheral nerve fibers. Furthermore, eyedrops containing NGF promote resurfacing of persistent corneal epithelial defects in animals and humans.

**Stromal maintenance**

**Extracellular matrix and stromal repair**

Structural and biochemical homeostasis of the ECM in the corneal stroma is thus maintained by a balance in the keratocyte regulation of the synthesis and degradation of ECM components. In response to corneal injury, keratocytes transdifferentiate into myofibroblasts and actively produce matrix components for healing of the injured stroma, with each newly expressed macromolecule appearing to play an
important role in the repair process.

During infectious ulceration of the corneal stroma, enzymes that degrade the ECM of the stroma are released by both host cells and the infecting bacteria. Furthermore, pseudomonal elastase degrades collagen directly as well as promotes collagen degradation by keratocytes, in part, via activation of pro-MMPs. Thus, there appear to be at least three different pathways for the degradation of stromal collagen fibers in individuals with infectious corneal ulceration: (1) direct degradation by bacterial collagenase, (2) degradation by MMPs released from keratocytes (or myofibroblasts) activated by bacterial factors such as elastase, or (3) activation by infiltrated inflammatory cells.

Cytokines and growth factors

Both keratocytes and infiltrated cells, such as lymphocytes, neutrophils, and macrophages, secrete cytokines or growth factors and modulate behaviors of cells in the healing corneal stroma. Each cytokine or growth factor activates signal transduction pathways that regulate the expression of specific genes that contribute to the inflammatory response. Targeting of such regulation at the ligand or signaling level may provide new strategies for treatment of wound-related pathology. TGF-β is thought to play a key role in healing of the corneal stroma. It is expressed by both epithelial cells and stromal cells (keratocytes or scleral fibroblasts) as well as by inflammatory cells that activate stromal cells and promote their transdifferentiation into myofibroblasts. Myofibroblasts contribute not only to wound repair but also to post-injury stromal scarring in the cornea and sclera as a result of the overproduction of matrix components. Blockade of TGF-β signaling effectively reduces the fibrogenic reaction and consequent scarring and opacification in a mouse model of corneal alkali burn.

The proinflammatory cytokine tumor necrosis factor (TNF)-α is also up-regulated in response to injury. TNF-α induces various effects in the cornea under pathological conditions such as injury, allergy, and infection. However, the complete loss of TNF-α in the cornea of knockout mice results in enhancement of post-alkali burn inflammation, suggesting that the role of TNF-α in the cornea might depend on the specific condition.

Neovascularization in the corneal stroma

The cornea is an avascular tissue and must remain transparent in order to refract light properly. Neovascularization in the cornea resulting from inflammation associated with various conditions such as trauma, microbial infection, or alkali burn or from limbal stem cell deficiency can thus impair vision. Cytokines and growth factors orchestrate cell behavior associated with the development of corneal neovascularization. Vascular endothelial growth factor (VEGF) and TGF-β thus contribute to injury-induced neovascularization, with ECM components supporting the growth of new vessels. These factors are up-regulated in the corneal stroma (in both inflammatory cells and resident cells) during wound healing or inflammatory disorders.
Development of the Anterior Eye Segment

Characterization of the development of ocular tissues during embryogenesis is important for understanding the pathogenesis of congenital anomalies of the cornea and anterior eye segment (Fig. 1.14). Morphogenesis of the eye is achieved by cell lineages of various origins including the surface and neural ectoderm during embryonic development. Epithelial cells of the cornea are derived from the epidermal ectoderm, whereas keratocytes, scleral fibroblasts, and endothelial cells are of neural crest (neuroectodermal) origin. The surface ectoderm above the neuronal optic cup invaginates to form the crystalline lens. After the lens vesicle has separated from the surface ectoderm, the epithelium on the immature lens differentiates into the corneal epithelium. Neural crest-derived mesenchymal cells migrate in the space between the lens and primitive corneal epithelium and develop into the corneal stroma, endothelium, iris, and trabecular meshwork. Many anomalies of the anterior eye segment result from impaired differentiation of these neural crest-derived tissues.
The surface ectoderm above the optic cup invaginates during the fifth week of gestation in humans, and the primitive corneal epithelium develops junctional complexes by the sixth week. Most scleral fibroblasts differentiate from neural crest cells that surround the optic cup during the sixth week. Mesodermal cells also contribute to development of the sclera and the extraocular muscles. The neural crest cell-derived mesenchyme migrates into the space between the primitive corneal epithelium and lens vesicle in three waves during the seventh week. The first wave of migration results in formation of the corneal endothelium and trabecular endothelium; the second wave of cells differentiates into keratocytes; and the third wave gives rise to the iris. During the eighth week, the keratocytes form five to eight layers of collagen lamellae and the corneal endothelium starts to form Descemet's membrane. Defects in the migration of neural crest-derived mesenchymal cells are responsible for anomalies of the cornea and anterior eye segment including Peters' anomaly. Several genes, including those encoding TGF-β2 and the transcription factor FOXC, have been implicated in the differentiation of neural crest cells into the primitive corneal stroma in mice.[198]

The spaces among collagen fibers become occupied by proteoglycans that are formed as a result of the
binding of glycosaminoglycan chains to previously accumulated core proteins. Even by the sixth month of gestation, the cornea is still not fully mature. The epithelium has only three or four layers of cells, and keratan sulfate proteoglycans continue to accumulate. During the seventh month, however, the cornea is well developed, with the epithelium consisting of four or five layers with readily recognizable basal, wing, and superficial cells. The stroma is also almost fully developed at this time, with the accumulation of keratan sulfate proteoglycans among collagen fibers being virtually complete. Hyaluronan is a major glycosaminoglycan in the corneal stroma during the early stages of embryonic development, but its abundance declines concomitantly with the increase in that of keratan sulfate, chondroitin sulfate, and dermatan sulfate, giving rise to a glycosaminoglycan composition similar to that of the adult stroma.[199]

Recent advances in transgenic and gene knockout technologies in mice have provided important insight into the role of specific genes in the development and homeostasis of corneal tissue as well as into congenital anomalies in humans.[199],[200] Interpretation of such studies also depends on an understanding of the normal process of eye development in the mouse (see Fig. 1.14). The surface ectoderm invaginates into the optic cup at embryonic day (E) 10.5 in mice. At E12.5, the primitive lens has already separated from the surface ectoderm, which will become the corneal epithelium, and the neural crest-derived mesenchyme has begun to migrate into the space between the primitive corneal epithelium and lens. In contrast to the human embryo, the neural crest-derived cells migrate into this space in a single wave. At E14.5, the embryo has already developed the epithelium, stroma, and endothelium of the cornea, and at E18.5 the corneal stroma has increased in thickness as a result of the synthesis of matrix macromolecules. The upper and lower eyelids fuse to each other between E14.5 and E16.5; the eyelids separate and the eyes reopen after birth, and the corneal epithelium then undergoes final maturation.
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Chapter 2 – The Conjunctiva: Anatomy and Physiology

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Critical to maintaining the integrity of the eye, the conjunctiva is a mucous membrane that protects the soft tissues of the eyelid and orbit, allows extensive movement of the eye and is the main site for the production of the aqueous and mucous components of tears. The sebaceous glands of the eyelid produce the third component of the tear film. The conjunctiva also provides a source of immune tissue and antimicrobial agents to protect the ocular surface. Abnormalities of the conjunctiva may lead to restriction of ocular movement, deficiency of the tear film, and decreased host resistance to infection. In addition, the cornea may ultimately be adversely affected because of conjunctival disease.

Embryology

The conjunctiva arises from surface ectoderm and neural crest tissues in the region of the optic vesicle. At 8 weeks (32- to 37-mm stage) the eyelids form from folds of the surface ectoderm and fuse together. The conjunctiva develops within the lid folds from surface ectodermal and neural crest tissue along the posterior surface of the lids and from similar tissues around the developing cornea. The conjunctival epithelium differentiates from the cutaneous epithelium and corneal epithelium as early as the tenth week and definitely by the twelfth (60- to 70-mm stage). Budding of the epithelium in the conjunctival fornices forms the lacrimal gland superotemporally and accessory lacrimal glands of Wolfring and Krause in the inferior and superior fornices (12 weeks, 50- to 55-mm stage). The caruncle arises as a sequestration of the medial lower eyelid to accommodate the development of the nasolacrimal duct. The caruncle is composed of tissues found both in the conjunctiva and skin; however, the surface epithelium is nonkeratinized. The plica semilunaris (semilunar fold) lies between the caruncle and globe. It is similar to the nicatating membrane of certain mammals but does not contain cartilage.
Anatomy

The conjunctiva extends from the corneoscleral limbus to the mucocutaneous junction on the eyelids. The conjunctiva reflects to form a fornix on three sides and an extendible plica medially. The redundant conjunctiva in this region allows for independent movement of the eye and eyelids. Conjunctival surface folds increase the surface area of the conjunctiva, decrease the area of contact, and reduce friction between the bulbar and tarsal conjunctiva (Fig. 2.1).
The larger superior fornix is maintained by fine smooth muscle slips passing from the deep surface of the levator palpebrae muscle to insert into the conjunctiva. These effectively prevent the superior fornix conjunctiva from falling down and blocking vision during upward gaze. The temporal conjunctiva is attached by fine fibrous slips to the lateral rectus tendon, which maintains the position of the conjunctiva during horizontal gaze. A true fornix does not exist medially except in adduction. Fine fibrous strips from the medial rectus tendon insert deep into the plica and caruncle. With contraction of the medial rectus, these slips tighten and form a cul-de-sac medially as the eye adducts. The total surface area for the adult conjunctival sac including the cornea averages 16 cm² for one eye.

The plica semilunaris is a crescent-shaped fold of conjunctiva with its free lateral border lying 3–6 mm lateral to the caruncle. On adduction, a cul-de-sac of approximately 2–3 mm in depth is formed that mostly disappears on abduction. The epithelium contains goblet cells, Langerhans cells, and dendritic melanocytes. The substantia propria, or conjunctival stroma, is highly vascularized and may contain nonstriated muscle, sympathetic nerves, cartilage, and fatty tissue. The caruncle measures 4–5 mm horizontally and 3–4 mm vertically and is located at the most medial aspect of the interpalpebral fissure. The caruncle is attached to the medial rectus and moves with the plica semilunaris during movement of the globe. The caruncle is composed of pilosebaceous units, accessory lacrimal gland tissue, fibrofatty tissue, occasional smooth muscle fibers, and eccrine glands. Deep to the caruncle there may be several large sebaceous glands without cilia, similar to meibomian glands, which open onto the surface. At the mucocutaneous junction of the eyelid margin, there is an abrupt transition from the keratinized, stratified squamous epithelium to the nonkeratinized stratified squamous epithelium of the palpebral conjunctiva. Meibomian glands of the eyelid are seen easily through the transparent palpebral conjunctiva as yellow lobulated structures separated by vascular arcades in the tarsus of the upper and lower eyelids running perpendicular to the eyelid margin. Overlying the mucocutaneous junction is a hydrophobic strip of lipid secreted by the meibomian glands, which separates the dry anterior keratinized portion of the eyelid from the wet posterior, nonkeratinized part. The exact position of the mucocutaneous junction is determined by the air–fluid interface of the tear film meniscus. Ectropion will cause the mucocutaneous junction to move posteriorly, while entropion will cause the mucocutaneous junction to move anteriorly.

The tarsal conjunctiva is tightly adherent to the substance of the tarsus to present a smooth surface to interface the anterior corneal surface. Consequently, there is no accessible subconjunctival tissue plane for dissection of the tarsal conjunctiva. Along the tarsal surface, 2 mm posterior to the lid margin, lies a
shallow subtarsal groove less than 1-mm deep. The subtarsal groove is situated parallel to the eyelid margin for most of the length of the tarsus. In this region there is transition from the nonkeratinizing stratified epithelium of the lid margin to the more cuboidal epithelium of the tarsal conjunctiva (Fig. 2.2). Between the eyelid margin and the tarsal groove are multiple ridges and grooves that communicate with goblet cell-lined invaginations of the conjunctival epithelium (the pseudocrypts of Henle) (Fig. 2.3). Few crypts are present at birth; most develop at puberty. After age 50 years the crypts are found in about one-third of specimens.[2] The crypts are more numerous in the nasal conjunctiva and around the plica. Accessory lacrimal glands are located in the fornical conjunctiva (glands of Krause) and in the palpebral conjunctiva above or within the tarsus (glands of Wolfring) (Fig. 2.4).
Fig. 2.2 Histologic section through the upper eyelid. Meibomian glands (1) and the mucocutaneous junction (2) can be seen.

Fig. 2.3 Histologic section of tarsal conjunctiva showing the pseudocysts of Henle (1).
The bulbar conjunctiva is smoother and more loosely adherent to underlying tissues than the tarsal conjunctiva. At the corneoscleral limbus there is a series of fibrovascular ridges perpendicular to the corneal margin (palisades of Vogt). These arcades are formed by the epithelial rete ridges and the stromal condensations beneath them and may become accentuated to form peripheral corneal neovascularization (corneal pannus).
Histology

The conjunctival surface is composed of stratified nonkeratinizing epithelium and varies in thickness and appearance from the eyelid margin to the limbus. Unlike any other stratified squamous epithelium, goblet cells are dispersed among and attached to neighboring epithelial cells. There is some controversy as to the location of the conjunctival stem cells. They may be uniformly distributed throughout the bulbar and fornical conjunctiva,[3] located at the mucocutaneous junction on the eyelid,[4] or at the corneal–scleral limbus and the mucocutaneous junction.[5] Recent work further supports the uniform distribution of conjunctival epithelial stem cells in the bulbar conjunctiva.[6] Ectopically, corneal epithelial cells may reside in the conjunctival epithelium and participate in corneal re-epithelialization in cases where the corneal stem cells are compromised.[7]

Palpebral and fornical conjunctiva

The forniceal conjunctival epithelium is two to three cell layers thick over the superior tarsus and four to five cell layers thick over the inferior tarsus. The forniceal conjunctival epithelium tends to be more columnar in nature, whereas the palpebral conjunctival epithelium is more cuboidal. Subepithelial cysts often arise from invaginated areas of surface palpebral conjunctival epithelium or crypts that have closed off (pseudocrypts of Henle). These cysts are lined by surface epithelial cells and contain mucin secreted from goblet cells. Near the eyelid margin, the stratified columnar epithelium of the conjunctiva contains more tonofilaments and merges imperceptibly with the keratinized, stratified squamous epithelium of the eyelid skin (see Fig. 2.2). The surface epithelium beyond the eyelid margin becomes keratinized where the surface is not covered by fluid and is continuously exposed to air.

Bulbar conjunctiva

The bulbar conjunctival epithelium consists of six to nine layers of stratified nonkeratinizing squamous epithelial cells arranged in an irregular fashion in contrast to the more regularly arranged corneal epithelium. Cytoplasmic organelles are similar to those of the cornea but more abundant. The basal and intermediate epithelial cells contain more and larger mitochondria than the corneal epithelium, suggesting a higher level of oxidative metabolism. Cytoplasmic tonofilaments are present and form dense bundles, some closely associated with desmosomes. Fewer desmosomes are present than in the corneal epithelium. The epithelial cellular membranes show marked infoldings, with incomplete interdigitation with adjacent cells. This configuration produces wide intracellular spaces in which antibodies and other plasma constituents and inflammatory cells from underlying vessels can accumulate. In addition, infectious and topically applied substances can gain access to the intracellular spaces and then to the subconjunctival capillaries and systemic circulation. Apically, a glycocalyx is secreted from mucin-containing intraepithelial vesicles[8] (Fig. 2.5). The vesicles release their contents by fusing with the apical membrane forming the glycocalyx, consisting of transmembrane mucins (MUC1, MUC4, MUC16). The long-chain glycoprotein molecules maintain tear film stability by anchoring the mucin produced by the goblet cells (MUC5AC) to the conjunctival surface and also bind immunoglobulins. The bulbar conjunctival epithelium is attached to a thin basement membrane, which is discontinuous in some places, by relatively few hemidesmosomes. This configuration allows wandering cells access to the conjunctival stroma. Lymphocytes, dendritic melanocytes, and Langerhans cells may be seen in the suprabasal region.
of the epithelium.

Fig. 2.5 Electron micrograph of the conjunctiva showing the microvilli (1) and glycocalyx (2, inset).

The conjunctival basement membrane zone (BMZ) does not normally show immunochemical reactivity to any immunoglobulins, complement components, or albumin. The superficial cells in normal subjects do show variable amounts of IgA and IgG reactivity. BMZ immunoreactivity to IgM, IgD, and IgE may be seen in patients with ocular cicatricial pemphigoid but is not found in normal conjunctiva. Fibrinogen is normally found at the BMZ and can serve as a positive control when processing conjunctival specimens for immunoreactivity.[9]

**The corneoscleral limbus**

Similar to the palpebral eyelid margin, there is a gradual transition at the limbus from the stratified, nonkeratinized columnar epithelium of the conjunctiva to the stratified, nonkeratinized squamous epithelium of the cornea (Fig. 2.6). There are seven to ten layers of cells at the limbus, which have cell-to-cell and cell-to-substrate attachments similar to those of the cornea. The areas of stratified nonkeratinizing squamous epithelium at the eyelid margin and limbus correspond to the areas of most common contact and greatest pressure between the palpebral and bulbar surfaces maintained by the muscle of Riolan of the eyelid margin. This mechanical appositional pressure, along with eyelid movement, has been suggested as the greatest stimulus for the formation of stratified squamous epithelium at the eyelid margins and limbus.[10] The stem cells for corneal epithelium reside at the limbus in the basal layer. The limbus may also be a source of conjunctival stem cells.[5]
Conjunctival goblet cells

Goblet cells are unicellular, mucin-secreting glands that account for approximately 5% to 10% of basal cells. They are likely apocrine in nature, with all the secretory granules secreted once the cell has been activated. They are the primary source of the large soluble mucins in the tear film. Goblet cells release their secretory granules in response to activation of the parasympathetic nerves that surround them. The neurotransmitters acetylcholine and vasoactive intestinal peptide (VIP) are known neurotransmitters that stimulate goblet cell mucin secretion. Sympathetic nerves also surround the goblet cells. While sensory nerves surround conjunctival squamous epithelial cells, they do not appear to surround the goblet cells. The mucin synthesized by goblet cells of normal human conjunctiva is identified as MUC5AC. The cysteine-rich domains at the N and C termini lead to a viscous mucus (gel-forming) that provides a scaffolding for the mucin layer of the tear film. Goblet cells may also secrete other proteins. Transmembrane mucins MUC1, MUC4, and MUC16 are expressed at the ocular surface. These mucins function to protect, hydrate, and lubricate the ocular surface. They are also involved in cell signaling and may be abnormally released from the ocular surface in conditions associated with inflammation (e.g., dry eye).

Goblet cells likely arise from common bipotential progenitor cells distributed in the bulbar and palpebral conjunctiva. The nucleus and cytoplasmic organelles are displaced toward the basal aspect of the cells with mucin packets located apically, accounting for its goblet-like appearance (Fig. 2.7). Although tonofilaments are present, they are not highly differentiated. Tight junctions attach goblet cells with adjacent epithelial cells. The density of conjunctival goblet cells is between 1000 and 56 000 cells/mm². They are not distributed uniformly, may occur singly or in random groups, and are more numerous over the tarsal and inferonasal bulbar conjunctiva and less dense temporally and at the limbus. The density of goblet cells peaks in young adults and then decreases until age 30 years, remaining constant thereafter. The density of goblet cells is influenced by local ocular disease such as keratoconjunctivitis sicca, ocular pemphigoid, Stevens–Johnson syndrome, and chemical injuries, and by factors in the external environment such as humidity, temperature, and pollution.
In ocular cicatricial pemphigoid, vitamin A deficiency, and atopic keratoconjunctivitis there is an increase in the conjunctival epithelial mitotic rates. Although conjunctival goblet cell density is decreased in ocular cicatricial pemphigoid, it is increased in atopic keratoconjunctivitis. Recent evidence suggests that goblet cells are innervated by cholinergic and sympathetic nerves. The hormonal control of goblet cells is not known. In vitamin A deficiency, goblet cells are lost and epithelium becomes keratinized (squamous metaplasia). This suggests that vitamin A may play an important role in conjunctival differentiation and in the pathogenesis of some ocular surface diseases. The loss of goblet cells is an early sign of squamous metaplasia.
**Substantia propria**

The conjunctiva rests on fibrovascular connective tissue, which varies in thickness and density (substantia propria). In the tarsal conjunctiva, the substantia propria is thin and compact and is attached to the tarsus more firmly superiorly than inferiorly. In the fornices, it is thick and loosely attached to the globe and orbital septum. The substantia propria extends temporally behind the canthus and nasally to the semilunar fold. At the limbus it is thin and compact, and merges with Tenon's fascia and episcleral tissues.

The substantia propria can be divided into superficial and deep layers. The superficial layer of the substantia propria consists of loose, interconnected connective tissue. This layer is not present at birth and begins to form at 8 to 12 weeks of age. In adults there is a 50- to 70-mm thick layer of lymphocytes (adenoid layer), which is more prominent inferiorly. In normal, noninflamed conjunctiva, there are no true follicles with germinal centers; however, lymphocytes can be stimulated by viral or chlamydial infections or by a toxic reaction to certain topical medications to form follicles with reactive germinal centers. Follicles tend to elevate the conjunctival epithelium, producing a round, fish-egg-like mound. Similar to other lymphoid organs, sensory nerves have been found in association with the conjunctival mucosa-associated lymphoid tissue in monkeys.[29] Papillae form from a reactive, histamine-mediated vascular reaction. Papillae are characterized by chronic inflammatory cells (lymphocytes and plasma cells) and the presence of a central vascular core.[2]

The deeper, fibrous layer contains vessels, lymphatics, and nerves. Capillaries arise from the anterior ciliary arteries, which are branches of the ophthalmic artery, and drain into the episcleral venous plexus. Lymphatics drain into the episcleral plexus, which joins the drainage system of the eyelids, draining into the submandibular and preauricular lymph node systems. There is no lymphoid drainage posterior to the orbital septum. Sensory nerves arise from the ophthalmic division of the trigeminal nerve (V1).
Vascular Supply

The palpebral conjunctiva and lids share a common arterial blood supply that arises from terminal branches of the ophthalmic artery: the dorsal, nasal, frontal, supraorbital, and lacrimal arteries. The facial, superficial, temporal, and infraorbital branches from the facial artery provide supplemental blood supply. In the bulbar conjunctiva, branches from the anterior ciliary arteries, which are a continuation of the muscular branches supplying the rectus muscles, form a superficial marginal plexus at the limbus, giving rise to the terminal vessels of the peripheral arcades and the palisades of Vogt. Branches of the bulbar anterior ciliary arterial system anastomose in the fornices with recurrent vessels from the palpebral conjunctiva. Conjunctival vessels maintain their superficial position, and a deeper circulation furnishes blood supply to the peripheral corneal arcades, iris, and ciliary body. Inflammatory processes of the conjunctiva result in prominence of the superficial vessels, which increases away from the limbus. Inflammatory processes of the cornea, iris, or ciliary body result in prominence of the deep vessels, which increases toward the limbus. Clinically, this process is manifest as coronal or ciliary flush.

Conjunctival capillaries are fenestrated. Fenestrae are specialized plasma membrane microdomains in endothelial cells that are involved in vascular permeability. They appear as circular discontinuities of ≈60 nm in diameter and usually occur in clusters in the most attenuated regions of the endothelial cell.[30] Each fenestration is covered by the plasma membrane of the underlying endothelial cell. Some of the deeper vessels are not fenestrated. Fenestration allows more rapid passage of luminal contents in inflammation. After intravenous injection of fluorescein, conjunctival vessels can be seen to leak in a time and concentration sequence similar to that of the choroidal capillaries. The vessels at the palisades of Vogt may be more competent and leak less than conjunctival vessels elsewhere.[31] Conjunctival inflammation, infections, irritation, or severe intraorbital inflammation cause the conjunctival capillaries to leak plasma proteins faster than the fluid can pass between the epithelial cells. This process causes thickening of the epithelium and chemosis of the conjunctiva.[32] Vessel engorgement varies during the menstrual cycle.[33]

Venous drainage from the palpebral conjunctiva joins the post-tarsal veins of the eyelids and the deep facial branches of the anterior facial vein and pterygoid plexus. The bulbar conjunctival veins drain into the episcleral venous plexus, which drains into the intrascleral plexus. Wind, cold, heat, and endocrine changes associated with menstruation and early pregnancy dilate the venous side of circulation.[33]
Lymphatic Drainage

The conjunctiva contains a rich anastomotic network of lymphatic channels that drain into the episcleral lymphatic plexus. Many small, irregular lymphatic channels arise 1 mm peripheral to the limbus and anastomose to form larger collecting channels in the deep layer of the substantia propria. Occasionally, these can be seen as irregular, dilated, sausage-shaped channels (lymphangiectasia). Lymphatics of the conjunctiva join the lymphatics of the eyelids and drain medially to the submandibular lymph node and laterally to the preauricular (intraparotid) lymph node system.
Nerve Supply

Proper sensory innervation of the conjunctiva is essential to maintain its health and ultimately the health of the eye. The conjunctiva is richly supplied with free nerve endings that arise from the lacrimal, supraorbital, supratrochlear, and infraorbital branches of the ophthalmic branch of the trigeminal nerve (V1). The threshold for tactile conjunctival sensitivity is 100 times greater than that of the center of the cornea. This is likely due to lower innervation density and that the nerve endings innervating the conjunctival epithelium are further away from the surface and less exposed to stimuli compared to the cornea. It is least sensitive in the perilimbal area and most sensitive along the marginal palpebral conjunctiva. Pain can occur with inflammation, an epithelial defect, hypoxia, and osmotic shock, all of which cause deformation of the nerve endings. Small peptides produced by inflammation may stimulate free nerve endings and increase pain. Inflammation can also lower the threshold to pain (primary hyperalgesia). When the pain threshold is exceeded, pain may become more intense and severe (secondary hyperalgesia). The most common sensations are foreign body sensation, burning, and itching. The conjunctiva is also capable of low-threshold temperature sensitivity.[34]

In the rat, conjunctival nerve fibers contain neuropeptide Y, vasoactive intestinal peptide (VIP), histidine, isoleucine, helospectin, substance P, and calcitonin gene-related peptide. The superior cervical ganglion (sympathetic) contributes the most to innervation via neuropeptide Y-containing fibers. VIP-containing fibers (parasympathetic) arise from the sphenopalatine ganglion. Substance P-containing fibers (sensory) travel to the trigeminal ganglion.[35] In humans, the accessory lacrimal glands of Zeiss and Wolfring, the glands of Moll, and goblet cells are innervated by VIP-containing nerve fibers.[36] Parasympathetic nerves and M(1), M(2), and M(3) muscarinic receptors as well as sympathetic nerves are present on mouse and human goblet cells.[15] Adrenergic receptors are also present on mouse and human goblet cells.[15]
Normal Flora

The conjunctiva is well protected from infectious disease. The mechanical sweeping of the lids and the presence of tear lysozyme and lactoferrin, as well as other antimicrobial factors in the tears, are available defense mechanisms. In addition, the migration of antibodies and inflammatory cells, which are supplied across the conjunctival epithelium from the indigenous lymphocytic population and from the systemic circulation, participate in this defense. There is also a delicate balance between host tolerance and parasitic invasiveness. Any alteration of this delicate balance can lead to tissue destruction. The normal conjunctival flora is relatively consistent worldwide. If an organism is present in one eye, it is usually cultured from the other eye. Organisms cultured from the conjunctival sac are almost always found on the eyelids. When organisms are found on the eyelids, however, only about one-half are cultured from the conjunctival sac.[37]

The ocular surface of healthy individuals supports a relatively small population of bacteria, typically coagulase negative staphylococci, of which *Staphylococcus epidermidis* is the most common isolate. Bacteria are more frequently isolated from individuals with dry eye compared to normals.[38] A greater diversity of conjunctival bacteria are isolated from normals by using molecular cloning and DNA sequencing, including *Corynebacterium*, *Propionibacterium*, *Rhodococcus erythropolis*, *Klebsiella* spp., *Erwinia* spp.[38] Ten percent of adults’, 5% of children’s, and 1% of infants’ eyes are culture positive for fungi.[39] Conjunctival cultures of adults have shown a greater number of aerobic and anaerobic bacterial species than those from children, while *Streptococcus* spp. were more commonly isolated from children.[40]
Physiology of the Conjunctiva

The conjunctiva provides a barrier to exogenous infectious agents and foreign bodies and allows rotation of the globe. The human conjunctiva occupies 17 times more surface area than the cornea, is more permeable than the cornea, and likely exerts more effect on the tear film than the cornea.[41] The conjunctival epithelium has sufficient water permeability and the transporters necessary to contribute significantly to tear film volume that may represent basal tear secretion.[42],[43] The conjunctiva not only secretes electrolytes, water, and mucin into the tear film but also is capable of absorbing electrolytes, water, and other compounds from the tear film. It plays an important role in the absorption of ophthalmic drugs applied to the ocular surface.[44] Under pathological conditions such as inflammation or the application of substances that increase vascular permeability, there is leak of plasma, electrolytes, water, and proteins, which can alter the composition of the tear film. Under normal conditions there is fluid transport across the epithelium. This secretion is regulated by nerves, growth factors, and other types of agonists such as the P2Y2 agonists UTP and ATP. Mucin secretion by goblet cells and fluid secretion by stratified squamous cells is likely controlled by different nerves.[44]

Nutrition to the conjunctiva comes from underlying blood vessels and the tear film. Conjunctival epithelium differs from corneal epithelium in gross and histologic appearance and in its biochemical functions. The cornea is a clear, regular, refracting and reflecting surface without blood vessels. The conjunctiva, in contrast, is translucent, irregular, and vascularized. The cornea is devoid of goblet cells; the conjunctiva has numerous goblet cells. The corneal epithelium is five to six layers thick with an orderly progression from basal to wing to superficial cells on an avascularized stroma, whereas the conjunctiva consists of six to nine layers of cells arranged in an irregular fashion on a vascularized stromal bed. Nutrition for the cornea must diffuse across a great distance through the corneal epithelium, endothelium, and stroma. Conjunctival nutrition comes directly from nearby blood vessels. The corneal epithelial cells maintain and require large stores of glycogen for epithelial wound healing; the conjunctiva does not (Table 2.1).

Table 2.1 -- Comparison of conjunctiva and corneal anatomy, histology, and physiology

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conjunctiva</th>
<th>Cornea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity</td>
<td>Translucent</td>
<td>Clear</td>
</tr>
</tbody>
</table>
Epithelium | 6–9 less orderly layers | 5–6 orderly layers
--- | --- | ---
Goblet cells | Present | Absent
Stromal bed | Vascular | Avascular
Source of nutrition | Conjunctival vessels, tear film | Anterior chamber, tear film
Glycogen content | Low | High
Dependence on glycogen | Low | High

The conjunctiva is an important source of tear mucin, which arises from the goblet cells (MUC5AC) and conjunctival epithelium (MUC1, MUC2, MUC4). Goblet cells account for 5% to 10% of all ocular surface cells.[11] Carbohydrates, amino acids, and other nutrients are readily available from nearby conjunctival vessels. Small amounts of glycogen are present, since less stored glycogen is needed to meet metabolic needs in case of a crisis. The conjunctiva depends much less on oxidative pathways than does the cornea.[45] There are high levels of glycolic, tricarboxyacetic acid, and respiratory chain enzymes, and low hexose monophosphate shunt activity.[46]

Limbal corneal epithelium with its stems cells act as a barrier that prevents migration of conjunctival epithelial cells on to the cornea.[47] With corneal abrasions that involve the limbus or in ocular surface conditions that result in loss of corneal limbal stem cells (e.g. ocular cicatricial pemphigoid, Stevens–Johnson syndrome, and alkali burns), conjunctival epithelium can migrate onto the cornea. ‘Conjunctivialization’ of the cornea is usually accompanied by blood vessels and is characterized cytologically by the presence of goblet cells.[48] Conjunctival epithelium on the cornea is not stable, does not tolerate trauma well, and is prone to epithelial defects. It has been thought that conjunctival epithelium on the denuded corneal surface eventually undergoes transdifferentiation from conjunctival to corneal epithelium. Recent work suggests that transdifferentiation does not occur in humans and that conjunctival epithelium is instead replaced by normal corneal epithelium from remaining limbal stem cells[49] or from ectopic corneal epithelial cells in the conjunctival epithelium.[7]
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Chapter 3 – Tear Film
Michael A. Lemp, Roger W. Beuerman

Overview and Function

The tear film is a complex composite whose components have multiple sources, which include the lacrimal gland, meibomian glands, goblet cells, and accessory lacrimal glands of the ocular surface. Additional secretory contributions from the ocular surface, which contains several types of embedded tissues, such as the glands of Krause, Moll, and Wolfring, have a structure very similar to that of the main lacrimal gland.[1],[2] The base of the tear film is the outer surface membrane of the corneal or conjunctival epithelial cells. The membrane of the corneal cells is striking in appearance. The membrane is thrown into folds called microplicae or microvilli and the membrane leaflet touching the tear film is very osmophilic. It is usually assumed that the reason for the elaborate folds and filaments extending into the tear film is to aid adherence of the tear film. However, the tear film extends over the conjunctiva as well, and the outer surface of those cells do not show the same elaborations to the extent seen for the corneal cells (Fig. 3.1), but both structures serve to increase the surface area, presumably aiding in tear–epithelial adhesion. The tears have been reviewed often and there are classic texts that detail the well-accepted concepts of the tears; in the present work the emphasis will be on the evolving concepts of the tears.[3],[4]
The function of the tear film includes lubrication, protection from disease, nutrition of the cornea, and a critical role in the optical properties of the eye. In fact, the crisp optical (corneal) reflex commonly seen in clinical or casual photographs of the eye provides evidence of the mirror-like quality of the optical function of this surface and an indication that the tear film is intact. Normal tear volume is around 6 µL and production is about 1.2 µL/minute with a turnover rate of about 16% per minute. The precorneal tear film is a meta-stable structure between blinks allowing for clear vision; this limited stability is compromised in dry eye disease, leading to optical image degradation between blinks.

Although early studies separated the tear film into three discernable layers, that structural rigidity has changed with time, and the tear layers are considered to be more of a continuum with the lipid layer most anterior to the aqueous and mucin components. The aqueous component of the tear film contains proteins, and electrolytes of lacrimal gland origin, and other ocular surface sources. Normal tears contain 6–10 mg/mL total proteins and almost 500 proteins have been reported. Major tear proteins include lysozyme, lactoferrin, secretory immunoglobulin A (sIgA), serum albumin, lipocalin (previously called tear-specific prealbumin), and lipophilin. Tears are a dilute protein solution and both the electrolyte and protein content of the tears varies from that of the serum. Chloride and potassium are higher in the tears (tears, 120 mEq/L and 20 mEq/L; serum, 102 mEq/L and 5 mEq/L, respectively) and glucose concentration is lower in the tears (about 2.5 mg/100 mL) compared to plasma (85 mg/L). The osmotic pressure of the tears in normals ranges between 280 and 305 mOsm/L, whereas in plasma the value is about 6 atm.

Tear proteins vary with the state of health of the ocular surface. Measured levels of proteins such as β2-microglobulin (Mw = 11.8 kDa), cystatins (Mw = 14 kDa), epidermal growth factor (EGF), transforming growth factor β1 (TGF-β1) and β2 (TGF-β2), plasmin, trypsin, and α1-antitrypsin change depending on conditions, although how these change is not always clear. Examination of the levels of IgE and IgA antibodies in serum and tears in a series of patients showed the same specificity for many allergens. Elevated tear plasmatic concentrations were observed after anterior keratectomy and tryptase levels were elevated in tears of patients with active ocular allergy. Growth factors, EGF, TGF-β1, and TGF-β2, are present in normal tear fluids and have been associated with corneal wound healing. In addition to these components of the tear proteome, genetic markers for both ocular and systemic diseases have been reported to be present in tears. Rapid, reliable analysis of tear properties such as tear osmolarity and individual components such as proteins may be useful in the clinical diagnosis of eye diseases such as dry eye disease and conjunctivitis. Sampling of tears, however, presents certain challenges and almost certainly affects results.
Control of Tear Secretion

Control of the tears and hence the activity of the tears has recently been suggested to be under constant neural regulation: a somewhat different concept from the more traditional one that thought only reflex tears are a result of neural participation and that normal tears were the results of intrinsic lacrimal gland activity. Obviously, either too little or too much aqueous secretion will present a problem with visual function. Thus, there is a means for the ongoing homeostatic regulation of the ocular surface, which is under a control mechanism whose components include afferent nerves from the cornea and other ocular surface tissues, central nervous system relay nuclei, and efferent nerves which comprise the autonomic innervation to secretory tissues whose products contribute to the tear film (Fig. 3.2).[28–30] This mechanism is suggested to supply a relatively constant level of neural signals that precisely meter the amount of tears secreted by the main lacrimal gland, but also may mediate lipid production by the meibomian glands and mucin secretion from the goblet cells. The accessory lacrimal glands have been shown to have local innervation, but it is not known if these nerves are included in the homeostatic mechanism or if secretion from the accessory lacrimal glands can be stimulated by transmitter release as part of the lacrimal reflex (Fig. 3.3). Interruption of the neural pathway by different means such as LASIK or anesthesia of the cornea decreases tear flow.[31],[32]
Fig. 3.2 The small sensory nerve endings located just below the epithelial surface of the cornea, lid margin, and conjunctiva constantly respond to drying and temperature change as well as contact and chemical changes, by sending intensity-coded neural signals to the spinal trigeminal nucleus located in the brain stem. A multisynaptic pathway to the preganglionic parasympathetic nuclei in the superior salivatory nucleus forms the output to the secretory tissue. An irritation to the ocular surface gives rise to a large neural input, which provides the neural signal for reflex tearing. The loop is reset when the irritation is removed by copious tearing. For simplicity, some of the components of the neural pathways such as the trigeminal ganglion, sympathetic nerves, and meibomian glands, have been omitted.
**Fig. 3.3** Transmission electron micrograph of the accessory lacrimal gland of the tarsal conjunctiva of the upper eyelid from a nonhuman primate (× 6000). The basic secretory morphology is very similar to that of the orbital lacrimal gland. Small unmyelinated axons (asterisks) are seen adjacent to the basal aspect of the acinar cells.
Tear Layer Thickness

Although its importance may not be immediately apparent, the thickness of the tear layer has received a great deal of attention. It is of interest to know the volume of the tears over the surface of the eye, particularly the cornea, as it is a reservoir for drugs that have been delivered by either topical or systemic routes for penetration into the eye. As a major risk to vision, the lack of a sufficient amount of tears is the primary problem in aqueous-deficient dry eye. The contact lens industry has been interested in the thickness of the tear layer as contact lenses need the support of the tear layer for both optical placement and comfort. From earlier studies, the thickness of the tear layer was found to be about 7–8 µm.[33]

Studies by Prydal using confocal microscopy and interferometry, however, estimated tear film thickness to be over 40 µm.[34] The use of lipid interference patterns to monitor the lipid component of tears have produced interesting new insights and it has been found that several orders of interference patterns could be used to detect changes in the lipid layer and that a dry eye patient was deficient in this regard.[35] Recent studies have reported that the lipid layer of the tears is thinner in many patients with dry eye disease.[36]

However, the controversy has continued. Making use of innovative methods, additional thickness values are still being offered. Using reflection spectra of the human tear film, it was found that there were no oscillations that compared to Prydal's measurements or of earlier estimates. Rather, the results of this study suggested a tear film of about 3 µm.[37] A study of the mouse tear film using a microelectrode technique found the tear film to be about 7 µm.[38] In infants, the lipid layer was found to be thicker than in adults, which may be a response to a thinner aqueous layer.[39]
Analytical Methods

The tears are an attractive source for sampling due to their accessibility, rich content, and largely acellular structure. This latter quality means that unlike the processing of blood samples necessary to separate the cells from serum, direct measurements of tear samples are possible. As mentioned above, tears have an extensive proteome and genomic markers have been reported. There are, however, challenges to sampling tears. Most tear samples are collected using a glass capillary tube applied to the inferior marginal tear strip. Since the entire volume of minimally stimulated tears (ordinary environmental stimulation) is around $7 \mu L$ and only about $2–3 \mu L$ are in the inferior marginal tear strip, and there is minimal exchange between the marginal tear strip and other compartments of tears, collection of more than $2 \mu L$ samples at a single sampling implies reflex tearing. The lacrimal functional unit is composed of the cornea, conjunctiva, tears, lids, and drainage pathways. As outlined above, this neurally controlled unit responds to perturbations in an attempt to maintain a homeostatic environment for cells of the ocular surface. Both changes in volume and composition are induced. Increased secretion from the lacrimal glands and increased passage of transconjunctival fluid result in reflex tearing. Tear composition changes with an increase in some tear proteins and a decrease in others. This leads to great variability in many components of tears, creating clinical variances depending on the degree of induced reflex tearing. Attempts to measure glucose, for example, have been plagued by large differences which are thought to be due to influx of glucose from serum across the conjunctiva induced by sampling stimulation.[40]

Despite the sampling problems described above, a number of analytical methods that couple microliter sample sizes with high sensitivity and resolution have prompted more detailed studies of event-related changes in tear composition. Qualitative and quantitative techniques include one- and two-dimensional polyacrylamide gel electrophoresis (PAGE),[11,41–43] isoelectric focusing (IEF),[11,41] crossed immunoelectrophoresis, enzyme-linked immunosorbent assay (ELISA), and high-pressure liquid chromatography (HPLC) techniques such as size-exclusion HPLC,[44–46] reversed-phase HPLC,[12] and ion-exchange HPLC.[12] Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has also been applied to study changes in tear proteins before and after corneal wound healing,[47] and proteomic methods have been used to map tear protein profiles.[48],[49]

Electrophoresis and 2D-PAGE have been used extensively for tear protein analysis. However, these methods are limited due to the large volume of tears required and the time required to perform the analysis. Furthermore, 2D-PAGE is limited in its ability to analyze small proteins, extremely acidic or basic proteins, or hydrophobic proteins. MALDI provides mass information, but using this technique for quantification can be difficult, although progress is being made in this area.[49]

Surface-enhanced laser desorption-time of flight (SELDI-TOF) ProteinChip technology has recently been introduced as an alternative to 2D-PAGE.[50–52] This technology utilizes affinity surfaces to retain adherent proteins based on their physical or chemical characteristics, which is then followed by direct analysis using TOF-MS. It is a rapid and reproducible technique used to generate protein expression profiles known as ‘phenomic fingerprints.’ SELDI-TOF is more sensitive and requires smaller amounts of tear samples (2–3 \mu L) than 2D-PAGE (Fig. 3.4). This system has enabled detection of critical proteins directly from crude mixtures without time- and labor-intensive preprocessing and has been proven to be a very useful tool to identify biomarkers in various cancers and biomarkers of disease and trauma.[53],[54]
This method is promising for a rapid scan of tear proteins; however, it requires some additional precision to accurately determine mass and, hence, exact protein information.

Fig. 3.4 Tear mass spectrogram from a 3 µL sample of human tears of a normal eye from a SELDI chip. Some of the common tear components are noted as part of identified peaks. The SELDI chip is a rapid method for obtaining a composite spectrogram of many peptide peaks that can undergo further analysis of specific peptide components.
Peptide Components of Tears

As seen in Table 3.1, peptides in the tear film include a heterogeneous variety of bioactive molecules, including a wide range of growth factors with multicellular targets and neuropeptides. There has been a longstanding interest in molecules which augment corneal wound healing, as well as understanding how some of these growth factors (shown in Table 3.1) may complicate wound healing by stimulating scar formation. Epidermal growth factor (EGF) has been shown to stimulate migration of corneal epithelial cells in tissue culture. However, it was found that EGF is a naturally occurring component of the tears.[55],[56] Uncovering antimicrobial peptides in tears was an early initiative due to the vulnerability and devastating effect of infectious disease on the cornea, and continues with additional vigor today, augmented by more sensitive instrumentation. In fact, activity in this area has increased as it has become clear that these peptides have a number of properties in addition to their antimicrobial properties and may in fact be active in the wound response. The cornea and ocular surface, although small in area, are critical for vision and extremely vulnerable to airborne and contact-transmitted pathogens. Accordingly, there has been a great deal of work to determine the presence and activity of the members of these molecular species. Antimicrobial peptides form the system of innate immunity of the ocular surface and are evolutionarily old. These naturally occurring antibiotics act against a wide range of viruses, bacteria, and fungi; however, recently these have been suggested to directly participate in wound healing. The tears have been shown to have lysozyme, lactoferrin, and both α- and β-defensins. [57–89] PMNs which contribute to some of the tear peptides are not found in great numbers in normal tears, but following stress of the ocular surface these are abundant in tears and clearly contribute to the protein milieu. These references represent a good survey of the reports of protein substances within tears thought to play a role in mediating responses to environmental, infectious challenges and disease states.

<table>
<thead>
<tr>
<th>Table 3.1 -- Functional peptides of tears</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>References</strong></td>
</tr>
<tr>
<td><strong>Growth factors</strong></td>
</tr>
<tr>
<td>Epidermal growth factor (EGF)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Transforming growth factor alpha (TGF-α)</td>
</tr>
<tr>
<td>Protein/Peptide</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Transforming growth factor beta-1 (TGF-β1)</td>
</tr>
<tr>
<td>Transforming growth factor beta-2 (TGF-β2)</td>
</tr>
<tr>
<td>Hepatocyte growth factor (HGF)</td>
</tr>
<tr>
<td>Basic fibroblast growth factor (FGF-2)</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF)</td>
</tr>
<tr>
<td>Platelet derived growth factor-BB</td>
</tr>
</tbody>
</table>

**Neuropeptides**

<table>
<thead>
<tr>
<th>Neuropeptide</th>
<th>Reference(s)</th>
<th>Activity/Phenomenon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substance P</td>
<td>15, 71, 72</td>
<td>Wound healing, neurogenic inflammation</td>
</tr>
<tr>
<td>Calcitonin gene related peptide</td>
<td>64, 73</td>
<td>Wound healing, neurogenic inflammation</td>
</tr>
</tbody>
</table>

**Interleukins**

<table>
<thead>
<tr>
<th>Interleukin</th>
<th>Reference(s)</th>
<th>Activity/Phenomenon</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>76</td>
<td>Increases in vernal conjunctivitis</td>
</tr>
<tr>
<td>IL-1α, IL-1β</td>
<td>77, 78</td>
<td>Elevation of IL-1 in dry eye patients</td>
</tr>
<tr>
<td>IL-2, IL-4, IL-6, IL-8, IL-10</td>
<td>79, 80, 81</td>
<td>Increases with contact lens wear, ocular allergy</td>
</tr>
</tbody>
</table>

**Immunoglobulins**

<table>
<thead>
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<th>Immunoglobulins</th>
<th>Reference(s)</th>
<th>Activity/Phenomenon</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA, IgE, IgG(1–4) and complement</td>
<td>21, 82</td>
<td>Ocular allergy</td>
</tr>
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</table>

**Proteases**

<table>
<thead>
<tr>
<th>Protease</th>
<th>Reference(s)</th>
<th>Activity/Phenomenon</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1, MMP-3, MMP-9, TIMP-1, cathepsin, alpha2-macroglobulin</td>
<td>83, 84, 85, 86</td>
<td>Role in pterygium migration and vernal keratoconjunctivitis, protection of the ocular surface</td>
</tr>
</tbody>
</table>

**Antimicrobial peptides**

<table>
<thead>
<tr>
<th>Antimicrobial peptides</th>
<th>Reference(s)</th>
<th>Activity/Phenomenon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme, lactoferrin, α and β defensins, phospholipase A2</td>
<td>87, 88, 89, 90, 91, 92</td>
<td>Increases in infections, wound healing, may decrease in dry eye</td>
</tr>
</tbody>
</table>

The defensin family of peptides has received a great deal of attention as there is considerable evidence for multifunctionality of antimicrobial and wound healing effects.

Defensins are a family of small, cationic antimicrobial peptides containing an average of 35 amino acids with molecular weights around 3–4 kDa. They possess six cysteine residues that form three intramolecular disulfide bonds and have broad-spectrum antimicrobial action against Gram-positive and Gram-negative bacteria, fungi, and some viruses. More recently, human α-defensins (HNP-1, HNP-2, and HNP-3) have been shown to possess anti-HIV activity for human CD8+ T lymphocytes. In humans, six α-defensins (HNP-1–4, HD-5, and HD-6) and three β-defensins (hBD-1-3) have been identified. The α-defensins are most probably released into the tear fluids by resident or passing neutrophils and secretion from lacrimal ductular epithelia. However, other biological functions of defensins have been reported or suggested, such as accelerating epithelial wound healing and mediating inflammatory process. Tumor necrosis factor has already been found to be a tear component in vernal conjunctivitis, but the interactions between these many indicators of immune disease are not yet clear.

Tear research should continue the present resurgence of interest over the next several years and, with the ability to measure a wide range of peptides in a single small volume sample, the diagnostic value of tears may become a reality for diseases such as KCS, immune disease, and for a wide range of corneal infections. Certainly, working out the tear proteome...
would be a much-needed early step to understand how these peptides affect the health of the ocular surface and vision and to develop the research background for targeted therapies. At this time, however, the clinical utility of tear levels of individual proteins and peptides is limited by the volume of tears necessary for most analyses requiring reflex tear samples. Progress in this field may depend on the development of new methods of sampling which collect small samples without inducing reflex tearing and nano-assay techniques for quantitative analysis. Identification of proteins which are not normally present in tears, e.g. IgE in ocular allergy, presents a binary approach which may not be as dependent on quantitative assays.

Another approach is to measure properties of the whole of the tear film rather than individual components, e.g. tear stability and tear concentration of electrolytes or its surrogate, tear osmolarity. Tear film break-up time is a measure of the stability of the tear film; a recently reported new technology for sampling nanoliter quantities of tear atraumatically and measurement of tear osmolarity on samples of 40 nL size may represent a new platform on which to develop diagnostic tests without prior sample processing and suitable for use in the clinical setting.[99]
Acknowledgments

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The eyelids are a thin, complex, and dynamic structure, whose primary function is to protect the ocular surface of the eye. They cleanse and lubricate the eye, protecting it from desiccation as well as damage from foreign bodies and, in doing so, maintain optical visual clarity of the cornea. They serve as both a physical and immunological barrier providing a crucial means of defense against infection. In addition, the eyelids also serve as an important facial aesthetic subunit, and play an essential part of facial expression and cultural identification. It is for these reasons that the eyelids are not only critical for human survival but also for maintenance of quality of life.

The eyelid can be conceptualized as a trilamellar structure. The anterior layer is composed of the eyelid skin and orbicularis oculi and is contiguous with the skin and superficial muscular aponeurotic system of the face and the galea aponeurosis of the scalp. The posterior layer consists of the palpebral conjunctiva and underlying smooth muscle fibers and is contiguous with bulbar conjunctiva by way of the fornix. The anterior and posterior lamellae are separated by a middle tarsofascial layer, which is composed of the orbital septum anteriorly and the retractors of the eyelid posteriorly. The septum and retractors are separated by orbital fat, bound near the eyelid margin by the tarsus. Near the eyelid margin, the orbital fat is attenuated and the septum and eyelid retractors are fused. In the upper lid, the lid retracting structures are the levator palpebrae and its aponeurosis, while in the lower lid they are the inferior retractors and capsulopalpebral fascia.\[1\],\[2\]
Embryology

The formation of the eyelid starts the first week of gestation and continues until birth. During the first nine weeks of gestation, the primitive optic vesicle is covered with a layer of surface ectoderm that is destined to eventually form the future eyelids, conjunctiva, and cornea. Despite their close proximity, the presence of an eye is not required for lid formation.[3]

During the second month of gestation, the surface ectoderm further proliferates and divides to form a multilayered epithelium. A rudimentary fold forms above and below the eye, which is the primitive ‘bud’ of eyelid formation.[4],[5] The fold destined to become the upper eyelid appears slightly before that of the lower eyelid. As these folds continue to grow and lengthen, the outer layer of epithelium forms the skin, while the inner layer becomes the conjunctiva.

Within the primitive eyelid is a layer of mesoderm. The upper lid mesoderm is derived from paraxial mesoderm, while the lower lid develops from visceral mesoderm. Shortly following ectodermal development, the mesoderm begins to thicken and form the basement membrane under the epithelium. Blood vessels then invade the basal portion of each eyelid. The mesoderm will ultimately become the muscles, connective tissues, and tarsus of the eyelids.[5],[6]

Around the eighth and ninth week of gestation, the lacrimal glands are formed, and glandular secretion begins. At week nine, the eyelids have elongated enough to abut each other. Lid fusion then occurs, starting from the medial and lateral canthal regions and moving centrally, progressing from the inner eyelid outward. This is thought to be a true epithelial junction as electron microscopic studies have demonstrated evidence of desmosome formation.[7] After fusion of the eyelids is complete, the closed conjunctival sac becomes filled with lacrimal secretion, and is separated from the amniotic sac.[5]

Upon complete fusion of the eyelids, glandular formation begins. Epithelial invaginations from the fused lid margins form the primitive cilia hair bulbs. This formation begins anteriorly on the lid margin and proceeds posteriorly. Also around the tenth week of gestation, primitive muscle is developing underneath the skin, and the tarsus is beginning to form.[5],[8] Major glandular formation occurs at the fourth month of gestation. The meibomian glands develop as epithelial cell invaginations into the tarsus along the posterior region of the fused lid margins. Lateral outgrowths of epithelial cells from hair follicles initiate the formation of the glands of Zeis and Moll. The glands of Zeis are holocrine glands with sebaceous secretion, while the glands of Moll are modified apocrine sweat glands whose secretions efflux at the base of eyelashes and appear to have immunoprotective properties.[6],[7] Further
differentiation of meibomian glands and the glands of Zeis and Moll occurs later in gestation, including acinar formation and maturation into functional, secreting glands.[5],[6]

Around twenty weeks' gestation, the eyelids begin to separate. This is preceded by the production of lipid in the glands of Zeis and Moll at the lid margin and keratinization of the walls of the glandular ducts.[5] Eyelid disjunction begins anteriorly and progresses in a posterior direction, requiring three weeks for full separation. By this time, the major elements of the lid are present. Glandular differentiation and maturation continues after lid separation, to produce the developed eyelid.[4–6,8]
Anatomy

Epithelium

The skin of the eyelid is the thinnest and most pliable on the human body, ranging from 500 to 1000 µm in thickness. It is thinnest near the lid margin and thickest near the orbital rims, where it is contiguous with infrabrow skin superiorly and malar skin inferiorly. Because of its physical characteristics, the eyelid skin contains more wrinkles than infrabrow or cheek skin, and replacement of eyelid skin may pose a challenge in finding donor sites with comparably thin skin.

Histologically, the skin of the eyelid closely resembles other facial skin, composed of keratinized stratified squamous epithelium. At the mucocutaneous junction of the eyelid (Marx's line), which is located on the eyelid margin just posterior to the orifices of the meibomian glands, the stratified squamous epithelium becomes nonkeratinized (Fig. 4.1).[9]
The dermis of the eyelid is also very thin when compared to skin on the rest of the face and body, and nearly nonexistent. It is composed of loose collagenous fibers interspersed with a rich elastic fiber network. Sparse glands and hair follicles, when present, are contained in the dermis.

The eyelids are relatively hairless, except for laterally where nonpigmented vellus hairs may be found. These vellus hairs may convert into pigmented hairs on hirsute people. Eyelashes are a relatively sparse, specialized type of hair, which may serve as sensory structures causing a reflex eyelid closure when dust or foreign bodies hit them. There are approximately 100 eyelashes in the upper eyelid and 50 in the lower eyelid. In addition, eyelashes serve an important role for eyelid aesthetics. Their limited length, increased shaft diameter, and unique curvature set them apart from any other hair on the body.

Glandular structures contained within eyelid skin include sebaceous glands and sweat glands, which are present at higher density at the peripheral portions of the eyelid, away from the eyelid margin. The lash line also contains two types of glandular structures. The glands of Zeis are holocrine sebaceous glands which secrete their contents into the eyelash duct, which is lined by epithelium continuous with the eyelash follicle. Clinically, these glands are significant due to their potential for malignant transformation into sebaceous cell carcinoma. The glands of Moll are apocrine sweat glands, which empty into the follicle of the eyelash or directly onto the surface of the lid margin. These glands are less numerous than the glands of Zeis and have the potential of forming cystic tumors (sudoriferous cysts).

The subcutaneous space of the eyelid is essentially devoid of fat. The eyelid skin is directly attached to the underlying orbicularis muscle by way of loose connective tissue. At the upper eyelid crease, the canthal angles, and the eyelid margin, the skin is more firmly adherent to the orbicularis.
**Orbicularis oculi**

The orbicularis oculi is a sphincter-like muscle that lies directly beneath the skin of the eyelids and extends from the lid margin and eyelash follicles to beyond the superior and inferior orbital rims. The muscle originates from and inserts into medial and lateral canthal tendons. It is densely adherent to the lower tarsal plate and the superior tarsal plate.

The muscle is divided into three concentric portions, named anatomically according to the underlying structures of the eyelids: pretarsal, preseptal, and orbital orbicularis muscle. The orbital and preseptal orbicularis are under voluntary control, whereas the pretarsal muscle is almost exclusively responsible for the involuntary corneal blink reflex, which plays an important role in lubricating the cornea. The orbicularis is a striated muscle with diffuse innervation by multiple branches of the facial nerve, including frontal, zygomatic, and buccal branches. This muscle is the first of the facial nerve-innervated muscles to begin contracting in utero, which is observed between the eighth and ninth weeks of gestation.[9],[14]

The orbicularis muscle is crucially important for eyelid closure, protection of the globe, and maintenance of corneal moisture. Horner's muscle is a branch of the orbicularis oculi muscle that passes posterior to the lacrimal sac. It divides the lacrimal sac into upper and lower compartments, and covers the lateral component of the lacrimal canaliculus, and contributes to the lacrimal pump.[15] The muscle of Riolan consists of marginal fibers of the palpebral portion of the orbicularis muscle, and is believed to form the 'gray line' at the margin of the eyelid. It functions to hold the lacrimal punctum against the sclera for proper drainage of tears.[16]

**Orbital septum**

The orbital septum is a thin membranous structure that originates from the arcus marginalis of the orbital rim and fuses with the lid-retracting structures (levator palpebrae superioris aponeurosis superiorly and capsulopalpebral fascia inferiorly). It serves as a demarcation point, separating the preseptal space anteriorly and the orbit posteriorly. Anteriorly, the orbital septum lies subjacent to the thin, areolar connective tissue known as the postorbicularis fascia. Retro-orbicularis fat may be found within this space. Deep to the orbital septum lie the upper and lower orbital fat pads. In the lateral superior orbit, the orbital septum abuts the orbital lobe of the lacrimal gland. The orbital septum does not contribute to movement of the eyelid, but rather acts as a retinacular membrane that prevents prolapse of orbital contents. Indeed, its attenuation with age is associated with steatochalasis of the upper and lower eyelids.

**Retractors**

**Levator palpebrae superioris**

The upper orbit has two distinct muscles for supraduction of the globe (superior rectus) and for retraction of the eyelid (levator palpebrae superioris). The inferior orbit has a solitary muscle (inferior rectus) that subserves movement of both the globe and eyelid. Consequently, the upper eyelid is capable of 10–14 mm of excursion, but the lower eyelid is capable of only 4–5 mm.[17]

The levator palpebrae superioris arises from the annulus of Zinn above the superior rectus muscle. It is innervated on its undersurface by the third cranial nerve. The levator muscle passes along with the superior rectus muscle through the posterior orbit, loosely attached to the rectus muscle by intermuscular septa. Where the superior rectus attaches to the globe, the levator muscle transitions to a fascial sheet known as the levator aponeurosis. The levator aponeurosis is a fan-like structure that securely inserts at multiple points on the anterior surface of the tarsus, and to the canthal tendons via more loose attachments. Moreover, extensions of the levator aponeurosis attach to the orbital septum, orbicularis muscle, and pretarsal skin near the upper tarsal border, forming the upper eyelid crease.

**Müller's muscle**

Just deep to the levator aponeurosis lies the Müller's muscle. The Müller's muscle is composed of smooth muscle fibers and is innervated by sympathetic input. It inserts upon the superior tarsal border and is
densely adherent to the underlying conjunctiva. At resting tone, it is responsible for approximately 1–2 mm of vertical elevation of the upper eyelid, whereas at times of stress, its contraction is responsible for creating a look of surprise or shock.[18]

Capsulopalpebral fascia and inferior retractors

In the inferior orbit, the inferior rectus muscle is responsible for inferior displacement of the globe and lower eyelid. A fibrous tissue expansion known as capsulopalpebral fascia extends from the inferior rectus muscle to the anterior surface of the lower tarsus, transmitting pull from the inferior rectus muscle. This fascia is analogous to the levator aponeurosis, and is capable of moving the lower eyelid approximately 4–5 mm from extreme upward gaze to extreme downward gaze. This is under control by cranial nerve III and its action is yoked to the action of the inferior rectus. The inferior orbital septum fuses to the capsulopalpebral fascia 3–4 mm inferior to the tarsus. In addition, a narrow strip of nonstriated muscle known as the inferior tarsal muscle, analogous to Müller's muscle, attaches to the inferior tarsal border. The inferior tarsal muscle is innervated by sympathetic fibers.[18]

Tarsal plates

The tarsal plates are composed of a firm, densely packed collection of collagen fibers. They provide structural stability to the eyelids and also serve as a platform upon which the orbicularis muscle and levator muscle insert. The tarsal plates are tethered medially and laterally by their respective canthal tendons, forming a tarsoligamentous sling upon which the forces of the protractors and the retractors act. The tarsal plates measure approximately 25–30 mm in horizontal length and 0.75 mm in thickness. The vertical length of the tarsal plates is generally 10 mm centrally in the upper eyelid and 4–5 mm in the lower eyelid (Fig. 4.2).
Fig 4.2  Sagittal section of the upper and lower eyelid.

Contained within tarsal plates are the meibomian glands. These glands contribute the oily portion of the tear film, which is important for stabilizing the tear film, and preventing rapid evaporation. The meibomian glands are found in greater numbers in the upper eyelid compared to the lower eyelid (approximately 40 versus 25). Also, the glands are longer in the upper eyelid. Both of these factors may explain the increased production of lipid material by glands of the upper eyelid relative to the lower eyelid. The meibomian glands are a frequent site of chronic granulomatous inflammation of the lids, and rarely may undergo malignant transformation into sebaceous cell carcinomas.

**Conjunctiva**

The conjunctiva is the mucus membrane that lines the inner surface of the eyelids and the anterior surface of the globe. Histologically, it is composed of a nonkeratinized stratified epithelium with goblet cells. The underlying substantia propria, or stroma, is richly vascularized and contains numerous immune defense cells. The histology of the epithelium varies depending on location, from squamous epithelium near the lid margin to columnar epithelium in the tarsal area. In the fornix, the conjunctiva transitions to a prismatic cell type, and to a cuboidal cell type in the bulbar area. Finally it transitions back to squamous epithelium near the limbus. The conjunctiva is continuous with the eyelid skin through the mucocutaneous junction at the lid margin (Marx's line), with the corneal epithelium at the corneoscleral limbus, and with the respiratory mucosa through the lacrimal puncta. The marginal mucosa of the conjunctiva is responsible for spreading the tear film.
At the superior and inferior fornices, the conjunctiva is adherent to underlying structures through the attachment of fibrous extensions from the superior and inferior rectus muscles, respectively. These attachments maintain the shape and integrity of the fornices and prevent prolapse of the conjunctival tissue into the lid aperture. The fornices also contain numerous accessory lacrimal glands of Wolfring and Krause within its submucosal tissue. These lacrimal structures are more numerous in the superior fornix than inferior fornix.

**Eyelid margin**

The lid margin is a short 2-mm wide segment that contains the mucocutaneous junction that demarcates keratinized epithelium anteriorly and conjunctival nonkeratinized epithelium posteriorly, and is composed of pseudostratified squamous mucosa. There are three clinically apparent lines on the eyelid margin. At the posterior eyelid margin, where the horizontal component of the eyelid ends and the vertical component begins, the mucosa makes an abrupt transition into stratified columnar epithelium. Anterior to this lies the gray line which represents an optical reflection of the marginal portion of the orbicularis (muscle of Riolan), and which lies coincident with meibomian gland orifices. The mucocutaneous junction itself lies just posterior to the gray line and is not clinically apparent. At the anterior eyelid margin is the lash line. Chronic inflammation, as in the case of blepharitis, can lead to disruption of these distinct transitions as well as effacement and anterior migration of the mucocutaneous junction.[9],[16]

**The suspensory system of the eyelids**

The medial and lateral commissures are located at either corner of the eye where the upper and lower eyelids meet. These important structures are supported by the medial and lateral canthal tendons, which create a sling-like structure to support the lid margin and maintain apposition against the globe.

The medial canthal tendon has two limbs, an anterior and a posterior limb. The anterior limb is a broader band arising from the superficial head of the pretarsal orbicularis muscle and inserting onto the anterior lacrimal crest. The posterior limb of the medial canthal tendon arises from the deep head of the pretarsal and preseptal orbicularis muscles and inserts onto the posterior lacrimal crest.

The lateral canthal tendon has a superior crus arising from the superior tarsus and an inferior crus arising from the inferior tarsus. The superior and inferior crura fuse at the lateral border of the tarsal plates to join the lateral retinaculum which attaches to Whitnall's tubercle, located 2–4 mm posterior to the lateral orbital rim, and 9–12 mm below the zygomaticofrontal suture.[21]

When observed straight on, the lateral canthus appears about 2 mm higher than the medial canthus, subtending a 15-degree inclination from the medial canthus to the lateral canthus.[9]

Whitnall's ligament is a superior transverse ligament that acts as the main suspensory ligament of the upper eyelid, as a support ligament for the conjunctival fornix, and also as a check ligament for the levator complex. It extends from the periorbita of the trochlea medially to the frontozygomatic suture laterally. It is located 15–20 mm superior to the superior border of the tarsus.

Lockwood's ligament acts as a suspensory sling or 'hammock' for the globe. It also anchors the inferior conjunctival fornix. It is composed of a fibrous condensation of the capsulopalpebral fascia, Tenon's capsule, intramuscular septa, check ligaments, and fibers from the inferior rectus sheath. It attaches medially to the medial canthal tendon and laterally to the lateral canthal tendon.[21]

**Orbital fat compartments**

Superior orbital fat contains two separate compartments, the preaponeurotic and the medial fat pads, separated by the trochlea. The medial fat pad is firmer and pale white in color, and is associated with the medial palpebral artery and infratrochlear nerve. The preaponeurotic fat pad is more yellow in color due to increased carotenoid content[22] and extends laterally over the lacrimal gland. Due to their close proximity to the trochlea, superior oblique palsy and Brown syndrome have been reported following excision of fat during upper eyelid blepharoplasty.[23] The superior orbital fat compartments are bordered posteroinferiorly by the levator aponeurosis, anteriorly by the orbital septum, and inferiorly by the fusion of both.
The inferior orbital fat contains three distinct compartments. The medial and central fat compartments are separated by the inferior oblique muscle. The central and lateral fat compartments are separated by the arcuate expansion of the inferior oblique muscle. The inferior orbital fat compartments are bordered posterosuperiorly by the capsulopalpebral fascia and anteriorly by the orbital septum.[24]

**Vascular supply**

The eyelids are encircled by superior and inferior palpebral vascular arcades with extensive contributions from both the internal and external carotid artery systems. The lower eyelid has a single arcade that lies between the inferior tarsal muscle and the confluence of the orbital septum and capsulopalpebral fascia, just below the inferior limit of the tarsus. The upper eyelid contains two discrete arcades: the marginal, which lies 2 mm from the eyelid, margin and peripheral arcade, which lies between the levator aponeurosis and the Müller's muscle just above the superior limit of the tarsus.

At the medial canthus, the superior and inferior palpebral arcades receive contributions from the medial palpebral arteries, arising from the ophthalmic branch of the internal carotid artery. The superomedial portion receives contributions from the supratrochlear and supraorbital arteries, which are also derived from the ophthalmic artery. At the superolateral aspect of the orbit, the superior palpebral arcade anastomoses with the zygomatico-orbital branch of the superficial temporal artery. Near the lateral canthal area, the superior and inferior palpebral arcades anastomose with the two lateral palpebral branches from the lacrimal artery, a branch of the ophthalmic artery. The inferolateral lower eyelid receives most of its contributions from the transverse facial artery, whereas the inferomedial lower eyelid receives major contributions from the angular artery, a terminal branch of the facial artery. The superficial temporal artery, transverse facial artery, and facial artery are derived from the external carotid system.

Venous drainage of the eyelids is through the anterior facial and superior temporal veins into the external jugular system and through the ophthalmic vein into the cavernous sinus and internal jugular system.[25],[26]

**Lymphatic drainage**

The lateral aspect of the upper and lower eyelids drains into the preauricular nodes and to a lesser extent into the parotid gland nodes. The medial aspect of the upper and lower eyelids drains into the submandibular nodes and to a lesser extent into the submental nodes.[1]

**Sensory innervation**

The sensory innervation of the upper lid is derived from the ophthalmic division of the trigeminal nerve (CN V) and its branches: supraorbital, supratrochlear, lacrimal, and infratrochlear. Sensation to the lower lid is supplied by the maxillary division of the trigeminal nerve via the infraorbital branch. The medial and lateral canthi are supplied by overlapping branches of the ophthalmic and maxillary divisions of the trigeminal nerve.

**Blink reflex**

Closure of the eyelids can be under voluntary or involuntary control. The eyelids serve the critical role of distributing the tear film over the anterior surface of the globe to maintain moisture. Continuous spreading of the tear film depends on the subconscious blink reflex, which occurs every 6 to 10 seconds. The afferent pathway of this reflex is dependent on the trigeminal nerve, while the facial nerve controls the efferent pathway through the pretarsal portion of the orbicularis muscle.[27]
References


Chapter 5 – A Matrix of Pathologic Responses in the Cornea

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Anatomical Regions of the Cornea

Anatomically, the cornea consists of two cellular layers, the epithelium and the endothelium. Each rests on a basement membrane: the epithelial basement membrane and Descemet membrane, respectively. These two cellular layers sandwich a thin layer of acellular connective tissue (Bowman’s layer) and a thicker, cellular layer of connective tissue (stroma). For the purpose of discussing pathologic responses in the cornea, we can divide the cornea into four regions (Fig. 5.1):

1. Epithelium
2. Subepithelial zone
   - a. Epithelial basement membrane
   - b. Bowman’s layer
   - c. Superficial stroma
3. Stroma
4. Endothelium and Descemet’s membrane

A whole spectrum of pathologic processes can disrupt the structure of these four zones and interfere with corneal function. However, the cornea can generate only a limited number of responses to these insults. Although there is some overlap among them, these responses can be grouped conveniently into six categories (see Fig. 5.1):

1. Defects (and their repair)
2. Fibrosis and vascularization
3. Edema and cysts
4. Inflammation and immune responses
5. Deposits
6. Proliferation

To provide the ophthalmology resident, the corneal fellow, and the practicing clinician with a useful perspective, the authors describe concisely the patterns of tissue response that characterize each zone, and provide representative clinicopathologic examples, as originally presented by Waring and Rodrigues[1] and elaborated by Freddo and Waring[2] (Fig. 5.2).
Fig. 5.2 This matrix of the four corneal zones and six types of pathologic responses can include almost all corneal diseases, as demonstrated by the listed examples. (Modified from Waring GO 3rd, Rodrigues MM. Patterns of pathologic response in the cornea. Surv Ophthalmol 1987; 31:262. Copyright Elsevier 1987.)

Clinical details and the pathophysiology – including the molecular biology – of the disorders and the pathologic processes discussed here are found in relevant chapters throughout this textbook.
General Pathologic Responses of the Cornea

1 Defects and their repair

Defects are a partial or complete absence of a portion of corneal tissue. A defect is acute if it appears suddenly and heals (e.g. corneal abrasion and breaks in Descemet's membrane and the endothelium in acute hydrops of keratoconus). It is recurrent if it appears repeatedly (e.g. recurrent epithelial erosion). A defect is chronic or persistent if healing stops and the defect remains (e.g. sterile, indolent epithelial ulcer associated with herpes simplex keratitis).

2 Fibrosis and vascularization

Fibrosis and vascularization are part of the normal repair process in connective tissues. In most tissues, these processes have a beneficial effect; in the cornea, however, fibrosis and vascularization lead to stromal scarring with opacification and disruption of optical function. Because the normal cornea is avascular, the appearance of blood vessels in the cornea is always abnormal.

3 Edema and cysts

Edema and cysts are grouped together for simplicity and because they often resemble each other clinically. When edema (i.e. excess fluid in or between cells) occurs, the normal architecture is disrupted, leading to opacification. The edema can be diffuse (stromal edema) or focal (epithelial bullae). Corneal cystic areas are focal collections of fluid or solid material without an epithelial lining.

4 Inflammation and immune responses

Inflammation and immune reactions result from a variety of insults which can lead to reversible or irreversible changes. In general, three basic steps are involved: (1) an inciting pathologic event, either exogenous (e.g. infection) or endogenous (e.g. autoimmune), acute or chronic; (2) a host cellular and humoral inflammatory and/or immune response; and (3) a repair process. These three steps are beneficial when they contain and control the pathologic
process (e.g. eliminate an epithelial viral infection); however, they can be harmful if they concurrently damage the cornea. Details are presented in the latter part of this chapter.

**5Deposits**

Abnormal types or amounts of material can be deposited in the cornea. Exogenous sources include drugs (more than 50); endogenous sources include excess material from metabolic diseases (e.g. Wilson's disease with deposits of copper in Descemet's membrane), and corneal dystrophies and degenerations (e.g. excess glycosaminoglycans in the stroma and endothelium in macular dystrophy), and autologous breakdown products (e.g. iron deposits which occur as iron lines in the basal epithelium).

**6Proliferation**

There are three basic types of abnormal proliferative responses: (1) abnormalities of growth and maturation (e.g. hypertrophy, hyperplasia, metaplasia, and dysplasia/neoplasia – such as corneal intraepithelial neoplasia [CIN]); (2) ectopic migration such as epithelial ingrowth; and (3) corneal stem cell deficiency, leading to conjunctivalization of the ocular surface.
Specific Pathologic Responses of the Cornea

A summary of the six pathologic responses as each relates to the four corneal zones is presented in Figure 5.2. Representative disorders occupy each box of this pathophysiologic matrix. The amount of functional deficit inflicted by a disease process depends on:

- The type of insult
- The duration of the insult
- The severity of the insult
- The portion of the cornea affected
- The cornea's ability to repair and restore normal structure and function

In managing corneal disease, the clinician seeks to inhibit repair responses in some cases and to encourage them in others. For example, in the management of herpes simplex disciform keratitis, the goal is to prevent scarring and vascularization of the stroma in order to protect the refractive and image transmission functions of the cornea. In contrast, in the management of an alkali burn of the cornea, the goal often is to encourage scarring and vascularization in order to maintain the structural and protective functions of the cornea.
Pathologic Responses of the Corneal Epithelium

1. Defects (and their repair)

The normal corneal epithelium is replaced continuously every 4 to 7 days, involving three processes: (1) differentiation of the basal cells toward the surface; pathologic example: epidermalization and keratinization in vitamin A deficiency; (2) centripetal movement of limbal and peripheral cells; pathologic example: chemical damage of the limbal epithelium; (3) desquamation of epithelial cells from the surface; pathologic example: extended-wear soft contact lenses interfering with normal desquamation. Among the causes of epithelial defects are acute corneal abrasions (including excimer laser photorefractive keratotomy), focal foreign bodies, viral invasion by herpes simplex and herpes zoster, and sloughing of cells in recurrent epithelial erosion, neurotrophic keratopathy (Fig. 5.3). Healing of a defect in the corneal epithelium involves four major stages: sliding of cells to cover the defect, mitosis of cells to restore normal thickness, attachment of cells to the basement membrane, and remodeling to establish normal architecture.[3],[4]

2. Fibrosis and vascularization

The corneal epithelium contains no connective tissue and therefore is not subject to fibrosis or vascularization. However, either process can occur subepithelially and may adversely affect epithelial repair, particularly after complete epithelial debridement, following which either limbal stem cell differentiation or conjunctival transdifferentiation is required to resurface the cornea.[5]

3. Edema and cysts

The epithelium takes on a cystic appearance when edema develops within or between the cells (e.g. endothelial dysfunction) and when changes in epithelial maturation create small, debris-filled cystic spaces (e.g. Cogan's microcysts in epithelial basement membrane degeneration). These changes reduce visual acuity if they create an irregular surface or if they refract and scatter light. If they cause a break in the epithelial surface or loosen the epithelium so that it shifts during blinking, pain can result from sensory nerve stimulation.

Epithelial edema

There are two common causes of epithelial edema: endothelial dysfunction and epithelial hypoxia and trauma. When fluid lifts cells from the basement membrane, blister-like bullae appear. At this stage, the epithelial sheet is held together by desmosomal connections (Fig. 5.4).
Epithelial hypoxia and trauma

Contact lens-induced edema is caused by epithelial hypoxia, hypercapnia, trauma due to improper fitting or overwear, or a combination of these. Hypoxia causes depletion of glycogen stores and an increase in lactate accumulation, indicative of a conversion to anaerobic metabolism. Intracellular edema results when the compensatory abilities of the epithelium are exceeded. Chronicity can stimulate angiogenesis.

Changes in epithelial maturation

Cysts can result from accumulation of rapidly multiplying (e.g. Meesmann’s dystrophy) or degenerating (e.g. epithelial basement membrane degeneration) epithelial cells.

In recurrent epithelial erosions, chronically regenerating epithelium often manifests clusters of clear, pinpoint microcysts in the area of a previous erosion. Many disorders produce a punctate epithelial keratopathy (PEK; sometimes called superficial punctate keratopathy [SPK]) that often takes on a cystic appearance, especially in retroillumination, as a result of focal accumulation of dead or dying epithelial cells.

Inflammation and immune response

In corneal allograft rejection, the donor epithelium may be attacked by sensitized cytotoxic T lymphocytes. This is a specific response to foreign antigens (e.g. the human leukocyte antigens [HLAs] in the epithelial cells or in Langerhans cells) and appears clinically as a serpentine line that spreads from the graft-host margin toward the center of the transplant. Fortunately, epithelial healing often keeps pace with cell death; in this instance, the epithelial rejection lines are a passing, asymptomatic phenomenon and no surface defect occurs. In contrast, Thygeson’s superficial punctate keratitis is a recurrent disorder characterized by focal, intraepithelial white infiltrates that appear clinically as fine spots of crushed chalk.

Herpes simplex epithelial dendriform keratitis is a combination of epithelial defect and infection-inflammation (Fig. 5.5).
5Deposits

Epithelial deposits can be divided into four categories based on their origin: (1) elements (iron, copper), (2) drugs (topical and systemic), (3) systemic diseases and (4) corneal dystrophies and degenerations.

Elements: iron deposits

The most common intraepithelial deposit is an iron line where hemosiderin pigment is deposited in lysosomes of the basal epithelial cells. Iron lines also commonly appear adjacent to elevated areas of the cornea (Fig. 5.6).
Drugs: topical and systemic
Numerous systemically administered drugs accumulate in the epithelium, including the antiarrhythmic drug amiodarone with its whorl-hurricane pattern (90% incidence in patients on long-term therapy) the psychotropic drug chlorpromazine, the antiinflammatory drug indomethacin (rarely), the antimetabolite tilmorone, and the numerous antimalarials including chloroquine; other drugs include naproxen, perhexiline, suramin, the thioxanthines, and tamoxifen. These drugs probably gain access to the cornea through the tears. The severity of the deposits is directly proportional to the total drug dose. Generally, when the drug is withdrawn, the corneal deposits disappear gradually. These drugs enter the cytoplasmic lysosomes where they become trapped, combine with bipolar lipids, and produce lipid–drug lamellar complexes refractory to enzymatic digestion.

Systemic diseases
Epithelial deposits from systemic diseases seldom reduce visual acuity. Exceptions include certain of the inherited metabolic disorders (e.g. mucopolysaccharidosis type VI-A, Maroteaux-Lamy, and the sphingolipidosis of Fabry’s disease with its whorl pattern (vortex or cornea verticillata). Multiple myeloma and other dysproteinemias may deposit fine, grayish crystals of immunoglobulins in the cytoplasm. Intraepithelial crystals of cystine are found in cystinosis.

The limbal conjunctival melanocytes of non-Caucasians frequently migrate into the epithelium, especially in eyes with a chronic superficial keratopathy, forming a streaming whorl called striate melanosis.

Corneal dystrophies and degenerations
Few of the corneal dystrophies produce deposits within the epithelium. Exceptions include Meesmann’s epithelial dystrophy (see Edema and cysts above).

Proliferation
The epithelium manifests a full spectrum of disorders of growth and maturation, including hyperplasia, metaplasia, and dysplasia-neoplasia. Because the epithelium conforms to the contour of the underlying basement membrane and stroma, its thickness varies. Areas of atrophy or thinning occur over elevations (e.g. over Salzmann’s nodules) whereas areas of hyperplasia or thickening occur when the epithelium fills in focal defects (e.g. a facet) or in a broader defect (e.g. that caused by excimer laser photorefractive keratectomy). These adjustments in epithelial thickness appear to be efforts to preserve a smooth corneal surface in order to maintain optimal optical function, but the mechanisms through which this adjustment is accomplished are unknown.

Epithelial filaments are caused by the attachment of strands of mucus to punctate epithelial defects, followed by abnormal proliferation of epithelium and

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Fig. 5.6 (A) Nine types of iron lines in the corneal epithelium.

(B) Irregular epithelial iron line with whorl-shaped brownish deposits in Fuchs’ dystrophy with chronic corneal edema and an irregular epithelial surface. (C) Histopathology demonstrates iron deposits in the basal layer of corneal epithelium.

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basement membrane around the strands. After an accidental or surgical perforating trauma, proliferating corneal epithelium can invade the anterior chamber through a fistula and form a cyst or a sheet. Epithelium can proliferate as ingrowth under a laser in situ keratomileusis (LASIK) flap. Metaplasia from a normal to a keratin-forming abnormal epithelium can occur in severe ocular inflammation such as ocular cicatricial pemphigoid and Stevens-Johnson syndrome. The epithelium is the only layer of the cornea that can become neoplastic, giving rise to squamous cell carcinoma, predominantly at the limbus.[8]

Because the epithelia of the cornea, conjunctiva, and limbus are contiguous, benign and malignant neoplastic disorders commonly affect all three epithelia simultaneously. The full spectrum of changes, from mild dysplasia through carcinoma in situ, is grouped under the term 'intraepithelial neoplasia.'[9][10] It appears as a gray intraepithelial sheet advancing onto clear cornea as a sharply demarcated margin with finger-shaped extensions and clusters of islands (Fig. 5.7) Invasive squamous cell carcinoma of the cornea/conjunctiva is amenable to resection under frozen section control.
Histopathology demonstrates thickened epithelium with loss of normal maturation and basilar neoplastic cells.

(From Waring GO 3rd, Roth AM, Ekins MB. Clinical and pathological description of 17 cases of clinical intraepithelial neoplasia. Am J Ophthalmol 1984; 97:547.)

Fig. 5.7  (A) Corneal intraepithelial neoplasia. A flat limbal mass extends onto the cornea as a gray, opaque sheet with a sharply margined, fimbriated leading edge.

The Pathologic Responses of the Subepithelial Zone

1 Defects (and their repair)

The epithelial basement membrane and Bowman's layer are both acellular, but their healing responses differ. The basement membrane is secreted by the basal epithelial cells and, therefore, can be regenerated or produced in excess or altered form. Bowman's layer, once damaged or destroyed, does not regenerate. A defect in Bowman's layer fills with fibroblasts and connective tissue, creating a permanent scar (e.g., keratoconus).

2 Fibrosis and vascularization

Neither the epithelial basement membrane nor Bowman's layer can become fibrotic or vascularized; however, fibrous or vascular tissues can spread between the basement membrane and Bowman's layer and replace the latter or grow into the anterior stroma immediately posterior to Bowman's layer as a pannus (Latin: carpet), either as avascular fibrosis or vascular fibrosis.

Subepithelial avascular fibrosis

Patches of avascular fibrous tissue appear beneath the epithelium as a nonspecific response. Examples include (1) corneal edema after cataract surgery, (2) advanced Fuchs' endothelial dystrophy, (3) granular and lattice corneal dystrophies, and (4) chronic superficial keratitis (e.g., phlyctenulosis). Another example is the subepithelial opacity from excimer laser photorefractive keratectomy. At 4 to 8 weeks after photorefractive keratectomy, a subepithelial haze may occur, which corresponds to a layer of subepithelial collagen and proteoglycans[11] that gradually is remodeled so that the subepithelial zone becomes clearer, although permanent scars can occur (Fig. 5.8).

Fig. 5.8 Subepithelial central scar from excimer laser photorefractive keratectomy.


Salzmann's nodular degeneration is a distinctive type of nonspecific avascular subepithelial fibrosis. The bluish-gray nodules usually appear in corneas afflicted by chronic, superficial inflammation that began in childhood, such as vernal keratoconjunctivitis. The nodules of hyaline and basement membrane material accumulate between Bowman's layer and the thinned but continuous epithelium and can be scraped off readily, with minimal
chance of recurrence.

**Subepithelial vascular fibrosis**

Subepithelial vascular fibrosis involves three basic cells: leukocytes, proliferating vascular endothelial cells, and active fibroblasts that secrete an extracellular connective tissue matrix. This process occurs (1) after mild insults (e.g. hypoxia beneath an extended-wear soft contact lens), in which a very fine sheet of fibrovascular tissue slowly migrates in from the limbus, (2) during chronic long-lasting insult such as trachomatous eyelid scarring and entropion where a progressive dense pannus spreads centrally, as well as (3) after devastating insults (e.g. alkali burns) in which a thick layer of exuberant fibrovascular tissue can progress across the entire cornea (Fig. 5.9).

**Fig. 5.9** (A) Set of images showing subepithelial vascular fibrosis in an alkali burn of the cornea. (B) Histopathology of subepithelial vascular fibrosis demonstrates thickened epithelium. (From Leibowitz HM, Waring GO, III. Corneal Disorders. Clinical Diagnosis and Management, 2nd edn. Philadelphia, W.B. Saunders Company, 1998. Copyright Elsevier 1998.

3Edema and cysts

Subepithelial edema arises from endothelial dysfunction, as described above. Diffuse stromal edema can throw the epithelial basement membrane into folds, referred to as shift lines.

4Inflammation and immune responses

Damage to the subepithelial zone from inflammation and innate immune response accompanies severe bacterial or fungal infection or trauma and usually is characterized by an epithelial defect and focal white superficial infiltrate that damages Bowman's layer and the superficial stroma.

Subepithelial infiltrates can also be caused by antigens and toxins that pass through the intact epithelium into Bowman's layer and the superficial stroma, where they elicit immune and inflammatory responses that cause focal areas of infiltration and edema, generally in the absence of concurrent epithelial defects or ulcers.

There are two general locations of subepithelial infiltrates: the first is central and paracentral, where chronic, focal, ground-glass spots accumulate following acute adenoviral keratoconjunctivitis[12] and protein-coated, extended-wear soft contact lenses or dendriform or geographic pattern infiltrates occur after herpes simplex epithelial keratitis, and herpes zoster keratitis. Following penetrating keratoplasty, chronic 0.5-mm subepithelial infiltrates, confined to the donor button, reflect a mild form of allograft rejection. The second location of subepithelial infiltrates is paralimbal. These acute focal, dense, flat marginal 'staphylococcal catarrhal' corneal infiltrates are separated from the limbus by a clear zone, and those of phlyctenular keratoconjunctivitis have no intervening clear zone, becoming vascularized.

5Deposits

Deposits in the epithelial basement membrane, such as silver granules from topical medications, are seldom visible clinically. Topical and systemic drugs rarely accumulate subepithelially. One exception is epinephrine, which deposits within and below the epithelium as adrenochrome pigment.

Systemic diseases rarely leave deposits selectively in Bowman's layer. Superficial, iron-containing foreign bodies embedded in the cornea can deposit a rust ring in Bowman's layer and the superficial stroma.

Reis-Bücklers and related corneal dystrophies produce deposition of fine, curled filaments 10 nm in diameter that replace Bowman's layer. These filaments give the cornea a central fish-net appearance and disrupt the epithelial basement membrane, causing painful erosions.
In Avellino corneal dystrophy, the granular dystrophy-like deposits are most dense in the anterior stroma.

A form of amyloid is deposited subepithelially in primary, gelatinous, droplike dystrophy, in which milky white, nodular opacities create mulberry-like lesions.

Calcium deposited in Bowman's layer as band-shaped keratopathy represents a degeneration that usually begins as a turbid haziness and gradually progresses within the palpebral fissure as a chalk-white plaque. Peripheral, arcuate calcific anterior stromal deposits may result from hypercalcemia.

Spheroidal degeneration of the cornea with its yellowish, round deposits in Bowman's layer and the anterior stroma within the palpebral fissure is also known as climatic droplet keratopathy and occurs in people who work in climatic. Focal spherules of a proteinaceous, autofluorescent material accumulate in Bowman's layer consisting of some constituent of plasma or tears in addition to elastotic fibrillar degeneration of collagen.[13]

**Proliferation of the epithelial basement membrane**

The basal corneal epithelial cells can secrete exuberant amounts of basement membrane, both subepithelially and within the epithelium. This excess tissue appears in primary epithelial disorders (e.g. epithelial basement membrane degeneration/dystrophy[14] with its patterns of maps, gray lines, refractile parallel lines (fingerprint lines), and gray putty-like intraepithelial cysts) (Fig. 5.10), as a nonspecific response (e.g. chronic corneal edema, Salzmann's degeneration), and as a manifestation of systemic diseases (e.g. diabetes mellitus).

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**Fig. 5.10** Epithelial basement membrane degeneration. (A) An irregular, gray maplike pattern of epithelial basement membrane. (B) Histopathology demonstrates basement membrane cells that show thickening and degeneration (the basis of the map figure, clinically) and focal cyst-like formation (white area) which create 'Cogan's microcysts.'

Pathologic Responses of the Corneal Stroma

The structural integrity, tensile strength, and contour of the cornea are derived primarily from stromal collagen, predominantly type 1.

**Defects (and their repair)**

Stromal defects often occur acutely after accidental or surgical trauma and are repaired according to the principles of normal corneal wound healing. Acute stromal defects also result from ulceration due to microbial invasion; their repair requires elimination of the microorganism and control of the inflammation. Chronic defects often are progressive and fall into three categories: (1) stromal thinning without ulceration, (2) sterile stromal ulceration, and (3) congenital posterior corneal defects (see Endothelium).

Stable or progressive thinning of the stroma without epithelial ulceration or stromal inflammation occurs in keratoconus (Fig. 5.11), keratoglobus, pellucid marginal degeneration, Terrien’s marginal degeneration, and LASIK – especially with postoperative corneal ectasia. Stromal thinning alters corneal curvature and may create irregular astigmatism. The thinner the cornea, the less protection it affords the intraocular contents, the less stable it is, and the less it is amenable to optical or surgical vision correction. In general, a thickness of 250–300 µm is required to preserve corneal integrity and normal contour.

**Fig. 5.11** Keratoconus. (A) The red fundus reflection highlights the central cone, giving an oil droplet appearance. (B) Histopathology of the central cornea in keratoconus. Left side...
Keratoconus is a variably progressive disorder of central corneal thinning and ectasia with a decrease in the number of stromal collagen lamellae, from about 350 to about 150 (see Fig. 5.11). Despite thinning, corneas with this disorder rarely perforate, even after a rupture of Descemet's membrane and the endothelium in acute hydrops. The term keratoglobus is applied colloquially to advanced keratoconus with total corneal thinning. The most extreme corneal thinning occurs in keratoglobus as part of the autosomal recessive connective tissue disease Ehlers-Danlos type VI A; minor trauma can rupture these corneas.

A variety of corneal diseases – including herpes simplex keratitis, alkali burns, systemic connective tissue disease such as rheumatoid arthritis (Fig. 5.12), bacterial and fungal keratitis, neurotrophic keratitis, and drug toxicity – may set off a chain of destructive inflammatory and enzymatic events that result in a persistent, sterile, sharply demarcated stromal defect, sometimes progressing to a descemetocele and corneal perforation.

2Fibrosis and vascularization

Two of the most common and nonspecific pathologic processes that opacify the stroma are fibrosis (scarring) and vascularization. Wound healing in the corneal stroma occurs slowly, presumably because the tissue is avascular and its rate decreases with age. The resultant scar tissue is weaker than the normal stroma, as evidenced by traumatic dehiscence of penetrating keratoplasty wounds many years after surgery, a circumstance obviated by lamellar corneal transplant techniques such as deep anterior lamellar keratoplasty (DALK) and endothelial keratoplasty techniques.

There are three basic phases in the healing of stromal wounds: (1) the destructive phase involves removal of abnormal tissues by polymorphonuclear (PMN) leukocytes and macrophages, aided by collagenases and proteoglycanases from epithelial cells, fibroblasts, and inflammatory cells; (2) the synthetic phase involves closure of the wound through synthesis of new collagens and proteoglycans by stromal fibroblasts, aided by epithelial cells; and (3) in the remodeling phase, the newly synthesized materials are initially assembled into a fine scar that is slowly remodeled into a clearer structure that more closely resembles normal cornea but never achieves total transparency or normal strength.

If the destructive phase is not constrained, melting of the corneal stroma can lead to corneal perforation. If the synthetic phase is inhibited by drugs (e.g. corticosteroids) or disease (e.g. rheumatoid arthritis), healing is delayed and wound strength may be decreased. In contrast, if the synthetic phase proceeds uncontrollably, optically destructive scars can result. If the remodeling phase is incomplete, a larger scar persists.

Within hours of anterior stromal wounding, a fibrin clot fills the defect, fluid from the tears and aqueous humor produces swelling of the adjacent stroma, PMNs migrate into the wound from the tears, keratocytes at the edge of the wound die, and the epithelium migrates toward the wound. Epithelial–stromal interaction is important in corneal wound healing. The healing epithelium elaborates cytokines (e.g. interleukin (IL)-1, tissue growth factor (TGF)-β), which stimulate stromal keratocytes to transform into fibroblasts and myofibroblasts and to secrete extracellular matrix. This response is seen after excimer laser photorefractive keratectomy, where the healing epithelium is in direct contact with the underlying stroma (without Bowman's layer) (see
Photocoagulation (preferably yellow dye) or with photodynamic therapy, but this cannot be done effectively for diffuse fans of vessels. Topical or subconjunctival bevacizumab—and possibly other vascular endothelial growth factor (VEGF) inhibitors—can inhibit stromal vascularization.

Stromal fibrosis

Alterations in the regular alignment of collagen fibrils greater than a 20-nm distance (i.e. one-half the wavelength of visible light) cause scattering of light. The observer sees this as a stromal opacity (back scatter), and the patient experiences glare (forward scatter). The opacity can take the form of a nebula (a mild, diffuse cloudiness), a macula (a moderately dense spot), or a leukemia (a markedly white opacity). In general, it is not the specific disease but rather its severity and duration and the extent of healing that determine the degree of corneal scarring.

The pattern of a corneal scar usually is not diagnostic, but some processes leave characteristic scars. Bacterial and fungal keratitis usually create a focal, sharply demarcated scar whose depth reflects the degree of penetration of the stromal abscess. Vernal keratitis creates a discrete, shield-shaped anterior opacity, generally in the superior half of the cornea. Alkali burns leave diffuse, opaque, marbleized scars. Syphilitic interstitial keratitis is characterized by deep stromal scarring with ghost vessels and lipid deposits. Surgical scars, such as those produced by radial keratotomy, clear cornea cataract surgery, and penetrating keratoplasty also are characteristic.

Stromal vascularization

Vascularization of the corneal stroma is a nonspecific pathologic response; the location and number of vessels reflect the location and severity of the inflammatory response. Stromal vessels can reduce vision in three ways: (1) they disrupt the normal stromal architecture, (2) they allow leakage of lipid into the stroma, and (3) they increase the potential for allograft rejection in corneal transplantation. In most instances, the clinician tries to prevent stromal vascularization to preserve vision. At times, however (e.g. stromal melting), ingrowth of blood vessels is desirable because it helps prevent corneal perforation by facilitating the transport of nutrients, immunoprotective factors, antimicrobial factors, antiproteases, and fibroblasts to the stroma.

Stromal vessels characteristically grow at three levels: (1) subepithelial and superficial stromal vessels appear in response to superficial corneal disease (e.g. chronic blepharitis, phlyctenulosis, contact lens wear, recurrent epithelial defects; see Subepithelial Zone); (2) vessels appear in the middle layers of the stroma in response to chronic inflammation (e.g. necrotizing stromal herpes simplex keratitis, bacterial or fungal abscesses, chemical burns); and (3) vessels appear in the deep stroma, anterior to Descemet’s membrane, and in eyes with keratouveitis (e.g. syphilitic interstitial keratitis).

Blood vessels that invade the stroma arise from superficial conjunctival vessels, deep scleral vessels, or iris vessels when the iris is in contact with the cornea. The vessels spread along the natural collagen lamellar planes but do not grow in an anterior–posterior direction unless a scar is present along which they can migrate.

In inflammatory conditions, the pattern of the vessels often follows that of the leukocytic infiltrate. Triangular tufts grow toward focal inflammation (e.g. infiltrates in rosacea keratoconjunctivitis). In contrast, a ring of vessels surrounds the cornea in limbal vernal conjunctivitis and the host–graft junction in penetrating keratoplasty. Generally, no single pattern characterizes a particular disease; exceptions include the superior limbal pannus of trachoma and superior limbic keratoconjunctivitis and the 360-degree limbal tufts from excessive soft contact wear. During active inflammation, stromal vessels dilate; when inflammation subsides, the vessels gradually shrink to endothelium-lined tubes without blood flow (i.e. ghost vessels), which refill with blood if inflammation recurs or if ischemia develops (e.g. contact lens wear). Large single stromal vessels can be treated by occlusion with argon laser photocoagulation (preferably yellow dye) or with photodynamic therapy, but this cannot be done effectively for diffuse fans of vessels. Topical or subconjunctival bevacizumab—and possibly other vascular endothelial growth factor (VEGF) inhibitors—can inhibit stromal vascularization.

3Edema and cysts

Edema of the stroma is a common clinical sign (e.g. epithelial-lined cysts of the corneal stroma occur rarely. Edema of the corneal stroma occurs when its water content rises above the normal 78%. In most cases, corneal stromal edema results from disruption...
of endothelial or epithelial functions and manifests itself as an increase in corneal thickness. Fluid accumulates in the glycosaminoglycans (not in the collagen fibrils) of the stroma, altering the regular arrangement of collagen fibrils. Clinically, stromal edema appears as a gray, ground-glass haze that varies from a fine, diffuse granularity to a dense, gray opacity, with clear, cystlike lakes of fluid sometimes present. As the stroma swells, the anterior curvature of the cornea, as established by Bowman’s layer, remains fixed, whereas the more elastic Descemet’s membrane is displaced posteriorly toward the anterior chamber, developing folds, clinically termed corneal striae. As the glycosaminoglycans expand and the collagen fibrils separate, the cornea thickens from its normal central value of approximately 530 µm to as much as 1500 µm, as measured by optical or ultrasonic pachymetry or optical coherence tomography.

Disruption of the endothelium is the most common cause of stromal edema. It occurs frequently (1) after the trauma of intraocular surgery, (2) as part of Fuchs’ endothelial dystrophy or the ICE syndrome, (3) in cases of severe iridocyclitis, herpes simplex disciform keratitis, and acute angle-closure glaucoma, and (4) in the most extreme circumstance, when there is a defect in Descemet’s membrane and the endothelium is absent in that location either acutely (e.g., hydrops in keratoconus, forces injury during birth) or congenitally (e.g., Peters’ anomaly).

Stromal edema usually remains confined to the area in which the endothelial or epithelial damage has occurred, presumably because the functioning endothelium in the other areas continues to pump fluid from the stroma. For example, edema often remains central in Fuchs’ endothelial dystrophy, superior or temporal adjacent to a cataract incision, and inferior in association with a retained anterior chamber foreign body.

With corneal edema, so-called ‘cystic’ spaces are fluid-filled lacunae caused by extreme stromal edema, and do not have an epithelial lining. An extreme example is the fluid interface syndrome after LASIK, in which high intraocular pressure and/or a dysfunctional endothelium allow fluid accumulation between the corneal flap and the stromal bed (Fig. 5.14).[27]

Epithelium-lined intrastromal cysts occur rarely after penetrating corneal trauma.[28] Sometimes they contain cloudy material, probably accumulated epithelial cells and debris.

4Inflammation and immune responses

Numerous infections, immunologic diseases, and traumatic disorders have as their common denominator the aggregation of leukocytes in the corneal stroma. Details of corneal inflammation are presented in a later section of this chapter. On clinical inspection, leukocytes migrating through the corneal stroma have a faint, gray-brown, granular appearance. When leukocytes congregate at the site of attack, they create focal or diffuse white inflammation. If corneal damage secondary to leukocytic infiltration is severe, the stroma thickens with edema and pus, becomes gelatinous, and begins to melt. The destructive activity of leukocytes usually is repaired by fibrosis with or without vascularization, and the resulting scar causes decreased vision if it is central or paracentral.

Histopathologically, leukocytes migrate along stromal lamellae and congregate with varying density. Bacteria, particularly Gram-negative bacteria, cause severe stromal suppuration and destruction. An extreme example is Pseudomonas aeruginosa keratitis, in which both the PMNs and the bacteria...
secrete proteolytic enzymes that can lead to corneal perforation in a short time.

Immune-based stromal inflammation is more complicated and stems from deposition of antigen–antibody complexes and complement-mediated hypersensitivity as well as from alteration of stromal cell surface antigenicity through previous exposure to an infectious agent, such as herpes simplex virus (HSV).

As one example, the peripheral corneal stromal melting associated with adult rheumatoid arthritis (see Fig. 5.12) likely results from deposition of immune complexes that activate the complement cascade, which results in chemotaxis of PMNs, leading to lysosomal enzyme release and stromal melting.

Herpes simplex stromal keratitis probably is mediated not by active viral replication but by deposition of viral antigens into the stroma and subsequent immune complex hypersensitivity with the migration of PMNs and lymphocytes to form a centripetally migrating immune ring (Wessley ring). In contrast, herpes simplex disciform keratitis more likely represents a delayed-type hypersensitivity response prompted by HSV-induced modification of membrane surface expression in corneal stromal cells or by damage to the underlying endothelium.

5Deposits

Deposits of substances in the stroma disrupt image formation in proportion to their central location and density.

Topical and systemic drugs

Few drugs accumulate in the stroma. One example: the compounds of gold sometimes used in the treatment of rheumatoid arthritis can accumulate in the cytoplasm of keratocytes, appearing as myriad, fine, distinct, round, ashlike particles varying in color from gold to violet (ocular chrysiasis).[29]

Ocular diseases

A foreign body that is retained in the corneal stroma, such as a fragment of wood or dirt, if not removed surgically, is expelled as part of the inflammatory response. Other less reactive foreign bodies are encased in subsequent scarring: glass, sand, pencil lead, and nylon or polypropylene sutures. These need little attention unless they lie in the central cornea and interfere with light transmission.

Lipid deposits in the cornea are common,[30][31] not only in humans but also in dogs.[32] Lipids can leak from stromal blood vessels. These deposits vary from refractile crystals at the tip of a vessel to a full-thickness stromal mass that pushes Descemet’s membrane posteriorly. Laser photocoagulation sometimes can occlude feeder vessels, allowing the lipids to regress. Only a small percentage of vascularized corneas manifest lipid deposits. Stromal keratocytes are capable of synthesizing lipids, which suggests that lipid precursors can leak from vessels and may be absorbed by the keratocytes, which synthesize and secrete cholesterol and fatty acids. Lipids can deposit around a corneal inlay, such as ring segments for myopia and keratoconus[33] but not all inlays manifest these deposits. Corneal arcus is the most common lipid deposition in the cornea and is considered a normal change of aging unless it appears before the mid-30s, when it is suggestive of hyperlipoproteinemia.[34][35]

Blood staining of the cornea occurs after anterior chamber hemorrhage (hyphema), particularly if there is a persistent increase in the intraocular pressure (IOP) or damage to the corneal endothelium (Fig. 5.15).
Systemic diseases

In certain systemic diseases, nonimmune deposits can appear in the corneal stroma, either because the keratocytes are involved in an inherited metabolic disorder (e.g. disorders of lipid metabolism, the mucopolysaccharidoses, cystinosis) or because the corneal stroma is a repository for abnormal, circulating substances (e.g. globulin crystals in multiple myeloma, benign monoclonal gammopathy).

Central corneal lipid deposits are found as part of rarer genetic disorders of high-density lipoprotein (HDL) metabolism such as lecithin-cholesterol acyltransferase (LCAT) deficiency.

In the mucopolysaccharidoses, corneal deposits of excess dermatan sulfate and keratan sulfate create a diffuse, ground-glass appearance in the stroma.

Deposits from dystrophies and degenerations of the stroma

Deposits of abnormal substances or abnormal amounts of normal substances can create opacities in corneal stromal dystrophies, such as a form of amyloid in lattice and Avellino dystrophies, phospholipids in granular and Avellino dystrophies, glycosaminoglycans in fleck and macular dystrophies, and lipid in Schnyder's central crystalline dystrophy.

Proliferation

Stromal proliferation usually occurs in the peripheral cornea and can be congenital or acquired. The congenital type includes dermoid choristomas, which are histologically normal tissues in an abnormal location, appear most commonly at the limbus, either as an isolated finding or as part of a systemic syndrome (e.g. Goldenhar's syndrome, organoid nevus syndrome), vary from small white spots to large masses, occupy the anterior one-third to one-half of the stroma, and do not enlarge after birth.

The acquired type includes connective tissue elements of the stroma that proliferate at a surgical or accidental wound without vascularization. This can occur anywhere in the posterior surface of the cornea. The posterior lamellar graft wound or along a keratoprosthesis across the posterior surface of the cornea, possibly with the assistance of fibroblast-transformed corneal endothelial cells, can form a thick gray layer, a retrocorneal membrane. The potential for posterior stromal proliferation ceases if the endothelium secretes a new basement membrane over the posterior surface of the wound.
Pathologic Responses of the Corneal Endothelium and Descemet's Membrane

**1 Defects (and their repair)**

Normal adult endothelial cell density is approximately 2500 cells/mm² and normal cell size is approximately 250 µm. Defects in the endothelium can occur alone or in combination with defects in Descemet's membrane. In either case, aqueous humor rushes through the defect into the corneal stroma, producing stromal and epithelial edema that persists until a functioning endothelial monolayer reestablishes itself.

**Defects in the endothelium**

Defects in the endothelium may occur acutely (after accidental or surgical trauma by a phacoemulsification tip or an intraocular lens [IOL]) or chronically (in diseases that cause gradual attrition of endothelial cells with 'micro-defects' between the sick cells – as in Fuchs' dystrophy). The endothelial defect is difficult to visualize with the slit lamp or specular microscope if there is overlying corneal edema.[42]

The wounded corneal endothelium repairs itself primarily through limited migration and hypertrophy and minimally through cell division.[43] The corneal endothelium does not divide under normal circumstances but can be stimulated by injury to divide. The regenerative potential of the endothelium in children is substantial and can produce excess Descemet membrane, but it decreases with age. After an injury, only cells adjacent to the defect participate directly in wound healing; those farther from the site retain their normal configuration, although the limbal endothelium may be a source of regenerative cells. Stromal edema resolves when the endothelial monolayer and barrier and pump functions are reestablished.

Alterations in individual endothelial cell area and shape occur during healing. Enlarged cells represent those that spread out to cover the defect, while smaller cells represent those that result from mitotic division or are still in the process of desquamating. In normal corneas, 48–90% of endothelial cells are hexagonal; as the cells spread and heal, the number of hexagonal cells decreases. Thus, variation in cell size and shape reflects the severity of the damage. There is a poor correlation between the size of an endothelial cell and its function. Presumably, enlarging endothelial cells develop more pump sites in their lateral plasma membranes, and barrier and pump functions remain at a normal level. Corneas with cell densities as low as 500 cells/mm², a figure that corresponds to an average cell size of approximately 2000–3000 µm², can remain clear.

Acute damage to the endothelium is most commonly surgically induced during cataract extraction[44] or corneal transplantation – especially posterior lamellar endothelial keratoplasty techniques that involve the folding and manipulation of the posterior stromal/endothelial donor disc.[45]

Chronic diseases of the endothelium, such as Fuchs' endothelial dystrophy[46] and chronic touch by an anterior chamber IOL (pseudophakic corneal edema)[47] cause a progressive loss of endothelial cells. As cells are lost, the remaining cells progressively enlarge and flatten to maintain a continuous covering over Descemet's membrane. If cell loss continues, however, the capacity of the remaining cells to maintain
corneal deturgescence is exceeded and corneal decompensation results, with stromal and epithelial edema. After penetrating keratoplasty, endothelial cell density drops for about 5 years and then becomes relatively stable.[48]

**Defects in Descemet's membrane**

Descemet's membrane has less tensile strength than full-thickness stroma; therefore, conditions in which the cornea is stretched may produce breaks in this membrane. The size of these defects is enlarged by retraction and coiling of Descemet's membrane along the edge of the break. For example, birth forceps injury compresses the globe vertically, stretching Descemet's membrane horizontally and creating vertical or oblique breaks; elevated IOP in infantile glaucoma stretches the cornea, creating serpentine or circular breaks in Descemet's membrane; and the thin, ectatic keratoconic cornea can stretch sufficiently to produce a focal spontaneous, elliptical rupture in the endothelium and Descemet's membrane (acute corneal hydrops) (see Fig. 5.4).

In all of these disorders, Descemet's membrane can separate from the overlying stroma to form a ledge or strand in the anterior chamber. Because most of these disorders occur in children and young adults, the corneal endothelium can repair and cover the defect, usually with production of a thick subendothelial fibrillar matrix (posterior collagenous layer). The retracted, coiled ends of the ruptured Descemet's membrane do not reapproximate, even when endothelial continuity is reestablished.[49]

Congenital, focal defects in Descemet's membrane and the endothelium are present in most cases of Peters' anomaly and its variants. These range from a slight indentation (posterior localized keratoconus) to an excavation that reaches Bowman's layer and is accompanied by focal stromal defects and scarring.

**2Fibrosis and vascularization posterior to Descemet's membrane**

Like stromal keratocytes, with which the healing corneal endothelium has a common mesenchymal origin in the neural crest, endothelial cells also can transdifferentiate into epithelium-like cells (e.g. posterior polymorphous dystrophy).

The endothelium and Descemet's membrane contain no connective tissue and do not respond to adverse stimuli with classical fibrosis or vascularization. However, when the endothelium is damaged or diseased, it secretes a layer of abnormal fibrillar tissue on the posterior surface of the original Descemet's membrane (Fig. 5.16).

**Posterior collagenous layer**

Clinically, this tissue appears as a gray sheet at the level of Descemet's membrane and has been called a 'thickened' or 'multilaminar' Descemet's membrane or retrocorneal membrane. The term 'posterior collagenous layer' (PCL) is preferable because: (1) it designates the tissue as a distinct abnormality; (2) it locates the tissue in the posterior cornea rather than mislabeling it 'behind' the cornea, since endothelial cells are often present on its posterior surface; (3) it describes the layered architecture; and (4) it indicates that collagen is a major component.

A posterior collagenous layer has been described by various names in more than 30 different corneal disorders. Examples include cornea guttata and Fuchs’ endothelial dystrophy, the refractile ridges in syphilitic interstitial keratitis, and the gray ‘thickened Descemet's membrane’ apparent in pseudophakic corneal edema. Clinically, the posterior collagenous layer is best seen in a broad slit beam that sweeps tangentially across the posterior surface of the cornea to reveal the plaques or sheets of gray, swirling, crinkled tissue.

With light microscopy, the periodic acid-Schiff (PAS) stain demonstrates the original uniform Descemet's membrane adjacent to the stroma, with the posterior collagenous layer behind it, consisting of multiple lamellae of varying thickness and staining. Immunohistochemistry identifies five different collagen types and proteoglycans in the abnormal layers.

Using the posterior collagenous layer to date the onset of endothelial or Descemet's membrane disease with transmission electron microscopy

Under normal conditions, Descemet's membrane thickens throughout life, increasing from approximately 3 µm at birth to approximately 18 µm by age 90 years. When viewed by transmission electron
microscopy, the anterior banded portion of Descemet's membrane is present at birth. The posterior, homogeneous, nonbanded layer is produced and thickens throughout life.

The multiple lamellae of the posterior collagenous layer that result from disease in or trauma to the endothelium accumulate as a historical record, like geologic strata or tree rings. By noting whether abnormalities exist in the anterior banded or posterior nonbanded portion of Descemet's membrane, one can reasonably infer whether a corneal disease process is congenital or acquired. For example, in corneas affected by the iridocorneal endothelial (ICE) syndrome, the layers of normal banded and nonbanded Descemet's membrane are present, bounded posteriorly by abnormal posterior collagenous layers, indicating an acute onset of the endothelial disorder in adulthood. A contrasting example is Fuchs' dystrophy, where abnormal wide-spacing collagen bundles are present throughout the nonbanded layer, indicating it is onset congenitally.

Vascularization does not occur in Descemet's membrane or in the posterior collagenous layer. Certain thick, fibrocellular membranes that are truly retrocorneal can become vascularized, but most of these are connected to the stroma through a wound, often with adherent iris.

3 Edema and cysts

Edema of the endothelium is usually associated with decreased endothelial function and overlying stromal edema. True cysts do not occur in these layers. Accumulated fluid within and between endothelial cells forms dilated spaces, creating a dewdrop, beaten-metal appearance sometimes called 'pseudo-guttata.' Specular photomicrographs show the swollen endothelial cells as a patchy array of dark spots that do not have the central white reflection characteristic of cornea guttata.

Inflammation affecting the endothelium is the most common cause of endothelial edema; herpetic disciform edema is believed to result from an endothelitis.

Because Descemet's membrane is a compact tissue that is readily permeable to water and contains only small amounts of glycosaminoglycans, it does not become edematous. However, Descemet's membrane can be displaced from the posterior stroma by edema, focal hemorrhage or a pocket of pus to form a posterior bulge, folds, or ledges. Posterior polymorphous dystrophy is characterized by focal, round, small lesions that resemble a group of vesicles or blisters. However, these are not true vesicles but are small pits in the posterior stroma lined by a thin Descemet's membrane.

4 Inflammation and immune responses

The endothelium indirectly becomes involved in inflammatory processes in disorders such as microbial keratitis and iridocyclitis, in which vasodilatory and chemotactic factors bring it into contact with leukocytes, forming various patterns of keratic precipitates. Keratic precipitates form a variety of patterns, including (1) a nonspecific spattering on the posterior cornea (e.g. ankylosing spondylitis), (2) a focal aggregation (e.g. disciform herpes simplex keratitis), and (3) a central, inferior, elliptical or triangular pattern (e.g. sarcoid uveitis). The endothelium also becomes directly involved in inflammatory processes in disorders such as herpetic disciform keratitis and allograft reactions, in which antigens on the endothelial cell surface stimulate the inflammatory process, for example an endothelial rejection line on the donor.

PMNs and mononuclear leukocytes adhere to the endothelial cell surface, penetrate between the cells, and intersperse themselves between Descemet's membrane and the endothelium. If the inflammatory process is mild to moderate or is appropriately treated, the leukocytes migrate back into the anterior chamber, and the endothelial monolayer recovers functional viability. If the inflammatory process is more severe and prolonged or is inadequately treated, endothelial cells show increasing vacuolization, separation from Descemet's membrane, desquamation into the anterior chamber, and death.

Descemet's membrane is remarkably resistant to the proteolytic enzymes elaborated by microorganisms, leukocytes, and epithelial cells. It resists destruction in the presence of severe keratitis, iridocyclitis, and endophthalmitis, and acts as a barrier that prevents the passage of leukocytes and most microorganisms
between the anterior chamber and the stroma.[58] Fungi are an exception; many elaborate enzymes that enable them to penetrate Descemet's membrane. After severe stromal melting, Descemet's membrane, bulging anteriorly as a descemetocele, may persist as the only intact structure in the cornea.

Inflammation directed specifically at Descemet's membrane is rare. A granulomatous reaction can occur around a fragmented Descemet's membrane, most commonly in chronic ulcerative herpes simplex keratitis, although it also is encountered in other inflammatory disorders.[59]

5Deposits

Topical and systemic drugs

Drugs and metals deposit in Descemet's membrane, whereas melanin pigment selectively deposits in or on the endothelium. Although they are seldom used today, prolonged topical administration of silver-containing medications (e.g. Argyrol) historically was the most common source of deposits in Descemet's membrane.

Ocular and systemic diseases

The most common deposit in Descemet's membrane resulting from a systemic disease is copper, which appears as the Kayser-Fleischer ring in Wilson's disease (hepatolenticular degeneration).[60] Clinically, the copper accumulates in the peripheral part of Descemet's membrane, initially in superior and inferior arcs that eventually become confluent and form a 360-degree greenish brown deposit).

Certain ocular diseases result in deposition of melanin pigment in or on the endothelium. This occurs from four different sources within the eye, each with distinctive clinical and histopathologic features.

1. Endothelial cells phagocytose pigment in disorders such as pigment dispersion syndrome and Fuchs' endothelial dystrophy, creating the clinical appearance of fine dusting of the posterior surface of the cornea, often in the form of a vertical, central Krukenberg's spindle. The vertical pattern of distribution comes about as the result of aqueous humor rising posteriorly in proximity to the warm iris and falling anteriorly on contact with the cooler cornea.

2. Iris stromal melanocytes can migrate over the posterior cornea, particularly in areas where there has been endothelial damage or where iris adhesions are present, forming a faint, brownish membrane that often consists of individual dendriform-shaped cells.

3. Iris pigment epithelial cells migrate onto the posterior surface of the cornea, especially in areas where the endothelium is damaged or absent or where iris adhesions are present, and create sharply marginated, rounded patches of dense, round, dark-brown pigment.

4. Pigmented macrophages also may be found in the endothelium but generally are not visible clinically.

Other types of material deposit on the endothelial surface: lymphocytes and keratic precipitates in inflammation, clumps or sheets of red blood cells in anterior chamber hemorrhage, tumor cells in lymphoproliferative disorders, white flakes in the exfoliation syndrome, and pieces of lens cortex or capsule after extracapsular cataract extraction.

Corneal dystrophies and degenerations

Among corneal dystrophies, only macular dystrophy produces deposits in the endothelium and Descemet's membrane.[61]

The most common material deposited in Descemet's membrane is lipid as part of a corneal arcus. Because Descemet's membrane ends abruptly at Schwalbe's line, the lipid material appears with a sharp outer margin and a diffuse inner margin.

6Proliferation

The production of excess basement membrane and collagenous tissue by the endothelium (posterior collagenous layer) is discussed in the section on fibrosis. There are no neoplastic or dysplastic disorders of the endothelium, as might be expected in a tissue with minimal regenerative capacity.
Endothelial cells are capable of transforming into both fibroblast-like and epithelium-like cells. In fibroblastic metaplasia, endothelial cells secrete extracellular collagenous tissue (the posterior collagenous layer). Patches of keratin-containing, epithelium-like cells occupy the posterior cornea in posterior polymorphous dystrophy, the ICE syndrome, congenital hereditary endothelial dystrophy, and Fuchs’ endothelial dystrophy.

Despite minimal regenerative capacity, the endothelium can proliferate over the surface of the trabecular meshwork, iris, and vitreous under specific circumstances, especially in children. When this occurs, the endothelium itself is not visible but the basement membrane it produces (ectopic Descemet's membrane) is visible and is often described as a glass, cuticular, or hyaline membrane. The process is sometimes referred to as endothelialization or descemetization of the anterior chamber and can result in glaucoma and distortion of the iris and pupil.

The ICE syndrome has been called ‘primary proliferative endothelial degeneration.’ Its common feature is an ectopic proliferation of the endothelium across the trabecular meshwork and onto the iris, where it secretes an ectopic basement membrane that contracts, forming unilateral peripheral anterior synechiae, distorting the pupil and creating iris stromal tufts or nodules.
The Immune Response: Components and Reactions in the Eye

Overview

The ocular immune response involves a complex set of interactions between local and systemic immunocompetent and parenchymal cells that communicate through specialized cell surface receptors and soluble mediators to protect the delicate functional and structural integrity of the eye. Immunocompetent cells include those of the lymphoid system (lymphocytes) and those of the myeloid system (macrophages, polymorphonuclear leukocytes, eosinophils, basophils, and antigen presenting cells [APCs]). Antigen presenting cells include macrophages, Langerhans cells, and B lymphocytes. Soluble components of the immune system include immunoglobulins, cytokines (peptide/glycopeptide intercellular mediators), chemokines (molecules with chemoattractant and cytokine properties), and complement.[65]

The immune system has primary and secondary tissue components. Primary lymphoid tissue includes the thymus, spleen, and bone marrow. Lymph nodes and the mucosa-associated lymphoid tissue (MALT) comprise the secondary tissue. The eye has its own MALT and is composed of the conjunctival lymphoid elements and lacrimal gland.

Adhesion molecules are a family of cell-surface glycoproteins located on a variety of circulating and fixed cells that mediate cell communication and migration. Special receptors on the surface of some lymphocytes (homing receptors) regulate the traffic of sensitized cells across the vascular endothelium into local lymphoid tissues of the MALT.[65] During the immune response, several families of adhesion receptors participate in a cascade of binding events that control cell migration out of the vascular compartment and also within the tissues themselves. Expressed at low levels under normal conditions, adhesion molecules can be dramatically up-regulated during an immune or inflammatory response.

There are two broad types of immune response to antigens: the innate or natural immune response and the adaptive or acquired immune response.[65] The innate response is the first line of defense against foreign agents. It is rapid in onset (minutes), lacks memory, and does not have the capacity for a more aggressive immune response following subsequent exposure to a specific agent (anamnestic response). Elimination of antigen occurs through cellular elements such as macrophages, polymorphonuclear leukocytes, and certain lymphocytes (natural killer [NK] cells). Soluble factors also participate, and in the eye they include complement, lysozyme, and some inflammatory mediators.

The more complex adaptive immune response has both humoral (antibody) and cell-mediated immune (CMI) pathways and occurs over a longer time frame (hours, days). Both cells (B and T lymphocytes) and soluble components such as antibodies and cytokines also participate in this specific response to antigen. In humans, the major histocompatibility complex (MHC) gene codes for MHC surface proteins whose primary function is to distinguish between self and nonself.[65] These MHC molecules play an integral role in antigen recognition and presentation.

The adaptive immune response has three phases. First, the afferent arm involves an initial antigen recognition process followed by antigen presentation to host T lymphocytes. Antigen presentation involves the interaction between an APC and a helper T lymphocyte. The second phase involves antigen...
processing and activation of lymphocytes (B cells and T cells), as well as differentiation and proliferation of specific effector lymphocytes. Finally, mature specialized cells interact with their specific target antigens. Subsequent exposure to antigen generates a more aggressive (anamnestic) response through the activation of memory cells that have been sensitized to that specific antigen.[65]

Regulation of the immune response is complex and involves both soluble and cellular factors. Many immunoregulatory phenomena occur in the anterior segment, including tissue transplantation (corneal, stem cell), immune privilege, immune tolerance, and autoimmunity.[66]

Although the immune response is usually protective, tissue injury may occur subsequent to an exuberant immune reaction. Four well-characterized hypersensitivity reactions account for a variety of immunopathologic mechanisms in the anterior segment, although a single pure hypersensitivity reaction in the eye is uncommon.[65]

Finally, the ocular immune response, in particular, occurs through a complex and integrated system involving many cellular and tissue structures of the eye. This chapter discusses basic principles of the immune response in general and focuses particular attention on special features of immune privilege and the ocular immune response.[66–68] The cascade of events is illustrated in Figure 5.17 for acute inflammation and Figure 5.18 for chronic inflammation.
Fig. 5.17  Acute inflammation. Following an acute stimulus, cells release IL-1, IL-6, IL-8, and TNF-α, which stimulates the migration of limbal Langerhans cell into the central cornea. These cytokines also upregulate ICAM-1, E-selectin, L-selectin, PECAM-1 on the vascular endothelium of the limbus, and facilitate PMN (PMN-1) infiltration. Complement becomes activated and local receptors regulate the complement response. Specific growth factors and angiogenic factors are also released. Keratomalacia may result from IL-1-stimulated IL-8 release and activation of PMNs (PMN-2), which release metalloproteinase (MMP) and other lysozomal enzymes that cause corneal ulceration. IFN-γ and IL-2 release may extend PMN (PMN-3) and recruitment of NK cells.

Fig. 5.18  Chronic immune/inflammatory response. In the chronic phases of immune/inflammatory responses, infiltrating lymphocytes (NK cells) release IFN-γ, which stimulates an upregulation of ICAM-1 and HLA-DR coexpression on the corneal stroma/endothelium, thereby providing the mechanism for HLA-DR-dependent cell-mediated cytotoxicity. Macrophages may play either a proinflammatory or antiinflammatory role depending on the cytokines released. Specific immune responses
through antigen processing (between the macrophage and T lymphocyte) result in the production of Th1 or Th2 lymphocytes. The Th1 responses result in release of IL-2, IFN-γ associated with viral infections, graft rejection, and dry eye. Th2 responses result in the release of IL-4, IL-5, and IL-13. These are associated with allergic and parasitic reactions. In addition, keratocytes through the action of bFGF and PDGF transform into fibroblasts and through TGF-β become myofibroblasts producing aberrant collagen (types V, VIII) leading to stromal scarring. Angiogenic factors also result in abnormal stromal vascularization.

**Cells of inflammation and the immune response**

The major types of immunocompetent cells include lymphocytes (B cells, T cells, and non-B, non-T cells), cells of the mononuclear phagocytic system (monocytes and macrophages), cells of the myeloid system (polymorphonuclear leukocytes), and auxiliary cells. The latter group includes a variety of constitutive and facultative antigen presenting cells, dendritic cells, platelets, and endothelial cells.[65]

Lymphocytes, which cannot be distinguished morphologically, are defined on the basis of their development, cellular products, and characteristic cell membrane receptors.[66] These surface receptors are characteristic of different cell lines, stages in development of a specific cell line, or activation levels of a specific cell type. The identification of a growing number of specific lymphocyte subsets has been facilitated by the use of monoclonal antibodies that bind to specific cell surface glycoproteins. The current nomenclature utilizes a universal system based on ‘clusters of differentiation’ (CD) designations.[65] Table 5.1 lists the major soluble mediators and receptors of inflammation.

<p>| Table 5.1 -- Soluble mediators and receptors of inflammation (important examples) |
|--------------------------------------------------|------------------|-----------------|---------------------------------|
| <strong>Group</strong>                                       | <strong>Example</strong>      | <strong>Source/cell</strong> | <strong>Target/ligand/action</strong>       |
| Adhesion molecule                               | Intercellular adhesion molecule 1 (ICAM-1) | Endothelial cells (EC) | Lymphocyte function associated antigen 1 (LFA-1) Promote leukocyte recruitment |
|                                                 | Very late antigen 1 (VLA-1) | T cells | Collagen, fibronectin, laminin |
|                                                 | Vascular cell adhesion molecule (VCAM) | Endothelial cells (EC) | Very late antigen 4 (VLA4) |
|                                                 | Platelet endothelial cell adhesion molecule (PECAM) | T cells | Endothelial cells (EC) Platelets |
|                                                 | Fas ligand (Fas L) | Many cells | Fas ligand receptor (FasR) Apoptosis Cytotoxic T-cell activity Corneal immune privilege |
|                                                 | Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) | T cells | Apoptosis Death receptors DR4 (TRAIL-RI) and DR5 (TRAIL-RII). |
|                                                 | Mucosal adressin cell adhesion molecule 1 (MAdCAM-1) | T lymphocytes (T) | Lymphocyte Peyer's patch HEV adhesion molecule 1 (LPAM-1 or integrin αβ7) |
|                                                 | P-selectin | Endothelial cells (EC) | White blood cells (WBC) |
|                                                 | E-selectin | Endothelial cells (EC) | White blood cells (WBC) |</p>
<table>
<thead>
<tr>
<th><strong>L-selectin</strong></th>
<th><strong>White blood cells (WBC)</strong></th>
<th><strong>Endothelial cells (EC)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemokines</strong></td>
<td>Chemokine ligand 5 (CCL5), Regulated in activation of normal T cells expressed and secreted (RANTES)</td>
<td>T cells, Basophils (Φ), Eosinophils (EpC)</td>
</tr>
<tr>
<td><strong>CXCR1,2</strong></td>
<td>Natural killer (NK) cells, basophils (Φ)</td>
<td>IL-8</td>
</tr>
<tr>
<td><strong>Interleukin 8 (IL-8)</strong></td>
<td>Fibroblasts, Corneal epithelial cells (EpC)</td>
<td>Corneal neovascularization, Attract neutrophils</td>
</tr>
<tr>
<td><strong>Chemotactic factors</strong></td>
<td>Eosinophil chemotactic factor</td>
<td>Mast cells (MC), Attract eosinophils</td>
</tr>
<tr>
<td>Neutrophil chemotactic factor</td>
<td>Mast cells (MC), Attract neutrophils</td>
<td></td>
</tr>
<tr>
<td><strong>Eotaxin</strong></td>
<td>Eosinophils (EpC), Attract eosinophils</td>
<td></td>
</tr>
<tr>
<td><strong>Macrophage migration inhibiting factor (MIF)</strong></td>
<td>T cells</td>
<td>Cell-mediated immunity (CMI), Immunoregulation, Inflammation</td>
</tr>
<tr>
<td><strong>Platelet activating factor (PAF)</strong></td>
<td>Mast cell (MC)</td>
<td>Vasodilatation, Increase permeability</td>
</tr>
<tr>
<td><strong>Clotting and fibrinolytic factors</strong></td>
<td>Fibrin (factor Ia)</td>
<td>Fibrinogen, Clotting, Inflammation</td>
</tr>
<tr>
<td>Thrombin (factor XIII)</td>
<td>Endothelial cells</td>
<td>Converts fibrinogen to fibrin</td>
</tr>
<tr>
<td>Fibrinogen (factor I)</td>
<td>Liver</td>
<td>Fibrin precursor</td>
</tr>
<tr>
<td>Laminin</td>
<td>Basal epithelial cells</td>
<td>Integrins</td>
</tr>
<tr>
<td><strong>Fibronectin</strong></td>
<td>Macrophages (ΜΦ)</td>
<td>Anaphylatoxin</td>
</tr>
<tr>
<td><strong>Complement</strong></td>
<td>Complement factor C5a</td>
<td>Hepatocytes, APC, Histamine release from mast cells, Neutrophil chemotaxis</td>
</tr>
<tr>
<td>Complement factor C3a</td>
<td>Macrophages</td>
<td>Chemotaxis, Anaphylatoxin</td>
</tr>
<tr>
<td>Complement factor C3b (opsonin)</td>
<td>Macrophages (ΜΦ), fibroblasts (FB)</td>
<td>Opsonize bacteria by macrophage (ΜΦ)</td>
</tr>
<tr>
<td>Decay accelerating factor (DAF)</td>
<td>Corneal epithelial cells (EpC)</td>
<td>Complement regulation prevents the assembly of the C3bBb complex</td>
</tr>
<tr>
<td><strong>Granulocyte-macrophage colony-stimulating factor (GM-CSF)</strong></td>
<td>Macrophages (ΜΦ), fibroblasts (FB)</td>
<td>Macrophage activation</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td>Interleukin 1 (IL-1α, β)</td>
<td>Corneal epithelial cells (EpC), Macrophages (ΜΦ), Langerhans cells (LC)</td>
</tr>
<tr>
<td>Interleukin 1 receptor (IL-1R)</td>
<td>T helper 1 cells (Th1), Natural killer (NK) cells, Keratocytes</td>
<td>T: proliferation and lymphokine secretion, Th2: induces interferon gamma (IFN-γ), secretion</td>
</tr>
<tr>
<td>Interleukin 2 (IL-2)</td>
<td>T helper 2 cells (Th2)</td>
<td>Increase IgE, Decrease</td>
</tr>
<tr>
<td>Cytokine</td>
<td>Target Cells</td>
<td>Function</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Interleukin 4 (IL-4)</td>
<td>Natural killer (NK) cells, Mast cells (MC)</td>
<td>proinflammatory cytokines, Suppress T helper 1 (Th1)</td>
</tr>
<tr>
<td>Interleukin 6 (IL-6) (IFN-β2)</td>
<td>T helper 2 cells (Th2), Macrophages (MΦ), Dendritic cells (DC), Mast cells (MC)</td>
<td>T-cell activation, B-cell Ig secretion, Macrophages (MΦ) differentiation</td>
</tr>
<tr>
<td>Interleukin 10 (IL-10)</td>
<td>T helper 2 cells (Th2), Macrophages (MΦ), Mast cells (MC)</td>
<td>Th1: inhibit IL-2, IL-3, interferon gamma (IFN-γ) synthesis, Th1: inhibit DTH, Macrophage (MΦ): inhibit TNF, IL-1, IL-12 production</td>
</tr>
<tr>
<td>Interleukin 12 (IL-12)</td>
<td>Macrophages (MΦ), Dendritic cells (DC)</td>
<td>T helper 1 (Th1) differentiation, Interferon gamma (IFN-γ) production</td>
</tr>
<tr>
<td>Interleukin 18 (IL-18)</td>
<td>Macrophages (MΦ)</td>
<td>Cell-mediated immunity (CMI), Inflammation</td>
</tr>
<tr>
<td>Tumor necrosis factor alpha (TNF-α)</td>
<td>T cells, Macrophages (MΦ), Mast cells (MC)</td>
<td>T-cell stimulation, Matrix metalloproteinase (MMP) induction, Adhesion molecule expression</td>
</tr>
<tr>
<td>Interferon gamma (IFN-γ)</td>
<td>T cells, Natural killer (NK) cells</td>
<td>HLA-DR expression, Activation of: T cells, natural killer (NK) cells, macrophages (MΦ)</td>
</tr>
<tr>
<td>Interferon alpha (IFN-α) (14 subtypes)</td>
<td>Macrophages (MΦ), Leukocytes</td>
<td>Innate immune response (virus), IFN-α receptor (IFNAR)</td>
</tr>
</tbody>
</table>

**Eicosanoids**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Target Cells</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukotriene B4</td>
<td>Mast cells (MC)</td>
<td>Promotes inflammation and breakdown blood–ocular barriers</td>
</tr>
<tr>
<td>Leukotriene C4</td>
<td>Eosinophils (ΕΦ)</td>
<td>Increase capillary permeability</td>
</tr>
<tr>
<td>Prostaglandin D2 (PGD2)</td>
<td>Mast cells (MC)</td>
<td>Vasodilation</td>
</tr>
</tbody>
</table>

**Growth factors**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Target Cells</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular endothelial factor (VEGF-A, B, C, D)</td>
<td>RPE and neurosensory retinal cells</td>
<td>Angiogenesis, Lymphangiogenesis, Macrophage chemotaxis, Vasodilation</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Many cells</td>
<td>Fibroblast proliferation, Collagen synthesis, Decrease matrix metalloproteinases</td>
</tr>
<tr>
<td>Compound/Protein</td>
<td>Cells/Matrix/Receptors</td>
<td>Function</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Macrophages (MΦ)</td>
<td>Epithelial growth, Neural cell development</td>
</tr>
<tr>
<td>Nerve growth factor (NGF)</td>
<td>B cells, T cells, Fibroblasts</td>
<td>Nerve proliferation and development</td>
</tr>
<tr>
<td>Basic fibroblast growth factor (bFGF)</td>
<td>Basement membrane, Vascular subendothelial matrix</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td>Epidermal growth factor (EGF)</td>
<td>Macrophages, Platelets</td>
<td>Epidermal growth factor receptor (EGFR), Epithelial migration</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Platelet derived growth factor (PDGF)</td>
<td>Angiogenesis</td>
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<tr>
<td>Immunoglobulin A (IgA)</td>
<td>B lymphocytes</td>
<td>Mucosal immunity</td>
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<tr>
<td>Immunoglobulin D (IgD)</td>
<td>Immature B cells</td>
<td>B-cell activation</td>
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<tr>
<td>Immunoglobulin E (IgE)</td>
<td>B lymphocytes</td>
<td>Allergy, type I reactions, Binds to Fc receptors on mast cells (MC)</td>
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<tr>
<td>Immunoglobulin G (IgG)</td>
<td>B lymphocytes</td>
<td>Ig2a fixes complement</td>
</tr>
<tr>
<td>Immunoglobulin M (IgM)</td>
<td>B lymphocytes</td>
<td>Complement activation</td>
</tr>
<tr>
<td>Kinin forming system</td>
<td>Bradykinin</td>
<td>Increase vascular permeability, Vasodilator</td>
</tr>
<tr>
<td>Leukocyte oxidants</td>
<td>Hydrogen peroxide</td>
<td>Oxidizes free radicals</td>
</tr>
<tr>
<td>Neuropeptides</td>
<td>Substance P</td>
<td>Inflammation and pain, neurokinin 1 receptor (NK1-receptor, NK1R)</td>
</tr>
<tr>
<td>Proteases/enzymes</td>
<td>Collagenase (MMP-1,8,13,18)</td>
<td>Degrade collagen and stromal matrix</td>
</tr>
<tr>
<td>Membrane type matrix metalloproteinases (MMP, 14–17)</td>
<td>Keratocytes (K)</td>
<td>Activate progelatinase A</td>
</tr>
<tr>
<td>Gelatinases (matrix metalloproteinases [MMP] 2,9)</td>
<td>Keratocytes (K)</td>
<td>Native type IV, V, VII collagens, Fibronectin</td>
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<tr>
<td>Matrilysins (matrix metalloproteinases [MMP] 7,12,19,20)</td>
<td>Keratocytes (K)</td>
<td>Gelatin, Fibronectin, Elastin</td>
</tr>
<tr>
<td>Stromelysins (matrix metalloproteinases [MMP] 7,12,19,20)</td>
<td>Keratocytes (K)</td>
<td>Proteoglycans,</td>
</tr>
<tr>
<td>Metalloproteinases [MMP] 3,10,11</td>
<td>Keratocytes (K)</td>
<td>Fibronectin, serine protease inhibitors</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------</td>
<td>----------------------------------------</td>
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<tr>
<td>Cathepsins (A and B)</td>
<td>Lysosomes</td>
<td>Protease activity</td>
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<tr>
<td>Tryptase</td>
<td>Mast cell (MC)</td>
<td>Complement activation</td>
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<tr>
<td>Peroxidase</td>
<td>Eosinophil (ΕΦ)</td>
<td>Epithelial cytotoxicity</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Macrophages (ΜΦ) Lacrimal acinar cells (AC)</td>
<td>Degrade bacterial cell walls</td>
</tr>
<tr>
<td>Vasoactive amines</td>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>Mast cell (MC)</td>
<td>Dilate blood vessels</td>
</tr>
<tr>
<td>MBP</td>
<td>Eosinophil (ΕΦ)</td>
<td>Mast cell degranulation</td>
</tr>
<tr>
<td>Heparin</td>
<td>Mast cell (MC)</td>
<td>Anticoagulation</td>
</tr>
<tr>
<td>Cationic protein</td>
<td>Eosinophil (ΕΦ)</td>
<td>Epithelial cytotoxicity</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Lacrimal acinar cells</td>
<td>Monocytes, macrophages, PMN Antimicrobial activity Binds divalent cations</td>
</tr>
</tbody>
</table>

**Cells of the lymphoid system**

**B lymphocytes**

B lymphocytes constitute 5–15% of the circulating lymphocytes and are primarily responsible for the humoral (antibody) arm of the adaptive immune response. B cells manufacture a large number (20,000 to 200,000) of specific immunoglobulins, which are expressed on their cell surface. There are five subclasses of B lymphocytes: IgG, IgA, IgM, IgE, IgD.

There are many surface markers on B cells. Most human B cells in the peripheral blood express IgM and IgD. The receptor for the Fc portion of IgG (FcγRII, CD32) is also expressed on B cells. Major histocompatibility class (MHC) II antigens are also located on most B cells and provide the ‘antigen-presenting’ capacity of these cells. B cells can endocytose and present antigen to helper T lymphocytes in the context of their surface MHC class II molecules.

**T lymphocytes**

T lymphocytes make up 65–85% of peripheral blood lymphocytes and direct the cell-mediated arm of the adaptive immune response.[65] T cells develop from cell precursors within the thymus where 90% of cells are T lymphocytes. During intrathymic differentiation, the repertoire of T-cell antigen receptor (TCR) specificities is generated. The T-cell antigen receptor is the definitive marker for T lymphocytes. Each T-cell antigen receptor is also associated with the CD3 or T-cell differentiation antigen, which is made up of five polypeptides. T cells have MHC class II HLA-DR surface antigens.

Three major functional subsets of helper T cells have been characterized: Th1, Th2 and Th17. The differences between the three major subtypes are defined primarily by their unique patterns of cytokine secretion. Regulation of the immune responses through these three subsets of T helper cells is depicted in Figure 5.19. Th1 cells manufacture IFN-γ and TNF-β. IFN-γ increases the production of IL-12 by dendritic cells and macrophages, and via positive feedback, IL-12 stimulates the production of IFN-γ in helper T cells, thereby promoting the Th1 profile. Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13. The Type 2 response promotes its own profile using two different cytokines. IL-4 acts on helper T cells to promote the production of Th2 cytokines, while IL-10 inhibits a variety of cytokines including IL-2 and IFN-γ in helper T cells and IL-12 in dendritic cells and macrophages. T helper 17 cells (Th17) are a newly discovered subset of T helper cells producing IL-17, IL-6, IL-22, and G-CSF. They are considered developmentally distinct from Th1 and Th2 cells and excessive amounts of the cell are thought to play a key role in inflammation and autoimmune disease such as autoimmune uveitis, juvenile diabetes, rheumatoid arthritis. IL-22 may play an important role in mucosal immunity.
Cytokine regulation of the acquired immune response. Hypothetical activation pathways of proposed Th1, Th2 and Th17 cells leading to effector cell stimulation are depicted. Hypothetical stimulatory pathways (solid arrows) and inhibitory effects (dashed arrows) are shown. Th1 cells release IFN-γ, which inhibits Th2 cells. Th2 cells release IL-10, which may inhibit IFN-γ production and APC activation of Th1 cells. Th17 cells produce IL-17, IL-6 and G-CSF. Allergens seem to preferentially activate Th2 cells, which stimulate IgE-mediated allergic responses (including IgE Fce receptors). IL-3 and IL-4 release would also activate mucosal mast cells, and IL-5 stimulates eosinophil proliferation. Activation of Th1 cells would also be inhibited. On the other hand, APCs seem to present antigens more to the Th1 side of the immune response. These cells release IL-2 and IFN-γ, mediating CTL and macrophage activation and IgG2a production. Antibody-dependent, cell-mediated cytotoxicity (ADCC) and delayed-type hypersensitivity (DTH) responses are also mediated by this pathway. This response is characteristic for responses to intracellular (viral, parasitic) antigens.


Cytotoxic T cells (Tc) are CD8+ positive and carry the TCR-2 receptor. They participate in reactions related to cell destruction. They are MHC class I restricted and destroy viral infected cells and foreign allogeneic cells.

Null lymphocytes

Natural killer (NK) cells are a heterogeneous population of granular, nonadherent, nonphagocytic lymphocytes found in the peripheral blood, spleen, and lymph nodes. They represent 10–15% of circulating lymphocytes or 5% of all white cells. There are more NK cells in circulation than B cells.

NK cells function in immune surveillance. They destroy cells without prior sensitization or interaction with
antigen presenting cells. NK cells participate in the innate arm of the immune response and kill tumor cells, viral infected cells, and xenogeneic cells. NK cells release IFN-γ, TNF-α, and IL-1 as well as other soluble cytotoxic factors, including NK cytotoxic factor. The cytolytic activity of NK cells may be enhanced by several lymphokines including IFN-α, IFN-β, IL-2, and IL-4.[65]

**Cells of the myeloid system**

**Macrophages and the mononuclear phagocytic system**

The mononuclear phagocyte system consists of a single population of cells called macrophages (MΦ). Macrophages are located throughout the body and provide a number of important functions in host defense. These bone marrow-derived cells develop from a myeloid progenitor cell, enter the bloodstream as monocytes, and migrate into various tissues as macrophages.

Macrophages are the preeminent APC. They serve as a link between the innate and adaptive immune responses, actively participating in innate immune responses through phagocytosis of foreign material. Macrophages mediate the initiation and effector phases of immune responses. They also influence lymphocyte responses to antigen and can stimulate T lymphocytes directly.

Macrophages produce a variety of important secretory factors including proteases, collagenases, angiotensin-converting enzyme, lysozyme, fibrofetin, platelet activating factor, arachidonic acid derivatives, prostaglandins, leukotrienes, and oxygen metabolites. Macrophages also release soluble products called monokines, which include IFN-α, IL-1, IL-6, and TNF-α.[65]

**Dendritic cells**

Dendritic cells (DC) make up a system of specialized APCs within the mononuclear phagocytic system. This network of highly motile cells initiates a variety of immune responses, particularly antigen recognition and processing. Dendritic cells migrate between tissues and home to specific T-cell-dependent areas of lymph nodes and other lymphoid structures. Dendritic cells contain specialized cell surface adhesion molecules (β2 integrins), which are associated with their homing function. Dendritic cells are found in a variety of nonlymphoid tissues including the epithelium of the skin, the ocular surface, iris, ciliary body, and other mucosal epithelia.[67–69]

**Langerhans cells**

Langerhans cells (LC) are bone marrow-derived DC that are also part of the monocyte/macrophage family. Langerhans cells are found in the thymus, lymph nodes, and epithelial layers of the skin, oral cavity, esophagus, nasopharynx, cervix, conjunctiva, and cornea.[67–70] They are important immunocompetent cells of the ocular surface and mucosal immune system. Langerhans cells have been extensively studied for their capacity to present antigen to T lymphocytes and trigger T-cell proliferative responses.

**Other cells of the myeloid system**

**Polymorphonuclear leukocytes**

The myeloid system is made up of erythrocytes, platelets, monocytes, and granulocytes. Polymorphonuclear (PMN) leukocytes (also called granulocytes) are divided into three categories: neutrophils, basophils, and eosinophils. Granulocytes represent 60–70% of circulating white cells and are relatively short lived. They participate in the innate immune response by migrating into tissues at sites of inflammation and releasing mediators.[65]

The neutrophil is the first cell type to appear at sites of inflammation and infection. It possesses two types of cytoplasmic granules: primary (azurophilic or lysosomal) and secondary (specific) granules. These granules contain a variety of enzymes including myeloperoxidase, acid and alkaline phosphatases, and lysozyme. They phagocytose organisms and degrade them through their lysosomal enzymes. The cell surface of neutrophils contains many types of adhesion molecules that regulate their migration out of the vascular compartment and into the tissues.
**Eosinophils**

Eosinophilic granulocytes (eosinophils) represent about 2–5% of peripheral leukocytes. They possess intracellular granules rich in acid phosphatase and peroxidase and have the capacity to activate a wide variety of other cells including basophils, neutrophils, and platelets. Eosinophil major basic protein (MBP) is released by these cells and induces the production of IL-8 by other eosinophils, macrophages, and T cells.[65] Eosinophils are phagocytic and participate in the ingestion of antigen as well as antibody complexes. They can present antigen through their cell surface MHC class II antigen.

**Basophils**

Basophilic granulocytes (basophils) make up less than 0.5% of all circulating leukocytes. Mast cells have characteristics similar to basophils, including receptors for Ig. However, basophils release different cytokines (IL-4, IL-13) and have different cell surface receptors for cytokines (IL-1 to IL-5).[65] Basophils circulate in the peripheral blood, and have life spans of several days, like other granulocytes. They migrate to sites of inflammation, particularly during the late phase of allergic reactions and possess a wide variety of cell surface receptors for adhesion molecules.[71]

**Mast cells**

Mast cells play active roles in both innate and adaptive immune responses.[65],[66] Mast cells have receptors for IL-4, IL-6 and release TNF-α, IL-3 to -6, -10, -13, -16, VEGF, and GM-CSF (see Table 5.1). Mast cells are present only in mucosal epithelia and connective tissue and have life spans of months. They participate in all four types of hypersensitivity responses.

Two functionally and structurally distinct types of mast cells are characterized by their surface receptors, tissue distribution, and cell products. Mucosal mast cells (MMC) are located primarily in the gut, lung, and eye.[71],[72] The connective tissue mast cells (CTMC) are found in the skin and peritoneum. MMCs are regulated in part through IL-3 released by Th2 lymphocytes. CTMC, however, are not T cell/IL-3 dependent. MMC contain IgE both on their surface and within the cell, whereas CTMC contain IgE only on the surface. MMC release tryptase and chondroitan sulfate, whereas CTMC release tryptase and chymase as specific proteases.
Adhesion molecules

Adhesion molecules are cell-surface proteins that regulate cell–cell interactions, as well as cellular contact with intercellular matrix proteins such as collagen and fibronectin. Adhesion molecules participate in a variety of processes including antigen presentation, migration of leukocytes to inflammatory sites, lymphocyte homing to specific tissues, and adherence of immunocompetent cells to resident (target) cells. Adhesion molecules are selected to perform distinct effector functions based on their cell background and factors present in the local environment.

A multistep process occurs for neutrophil and monocyte migration out of the vascular system into sites of inflammation. This combination of steps and multiplicity of ligand pairs provides the diversity for regulating the multitude of leukocyte functions in vivo. This cascade of overlapping but successive phases is similar to the multistep process involved in blood clotting and complement-mediated killing.

Adhesion molecules are classified into several groups of similar structures: selectins, integrins, and immunoglobulins. The selectins regulate the first phase of ‘tethering and rolling’ of leukocytes along the margin of the vascular endothelium. The slowing down of cells ends with the more firm adhesion of the cell to the endothelium. The second ‘activation’ phase results from chemoattractants, or chemokines, released from the vessel wall, which activate the second group of adhesion molecules, the integrins, on the leukocyte to mediate ‘firm adhesion’ to their immunoglobulin superfamily ligands on the vascular wall. The final phase of ‘transendothelial migration’ of leukocyte entry into inflamed tissue is also mediated by integrin molecules. Certain endothelial adhesion molecules also demonstrate organ- and tissue-specific expression and specific leukocytes also have specific homing molecules with differential expression. Blocking the expression of these molecules may inhibit inflammatory processes.

Cytokines

The term cytokine refers to any intercellular peptides or glycopeptides secreted by immune
Cytokines act on other hematopoietic cells to modulate immune and inflammatory responses. They differ from hormones and growth factors, which act on nonhematopoietic cells. The group of cytokines includes the interleukins, TNF, chemokines, colony-stimulating factors, interferons, and growth factors (see Table 5.1).

Different cytokines can act on the same cell type to mediate similar effects (redundancy). Cytokine receptors generally consist of two polypeptide chains: a ligand binding receptor, and a nonbinding signal transducer. Different ligand binding molecules may share the same signal transducer, which may explain in part the redundancy in cytokine effects. The effects of each cytokine also depend on the specific target cell (pleiotropism). Many cytokines function as part of a complex cascade of cytokine responses between cells and can act synergistically as well.

Because cytokines depend on a variety of factors, including the specific combination and concentration of each cytokine, the effects of the cytokine network is determined to a large extent by the local environment. Because of their role in inflammation, synthesis of most cytokines is highly regulated, especially in the cornea where unchecked inflammation could lead to significant functional loss.[65,66,68]

Chemokines

Chemokines are small secreted molecules which have both chemoattractant and cytokine properties.[65,66,73] They have four families, comprising: CXC (α), CC (β), XC (γ), and CX3C (δ) (see Table 5.1). Chemokines can be divided into constitutive (SDF-1, TARC, SLC, etc.) and inducible (RANTES, MIP1s, IL-8, and MCP). Constitutive chemokines are expressed in primary and secondary lymphoid organs and regulate lymphocyte traffic in physiologic conditions while the inducible chemokines play roles in response to inflammatory conditions. One of the important features of chemokines is their redundancy: most receptors interact with multiple chemokines and most chemokines bind to most receptors.[65,74]

Complement

The complement system is a potent mechanism for initiating and amplifying the inflammatory host response against bacteria and foreign antigens. The complement system involves a set of proteins numbered C1 to C9, which interact in a cascade-like fashion determined by a series of enzymatic steps. It is an integral component of the humoral immune response and participates in types II and III hypersensitivity responses.[65] It also participates in discrimination between self and nonself. Both recognition and effector pathways promote the inflammatory response, assist in immune complex formation, and alter the plasma membrane of cells leading to cell death. Three major functions of complement are opsonization of bacteria/immune complexes, target cell lysis, and activation of phagocytosis.

Two pathways can activate the complement cascade. The classic pathway is activated by IgG or IgM bound to a specific target. C1 has three components: C1q, C1r, and C1s. C1q binds with the Fc receptor on the Ig molecule and becomes activated. Activated C1 then initiates the cascade of proteolytic events.

The alternative pathway (also called the properdin system) directly activates the complement system without the participation of antibody. This process occurs through stimulation by several factors including the Fab (in contrast to the Fc of the classic pathway) area of immunoglobulin complexes (IgA, IgE, IgG), zymosan, endotoxin, and bacterial cell walls.
Formation of C3 convertase is a critical step in both classic and alternative pathways. This enzyme stimulates the formation of C3b (the opsonin component) and C4b, which bind to cell membranes. The final common pathway of the complement system is cell destruction by osmotic lysis, which is mediated by the formation of membrane attack complex (MAC) (factors C5–C9). C5a, the chemoattractant component, serves to recruit other inflammatory cells.

In the eye, activation of the complement cascade must be controlled to focus on foreign targets and not on host cells, and mechanisms are in place to regulate this process (see Fig. 5.17). C1–7 and factors B and P have all been identified in the cornea.

There are three principal locations of complement regulatory proteins: fluid phase (C1-INH, Factors I and H, S protein-40 [SP-40]); cell membranes (decay accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46), and CD59, and membrane C3 proteinases; and matrix (decorin).[65] All cell membrane regulatory proteins (DAF, MCP, CD59, and decorin) are expressed differentially in the normal human cornea (see Fig. 5.17). CD59 blocks the interaction of C9 and C8, preventing the formation of membrane attack complexes and subsequent polymerization of C9. DAF, MCP, and CD59 are strongly expressed in the corneal epithelium and limbus, whereas CD59 is expressed more than DAF or MCP on the keratocytes, suggesting that a complement regulatory system in the eye inhibits destruction of normal tissue. Decorin, a dermatan sulfate proteoglycan, binds C1q with high affinity.
Tissue Components of the Ocular Immune System

**Mucosa-associated immune system (MALT)**

Granulocytes and monocytes are eliminated during inflammation. Lymphocytes, APCs (macrophages), and DC, however, recirculate from the spleen and lymph nodes through the blood and lymphatic system to sites where specific antigen was first encountered. More than 1% of the total lymphocyte pool circulates every hour. Lymphocyte migration from the blood vessels into specific tissues is controlled by the expression of complementary pairs of homing receptors (HR) on lymphocytes and vascular addressins (VA) on endothelial cells. Addressins are tissue- and organ-specific endothelial cell glycoproteins that bind to specific adhesion molecules on specific lymphocytes. The vascular addressins are constitutively expressed on various tissue components of the mucosa-associated lymphoid tissue (MALT).

MALT is a distinct network of diffuse aggregates of lymphoid tissue located in a variety of mucosal surfaces including the gut (GALT), bronchus (BALT), conjunctiva (CALT), nasal mucosa (NALT), and mammary gland.[75] Because mucosa-associated lymphocytes actually recirculate throughout the many sites, the various components actually compose a distinct lymphoid structure. Antigenic access is augmented through specialized epithelial cells which work with other APCs in the conjunctiva, particularly Langerhans cells.[67],[68] The processes of antigen presentation, Ig production and T-cell activation then take place. T-suppressor cells predominate over Th cells in the conjunctiva.

**The lacrimal functional unit (LFU)**

The lacrimal gland, tear film, ocular surface epithelium (cornea and conjunctiva, and the meibomian glands), eyelids, and the interconnecting sensory and motor nerves comprise a complex functional unit which modulates the homeostasis of the ocular surface.[73,75,76] The local immune pathways are determined by a wide variety of factors including the products of the lacrimal gland. In the normal lacrimal gland, the predominant lymphocytic cell type in the lymphocytic aggregates of the interstitium is the plasma cell (IgA and IgD), important vehicles of the adaptive immune response. Tc cells constitute the predominant cell type in the interstitium away from the lymphoid aggregates. With age, the acinar elements undergo degenerative changes, atrophy, and decrease in number. The IgA secretory piece, which
binds two IgA molecules, is produced by the acinar epithelial cells.
The Cell-Mediated Immune (CMI) Response

Major histocompatibility complex\footnote{65}

The major histocompatibility complex (MHC) is a region on human chromosome 6p21.31 which is the most gene-dense region of the human genome. The MHC is divided into three regions: class I, class II, and class III. These genes code for cell membrane glycoproteins important in immune regulation. Class I antigens consist of a single glycoprotein chain that is noncovalently associated with a smaller protein, α2 microglobulin. These antigens code for the HLA-A, HLA-B, and HLA-C antigens found on all nucleated cells. Class I molecules present peptides from endogenous antigens to CD8+ T cells. Class II antigens consist of two noncovalently linked glycoprotein chains, α and β. These code for HLA-DP, HLA-DQ, and HLA-DR antigens, which are present on several important immunocompetent cells including monocytes, macrophages, dendritic cells (including Langerhans cells), and B lymphocytes. These molecules present peptides from exogenous antigens to CD4+ T cells. Cells not normally expressing class II antigens may be stimulated to express them by certain cytokines such as IFN-γ (from Th1, NK cells). Class II antigens function in regulating the immune response primarily through interactions between lymphocytes and macrophages or antigen presenting cells. The class III region contains genes which code for molecules of the complement system, inflammation, and other system functions.

Antigen presentation and T-cell activation\footnote{65}

Antigen processing is a complex sequence of events between a T lymphocyte and an antigen processing cell. This involves a complex process of antigen recognition, antigen uptake, intracellular processing, and finally presentation to resting Th lymphocytes. Activated Th cells then interact with and sensitize other cells to bring about immune responses. Although macrophages, monocytes, and DC are the most important antigen processing cells, other parenchymal cells may be stimulated by IFN-γ to acquire antigen-presenting capacity. The activated Th cell then stimulates the differentiation and clonal expansion of a variety of committed antigen-specific effector cells through the secretion of a variety of cytokines including IL-2 to IL-6, IFN-γ, and TNF-β. IL-2 stimulates antigen-respoding cytotoxic T cells (Tc) to mediate the direct tissue destruction and cells of delayed-type hypersensitivity to mediate DTH responses. IL-2, IL-4, and IL-5 also stimulate B cells to produce memory cells
and antibody-producing plasma cells.

**Cell-mediated immune response**

A variety of cell-mediated immune (CMI) responses can be T-cell dependent or T-cell independent.[65] T-cell-independent responses constitute the innate immune response and include phagocytosis by PMNs, complement-mediated cell destruction, and cytotoxic activity of NK cells and macrophages. Th-cell-dependent responses are more complex, and the mechanism by which specific pathways are selected is unknown. There is some evidence that allergens may preferentially activate Th2 cells, whereas antigens mediate their effects through Th1 activation (see Fig. 5.19). Th cells contribute to the differentiation and proliferation of effector cells and the final mechanism of target cell/antigen destruction/elimination. The main effector cell pathways take place via cytotoxic lymphocytes (Tc, NK, K), but also through mast cells and eosinophils through antigen-specific IgE. In antibody-dependent cell-mediated cytotoxicity (ADCC), cytotoxic cells that possess the Fc receptor for IgG on their cell membrane mediate cell destruction through the release of cytotoxic cytokines (TNF-α, TNF-β, IFN-γ). Complement also may play a role in this mechanism. Finally, lymphokine-mediated macrophage activation also occurs through TDTH cells. If the CMI response fails to effectively eliminate the antigen, tumor cells, or transplant antigen, then the localization of T cells, immune complexes, macrophages, and PMN may lead to chronic inflammation and granuloma formation.
The Humoral (Antibody-Mediated) Immune Response

Binding of antigen to antibodies located on the plasma membrane leads to B-cell activation. After contact with antigen, Ig actively migrates within the cell membrane to form a ‘cap,’ which is then either internalized or shed. B cells respond to specific antigenic stimulation, with help from T-helper cells (Th cells), by blastogenic transformation. This transformation is associated with increased protein and DNA synthesis, antibody synthesis, and finally differentiation into plasma and memory cells. Plasma cells are immunoglobulin 'factories' that manufacture a specific antibody. Memory cells are previously sensitized cells that manufacture a specific antibody. They account for the more rapid and effective immune response following reexposure to antigen.

Immunoglobulins

Characteristics of immunoglobulins[65]

Antibodies are immunoglobulins (Ig) produced by B lymphocytes in response to antigenic stimulation. They play a key role together with the T-cell antigen receptors in providing the characteristic specificity of the adaptive immune response. Immunoglobulins are composed of four polypeptide chains linked together by disulfide bonds. Two of the chains are longer than the others and are termed heavy (H) chains; the two shorter chains are termed light (L) chains. Similar amino acid sequences are termed constant (C) regions, while other sequences are variable (V). A small sequence that is quite variable is termed hypervariable. This region is associated with the antigen-binding portion of the immunoglobulin. Antibody-mediated immunity requires noncovalent contact between the antigen and antibody. Antigenicity is the physicochemical binding of an antigen to an antibody while immunogenicity is the ability to induce the biosynthesis of antibody in a physiological property.

There are five classes of immunoglobulins in humans: IgG, IgA, IgM, IgE, and IgD. IgG accounts for about 75% of the serum Ig and it is the principal antibody of the secondary immune response. It fixes complement (IgG2a) and plays an important role in mediating inflammation and fighting infection through types II, III, and IV hypersensitivity reactions. By binding to Fc receptors on macrophages, NK cells, mast cells, and basophils, it also
functions in the cytotoxic arm of the immune response through ADCC.

IgA is the next most common serum Ig and makes up about 15–20% of circulating Ig molecules. IgA functions primarily in opsonization, neutralization of toxins, and agglutination. IgA is a dimer with a secretory form containing a stabilizing secretory component that is synthesized by glandular epithelial cells (lacrimal). The secretory component protects the Ig from proteolysis by enzymes usually found on the mucosal (ocular) surface. IgA, and more specifically secretory IgA (sIgA), is the predominant Ig found in external secretions such as tears, saliva, milk, and the mucosa of the respiratory and digestive tracts. Therefore, it participates in the peripheral surveillance system of the mucosa-associated lymphoid tissue (MALT) where there is frequent exposure to a wide variety of foreign antigens.

IgM is the largest Ig and is composed of five Ig molecules. It constitutes only 5–10% of the total serum Ig. IgM is the predominant Ig formed after initial exposure to antigen and plays a dominant role in agglutination, complement fixation, and cytolysis. Because of its size and structure, IgM has a high antigen-combining capacity and does not migrate across the placenta.

IgE is an important mediator of anaphylactic responses and ocular allergy. The IgE molecule is fixed to mast cells and basophils through the Fc receptor. There are several types of Fc receptors with variable affinities. In an immune response to allergen, Th2 cells respond by releasing IL-4, which promotes an isotype switching to IgE production (see Fig. 5.19). After binding with antigen, IgE mediates the type I hypersensitivity immune response characterized by histamine and vasoactive mediator release.
Anterior Chamber Associated Immune Deviation (ACAID)

There are many physiological and regulatory phenomena which provide 'immune privilege' to the anterior segment. These include anterior chamber associated immune deviation (ACAID), and soluble and cell membrane-bound immunosuppressive factors in the anterior segment.[66]

ACAID is an unusual systemic immune response, whereby, following foreign antigen injection into the anterior chamber of the eye, a signal is produced that communicates with the immune system through the spleen. A series of events occurs that has several important features: (1) an inhibition of systemic DTH; (2) an inhibition of a complement-fixing antibody response; (3) the maintenance of a normal cytotoxic T-cell and humoral immune response; and (4) the capacity to adoptively transfer ACAID through antigen-specific splenic suppressor T cells, both CD4+ and CD8+, to immunologically naive recipients. As DTH and complement fixing antibodies would generate intense local immunogenic inflammation, the eye has developed a mechanism to reduce this type of immune response.

Niederkorn and Kaplan describe three phases of ACAID.[66] The first ‘ocular phase’ involves an antigen-specific signal which is generated in the eye and delivered to the systemic immune system. F4/80+ APC in the anterior chamber constitute this signal. These cells are exposed to a variety of immunosuppressive agents which lead to: (1) a reduced capacity to produce Th1 inducing IL-12; (2) enhanced production of the Th2 cytokine IL-10; (3) reduced expression of CD40 costimulatory molecule; and (4) autocrine production of TGF-β. The presence of Fas ligand (CD95L) in the eye is also essential for the generation of these cells. In the second ‘thymic’ phase, F4/80+ APC migrate to the thymus where they generate NK1.1+, CD8+CD4- efferent cells which suppress DTH. Finally, the ‘splenic’ phase requires the spleen for at least 7 days. F4/80+ APC also migrate to the spleen where they generate CD8+ regulatory suppressor cells. B cells, T cells bearing the γδ T-cell receptor, and B cells are also required for ACAID.

Two cell populations are responsible for ACAID. The first are CD4+ cells which produce increased amounts of IL-10 and decreased amounts of IFN-γ. These Th1-type cells are termed ‘afferent suppressor cells.’ These cells are required to generate a second population of CD8+ cells which inhibit the expression of DTH responses and are termed ‘efferent suppressor cells.’
In addition to ACAID, there are many immunoregulatory phenomena in the anterior segment which contribute to ocular immune privilege. The blood–ocular barrier, located at the tight junctions of the ciliary epithelium of the ciliary body, physically provides a barrier to cellular infiltration. Soluble factors also inhibit a variety of immunological processes including: (1) T cell proliferation; (2) IFN-γ production by Th1 cells; (3) proinflammatory factors secreted by macrophages; (4) NK cell activity; (5) DTH response; and (6) infiltrating cells by FasL.
Immune Hypersensitivity Reactions

When the adaptive ocular immune response occurs in an excessive or inappropriate form and results in damage to ocular tissue, it is termed a hypersensitivity response. In 1968, Gell and Coombs described four classic types of hypersensitivity responses. Types I through III are antibody mediated, and type IV is cell mediated (T cells and macrophages). Many clinical entities probably result from a combination of mechanisms.

**Type I hypersensitivity response (atopic, allergic reactions)**

After exposure to antigen, an antigen presenting cell (APC) presents antigen to a helper 2 T lymphocyte (Th2), causing the release of cytokines interleukins 4 and 5, which stimulate the (excessive) antigen-specific synthesis of IgE antibodies by B lymphocytes. IL-3 and IL-4 also stimulate the proliferation of FcεRI + mucosal mast cells. After secondary exposure to antigen, eosinophils and mast cells with antigen-specific IgE respond to antigen by bridging two immunoglobulin molecules. An aggregation of receptors in the membrane then causes a rapid membrane-coupled activation of adenylate cyclase, which leads to the increase in cyclic adenosine monophosphate (cAMP). This leads to the degranulation of preformed mediators of inflammation and allergy from storage granules. Newly synthesized mediators are also generated.

Mast cells and basophils release a variety of mediators. Some are preformed, and others must be synthesized. Preformed mediators include an amine (histamine or serotonin), proteoglycans (heparin or chondroitin sulfate), and many different neutral proteases, including aryl sulfatase. Newly formed mediators are usually produced following an IgE-mediated activation. Different profiles of newly formed mediators are probably produced by different populations of mast cells. The newly formed mediators include arachidonic acid metabolites, prostaglandins (PGD2), products of the cyclooxygenase (thromboxanes) and lipoxygenase pathways (leukotriene C4, D4, B4), and cytokines including TNF-α, IL-3 to IL-6, IL-10, IL-13, and VEGF. The release of vasoactive mediators results in the familiar clinical signs of chemosis, vascular injection, itching, and increases in local (tear) IgE levels.

**Type II (cytotoxic) hypersensitivity response**
A type II hypersensitivity response results from complement-fixing antibodies (IgG1, IgG3, or IgM), which bind to endogenous (acetylcholine receptor in myasthenia gravis, basement membrane zone in ocular cicatricial pemphigoid) or exogenous (microbes, transplanted cells) membrane antigens.

Cell damage is mediated by several mechanisms. Through one mechanism, a variety of phagocytic effector cells (macrophages, neutrophils, eosinophils, NK cells) can bring about cell destruction through binding via their Fc receptor and release proteolytic and collagenolytic enzymes. Significant ‘bystander’ damage may result when the target tissue (basement membrane) is too large to be engulfed by the phagocyte. Neutrophils play an important role in this reaction.

Through antibody-dependent cell cytoxicity (ADCC), rather than through enzymatic membrane destruction, NK cells cause direct cell damage through nonspecific binding of antibody to their Fc receptor. These cells release proteolytic enzymes, resulting in target cell destruction.

Finally, antibodies also may activate complement through the classic and lytic pathways, resulting in the deposition of the C5b-9 membrane attack complex. C3b can also bind to target cells and mediate membrane damage via the C3b receptor on phagocytic cells. C3a and C5a are also powerful chemoattractants of inflammatory cells including mast cells, macrophages, and T lymphocytes. These cells also release their own inflammatory mediators. Mast cells release IL-8, ECF, LTB4, and vasoactive amines. Macrophages release LTB4, IL-1, and TNF-α, and T cells release IFN-γ, IL-8, and TNF-α/β (see Table 5.1). These mediators further attract inflammatory cells.

**Type III hypersensitivity response (immune complex)**

In the type III hypersensitivity response, soluble antigen–antibody complexes bind complement and either it becomes deposited into blood vessels throughout the body or the antigen combines with the antibody in the extracellular space. PMNs and phagocytes are attracted into the tissue and directly or indirectly destroy it. The same complement-fixing antibodies in the type II response (IgG and IgM) participate.

Antigen–antibody complexes are normally eliminated through the reticuloendothelial system (larger complexes). This system may become overloaded, resulting in the deposition of complexes into tissues. The outcome usually depends on the size of the complexes, with smaller complexes not deposited in the tissues and larger ones being cleared by the reticuloendothelial system. The intermediate-sized complexes are the most likely to lead to deposition.

Persistent antigen exposure to specific body sites may generate a systemic circulating antibody response with local deposition. Increases in vascular permeability through vasoactive amine release or previous damage to the endothelium is necessary for the complexes to exit the circulatory system and deposit into the tissues. Potential inciting antigens include microbes, drugs, or autoantigens.

**Type IV (delayed-type hypersensitivity [DTH]) response**

Type IV reactions are mediated by macrophages and antigen-specific T lymphocytes rather than antibodies. They are different from the other hypersensitivity reactions in that they are
reactions to fixed, rather than soluble, antigens. These include infectious agents, tumors, and foreign grafts. Antigen is presented to T cells by an antigen presenting cell (APC), which then migrates to lymphoid tissue where it presents to resting T lymphocytes. Once activated, these antigen-specific sensitized cells respond by direct cytotoxic attack or through the release of cytokines that have secondary effects including macrophage chemotaxis and activation. It requires about 48 hours to elicit a maximum response through antigen-specific T cells. Tc and TDTH cells directly attack the target cell. Macrophages are also recruited through cytokine release by these lymphocytes and participate in the elimination of the fixed tissue antigen or organism. Three types of type IV hypersensitivity responses are currently recognized: contact hypersensitivity, tuberculin-type hypersensitivity, and granulomatous hypersensitivity. Corneal allograft rejection results from this process.
References


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As with all other aspects of ophthalmic examination, having a routine aids in ensuring that the examination has been both complete and expeditious. One example routine is provided in Box 6.1, and although it covers the major aspects of the examination it should be modified as necessary.

**Box 6.1**

A recommended order for examination of the eyelids

- Take history and observe patient's behavior and habits
  - Note eye rubbing, blink rate, blotting of tears

- Examine face and eyelids in ambient lighting
  - Note signs of atopic or contact dermatitis, rosacea, tumors, infection, entropion, ectropion, scarring or other signs of trauma

- Examine tear meniscus and puncta with slit lamp (before administering drops or dyes of any kind)
- Note the height of the meniscus, the presence of debris or bubbles, and any punctal ectropion or stenosis

- Examine anterior eyelid
  - Note any eyelash changes and crusting, tumors, nodules

- Examine posterior eyelid
  - Note erythema, telangiectasis, pouting of meibomian gland orifices, distichiasis, rounding of posterior eyelid border

- Express the meibomian glands
  - Note the quantity and character of the fluid expressed

- Step back again and examine the mechanical properties of the lids
  - Note eyelid laxity, and measure the interpalpebral fissure

- Instill dye into the eye (fluorescein, lissamine green or rose Bengal)
  - Use slit lamp again to note the presence and location of the Marx line (mucocutaneous junction) relative to the meibomian orifices

- Consider imaging studies as appropriate (generally for research applications)
Symptoms from eyelid disease are frequently quite vague and non-specific; nevertheless, the customary questions about onset, severity, duration, exacerbation, localization, and history of previous treatments are still appropriate.

Of course, the examination begins during the history taking. As patients are often distracted, this is an excellent time to observe such behaviors as eye rubbing, scratching itchy skin, or wiping away excess tears. One should also observe the manner and rate of blinking, particularly noting whether blinks are forced or incomplete. Patients taking psychotropic drugs and those with central nervous system disease, for example, may blink much less frequently.
Dermatologic Examination

A general examination of the lids begins with the skin around the orbit and face. This is aided by examining the patient in fairly bright, diffuse lighting as close in color to daylight as possible; darkened rooms and artificial light will distort the true color and translucency of tissues. Many patients have dermatologic conditions of which they are unaware.

Contact dermatitis involving the eyelids is quite common and is associated with other ocular allergies.[1] In this condition the skin slightly away from the lid margin is usually more involved. The skin may be quite erythematous, edematous, and display considerable scaling. A recent history of use of lotions, creams, or any topical application to the area should be diligently sought. Atopic dermatitis, or eczema, can be associated with severe keratoconjunctivitis. The periorbital skin may be thickened, scaly, erythematous, and even fissured. Patients may also be aware of lesions elsewhere on their skin, but may not have associated their dermatitis with their eye condition.

Rosacea is a common dermatologic condition of unknown etiology that affects up to 10% of the population and is most commonly found in those of northern European origin. Rosacea dermatitis is characterized by malar flushing, telangiectasias, papules, pustules, and sebaceous gland hypertrophy.

Bacterial infections can also occur, causing preseptal cellulitis, or even progressing to orbital cellulitis. Xanthelasma can also provide evidence of lipid abnormalities.
Eyelid Position

It is important to pay close attention to the position of the eyelids, since many patients with exposure keratopathy do not complain of their condition.[2] The distance between the upper and lower eyelids in the center of the cornea should be evaluated for symmetry. Eyes with large interpalpebral fissures have a much greater surface area. Because evaporation is a direct function of surface area, patients with larger interpalpebral apertures and somewhat compromised tear production are more likely to experience dry eye from increased exposure. One should also observe the position of the eyes when the lids are closed, and look for the presence of corneal and conjunctival exposure. It is important not to allow the patient to force lid closure. Thus, it is best to wait at least 1 minute with the lids closed to allow time for the patient to relax and reveal the true position of the lids.

The examination for ectropion and entropion is important because symptoms are often non-specific.[3] Ectropion often produces abnormalities in the tear film and dryness even to cause keratinization of the conjunctiva. Entropion produces irritation and vascularization of the conjunctiva and corneal surface, both of which also increase evaporation and make any dry eye condition worse. Sometimes ‘spastic’ entropion, which occurs after a forced blink, can be observed following ophthalmic surgery. It is also important to note the position of the lashes with attention to trichiasis and distichiasis.

Eyelid laxity is relatively common, and is easily evaluated by manipulating the lids themselves. Gently pulling the lid straight away from the eye surface tests how much it can be displaced, while pulling a lower lid downward toward the cheek and releasing tests the ability of the lid to snap back to its original location. Excessive laxity may precede ectropion and is often associated with corneal exposure and dryness. Occasionally the lids are relatively tight for the given eye, and this can be associated with problems with diseases such as superior limbic keratoconjunctivitis and exopthalmos.

Floppy eyelid syndrome (Fig. 6.1) results from excessive eyelid elasticity and usually presents with conjunctival injection, mucous discharge, and irritation that is often worse in the morning and not associated in the patient’s mind with eyelid disease.[4–7] These patients are usually obese and frequently report snoring and sleeping face-down. Examination involves first having the patient look down. The examiner then places both thumbs on the superotemporal orbital rims and draws the upper eyelid up and temporally. Floppy eyelids are diagnosed when the upper lid stretches excessively, often to the superior orbital rim, and the tarsal plate everts exposing the palpebral conjunctiva. The syndrome involves not only the presence of floppy eyelids but also signs and symptoms of chronic irritation, including conjunctival injection, thickening, and a papillary response. The cornea also may show mild to moderate vascularization, particularly in the inferior and temporal limbal area.
**Fig. 6.1** Floppy eyelid syndrome depicting eversion of the upper lid with exposure of the conjunctiva.
Tear Meniscus and Puncta

When performing biomicroscopy, the tear lake should be examined first. The tear meniscus should be examined with a slit lamp, but with the light turned off and the ambient light just sufficient to reveal the size of the meniscus. It is also best to refrain from manipulating the eyelids before examining the tear lake. Once the tear meniscus has been carefully examined, slit lamp illumination can be turned on and the degree of reflex tearing noted. The intersection between the lid margin and the ocular surface contains the tear meniscus. The height and volume can reveal the relative severity of dry eye and the amount of debris in the tear film.\[8–10\] Foamy tears generally indicate meibomian gland dysfunction. Patients with a very small tear meniscus who are unable to generate a response to the slit lamp light are much more likely to have difficulty with dry eye than a person who is still capable of a significant reflex tearing.\[10–12\]

The positions of the upper and lower puncta are important for normal function. The lower punctum may be everted even if the position of the central part of the eyelid is relatively normal. Punctal ectropion will reduce its ability to drain the tear lake, leading to epiphora. A punctum that is rotated inward rarely causes a problem. Puncta may be scarred closed from a variety of conjunctival diseases or as a treatment for dry eye, although patients may be unaware of previous occlusions. Pemphigoid and alkali burns frequently lead to occlusion of the punctum.\[13\],\[14\]
Anterior Eyelid

Although it is best to examine the patient first in ambient lighting with attention to color, transparency, induration, and other general characteristics, any nodules or other suspicious lesions should be examined with the biomicroscope as well. Eyelid scaling, separation, scarring, and atrophic changes are also more easily seen with magnification.

Examination of the lashes is most readily performed with the biomicroscope. The length of the lashes and the number of white, broken and missing lashes should be noted. Particular attention should be paid to the presence of very small lashes that may be directed posteriorly (Fig. 6.2). In some patients, particularly those with Stevens–Johnson syndrome and related diseases, trichiasis of very small lashes is a very difficult problem and may lead to severe symptoms, inflammation, and scarring of the corneal surface.[15]
Fig. 6.2 Trichiasis from lid margin scarring can be seen in blepharitis as well as in Stevens–Johnson syndrome and other scarring diseases of the conjunctiva.

A collarette, which forms in areas of inflammation or hyperkeratinization, is simply mucous debris that adheres to the lash and becomes visible as the lash grows. Collarettes are a relatively non-specific sign of inflammation. The lashes also should be examined for signs of infestation. Lice are relatively easy to see on the lashes, whereas Demodex organisms are much smaller and more difficult to identify.\textsuperscript{[16–19]} Infectious processes may occur at the lashes and are usually evident by swelling and pus noted at the base. Such hordeola of the hair follicles are often associated with a more generalized bacterial infection of other lid structures.\textsuperscript{[20],[21]}

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Posterior Eyelid

Inflammatory stimuli or infections of the eyelid may induce rounding of the posterior lid margin, which normally has a squared edge in profile.[22] In normal, noninflamed lids very small capillaries can be identified, but large vessels are not seen. Atrophy and inflammation of the entire lid margin will also cause the appearance of hypervascularity, in part because atrophy causes an increased transparency of the lid margin which makes the deeper vessels more visible. These vascular changes are relatively nonspecific but are often associated with obstructive meibomian gland dysfunction, rosacea, or infections, but not with seborrheic meibomian gland dysfunction (Fig. 6.3).

Fig. 6.3  Vascularization and hypertrophy along the lid margin which alters the normal contours and obscures landmarks.

Besides trauma, chalazia are the most common cause of lid scarring, and may leave notches that distort the smooth contour of the lid. These notches are also associated with trichiasis and are indicative of obstructive meibomian gland dysfunction (Fig. 6.4).
Fig. 6.4 Scarring line on the lid margin from chalazia formation may produce a loss of tissue, which appears as a notch on the lid margin. Lashes are often found within the notch (1).

Allergic processes cause thickening of the conjunctiva and may also cause chronic changes to the lid margin. In severe disease, deep furrows in the skin and conjunctival surface of the lid margin develop which may become secondarily infected and lead to frank ulceration.[23] The openings of the meibomian glands should be inspected carefully for signs of chronic disease. Periglandular atrophy renders the ducts more evident as the lid margin recedes around the keratinized duct.[24] Hyperkeratinization of the ductal epithelium also may occlude the meibomian orifice entirely.[25–27] In some instances, partial occlusion from keratinization will add to obstruction from dry and hardened inflammatory debris and further obstruct the meibomian gland. Chronic changes also occur simply from aging, and are exacerbated by the effects of long-term obstructive meibomian gland dysfunction and dry eye.[28]
Meibomian Gland Expression

Meibomian gland expression is an essential part of the lid examination.[28],[29] Digital pressure is applied to the meibomian gland through the skin just distal to the opening of the gland duct with the patient in upward gaze. A cotton swab may be used to press on the lid. Firm pressure sufficient to indent the contour of the globe is usually required to express meibomian gland excreta. The pressure may need to be maintained for several seconds to evaluate adequately the appearance of the expressible lipid. Usually 20–25 meibomian glands are present in the lower lid; two or three can be compressed at one time. The entire lid margin should be examined and the volume and viscosity of the excreta noted. Particular attention should be directed at those glands with abnormal findings, as normal glands are commonly found next to glands with severe disease.

The volume of the meibomian excreta can be recorded as the diameter of the lipid dome that forms after several seconds of pressure. The normal diameter of each dome is 0.5–0.7 mm. The volume of lipid is increased if any of the lipid domes are 0.8 mm or larger; this finding is sufficient to diagnose seborrheic meibomian gland dysfunction (Fig. 6.5). Meibomian gland lipid production may also be measured by evaluating the area of increased transparency of a paper strip placed against the meibomian orifices.[30],[31] Smaller lipid volumes or totally obstructed glands that cannot be expressed with digital pressure are associated with obstructive meibomian gland dysfunction.
The viscosity and opacity of the expressed lipid are important signs of eyelid disease. Normal meibomian lipid is liquid at body temperature, flows easily, and is completely transparent. Seborrheic meibomian gland dysfunction is associated with a more opaque lipid that remains liquid. In obstructive meibomian gland dysfunction the viscosity increases, the transparency of the liquid declines, and the volume usually declines as well. At the highest level of viscosity lipid will emerge slowly like toothpaste, will not flow except under pressure, and will be totally opaque, with a white or light yellow color (Fig. 6.6).[28] Although such increased viscosity is usually associated with obstructive meibomian gland dysfunction, it is also found in some subjects with rosacea.[29] The differences in consistency of meibomian excreta have been found to be due to changes in lipid composition.[32]
Meibomian glands may also become infected, whereupon expression will often produce pus from an orifice and will be quite tender. This condition should not be confused with staphylococcal blepharitis, which is usually the result of an immune response to heavy staphylococcal overgrowth on the eyelids.[33] *Staphylococcus* and *Streptococcus* organisms are usually responsible, and culturing the organism for antibiotic sensitivity may be helpful.[34–36] Because nearly all eyelids harbor such organisms, it may be difficult to determine the significance of the bacteria found with eyelid cultures. There is some evidence that different strains of bacteria are involved in different forms of blepharitis.[34] Blepharitis patients may also have heavier growth of bacteria on their lids.[33] The relative contribution of bacterial overgrowth, infection, bacterial toxins, and abnormal immune responses towards the development of blepharitis and meibomian gland dysfunction is a subject of continuing controversy and investigation.

In clinical practice, although much is often made of examination techniques to distinguish between infectious and inflammatory blepharitis, such distinctions may not be critical as the use of topical antibiotics, steroids, and systemic tetracyclines reduces the bacterial load and alters the immune response for most patients.[37] It is important, however, to recognize the presence of meibomian gland...
disease to enable appropriate treatment.
Mucocutaneous Junction

The mucocutaneous junction is where the keratinized squamous epithelium of the skin meets the moist, nonkeratinized squamous epithelium of the conjunctiva, and it normally lies just posterior to the opening of the meibomian orifices.[22],[38] After instilling lissamine green, rose Bengal or fluorescein onto the ocular surface, a visible line of demarcation, called the Marx line, is often apparent on the lid margin. This line is thought to represent the mucocutaneous junction, and anterior displacement relative to the meibomian gland orifices may correlate with gland dysfunction.[38] Other authors have disagreed, finding that, although meibomian secretion declines with age, no age-related changes occur with the position of the mucocutaneous junction.[22]
Meibomian Gland Imagery

Albeit often reserved for research settings, transillumination of the lids can clearly image the morphology of individual glands. Either lid can be transilluminated, but the lower is considerably easier to evert. Digital infrared images of the entire lid can now be obtained with resolution approximately equal to that of infrared film.[39–41]

In humans, one can see evidence of gland loss, ductal dilation, chalazion formation, microcyst formation, and aberrant gland development (Fig. 6.7). Animal investigations demonstrate similar pathophysiologic processes.[25],[27] The most obvious change seen with transillumination is gland dropout. Dropout is associated with obstructive meibomian gland dysfunction and is not associated with infectious blepharitis, allergic phenomenon, or seborrheic meibomian gland dysfunction. Seborrheic meibomian gland dysfunction shows no abnormalities of gland morphology. Patients with rosacea often reveal a mixed picture. Some areas of the lid will reveal gland dropout and increased lipid viscosity, whereas other areas will have increased lipid volume without gland dropout. This is consistent with the report that chalazia are more common in patients with rosacea than in normals.[42] Active chalazia often induce such thickening and induration of the lid that the details of meibomian glands cannot be seen with transillumination. Following resolution of the acute inflammation, a scarred area that may contain small cystic structures is usually evident.

Fig. 6.7 Meibomian gland imagery. A, Transillumination of a normal eyelid showing evenly spaced glands. B, An infrared image with transillumination of the lower lid showing loss of glands.

Patients receiving isotretinoin (Accutane) therapy will have diminished lipid density in their meibomian glands, and the images will be much fainter, especially when the dosage has been relatively high. In such cases the meibomian glands seem almost to disappear, but will return to a normal appearance several weeks to months after cessation of therapy.[43],[44]

Radiation to the orbit also damages meibomian glands, which are quite radiosensitive; in such cases transillumination shows that the number of glands is reduced, as is the volume of lipid secretion, and the viscosity is often increased.
In vivo confocal microscopy has also been used to image living meibomian glands in great detail both in normal patients and in cases of inflammatory meibomian gland obstruction. The obstruction causes a reduction in the number of acini and dilation of the ones that remain, as well as glandular atrophy and surrounding fibrosis in advanced cases.[45] This provides useful insight into the sequence of events that leads to gland dropout, and may become more clinically useful in predicting those patients who are most likely to benefit from lid margin treatment. To that end, the same technique can also be used to monitor the meibomian glands for treatment effects.[46]
References


Chapter 7 – Slit Lamp Examination and Photography

Csaba L. Mártonyi

‘On August 3, 1911, Alvar Gullstrand presented his first rudimentary model of the slit lamp ... and explained its optics and applications.’*

An occasion of tremendous significance to ophthalmology had taken place. Gullstrand had introduced a device with the potential to advance the understanding of the eye and its problems as profoundly as did the direct ophthalmoscope 50 years earlier.

This chapter will deal primarily with techniques of examination (all applicable to the photographic process) and will address special considerations required for photodocumentation under the heading Photography, below.

At present, only an appropriately equipped photo slit lamp biomicroscope (PSL) is able to reproduce the information seen at the clinical slit lamp.

The Instrument: Examination and Photography

The principle underlying the slit lamp biomicroscope is isolation. This instrument provides precise and modifiable illumination plus magnification with which to isolate, and thereby make visible, fine detail (Fig. 7.1).
The magnified optic section is the most important capability of the slit lamp biomicroscope.

(From Mártonyi CL, Bahn CF, Meyer RF. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Fig. 7.1 (A) Slit illuminator and biomicroscope.

(Redrawn from Mártonyi CL, Bahn CF, Meyer RF. Clinical slit lamp biomicroscopy and photo slit lamp biomicrography. Ann Arbor: Time One Ink, Ltd; 1985.)

Composed of two primary components, the biomicroscope and the slit illuminator, today's slit lamp is both highly efficient and accommodating. The addition of available accessories provides for an impressive array of functions.

Most biomicroscopes consist of a parallel, Galilean telescope design. Utilizing optical changers, interchangeable oculars, or both, these instruments produce an effective range of magnification with excellent resolution. Many offer optional beam splitters to accommodate one-to-one teaching or to accept a video camera for real-time display or recording for later use.

The slit beam delivery system is basically a projector, with the slit aperture as the actual 'object' focused
on a plane corresponding to the focal length of the biomicroscope. The foremost prowess of the slit lamp is its ability to create a focused, well-delineated, narrow slit beam that forms an optic section in transparent and translucent tissue. Not restricted to a single configuration, however, this beam is highly malleable through the use of simple controls that dictate its size and shape. It finds many additional applications in its various forms.

The biomicroscope and the illuminator are mounted on a common axis in a copivotal arrangement. This arrangement facilitates the parfocal (biomicroscope and slit beam are focused on the same plane) and isocentric (the slit beam is centered in the field of view) relationships essential for practical function. A departure from these relationships can be purposely created for certain techniques of examination; otherwise, the absence of isocentricity or parfocality indicates a faulty condition requiring adjustment or repair.

The Instrument: Photography

Good photographic results require the use of a PSL equipped with the following additional elements:

**Beam splitter:** A beam splitter provides the necessary coaxial view shared by the examiner and the camera back. That is the only arrangement whereby complete control over the image can be exercised before it is recorded. Beam splitters will divert from 50% to 85% of the light to the camera to ensure satisfactory exposures with most forms of illumination. As more light is diverted to the camera back, less remains for the examiner. A suitable compromise must be established for practical use, especially when the same instrument serves both examination and photography.

**Electronic flash:** Electronic flash produces light of high intensity at an effective duration of exposure of approximately 1 ms, the speed required to arrest the motion of the eye at high magnifications. The flash delivery system must be coaxial with the ambient light from the slit beam illuminator to reproduce the effect of lighting established by the examiner.

**Fill light:** The fill light is an accessory source of diffuse illumination unique to the PSL. It provides partial compensation for the loss of the dynamic, three-dimensional character of an examination by contributing the important element of perspective in situations that call for limited direct focal illumination. The addition of diffused, overall light places isolated elements into context. In a single image, the fill light provides overall, general information about the eye, and the slit beam is used to highlight specific changes in the cornea (Fig. 7.2). The fill light also must be equipped with electronic flash proportioned to an output of approximately two stops lower than that of the slit illuminator. This relationship provides the necessary contrast between the diffusely illuminated background and the bright, narrow slit beam.

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Fig. 7.2 (A) Diagrammatic representation of the slit lamp illuminator, biomicroscope objective lens, and fill light. (B) Chemical injury to the cornea seen in optic section combined with diffuse illumination from the fill light.

Preparing for Photography

The basic protocol for photodocumentation is essentially the same as for slit lamp biomicroscopy. One begins with an overview and proceeds to isolate further with illumination and magnification the salient features of the condition under consideration. Techniques of illumination that produce specific information over a wide area of distribution should be considered whenever applicable. The most utilized images are those that present findings in recognizable context.

Essential detail, however, should not be compromised in an attempt to include everything in a single view, and the fill light should not be used in conjunction with indirect forms of illumination. While such photographs are often used to obviate the need for multiple images, the results will always be compromised.

Although the fundamental principles of clinical slit lamp biomicroscopy and photo slit lamp biomicrography are essentially the same, additional considerations are necessary for successful photodocumentation. Chief among these considerations is the conscious awareness that slit lamp illumination, by its very nature, is a compromise. The larger the area of simultaneous illumination, the less fine detail is seen. Conversely, the more detail elicited by selective illumination, the more out of context that information will be. During a dynamic, three-dimensional examination of the eye, these limitations have little effect on the process of gathering information. The result of a thorough examination is a complete mental image of the condition of the eye. By comparison, a static, two-dimensional photograph is not only deprived of the elements of motion and the third dimension, but is also limited to a single moment of such an examination. As such, it is amazing how effective a single photograph can be.

Several components have been discussed as essential to the PSL. Additional factors must be considered to produce consistently accurate and pleasing photographs. Correct mechanical focus, format, magnification, centration, control of artifacts, and optimum exposure are elements that combine to reproduce visual impressions most accurately.

Focus

The maintenance of a sharp image in the biomicroscope is a continuous element of a
dynamic slit lamp examination. Focus is a perpetual, flowing transition as the slit beam is played over the gently curving surfaces of the eye. In a practical sense, there are no specifically individual images, but rather a compendium of infinite, transitional views that produce an aggregate impression. Each photograph, however, is but a single slice of that examination. Therefore, preparations for photo documentation must include the selection of the most informative single view (or the first in a series of views) and the perception of its appearance as a static, two-dimensional image. To ensure a sharp image, precise mechanical focus of the biomicroscope at the time of exposure is critical.

The view seen in the biomicroscope is an aerial image. An aerial image is suspended in space rather than projected onto a flat, immovable plane (as the focusing screen of a single lens reflex camera). Because the view through the biomicroscope would be unacceptably diffused by such a focusing screen, it is not used. The aerial image system, therefore, is the most practical in such an application, but not without limitation. With the image literally floating in air, the mechanical position of the biomicroscope (and the camera back) can be unwittingly altered from its correct focal distance through simple accommodation. Although seen as a sharp image by the examiner, the resultant photograph may be so unsharp as to be unusable. To produce a sharp image on film, a specific protocol is followed.

To facilitate the correct focus of the biomicroscope, a ‘cross-hair’ reticle is used in one ocular for reference. To use the reticle correctly, the ocular must be adjusted for the user’s refractive error. That is best achieved by first turning the eyepiece adjustment to its maximum plus setting. Then, while the user looks through the eyepiece, with accommodation completely relaxed, the setting is adjusted toward the minus side while observing the reticle. This rotation toward the minus should be relatively brisk and should result in a sharp impression of the reticle at or near the user’s refractive error, or at zero for an emmetrope. This exercise should be repeated until consistent results are achieved. The image in the biomicroscope should be treated as an object at infinity and viewed with the accommodation completely relaxed. The reticle is always used in the ocular that shares the image with the camera back.

**The 35-mm format**

The circular field seen in the biomicroscope must be reduced to a corresponding rectangle within that circle. The areas beyond the rectangle, which had been used for examining the eye, must now be disregarded and the intended picture area confined to the photographic format. As a guide, a reticle eyepiece including the rectangular outline is an ideal solution.

**Magnification**

Many PSLs demonstrate an apparent difference between the image size seen in the microscope and the recorded image. Although the actual magnification is the same, the resultant photographic image appears disappointingly small. This discrepancy results from the larger physical area of the film or sensor as compared with the area within the oculars. To obtain a photograph that matches more closely the image seen in the oculars, the photograph must be taken at one magnification setting higher. On some PSLs, this can be accomplished by placing a 2× optical magnifier between the microscope and the camera back. When a 2× magnifier is not used, the image of the eye to be photographed is viewed at the magnification desired; then, just before taking the photograph, the magnification changer is advanced to the next higher setting. Once the image is recorded, the magnification can be returned to the setting that best delineates visually the next field under consideration.
**Centration**

The need to ensure that the principal subject is in the center of the photographic field seems obvious. Nevertheless, this element is frequently compromised. The most common cause may be a momentary disregard of the rectangular format in favor of the full, circular field seen in the oculars. Additionally, when the isocentric relationship between the slit illuminator and the biomicroscope is left intact when using indirect forms of illumination, the subject area becomes decentered in favor of the isocentric incident beam. The beam must always be decentered to allow centration of the principal subject area when indirect forms of illumination are used.

**Control of artifacts**

To be effective, a photograph must first include the principal subject area. Of equal importance is the elimination of unwanted elements that either obscure the desired detail or distract attention from the principal subject.

The most distracting artifacts of illumination are the ubiquitous specular reflections seen during the course of an examination. They are of little concern as they come and go, because their effect is nullified by their momentary presentation during the dynamic examination. In a static photograph, however, such artifacts can considerably compromise an otherwise excellent image. When final adjustments are made preparatory to making an exposure, it is important to view the image only through the ocular that shares that image with the camera back. On most photo slit lamps, the camera back is mounted to share the view seen with the right eye. By closing the left eye, the monocular image on the right, as seen by the camera, will be much more manageable in terms of minimizing artifacts and maximizing desirable elements before its capture.

**Exposure**

A good exposure results from correct white balance, media sensitivity, intensity of illumination, subject reflectivity, and duration of exposure.

**Color balance and sensitivity**

Unlike film, digital cameras provide for easy adjustment to match the color temperature of various light sources. This process is called 'white balance' and should be set following the instructions for the camera back used. It will ensure accurate color reproduction in captured images. For electronic flash, the setting is approximately 5400K (degrees Kelvin).

Sensitivity refers to the responsiveness of film and digital media to light, stated in ISO numbers. Low ISO settings will provide better image quality when there is sufficient light for a good exposure. When the captured image is too dark and the flash intensity is already set to maximum, the ISO should be increased sufficiently to obtain good results.

**Intensity of illumination**

The light output, or flash intensity, of the PSL, while seemingly very bright, is the limiting
factor in the use of low ISO values for certain forms of illumination. Flash intensity should be set to maximum for demanding situations before increasing ISO settings for better image quality.

**Subject reflectivity**

Subject reflectivity is an element of great influence. In recording conditions by direct illumination, levels of subject reflectivity may dictate an adjustment of one to two f-stops for a good exposure at either extreme. In certain applications of indirect illumination, however, a range of four to five f-stops may have to be considered.

**Duration of exposure**

Duration of exposure is dictated by the electronic flash source, as mentioned earlier. Of very short duration, it makes the effective ‘shutter speed’ approximately 1/1000th of a second, an ideal speed to arrest motion. This flash duration is not variable. The shutter speed setting on the camera back must be set to the speed prescribed by the manufacturer for electronic flash synchronization – the maximum speed at which both shutter curtains are simultaneously clear of the full frame to permit the short-duration flash to expose the entire image area. Using higher shutter speeds results in the loss of all or part of the frame.

*Exposure is everything!* The most skilled and experienced clinician will fail to produce good slit lamp images if exposure is miscalculated or neglected. The ability of our eyes to adapt over a wide range of light intensity is not even remotely shared by the camera. Elements of interest that are easily appreciated visually may be completely off the scale from the standpoint of exposure. The development of a thorough exposure guide is an excellent investment, as it provides predictable exposures for most situations. The successful photographer will also have cultivated an intuitive sense for differences beyond the obvious —differences that are subtle visually but require exposure compensation for best results.
Forms of Illumination: Examination and Photography

While there are only a few basic forms of illumination, most have variable uses and are highly effective in specific applications. This chapter concentrates on those forms of illumination and techniques of examination and photography that specifically address the eyelids, conjunctiva, cornea, sclera, and iris. Other structures are mentioned as their examination may be helpful in establishing a diagnosis of conditions involving the principal structure under consideration.

Techniques of illumination are broadly divided into direct and indirect forms. Direct illumination, as the term implies, describes any situation where the beam of light is directed to strike the principal subject area. Direct illumination may be diffused or focal. Indirect illumination techniques use a secondary surface that reflects light onto the principal subject area or light transmitted through tissue from an area of adjacent illumination.
Direct Illumination

Diffuse illumination: examination

Diffuse illumination facilitates simultaneous observation of large areas at low magnification. The area surrounding the eyes, the eyelids, conjunctiva, sclera, cornea, and iris can be quickly reviewed for gross abnormalities. Initiating the slit lamp examination in this manner generates an early, overall impression and provides a unifying matrix for the more isolating magnifications and forms of illumination to follow. With the slit illuminator set at its largest aperture and the diffuser in place, the illuminator is rotated through its arc of travel from side to side. The effect is to create alternating axial and tangential illumination. Tangentially applied light, even when diffused, produces highlights and shadows and enhances the visibility of many changes. As shadows and highlights wax and wane with the oscillating illumination, alterations from normal topography become exaggerated and more readily apparent. A subtle presentation of molluscum contagiosum, for example, possibly hidden by cilia, may elude detection in static light, but may become quite obvious through the motion of the illuminator and the biomicroscope. Abnormalities of the lashes, such as colarettes, scales, and broken or missing lashes, are well enhanced with this approach. Because of shadows cast by cilia and foreign matter and the generally translucent nature of such deposits, static light may not provide adequate discrimination. The dynamic travel of light, however, animates shadows and cascades highlights to fully dimensionalize and identify even mild expressions of various conditions (Fig. 7.3).

![Fig. 7.3](image)

Focal alterations in skin color (e.g. hyperemia, hyperpigmentation, or hypopigmentation) also present more readily under diffused and dynamically altered light. Focal illumination tends to isolate an area with a proportionate loss of perspective. Additionally, the brightness of focal illumination, with its inherent contrast, makes slight differences in color difficult to appreciate. Another factor to limit the usefulness of focal illumination in this application is the possibly enhanced reflectivity of the skin of the eyelids. Secretions from resident sebaceous glands can engender considerable specular reflections, greatly limiting a view beyond the episurface.
As stated earlier, the initial survey of the conjunctiva, sclera, cornea, and iris in diffuse illumination provides a useful introduction to overall condition (Fig. 7.4). Many abnormalities are easily visualized with this technique. Findings such as conjunctival injection, follicles, papillae, chemosis, membranes/pseudomembranes, and scarring are recognizable in diffused light and should prompt examination with additional forms of illumination. The inferior and superior palpebral conjunctivae and much of the fornices can be given a preliminary review in the same manner. Tangentially applied diffuse illumination, with increased magnification, is an excellent technique for initial examination of these surfaces (Fig. 7.5).

Fig. 7.4. Staphylococcal keratoconjunctivitis and blepharitis are presented in diffuse illumination. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Fig. 7.5. Trachoma with linear scarring seen in diffuse illumination. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Inspection in diffuse light often provides the first indication of abnormalities present in the cornea (Box 7.1). Gross opacification or changes that affect its topography present with little coaxing. After such an overview, further investigation can continue with more selective illumination and magnification.

**Box 7.1**

**Examples of conditions seen in diffuse illumination**

- Sclerocornea
- Band keratopathy
- Trichiasis
Diffuse illumination: photography

Diffuse illumination is required to show large areas simultaneously at low magnification. By diffusing the light from the slit illuminator, along with the fill light, two sources of diffuse light are available to produce even illumination of the external eye (Fig. 7.6). Such overviews are useful for demonstrating the general condition of the eye and serve as introductions for more isolated views.
Focal illumination

Broad-beam illumination: examination

The term broad-beam illumination is variably used and highly subject to interpretation. It can vary from a beam width of 1 mm to its full size of approximately 11 mm. In this discussion a flexible width is assumed. As with the recommended oscillation of the slit illuminator, a dynamically altered beam width is also beneficial. In this application, the beam is intended only as a source of bright, focal illumination, with the width adjusted to maximize information within the area under study. As the light strikes tissue interfaces, it is reflected, refracted, transmitted, scattered, and absorbed in a highly variable fashion. Thus, a given width of beam, with its corresponding overall intensity, may provide good information to confirm a particular entity, but may overpower findings associated with another. A beam width of 2–3 mm can provide a good starting point (Fig. 7.7). Although width is an important factor in the beam’s efficiency in specific applications, its intensity also affects its usefulness. A beam that is too bright will produce scatter, reducing the examiner’s ability to discriminate. Conversely, a beam intensity that is too ‘gentle’ (in deference to patient comfort, for example) may preclude detection of mild departures from the normal.

As various tissues are examined, suspected alterations from the normal will either be confirmed or will prompt the examiner to use more or less light in their continued pursuit. As a general rule, forms of illumination should be exaggerated in both directions beyond the ideal setting, especially with the use of broad-beam illumination. The beam should be narrowed to the point of diminished width and then increased in width beyond the ideal setting to the point at which information loss occurs once again. Only by testing these limits will optimum size and intensity become apparent.

Many conditions are seen best using broad-beam illumination (Box 7.2). All changes that are fairly opaque and reflect or absorb considerable amounts of light can be visualized easily. The light should be applied tangentially for maximum effectiveness. Topographic changes will become dramatically sculpted by the raking light. Additionally, oblique illumination will obviate the dazzling specular reflections resulting from axial lighting (Fig. 7.8).

Tangentially applied broad-beam illumination is one of the most effective forms for examining the iris surface (Fig. 7.9).

<table>
<thead>
<tr>
<th>Box 7.2</th>
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<tr>
<td>Examples of conditions seen in broad-beam illumination</td>
</tr>
<tr>
<td>Corneal vascularization</td>
</tr>
<tr>
<td>Basement membrane dystrophy</td>
</tr>
<tr>
<td>Reis-Bücklers’ dystrophy</td>
</tr>
<tr>
<td>Schnyder’s crystalline dystrophy</td>
</tr>
<tr>
<td>Terrien’s marginal dystrophy</td>
</tr>
<tr>
<td>Amiodarone vortex dystrophy</td>
</tr>
<tr>
<td>Prominent corneal nerves</td>
</tr>
<tr>
<td>Salzmann’s nodular degeneration</td>
</tr>
</tbody>
</table>
Posterior embryotoxon
Corneal scars
Lisch nodules
Keratic precipitates
Granular dystrophy
Iris atrophy
Pterygium
Band keratopathy
Macular dystrophy
Arcus senilis

Fig. 7.8 Broad-beam illumination demonstrating a luxated, mature lens nucleus. The tangentially applied light dramatizes dimension and minimizes reflections from overlying surfaces. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Fig. 7.9 Broad-beam illumination of the iris and anterior lens surface in Rieger's syndrome. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

When no abnormalities are seen with this technique, more selective forms of illumination are indicated. The absence of findings under broad-beam illumination should never encourage the conclusion that abnormalities are not present. However useful in the applications described above, broad-beam illumination can be quite counterproductive to the detection of many alterations of subtle expression.

Broad-beam illumination: photography
When diffused light causes too much scatter or when a specific element of a condition must be emphasized, a broad beam, without fill light, can be used. Beam size is important to the outcome. Although desirable from the standpoint of an all-inclusive photograph, when beam size is enlarged to include too much, the results are often compromised. The effect of illumination must be observed carefully while dynamically altering beam width to determine the optimum setting (see Figs 7.7–7.9). Exposure is not a problem because this form of illumination returns the largest percentage of the light available. Magnification should be increased to make best use of the photographic format.

The presentation of the beam should be as oblique as possible. Axial light is reflected axially from surfaces overlying the condition or object to be photographed and further reduces dimensional information within the subject area (Fig. 7.10). The tangential presentation of light, however, facilitates illumination of the subject without overlying reflections to diffuse information and simultaneously enhances its topography (Fig. 7.11). This form can also be used to isolate abnormalities of high reflectance in transparent tissue. When photographing an object within the lens or the anterior vitreous, a well-dilated pupil is necessary to accommodate a sufficiently tangential presentation of light to obviate reflections from overlying surfaces (Fig. 7.12).

Fig. 7.10 (A) and (B) This iris lesion is de-emphasized by the diffusing axial reflection of light and the lack of highlights and shadows to demonstrate its dimensional nature. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd, 2007.)
Fig. 7.11 (A) and (B) A tangentially applied beam will obviate the reflections inherent in axial illumination and will dimensionalize the subject with the highlights and shadows it creates. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd, 2007.)
Optic section: examination

This narrowest slit beam is, in effect, a fine blade of light that makes possible the virtual serial sectioning of transparent tissues in the living eye. The tangential presentation of these ‘light slices’ facilitates an essentially cross-sectional view of the cornea and lens, even though these structures are largely parallel with the plane of observation. The sharply focused light is completely confined to the optic section, reducing scatter and maximizing contrast between the illuminated section and the dark, unilluminated surround. The result is a clear, basically uncompromised view of the tissue within the beam. As the slit beam is projected from an increasingly lateral position (away from the axis of the biomicroscope), the greater angular presentation has the effect of increasing the distance between the anterior and posterior surfaces of the structure under study. This increase serves to clarify intrastructural relationships and localization of abnormalities within.

This capability represents the most selective and most isolating manner in which such tissue may be illuminated and observed (Box 7.3). For maximum effectiveness, the light intensity is set to maximum, and the slit beam is diminished in width to a point just before the optic section loses structural integrity. The thinner the beam, the more selective the optic section, thus producing finer delineation of information within that section. Beam width, however, should never be reduced to the point at which information is compromised because of light loss.

Box 7.3

Examples of conditions seen in optic section

<table>
<thead>
<tr>
<th>Edema</th>
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<tbody>
<tr>
<td>Stromal opacities</td>
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<tr>
<td>Marginal dystrophy</td>
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<tr>
<td>Kayser-Fleischer ring</td>
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<tr>
<td>Fuchs' dystrophy</td>
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<tr>
<td>Corneal pannus</td>
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<tr>
<td>Epithelial defect</td>
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<tr>
<td>Corneal infiltrates</td>
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<tr>
<td>Furrow dystrophy</td>
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<tr>
<td>Lens opacities</td>
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<tr>
<td>Dellen</td>
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<tr>
<td>Microcysts</td>
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<tr>
<td>Bullae</td>
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<tr>
<td>Ectatic changes</td>
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<tr>
<td>Anterior chamber depth</td>
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<tr>
<td>Tear film deficiency</td>
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<tr>
<td>Corneal thinning</td>
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</table>

The transparency of the cornea, coupled with its propensity for both primary and secondary expressions of numerous diseases, makes it the most important component of the eye to section with light. Although remarkably transparent, normal corneal tissue is sufficiently translucent to reflect the narrow slit beam and articulate the optic section.

A high-magnification view of the optic section will produce excellent discrimination of the substantial corneal layers. Beginning with the tear film, each layer may be selectively examined for departures from the normal (Fig. 7.13). The normal tear film will ‘flow’ dynamically within the slit beam following each blink of the eyelids. Its reflectivity will alter with the amount of refreshed, protective lipid on its surface, but will maintain a constant thickness and a smooth anterior face. The epithelium is seen as a line of nonreflectance or greatly diminished reflectance between reflections from the tear film and Bowman’s layer, which is contiguous with (and largely indistinguishable from) the anterior-most reflection from the stroma. The stroma itself is quite transparent. By encroaching on the zone of specular reflection, however, its reflectance can be enhanced considerably for better visualization of its structure. The optic section will terminate with the heightened reflection from the endothelium.
As corneal transparency is lost to disease or injury, an increased amount of light is reflected. (The condition of a sclerotic, totally opaque cornea represents the extreme, or terminal, end of this transmission spectrum. In this condition, much of the light is reflected by the surface, limiting visual access to deeper layers. In such cases, moderate amounts of illumination will be considerably more informative, and a thorough examination will necessitate the use of indirect techniques, as discussed later in this chapter.)

An inadequate tear film will present as a compromise to the normally smooth, unbroken reflection of the beam (Fig. 7.14). An edematous epithelium will become dimensional in the optic section and reflect increasing amounts of light (Fig. 7.15). Focal density changes will be isolated to the anterior, mid, or posterior cornea (Figs 7.16 and 7.17). Descemet’s membrane will become visible when abnormal mechanical forces alter its normal topography (Fig. 7.18). The endothelium will reflect increased amounts of light when affected by changes such as Fuchs’ dystrophy (Fig. 7.19). Within the visual axis, relatively mild expressions of tissue compromise can cause notable symptoms. Determining exact location and distribution is significant for arriving at a diagnosis.
Fig. 7.14 The fragmented reflection from the corneal surface indicates an inadequate tear film, an uneven epithelial surface, or both.
(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
Fig. 7.15 The normally nonreflective epithelium is visible when edematous. The reflection is contiguous with the reflections from the tear film and the stroma. Two small edema clefts are seen in optic section.

(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Fig. 7.16 Anterior to mid-stromal deposits are seen in a patient with dystonia.

(Copyright Mártonyi CL, WK Kellogg Eye Center, University of Michigan.)

Fig. 7.17 (A) and (B) Two images demonstrating the right-to-left, anterior-to-posterior track of a penetrating foreign body.

(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
Fig. 7.18 The normally nonreflective Descemet's membrane layer is made visible by the reflection of light from its disturbed architecture in pseudophakic bullous keratopathy.
(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Fig. 7.19 Abnormal amounts of light are seen reflected from the endothelial layer in Fuchs' dystrophy.
(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
As the cornea is scanned in optic section, the relationship of the anterior to the posterior surface is also observed for changes in normal thickness and curvature. Ectatic changes, such as keratoconus or keratoglobus, will become readily apparent (Fig. 7.20). Focal elevations or depressions also become obvious as the slit beam deviates toward or away from the light source (Fig. 7.21).

Fig. 7.20 Central thinning is obvious in this optic section of keratoconus.
(From Mártonyi CL et al. Silit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
The narrow slit beam is important to apply to all ocular surfaces. A thorough scan of the lid margins, the bulbar and palpebral conjunctiva, the plica, caruncle, and corneal limbus will provide confirmatory information or add to what was gleaned using the modalities described earlier. The narrow slit beam is most effective in detecting topographic changes in these structures (Fig. 7.22). Follicles, papillae, or other dimensional alterations are well stated in this manner. All tissue will demonstrate some penetration by the beam. The degree of penetrativeness is variably limited by the optical density of the tissue under study. Although actual penetration may be minimal, the information produced can be valuable. Of equal or greater importance is the indirect, proximal illumination simultaneously achieved (see section entitled Indirect Illumination).

The relationship between the cornea and iris is also evaluated with a narrow slit beam. By projecting the light from a moderate angle and observing the distance between the reflections from the cornea and the iris, a good estimate of anterior chamber depth can be obtained. Observing this relationship at the limbus provides information regarding the grade of the angle.[1] A completely closed segment of the angle is indicated by a contiguous presentation of corneal and iris reflections (Fig. 7.23). In a similar fashion, anterior synechiae may present as focal areas of contact between the reflected beams at the posterior corneal surface. This condition is confirmed by observing the slit beam coursing up the side of the tented iris tissue to make contact with the reflection from the posterior corneal surface (Fig. 7.24).
Fig. 7.23  (A) A very shallow anterior chamber is evidenced by the proximity of reflections from the iris and the corneal endothelium. Superiorly, contiguous reflections indicate an area of closed angle. (A, From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007. B, © CL Mártonyi, WK Kellogg Eye Center, Univ.)

Fig. 7.24  Iris tissue adherent to the posterior corneal surface in an eye that, following successful penetrating corneal transplantation, suffered a penetrating foreign body injury. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
Optic section: photography

Easily applied at the clinical slit lamp, the optic section remains a challenge to reproduce photographically. The primary problem is insufficient light. Most PSLs do not generate sufficient flash power to adequately expose a thin slit beam in relatively clear cornea. As a result, the most refined sectioning capabilities of the slit beam may have to be sacrificed in the interest of exposure. A wider than optimum beam may have to be used as the standard; however, the beam should not be widened to the point at which the optic sectioning capability is lost. Rather than settling for a beam too wide, one should consider increasing the ISO setting. Once the best compromise between beam width and exposure is achieved, the instrument should be calibrated to reproduce that same width whenever the situation calls for an optic section. The use of maximum flash power is assumed.

Photographs of the optic section can convey precise information regarding the condition of the cornea and other structures (see Figs 7.13 through 7.24).

Combined direct focal and diffused illumination: photography

This type of illumination produces one of the most informative single images of the eye. It combines the narrow slit beam discussed above with diffuse illumination from the fill light. The fill light is responsible for mean exposure and, therefore, the slit beam is used at an intensity that technically constitutes an overexposure. This relationship is necessary to demonstrate adequate background information and a sufficiently brilliant slit beam to highlight information within the section. Numerically, the slit beam is approximately two f-stops (four times) brighter than the fill light. This combination is excellent for portraying conditions of the cornea and also applicable to numerous other situations (see Figs 7.20 through 7.24).

Tyndall’s light/anterior chamber cells and flare: examination

Based on Tyndall’s phenomenon, pinpoint illumination is maximally effective at isolating aqueous cells and flare. The anterior chamber is considered optically empty, as its contents do not reflect sufficient light to express the beam in the normal state. The cells and protein that present in response to local inflammation, therefore, can be seen readily when isolated within the well-defined, narrow tunnel of light produced by pinpoint illumination.

A small, round beam of high intensity is directed tangentially through the anterior chamber, and the focal point of the light (and the biomicroscope) is swept through the aqueous to determine the presence and density of cells and flare. For maximum contrast, when conditions permit, cells and flare should be observed against the dark background of a dilated pupil, while minimizing the light striking the iris.

Although the ‘pinpoint’ or ‘pencil of light’ configuration represents the most discriminating technique,[2] the standard grading system used to describe the concentration of cells and flare assumes the use of a beam approximately 1 × 3 mm in size. The number of actual cells seen simultaneously within that beam is the stated degree of the condition. The amount of abnormal protein is determined by the examiner’s impression of the reflectivity (or Tyndall effect) of the aqueous. The degree of expression of these two conditions is stated in terms of one to four-plus cells and/or flare (Fig. 7.25).

![Image of a dramatic expression of four-plus aqueous cells in a case of endophthalmitis.](From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Tyndall’s light/anterior chamber cells and flare: photography

Documentation of this condition is clearly the most challenging task in slit lamp photography. Although cells and flare are easily visualized, their low reflectivity makes it difficult to obtain an adequate exposure. Even ‘four-plus’ expressions will be marginally exposed at a sensitivity below 3200 ISO.[2]

The beam should be configured into a small spot or ‘pencil of light’ to produce maximum isolation.[3] With a fairly tangential presentation of the beam at moderate magnification, useful images can be achieved. For maximum contrast and best results, the focal point of the beam should be placed over the dark, unilluminated pupil (Fig. 7.26).
Specular reflection: examination

The bright, mirrored reflections of light sources are considered regular, or specular, reflections, as opposed to the more common, irregular reflections whereby most objects are seen. Specular reflections are subject to Snell's law of optics, which states that the angle of reflection equals the angle of incidence. That suggests a level of difficulty associated with the location of such a reflection that is simply not present when examining the eye. On the curved ocular surfaces, specular reflections are easily elicited. The convex cornea and the high reflectivity of its anterior-most layer, the tear film, combine to make such reflections more or less ever-present companions during the course of an examination. While at times annoying, these reflections are, in fact, of great value for gathering information about the condition of the eye.

As the eye is viewed in direct illumination, the zone of specular reflection is studied as an expression of surface integrity. This evaluation should be performed under a fairly low light level, so as to diminish scatter and make visible detail within the zone of specular reflection. The area of specular reflection is not only a mirror image of the source but also faithfully mirrors the topographic condition of the surface on which it rests. Therefore, a compromised corneal surface will produce an abnormal reflection of the light source. Broken or granular reflections may indicate an inadequate tear film, the presence of foreign material, or a compromise to underlying tissue expressed as an alteration from normal topography. A greatly diminished or irregular reflection is always a clear sign of abnormality (Fig. 7.27).
The most important application of the specular reflection is in the evaluation of the corneal endothelium. While not expressly difficult, this technique may prove initially challenging. The reflectivity of the endothelial surface is so much lower than that of the tear film layer that the specular reflection may not be appreciated even when present. An angular difference of 30 to 40 degrees between the slit illuminator and the biomicroscope will make the task easier by producing greater separation between the two reflections. Moving the incident beam laterally across the face of the cornea will elicit the bright specular reflection from the tear film layer. By observing the adjacent area on the side away from the light source, the more demure endothelial reflection is seen. Moving the biomicroscope forward approximately 0.5 mm will bring into focus the endothelial layer and cellular detail should become apparent. To obtain a clear view of endothelial cells, especially those that populate the uncompromised, young cornea, a magnification of 25× to 40× is required (Fig. 7.28). When such levels of magnification are not available, the endothelium can still be evaluated with this technique. The reflection is observed for continuity and uniform intensity as the light is played across the endothelium. When conditions such as guttae are present, the homogeneity of the reflection is interrupted (Fig. 7.29).
Fig. 7.28 (A) The bright specular reflection from the tear film layer is easily seen. The reflection from the endothelium is found just adjacent on the side opposite the light source. (B) Endothelium is appreciable.

(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
In severe expressions of disease-related endothelial compromise, such as advanced Fuchs' dystrophy, the reflection may become totally altered from the normal. Coalesced guttae may cause it to appear patchy or quite dark overall and, even when viewed at high magnification, information about individual cell borders may not be present (Fig. 7.30). In such conditions, even specular micrography may fail to produce satisfactory information regarding cell morphology or density.

The specular reflection can also be used to examine the surface of the conjunctiva and the anterior and posterior surfaces of the lens.

**Specular reflection: photography**

Although the reflection from the endothelium is much less intense when compared to the reflection from the epithelium (or more accurately, the tear film layer), there is more than adequate light with which to obtain a good exposure. Photographs should be taken at high magnifications (25× to 40×) to show cellular detail. Critical focus is essential (see Fig. 7.28).
Indirect Illumination

**Proximal illumination: examination**

In proximal illumination, the light is directed to strike an area just adjacent to the area to be examined. The principal subject, therefore, is illuminated by light transmitted through tissue. The effect is one of retroillumination from deeper layers. It is remarkably effective for observing subsurface changes in tissue of sufficient opacity to prevent light penetration to the desired level with direct illumination (Fig. 7.31). Similarly, proximal illumination can facilitate location and determination of size and shape of an imbedded foreign body or one obscured by soft tissue reaction. It also can be helpful in gathering additional information about abnormalities that are apparent in direct focal illumination. By observing conjunctival or skin alterations with this modality, the specular reflections produced by direct illumination are eliminated, and another, valuable perspective is obtained in what becomes a form of retroillumination (Fig. 7.32).

*Fig. 7.31 (A) and (B) Direct, broad-beam illumination of a Cardona keratoprosthesis is ineffective in demonstrating the flange of the device by light reflected by the sclerotic corneal tissue. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd, 2007.)*
The benefits of proximal illumination are probably exploited more frequently than may be realized. A scan of the conjunctiva in direct focal illumination (e.g., with a narrow slit beam) includes making use of the information seen in the adjacent area of proximal illumination. Proximal illumination may not be the conscious goal of the examiner, but information from this zone is nonetheless gleaned. In fact, without it, the examination would be incomplete. When high magnification is used in observations by proximal illumination, the subject area may become sufficiently decentered to make viewing cumbersome. In such cases, the slit illuminator must be decentered from its normal isocentric position to permit centration of the principal subject area within the field of view.

**Proximal illumination: photography**

All indirect forms of illumination require decenteration of the slit beam for the maintenance of a centered principal subject area. Although it may be unnecessary for most of a slit lamp examination, decenteration of the incident beam is essential to the success of each slit lamp photograph. Proximal illumination poses quite a challenge in achieving good exposure. The amount of light by which such changes are visible represents a small percentage of the light used to directly illuminate the adjacent area. For this reason, the exposure is frequently underestimated. The area of direct illumination must be dramatically overexposed as compared with the area of indirect illumination. This is an unavoidable by-product of this technique, which the photographic process exaggerates beyond the visual impression. In some cases, the distraction factor of this zone of overexposure may be tempered by increasing magnification to the exclusion of the directly illuminated area. The important goal, however, is to use sufficient incident light to adequately expose the principal subject area. Although somewhat variable because of differences in tissue reflectivity and absorption, an increase of approximately three f-stops of light is required (see Figs 7.31 and 7.32). [2]

**Sclerotic scatter: examination**

Specifically applicable to the cornea, sclerotic scatter permits the illumination of the entire cornea against a largely unilluminated background. An intense beam of moderate size is directed at the corneoscleral junction. The light travels the breadth and width of the cornea by total internal reflection. In the normal cornea, this light passes through the stroma undisturbed and is visible only as a ring of light at the limbus, where it intersects, and is reflected by the sclera. The brightest portion of this ring of light is located directly opposite the source. The normal cornea itself will appear unilluminated. Because of the extreme degree to which the cornea must be decentered to accommodate illumination of the limbus, the slit illuminator must be disengaged from its normal, isocentric relationship with the biomicroscope to allow centration of the cornea within the field of view (Fig. 7.33). In the abnormal cornea, the light that is axially reflected or refracted makes the abnormality visible. The degree to which this light is visible depends on the optical density and other characteristics of the abnormality and the size and intensity of the incident light beam (Fig. 7.34).
Fig. 7.33  A. The beam is decentered to facilitate centration of the cornea in the biomicroscope. (B) The light at the corneoscleral junction illuminates the cornea by total internal reflection and remains dark against an essentially dark background.

(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
Sclerotic scatter is quite remarkable for its sensitivity to subtle change while yielding information over a large area of distribution (Box 7.4). That combination is not possible with most forms of illumination. The ever-present compromise of ‘area versus detail’ limits each application. Generally, the larger the area of simultaneous illumination, the more light that will be scattered, producing a corresponding loss of fine detail. However, in sclerotic scatter, as the cornea is illuminated by a source that is comparatively small (the size of the beam directed at the limbus), this technique is not strictly subject to this limitation. In reality, sclerotic scatter provides a simultaneous view of a large expanse of cornea, making it useful in identifying certain disease entities through recognition of a characteristic, overall pattern. In certain instances, the incidental light that falls on the iris, especially one of light pigmentation, may become a significant negative factor. Thus, a condition of subtle expression (e.g. cornea verticillata in Fabry’s disease) can be best appreciated against the dark background of a dilated pupil (Fig. 7.35).

**Box 7.4**

**Examples of conditions seen in sclerotic scatter**

- Corneal foreign bodies
- Corneal edema
- Keratic precipitates
- Verticillata
- Interstitial keratitis
- Granular dystrophy
- Radial keratotomy scars
- Hydrops
Sclerotic scatter: photography

Sclerotic scatter can produce information over a wide expanse of cornea in a single photograph. It is best used to delineate alterations that are of low optical density. This technique requires a complete decentration of the slit beam to permit centration of the cornea in the final image (see Fig. 7.33). It also requires the maximum light output from the power supply. Obvious conditions, such as the bright foreign bodies seen in Figure 7.34, are easily exposed. More subtle entities, such as the verticillate pattern in Fabry’s disease may require a setting of 400–800 ISO. Dilating the pupil ensures a sufficiently dark background to provide the contrast necessary for good visualization (see Fig. 7.35).

Direct and indirect retroillumination from the iris: examination

Representing two distinct forms from the standpoint of how they function, direct and indirect retroillumination from the iris are most informative when used together. This combined technique is the most important to the thorough examination of the cornea. It actually produces three types of illumination with corresponding zones of information. With the beam applied tangentially, the area of the cornea observed against the directly illuminated iris (direct retroillumination from the iris) demonstrates alterations that are chiefly opaque. The zone of cornea that falls on either side of the illuminated background, i.e. cornea that lies in front of unilluminated iris or pupil (the zone of indirect retroillumination from the iris), demonstrates primarily refractile changes and changes of low optical density. Of greatest importance is the interface between light and dark backgrounds, where the most subtle changes may be seen. Abnormalities that both refract and reflect light become most dimensional in this zone between light and dark backgrounds.

Technically, this juncture is an interface rather than a true zone. Because the biomicroscope (and therefore the slit beam) is focused at the level of the cornea, however, the interface is formed by divergent rays, resulting in an unsharp image at the level of the iris. Because this unsharp image of an unsharp interface appears to occupy space by virtue of its broader appearance, it becomes a zone in the practical sense (Fig. 7.36). The entire cornea can thus be examined for both subtle and obvious alterations. The beam of light is applied tangentially and moved across the cornea while observing the three zones simultaneously, with particular attention paid to the interface of light and dark backgrounds.[2] To ensure that all information about the cornea has been gathered with this modality, the scan should be repeated, with the light applied from both the temporal and nasal sides.
The combination of direct and indirect retroillumination from the iris produces remarkable detail of subtle corneal findings, as seen in this example of lattice dystrophy. The zone of interface between light and dark backgrounds is the most informative.

(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Many entities are easily visualized and identified in this manner (Box 7.5). Lattice dystrophy, with its characteristic signature, is seen in all three zones of illumination (see Fig. 7.36). Folds in Descemet's membrane are best seen in indirect retroillumination from the iris, but close to the interface of light and dark backgrounds (Fig. 7.37). The classic, bubble-like microcysts characterizing Meesmann's dystrophy are most dimensionally described within the interface zone (Fig. 7.38).

**Box 7.5**

Examples of conditions seen in direct and indirect retroillumination from the iris

- Lattice dystrophy
- Corneal foreign bodies
- Meesmann's dystrophy
- Map-dot-fingerprint
- Cornea farinata
- Descemet's folds
- Keratic precipitates
- Thygeson's superficial punctate keratitis
- Corneal infiltrates
- Early edema
- Filaments
- Microcysts
- Fuchs' dystrophy
- Corneal scars
Fig. 7.37 (A) and (B) Wrinkles in Descemet's membrane are primarily refractile and are best seen against a dark background directly adjacent to the illuminated background. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
Fig. 7.38 Meesmann’s dystrophy is effectively demonstrated in indirect retroillumination from the iris. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

**Direct retroillumination from the iris: photography**

A beam of moderate width is projected onto the iris to create a light background against which opaque changes are visible. When the condition presents in an eye with a light iris, the exposure appropriate to the documentation of that iris in direct illumination is sufficient. When the condition presents against a dark iris, an increase in exposure is required. The beam should be wide enough to create an adequate background, without directly illuminating the corneal condition (Fig. 7.39).
Indirect retroillumination from the iris: photography

The photography involved in this technique is somewhat more challenging because the light available to illuminate the abnormal condition is but a small portion of the light striking the iris (Fig. 7.40). With increased pigmentation of the iris, light loss also increases, requiring a greater adjustment in exposure. Many changes are visible in this form of illumination. Alterations that are primarily refractile are especially well described in this manner. Such changes are the most striking at the interface of light and dark backgrounds, demanding careful attention to exposure (see Figs 7.36 through 7.38). As a general rule, the exposure used for direct illumination of the iris should be increased by one f-stop to provide good exposure of refractile changes at the interface generated by a light iris, and up to three f-stops when the condition is being photographed at the interface created by the surface of a darkly pigmented iris.
**Retroillumination from the fundus: examination**

Using the light reflected by the retinal pigment epithelium, the anterior vitreous, lens, and cornea may be examined in retroillumination. The slit lamp illuminator is placed into an axial position with the biomicroscope and the light is introduced through a dilated pupil to illuminate the fundus. With modest excursions of the illuminator to either side of center, the optimum position is established when the greatest retroillumination effect is achieved. A large pupil is required for maximum effectiveness. After configuring a moderately sized beam, the entire instrument can be moved from side to side to facilitate examination of most of the cornea. Decentering the slit to one side of the available pupil and then to the other facilitates a serial, uncompromised view of both sides of the subject stratum, and the area under study remains centered in the biomicroscope. Leaving the iris unilluminated ensures maximum visibility of the abnormality. Light striking the iris causes scatter and reduces the effectiveness of this valuable form of illumination.

One major advantage of this modality is that it produces excellent delineation of subtle changes over a wide area of distribution. In that regard, it is similar to sclerotic scatter. The principal difference between the two is that sclerotic scatter produces darkfield illumination (objects illuminated against a dark background), whereas retroillumination from the fundus is a brightfield technique (objects silhouetted against a bright background). Darkfield excels at demonstrating changes that primarily reflect light, and brightfield produces best contrast for opaque changes and those that are refractile (Box 7.6). Much of the cornea or lens may be visualized simultaneously, limited only by the size of the pupil and shallow depth of field. The classic findings in fingerprint dystrophy are beautifully displayed in this form of illumination (Fig. 7.41). Similarly, many lens changes are most easily identified in this manner. Cataract formation and subluxation of the lens are dramatically demonstrated (Fig. 7.42). The essentially colorless lens and cornea are transformed into structures that are seen in additional contrast by virtue of the color reflected by the retinal pigment epithelium.

**Box 7.6**

**Examples of abnormalities seen in retroillumination from the fundus**

- Lattice dystrophy
- Pseudoxfoliation
- Keratic precipitates
- Corneal scars
- Meesmann’s dystrophy
- Map-dot-fingerprint dystrophy
- Lens vacuoles
- Cataract
- Corneal rejection lines
Fig. 7.41 (A) and (B) Epithelial fingerprint dystrophy is best visualized in retroillumination from the fundus.
(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)

Fig. 7.42 A traumatically subluxated lens is seen with blood on its posterior surface, well demonstrated in retroillumination from the fundus.
(From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
**Retroillumination from the fundus: photography**

Exposure is generally not a problem. With a well-dilated pupil and clear media, excellent images of corneal or lenticular changes can be captured.

The slit beam is configured into a short rectangle or, when possible, into a half-moon shape to permit maximum illumination of the background (the retinal pigment epithelium) without allowing the light to strike the iris. The light beam is then displaced to the side of the pupil that causes the least compromise to the information to be recorded. A considerable decentration of the beam is necessary to maintain centration of the pupillary area (see Fig. 7.42). When necessitated by the presentation of the condition, the beam is moved to the other side of the pupil for additional photographs to provide complete documentation.

In the absence of optimum conditions, coupled with a heavily pigmented retinal pigment epithelium, exposure may need to be increased considerably. When maximum retroillumination is required, the eye is rotated to cause the incident beam to strike the optic nerve head, producing more intense retroillumination from that highly reflective surface.

**Transillumination of the iris: examination**

Iris transillumination is a simple extension of the technique just described (Fig. 7.43). An important difference is the optimum pupil size. A completely dilated pupil is counterproductive to iris transillumination; a pupil size of 2–3 mm is ideal. Through such an opening, a moderate beam of high intensity can be introduced to illuminate the fundus. In that presentation, the iris is still sufficiently attenuated to demonstrate even subtle expressions of transillumination (Fig. 7.44).

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Fig. 7.43  Cataract formation and iris atrophy, resulting from a contusion injury, are simultaneously observed in retroillumination from the fundus and transillumination of the iris. (From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
Transillumination of the iris: photography

Figure 7.43 required midrange intensity of illumination, whereas Figure 7.44 required the maximum light output of the PSL.

The peripheral cornea (gonioscopy): examination

The peripheral cornea cannot be examined without the use of a gonioscope. Indirect lenses provide the ideal view with a choice of mirror angles and reduced light scatter. Once the lens is placed, the optic section can provide information regarding the posterior surface of the cornea and the condition of the angle. A wider beam, which is conformed to the area under study, is useful for the simultaneous view of a larger area to assess structural relationships further. Confining the beam to the zone of immediate attention minimizes distracting reflections that inevitably reduce contrast and detail (Fig. 7.45). The anterior chamber angle is extremely reflective in most eyes, and moderate amounts of light are suggested for its examination.
The peripheral cornea: photography

Photography of the peripheral cornea requires the same basic techniques as photography of the filtration angle. Both areas are imaged simultaneously, and the delineation of the principal subject area is largely a matter of focus. The best access to this area is provided with the most oblique mirror in the Goldmann three-mirror lens, set at an angle of 59 degrees. In this application, the more oblique view provides better access to this zone of the anterior chamber. For some conditions, the 67-degree mirror produces a better overview, providing an enhanced perspective (Fig. 7.46). Because of the highly reflective nature of this area, the danger of overexposure is much greater than underexposure. Because findings in this zone are frequently subtle, it is doubly important to avoid overexposing the image. In some instances, a moderate underexposure produces a more saturated image containing more detailed information (see Fig. 7.45). Previewing the image with only the eye that shares the image with the camera back will help in managing unwanted reflections from the flat surface of the contact lens.
Vital dyes: examination

Vital dyes provide information important to a complete ocular examination. Their application is essential to determine the condition of the corneal and conjunctival epithelium. Because these dyes can cause irritation (rose Bengal, in particular) and because their presence may interfere with the assessment of deeper layers of the cornea, they may be best used toward the end of the examination.

Fluorescein is a good indicator of contact lens fit. With the blue exciter filter in place and the light intensity sufficiently increased, such information is easily obtained (Fig. 7.47). Similarly, tear film break-up time can be ascertained (Fig. 7.48). Conditions that disturb the normal tear film also can be confirmed (or detected\textsuperscript{4}) with this same technique (Fig. 7.49).
To determine the presence of epithelial compromise, the dyes can be used individually or mixed and applied in combination. The irritation caused by rose Bengal may warrant the use of a topical anesthetic before instillation. The cornea and conjunctiva should be examined for signs of staining with both white and blue light (Fig. 7.50). Because devitalized epithelium stains with rose Bengal and areas that are de-epithelialized stain with both rose Bengal and fluorescein, the subtle areas of rose Bengal staining may be better appreciated when viewed in a matrix of fluorescein. Since rose Bengal absorbs much of the incident blue light, its consequently dark appearance contrasts well with a brightly fluorescing background (Fig. 7.51).
The patient should blink repeatedly to help differentiate pooling from staining. Areas of staining become apparent as they move with the eye. Pooled dye appears somewhat static by comparison. A definitive differentiation can be made only by rinsing the pooled dye. A particularly dry eye will certainly require rinsing before an accurate assessment can be made regarding actual staining.

The Seidel test: examination

The Seidel test is used to determine corneal or conjunctival patency. When the escape of aqueous is suspected, fluorescein dye is applied directly to the site of suspected leakage. When present, escaping aqueous dilutes the fluorescein as it flows down the surface of the eye. The rate of dilution is the indicator of the dynamic of the positive test. The application of concentrated fluorescein results in a dark, nonfluorescing background, against which the diluted and now brightly fluorescing dye is highly visible, even in the presence of a modest flow (Fig. 7.52).
Vital dyes: photography

Vital dyes are best photographed in direct, broad-beam illumination. Of great importance is the removal of excess dye before photodocumentation. Thus, only actual staining is recorded. This is important to the accurate recording of both rose Bengal and fluorescein staining.

Rose Bengal staining is easily photographed, requiring only minor adjustments from the normal exposure used for broad-beam illumination. Since rose Bengal delineates subtle areas of epithelial compromise, care must be taken to preserve the subtle nature of the information. The light beam is applied tangentially to avoid obscuring information with overlying specular reflections. In most cases, a slight underexposure will better express subtle focal staining (Fig. 7.53).

Fluorescein is best viewed and photographed under blue light (approximately 480 nm) used to excite the dye to fluorescence. Because the blue filter diminishes overall light intensity, adjustment in exposure is required. About two f-stops of additional light are needed, depending on the actual filter used. For most applications, only an excitation filter is required (see Figs 7.47–7.49). For photographs that demonstrate extremely subtle staining, a barrier filter (approximately 520 nm) is added to provide adequate discrimination. Additional increase in exposure may be necessary.

Combining rose Bengal and fluorescein can add a further dimension to such coverage, as discussed above under ‘Examination’ (see Fig. 7.51).

The Seidel test: photography

To produce photographs of this technique, the blue filter is placed over the light source, which is set to produce full, broad-beam illumination, and the exposure is set for routine fluorescein photographs. The fluorescein is applied directly to the suspected site of leakage and the image captured as the diluted fluorescein cascades down the surface of the eye (Fig. 7.54).
Techniques specific to keratoconus: photography

Documenting the Fleischer ring

The Fleischer ring may be documented by using the blue filter described earlier for exciting fluorescein. In this case, the blue light is absorbed by the iron line, delineating the conus and causing it to appear dark in the resultant photograph. It is only effective, however, when the iron line is seen against a light iris (Fig. 7.55).

Munson’s sign

Munson’s sign is a simple and graphic means of demonstrating the abnormal corneal outline. By asking the patient to look down, the lid margin conforms to the cornea’s horizontal profile, boldly revealing the condition (Fig. 7.56).
A vertical profile can be documented by turning the patient’s head in the chin rest assembly sufficiently to obtain a temporal view. By directing a moderate beam of light to strike the nasal bridge behind the cornea, a light background is produced, against which the condition is presented in a dramatic and pleasing manner (Fig. 7.57).

![Fig. 7.57 A corneal profile of mild keratoconus seen against the illuminated nasal bridge.](From Mártonyi CL et al. Slit lamp: examination and photography. Sedona: Time One Ink, Ltd; 2007.)
The examination of the eyes is begun after establishing the history of the case. In making this examination, too much stress cannot be laid upon the necessity of proceeding systematically, since otherwise important matters can very readily be overlooked. We first examine the patient with regard to his general physical condition as well as with regard to the expression of his countenance, and then, in observing the eyes themselves, proceed gradually from the superficial parts - lids, conjunctiva, and cornea - to the deeper portions.**

The ideal examination includes a careful, highly dynamic analysis of all structures, using each applicable form of illumination. The result should be a fully detailed, three-dimensional mental image of the segments of the eye. Although many abnormalities are easily identified, some of subtle expressivity cannot be ruled out without having exercised fully the capabilities of the slit lamp. In the absence of clear clinical signs, with only vague symptomatology reported, the examiner must exhaust all possibilities. After the abnormality is identified, additional information about its severity, extent, or particular characteristics can be gleaned through observation under all forms of illumination.

The importance of a dynamic approach cannot be sufficiently stressed. Observing the eye in static light deprives the examiner of much of the available information. The process of examining the cornea in direct and indirect retroillumination from the iris, for instance, requires a scan of the cornea from one limbus to the other. This motion itself will reveal information that may otherwise go unnoticed. It enhances the dimensional qualities of the information observed and results in a more accurate and complete impression of the extent and severity of the abnormality present. Similarly, observing the motion of the eye and the eyelids can provide important clues to normal or abnormal function.[7]

Establishing a routine protocol will minimize the time required to complete an examination and provides a fail-safe measure to ensure its completeness. As the examiner gathers experience, an individualized routine emerges. Type of practice and attendant patient population may be influencing factors in establishing a protocol. When circumstances permit, steps of an examination that may cause somewhat greater discomfort (e.g. eversion of the upper eyelid, application of vital dyes) may be best carried out toward the end of the routine to help ensure the patient's ability to cooperate throughout the examination.

Further reading

References


Chapter 8 – Tear Film Evaluation

Michael A. Lemp

The tear film is a critical component in maintaining the health of the ocular surface and as a pathway for repair. Oxygen captured from the atmosphere during the day and from the capillaries of the conjunctiva lining the upper lid during sleep supplies the cornea and conjunctiva, supporting the cellular turnover and maturation necessary to maintain a clear cornea for vision.[1] The tear film, moreover, provides an exit pathway for cellular debris, metabolic waste products, microbes, and other particulate matter in the tear film via drainage through the nasolacrimal ducts.[2] In addition, the ocular surface and the tear-producing structures (lacrimal glands, meibomian glands of the eyelid, and mucin-producing cells of the conjunctiva and the mucosal lining of the nasolacrimal ducts) are linked by a neural pathway forming an integrated functional unit that regulates epithelial cell turnover in health and epithelial repair processes in response to trauma or pathophysiological processes.[3],[4] The tear film contains water, electrolytes, proteins (which form cytokines directing epithelial cell activities), mucins, sugars, and other water-soluble substances. Overlying this aqueous phase produced primarily by the main and accessory lacrimal glands (and to a lesser extent by the conjunctiva) is a thin lipid layer. The lipid is produced by the meibomian glands of the eyelids and serves to stabilize the tear film, retard evaporative tear loss, and prevent contamination of the ocular surface with skin lipids[5],[6] The ocular surface is covered by a mucin layer consisting of two parts: a thin membrane-associated mucin produced by the epithelial cells and a thicker mucin blanket, the product of the goblet cells of the conjunctiva.[7] Mucin serves to render the epithelial cells wettable by aqueous tears and interacts with the overlying lipid layer to stabilize the tear film.

In dry eye disease there are qualitative and quantitative alterations in the volume, composition, and structure of the tear film. In this chapter we will consider examination techniques and clinical tests designed to aid in the diagnosis of dry eye disease.

General Inspection

Gross examination of the ocular adnexa can reveal significant structural changes important in the pathogenesis of dry eye disease. Alterations in the eyelid structure and function can be observed with bright natural or artificial light. The eyelids should approximate the ocular surface, and the upper lid travel over two-thirds of the cornea with each blink. Interpalpebral fissure widths vary greatly but an excessively wide interpalpebral fissure, e.g. in thyroid eye
disease, is associated with increased evaporative tear loss. Trichiasis, ectropion, or entropion can interfere with normal tear film dynamics, and incomplete closure of the lids can lead to localized areas of drying on the ocular surface. Bell’s phenomenon in which the cornea rotates upward on lid closure ensures protection for the corneal surface. About 5% of the normal population will have an absent or deficient Bell’s reflex. This can be estimated by asking the patient to close the eye while holding the lid and observing the cornea. A deficient Bell’s reflex can lead to exposure keratopathy.
Examination of the inferior marginal tear strip can yield information about the volume of tears present on the ocular surface. The tear strip is a line of tears just above the lower lid (Fig. 8.1). It is normally about 0.5 mm in width and has a concave upper aspect. When this strip is thin or discontinuous, it is evidence of deficient aqueous tear volume. The tear strip is better visualized by fluorescein staining but care must be taken not to flood the surface by overwetting the fluorescein strip; it should be just barely moistened. While thinning of the marginal tear strip is a relatively late sign of aqueous tear deficiency (ATD), attention to this area can yield valuable information.
Another feature frequently seen in dry eye is increased debris in the tear film. Bits of mucus, fragments of sheets of sloughed epithelial cells, and other foreign material trapped in the tear film are suggestive of delayed tear clearance seen in dry eye.\[9\] Examination of the ocular surface with the slit lamp can also reveal alterations in the morphology of the conjunctiva such as redundant folds in the bulbar conjunctival epithelium (conjunctivochalasis). This finding has been reported to be characteristic of dry eye.\[10\]

There are a variety of objective tests of tear film characteristics and function. Although most of these have some clinical utility, many have been relegated to a research setting or have not gained wide clinical acceptance. This chapter will confine itself to objective tests which are either in wide clinical use or are of such importance that they may become essential elements of routine clinical examination.
Tear Stability

In dry eye disease the tear film is unstable, resulting in an abnormally rapid break-up of the precorneal tear film between blinks.[11],[12] After tears are surfaced by the action of the lids, a meta-stable tear film is established. Over time (usually 10–30 seconds) the tear film thins, leading to the development of randomly distributed dry spots in the precorneal tear film (Fig. 8.2). The interval between the last complete blink and the appearance of the first random dry spot is the break-up time (BUT). This is generally measured after a small amount of fluorescein has been instilled or a slightly moistened fluorescein strip has been applied to the superior aspect of the bulbar conjunctiva. A wide slit lamp beam with the cobalt blue filter is used to scan the cornea; the patient is instructed to blink several times and then not blink. A hand-held timer is used to measure the seconds until the appearance of the first randomly distributed dry spot in the fluorescein-stained precorneal tear film. This is repeated several times and averaged. Values of less than 10 seconds are considered abnormal.[13]
Fig. 8.2 Fluorescein-stained tear film break-up.

There are nonfluorescein (noninvasive) measurements of BUT that employ reflective devices with a grid projected onto the corneal surface.\(^{[14]}\) These values are slightly higher and require equipment not widely available.

BUT is a measure of the stability of the tear film; abnormally low values are seen in aqueous tear deficient and in evaporative dry eye. Abnormal BUT values are reflective of a tear film abnormality but do not specify the type of dry eye. The tear film will break up rapidly over an underlying epithelial irregularity such as superficial punctate keratopathy. The presence of corneal staining will result in a rapid BUT that is not necessarily evidence of an intrinsic tear abnormality but rather the epitheliopathy. BUT has been criticized as being quite variable in an individual.\(^{[15]}\) This inter-test variability is probably due to the method with which the test is performed, variations in blink patterns, and the dynamics of tear production and flow. Consistent results below 10 seconds are, however, pathognomonic of a pathologically unstable tear film. More recently, a newer method of measuring and recording tear film break-up has been developed and is being used in clinical drug trials.\(^{[16]}\) In this method a quantified amount of sodium fluorescein solution 1% is instilled into the conjunctival sac, blinking occurs and the appearance of the first randomly occurring corneal dry spot is video-recorded with a timer recording the time in 0.1 second increments. Three measurements are recorded and the timing measured by three independent observers. The authors have reported a new reference value for this technique. Values below 7 seconds are considered abnormal and reflective of the presence of dry eye disease.\(^{[16]}\) The same authors have combined their BUT measurements with an assessment of the blink rate, which is calculated by dividing 60 by the number of observed blinks per second. The ratio of the tear film BUT over the interblink interval (IBI) is referred to as the Ocular Protection Index (OPI): \(\text{OPI} = \frac{\text{BUT}}{\text{IBI}}\). Values below 1 are characteristic of tear film instability and dry eye disease.

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Tear Production

The most widely used test to measure aqueous tear production is the Schirmer's test. In this test a standardized size strip of filter paper is inserted over the lower lid margin into the cul-de-sac, usually in the temporal one-third of the lid (Fig. 8.3). The patient is instructed to close the eyes and the strip is removed at 5 minutes; the extent of wetting of the strip is measured. Values below 5.5 mm of wetting are diagnostic of aqueous tear deficiency.\[17\] This test is performed both with and without the use of topical anesthesia. The so-called Schirmer's II (with anesthesia) has been purported to measure 'basal' tear secretion, i.e. nonstimulated tears.\[18\] It has been demonstrated that, even with anesthesia of the cornea and conjunctiva, tear secretion is driven by sensory stimuli, e.g. the lids, lashes, air currents, and light.\[19\] The whole concept of 'basal' or unstimulated tears has been called into question. A Schirmer's I (without anesthesia) has become the generally accepted method for assessing aqueous tear production.
This test has been criticized for its variability.\textsuperscript{[20]} Differences in performance of the test will greatly influence the sensory stimuli. The Schirmer's test, however, is a useful estimate of aqueous tear production because of its ease of performance, wide availability, and low cost. As aqueous tear-deficient dry eye disease progresses and the lacrimal glands lose their ability to respond to sensory stimuli or the sensory receptors on the ocular surface are compromised, results of the Schirmer's test become more consistent. Serially consistent Schirmer's I results below 5 mm of wetting at 5 minutes are highly suggestive of dry eye disease.

An alternative method of measuring aqueous tear production has been proposed – the phenol red test.\textsuperscript{[21]} This involves the use of a special cotton thread that has been impregnated with a dye – phenol red. The thread is inserted over the inferior lid margin into the temporal conjunctival sac. At the end of 15 seconds, the dye, which is pH sensitive, turns color from yellow to orange, indicating the length of the thread wetted by tears. This test has been reported to be less uncomfortable and more specific in the diagnosis of aqueous tear-deficient dry eye disease.\textsuperscript{[22]}
Tear Composition and Characteristics

Of the more than 200 proteins that have been identified in tears, several have been used as surrogate measures of aqueous tear production, i.e. lysozyme and lactoferrin. Lysozyme was first of interest because of its antibacterial activity. It has been demonstrated that tear lysozyme levels are decreased in aqueous tear-deficient dry eye disease.[23] Lysozyme is one of the principal protein components of tears. Its measurement is based on the enzyme’s ability to lyse a suspension of the bacterium, *Micrococcus lysodeikticus*. When this suspension is placed in an agar gel, a tear sample is collected by micropipette and placed in a well in the suspension containing gel. The plate is incubated and the area of lysis noted. The larger the area of lysis, the greater the concentration of the enzyme. This method is not used very often because of lack of availability of the plates, cost, and the lack of specificity of the results. Decreased tear lysozyme levels are also seen in a number of inflammatory conditions.[24]

Of more recent interest is the tear protein lactoferrin, which also possess antibacterial activity.[25] In addition, it has a protective effect on the corneal and conjunctival epithelium.[26] Previously, an assay was based on a commercial solid-phase ELISA methodology but more recent reports of a colorimetric analysis of microvolumes of tears have shown good diagnostic utility.

In this method (Touch MicroAssay), a micropipette is used to collect a small volume of tears, which is then transferred to a cell where the tears are exposed to a reactive reagent that is colorimetrically tagged; the resultant sample is read in a commercially available colorimeter.[27] Clinical experience has, however, shown tear lactoferrin levels to be scattered over a broad area. Decreased lactoferrin secretion in aqueous tear-deficient dry eye disease would be expected to be counterbalanced by the tear-concentrating effects seen in both aqueous deficient and evaporative dry eye disease, yielding variable results. Ways to compensate for the increased tear concentration characteristic of dry eye disease might improve the diagnostic value of this marker for aqueous tear-deficiency dry eye disease.

**Tear ferning**

It has been observed that tear samples dried on a slide and examined under a microscope display a crystalline pattern of tear mucin. In aqueous tear deficiency, this pattern resembles ferns. A grading system has been developed and this test has been reported to have greater
specificity and sensitivity than the Schirmer's test, particularly for more severe forms of dry eye disease.[28]

**Tear osmolarity**

It has been known that in dry eye disease the tear film is in a hyperosmolar state. This is true for both aqueous deficient and evaporative dry eye disease.[29] Tear film osmolarity has been measured using freezing point depression and vapor pressure measurement. Unfortunately, these methods have been limited primarily to a research setting, owing to their complexity, the high operator skill required and, most importantly, the need for relatively large volumes of tears, which necessitates stimulating tears for collection, and even this is insufficient in many dry eye subjects.[30] A large, recently published meta-analysis of the literature over the last 25 years identifies tear hyperosmolarity as the single diagnostic test with the highest accuracy in identifying patients with dry eye disease.[31] The advent of a new technology requiring tear samples of less than 50 nanoliters and measuring tear osmolarity easily and quickly in a clinical setting promises to provide a new practical diagnostic test suitable for clinical use. A recent report suggests very high sensitivity and specificity and positive predictive values for this TearLab technology, making this a new 'gold standard' in the diagnosis of dry eye disease.[32]
Meibomian Gland Structure and Excreta

The meibomian glands of the eyelid number between 20 and 25 in each lid. They secrete a lipid mixture which is discharged onto the tear film: excretion of the lipid is effected primarily through the muscular contraction associated with blinking. Meibomian gland lipid forms the top layer of the tear film and both stabilizes the tear film and retards evaporative tear loss.[33] Evaluation of the functional status of these glands involves slit lamp inspection of the lid margin and an estimate of the quantity and quality of the excreted lipid (meibum). Evidence of altered meibomian gland structure includes increased vascularity of the lid margin, plugging of the orifices, and loss of orifices.[34] Increased vascularity of the lid margins occurs with advancing age and is not, by itself, a reliable indicator of meibomian gland disease.

Meibum can be evaluated by pressing against the lower lid with a finger about 1 mm below the lid margin. In the normal subject, it will be possible to express some lipid from about two-thirds of the glands at a given time. This excretion is normally fluid and clear. Lack of expression from the glands, and/or alteration in the character of the excretion, is critical in the diagnosis of meibomian gland dysfunction (MGD). As the disease process advances, the excretion will vary from turbid to coagulated (toothpaste-like). Such meibum is pathognomonic of MGD. A grading scale for assessing severity of meibomian gland dysfunction has been developed for use in clinical trials but is equally suitable for clinical practice.[35]

Another method of assessing meibomian gland dysfunction involves transillumination of the eyelid. Using an examining muscle light placed inside the lower eyelid (after topical anesthesia), it is possible to visualize the outline of the glandular structure. This visualization can be enhanced and recorded with the use of infrared film.[36] The normal pattern is that of branching ductules coming off a central vertical core. Obliteration of this structure is evidence of chronic inflammation and glandular dysfunction.
Coincident with a decrease in aqueous tear production, there is a decrease in tear turnover, which is defined as the rate at which newly secreted tears reside within the tear film before they are lost either to evaporation or drainage through the lacrimal punctae and the nasolacrimal ducts. Tear volume and turnover are most accurately measured by dye dilution studies. In this methodology, a small amount of fluorescein dye is instilled into the tear film and the concentration of the dye is measured over time. Special fluorophotometers have been built to accurately measure dilution of the dye as new tears enter the tear film and old tears exit. Expense has limited the availability of this methodology. An alternative, inexpensive method of semiquantitatively grading fluorescein dilution has been proposed and is in use.[37] In this method (Fluorescein Clearance Test [FTC]), 5 \mu L of 1% fluorescein dye is instilled into the tear film. The patient is asked to blink to distribute the dye and serial 1-minute Schirmer's tests are performed every 10 minutes. Initially, the staining of the paper strip with the dye will be intense. Persistent staining (beyond 10 minutes) indicates delayed tear clearance (DTC).

A combined use of the Schirmer's II test with the FTC has been proposed.[38] This tear function index (TFI) is the ratio of the value of the Schirmer's test over the tear clearance rate. The use of the TFI in the diagnosis of dry eye disease is reported to demonstrate a specificity of 91% and a sensitivity of 79%.
Staining of the Ocular Surface

The normal ocular surface does not take up water-soluble dyes instilled into the tear film. With disruption of the mucin coating protecting the surface epithelial cells and/or damage to the epithelial cell walls, water-soluble dyes will diffuse into the surface cells. The three most commonly used dyes are fluorescein, rose Bengal (RB), and lissamine green (LG). Fluorescein, which stains damaged epithelial cells, is best visualized on the corneal surface. A 1% solution or a filter paper strip impregnated with fluorescein is used to introduce the dye into the tear film. The patient is instructed to blink to distribute the dye. The ocular surface is scanned using the broad beam of the slit lamp with the cobalt blue filter (or Wratten 47 blue filter). The extent and intensity of the stain are assessed. There are a number of grading scales, including the Van Bijsterveld, NEI/Industry Workshop, and Oxford systems.[39] The NEI/Industry Workshop grading system has the advantage of collecting data on five discrete subareas of the cornea separately, e.g. the central cornea.

Staining of the conjunctiva is seen when there are disruptions in the protective mucin coating; RB and LG are used. A 1% solution of either is instilled into the tear film, and the patient is asked to blink. The surface of the conjunctiva can be viewed within 10 seconds with RB but one should wait at least 2–3 minutes before viewing LG stain, and low light should be used. RB is more irritating to the patient and LG is gaining wider acceptance for this reason. Staining of the ocular surface is evidence of ocular surface damage and is characteristic of more severe dry eye.
Recently, attention has been directed to optical aberrations which have been identified in patients with dry eye disease. Although severe dry eye with significant staining of the central cornea has long been known to reduce visual acuity, recent studies have demonstrated that even in the absence of significant central corneal staining, the instability which is characteristic in all forms of dry eye disease results in rapid break-up of the tear film between blinks, compromising image quality. This effect on visual acuity is not captured during ordinary Snellen chart measurement of acuity because the patient can blink, momentarily improving vision. More rapid break-up occurs within 3 second of a blink in many dry eye patients, reducing their inter-blink acuity to levels of 20/60 or less.[40]

Recent work has developed two instruments to detect these changes. In one, the tear stability analysis system (TSAS), serial videokeratographic images are collected each second between blinks.[41] In another approach, a functional visual acuity (FVA) device has been developed which measures visual acuity by way of rapid presentation of optotypes. Both of these technologies promise to add to our armamentarium of diagnostic technologies in the near future.[42]
Conclusion

The various objective methods of examining the tear film can provide useful information, diagnosing, classifying, and grading the severity of dry eye.
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Chapter 9 – Refraction of the Abnormal Cornea

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Refractions of the abnormal cornea are as much art as they are science. The refractionist must often employ techniques not routinely used in the normal eye. If responsive, the patient can play a significant role in determining the final prescription, while patients with abnormal corneas who cannot provide subjective input, such as infants, present a greater challenge.

There are principles that, if understood, may enhance successful refraction of the abnormal cornea. The air–tear interface is the main refracting element of the eye. Tears assume the curvature of the underlying corneal tissue. There is a much greater change in the index of refraction between the air and tears than between tears and cornea, cornea and aqueous, aqueous and the crystalline or pseudophakic lens, lens and vitreous, or vitreous and retina. Therefore, pathology that causes even minor alterations in the surface curvature of the central cornea can result in significant degradation of vision.

Stromal opacities, on the other hand, that are not accompanied by irregularities of the corneal surface may be consistent with surprisingly good visual acuity. Even so, stromal opacities can reduce both visual acuity as well as contrast sensitivity, so that vision may be impaired both qualitatively and quantitatively.

Instrumentation

Four devices increase accuracy and reduce total time needed for refraction of the abnormal cornea. They are the retinoscope, the keratometer, computed topography, and the trial frame.

Retinoscopy

Retinoscopy is one of the most important tools in ophthalmology for determining refractive error and alterations in the corneal surface. A skillful retinoscopist understands what component the ocular media – cornea, anterior chamber, lens, and vitreous – play in vision. Opacities and irregularities in these structures degrade the image falling on the retina. Retinoscopy reveals critical and sometimes subtle anterior corneal abnormalities, axial corneal opacities, significant anterior chamber inflammation, opacities in and multiple refractive layers of the lens, as well as optically significant vitreous pathology. After years of experience, the skilled retinoscopist can predict best-corrected visual acuity assuming good neurologic ( retina, optic nerve, visual pathway) health.

Subtle corneal anterior membrane pathology degrades the quality of the red reflex. Corneal curvature irregularities such as in keratoconus and pellucid marginal degeneration are recognized by characteristic scissoring of the retinoscopic streak. Indeed, the retinoscope is the instrument that is most sensitive to the early changes found in the noninflammatory ectatic diseases of the cornea.

Two refractive abnormalities in which retinoscopy is particularly useful are high astigmatism and high myopia or hyperopia. In the case of high astigmatism, retinoscopy can both determine, with remarkable precision, the amount of as well as the axis of the cylinder. Occasionally, moving closer to the patient with
the retinoscope helps in this determination.

In high myopia, even in the presence of a relatively clear cornea, the red reflex is dull or dark. A high spherical error can be uncovered if the retinoscope sleeve is dropped; the red reflex will become brighter, and movement of the streak will be far more noticeable. Of course, in this circumstance the retinoscopist must remember to add corrective lenses in the opposite manner. For instance, with the sleeve dropped, minus lenses will be added if there is with motion rather than the usual with motion observed with plus lenses when the sleeve is in the up position.

There are times when the retinoscopic reflex is so poor, due to abnormal media, that retinoscopy can only be used as a diagnostic tool rather than a refracting device.

**Keratometry**

The Bausch & Lomb Keratometer™ is an extremely useful tool for quantification of corneal astigmatism and the diagnosis of curvature irregularities. It may help to predict the best-corrected visual acuity, assuming that the remainder of the eye is normal. Practitioners who examine a large number of patients with corneal abnormalities should consider including this instrument, in addition to a phoropter, in every examination room.

The keratometer can be used to evaluate the quality of the corneal surface as well as the dioptric curvature of the anterior cornea. It is important to remember that the keratometric mires that reflect from the cornea fall on a very small area (3.0–3.5 mm) and do not represent the curvature of the entire cornea. For example, on a cornea of 45 spherical diopters, the diameter of the mire is 3.2 mm. If a 3.0 mm diameter drill were used to produce a hole through a steel ball having a 45-diopter curvature, and readings were taken with an axis through the center of the hole, the mires would be perfect. The mire would fall on the unaffected steel ball just outside the hole because its diameter on a 45 diopter curvature would be 3.2 mm. In the case of the human cornea with pathology in the central visual axis, the result is surface irregularity radiating from that pathology. In such a case, keratometric mires would be irregular. Therefore, even though the keratometer K measures a small, defined area, regularity of the mires is significantly affected by adjacent central pathology.

Every time K-readings are taken, the quality of the mires should be assessed and described. A useful convention is to describe them as regular (Fig. 9.1), mildly irregular (Fig. 9.2), moderately irregular (Fig. 9.3), or markedly irregular (Fig. 9.4). Mires are regular if they overlap perfectly. A patient can have 5 diopters of corneal astigmatism with perfectly overlapping mires. This would be described as ‘regular’ astigmatism. If keratoconus is present and the patient has 5 diopters of astigmatism, the mires will not overlap perfectly and can be described as mildly, moderately, or severely irregular. Other factors may contribute to irregularity of keratometric mires, including dry eye, eye drops used as part of the eye examination, and post-tonometry surface irregularities.
Fig. 9.1 Regular keratometry mires.
Fig. 9.2 Mildly irregular keratometry mires.
Fig. 9.3 Moderately irregular keratometry mires.
The skilled retinoscopist and examiner, adept in the use of the keratometer, can often predict best-corrected visual acuity and can judge from the anterior corneal surface abnormalities the visual potential. Perfect mires are compatible with 20/20 or better visual acuity. Mild irregularity may be compatible only with 20/25 to 20/40 visual acuity, moderate irregularity with 20/40 to 20/60 visual acuity, and severe irregularity with 20/60 to 20/200 visual acuity. These parameters are, of course, arbitrary, but with experience, each examiner develops his or her own empirical classification. Even with the advanced technologies of the current systems of topographic analysis (see Ch. 160), the keratometer can provide subtle visual information that is missed by the automated analyzers.

In the majority of cases of keratoconus, the earliest finding is steepening of the cornea below the visual axis. This inferior corneal steepening is easily observed by taking K-readings with the patient looking straight ahead and then asking the patient to look up slowly. The vertical mires slowly spread apart as they get smaller, indicating inferior steepening. At the point of maximum separation, a new reading can be taken, and the amount of dioptric steepening can be recorded.

When corneal steepening measures greater than 52 diopters, a +1.25 diopter trial lens can be taped over the opening on the front of the keratometer, and new readings are taken. One then adds 9 diopters to the reading for the final result. For example, if the curvature is greater than 52 diopters and a +1.25 diopter lens is taped over the front, a new drum reading of 49 diopters would be extrapolated to 58 diopters.
When keratometer readings are less than 36 diopters, a −1.00 diopter lens can be taped to the keratometer and 6 diopters subtracted from the new reading. In this case, if the reading using a −1.00 diopter lens is 38 diopters, the reading would be extrapolated to 32 diopters. Standard nomograms for extension of the keratometer are available in tabular format from the manufacturer.

**Computer-assisted topographic analysis**

Of equal utility to the keratometer in facilitating the refraction of the abnormal cornea is computer-assisted topographic analysis. A standard topogram will often provide the refractionist with a clear cylinder axis, even when this is hard to determine with retinoscopy. Using the topogram, the refractionist can dial in both the axis of the cylinder as well as the approximate amount of astigmatism in diopters. As a general rule, the refractive cylinder is often roughly two-thirds of the topographically measured cylinder. Using this as a base, particularly in patients with high cylinder, one can often start the refraction with considerably less guesswork. Moreover, the topogram provides a reasonably accurate assessment of the degree of irregular astigmatism. This information can be gleaned by observing the color map or by using the numerical indices (e.g. surface regularity index) provided by some analyzers. While topographic analysis does not provide significantly greater refractive information than does a keratometer, the information is very easily accessible and is not subject to interpretation of the operator as is, to some extent, keratometry. Much in the same way in which topography aids in the determination of selective suture removal, it may be of use to the refractionist when the graft patient is ready for spectacle or contact lens prescription. Likewise, in the patient with an irregular cornea (e.g. keratoconus or scarring from trauma or infection), the topogram may explain why best-corrected acuity is not achieved even when retinoscopy provides distinct information about sphere and cylinder but acuity does not improve with spectacle refraction. (See Ch. 160 for a detailed analysis of contemporary computer-assisted topographic analysis systems.)

**Trial frame**

The patient with a high refractive error, significant corneal pathology, or inability to cooperate, is best refracted using a trial frame instead of a phoropter. A useful technique that may help define the axis of astigmatism preferred by the patient is to ask the patient to turn the dial on the trial frame that changes the axis (Fig. 9.5). The patient can accurately determine the axis by rotating the lens around the axis until the best vision is obtained. This technique is especially helpful in cases where the retinoscopic reflex is poor or when K-readings are moderately or severely irregular. It does, however, require a reasonably cooperative and observant patient. Moreover, vertex distance – especially important in high refractive errors – can be more accurately determined with the trial frame than with phoropter.
Fig. 9.5 Patient with high astigmatism helping with manifest refraction by turning cylinder screw of trial frame.

In patients with abnormal corneas, the manifest refraction found in the trial frame should always be used to test whether the expensive glasses to be purchased by the patient are likely to work. A 15-minute walk around the clinic while wearing the trial frames usually answers this question. If the power in either eye or using both eyes together is not acceptable, changes in the sphere or cylinder may result in a better tolerated prescription, even if the visual acuity is not quite as good. Some patients, especially those with keratoconus, will accept a surprisingly high amount of anisometropia.
How, then, are the retinoscope, keratometer, topography unit, and trial frame used together to refract the patient with an abnormal cornea? Although there is more than one strategy for use in the abnormal cornea, the approach here employs the procedural paradigm seen in Figure 9.6. Other strategies reflect the fact that there are different approaches which accomplish a successful result.[1],[2]
One first uses the retinoscope to evaluate the quality of the red reflex and streak. At the same time, the refractionist can assess what the best-corrected visual acuity should be, assuming the remainder of the eye to be normal. If the retinoscopic reflex is reasonably good, one uses the trial frame to complete a manifest refraction. If the reflex is poor, one then uses the keratometer or topography unit to measure the curvature and evaluate the quality of the anterior surface, another predictor of best-corrected visual acuity. If there is minimal cylinder, one uses the measurement obtained along with retinoscopy and trial lenses to determine the refraction. The best-corrected visual acuity obtained with the manifest refraction should have been predicted with the retinoscope and keratometer or topography unit.

If the cylinder is moderate or high, one should start with three-quarters of the amount in a trial frame. For example, if there are 6 diopters of astigmatism by keratometry or topography, and the cylinder axis for the steeper reading is at 90 degrees, one can place a +4.5 diopter lens at 90 degrees in the trial frame. Retinoscopy is then used to determine the sphere. The examiner asks the patient to refine the axis by turning the screw on the trial frame. The manifest refraction can then be completed using cylindrical and spherical lenses. Once again, the best-corrected visual acuity should make sense from information gained by retinoscopy and keratometry. In the special case of the presence of a corneal graft, pre-refraction topography is often a great time saver for the refractionist. And in the case of a reasonably clear cornea with moderate to marked irregular astigmatism, as may be found in the post-keratoplasty patient, the use of a trial rigid contact lens with over-refraction can be very helpful in determining the role of irregular astigmatism in the decreased acuity.

An autorefracting device can sometimes be a useful piece of equipment but often provides erroneous readings due to the irregularity of the refracting surface.
Conclusion

Refracting the patient with an abnormal cornea is a challenging, interesting, but most especially a rewarding experience for both patient and refractionist. Successful refraction of the abnormal cornea may require both the use of additional instrumentation as well as a change of the order in which the steps in refraction are employed.
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Chapter 10 – Corneal Diagnostic Techniques

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Corneal diagnostic techniques are specialized methods of examination that may involve simple or complex aids to yield valuable information for the diagnosis and treatment of ocular disease. These techniques are commonly but selectively used, depending on the patient's history and the goals of the examination. External examination of the lids, slit lamp biomicroscopy techniques, and tear film evaluation usually precede other diagnostic tests and have been discussed in previous chapters. Techniques discussed here are corneal staining, pachymetry, tests for corneal sensation, and osmolarity.

Corneal Staining

Corneal stains are diagnostic tools to assess the integrity of superficial cell layers of the cornea and the surface environment. These may be the most commonly performed tests in routine slit lamp biomicroscopy. Characteristic staining patterns aid in the diagnosis and management of corneal and external disease. Staining should be documented, noting depth and extent. Descriptions may specify micropunctuate (resembling small dots), macropunctuate (larger dots), or coalescent (a patch). Depth may be limited to the epithelium or include stroma.

Fluorescein and rose Bengal are the most common dyes used to evaluate the ocular surface. Both are halide derivatives of the hydroxyxanthene dye family. The addition of seven halogen atoms (three iodide and four chloride) to the hydroxyxanthene skeleton is responsible for the photophysical differences of rose Bengal. The spectroscopic absorption undergoes a red shift that contributes to the rose Bengal dye color.

Recently Feenstra and Tseng have demonstrated that the original concepts of fluorescein and rose Bengal staining have not been entirely correct.[1] While both dyes can stain living cells, rose Bengal does so more effectively and is intrinsically toxic. However, a healthy precorneal tear film will block rose Bengal staining of healthy and damaged cells. The lack of a healthy precorneal tear film in keratoconjunctivitis sicca explains the clinical usefulness of rose Bengal staining in that disorder. Cell degeneration or death increases membrane permeability to both dyes, but rose Bengal diffusion into the stroma is limited. Its clinical usefulness is recognized in the evaluation of keratoconjunctivitis sicca, the interpretation of epithelial dendrites (Fig. 10.1), and dysplastic or neoplastic lesions.
Because of the fluorescence property of fluorescein, examination of a fluorescein-stained cornea is enhanced by the use of a cobalt (blue) filter along with a yellow (Wratten # 12) barrier filter. Conjunctival staining, otherwise often difficult to appreciate, becomes more visible. Fluorescein staining of healthy cells is limited, but fluorescein diffuses rapidly into the intercellular spaces or stroma when disruption of cell–cell junctions occurs. This diffusion property is responsible for the need to examine the cornea very soon after fluorescein is applied. Fine details of fluorescein staining may be lost after as little as 2–3 minutes (Fig. 10.2). One technique for visualizing staining (according to Korb) is as follows: instill a drop, have the patient blink three times, and wait 1–2 minutes. This is enough time for the stain to penetrate damaged epithelial cells but also leach out of the tear film. Some pathologic conditions such as diabetes mellitus, as well as some medications, may increase epithelial permeability. As noted earlier, cell degeneration or death increases membrane permeability to both dyes, and, given time, fluorescein will also stain dead cells. These properties of fluorescein dye are responsible for its usefulness in the various forms of epithelial defect, in evaluating the status of precorneal tear film, in contact lens fitting, in detection of aqueous humor leakage, and to measure epithelial or endothelial permeability.
The technique of applying stain to the cornea can influence the information gathered for fluorescein. The clinician quickly learns ‘less is more.’ A very small amount of concentrated dye yields much better diagnostic information than a full drop. In fact, a full drop may overwhelm the cornea and mask subtle findings. Therefore, dye strips may be more useful, as well as more sanitary, than corresponding solution. Placing a small drop in the middle or proximal end of the strip and letting it run down to the end provides a small but highly concentrated volume of corneal stain.

Gross evidence of epithelial discontinuity can easily be seen after the instillation of dye but must be distinguished from ‘pooling.’ Pooling of the fluorescein tear film occurs in a depressed or irregular area of the cornea. The easiest method to distinguish pooling from staining uses a wisp of cotton from a cotton-tipped applicator. In an anesthetized cornea, without blinking, the cotton wisp is used to absorb the fluorescein tear film in the area of concern. If the epithelium is intact, the pool of fluorescein will be removed and no staining in the base will be found (Fig. 10.3).

Ocular surface staining grading with fluorescein (yellow) filter is one of the four most common valuable examination techniques in assessing dry eye. Others include fluorescein break-up time (BUT), Schirmer’s test, and meibomian examination. The monitoring and assessment of staining can be greatly enhanced by the use of a grading scale and standardized dye instillation and evaluation techniques. At least three grading systems (the Van Bijsterveld system, the Oxford system, and a standardized version of the NEI/Industry Workshop system) are in current use or discussed in the International Dry Eye WorkShop (DEWS) report, 2007 (www.tearfilm.org).[4] Part of the difficulty in ocular surface disease diagnosis is the common scenario of a mismatch in the signs and symptoms. Indeed, the repeatability of both
fluorescein and rose Bengal staining has been found to be poor.[5] In contrast, the repeatability of serial Schirmer's test was moderate, repeatability of tear break-up time was substantial, and repeatability of subjective symptoms (dryness and grittiness) was moderate to high. No single diagnostic test is a gold standard for diagnosis, but various combinations of tests have been recommended and shown to be more valid.

Another biological stain commonly used is lissamine green (Fig. 10.4). At least as effective in evaluating the ocular surface as rose Bengal, Manning et al. showed that it was better tolerated than rose Bengal by patients. Mean sensation score was significantly lower and duration of symptoms was shorter.[6] The effects of lissamine green and rose Bengal were compared on proliferating human corneal epithelial (HCE) cells in vitro. Rose Bengal stained normal proliferating HCE cells and adversely affected HCE cell viability, unlike lissamine green, which demonstrated neither of these characteristics.[7] When lissamine green is used, a relatively large volume (10–20 mL) is necessary to maximally view staining. Staining is enhanced by a red filter (Wratten # 25) as a barrier device on the slit lamp.
Characteristic corneal staining patterns may occur with corneal infections, inflammation, toxic changes, degenerative changes, and allergic conditions. Staining may be diffuse, regional, or focal depending on the underlying cause. Both the location and the pattern of corneal staining aid in diagnosis and management of corneal diseases (Fig. 10.5). For example, linear staining in the superior third of the cornea is typically found with a foreign body on the superior tarsal conjunctiva.[8] Linear staining in a contact lens wearer indicates a foreign body beneath the lens (Fig. 10.6). Superior bulbar conjunctival staining is characteristic of Theodore’s superior limbic keratoconjunctivitis.
Fig. 10.5  Staining patterns of the cornea and conjunctiva in various disease states. TRIC, trachoma and inclusion conjunctivitis.
While some prefer to indicate any corneal staining as superficial punctate keratitis (SPK), it is more helpful to describe corneal staining precisely. Minute focal defects visualized at the slit lamp with fluorescein as small green dots are best described as punctate epithelial erosions (PEEs). While these are often the earliest stage of tear film instability or desiccation (Fig. 10.7), they are also found in some infectious disorders. Epithelial lesions with focal inflammatory infiltrates within the epithelium have punctate staining but also have areas of negative stain and are known as punctate epithelial keratitis (PEK). Finally, subepithelial infiltrates (SEIs) are deep to the epithelium and do not stain. Complete evolution of these staining patterns (PEE → PEK → SEI) occurs typically in some cases of adenoviral conjunctivitis and on occasion in herpes simplex, herpes zoster, chlamydia, and rosacea keratitis.
Negative staining patterns may provide as much information as positive staining of the cornea. Negative staining refers to an elevated or irregular area of the cornea with intact epithelium in which the normal fluorescein tear film quickly dissipates (Fig. 10.8). These patterns have both diagnostic and therapeutic implications in cases of recurrent corneal erosion. Negative staining may also demonstrate elevated areas of the cornea that may be contributing to irregular astigmatism such as corneal scars, Salzmann's degeneration, or corneal striae after laser-assisted in situ keratomileusis (LASIK).
Fig. 10.8 Mixture of positive and negative staining in a patient with drug-induced epithelial toxicity.
Pachymetry

Pachymetry, the measurement of cornea thickness, has become routine and is increasingly important in ophthalmic practice. Refractive surgeons invariably use central corneal thickness (CCT) in planning surgery, as adequate thickness is key in avoiding postoperative ectasia. Glaucoma specialists have learned that cornea thinning is a cardinal risk factor for development of the disease, and pachymetry is therefore performed as a standard in glaucoma consultation.

Pachymetry is an important indicator of corneal health but varies widely in 'normal' patients. The thinnest part of the cornea is usually located about 1.5 mm temporal to the center of the cornea. Rapuano et al. measured 303 normal corneas and found a range of 410 to 625 µm. Mean thickness was 515 µm in the central cornea. In the paracentral region, thickness varied from 522 µm inferiorly to 574 µm superiorly. In the peripheral zone, thickness was 633 µm inferiorly and 673 µm superiorly. No significant differences were noted in readings between right or left eyes, males or females, time of day, month of year, or systemic medication use. Paracentral and peripheral, but not central, measurements tended to become thinner with age, but this trend was not statistically significant. Since the absolute central value can vary significantly and still be 'normal,' the relationship of central, midperipheral, and peripheral corneal thickness is important and should remain constant. The central area (within a 4 mm optical zone) is typically thinner than the midperipheral cornea (4–9 mm optical zone), which is thinner than the peripheral cornea (outside a 9 mm optical zone). Therefore, a cornea with a central thickness greater than the thickness in the midperiphery should be considered suspicious for endothelial dysfunction centrally or thinning in the midperiphery, irrespective of the absolute values. In fact, a patient with early endothelial compromise may have a CCT equal to the midperipheral corneal thickness.

Corneal pachymetry abnormalities include both thinning disorders, such as keratoconus and pellucid marginal corneal degeneration, and thickened corneas with endothelial compromise, such as Fuchs’ endothelial dystrophy and pseudophakic bullous keratopathy. While corneal thickness is an indirect measurement of the endothelial pump function, it is also affected to a lesser degree by the intraocular pressure.

One of the most common uses of corneal pachymetry is in assessing the extent of functional impairment of the endothelial pump in patients with Fuchs’ endothelial dystrophy or previous intraocular surgery before a planned intraocular procedure. If the intraocular pressure is
normal, epithelial edema develops when the stroma has swollen about 40%, to a corneal thickness greater than 700 µm. If, however, swelling is only 20% or pachymetry demonstrates corneal thickness greater than 620 µm, the risk of corneal decompensation after cataract surgery is significant.[13] Other uses of corneal pachymetry include determining the ‘health’ of a corneal transplant, evaluating a patient with keratoconus, and monitoring the degree of stromal edema in herpetic disciform keratitis. In contact lens wear, corneal edema and hypoxia can be assessed in daily wear, extended wear, or therapeutic lens patients. Corneal swelling averages 4% during eye closure, 9–10% with extended wear lenses, 11–14% during sleep, and up to 18% with contact lens wear. On slit lamp examination, corneal striae become visible at 4–8%, folds are seen at 11–12% swelling, and loss of transparency can occur at greater than 20% swelling.[14] High altitude causes a significant increase in CCT in healthy volunteers with normal corneas likely due to endothelial dysfunction.[15]

Techniques for measuring CCT include optical pachymetry, ultrasound pachymetry, confocal microscopy, ultrasound biomicroscopy, optical ray path analysis or scanning slit corneal topography, and optical coherence tomography.[16]

Optical methods of pachymetry were first described as early as 1951 by Maurice and Giardini.[17] Donaldson[18] and Mishima[19] also described manual slit lamp techniques to view the tear film or anterior corneal surface and the endothelial surface of the cornea. The Mishima-Hedbys fixation device for the Haag-Streit slit lamp reduced alignment problems. Various equations were used to calculate corneal thickness. Variables in these equations were the cornea’s refractive index and the anterior radius of curvature. These variables, along with the subjective nature of optical pachymetry readings, led to imprecise measurements and further investigation.[12] Because of its subjective endpoints, the accuracy of optical pachymetry is partly dependent on the skill of the examiner. Advantages, however, include relatively low cost and noncontact technique.[20]

Some specular microscopes designed to evaluate the corneal endothelial cell count also measure corneal thickness using electromechanical devices. These were designed to measure central and apical readings only. The measurement derived is based on the distance from the posterior surface of the tear film to the posterior surface of Descemet’s membrane, thus inducing an error of as much as 20 or 30 µm. In the contact mode, corneal touch is involved and compression may be another source of error.[20]

Another optical method of measuring corneal thickness utilizes the Orbscan II Anterior Segment Analysis System. While Orbscan central thickness measurements are statistically thinner than with ultrasound, there is a conversion factor to allow a surgeon to adjust the Orbscan value.[21] An advantage of the Orbscan data is the display of corneal thickness in a ‘map’ that facilitates evaluation of regional changes in corneal thickness.

Since its introduction in 1980, ultrasound technology has improved tremendously. Early units were more expensive, difficult to use, variable, and subject to alignment errors. Salz et al.[22] compared optical pachymetry with three ultrasonic pachymeters and concluded that optical pachymetry had more intersession variation, significant intraobserver variation, and significant right–left thickness differences.

Ultrasound pachymetry is not without disadvantages. Topical anesthesia is necessary due to direct contact with the cornea. Contact is undesirable in the early post-op period and the handheld nature of the probe limits measurement accuracy.[23] Ultrasound measurements generally read thicker than Orbscan II.[24] Recent studies reveal that optical methods of measurement (partial coherence interferometry) not only measure thinner than ultrasound
but also have been quoted as 'more reproducible, more reliable',[25] and with the least intraobserver and interobserver variability.[26]

Sources of error in pachymetry may be 'systematic' or inherent in the methods used in the procedure. Stucchi et al.[27] studied several factors including repeated measurements, drying of the cornea, patient positioning, and marking. Repeated measurements of the same corneal point showed small variability (<1.5%) and were lower with blinking after each measurement (<1%). After lying down for 3 hours, thickness increased by 2.21%. Pachymetry performed after marking the cornea underestimated thickness in the marked area by an average of 2.7%.[27]

The evolution and growth of laser refractive surgery and the importance of preoperative knowledge of corneal thickness has supported significant improvements in pachymeters. OCT is capable of measuring flap thickness with great accuracy and can differentiate femtosecond laser-created planar flaps from microkeratome-created meniscus flaps.[28] Modern ultrasound pachymeters are far superior to their predecessors. Significant engineering improvements include the solid-tipped probe, defining the speed of sound in human cornea, continuous-read measurement, automatic gain and angle sensitivity within 5 degrees of axis, advanced electronics and microprocessor control, battery-powered operation, memory storage capability, and 50 MHz high-frequency transducer. Modern ultrasound pachymeters are as light as 3.6 ounces, have a reported accuracy of ±5 microns and resolution of 1 micron, and have an IOP correction factor available.[29] Intraoperative measurements of Intralase flaps are possible as well as an epithelium mode helpful in monitoring regression.[30]
Aesthesiometry

Corneal sensation is a function of the ophthalmic branch of the fifth cranial nerve (trigeminal). Abnormalities such as trauma, tumors, surgery, infection, and inflammation can affect normal corneal sensation. Both the corneal epithelium and, to a lesser extent, the endothelium depend on normal corneal innervation. Anesthetic corneas may develop a characteristic interpalpebral horizontal band of punctate epithelial staining. Untreated, this may progress to an epithelial defect and subsequent stromal loss (neurotrophic ulcer). Interestingly, the endothelium also depends on ‘normal’ corneal innervation for its functions. In some cases, abnormalities in corneal innervation have been associated with cold-induced corneal edema.[31] Therefore, defects in corneal sensation can adversely affect the cornea.

Tests for corneal sensitivity may be very simple or more complex. Obviously, topical anesthetics should be avoided before these tests. The easiest and most widely used technique employs a cotton-tipped swab. A wisp from the cotton tip may be used to assess corneal sensation grossly. Care should be taken to avoid frightening the patient by bringing the wisp from the side. The patient’s response comparing the two corneas, as well as the examiner’s observation of the blink reflex, contributes to this gross assessment of corneal sensation.

A more accurate measurement of corneal sensitivity is obtained with the Cochet-Bonnet aesthesiometer. This device has a 6.0 cm-long adjustable nylon monofilament. To measure sensation, the tip is moved onto the corneal surface perpendicularly. The pressure (from 11 to 200 mg/mm²) exerted at the corneal surface directly relates to the length of the filament. Persons with normal corneal sensitivity sense touch at 6.0 cm length. If touch at 6.0 cm is not felt, the filament is reduced by 0.5 cm at a time until perceived. Measurements may be repeated and averaged.

Other methods of measuring corneal sensation primarily have research applications. Beuerman et al.[32] devised a technique based on warming the cornea with a jet of warm saline solution within a saline bath at ocular temperature. A 4.5°C increase in temperature elicited a response in a normal cornea.[33] Zaidman et al.[34] used a noncontact air puff technique to stimulate the cornea. Chemical stimulation of the cornea may be a reliable measure of sensation using the pungent substance capsaicin.[35] Brennan and Maurice stimulated the cornea thermally with a carbon dioxide laser, potentially a highly controllable technique.[36]
Ocular sensitivity is greatest in the central cornea\[31] except in elderly patients, in whom the peripheral cornea is the most sensitive.\[29] Sensitivity drops rapidly as distance from the limbus increases. The temporal limbus is significantly more sensitive than the inferior limbus. Short-term lens wear causes no significant loss of sensitivity; sensitivity falls with increasing age and is not affected by iris color.\[37]

Corneal sensitivity may be the most reliable test of long-term corneal compromise. In contact lens wearers, Brennan and Bruce recorded six ocular parameters: visual function, microcysts, polymegathism, edema, oxygen flux, and sensitivity. They found a change in corneal sensitivity of up to 150% over 6 months of extended lens wear, the most dramatic and easily detected change of all parameters.\[38] Long-term hypoxia is associated with loss of sensitivity. The mechanism in long-term contact lens wear includes altered acetylcholine levels with combined metabolic and respiratory acidosis and build-up of lactic acid and carbon dioxide.

After penetrating keratoplasty (PK), corneal sensitivity of the graft usually progressively improves with time. The peripheral cornea remains more sensitive than centrally, and some clear grafts remain completely anesthetic. Neither age, preoperative diagnosis, nor graft size was correlated with the recovery of sensitivity.\[39] Rao et al.\[40] found that the central graft never recovered normal sensitivity.

Topical medications may be associated with serious corneal problems in patients with decreased corneal sensation. Nonsteroidal antiinflammatory drugs (primarily generic diclofenac but also proprietary diclofenac and ketorolac) have been linked to punctate keratitis, erosions, sterile ulcers, and even perforations. The risk appears heightened in patients with compromised corneas from chronic ocular disease, decreased sensation, or severe dry eyes.

Corneal sensitivity may be reduced after radial keratotomy (RK). Shivitz and Arrowsmith observed this in 31% of RK patients after 6 months and 70% of astigmatic RK patients after 6 months, recovering to 9.5% and 47%, respectively, at 12 months.\[41] This reduced sensitivity may help explain the ability of former contact lens-intolerant patients to wear lenses after RK. It may also be a factor in late infectious keratitis occurring months or years after RK.

Corneal sensitivity has been compared in laser refractive surgery patients. In a masked study, Kanellopoulos et al.\[42] tested 40 consecutive patients 6 to 12 months following PRK and LASIK with the Cochet-Bonnet aesthesiometer. Patients undergoing LASIK had statistically better corneal sensation than those having PRK.\[37] However, studies on patients undergoing LASIK have shown a decrease in corneal sensation following LASIK for 3 weeks to 9 months.\[43],[44] The location of the hinge in LASIK may be important in decreasing postoperative dry eye signs and symptoms. Donnenfeld et al.\[45\] showed that nasal hinge location preserved corneal sensation better than superior hinged flaps. This was felt to be due to sparing of one of two horizontal nerves entering the cornea at 3 and 9 o'clock positions.

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MY ORIGINAL WORK DRAMROO...
Osmolarity

The etiology of a very common ocular surface disease, dry eye disease, has only recently been clarified. Indeed, from the 1997 first edition of this text, 'The mechanism for lacrimal gland dysfunction in this condition is unclear' (Vol. II, p. 647). Ten years later the DEWS report states, 'Tear osmolarity may reasonably be regarded as the signature feature that characterizes the condition of ocular surface dryness.'[4] The cutoff value of 316 mOsm/L is well validated. Although long used as a research tool, osmolarity measurement has until recently been only performed in a laboratory due to cost, lack of technology, and inconvenience. At least two separate methodologies are imminent, and one FDA application has been filed. Microscopic nanoliter tear volumes are required and readings are instantaneous.[46],[47] This may become the gold standard for diagnosis.
Summary

The use of corneal dyes, pachymetry, and tests for corneal sensation may greatly enhance our ability to diagnose and manage corneal and external disease problems correctly. A thorough understanding of these techniques allows us to maximize the information obtained.
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Section 2 – Laboratory Investigations

Chapter 11 – Practical Ophthalmic Microbiology for the Detection of Corneal Pathogens

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Introduction

Practical laboratory tests for the detection of corneal pathogens provide pertinent information that impacts patient care. Practical tests are highly sensitive, 100% specific, involve easy specimen collection and transport to the testing center, and provide testing results within 24 to 72 hours, if not sooner. Clinical laboratory testing is a dynamic relationship between technology and managed care. Although technologic advances will provide newer sensitive tests, laboratories are limited within budgetary restraints to provide only essential testing. Managed care resulted in significant reduction of laboratory personnel because insurance reimbursement for testing has decreased, as in other fields of medicine. Laboratories operate under high regulatory standards that are mandated by Medicare and other insurance carriers. Laboratories must be certified for diagnostic testing by CLIA (Federal), JCAH (hospital), State, and CAP (independent) regulatory agencies. Most laboratories process specimens for routine and frequent repetitive testing because of cost-effectiveness and the ease to validate testing proficiency, which is necessary for reimbursement. Infrequent testing is not cost-effective due to overhead costs (waste of perishable media) and the necessity of dedicating valuable laboratory personnel for a few tests. Infrequent testing is generally handled with batch testing (waiting for an accumulation of similar testing) that delays laboratory results or by referring the specimens to a reference laboratory (a large hospital or government laboratory). It is very difficult for a laboratory to validate testing for an infrequent pathogen because of the lack of true positive specimens that may require an extended time to collect.

Many ophthalmologists will find it difficult to locate microbiology laboratories that cater to specific ocular infectious disease testing in a timely fashion. As this chapter will demonstrate, ophthalmic microbiology is unique but not necessarily so high-tech that diagnostic testing is
not available. Ophthalmologists will have to investigate the availability of laboratory testing that is pertinent to their practice. This chapter will describe the best practical tests that are available for the detection of corneal pathogens. The ophthalmic microbiology tests presented in this chapter were established in a modern, fully certified, clinical laboratory that caters exclusively to detecting corneal and ocular pathogens.
Central Laboratory versus In-office Testing

Government regulations have made in-office testing an obsolete option for diagnosing ocular infections. All laboratory testing is regulated to assure that patient care is maximized, with qualified personnel who are certified or properly trained. Even if testing is provided free of charge, in-office testing must be regulated, which can be costly and invites a regulating agency to the office practice.
Communication: Ophthalmologist and Laboratory

Direct communication of the ophthalmologist with the diagnostic laboratory is essential. The laboratory must understand the specific needs of the ophthalmologist. The ophthalmologist must indicate the type of specimen, the possible pathogens involved in infection, and specific antibiotic susceptibilities that must be tested. Unusual pathogens (mycobacteria, Acanthamoeba, etc.) require planning and acquisition of media. Simply sending a single soft-tipped applicator to a laboratory and requesting isolation of all possible pathogens (bacteria – aerobic and anaerobic, virus, fungus, Acanthamoeba, etc.) is unreasonable, and valuable time is lost for the ophthalmologist, patient, and laboratory.
Corneal Specimen Collection

Corneal specimens can be collected with spatulas, jeweler's forceps, and surgical blades (Fig. 11.1). Cotton-tipped applicators can be used when less aggressive cultures need to be obtained or when less distinct areas of infection over a large area require culture. Sometimes, after an instrument is used, an additional specimen can be obtained with a soft-tipped applicator at the infected site. The instruments are used to collect specimens for planting media and placing on glass slides for cytologic examination. Each ophthalmologist should determine which collecting instrument is most comfortable and provides the best specimen for laboratory diagnosis.
Conjunctival cultures can be diagnostic when corneal cultures are negative due to antibiotic pretreatment (Fig. 11.1). Conjunctival specimens are collected with soft-tipped applicators with plastic handles and heads composed of cotton, Dacron, or calcium alginate (Fig. 11.1). Calcium alginate swabs have been reported to produce a higher yield of bacterial organisms but these may not always be available; thus, cotton and Dacron are alternatives.[1]

**Transport media**

Direct inoculation of isolation media is best but this approach is not always possible. There are many transport systems for supporting bacteria, virus, and chlamydia prior to direct inoculation on proper isolation media. The ophthalmologist should ask advice from the laboratory on suggestions for transport media and reliable delivery of specimens to the laboratory.

**Mailing of diagnostic specimens**
Diagnostic specimens can be easily delivered through couriers and public mail with a few simple requirements as mandated by federal law. These samples must be double-sealed to prevent any leakage of sample. A proper amount of desiccant to absorb any leakage due to damage should also be packed within the double-sealed specimen. The sample should be marked to indicate that the package is a diagnostic specimen. For example, a corneal specimen collected on a soft-tipped applicator and placed in a plastic tube (transport sleeve, culturette™ (Becton Dickinson, Sparks, MD), wrapped in a paper towel, enclosed in a sealable vinyl zip-locked bag, placed in a bubble mailer addressed to the laboratory, and marked as 'Diagnostic Specimen (Not Restricted) – Packed in Compliance with IATA Packing Instructions 650' would be a properly sent diagnostic sample (Fig. 11.1).
Stains and Cytologic Specimens

The acquisition of tissue from infected corneas for cytologic examination is not only a rapid method for determining the presence of an infectious agent but also may be the only positive indication of a pathogen. Microscopic examination of cornea specimens is a function of an adequate specimen collected and the laboratory’s expertise in interpreting the stained specimen. The ophthalmologist must be proficient in obtaining at least two corneal specimens for laboratory examination. One slide allows for the examination of microorganisms and the other for cytology. Additional samples may be useful for additional staining procedures (i.e. acid-fast). Specimens should be collected with a spatula, forceps, or blade, placed in the middle of a glass microscopic slide, and circled with a wax pencil for location. No immediate fixation is necessary and the slides should be delivered to the diagnostic laboratory with directions indicating the differential diagnoses. Gram stain (Fig. 11.1) is the standard stain for determining the presence of Gram-positive (Staphylococcus, Streptococcus) and -negative (Pseudomonas, Haemophilus) bacteria, and other microbes (i.e. fungi, Acanthamoeba). Other stains for detecting microorganisms are acridine orange, calcofluor, and acid-fast. The ophthalmologist must feel confident in the interpretation expertise of the laboratory. Confidence is gained with direct communication between ophthalmologist and microbiologist.

Historically, the Giemsa stain has been the standard for cytology examination and it may be necessary to use a pathology laboratory. Along with normal epithelial cells, inflammatory cells such as mononuclear, polymorphonuclear, eosinophils, basophils, plasma, leibers, etc., can be observed with smears stained by Giemsa (Fig. 11.2). In addition, abnormal epithelial cells from the upper tarsal conjunctiva can indicate malignancy, and multinucleated epithelial cells could indicate herpes simplex infection (Fig. 11.2). This powerful stain is excellent for depicting microorganisms, including chlamydia, which cannot easily be observed with other stains. Pathology laboratories may have other ideas for cytologic examination, with higher comfort levels using other stains and it may be advantageous to be receptive to other approaches.
Fig. 11.2 Giemsa stain cytology. (A) Polymorphonuclear cells; (B) mononuclear and epithelial cells; (C) plasma cell in center; (D) Acanthamoebae cyst in middle; (E) dark purple chlamydial inclusion caps peripheral to nucleus of epithelial cell; (F) corneal multinucleated epithelial cell from HSV keratitis.
Bacterial Laboratory Diagnosis

Laboratory studies for the isolation of bacterial pathogens from the cornea are widely available. Laboratories must be aware of the type (i.e. tissue, soft-tipped applicator) and location (i.e. cornea, conjunctiva, eyelid, vitreous, etc.) of submitted samples. The ophthalmologist must inform the laboratory of the possible pathogens that could be the etiologic agent in disease and provide enough time for the laboratory to acquire special media for infrequent pathogens (i.e. mycobacteria, Acanthamoeba). Ophthalmologists must inform laboratories that all bacteria isolated from the cornea should be considered pathogens and tested for antibiotic susceptibilities. It should be requested that inoculated culture media be held and examined for at least 5 days.

The routine media for isolating bacteria from the cornea, conjunctiva, and eyelids are broth-based agar with 5% sheep blood, chocolate agar, and mannitol salt agar (Fig. 11.3). The sheep blood agar will isolate most bacterial pathogens except Haemophilus species, Neisseria gonorrhoeae, and nutrient variant Streptococcus species that will grow on chocolate agar. Mannitol salt agar is an optional medium that is very helpful to the microbiologist for differentiating Staphylococcus species.[3] Enriched thioglycollate liquid medium will provide isolation for most bacteria, including anaerobes. Anaerobes are best isolated on special anaerobe media or a chocolate plate incubated under anaerobic conditions such as an oxygen-depleted jar or bag. Anaerobic cultures should be treated as special cultures, with preparation between physician and laboratory.
Mycobacteria
The detection of mycobacteria is a specialization within a large laboratory that is highly regulated and requires planning by the ophthalmologist. Mycobacteria can be quite fastidious and can require long incubation periods for isolation. The process of screening for all mycobacteria species is quite complex and probably unnecessary for ocular specimens. In general, mycobacteria keratitis is due to fast-growing species (growth within 7 days), Mycobacterium chelonae and M. fortuitum, which propagate on routine culture media (i.e. blood and chocolate agar).[4] Inoculating routine agar media plus a mycobacteria agar medium (i.e. Lowenstein-Jensen) and liquid medium (Middlebrook 7H9) should suffice. To culture for all species of mycobacteria, many laboratories prefer tissue for inoculating multiple media and for special stains (i.e. acid-fast). Submitting lone soft-tipped applicators for mycobacteria testing without previously notifying the laboratory will result in a delayed planted specimen that most likely will test negative or be designated as an insufficient specimen.

Nocardia and Actinomyces
Nocardia and Actinomyces are less frequent pathogens of the cornea. Nocardia are easily cultured on routine bacterial culture media and can be visualized on stained specimens as branching or partially branching filaments. Actinomyces are a more fastidious grower on culture media and require an extended
growth period. Cytologic examination of corneal specimens by Giemsa and other stains is the best diagnostic test for detecting *Actinomyces*. *Actinomyces* appear as long, thin filaments with branching and sometimes as long thin rods (Fig. 11.3).
Antibiotic Susceptibility Testing

Antibiotic susceptibility testing is performed on fast-growing bacterial isolates to assess whether an antibiotic will be successful in therapy. Laboratories should be informed of the required antibiotics for which corneal bacterial isolates should be tested. Some laboratories may not provide antibiotic susceptibility testing on ocular bacterial isolates because no susceptibility standards exist for topical antibiotic therapy. The interpretation of antibiotic susceptibility for ocular isolates can be based on systemic standards if laboratories assume that the antibiotic levels in the ocular tissues are equal to or greater than the antibiotic levels reached in the blood serum. In vitro antibiotic susceptibility is determined using the disk diffusion and MIC methods. Disk diffusion susceptibility is determined by placing paper disks impregnated with a set amount of antibiotics on a lawn of bacteria (see Fig. 11.1). After 24 hours of incubation, zones of inhibition are measured and compared to predetermined zone standards that represent susceptibility, intermediate susceptibility, and resistance. Methods of MIC determination include broth dilution, agar dilution, and E-tests. Using these methods, the minimum inhibitory concentration of antibiotic required to inhibit bacteria is determined and compared to predetermined concentrations that represent susceptibility. Bacterial corneal isolates should be tested for antibiotic susceptibility to ciprofloxacin, ofloxacin (levofloxacin), moxifloxacin, gatifloxacin, oxacillin (or cefoxitin for staphylococcal methicillin resistance), cefazolin, vancomycin, gentamicin, tobramycin, sulfacetamide, polymyxin B, and bacitracin. There are no current systemic susceptibility standards for bacitracin, but an older manufacturer standard could be used to assess susceptibility with reservation.
Laboratory Diagnosis of Fungal Infection

As with mycobacteria, fungus isolation and identification is a specialized section in a microbiology laboratory, with special certification requirements. Many laboratories can isolate fungi from ocular cultures but must refer the identification to a reference laboratory. Most fungi that infect the cornea can be isolated on routine culture media (i.e. blood agar, chocolate agar, and Sabouraud's agar supplemented with gentamicin). In general, molds (hyphal elements extending over the agar medium that have a fuzzy appearance) (see Fig. 11.3) and yeasts (pasty bacteria-like colonies) are isolated within 3–7 days after inoculation. The most common molds isolated from fungal keratitis are *Aspergillus* and *Fusarium*, but other genera have been isolated.[6] *Candida albicans*, *C. parapsilosis*, and other *Candida* species are the most common yeast pathogens of the cornea, but other yeasts have been implicated.[7] Corneal specimens for microscopic examination by Giemsa or specialized mycology stain should be obtained and are highly recommended as a rapid detection test (see Fig. 11.3). Ophthalmologists should discuss with the laboratory the fungal possibilities and the range of fungal isolation and identification that is required. More invasive culturing may require a tissue specimen, which may necessitate a corneal biopsy.
Laboratory Detection of *Acanthamoeba* and Microsporidia

*Acanthamoeba* can be easily isolated in any laboratory, but stocking media for such an infrequent pathogen is not cost-effective for most laboratories. Corneal specimens, contact lenses, solutions, and water that possibly contain *Acanthamoeba* can easily be sent to laboratories that offer testing without loss of viability.[8] Routine corneal specimens are planted on non-nutrient agar plates that are overlaid with a heavy slurry of live *Enterobacter aerogenes* or *Escherichia coli*. After a few days of incubation, *Acanthamoeba* trophozoites can be microscopically observed with movement across the agar leaving tracks. Once the food source is exhausted, the trophozoites will form hexagonal cysts (see Fig. 11.3). Cultures using an axenic liquid medium and cell culture are also used to isolate *Acanthamoeba*.[9] Corneal specimens for microscopic examination by Giemsa, calcofluor, and acridine orange stain should be obtained and are highly recommended as a rapid detection test. Polymerase chain reaction (PCR) testing can also be used to detect *Acanthamoeba* DNA.[10]

The detection of Microsporidia from corneal specimens is determined through the examination of stained specimens. A modified trichrome staining of epithelial cells will reveal pinkish to red organisms.[11] Other stains such as Giemsa, Gram, periodic acid–Schiff, Grocott's methenamine silver, calcofluor, and acid-fast methods have been equally successful for visualizing Microsporidia (see Fig. 11.3).[12],[13]
Laboratory Diagnosis of Adenovirus Infection

The laboratory diagnosis of adenovirus infection is a function of specimen collection after the onset of clinical presentation.[14] At the time of early clinical onset, the adenoviral titer is large in regards to live virus, capsid antigen, and viral DNA. This large titer decreases rapidly within days of clinical onset and dwindles to minute amounts within 1–2 weeks. Table 11.1 describes adenovirus laboratory testing as a function of the onset of clinical presentation. Adenovirus can be detected from ocular specimens using four methods: (1) cell culture isolation, (2) shell vial, (3) rapid antigen testing, and (4) PCR.

Table 11.1 -- Laboratory detection of adenovirus from ocular specimens as a function of clinical onset

<table>
<thead>
<tr>
<th>Test</th>
<th>Clinical onset of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoclone™</td>
<td>Reliable only if positive</td>
</tr>
<tr>
<td>Cell culture isolation</td>
<td>Positive in 1 to 7 days</td>
</tr>
<tr>
<td>Shell vial isolation</td>
<td>Positive in 3 days</td>
</tr>
<tr>
<td>Polymerase chain reaction</td>
<td>Positive in 1 to 3 days</td>
</tr>
</tbody>
</table>

The isolation of adenovirus in cell culture is the 'gold standard' for detecting adenovirus from ocular samples. In general, cell culture isolation will not provide timely results for patient care. Clinical specimens are obtained from the cornea and/or conjunctiva using soft-tipped applicators and transported to a clinical virology laboratory in viral transport medium (Bartels Chlamydia transport medium, Wicklow, Ireland) or a viral culturette (see Fig. 11.1). Adenovirus is a very hardy virus and can be transported without loss of viability through mail carriers under normal conditions.[15],[16] Most virology laboratories inoculate A549 monolayer cells (human lung carcinoma continuous cell line) for adenovirus isolation. Observance of characteristic cytopathic effect (cell rounding) is monitored for at least 3 weeks before finalizing cultures as negative with the immunoassay (Adenoclone®, Meridian, Cincinnati, OH) (Fig. 11.4). Ocular specimens obtained within the first 3 days of clinical onset will be positive in culture as early as 1 day (rare) but generally at 4–7 days with a few positive cultures at 8–10 days. Cultures obtained after 3 days of onset should include both eyes, since the fellow eye generally becomes involved at this time point. Specimens obtained at this later time of clinical onset generally require 10 days to 3 weeks or more for positive cell culture results.
The shell vial method is a cell culture technique that provides results in 3 days.[17] Viral specimens are inoculated on a glass cover-slip placed in a shell vial (small glass cylinder) layered with a monolayer of A549 cells. The inoculated shell vial is centrifuged and incubated for 3 days. Instead of waiting for the appearance of cell rounding, individual infected cells are stained with monoclonal antibodies conjugated with fluorescein isothiocyanate that appear apple-green using a fluorescent microscope (see Fig. 11.4). Shell vial detection is as sensitive as standard cell culture from patients cultured within 3 days of clinical onset of infection.[17]

The enzyme immunoassay, Adenoclone®, is a 90-minute antigen detection test that does not require the presence of live adenovirus. At its inception, Adenoclone® appeared to be a highly sensitive and specific test[18] but subsequent experience demonstrated a decrease in positive testing with a sensitivity of less than 50%.[19–21] Adenoclone® can provide rapid results when tests are positive,
but all negative results require confirmation by cell culture isolation. Another rapid test, the RPS Adeno Detector™ was developed as a ‘point of care’ test to directly detect adenovirus antigen from the conjunctiva. The sensitivity of testing was 88%, the specificity was 91%, and it was demonstrated to be less reliable for testing samples from transport medium in the laboratory setting.[22],[23]

An important advance in the detection of adenovirus is PCR technology. PCR tests for the presence of specific adenoviral DNA sequences by amplifying these sequences in a series of steps and then detecting the amplified product with DNA detection methods (i.e. gels, ELISA, real-time). Compared to cell culture isolation, the sensitivity of PCR is over 90% and equivalent to shell vial.[24] The lower sensitivity is probably due to the lower volume of sample tested. Only 5–10 µL of sample is tested for PCR, whereas 500 µL is used for cell culture isolation and 200 µL for shell vial. Advances in technology are making PCR more available but many molecular diagnostic laboratories may still be slow to offer adenoviral PCR testing due to validation testing. A dedicated molecular laboratory is capable of producing positive or negative results within 1–3 days of receipt of specimen. Real-time PCR for adenovirus DNA detection was determined to be 85% sensitive and 98% specific.[25]
Laboratory Testing for Herpes Simplex Virus

The laboratory diagnosis of active herpes simplex virus (HSV) from ocular specimens is widely available and positive results can be obtained within 24–72 hours of receipt at the testing center. In general, the diagnosis of HSV ocular disease is made at the initial slit lamp examination with characteristic epithelial presentations, but studies have reported that only 55–65% of patients with culture-positive proven infection are treated at this time.[26],[27] This would indicate that HSV ocular infection is not always classical in appearance and that laboratory diagnosis may be crucial. Clinical specimens are obtained from the cornea and/or conjunctiva using soft-tipped applicators and transported to a clinical virology laboratory in viral transport medium (Bartels Chlamydia transport medium, Wicklow, Ireland) or a viral culturette. Care should be taken not to delay the delivery of the specimens, since HSV, an enveloped virus, is not as hardy as adenovirus.[28] The key laboratory methods for detecting HSV from ocular specimens are: (1) cell culture isolation, (2) ELVIS (enzyme linked virus induced system) one-day cell culture isolation, and (3) PCR.

The gold standard for the laboratory testing of HSV is cell culture isolation. This is a sensitive test that is processed by all virology laboratories and generally produces positive results within 2–3 days, but isolation can be delayed to 1 week or more in rare occasions. Clinical samples are inoculated to monolayers of A549, Vero, or other susceptible cell lines in glass test tubes. The monolayers are monitored every other day for 2–3 weeks for the observation of characteristic cytopathic effect (CPE – cell rounding) (see Fig. 11.4). Experienced laboratories can distinguish HSV from other viral CPE, while others can confirm the CPE with complementing tests (i.e. ELVIS, PCR).

A more rapid cell culture isolation method that can produce positive results within 24 hours is ELVIS.[29] In a similar fashion to the adenovirus shell vial test, 0.2 mL of clinical specimen is centrifuged on a specially engineered cell line that produces beta-galactosidase when HSV virus is introduced into the cell. After the clinical specimen is allowed to incubate on the cell line for 24 hours, the cell line is fixed and a substrate is added to react with the beta-galactosidase to produce a blue color within the cells, which are observed with a light microscope under 10–40× magnification (see Fig. 11.4). ELVIS is an uncomplicated test that provides 85% sensitivity.[29] The reduced sensitivity compared to standard cell culture is probably due to the reduction in inoculation volume and the decreased incubation period.

Most molecular diagnostic laboratories offer PCR testing for HSV DNA. This is an excellent
alternative when central virology laboratories are unavailable and specimens must be sent through couriers to testing centers. Viable HSV is not necessary for PCR but negative tests should be confirmed with cell culture isolation because PCR may not be 100% sensitive.[27] Real-time PCR for HSV viral DNA detection was determined to be 98% sensitive and 100% specific.[25] In contrast, prospective studies indicated that PCR was more likely to detect the presence of HSV DNA with patients of suspected HSV ocular disease than cell culture isolation. Of 47 patients with positive PCR testing, only 28 tested for live virus in cell culture isolation.[30]

The laboratory diagnosis of HSV as an etiologic agent in pre-existing disease by submitting corneal buttons after keratoplasty for analysis is different than diagnosis for active infection. Corneal buttons from keratoplasty are generally negative for active infection but some success for detecting HSV from these samples has been had using PCR and immunohistochemistry methods.[31]
Chapter 12 – Molecular Genetics of Corneal Disease

John F. Stamler, John H. Fingert

The Value of Molecular Genetics Study of Disease

The identification of disease-causing genes provides information about the pathogenesis of heritable corneal disorders at the most fundamental level. The function of disease-causing genes may suggest involvement of important biologic pathways in a particular disorder. In addition to clarifying mechanisms of disease, the discovery of disease genes will likely provide insights into the normal function of the cornea.

The discovery of the genes responsible for corneal disorders has important implications for patient care. As disease-causing genes are identified, DNA-based tests can be designed to aid with diagnosis and to help differentiate between clinically similar disorders. Such genetic tests have become available for many diseases on both a fee-for-service and a research basis (www.genetests.org). Identification of the specific mutation responsible for a patient's disease not only solidifies the diagnosis but also may often allow one to accurately predict the course of the disease. Mutation-specific phenotypes of many hereditary eye diseases including glaucoma,[1] retinitis pigmentosa,[2] and Von Hippel-Lindau[3] have been described and will be of increasing utility to clinicians in counseling patients about their prognosis in the coming years.

The identification of disease-causing genes is crucial to the development of therapies for heritable disorders. In some cases, the biologic function of a disease-causing gene may suggest the novel application of known medical and surgical therapies. In other cases, new interventions will be designed to mitigate or repair a gene defect once it has been discovered. Such interventions may include custom pharmaceuticals, gene replacement (replacing a defective gene using viral vectors) or other molecular genetic techniques such as suppressing expression of abnormal gene products using ribozymes.[4],[5]

Understanding the molecular genetic basis of a disease not only suggests new avenues for medical and surgical interventions but also facilitates testing these therapeutics in animal models and human subjects. The most relevant animal models will be based upon genetic defects similar to those observed in human disease and will be used for testing the efficacy...
and safety of new interventions. While therapeutics are being tested in animal models, populations of human study patients with the same genetic basis of disease can be identified with diagnostic genetic tests and recruited for participation in future human studies. Clinically defined diseases, especially common ones like Fuchs’ endothelial corneal dystrophy, are often really a group of similar-appearing diseases with different underlying mechanisms of disease. For this reason, it is unlikely that all subtypes of such a clinical disease will respond equally to a single therapy. The use of mechanistically homogeneous animal and patient populations will help to maximize the likelihood of identifying useful new therapies and targeting them to the right patients.
Review of Genetics and Human Disease

Genes are the most basic units of heredity. More specifically, genes are segments of deoxyribonucleic acid (DNA) molecules that specify the production of proteins, which in turn perform structural or enzymatic functions necessary for development and homeostasis. Other molecules necessary for life, such as carbohydrates and lipids, are produced as needed by proteins. In this fashion, information stored in the DNA of each cell in an organism is used to create and organize cellular building blocks and on a larger scale to determine recognizable qualities or traits. Heritable traits (and genetic diseases) are determined by variations in the DNA sequence of genes and the proteins they encode.

The DNA sequence of the human genome encodes approximately 30,000 genes divided among the 23 pairs of chromosomes (22 autosomes and the X and Y sex chromosomes). Each chromosome consists of a single, extremely long molecule of DNA that is complexed with proteins and extensively folded and packaged into a condensed element. Every cell of an organism contains a complete copy of all of its genes stored in chromosomes.

Although all of an organism's genes are present in each of its cells, only a fraction of these genes are active in a given cell. Some genes that perform basic functions necessary for survival, such as energy production, are active in every cell of the body, while others are tissue specific. Tissue-specific activation of genes occurs because proteins are not produced directly from DNA. The DNA sequence of a gene is first converted into ribonucleic acid (RNA) in a process known as transcription. The nucleotide sequence of an RNA molecule specifies a series of amino acids that, when connected in succession, form a protein (translation). In a given cell of a complex organism, most genes lie dormant and are neither transcribed nor translated and the proteins which they encode are not produced. The subset of genes that are transcribed and subsequently translated into proteins give a cell specialized form and function, and is the basis of cellular differentiation and tissue formation.

The structure of a gene includes several domains necessary for the production of a particular protein in a coordinated fashion (Fig. 12.1). Only a portion of a gene's DNA sequence (the coding sequence) specifies the amino acids that compose the encoded protein. In eukaryotic cells, the coding sequence is usually divided into several segments called exons, which are separated by noncoding DNA segments called introns. ‘Upstream’ of the coding sequence is the promoter region of the gene which regulates transcription. The promoter contains DNA sequences that are binding sites for the enzyme (RNA polymerase) and cofactors that transcribe the gene from DNA into RNA. Tissue-specific activation of gene expression is primarily determined by differential binding of these cofactors to the promoter.

Fig. 12.1 The gene structure of the BigH3 gene. The coding sequence of BigH3 is divided into 17 exons (represented by the boxes) which are separated by 16 introns (represented by the space between the boxes). Upstream (to the left) of the coding sequences is the promoter region that regulates the transcription (expression) of the BigH3 gene. The positions of the amino acids most commonly mutated in BigH3-associated corneal disease (Arg124 and Arg555) are shown with arrows. The drawing is to scale except that introns greater than 500 nucleotides long have been truncated (indicated with a vertical slash.
Techniques Used to Identify Disease-causing Genes

The general approaches that are used to identify genes responsible for heritable corneal diseases are population-based techniques (association studies or candidate gene screening) and family-based techniques (positional cloning). In the population-based approaches, the frequency of different types of genetic variations are compared between large numbers of patients with a disease and normal control subjects. Lately, there has been great success in studying populations of patients with eye disease using genome-wide association studies. In such studies, cohorts of patients and controls are typed with hundreds of thousands of genetic markers in search of a cluster of markers that are seen more frequently in patients than controls. These groups of disease-associated markers may define a region in the genome that contains a genetic risk factor for disease. Genome-wide association studies have been successful in mapping the location and identifying important genetic risk factors for common eye diseases such as age-related macular degeneration[6–8] and exfoliation syndrome.[9]

Another population-based technique for finding disease-causing genes is the candidate gene screening approach. With this method, a hypothesis about the mechanism of disease is used to select and prioritize candidate genes for mutation screening without regard to the chromosomal locations of the candidates. Genes are studied as possible causes of corneal disorders based upon their function or expression pattern. While it is true that some ocular diseases, such as gyrate atrophy[10] and Leber's hereditary optic neuropathy,[11] are caused by genes expressed ubiquitously, it is rarely true that ocular diseases are caused by genes that are not expressed in the eye. Therefore, genes expressed in the cornea would be given higher priority as candidate genes for corneal dystrophies than genes not expressed in the cornea. Genes with known functions that suggest an association with a corneal disorder would also be selected for further study. For example, the sulfotransferase gene CHST6 was considered a good candidate for involvement in macular cornea dystrophy after biochemical studies identified a deficit of sulfated glycosaminoglycans in this disorder.[12],[13] Finally, genes that cause corneal disease when disrupted in an animal model are excellent candidates for involvement in human corneal disease. For example, a keratin 12 ‘knock-out’ mouse (i.e. a mouse genetically engineered to have a genome lacking the keratin 12 gene) has a fragile corneal epithelium and is predisposed to corneal abrasions and erosions.[14] This observation suggested that the keratin 12 gene was a good candidate for causing Meesmann corneal dystrophy. Candidate gene screening is a useful technique for identifying disease genes when only individuals or small families affected with a disease are available.
for study. Candidate genes are evaluated by screening large cohorts of patients for disease-causing mutations.

Family-based studies may also be used to identify disease-causing genes. Large families with many members affected with an eye disease may be studied using a positional cloning approach. With these studies, potential corneal disease genes are identified and evaluated, based on their chromosomal location. This method is dependent on the availability of large families transmitting disease in a mendelian fashion. The chromosomal location of the disease-causing gene in such pedigrees is determined by linkage analysis. Family members are typed with hundreds of genetic markers with known chromosomal locations in search of markers that are coinherited (or linked) with the disease more often than can be explained by chance. This coinheritance is related to their physical nearness on a chromosome because genetic markers in close proximity to the disease-causing gene are less likely to be separated by a meiotic crossover. The likelihood of a crossover occurring between a marker and a disease gene is proportional to the distance between them. The known position of a linked marker indicates the chromosomal location of the disease-causing gene. Positional cloning has some advantages over candidate gene screening. This approach requires no hypothesis regarding the pathogenesis of the disease studied or of the function of the disease-causing gene. This feature of linkage analysis is of great utility in studying disorders for which the disease pathways are only poorly understood.[15]
Disease-causing Mutations versus Nondisease-causing Sequence Variations

The human genome is composed of approximately three billion base-pairs of DNA and there are millions of DNA sequence differences between any two unrelated individuals. The vast majority of these differences are not associated with any detectable phenotype. Consequently, one of the challenges of studying the genetics of human disease is differentiating between disease-causing mutations and nondisease-causing sequence variations.

A variety of criteria have been used to judge which sequence variations are disease-causing and which are not. In general, even to be considered to be a disease-causing mutation, a variation must be present in patients more frequently than in control subjects and must either alter the expression level of a gene or alter the encoded protein sequence. Statistical methods, sequence analysis, and functional studies have been used to infer which of these variations are truly pathogenic.

One can use statistical approaches to demonstrate a significant association between a sequence variation and disease, but the statistical methods appropriate for studies of many unrelated patients are different from those used to study individual families with many members affected with disease. In population studies, the pathogenicity of gene variations may be strongly supported by demonstrating a significantly higher frequency of a certain variation among a large number of patients as compared to a large number of controls. A crucial aspect of this technique is that the subjects and controls are well matched. Some nondisease-causing variations are specific to certain ethnic groups and if the ethnicities of the subjects and controls are not well matched, such variations may incorrectly appear to be associated with disease. If a gene variation is found in affected members of a large family, one can use statistics to show that coinheritance of the variation and the disease occurs more likely than can be attributed to chance. Analysis of sequence homology can also lend support to the pathogenicity of a particular sequence variation. Alterations of portions of genes that are identical in disparate organisms are considered more likely to cause disease than those that occur in portions of genes not conserved during evolution. Similarly, variations within known functional domains of a gene are often considered more likely to cause disease than variations in other portions of a gene.

Perhaps the strongest support of the pathogenicity of a sequence variation is to show directly
that the variation harms the function of the protein encoded by the gene. This can be done
with in vitro assays as well as with various types of animal models. Using molecular genetic
techniques, the pathogenicity of a specific gene variation can be evaluated by creating a cell
line or an animal that has the gene defect of interest. If such a model expresses a phenotype
similar to the human disease, it is likely that the particular gene variation does cause
disease. The Meesmann corneal dystrophy-like phenotype of the keratin 12 knock-out
mouse\cite{14} is an excellent example of this type of evidence for disease causation.

The term ‘dystrophy’ has been used in the ophthalmic literature to refer to various types of
disorders. However, it has become generally accepted that ‘corneal dystrophy’ is used to
characterize ‘a group of inherited corneal diseases that are typically bilateral, symmetric,
slowly progressive, and without relationship to environmental or systemic factors’ (IC3D
classification). These are not absolute constraints, but rather general guidelines. Some
dystrophies appear more sporadic than inherited, such as epithelial basement membrane
dystrophy. Other disorders that are historically considered dystrophies are often asymmetric
or unilateral, such as posterior polymorphous dystrophy. Still others, such as macular corneal
dystrophy, may have systemic features.

Recently, the IC3D classification system for corneal dystrophies has been published.\cite{16} This
is the system that we have used here.

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Epithelial and Subepithelial Corneal Dystrophies

Epithelial basement membrane dystrophy (EBMD, MIM #121820)

Poor adhesion of epithelium resulting in symptomatic recurrent erosions and the visual disturbances of glare and blur associated with irregular corneal epithelium is most often thought to be sporadic or of traumatic origin. However, familial cases have been reported.[17] Typically, there are no signs until adulthood, when irregular patches of epithelium are seen as hazy islands of epithelium with scalloped borders (maps) interspersed among pockets of round or oval gray inclusions (dots). Edges of overlapping epithelial layers can be seen as curvilinear lines (fingerprints). Episodic spontaneous erosions produce lacrimation, glare, and pain.

While most cases are sporadic or arise from superficial trauma, a subset of patients have been linked to mutations in the \textit{TGFBI} gene on chromosome 5q31.[18]

Epithelial recurrent erosion dystrophy (ERED, MIM #122400)

The autosomal dominant ERED has an early onset in the first decade of life. Recurrent erosions occur spontaneously or after minimal trauma. A subepithelial haze may be present and, in the Smolandiensis variant, may be prominent enough to require corneal transplantation.[19] The genetic locus and genes are not known.

Subepithelial mucinous corneal dystrophy (SMCD)

A rare bilateral subepithelial haze involving the whole cornea, but most prominent in the center, has been described to occur in affected family members of this autosomal dominantly inherited dystrophy.[20] The opacities occur in the first decade of life and are accompanied by painful recurrent erosions. Only one family has been reported and the gene that causes corneal disease in this family has not been identified.

Meesmann corneal dystrophy (MCD, MIM #122100)

Multiple, tiny intraepithelial vesicles extend from limbus to limbus with intervening clear areas
primarily in the intrapalpebral cornea in MCD. The lesions may appear as gray cysts or clear vacuoles on indirect illumination.[21] In the Stocker-Holt variant of MCD, the punctate epithelial opacities stain with fluorescein and fine linear whorls of opacities may be present.[22]

Mutations in the keratin genes, keratin 3 (KRT3) on chromosome 12q13 and keratin 12 (KRT12) on chromosome 17q12 have been implicated in MCD and MCD-Stocker-Holt variant.[23],[24]

**Lisch epithelial corneal dystrophy (LECD)**

Localized gray punctate epithelial opacities radiate from the limbus to the center of the cornea in flame, band, or feather-like whorls in individuals affected with LECD. Patients are generally asymptomatic unless the opacities extend to the central cornea. The inheritance pattern is X-linked dominant with a locus at Xp22.3.[25] However, the disease-causing gene in this locus has not yet been identified.
Bowman Layer Dystrophies

Reis-Bücklers corneal dystrophy (RBCD, MIM #608470, TGFBI gene)

First described by Reis[26] and then by Bücklers,[27] RBCD presents as subepithelial reticular opacities that focally elevate the epithelium, appearing symmetrically in both corneas around 4–5 years of age. The opacities remain asymptomatic until they produce recurrent erosions. In the second and third decades of life the visual acuity is reduced as the corneal haze and irregular surface progresses. The inheritance is autosomal dominant and specific mutations in the TGFBI gene (Arg124Leu and Gly623Asp)[28],[29] have been shown to cause disease.

Thiel-Behnke corneal dystrophy (TBCD, MIM #602082, TGFBI gene)

Thiel-Behnke corneal dystrophy is similar to RBCD in that it is characterized by subepithelial corneal opacities associated with symptomatic corneal erosions. However, the opacities can be differentiated by their honeycombed pattern and a clear zone near the corneoscleral limbus. Subepithelial fibrous tissue accumulates in wavelike patterns and is seen as the characteristic ‘curly’ fibers on transmission electron microscopy.[30]

TBCD can be caused by mutations in the TGFBI gene at locus 5q31.[28] However, another locus on chromosome 10 (10q23-q24)[31] has recently been identified, which suggests the existence of at least one additional gene that can cause TBCD.

The distinction between Reis-Bücklers and Thiel-Behnke corneal dystrophies has been a focus of controversy. In several early reports, descriptions of Reis-Bücklers dystrophy were based on patients that actually had Thiel-Behnke dystrophy.[30],[32] Consequently, features of Thiel-Behnke corneal dystrophy (honeycomb opacities seen at the slit lamp and peculiar ‘curly fibers’ seen on electron microscopy) and those of Reis-Bücklers dystrophy (geographic opacities seen at the slit lamp and rodlike bodies on electron microscopy) were not precisely delineated. In an extensive review of the literature, Küchle et al. found that most American and British literature reports of Reis-Bücklers dystrophy (prior to 1995) were likely affected with Thiel-Behnke dystrophy.[30] More recently, several patients initially diagnosed with Reis-Bücklers corneal dystrophy were found to harbor an Arg555Gln TGFBI mutation[33] and later had their clinical diagnosis changed to Thiel-Behnke dystrophy (the dystrophy associated with this TGFBI mutation).[28] At present, histological confirmation may be necessary for a
certain diagnosis. However, as genotype–phenotype correlations are strengthened, the gold standard for diagnosing Reis-Bücklers or Thiel-Behnke may shift to \( \text{TGFBI} \) mutation screening.

**Grayson-Wilbrandt (GWD)**

One family has been described with variable patterns of subepithelial corneal opacities that range from diffuse mottling to scattered gray-white opacities. The inheritance appeared to be autosomal dominant, but no information about a chromosomal locus or causative gene is known.\[35\]
Stromal Dystrophies

**TGFBI Dystrophies**

Early in the molecular genetic investigation of corneal dystrophies it became evident that several different phenotypes can be caused by variations in a single gene. Linkage studies demonstrated that lattice, granular, and Avellino corneal dystrophies all mapped to the same chromosomal location at 5q31,[36] suggesting that they were caused by different variations in the same gene. Specific mutations in the beta-transforming growth factor induced gene (TGFBI, also known as BIGH3; MIM #601692) were later shown to be associated with each of these disorders. Later, TGFBI mutations were found to be associated with several additional corneal dystrophies (Fig. 12.2).
Commonly detected mutations in patients with CHST6 is produced in the corneal stroma and in other tissues of the body. Without CHST6 activity in the corneal epithelium, unsulfated keratan sulfate mutations appear to alter the tissue-specific expression of the gene, such that no CHST6 protein is produced in the corneal epithelium, while normal progression with age. Both Arg124Ser and Arg555Trp mutations in the TGFBI peptide have been identified to produce GCD1.[37]

It was more than a little surprising to many clinicians to learn that the breadcrumb-like corneal deposits of granular dystrophy and the branching lines of lattice dystrophy were caused by mutations in the same gene. Specifically, alterations involving the arginine amino acids at positions 124 and 555 of the encoded protein were found to be responsible for these diseases.[38] Additional study has shown that alterations of these two amino acids are the most commonly detected mutations in patients with TGFBI corneal dystrophies.[28]

The protein encoded by TGFBI contains a host of protein-binding domains, suggesting that it may have a role in cell adhesion and has been shown to bind to type I, II, and IV collagen.[39]

**Classic lattice corneal dystrophy (LCD1, MIM #122200, TGFBI gene)**

Classic lattice corneal dystrophy (LCD1) is a typical manifestation of the first decade of life and is characterized by branching, linear, central corneal amyloid opacities within the stroma of both eyes. Corneal sensation is reduced and recurrent erosions are common. LCD1 is autosomal dominant and the majority of reported cases have been associated with mutations of C to T at nucleotide 417 in exon 4 of the TGFBI gene causing an Arg124Cys mutation; however, many other mutations in TGFBI have also been shown to cause forms of LCD1.[28][37]

Historically, several variants of lattice corneal dystrophy have been described, based on differences in phenotype, and are not included in the IC3D. These include types II, III, and IV. These subtypes are distinguished by the characteristics of the amyloid deposits, the age of onset, and the frequency of corneal erosions.

**Lattice corneal dystrophy, gelosin type (LCD2, MIM #105120, GSN gene)**

The gelosin type of lattice corneal dystrophy is not a true corneal dystrophy, but rather a systemic amyloidosis (also known as familial amyloid polynuropathy type IV, Meretoja syndrome, MIM #105120).[40] In LCD2 the corneal opacities are small, radial, fine lines that are more peripheral with relative sparing of the central cornea and fewer in number than those seen in LCD1. Further differentiating features are a later onset in the third or fourth decade and infrequent erosions. Unlike LCD1, LCD2 has systemic features of cranial, autonomic, and peripheral neuropathies, and dry, lax skin. Also protruding lips, pendulous ears, and blepharochalasias are characteristic features.

The amyloid in LCD2 is a mutated form of gelosin that accumulates in the cornea between the epithelium and Bowman's layer. It is also found in heart, kidney, skin, nerves, vessel walls, and various other tissues.[41] The gelosin gene (GSN), which is located on chromosome 9q34 and encodes an actin-modulating protein, is responsible for LCD2. Mutative mutations in nucleotide 654 of G to A and G to T resulting in substitutions of asparagine or tyrosine for aspartic acid have been reported.[42]

**Granular corneal dystrophy, type 1 (GCD1, MIM #121900, TGFBI gene)**

The classic type of granular dystrophy, GCD1, generally presents in the first decade of life as discrete, bilateral, crumblike, corneal deposits in the anterior central stroma. Reduced visual acuity, increasing glare, photophobia, and recurrent erosions worsen as the corneal opacities gradually progress with age. Both Arg124Ser and Arg555Trp mutations in the TGFBI peptide have been identified to produce GCD1.[37]

**Granular (Avellino) corneal dystrophy (GCD2, MIM #607541, TGFB1 gene)**

The second type of granular dystrophy, GCD2, contains features of both granular and lattice corneal dystrophies. This disorder has also been called Avellino corneal dystrophy because many of the families with GCD2 came from that region of Italy.[43] In GCD2, white, snowflake-like opacities appear in the central cornea in the first two decades of life. Then lattice lines and interstitial haze gradually develop. Recurrent erosions are not common in GCD2 and the visual acuity is variably affected. GCD2 has been associated with the Arg124His mutation in TGFBI.[37]

**Macular corneal dystrophy (MCD, OMIM #217800, CHST6 gene)**

The autosomal recessive macular corneal dystrophy (MCD) usually presents early in childhood as a diffuse stromal haze extending all the way to the limbus. As the patient ages, opacities gradually coalesce in the stroma to form irregular white patches or macules that give the dystrophy its name. However, in distinction with granular dystrophy, there are no clear areas between the opacities. MCD is not entirely a stromal condition. The deposits extend through the stroma to Descemet’s membrane and the endothelium. Eventually, the endothelium decompensates and corneal edema develops, producing severe loss of vision in the second or third decades.[44]

MCD is characterized by intracellular accumulation of glycosaminoglycans (GAG) within the keratocytes and endothelium, but not the epithelial cells. Similarly, extracellular deposits of GAG are present throughout the stroma and Descemet’s membrane.

Mutations in the carbohydrate sulfotransferase 6 gene (CHST6) on chromosome 16q22 produce most, but not all, cases of MCD. Well over a hundred different mutations have been identified and most are either missense or nonsense mutations that alter a single nucleotide of the CHST6 gene. However, insertions and deletions in and around the coding regions of CHST6 have also been detected.

There are three subtypes of MCD distinguished by the presence of keratan sulfate (KS). In type I, there is no detectable keratan sulfate in the cornea or serum. In type II, there are normal amounts of KS in both cornea and serum. The third type, IA, has KS in keratocytes, but not in serum. Although, these subtypes are not distinguishable clinically, specific classes of CHST6 mutations are correlated with particular types of MCD. Patients with MCD type I have homozgygous mutations in the coding sequence of CHST6 that would be expected to inactivate the function of the sulfotransferase protein. The loss of CHST6 function is thought to cause the formation of unsulfated keratan sulfate and accumulation of opaque deposits in the corneal stroma.[18] Alternatively, patients with MCD type II were found to harbor homozygous promoter sequence rearrangements in the CHST6 gene. These promoter mutations appear to alter the tissue-specific expression of the gene, such that no CHST6 protein is produced in the corneal epithelium, while normal CHST6 is produced in the corneal stroma and in other tissues of the body. Without CHST6 activity in the corneal epithelium, unsulfated keratan sulfate is produced, leading to corneal opacification. The normal keratan sulfate levels observed in MCD type II are due to normal expression of CHST6 in the corneal stroma and other tissues of the body.[13]

**Schnyder corneal dystrophy (SCD, MIM #121800, UBIAD1 gene)**

Although Schnyder crystalline corneal dystrophy has been a common name for SCD, the IC3D classification scheme recommends the term 'crystalline'...
not be used since only about half of involved patients have corneal crystals.\textsuperscript{[16]} Early in life, central corneal haze or frank crystals become apparent in affected individuals. Often, a light yellow opacity forms a ring shape in the anterior stroma and Bowman's layer. The opacities increase with age, but remain primarily in the anterior cornea. Typically, SCD is bilateral, but often asymmetric.

The opacities correspond histologically to both intra- and extracellular crystals of cholesterol and other lipids. While most cases of SCD lack any significant systemic abnormalities, many have hypercholesterolemia and its associated manifestations of arcus lipoides and xanthelasma.

Numerous mutations in UbiA prenyltransferase domain containing 1 (\textit{UBIAD1}) gene on chromosome 1p36 have been found to produce autosomal dominant SCD.\textsuperscript{[44a–c]}

\textbf{Congenital stromal corneal dystrophy (CSCD, MIM #610048, DCN gene)}

Only four families of this rare autosomal dystrophy have been reported. CSCD is characterized by the presence of diffuse, bilateral, tiny flakelike corneal opacities. The opacities are spread throughout the stroma. It is stable or slowly progressive. The epithelium and endothelium appear normal.

Defects in the decorin (\textit{DCN}) gene on chromosome 12q21.33 have been associated with two families with CSCD.\textsuperscript{[45]}

\textbf{Fleck corneal dystrophy (FCD, MIM #121850, PIP5K3 gene)}

Fleck corneal dystrophy, an autosomal dominant corneal dystrophy, is characterized by multiple asymptomatic and nonprogressive tiny white opacities scattered throughout the cornea. The epithelium, Descemet's membrane, and endothelium appear normal. The flecks correspond to intracellular accumulations of glycosaminoglycans and lipids. Vision is not affected, with the possible exception of mild photophobia.\textsuperscript{[46]}

Mutations in the phosphatidylinositol-3-phosphate/phosphatidylinositol-5-kinase type III (\textit{PIP5K3}) gene on chromosome 2q35 cause FCD.\textsuperscript{[47]}

\textbf{Posterior amorphous corneal dystrophy (PACD)}

The characteristic sheets of diffuse gray-white opacities of PACD are most prominent in the posterior stroma. The opacity may be noted in early life and may be congenital. The associated anterior anomalies of prominent Schwalbe line, fine iris processes, irido-corneal adhesions, corectopia, and anterior stromal tags suggest that this may not be a true dystrophy, but more appropriately termed a mesodermal dysgenesis.\textsuperscript{[48]}

PACD shows an autosomal dominant inheritance pattern, but no chromosomal locus or gene has been identified.

\textbf{Central cloudy dystrophy of Francois (CCDF, MIM #217600)}

The asymptomatic central polygonal or rounded stromal opacities that are surrounded by clear zones of CCDF are clinically indistinguishable from the corneal degeneration posterior crocodile shagreen. The opacities may be mucopolysaccharide and lipid.\textsuperscript{[49]} Although apparent autosomal dominant patterns have been reported, the inheritance has not been clearly identified. No chromosomal locus or disease-causing gene has been identified.

\textbf{Pre-Descemet's corneal dystrophy (PDCD)}

The clinical and genetic features of PDCD have not been well defined. No definite inheritance pattern has been described and histopathologic studies have not been consistent. The corneal opacities have been described as fine, gray opacities in the deep stroma of various shapes along with more central, larger lesions. Typically, the vision is not affected and the patients are without symptoms.\textsuperscript{[50]} No chromosomal locus or causative gene has been identified and there is debate as to whether PDCD is best described as a dystrophy or as a degeneration.
Descemet's Membrane and Endothelial Dystrophies

Fuchs' endothelial corneal dystrophy (FECD, MIM #1368000)

Classic FECD: Fuchs’ endothelial corneal dystrophy (FECD) is characterized by progressive, bilateral accumulation of central, focal Descemet's membrane excrescences or guttae, loss of endothelial cells, leading to loss of vision from corneal edema. There are at least two clinical types of FECD. In the typical adult-onset or classic type, significant numbers of guttae usually do not become visible until the fourth or fifth decade of life. After the appearance of guttae, it usually takes one or two decades or more for significant compromise of endothelial function and corneal edema to develop.[51]

Late-onset FECD displays an autosomal dominant inheritance pattern with an unknown rate of incomplete penetrance.[52]

Recently, four different mutations in the SLC4A11 gene were found in four of 89 FECD Chinese and Indian patients, but not in ethnically matched controls.[53] Because of this association and since the SLC4A11 gene has been implicated in another endothelial cell dystrophy (CHED2), this gene is an appealing possible disease-causing candidate for FECD. In other studies, different FECD genetic loci (13pTel-13q12.13[54] and 18q21.2-q21.32[55]) have been identified with family-based research. Furthermore, a recent study of the candidate genes COL8A1 and COL8A2 detected no disease-causing mutations in late-onset FECD.[56] Consequently, it is likely that mutations in at least three different genes can cause the FECD phenotype.

Early-onset FECD: In contrast to the classic FECD presentation, a rare early-onset type of FECD has been described.[57] First signs appear as early as the first decade with significant edema and visual impairment by the third or fourth decade of life. In addition, the guttae differ in morphology between the two types. In the early-onset form they are small, rounded, and associated with the endothelial cell center. This is in distinction to the classic FECD guttae, which are larger, sharply peaked, and initially positioned at the edge of endothelial cells.

This early-onset form of FECD is also autosomal dominant in the reported families. Linkage analysis demonstrated a genetic locus of 1p34.3-p32.[58] The gene that codes for the alpha2 subunit of collagen VIII (COL8A2) is within this locus and is highly expressed in corneal endothelial cells. Two different mutations in separate families in the COL8A2 gene segregate
with the disease phenotype. In three families with early-onset FECD, a missense mutation of Gln455Lys was found to occur within the triple helical domain of the protein. In the original family described by Magovern et al.,[59] a mutation was identified that changes leucine to tryptophan at residue 450 of the protein.[57] This is convincing evidence for the disease-causing role of COL8A2 mutations in the rare early-onset form of FECD.

*Posterior polymorphous corneal dystrophy (PPCD, MIM #122000, #609140, #609141)*

Posterior polymorphous corneal dystrophy (PPCD) is a rare disease of the corneal endothelium that has autosomal dominant inheritance.[60] PPCD is typically bilateral, with signs appearing in the first or second decade of life, and ranges from rapidly progressive and visually disabling to asymptomatic and stable. The endothelial cells in PPCD have epithelial characteristics such as multilaminar growth and the expression of cytokeratins that are not normally seen in endothelial cells but are typical of epithelial cells.[61],[62] Additionally, Descemet's membrane is abnormal.[63]

Three distinct loci and two genes have been identified for PPCD. One large family was mapped to a 30-cM locus on 20q11.[64] Initial studies suggested that mutations in a candidate gene, VSX1, in this locus caused PPCD.[65] However subsequent studies have neither confirmed VSX1 as a PPCD gene nor identified other disease-causing genes within the locus.[66–68]

An association of PPCD with a mutation in the collagen type VIII gene (COL8A2) located on the short arm of chromosome 1 has been reported.[58] However, other investigators have failed to find mutations in the COL8A2 gene in PPCD families.[69–71]

A third PPCD 8.55-cM locus was found on chromosome 10.[72] A frameshift-causing 2-bp deletion (2916_2917delTG) in the last exon of the gene TCF8 that codes for a transcription factor ZEB1 located within this locus was found to segregate with the disease in a large family.[71] Further analysis of 10 other families revealed mutations that frameshift or terminate this reading frame in four probands.[71] Subsequently, 10 more families (six Czech and four British) have been studied. Four novel pathogenic mutations were identified in the TCF8 gene.[67]

In summary, there is compelling data that suggests mutations in the TCF8 gene cause PPCD. However, the initial reports implicating the VSX1 and COL8A2 genes in PPCD have not been confirmed.

*Congenital hereditary endothelial dystrophy (CHED)*

Congenital hereditary endothelial dystrophy (CHED) is a disease of the corneal endothelium that is characterized by bilateral, symmetric stromal haze, which is apparent at or soon after birth. Diffuse opacification is secondary to edema caused by abnormal endothelial development.[73] CHED may be inherited in an autosomal dominant (CHED1) or autosomal recessive (CHED2) pattern.

*Autosomal dominant congenital hereditary endothelial dystrophy (CHED1, MIM #121700)*
The autosomal dominant form of CHED (CHED1) is slowly progressive and associated with significant vision loss usually requiring treatment with penetrating keratoplasty.\[74\] The gene that causes CHED1 has been mapped to a chromosome 20 locus. However, the disease-causing gene in this locus region has not been discovered.\[75\]

**Autosomal recessive congenital hereditary endothelial dystrophy (CHED2, OMIM 217700)**

Patients with CHED2 differ from those with CHED1 in that they have nystagmus and a nonprogressive course. Linkage analysis of an autosomal recessive pedigree mapped a gene that causes CHED2 locus to a chromosome 20p13 locus that is distinct from the nearby CHED1 locus.\[76\] Vithana et al. later showed that mutations in one of the genes in this locus, the sodium borate transporter (\textit{SLC4A11}), are a likely cause of CHED2.\[77\] \textit{SLC4A11} is expressed in human corneal endothelium and mutations in \textit{SLC4A11} were identified in several CHED2 pedigrees.\[77\] Subsequent studies showed that mutations in \textit{SLC4A11} are a common cause of CHED2.\[78–83\] However, no \textit{SLC4A11} mutations were detected in some families with CHED2, which suggests that some cases may be caused by different genes.

**X-linked endothelial corneal dystrophy (XECD)**

Congenital bilateral diffuse hazelike ground glass or milky corneal clouding characterizes patients with XECD. The endothelium has indentations or ‘moon craters’ with missing endothelial cells. There are secondary epithelial changes of band keratopathy. The inheritance pattern is X-linked dominant and a gene that causes XECD has been mapped to chromosome Xq25;\[25\] however, no causative gene has been identified.

**Keratoconus (OMIM 148300)**

Keratoconus is a disorder of the corneal stroma in which progressive thinning of the central cornea induces myopia, irregular astigmatism, and conical surface contours.\[84\] The heritability of keratoconus has been suggested by twin studies\[85–90\] and by pedigrees demonstrating autosomal dominant transmission of the disease.\[91–96\] More recently, computerized corneal topographic methods have increased the sensitivity for detecting keratoconus and have provided additional evidence of mendelian inheritance of the disease.\[94,\[95\]\] The cause of keratoconus is unknown; however, it has been suggested that disturbances in protease regulation,\[97,\[98\]\] wound healing, or IL-1-mediated apoptosis might be involved.\[99,\[100\]\] Associations have also been reported between keratoconus and osteogenesis imperfecta,\[101\] mitral valve prolapse,\[102–104\] Down’s syndrome,\[105–108\] Leber congenital amaurosis (LCA),\[109,\[110\]\] and eye rubbing.\[106\] Mutations in the \textit{VSX1}\[65\] gene have been implicated in the pathogenesis of keratoconus; however, results from initial reports have not been replicated or confirmed.\[111\]

Recently, a locus for a putative disease-causing gene for keratoconus has been indentified on 13q32.\[112\]

**X-linked megalocornea (MGC1, OMIM #309300)**

Megalocornea is a heritable condition defined by bilateral enlargement of the corneas (greater than 12.5 mm in diameter). Most cases show X-linked inheritance, although there
have been reports of autosomal dominant and autosomal recessive transmission. Arcus juvenilis and mosaic corneal dystrophy are frequently associated with megalocornea.[113] The gene causing megalocornea in one pedigree was mapped with linkage analysis to chromosome Xq12-q26.[114] The megalocornea disease gene at this locus is unknown.

**Cornea plana (CNA2, OMIM #217300, gene KERA)**

Cornea plana is a rare, heritable disorder in which the cornea has an abnormally flat curvature resulting in extreme hyperopia. Mutations in keratocan (KERA) which encodes a corneal proteoglycan, have been associated with autosomal recessive cornea plana (CNA2).[115] The gene or genes that cause autosomal dominant cornea plana (CNA1, OMIM #121400) have not yet been mapped to chromosomal loci and mutations associated with CNA1 have been excluded in CNA2.[116]
Conclusion

Recent molecular genetics research has provided startling insights into the pathogenesis of corneal disease showing that what were once thought to be disparate phenotypes are caused by mutations of the same gene and mutations in apparently unrelated genes could cause clinically indistinguishable disease. These molecular genetic discoveries have allowed for a reclassification of the corneal dystrophies and continue to provide greater understanding of corneal diseases and fertile soil for continued research.
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Vision scientists have examined the shape of the cornea for over 300 years. The study of corneal curvature began in 1619 when Father Christoph Scheiner compared the reflections of window panes on marbles of known size to those reflections from the cornea to determine corneal curvature.[1] In 1839, Kohlraush introduced a telescope with adjustable mires to measure reflected images from the cornea.[2] In 1854, Helmholtz extended this work and constructed a complex instrument that he called an ophthalmometer,[3] which measured the curvatures of the cornea, the lens, and other dimensions of the eye. In 1881, Javal and Schiotz introduced a simplified ophthalmometer for clinical use.[4] This simplified the cumbersome Helmholtz instrument into a practical clinical tool to rapidly measure corneal astigmatism. The designation of the Javal instrument as an ophthalmometer was a misnomer since the adaptation of the Helmholtz instrument measured only the cornea and not the other parts of the eye.[5] Hence, the instrument was renamed the keratometer, which to this day is used to measure the curvature of the principal corneal meridians. In effect, many topographers currently used are based on the basic principles used by keratometers: the measurement of patterns reflected from the corneal surface. However, the real cornea is not well described by keratometry, which measures the curvature of the two principal meridians from only four locations on the cornea. Corneas are radially asymmetric, aspheric, and may be irregular.[6] This is particularly true for corneas of patients who have undergone keratorefractive surgery.

The rapid expansion of keratorefractive surgery over the last three decades has highlighted the need to measure corneal shape over a large area. Measuring corneal topography is now a fundamental part of evaluating patients preoperatively as well as understanding the basis for visual complaints after surgery. In this chapter, fundamentals of keratometry and corneal topography will be explored and their applications and limitations will be discussed. The discussion is illustrated with examples of corneal topography commonly seen in clinical practice.
Keratometry

Keratometry describes a method to measure the two principal meridional radii of curvature of the central cornea. The keratometer does this by measuring the size of mire reflections from the corneal surface. Each meridian of the central cornea is considered a section of a spherical convex reflecting mirror. From geometric optics, the magnification equation is:

\[
\frac{h'}{h} = \frac{f}{x}
\]

where \(h'\) is the height of the virtual image of the mires, \(h\) is the physical height of the mire, \(f\) is the focal length of the mirror, and \(x\) is the distance from the mires to the principal focus.

The focal length \(f\) of a convex mirror is given by:

\[
f = \frac{r}{2}
\]

where \(r\) is the radius of curvature. Then

\[
\frac{h'}{h} = \frac{r}{2x}
\]

or

\[
r = \frac{2xh'}{h}
\]

Since the distance \(x\) from the mires to the focal point of the mirror surface is not known, the distance from the mire to the surface is used \(d\).

\[
r = \frac{2dh'}{h}
\]

This dimension, \(d\), is generally called the working distance and is a reasonable approximation as long as the distance from the mire to the surface is large compared to the focal length of the surface. Since the distance \(d\) from the keratometer to the surface (the cornea) can be fixed and the height of the mire \(h\) is known, one can solve for the radius of curvature \(r\) by measuring the size of the virtual image \(h'\). The keratometer is calibrated with measurements on test balls. Since the central area of the normal cornea is nearly spherical, and the keratometer is designed to measure the curvature of the principal meridians at a 3–4-mm diameter, K readings can provide accurate measurements of curvature and cylinder within the pupil.

Although the radius of corneal curvature is useful for fitting contact lenses, corneal power is generally more useful for diagnostics and intraocular lens (IOL) calculations. Corneal power can be calculated from the radius of curvature:

\[
P = \frac{(n' - n)}{r}
\]

where \(P\) is the corneal power, \(n'\) is the corneal index of refraction and \(n\) is the index of refraction of air (1.000), and \(r\) is the corneal radius of curvature in meters. The index of refraction of the cornea is generally taken as 1.376. However, because the keratometer attempts to estimate the total refractive power of the cornea and not just the air–tear interface, a value of 1.3375 is generally used instead of the corneal index of refraction. This is called the keratometric index, an effective index of refraction, which accounts for the small negative power introduced by the endothelial surface. On average, the anterior cornea has a
refractive power of +48 diopters (D) of convergence and the posterior cornea of −5 D of divergence. Thus, equation (5) becomes:

\[ P = \frac{1.3375 - 1.000}{r} = \frac{0.3375}{r} \]

The keratometric index makes several approximations including the assumption of spherical radii of curvature for the anterior and posterior corneal surfaces. Corneal surgery or pathology that results in a significant alteration in corneal thickness or changes in the curvature of the anterior and/or posterior cornea will introduce errors in this power relationship. For this reason, K-values used in intraocular lens calculations should not be measured with keratometry in eyes that have undergone keratorefractive surgery or are irregular for any other reason; average central corneal power indices are available on several corneal topographers for this purpose.
Corneal Topography

To extend measurement coverage of the corneal surface beyond the capabilities of the keratometer, the modern corneal topographer emerged via a series of instrument developments that began with the keratoscope, followed by the photokeratoscope, and finally the videokeratoscope. The videokeratoscope combines video capture of corneal images with computer processing to provide maps of the corneal surface power distribution. This is now called the corneal topographer. All of these use a more complete target to examine a wider area of the cornea than the keratometer. The most common target configuration used is still the circular mire pattern that characterized the Placido disk introduced by Antonio Placido in 1880.[8] The Placido disk consists of a target with concentric rings that alternate black and white mires with a central aperture through which the clinician could view the virtual image.

Photokeratoscopes that captured the Placido reflections from the corneal surface were useful for demonstrating irregular astigmatism in corneal grafts and the surface distortion in moderate keratoconus, but only qualitative information could be obtained, and the more modest corneal shape distortions that had a visual impact could not be detected by simple visual inspection of the mires (Fig. 13.1). Doss and associates published one of the first methods for calculating corneal power quantitatively from a photokeratoscope.[9] Klyce extended this approach and explored methods of representing the results to a clinical audience.[10] Further advances led to instrumentation that resulted in the video capture of Placido disk images, the automatic detection of the mires, and calculation of corneal shape and power distribution. These led to the development of the modern corneal topographer and the representation of corneal power with the color-coded contour map introduced by Maguire and associates.[11]
The development of widespread keratorefractive surgery in the 1980s was the impetus for this progress in corneal topography. The introduction of the personal computer (PC) helped make these systems available and useful in diagnosing pathology as well as understanding visual complaints with the early techniques of radial keratotomy and photorefractive keratectomy. Corneal topography has been essential to the development and evaluation of new techniques for refractive surgery. In the screening of refractive surgical candidates, corneal topography has become the standard of care.

Since the introduction of Placido-based topography, other methods to measure corneal shape have been explored. These technologies included scanning slit technology, raster stereography,[12] scanning high-frequency ultrasound,[13] holography, Fourier profilometry, and optical coherence tomography.[14] Two approaches are in general use currently: the Placido disk or reflection-based topographers, and the scanning slit-based tomographers.*

**Placido disk-based topographers**

Corneal topographers capture their images of the cornea with digital video cameras. The resolution of the different Placido disk-based topographers depends on the number and width of the mires. In general, the larger the spacing between the mires, the more interpolation between samples data points will be necessary. However, in clinical practice, ‘fine’ mire and ‘wide’ mire topographers can produce very similar topography displays (Fig. 13.2).
There are two different design types of Placido topographers: those with large-diameter targets and those with small, cone-type targets. The former have longer working distances and their respective larger targets help minimize issues related to alignment and focusing. Instruments with smaller targets and shorter working distances generally have sensitive focusing aids to achieve accuracy and repeatability. Images from the systems with larger targets are more likely to be affected by shadows from the nose and eyebrows that can produce inferonasal and superior areas that are be analyzed.

Placido disk reflection topographers are sensitive to disruptions in the tear film. Excessively increasing the amount of time between a blink and the time of capture can cause normal spherocylindrical corneas to exhibit irregularities.[15] The corneal topography examination should be done before drops are used or intraocular pressure is measured to maintain a regular, intact tear film. However, accommodation will not affect corneal topography in normal or keratoconic corneas,[16] while there may be a small effect of eyelid pressure on the corneal shape.[17] The eyelids cause a small amount of corneal distortion, primarily confined to the superior and inferior extrapupillary regions, and therefore have minimal effect on central corneal shape during the blink.

**Slit scanning tomography**

Corneal shape and thickness can be measured with the scanning slit beam. Placido topography utilizes the first Purkinje image reflected from the corneal surface. Under these conditions, the second Purkinje image from the endothelial surface of the cornea is not detectable with current instruments. With slit beam technology, light scatter from both corneal surfaces can be viewed, as is routinely done during a slit lamp examination. Because the elevation of each surface can be measured directly with slit beam technology,
no shape approximation errors should arise, as with some of the early Placido disk-based devices. Slit scanning tomographers project a series of slit beams at regular intervals. The successive images are captured with a digital camera over the course of 1–2 seconds. Image analysis is used to determine locations of points on the corneal surfaces. These data can then be processed to determine shape characteristics of both corneal surfaces as well as the thickness of the cornea. There are several instruments commercially available that use this method. The more widely distributed are the Orbscan (Model IIz, Bausch & Lomb, Rochester, NY) and the Pentacam (Model HR, Oculus, Inc., Wetzlar, Germany). The Orbscan is a hybrid system — both a topographer and a tomographer — that uses Placido disk technology to display conventional corneal topography, while the Pentacam derives corneal topography from the slit beam elevation data. Both instruments measure anterior and posterior surface elevations with scanning slits for the determination of corneal thickness. The Orbscan uses the projection of slit beams at 45-degree angles 20 times on each side of the video axis. This is done in two 0.75-second intervals. Note, however, that the cornea is in constant motion from microsaccadic movements and thus the entire image acquisition should ideally take less than 30 ms.

The Pentacam uses a scanning slit but with Scheimpflug optics, which increases the depth of focus. In doing so, simultaneous imaging of the cornea, lens, and iris is possible; this permits corneal, anterior chamber, and lens geometry to be imaged and analyzed. The Scheimpflug camera and a monochromatic slit light source rotate around the eye 180 degrees in 2 seconds, producing 25 images of the front and back surfaces of the cornea. Up to 25 000 elevation points are used to give a 3-D representation of the cornea. As with the Orbscan, the data must be translated and aligned to reduce errors due to eye movement during acquisition.

* Strictly speaking, when slit data is used to calculate corneal power from shape measurements, this process is tomography.
Calculations and Surface Reconstruction

**Placido disk**

Since curvature is generally calculated along meridians, the image of the Placido target is digitized and the radial positions of points along the mires are determined every 1 degree (see Fig. 13.2A,C). Computer algorithms are then used to calculate corneal curvatures that are consistent with the shapes of the mires. Again, close spacing of the mires will indicate a relatively steep portion of the cornea, while broadly spaced mires overlie regions of low power. This relationship is very clear in Figure 13.2 (compare the mires and curvature displays within the superior portion of the pupil to those within the lower portion of the pupil in C and D). The algorithms used to calculate corneal curvatures vary considerably among the different Placido-based topographers, but with proper consideration, good accuracy can be achieved.[18]

The keratometer suffers from the assumption that corneas are spherocylindrical. Corneal topographers are also usually calibrated using spherical test surfaces. However, modern calibration and the test surfaces used include shapes that are spherical, elliptical, and toric.[19] Using these can help evaluate the adequacy of certain corneal topographers in faithfully reproducing the topography of aberrated corneas. Obscure details of corneal topography that are important to understand certain aberrations that occur after corneal surgery need to be presented accurately in order to manage difficult cases. In particular, accurate portrayal of corneal shape is essential as excimer laser surgery adopts algorithms for corneal topography-guided ablations.

**Slit scanning technology**

The scanning slit systems capture light that is diffusely scattered from the two corneal surfaces and the obtained elevation data are used to calculate power in diopters from equation (5) above. For these topographers to calculate curvature with sufficient accuracy to differentiate between two curves that differ by 0.25 D, the resolution of this system needs to be less than 1 microns (µm).[20] However, this is difficult to achieve since the sensitivity of the slit scanning systems to changes in corneal power is some 20 times less than that of a Placido-based corneal topographer.
An additional challenge using slit-derived elevation data for calculation of curvature and power for the posterior corneal surface is that there is a lack of calibration surfaces. These must have dual surfaces from materials that mimic corneal scattering; this presents a problem.[21] The accuracy of slit-based posterior elevation maps and pachymetric maps has not yet been resolved because of this.
Presentation Methods

Color-coded maps

A graphic representation of the topographic distribution of local corneal dioptic power values can be achieved by presenting the results as a color-coded topographic map.[22] The ‘warmer’ colors represent higher dioptic powers (steeper curvatures), while the ‘cooler’ colors are used to represent the lower dioptic powers (flatter curvatures). Similar color-coded maps can be used to present changes in elevation.
The Normal Cornea

In 1989, Dingeldein and Klyce studied normal corneas with uncorrected vision of 20/20. They found that the cornea does flatten towards the periphery and that the rate of peripheral flattening varies significantly amongst individuals. They also found that the color-coded contour maps of each individual have a unique pattern, like a fingerprint. Moreover, the topographies of fellow eyes tend to be mirror images of each other (enantiomorphs) as shown in Figures 13.3 and 13.4. A final characteristic of normal corneas is that even with these variations between individuals, normal corneas have relatively smooth power contours.

Fig. 13.3  Color-coded contour map from Placido-based topographies of an individual's normal corneas. Note the scale and color palette used are the universal standard scale from Smolek et al. Note the nonsuperimposable mirror image symmetry. The underlying image of the eye is important to relate topographic features to the corneal surface.
The scale used to display corneal topography is extremely important both for diagnostic purposes as well as to appreciate features that might reduce vision. Most corneal topographers have implemented adjustable scales that allow the user to increase or decrease the sensitivity as well as the range of dioptric power. In addition, most corneal topographers allow the user to choose a self-adapting or normalized scale which will display every cornea with the same color range irrespective of its condition. The use of smaller dioptric intervals and a narrower range of powers may lead to a distracting amount of noise. On the opposite extreme, scales with broad dioptric intervals can lead to smoothing and masking of irregular astigmatism. The most effective color palette and dioptric interval has been evaluated in several studies (the Absolute Scale,[11] the Klyce/Wilson Scale,[22] and finally the Universal Standard Scale[24]). The Universal Standard Scale has been adopted by the ANSI standard on corneal topography.[19] With this scale, features such as regular corneal astigmatism can be visualized (Fig. 13.5) while masking corneal details irrelevant to interpretation. This scale is compared to other scales and different methods for corneal power calculation in Figure 13.6.
Fig. 13.5 Placido-based topography of astigmatism. Top: with-the-rule cylinder both eyes. Bottom: against-the-rule astigmatism. Note the classic bow tie shape of astigmatism on Placido-based topography which makes it easily recognizable.
Fig. 13.6 Using different scales and different power calculations can confuse interpretation of corneal topography. The topographies in this figure are all presentations of the same data. (A) Normal corneal topography presented with axial power and the 1.5-D contour interval Universal Standard Scale.\(^{[1]}\) (B) Axial power map with 0.5-D contour intervals. This gives the appearance of irregular astigmatism in this normal cornea. (C) Power map using tangential or instantaneous power calculation. Again, details are shown that confuse interpretation. (D) Power map using the calculation of refractive corneal power with Snell’s Law. This true power calculation is useful for ray tracing, but obscures the relation of power to corneal shape: that the cornea flattens toward the periphery.
Axial Curvature Maps

The axial dioptic power map was the first standard method for presenting corneal topography. Axial power represents corneal curvature in a way similar to keratometry. The size of mires reflected from the corneal surface are determined and analyzed as if emanating from a sphere. Axial power is useful clinically because it relates corneal power to corneal shape. The cornea has a prolate shape, which means that it is steeper in the center than in the periphery; this helps compensate for spherical aberration. Axial power presents itself in the same manner: that is, higher in the center than in the periphery. For these reasons, axial power maps are the preferred clinical default for presenting corneal topography for routine diagnostic and screening purposes.
Refractive Power Map

Most clinicians are used to thinking of the cornea in terms of refractive power since that relates to corrective lenses as well as amounts of correction in refractive surgery. While axial power maps convey shape information, they are not a true representation of the refractive power of the cornea except centrally over the photopic pupil. Refractive power is calculated with Snell's Law; hence, as the angle of incidence of the incoming rays increases in the corneal periphery, the power increases. When this method is used to calculate corneal power, the normal cornea will have a higher calculated power peripherally than in the center (see Fig. 13.6D). This is due to the natural residual spherical aberration of the cornea. A spherical cornea would exhibit an even higher peripheral refractive power than the normal prolate cornea. The measurement of corneal spherical aberration is of importance in the choice of an aspheric intraocular lens.

Caveat: With the refractive power map, as noted above, there is an increased calculated power in the corneal periphery. Keratoconus and pellucid marginal degeneration are frequently expressed as inferior peripheral steepening; the use of the refractive power map can mask these pathologies. An example of such a case that resulted in ectasia following LASIK surgery is illustrated in Figure 13.7.
Fig. 13.7  The refractive power map can obscure the peripheral steepening characteristic of keratoconus and pellucid marginal degeneration. (A) Preoperative refractive power map of patient who developed ectasia subsequent to LASIK. The map can be interpreted as against-the-rule cylinder, but the inferior steepening is muted by the method. (B) Axial power map of a similar topography clearly showing the inferior steepening and ‘C’ shape in topography characteristic of pellucid marginal degeneration.
Instantaneous or Tangential Power Map

Another method for calculating corneal power is through the use of the instantaneous radius of curvature or tangential power map. The second derivative of the shape of the surface is used to calculate curvature in this method,[20] which can introduce mathematical noise or fluctuations in power values. The instantaneous power map is not recommended for routine clinical use because the measurement noise can obscure the true optical quality of a cornea. For example, the normal cornea shown in Figure 13.6C with this type of power calculation appears irregular even though the cornea has 20/15 visual potential. However, tangential power maps are extremely useful in the demonstration and measurement of the optical zone size in modern refractive surgery as they emphasize transition zone power changes (Fig. 13.8).

![Fig. 13.8 Corneal topography of a −2.00 D LASIK treatment. (A) With the standard axial map, details of the treatment are obscure. (B) Using the tangential map, the transition zone between the treated area and the peripheral cornea is clearly demarcated.](image-url)
Certain changes in corneal topography with time can be usefully explored by subtracting one map from another. For example, when following a keratoconus patient, it is of paramount importance, particularly with current corneal cross-linking therapy, to make a prediction as to whether a cornea should be stabilized before functional vision is lost. Progression of keratoconus can be followed with difference maps and the consequences of refractive surgery evaluated (Fig. 13.9). When using differences maps, careful registration of the data may be necessary to reduce the positional errors due to microsaccades.
Fig. 13.9 The difference map can be used to confirm the power change induced by laser treatment. (A) Preoperative map of a cornea showing with-the-rule corneal cylinder. (B) Note the circular nature of the profile in the postoperative LASIK map. (C) The difference clearly shows the characteristics of the toric ablation used to reduce cylinder.
Elevation Maps

Except in the case of advanced disease such as keratoconus, distortions of corneal shape displayed with elevation data cannot be seen without amplification. Hence, elevation maps are generally displayed with reference to some standard shape. Commonly, the best-fitting sphere or toroidal surface is subtracted from the elevation data. However, there is no single known geometric shape that can approximate the cornea over its entirety.[25]

The Orbscan allows a choice of reference surface type, shape, size, and alignment as well as elevation direction. The Pentacam allows the user to use a best-fit sphere or an ellipsoid. Elevation maps generally use green to indicate zero difference from the reference surface. Warmer colors (red) represent areas higher than the reference surface and cooler colors (blue) areas below the reference surface. For anterior and posterior elevation maps, Tanabe et al. addressed the issue of scale and suggested that 10-µm and 20-µm scales for the anterior and posterior elevation maps, respectively, appear most suitable.[26]

It has been suggested that changes in posterior corneal elevation might be the initial changes that can be detected in keratoconus.[27] Mean posterior corneal elevation has been studied in an effort to distinguish keratoconic corneas from normal corneas.[28] Posterior elevation values can be used to distinguish normal and keratoconic corneas, but posterior elevations are not sensitive enough a measure to separately classify forme fruste keratoconus and normal corneas.
Pachymetric Maps

The slit-based tomographers have shown that the thinnest areas of the corneal stroma are generally inferotemporal to the fixation-reflex axis. Orbscan pachymetric central measurements tend to correlate with ultrasound measurements although they are consistently thicker by about 30 µm.[29],[30] This offset may change for corneas after refractive surgery,[31],[32] very thick corneas,[33] and for corneas in their periphery.[34] The Orbscan allows the user to shift all pachymetric measurements by a single acoustic factor in order to more closely match ultrasound measurements. However, the relationship between Orbscan pachymetry and ultrasound pachymetry may not be proportionate throughout the corneal thickness. Therefore, a single acoustic factor cannot be used on all measurements made by the Orbscan to equate the measurements made by the ultrasound pachymeter.[34],[35] The factory-calibrated Pentacam may correlate better with ultrasound than the Orbscan after refractive surgery.[36] The important clinical advantage of slit scanning technology is that it provides a data-rich global map of corneal thickness which exceeds the normal capabilities of manual ultrasound measurements (Fig. 13.10).

Fig. 13.10 Correlation of axial topography (A) and pachymetry (B) with the Pentacam. The dashed circle indicates the pupil margin and its center is marked with a cross. The filled white circle is the axis of the instrument while the empty black circle indicates the thinnest point.

Quantitative indices
Color-coded contour maps are very useful for diagnostics by recognition of patterns and by color association. Examples include the bowtie pattern that denotes corneal astigmatism and the red areas associated with moderate to advanced keratoconus cones. However, in order to derive quantitative measurements from corneal topography examinations, a number of statistical indices have been derived. The first of these was Simulated Keratometry or the SimKs SimK1 and SimK2. To mimic keratometry, these indices report the power derived from the corneal topography principal meridians at the four points 3–4 mm apart, just where the keratometer mires would fall on a cornea. SimKs are important indices the clinician has been trained to use for numerous tasks from diagnostics to contact lens fitting.

Standard keratometry agrees with SimK values. Placido disk-based measurements of surfaces with marked and frequent curvature changes can provide repeatable measurements. On the other hand, slit scanning systems can have artifacts of asymmetric topography which can confuse interpretation.

Keratometry by itself is not a good predictor of the visual potential of the eye as it does not evaluate the central cornea. Moreover, the simple visual inspection of irregularities in the power contours of a topography map cannot be used directly to predict their effect on visual acuity. The irregularity of the corneal topography over the pupil was developed with the Surface Regularity Index (SRI). SRI is correlated to potential visual acuity and is a measure of local fluctuations in central corneal power. When the SRI is elevated, the corneal surface ahead of the entrance pupil will be irregular, leading to a reduction in best spectacle-corrected visual acuity. High SRI values are found with dry eyes, contact lens wear, trauma, and penetrating keratoplasty. Large amounts of corneal astigmatism that are induced with corneal grafts can be corrected to some extent with cylindrical lenses, but it is the irregular astigmatism (higher-order aberrations) that is often the major source of visual loss in these patients. SRI has been implemented on a number of corneal topography systems including models produced by Tomey Corp. (Nagoya, Japan) and by NIDEK, Inc. (Gamagori, Japan). Indices such as the Central Irregularity Measure, or CIM, displayed by the Humphrey Atlas (Carl Zeiss Meditec, Inc., Jena, Germany) corneal topographer have been developed as a similar means to understand the visual impact of irregularities in corneal topography. The Orbscan offers a surface irregularity index from its Placido data as well which is the statistical sum of the standard deviations of the curvatures in the mean and astigmatic power maps over a certain area.

Fourier transforms and Zernike polynomials are also quantitative descriptors of corneal surface and can be used to calculate the aberrations of the cornea. Point spread, optical transfer, and wave aberration functions have been used to measure optical quality of the eye, and these can be obtained from corneal measurements. Zernike coefficients can also be used to describe corneal aberrations. These are derived from conversion of corneal topography to corneal wavefront; the method is very useful for the presentation and evaluation of corneal optics.
Fig. 13.11  Corneal wavefront analysis of a LASIK procedure with the NIDEK Magellan topographer. Using an aperture of 4.5 mm simulating the pupil of this eye under photopic conditions, vision is 20/20 (fourth line up from the bottom of the Snellen Chart segment). The blue circle outlines the pupil on the corneal topography map in the upper left. Zernike analysis allows display of (left to right) spherical aberration, coma, trefoil, residual higher-order aberrations, and total higher-order aberrations. The color scale in the upper right of the figure has a range of $-6.5$ to $+6.5$ mm of RMS wavefront error and corresponds to the wavefront displays. The lowest set of displays is a polychromatic representation of the point spread function for each of the individual aberration displays.
Corneal topography indices and screening methods

Topographic indices have been developed and used for corneal classification and screening. One of the first topographic indices developed for keratoconus detection is the I-S value introduced by Rabinowitz and McDonnell.[43] This index is helpful to recognize levels of corneal topographic asymmetry that are abnormal and are consistent with suspect keratoconus or clinical keratoconus. Additional sophistication was added with the recognition that central cones would not be detected with the I-S index, and that some keratoconus patterns involved skewing of the radial axes.[44] Since the topography of disorders characterized by corneal ectasia such as keratoconus and pellucid marginal degeneration can take several appearances (Figs 13.13–13.18), other indices were developed to detect specific patterns seen in keratoconus, and artificial intelligence techniques were used to recognize not only keratoconus but also several other abnormal corneal conditions (Fig. 13.19).[45–49]
Fig. 13.13  The right eye of this patient is interpreted as keratoconus suspect while the left eye is advanced enough so that thinning or other clinical signs would confirm the diagnosis of clinical keratoconus.

Fig. 13.14  ‘Unilateral’ keratoconus. The right eye has a classic inferior cone, while there are no obvious signs of a cone in the left eye.
Fig. 13.15 Bilateral keratoconus in a patient who has marked skewing of the radial axes.

Fig. 13.16 Central keratoconus with a nearly circular pattern in the right cornea and a truncated bow tie in the left. Even if the bow tie were perfectly linear and symmetric, the truncated sign is important to recognize as one of the hallmarks of keratoconus.
This patient may be thought to have pellucid marginal degeneration in the right eye (note the abortive ‘C’ shape in the topography) and classic keratoconus in the left eye. However, the diagnosis of pellucid can only be made through careful examination of the thinning pattern: arcuate perilimbal thinning is pathognomonic for pellucid.

Unilateral pellucid marginal corneal degeneration. Note the characteristic ‘claw’ or C-shaped pattern in the left eye.
Fig. 13.19 This cornea has more asymmetry than would be found in the variations of normal corneal topography. It is recognized as having astigmatism, but also has characteristics associated with suspect keratoconus and clinical keratoconus. NIDEK Magellan topographer. This corneal topography has the characteristics associated with a cornea with 2.01 D of cylinder (AST = 99.0%). There are also features of this topography similar to keratoconus suspect (KCS = 91.8%). There are also features of this topography similar to clinical keratoconus (KC = 42.5%) with a severity index (KSI) of 2.7%.

As with most tests, patient history is of paramount importance. Contact lens warpage can mimic mild keratoconus and needs to be ruled out.[50] Patients who have with-the-rule corneal astigmatism are particularly at risk for the development of pseudo-keratoconus from contact lens wear, particularly from the wear of rigid lenses. To differentiate between contact lens-induced corneal asymmetry and true keratoconus, it is necessary for the patient to discontinue contact lens wear completely, and to be reexamined at intervals of 2–3 weeks until the corneal topography stabilizes. In general, if the asymmetry is due to contact lens wear, the cornea will become symmetrical; if the asymmetry is indeed owing to keratoconus, the corneal topography will become more asymmetric as contact lens wear tends to regularize an asymmetric cornea. Relaxation of the cornea from contact lens warpage can be a slow process taking weeks or months depending upon the degree of corneal distortion.[51] An example of this is shown in Figure 13.20.
Fig. 13.20  Corneal warpage resembling a cone and cornea after removing lens for 6 weeks.
Conclusion

Corneal topography now defines the standard of care in anterior segment surgery. Used wisely, it has guided the development of corneal surgery to reduce irregular astigmatism in corneal grafts, to minimize cataract incision size and to guide their placement, to understand the sources of night vision complaints after refractive surgery, to report the optical quality of the corneal surface in health, disease, and after surgery, and importantly in the early detection of corneal disease. Used well, corneal topography is a basic tool to prevent patients with signs of keratoconus or pellucid marginal degeneration from undergoing traditional laser refractive surgery.

In addition, describing corneal topography is essential to the understanding of its role in the optics of the eye. With over two-thirds of the total power of the eye, the corneal surface shape plays a major role in optical performance. The normal cornea is by no means a perfect optical component; yet some amount of astigmatism, residual spherical aberration, and even coma that are not compensated by the lens can be accommodated by neural adaptation. This becomes important when managing refractive corrective procedures, since eliminating all the measurable aberrations of the eye may not be acceptable to the patient, or even optically ideal even accounting for amelioration by neural readaptation. The new frontier in the management of refractive imperfections may be to discover what the optimal ocular wavefront should be for a given patient; most likely it will contain significant higher-order aberrations. The impressive feature is that the clinician now has the tools to produce the desired optical wavefront – once we discover what it is!
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Chapter 14 – Specular Microscopy

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The specular microscope is unlike most microscopes. Instead of imaging light transmitted through a substance, the specular microscope images light reflected from an optical interface. The optical interface giving the most interest is that between the corneal endothelium and the aqueous humor. The specular microscope, however, can also be used to image the corneal epithelium, stroma, and crystalline lens. Depending on the instrument used, the projected light can be in the form of a stationary slit, a moving slit, or a moving spot. The optical design can be either confocal or nonconfocal. The design of the equipment can be either contact or noncontact.

The young, normal corneal endothelium as seen by specular microscopy (Fig. 14.1) shows a quasi-regular array of hexagonal cells, all having nearly the same size. With aging, trauma, and corneal disease, this regularity is lost. In general, the more the endothelium differs from the normal appearance, the more difficult it is for the endothelium to maintain corneal clarity.

The first direct visualization of the endothelium was demonstrated by Vogt in 1918.[1] Using the slit lamp microscope, Vogt demonstrated that the endothelial mosaic could be visualized in the axis of the reflected light. In 1924, Graves used similar methods to describe Fuchs’ endothelial corneal dystrophy in elderly patients.[2] It was not, however, until 1968 that David Maurice described the first laboratory specular microscope that could be used to study excised living corneas.[3],[4] Modifications of this specular microscope were made by Laing et al.[5] and later by Bourne and Kaufman,[6] allowing routine clinical examination and photography of the corneal endothelium.

Specular microscopes have evolved from interest in wider field and higher-resolution contact microscopes[7],[8] to narrower field, high-resolution noncontact microscopes equipped with software to obtain automated or semiautomated determination of endothelial cell density and morphology, including coefficient of variation and percentage of hexagonal cells.[9–13] Currently, specular microscopes are available for both clinical and eyebank use.

![Fig. 14.1](image)

Fig. 14.1 Normal corneal endothelium as photographed by specular microscopy. A quasi-regular array of hexagonal cells, all having nearly the same area, is seen.

Optical Principles of Specular Microscopy

To properly interpret endothelial photomicrographs obtained clinically, it is helpful to understand the optical principles of the specular microscope. Light striking a surface can be reflected, transmitted, or absorbed. Generally, some combination of the three effects occurs. Of primary importance in clinical specular microscopy is the light that is reflected specularly (i.e. 'mirrorlike'), where the angle of reflection is equal to the angle of incidence. It is this light that is captured by the specular microscope and forms the image of interest.

For the normal transparent cornea, most visible light is transmitted through the cornea. Some light, however, can be absorbed by the tissue, and some can be reflected by various objects having a different index of refraction than the surrounding corneal tissue. Some of the reflected light is scattered (reflected at arbitrary angles) by cellular organelles. With an increase in corneal edema, the fraction of scattered light increases and can become the dominant element, giving rise to a 'hazy' cornea.

As light passes through the cornea, it encounters a series of interfaces between optically distinct regions. At each interface, some light is reflected back and some is transmitted deeper into the cornea. The greater the difference in index of refraction between the regions, the greater the intensity of reflected light. The more edematous the tissue, the more light is scattered. A portion of this reflected light is collected by the objective lens of the
specular microscope and forms, at the film plane of the microscope, an image of that part of the cornea on which the instrument is focused (Fig. 14.2).

Laing et al. have described the components of the images formed by reflected light from various interfaces in the cornea. Figure 14.3A shows a narrow slit of light from a contact specular microscope that is focused on the posterior corneal surface. Most light is reflected at the major optical interfaces, labeled 1, 2, and 3. The greatest index of refraction exists between the objective lens and coupling fluid. Therefore, much more light is reflected from this interface (0.36%) than the interface formed by the coupling fluid and epithelium (0.025%) or, most importantly, the interface formed by the endothelium and aqueous humor (0.022%).

**Fig. 14.2** Pathway of light from its source in the clinical specular microscope back to the film plane of the same instrument. Although both epithelium and endothelium are shown in focus on the film plane of the graphic representation, in practice only one layer is in focus at any one time because of the restricted depth of field of the specular microscope.
According to Laing, if the beam is narrowed sufficiently, four zones of reflection can be seen. Zone 1 is the brightest region and is formed by the interfaces formed by the lens, coupling fluid, and epithelium. Zone 2 is a larger region and represents light reflected from the stroma. Zone 3 is the endothelial region, and zone 4 represents light reflected from the aqueous humor. Unless considerable debris is present, little light is reflected from the aqueous. As a result, zone 4 is usually dark. The boundary, then, between the endothelial region (zone 3) and the aqueous (zone 4) is almost always dark and is termed the ‘dark boundary.’ In contrast, some light is scattered from the stroma (zone 2), making it brighter than the endothelial region (zone 3). The boundary, then, between the endothelial region (zone 3) and the stroma (zone 2) is usually bright and is termed the ‘bright boundary.’ When examining specular photomicrographs taken with a stationary slit of light, one can usually identify a brighter portion of the image (the bright boundary which, in the stationary slit images provided in this chapter, is the upper portion of the image) and a darker portion of the image (the dark boundary which, in the stationary slit images provided in this chapter, is the lower portion of the image).

If the angle of incidence of the illuminating source is increased, less overlap occurs and a wider slit can be used. As a result, a larger field of endothelial cells can be seen (Fig. 14.3B). The wider beam, however, illuminates more of the corneal stroma and epithelium, resulting in a greater amount of scattered light. The increase in scattered light from tissue overlaying the endothelium results in a decrease in contrast, obscuring endothelial cell detail. A washed-out picture results with a decrease in contrast and loss of cellular definition. Furthermore, as the angle of incidence is increased, normal endothelial cells appear shortened in one direction. This distortion should be compensated for in morphometric analysis.

With widefield scanning slit or scanning spot confocal microscopes, the slit or spot is made very small to provide less interference and increased image quality. With the help of sensitive recording systems, the spot or slit can be scanned over the tissue and recorded to provide a high-quality image and a larger field of view. When using such equipment, dark and bright boundaries are generally no longer identifiable. These advancements have improved the ability to visualize the endothelium in edematous corneas and have allowed better visualization of stromal nerves and keratocytes. However, with the development of commercially available confocal microscopes, the limitations of the specular microscope to image with the thick cornea have been overcome by confocal technology (see Ch. 15).
Commercially available contact and noncontact specular microscopes are easy to use with little, if any, patient discomfort. To obtain good patient cooperation and ultimately acquire quality images for analysis, it is important to first explain the procedure to the patient. The positioning of the head and establishing patient comfort are key to minimize movement and allow optimal images of the endothelium.

In noncontact specular microscopy, instructing the patient to blink to wet the cornea and then to hold still just prior to image capture improves image sharpness. Also of note with noncontact instruments is that imaging a thickened cornea will sometimes fail in the automated capture mode and may require the operator to use a manual mode. If using a contact specular microscope, one or two drops of proparacaine anesthetic should be used before the eye is contacted. Once the cornea is contacted, a few epithelial irregularities may be seen; however, these usually disappear within a few hours. Positioning the eye in a straight-ahead position is best achieved with an internal fixation point. Optimal photographs are obtained when the cornea is relatively thin and clear, with minimal scarring or edema. Light reflexes from the iris can obscure the endothelial mosaic and are best eliminated by dilating the pupil.
Standardization of Imaging Techniques

A standard approach to examining the corneal endothelium is necessary for observing change over time. When using a contact specular microscope, the beam of light is directed through the pupil to ensure the placement of the cone on the most central portion of the cornea. Systematic scanning superiorly, inferiorly, nasally, and temporally will ensure a thorough evaluation of the endothelium. Noncontact specular microscopes use internal fixation points to provide a more standardized approach to consistently imaging the central endothelium, midperiphery, and periphery. With these systems, the patient view is shifted when the technician selects the desired region.

To best evaluate small changes over time in any region, including the central endothelium, the technician should image the region three times at the same sitting and record the average of the three images' analyses. This is particularly important with the higher-magnification microscopes as so few cells of the total corneal surface are captured in the final image. It is also important for the technician to use the same image analysis method from baseline and throughout the follow-up period. Most specular microscopic studies of the corneal endothelium determine solely the endothelial cell density of the central endothelium in order to attempt to consistently examine the same endothelial cell area over time. However, this may be misleading and does not necessarily reflect the impact of a surgical procedure that primarily damages the peripheral cornea. Changes in the central corneal endothelium in both density and morphology may take some time (months, years) to be reflected. In addition, the paracentral and peripheral endothelium, in particular superiorly, has a higher cell density than the central endothelium and may assist in maintaining central endothelial density and function. Procedures in this area may therefore have an even greater impact on central endothelium over time.
Instrumentation

There are several specular microscopes commercially available, both contact and noncontact. In addition, confocal microscopes (see Ch. 15) are used clinically to capture and analyze endothelial cell density and morphology (Table 14.1). All the instruments have computer integration and semi- or fully-automated analyses. Some instruments allow manual analysis and/or adjustments to the automated analyses. The noncontact instruments use autotracking and focusing technology. Perhaps the most widely used clinical instrument in the United States is the Konan NonCon Robo Series (Torrance, CA). Using this instrument, tracking of the cornea and imaging of the endothelium are automated, requiring minimal intervention by the operator. Once the patient is aligned, the operator presses a button on the control box to start the imaging process. The optics of the instrument first objectively aligns themselves relative to the cornea by using the Purkinje images until the proper specular reflection mode is achieved. The instrument then objectively focuses back to the endothelial surface, the flash lamp is triggered, and the resulting endothelial photograph is displayed on the monitor. Other noncontact instruments image sequential images (Tomey, Inc., Phoenix, AZ) or provide a live view (HAI Labs, Inc., Lexington, MA), both automatically selecting the best images. As mentioned above, a limitation of the noncontact instruments is the difficulty of getting quality images when the cornea is thickened. HAI offers a contact specular microscope with a focal depth of 0–999 µm. Though less comfortable for the patient because it is a contact instrument, it obtains sharp, widefield images regardless of corneal thickness or disease.

Table 14.1 -- Summary of currently available specular microscopes and the key advertised features of each instrument

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Type</th>
<th>Model</th>
<th>Analyses options</th>
<th>Advertised features</th>
<th>Sample image</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAI Labs, Inc., Lexington, MA</td>
<td>Contact</td>
<td>CL-1000xyz</td>
<td>Automated Fixed Frame Variable Frame Corners</td>
<td>- f.o.v. 250 µm × 400 µm</td>
<td>- Focal depth of 0–999 µm</td>
</tr>
<tr>
<td>Company</td>
<td>Instrument/Module</td>
<td>Features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| HAI Labs, Inc. Lexington, MA    | Noncontact CL-1000nc Automated, Semiautomated          | - Live view of endothelium  
- Automated selection of good images  
- Optical pachymetry  
- Central and peripheral points |
| Heidelberg Engineering Vista, CA| Confocal Contact Immersion Corneal Module HRT-Semiautomated | - Layer by layer imaging  
- Uniform illumination, undistorted image  
- Movie capture  
- Manual pachymetry |
<table>
<thead>
<tr>
<th>Konan Medical USA, Inc. Torrance, CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CELLCHEK Series (CELLCHEK XL, SP, RU, SP-9000 PLUS)</td>
</tr>
<tr>
<td>Automated, Semi-automated, Center, Center Flex, Corner Method, Simple Grid, Screener</td>
</tr>
<tr>
<td>- Fully automated alignment and focus, easy to use</td>
</tr>
<tr>
<td>- Five fixation points</td>
</tr>
<tr>
<td>- Optical pachymetry at all five fixation points</td>
</tr>
<tr>
<td>- Integrated computer system and analysis software</td>
</tr>
</tbody>
</table>
Nidek Fremont, CA

Confocal Noncontact Confoscan 4 Automated (20x)

- Wide measurement area (up to 1000 cells/exam)
- Fully noncontact (12-mm working distance)
- Quality imaging through opacities and corneal haze

Nidek Fremont, CA

Confocal Contact immersion (40x)

Confoscan 4 Automated

- Combines live in-vivo confocal microscopy, endothelial microscopy, and pachymetry
- Gel immersion exam
- Automated alignment
- Multiple internal fixation mires
Tomey, Inc.
Phoenix, AZ

Noncontact EM-3000 Automated

- Easy 'touch alignment'
- Serial photography (15 shots)
- Wide field – f.o.v. 250 µm × 540 µm
- Seven capture positions
<table>
<thead>
<tr>
<th>Brand/Model</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topcon Medical, Inc.</td>
<td>- Three modes for image capture (automated, semiautomated, manual)</td>
</tr>
<tr>
<td>Noncontact SP-3000P Automated</td>
<td>- Fast 3-D auto alignment and centering</td>
</tr>
<tr>
<td></td>
<td>- Five fixation targets</td>
</tr>
<tr>
<td></td>
<td>- Integrated color monitor and data analysis</td>
</tr>
</tbody>
</table>

* Not available in the USA.
Qualitative Specular Microscopy

Epithelium

Corneal epithelial cells do not normally present a flat surface suitable for specular microscopy. If a contact lens is pressed against the anterior surface of the eye, however, the epithelial cells can be flattened and can reflect light in a mirror-like fashion. Tsubota and colleagues have described contact lens systems that enable photography of the superficial layer of epithelial cells using specular microscopy. The reflection of the epithelium is reduced by the application of a soft contact lens with nearly the same index of refraction as the cornea, permitting the observation of individual epithelial cells. Combining these techniques with the higher resolution provided by modern widefield specular microscopes allows the study of even greater epithelial detail.

The normal corneal epithelium contains polygonal cells of varying brightness (Fig. 14.4). Most cells can be placed into one of three groups: dark, medium, and light. Hexagonal, pentagonal, and triangular cells may be present, but rounded, enlarged, or elongated cells are considered abnormal.

Elongated or enlarged corneal epithelial cells can be observed in wound healing processes such as penetrating keratoplasty (Fig. 14.5) and epikeratophakia. This is thought to be a result of the normal migration of cells during the wound healing response. Various other conditions can result in elongated or enlarged epithelial cells, including daily or extended soft contact lens wear, dry eyes, neurotrophic keratitis, aphakia, and diabetes. Elongated cells are also seen in keratoconus patients, especially near the cone’s apex. This has been shown to be a result of a keratoconic condition and not from the secondary changes due to contact lens wear. Since the last edition of this text, there have been several updated methods to evaluate the corneal epithelium, such as the Pentacam comprehensive eye scanner and ultrasound pachymetry.
Such techniques may be better in analyzing corneal thickness of keratoconic corneas with higher reliability than the current specular microscope systems.\cite{35,36} Confocal microscopy has also provided new insights into appearance and changes in the basal epithelium, wing cells, and superficial cells in normals and in disease (Ch. 15).

![Specular microscopic image of the corneal epithelium after penetrating keratoplasty. Note the enlargement and elongation of the cells. The bar represents 100 μm.](image)

**Fig. 14.5** Specular microscopic image of the corneal epithelium after penetrating keratoplasty. Note the enlargement and elongation of the cells. The bar represents 100 μm.

**Endothelium: miscellaneous bright and dark structures**

A number of abnormal endothelial structures can be seen with specular microscopy.\cite{37} One of the most notable is corneal guttae (Fig. 14.6A). Guttae are excrescences of Descemet's membrane. They can be seen with the specular microscope much earlier than with the slit lamp. They begin as small, dark structures, but, in time, these structures grow, often becoming larger than individual endothelial cells. The excrencences can be dome shaped, or they can assume the shape of a mushroom. If the excrencence assumes a mushroom shape with a flat top, light can be specularly reflected from the surface much like a mirror. When this occurs, a bright spot can be seen within the dark structure. In the case of Fuchs' endothelial corneal dystrophy, the surrounding endothelial cells are often abnormally shaped. Guttae, however, can also be seen in the far periphery of young individuals. In this case, they are called Hassall-Henle warts. The excrencence is usually shaped more like a dome than a mushroom, and the surrounding endothelium does not appear as abnormal.
Fig. 14.6 Miscellaneous endothelial structures. Arrows indicate features mentioned. (A) Isolated smooth excrescences (corneal guttae). (B) Multiple coalesced excrescences. Only the bright reflection from the apex of each excrescence is clearly seen. (C and D) Intracellular bright structures possibly representing cell nuclei. (E) Pigmented endothelial deposits. (F) Dark structures possibly representing endothelial cilia. (G) Intracellular dark structures possibly representing intracellular vacuoles. (H) Intercellular dark structures believed to be invading inflammatory cells.

If the excrescences are very abundant, they may begin to touch each other and coalesce. It may become difficult to identify endothelial cells. This is often seen centrally in advanced stages of Fuchs’ endothelial corneal dystrophy (Fig. 14.6B).

Intracellular bright structures can sometimes be seen (Fig. 14.6C,D).[37] They seem to be associated with stressed cells such as those seen after corneal transplantation. The size of the bright structure often corresponds to the size of the endothelial cell (the larger the cell, the larger the bright structure). This structure is felt to represent the cell nucleus.

Some bright structures span several endothelial cells and have sharp borders, indicating they are likely very near the endothelial–stromal interface (Fig. 14.6E). These structures are believed to represent pigment deposits on the endothelial surface.

Various dark structures can also be seen in specular micrographs. One such structure appears to be intracellular, is small, and has sharp borders. This structure is thought to represent endothelial cilia (Fig. 14.6F). Another intracellular dark structure is much larger and less distinct, suggesting that it is located within the cell (Fig. 14.6G). These are thought to represent intracellular vacuoles or blebs.

Specular microscopy of patients with anterior uveitis has revealed well-demarcated, dark structures usually located at endothelial cell intersections (Fig. 14.6H).[37] These structures are uniform in size and
are thought to represent invading white blood cells.

**Endothelium: morphometry**

Analyzing specular micrographs can be done qualitatively by looking at the cellular morphology and giving an interpretation, or quantitatively by counting cell density and performing morphometric analysis. To properly recognize abnormal endothelium, however, requires knowledge of the appearance of normal endothelial (Fig. 14.7). Complete qualitative analysis requires knowledge of cell conformation, cell boundaries and their intersections, configuration of the dark boundary, and the presence of acellular structures.\[14\] Additionally, one must be able to recognize the presence of optical artifacts and eliminate them from consideration when performing both qualitative and quantitative analysis.

Fig. 14.7 Widefield confocal microscopic image of normal corneal endothelium (cell density 2933 cells/mm², mean cell area 341 mm², CV [coefficient of variation] 0.22, and hexagonal cells 80%).

The normal specular micrograph of a young person should show a regular endothelial mosaic of hexagonal cells of approximately the same size. Cell boundaries should be well defined. With age, however, endothelial cells become larger and the cellular pattern becomes distinctly pleomorphic. Figure
14.8A–F demonstrates various examples of abnormal cell shapes. Laing has also described the appearance of abnormal cell boundaries, cell shapes, and other cell structures seen in specular microscopy.[38] It should be noted that, even though the cell pattern can be pleomorphic, the cell size is not always increased.

![Images of cell configurations](image1.jpg)

**Fig. 14.8** Variations in the configuration of the corneal endothelium. (A) Enlarged and elongated cells. (B) Cells having scalloped edges. (C and D) Round cells. (E) Square cell. (F) Triangular cell. Arrows indicate the cell types mentioned.

One of the simplest methods to determine cell area and cell density is comparison cell analysis. When using this method, the endothelial cell mosaic is compared to a cell pattern of known size.[39–41] The mean cell area and cell density can then be subjectively estimated.
Quantitative Specular Microscopy

There have been limited attempts to perform quantitative specular microscopy of corneal epithelial cell density and morphology impaired by the analysis of a multilayer structure, artifact induced by the higher resolution contact microscopes, and questionable value in the analysis of dynamic cell population undergoing constant turnover. Confocal microscopy has shown much greater value with its precise determination of depth of focus and the three-dimensional construction of images, including the epithelium (Ch. 15). The lens epithelium has also been studied in a limited manner by specular microscopy in a group of normal volunteers and cataract patients by using a noncontact specular microscope.[42] There was a statistically significant decrease in the cell density of the lens epithelium in a group of cataract patients over the age of 80 years. Cell area variation and the number of large black spots that were observed were not related to aging or cataract formation. Cell density of the lens epithelium decreased after the age of 80, but cataract formation did not affect the cell density or the variation in cell areas until the age of 80.

There is an extensive literature on the quantitative analysis of the density and morphology of the corneal endothelium and this is where specular microscopy has played the greatest role in the clinical management of corneal conditions. Quantitative analysis of a specular photomicrograph is the objective description of the attributes of a selected cluster of individual endothelial cells from a specular photomicrograph. Examples include endothelial cell density (ECD) (measured as cells/mm²), mean cell area (measured as µm²/cell), coefficient of variation (CV) (standard deviation of cell areas/mean cell area), and pleomorphism (usually measured as a percentage of 6, <6 or >6-sided cells). Modern specular microscopes can calculate these values automatically or semiautomatically.

For quantitative analysis for the determination of ECD, at least one of the three methodologies are found in analysis software included with modern microscopes: fixed-frame, variable-frame or center methods (see Fig. 14.9A,B,D). In addition, many modern instruments offer automated or semiautomated (manual adjustment by reader) analyses in the determination of both ECD and morphometric parameters. In fixed-frame analysis (Fig. 14.9A), the technician counts all the cells lying completely within a given area (in this case a square frame). To avoid overestimating the number of cells present, not all of the cells lying on the boundary of the frame are added. Usually the total number of cells lying on the boundary is divided by two, or only cells lying on two sides of the square are counted. The estimate of the total number of cells within this given area is then used to calculate cell density. In variable-frame analysis, (Fig. 14.9B) the technician traces the boundary of the largest possible known area of cells after calibration of the magnification of the image, preferably incorporating a minimum of 100 contiguous cells. The number of cells available for analysis is dependent on the magnification of the image and the size of the cells. The variable-frame analysis is more accurate than fixed-frame analysis because only whole cells are counted and it is not necessary to include portions of cells located on the frame boundary.
Cell density alone is not the most sensitive measure of endothelial health, as the endothelium functions even at low ECDs (under 500 cells/mm²).[43] A number of authors have suggested that polymegathism (variation in cell area as determined by the CV) and pleomorphism (variation in cell shape as represented by the percentage of hexagonal cells) are a more sensitive measure of the endothelium under stress. As the CV goes up and the percentage of hexagonal cells goes down, this would indicate that the cell population has a less stable thermodynamic relationship between the individual cell and the adjoining neighbor cells, which correlates with declining endothelial function (loss of barrier function and pumping function). Yee et al.[44] have demonstrated the decreasing endothelial cell density and increasing pleomorphism seen with aging. These measurements, however, do not tend to vary greatly between paired human corneas.

The increased sensitivity of morphometric analysis can be illustrated in the following example. If only one cell is lost in a cluster of 100 cells, the mean cell area would increase maximally by 1%, a statistically nondetectable increase.[45] On the other hand, if a six-sided cell is lost in the cluster of 100 cells, at least two cells (2%) or possibly a maximum of six cells (6%) will show significant changes in cell pattern as adjacent cells stretch, slide, or even fuse together to repair the defect. It can, therefore, be seen how cell loss that is not detectable by cell density measurements alone may be detectable by quantification of cellular polymegathism and pleomorphism.

Most modern specular microscopes provide automated (Fig. 14.9F) or semiautomated morphometric analyses (Fig. 14.9A–E). For fully automated analyses, it is preferable to have the ability to make corrections (i.e. remove and redraw lines). The corners method (Fig. 14.9C) has been the standard method of morphometric analyses since the inception of specular microscopy more than 30 years ago.[6]
With this method, the corner of each cell is denoted, and the software then connects these points to outline the cell borders, and then determines cell area. The Center method (Konan Medical USA) (Fig. 14.9D) was introduced in the late 1990s and incorporated a semiautomated method of generating morphometric data where the center of each cell and its adjoining cells are identified. This method requires the technician to accurately mark the center of an area of contiguous cells. All peripheral cells along the edge of the marked cells are discarded from analysis because there are no adjacent outer cells next to them, and adjacent cells are required to determine the distance from their centers. Thus, even if 50 cells are marked, only 25 cells may actually be analyzed. For this reason, when performing the center method, the technician should mark the center of a minimum of 100 cells. Konan has recently developed a new analysis, the center-flex method (Fig. 14.9E), which capitalizes on the advantages of both the variable-frame and center methods. With this method, the technician traces an area of cells with visible cell borders, boundaries, and centers and then marks the center of the cells within the traced area.

The accuracy of any quantitative analysis, both for ECD determination and for the determination of morphometric parameters, is dependent upon image quality, how closely the area sampled represents the entire population of endothelial cells, the technician’s understanding of endothelial cell morphology, and the technician’s understanding of and technique in performing the specific analysis method. Image quality must be sufficient to enable the technician to identify the cell borders, boundaries, and centers. When using noncontact instruments, training is required to achieve optimal images, particularly on the thickened cornea. This typically requires the technician to take the instrument off automated into a manual capture mode. Sampling can be improved by capturing and analyzing three images taken at the same location (i.e. central corneal endothelium.) Technicians performing specular microscopy should be trained to recognize normal and abnormal corneal morphology. The most common errors seen in the clinical setting related to specular microscopy are related to improper application of the method of analysis or, in the case of automated analysis, not knowing it has failed. Examples of common errors (Fig. 14.10) are double counting or missing cells when performing the center method and failing to recognize when automated analysis has traced cell borders inaccurately.

![Fig. 14.10 Examples of common counting and analysis errors.](image)

A. Missed cells. B. Double counting. C. Inclusion of unanalyzable areas in frame. D. Automation failed to trace cell borders accurately.

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Specular Microscopy in Clinical Trials and the Value of a Reading Center

Specular microscopy plays a significant role in safety and efficacy studies of anterior and even posterior segment procedures, devices, and pharmaceutical interventions. To minimize variability in FDA multicenter clinical trials, the following guidelines have been recommended for specular microscopy and endothelial analyses: (1) all participating sites should use the same specular microscope; (2) each individual site should use the same technician who is reading center certified in their ability to obtain good-quality images throughout the course of the study; (3) a centralized reading center should confirm calibration of each specular microscope used in the study; and (4) a single reader should analyze all the images.[46]

The Cornea Image Analysis Reading Center (CIARC), formerly the Specular Microscopy Reading Center (SMRC), at Case Western Reserve University and University Hospitals Case Medical Center in Cleveland, Ohio, has been one example that follows these guidelines. The CIARC has published its methodology for dual grading and adjudication for the determination of ECD in a multicenter clinical trial using a variety of specular microscopes.[47] The CIARC has developed these protocols to maximize the number of eligible sites, incorporate sites with noncontact microscopes that are dependent on a manufacturer's internal calibration of the image (e.g. Konan) to validate the calibration externally with a calibration tool developed jointly with the manufacturer and the CIARC, and minimize variability inherent in multicenter clinical trials. The CIARC provides the participating sites with detailed instructions for quality image capture techniques and microscope-specific quality standards. Multiple technicians can be certified for study participation based on their ability to consistently produce quality images, export the images, properly deidentify the images of all protected health information, label the images per protocol, and properly submit the images to the reading center.

The CIARC has developed standardized procedures to externally calibrate each commercially available specular microscope for the purposes of analysis at the reading center. The CIARC performs detailed training and certification procedures for each method of analysis employed by its readers so that the data are consistent over the course of a study regardless of staffing changes. The CIARC provides dual assessment of ECD[47] and morphometric parameters with adjudication to address interobserver variability and a retesting procedure to address intraobserver variability, assuring consistent standards over an extended period of time by the readers. In addition, the CIARC image quality criteria
classification system[47] provides specific image quality criteria in order to evaluate factors that may influence image quality and affect endothelial analyses.

Through its work on the Specular Microscopy Ancillary Study (SMAS) and other multicenter studies, the CIARC has determined that, for multicenter clinical trials, a reproducible and reliable central reading center methodology for the assessment of endothelial image quality and ECD and morphometric analyses includes the use of certified readers, a defined image quality classification scheme, dual grading and adjudication, and masked quality control procedures are all critical for the conduct of large, multicenter, long-term studies.[47],[48]
Clinical Applications for Specular Microscopy

Clinical specular microscopy is a practical tool not only for the cornea subspecialist in evaluating donor corneas and corneal dystrophies but also for the ophthalmic surgeon in identifying subtle as well as macroscopic changes in the cornea prior to surgery. A clear cornea with a normal pachymetry reading is no assurance of a normal endothelial morphology or cell density. The ECD at which corneal edema occurs is quite variable, but has been estimated to be between 300 and 700 cells per mm².[40,49,95] A recent study, however, has shown that at least in penetrating keratoplasty grafts, 14% of the clear grafts (40 of 277 participants) at 5 years had an ECD below 500 cells/mm².[43] Assuming a cell loss in the range of 0–30% for any given intraocular surgical event, a patient should have at least 1000 to 1200 cells per mm² to safely undergo most anterior segment surgery without an increased risk of permanent postoperative corneal edema. Moreover, when the ECD approaches this arbitrary cell count range, it behooves the surgeon to make certain that the patient understands the increased risk of postoperative corneal edema and subsequent corneal decompensation. Most patients, including those over the age of 70, should have an ECD of at least 2000 cells per mm²; however, given the large variation of ECD among age groups, age alone cannot be used to predict ECD. Typically, there should be no significant difference in the ECD between eyes. With age, however, some people can develop a significant difference in the ECD. To be meaningful, this difference should be greater than 280 cells per mm².[51]

There is evidence that a polymegathic and pleomorphic corneal endothelium does not tolerate intraocular surgery as well as a more uniform endothelium. A cornea with a coefficient of variation greater than 0.40 or the presence of less than 50% hexagonal cells should be considered abnormal and at increased risk for postoperative edema. As in ECD measurements, there is a range of variation of polymegathism and pleomorphism in all age groups, and age alone cannot be used to predict the endothelial morphologic appearance.

When an endothelial abnormality is noted during the preoperative examination, specular microscopy can often provide valuable information that may affect management.[19] Such slit lamp abnormalities can include corneal guttae, keratic precipitates, pigmented and inflammatory cells, endothelial surface or Descemet's membrane irregularities, and increased corneal thickness. A history of possible endothelial abnormality, i.e. family history of corneal dystrophy,[52] trauma,[53] acute narrow-angle,[54–56] or chronic open-angle glaucoma, uveitis,[57–60] keratitis, graft rejection,[61] previous ocular surgery,[62][63] secondary intraocular lens implantation,[64] or corneal transplantation,[65] can also affect the surgical outcome. When evaluating postoperative corneas, it is particularly important to utilize multiple images in the central, midperiphery, and even periphery, because a regional disparity in ECD and morphology in these postoperative corneas has been reported.[66,67]

Aging

In most individuals, ECD decreases (or mean cell area increases) throughout life.[68] Cell loss is most rapid from birth to the first few years of life.[69,70] Part of this decrease in ECD may be due to the normal enlargement of the globe during early childhood.[71] ECD is rather stable from age 20 through approximately age 50 years.[72] After the age of 60 years, ECD decreases significantly in most people, but there is a great degree of variability between individuals. On average, age-related cell loss is approximately 0.5% per year.[72] A higher variability in polymegathism and pleomorphism has also been shown to correlate with age.[74][75]

Corneal guttae

Corneal guttae is a common condition, the incidence of which increases significantly with age.[76][77] Corneal guttae are focal accumulations of collagen on the posterior surface of Descemet's membrane that are abnormal products formed by stressed or abnormal endothelial cells. Microscopically, they appear as mushroom-shaped excrescences of Descemet's membrane. These guttae can easily be seen by specular microscopy.[76–81]

Fuchs' endothelial corneal dystrophy

In 1910, Ernst Fuchs[82] initially described bilateral corneal stromal and epithelial edema in elderly patients without the benefit of slit lamp microscopy. With the advent of biomicroscopy, it became possible to diagnose early endothelial involvement by the presence of guttae, making it unclear whether the term Fuchs' endothelial corneal dystrophy (FECD) should be used for the early stage with endothelial changes or only for advanced disease associated with corneal edema.[83–86] It seems that most ophthalmologists, however, reserve the term FECD for guttae that are confluent by more than 1–2 mm centrally or paracentrally advancing to great confluence and corneal edema.[86]

FECD is usually bilateral and is more commonly seen in women in their thirties to sixties.[86] It is a progressive endothelial dystrophy that results in endothelial dysfunction, leading to progressive corneal stromal edema and eventually epithelial edema and subepithelial fibrosis (Fig. 14.11). Lai et al.[78] have described the progressive morphologic changes of corneal guttae in FECD. Five specific stages in the development of excrescences can be discerned during the early evolution of the disorder. All five stages can occur in a cornea clinically free of edema. Several stages can be observed in the same cornea at a given time, although in most cases the majority of corneal guttae seem to have progressed to the same stage of development. More recently, there has been an early-onset form of FECD recognized, that has an equal representation in males and females, occurring below the age of 50, which notably has marked thickening of the anterior banded layer of the Descemet's membrane with a normal posterior nonbanded layer, unlike the more common late-onset disease in which this layer is predominantly affected.[87][88] This early-onset disease is noted to be due to a pathogenic mutation in COL8A2 gene, which encodes the alpha2 subtype of collagen VIII, a major component of Descemet's membrane.
Specular microscopy of the common late-onset form of FECD demonstrates that an individual excrescence begins as a very small structure, much smaller than an individual endothelial cell. Adjacent endothelial cells appear normal. In time, however, the excrescence grows and begins to distort overlying and adjacent endothelial cells, making the borders of these cells indistinct. The guttae themselves appear as dark spots, sometimes with bright central reflections. In the case of FECD, they are usually more numerous centrally. If the guttae are confluent, slit lamp microscopy can reveal a beaten-silver appearance of the posterior cornea, similar to that seen in the iridocorneal endothelial (ICE) syndrome. The images of the guttae and endothelium in the early-onset form of FECD have a distinctive difference from the late-onset form with every endothelial cell associated with a single, low-elevation gutta (Fig. 14.12), a pattern of high density that alternates with large areas devoid of guttae (early onset) compared to sharply raised guttae with higher elevation and areas of coalescence (late onset).[87]

Ultrastructural studies in late-onset FECD have revealed both smooth, rounded excrescences and excrescences with flat, broad posterior surfaces containing a central depression.[89],[90] The latter umbilicated excrescences tend to be larger than the rounded excrescences, suggesting that the irregular posterior surface may represent a maturational change.

Many surgeons assess corneas with FECD before intraocular surgery to help predict the prognosis for postoperative corneal clarity. This specular evaluation, in addition to the clinical history of frequent fluctuating vision, abnormal pachymetry readings, and formation of microcystic bullae, and/or subepithelial fibrosis, can aid in deciding whether penetrating or endothelial keratoplasty is necessary at the time of intraocular surgery. If there are a significant number of peripheral endothelial cells and the guttae are primarily located centrally, there is a decreased likelihood that corneal transplantation will be necessary. On the other hand, if there is complete confluence of guttae even in the peripheral cornea associated with increased corneal thickness of over 0.68 microns (µm), one might consider a combined procedure.
Despite the advantages offered by specular microscopy, the likelihood of postoperative corneal clarity can be difficult to judge. For this reason, some surgeons do not consider keratoplasty combined with another intraocular procedure unless evidence of significant stromal edema is already present preoperatively.

**Lattice corneal dystrophy**

Lattice dystrophy is inherited in an autosomal dominant manner. Clinically, the dystrophy appears quite early – at the age of 2 to 7 years. As the disease progresses, the cornea becomes progressively cloudy, causing a marked reduction in acuity by age 20–30. The disease is caused by amyloid deposition throughout the stroma. Recurrent erosions sometimes result from the superficial nature of some of the deposits.

Specular microscopy of patients with lattice corneal dystrophy has revealed the presence of linear structures described as branching lines which crisscross the stroma. These lines are believed to be amyloid deposits or lesions caused by amyloid deposition.[91–93] A crater-form appearance has also been described.[94] Although abnormalities of both Bowman's layer and the epithelium have been documented histologically, no endothelial abnormalities have been found.[92]

**Iridocorneal endothelial syndrome**

The iridocorneal endothelial (ICE) syndrome is a progressive, nonfamilial group of disorders with female predominance. It consists of three variants, previously believed to be independent entities, but now grouped under the term ICE syndrome. These three variants are Chandler’s syndrome, essential iris atrophy, and Cogan-Reese syndrome (the iris nevus syndrome).[94][95] All three are characterized by a fundamental abnormality of the corneal endothelium that is responsible for variable degrees of iris atrophy, secondary angle-closure glaucoma, and corneal edema.[96–98] The disease may be related to a herpes simplex virus (HSV) infection of the corneal endothelium.[99] This helps explain its unilateral clinical presentation; however, studies have demonstrated subclinical involvement of the contralateral eye, evidenced by a degree of endothelial cell pleomorphism and polymegathism that is inconsistent with the patient's age.[100–104]

Ultrastructural studies of the more involved eye reveal abnormal endothelium covering a thickened, multilayered Descemet’s membrane which can extend over the inner surface of the trabecular meshwork and anterior iris surface.[96,105–108] Glaucoma may result from contraction of the membrane, which can also result in peripheral anterior synchiae with secondary glaucoma and various iris abnormalities.[98] The affected endothelial cells can also take on features of epithelial cells and can even become multilayered.[108]

The specular microscopic appearance of the endothelium is characterized as a rounding off of cell angles.[109,110] There is a loss of cellular definition and hexagonal shape, and many pentagonal cells are evident.[110] There can also be an increased granularity of the intracellular details, and small, centric dark areas in individual cells which can enlarge and become completely blacked-out areas within the cell.[110] Slit lamp examination will often reveal a characteristic hammered-silver appearance of the posterior cornea, which can involve the entire posterior corneal surface or only part of it.[97,108,110] As the disease progresses, the endothelial monolayer may no longer be recognizable as a mosaic of cells. A ‘reversal appearance’ may develop, with black central areas and white borders (Fig. 14.13).[103,108,110]

**Posterior polymorphous corneal dystrophy**

Posterior polymorphous corneal dystrophy (PPCD) is a disorder of the posterior, nonbanded layer of Descemet’s membrane. It is usually bilateral and is usually inherited as an autosomal dominant trait with variable expressivity. The clinical manifestations of PPCD are similar to those of the ICE syndrome, which complicates the diagnosis. Unlike ICE syndrome, however, PPCD is generally bilateral, nonprogressive, and only occasionally associated with corneal decompensation, visual dysfunction, and glaucoma.[99]

A review of PPCD by Waring et al.[90] divides its appearance, as seen with the slit lamp, into three basic forms: vesicular, band, and diffuse. All forms demonstrate the classic posterior polymorphous vesicle, a small, round, blister-like lesion at the level of Descemet’s membrane, surrounded by a ring of opacity (Fig. 14.14A).[111] Using specular microscopy, this vesicle can be used to differentiate PPCD from ICE syndrome. When examined with the specular microscope, the vesicle has a thick, dark border yielding a doughnut-like appearance (Fig. 14.14B). This structure appears to lie anterior to undistorted endothelial cells, which are generally more recognizable toward the center of the lesion.[113–115] A similar appearance can be seen when an isolated patch of ICE cells (also known as an ICE-berg) is identified in a patient with ICE syndrome. In ICE syndrome, however, the lesion does not appear anterior to the endothelium as in PPCD, but appears to lie within the endothelium. Furthermore, the endothelial cells adjacent to the structure are not normal in appearance as in PPCD, but are distorted and commonly smaller than normal.[113][115]

![Fig. 14.13 Iridocorneal endothelial syndrome. (A) Slit lamp appearance of ICE (Chandler’s) syndrome. (B) Specular micrograph showing more normal endothelium in the upper left quadrant in Chandler’s syndrome.](image-url)
Specular microscopy has also been useful in distinguishing PPCD from other problems causing corneal opacities. The parallel "rail track" (straight) borders from old Descemet's tears, such as those from Haab's striae, can be distinguished from the 'snail tracks' (irregular) seen in the band presentation of PPCD.[115][116]

**Keratoconus**

Specular microscopy of keratoconic corneas has revealed two populations of endothelial cells, one larger and one considerably smaller than normal. The most striking abnormality in keratoconus, however, is elongation of endothelial cells.[117] The cells appear to have been stretched by the ectatic process with their long axis in the direction of the apex of the cone. This lends support to the concept that acute corneal hydrops is a result of stretching of the endothelium and Descemet's membrane. These structures are thought to be stretched to the point of rupture, allowing the passage of aqueous into the stroma. Dehydration of the cornea usually occurs, presumably a result of endothelial cell migration and enlargement to cover the defect. This concept is supported by the finding of localized areas of endothelial cells that are 7 to 10 times larger than normal near the site of rupture.[117][118] Away from the site of rupture, however, the endothelial cells are of a more normal size and morphologic appearance, suggesting that the area of enlarged cells represents the site of endothelial and Descemet's rupture and the subsequent changes necessary to repair the defect.

Other abnormal findings seen in keratoconus include the presence of dark bodies completely contained within an otherwise normal-appearing cell. These dark bodies occur less frequently in larger endothelial cells and are consistent in appearance with blebs or vacuoles seen with the electron microscope.[118] Their role in the pathogenesis of keratoconus is not presently understood.

**Glaucoma**

Persistently elevated intraocular pressure likely results in gradual loss of endothelial cells and progressive loss of endothelial function.[120] It is evidenced by the decreasing pressure at which the cornea becomes edematous in many glaucoma patients as their disease progresses.[120] In vitro experiments, however, revealed no morphologic changes despite elevated pressures, as long as normal aqueous flow was maintained.[120] These experiments suggest that endothelial cell loss is not a direct result of increased pressure, but rather some other disturbance such as prolonged low oxygen concentration in the aqueous humor. Furthermore, in patients with unilateral glaucoma or a history of unilateral attacks of glaucomatocyclitic crisis, specular microscopy reveals a decreased endothelial cell density in the eye affected with glaucoma.[122][123] If the pressure is medically controlled, cell loss is reduced.[121] Studies have shown varying cell loss connected with various glaucoma procedures, in particular trabeculectomy and tube shunts.[125][128]

**Intraocular inflammation**

During an acute episode of anterior uveitis, mononuclear inflammatory cells penetrate apical junctional complexes and infiltrate both between endothelial cells and between endothelial cells and Descemet's membrane (see Fig. 14.6H). Endothelial cells, however, are not generally harmed by this process.[56] but in the most severe case can become dislodged and float free in the aqueous humor.[120]

**Cataract extraction with intraocular lens implantation**

Numerous investigators have reported findings on specular microscopy after cataract extraction.[63][130][139] Reports of endothelial cell loss after cataract surgery using a variety of surgical approaches have demonstrated variable cell loss ranging from no detectable cell loss to as much as 40% cell loss. Methods of cataract surgery, however, have changed dramatically through the years and are now much less damaging to the endothelium. Endothelial cell loss following uncomplicated phacoemulsification and posterior chamber intraocular lens implantation using viscoelastic and modern, small-incision techniques is quite low, ranging from no detectable cell loss to 20%.[131][132][133][134][135] There does not appear to be a statistical difference between endothelial cell loss resulting from phacoemulsification versus extracapsular cataract extraction.[135][138][140][159] Furthermore, suture fixation of a posterior chamber intraocular lens[160] or placement of an intraocular lens in the sulcus[162] appears to be no more traumatic and results in no more endothelial cell loss than a posterior chamber intraocular lens placed in the capsular bag. The implantation of an anterior chamber lens, however, has been shown to result in not only higher endothelial cell loss due to the procedure itself but also continued endothelial loss which is greater than that of posterior chamber intraocular lenses.[64][148][161][162]

Endothelial damage during phacoemulsification has been attributed to mechanical injury caused by anterior chamber instrumentation and/or anterior chamber manipulation of a lens nucleus, heat generation, or prolonged intraocular irrigation.[164][165] Endothelial loss, however, also correlates with ultrasound time and power.[140][143][145] and is greatest near the wound, which is also the area of maximal manipulation.[167][168][169] The endothelium suffering the least damage is the greatest distance away from the wound. Despite the ability of the endothelium to migrate during many forms of endothelial insult, this endothelial disparity may not correct with time.
In the past, significant endothelial damage could result from even momentary contact between the hydrophobic intracoroidal lens surface and the endothelium. The development of viscoelastics significantly limited such injury. Additionally, modern IOLs have a hydrophilic surface that produces minimal endothelial damage, especially when used with viscoelastic. The choice, however, of a dispersive viscoelastic over a cohesive viscoelastic does not appear to result in any less endothelial cell loss.[133,139,149,150,173] even though one could reason that a dispersive viscoelastic might provide more endothelial protection.[170] Other surgical materials, such as stainless steel, also cause endothelial damage on contact. The natural crystalline lens, however, appears to cause minimal damage when it is in contact with the endothelium for short periods.

Refractive surgery

Numerous investigators have studied the effects of photorefractive keratectomy (PRK)[174–179] and laser-assisted in situ keratomileusis (LASIK)[174,180–184] on the corneal endothelium. Most studies have shown that neither LASIK nor PRK results in a decreased endothelial density.[180] Laser ablation of the stroma within 200 µm of the corneal endothelium, however, will result in endothelial structural changes and the formation of the amorphous substance deposited onto Descemet's membrane.[185] Only two clinical studies have reported endothelial cell loss after LASIK or PRK.[174][186] Each of these studies, however, was composed primarily of patients with high myopia requiring very deep ablations.

Interestingly, many investigators have described an increase in central endothelial cell density after PRK and LASIK.[179,180,184] This is felt to be a result of endothelial migration initiated by the discontinuation of contact lens wear[174] and not a result of refractive surgery.[179,180,184]

Several studies have been conducted on the effects of PRK, LASIK, and laser-assisted subepithelial keratectomy (LASEK) on ECD. Diakonis et al.[187] studied the effects of mitomycin-C (MMC), an intraoperative agent used to decrease haze and regression, on ECD after use during PRK. While there was a decrease in ECD at 1 month and 3 months postoperatively, the authors concluded this may be due to the issues encountered with the repeatability of ECD determination.[187] Other studies on the effects of mitomycin-C during LASEK have also found no significant changes in the corneal endothelium.[186,189]

Azar et al.[190] studied the effects of uncomplicated intrastromal corneal ring segment placement on the corneal endothelium. ECD was determined preoperatively and at 6, 12, and 24 months. Significant endothelial cell loss was not present at the 6- and 12-month visits, but a decrease in ECD was found at the 24-month visit, which may or may not be related to surgery.

Previously, implantation of phakic intraocular lenses were thought to result in significant endothelial cell loss.[190–196] More recently, long-term studies on the FDA-approved Artisan/Verisyse phakic intraocular lens (IOL) have found acceptable mean cell loss rates of 1.3% per year after insertion to correct high myopia.[197] Hexagonality and coefficient of variation during a 4-year follow-up study were comparable to preoperative numbers.[188] Candidates who narrowly meet the minimal ECD of 2000 cells/mm²[198] are suggested to have greater anterior chamber depth due to greater cell loss.[199] Additionally, increased aqueous flare,[191] decreased crystalline lens transmittance,[191,195] pupillary ovalization,[192,193] and chronic inflammation[192,195] have been described after implantation of phakic intraocular lenses but these have been minimized with the use of iris-fixated IOLs, proper training, and strict exclusion criteria.[200]

Penetrating keratoplasty

Specular microscopy of successful penetrating keratoplasty (PKP) has revealed substantial but variable endothelial cell loss occurring during and shortly after surgery.[44,201–203] Furthermore, endothelial loss well above that found in normal eyes appears to continue throughout the life of the graft.[203–205] Ing et al.[204] have demonstrated that, 5 to 10 years after successful PKP for a variety of indications including keratoconus and endothelial dysfunction disorders, endothelial cell loss progresses at a rate seven times faster than normal. However, between 10 and 15 years, this rate substantially slows and approaches the rate of loss of endothelial cells in normal aging.[201] In the Specular Microscopy Ancillary Study (SMAS) of the multicenter Cornea Donor Study (CDS) examining the impact of donor age on graft survival and endothelial cell loss following PKP for endothelial dysfunction conditions only (FECD, pseudophakic/aphakic corneal edema), a substantial 70% median endothelial cell loss from baseline to 5 years postoperatively overall was noted (Fig. 14.15).[48] The younger age group (12–65 years old) experienced a substantial but slightly lower 69% median percentage cell loss, compared to the older group (66–75 years old) of 75% cell loss; however, graft clarity was equal in both groups at 5 years, at 86% of the 1090 grafts performed.[204] In part because of this difference between cell loss with comparable graft clarity, the study has now been extended to 10 years. Another longitudinal study following PKPs for a variety of conditions done by one surgeon has shown that of the 388 initial grafts used for analysis, the 67 cases available for analysis at year 15 had a 71% endothelial cell loss, which was unchanged from the 10-year analysis.[207]
Fig. 14.15 Endothelial cell loss following PKP for endothelial dysfunction in the Specular Microscopy Ancillary Study with clear graft at 5 years. (A) baseline, (B) one year, (C) two years.

Besides the contribution of the SMAS to our understanding of endothelial cell loss following PKP for at least the endothelial dysfunctions, the study also provided insight as to the importance of the quality of the specular image in achieving the most accurate cell count by the eye bank and also pointed out the variability of cell counting among eye banks and the importance of a reading center to provide the most accurate, standardized endothelial cell density (ECD) determination.[47],[206a] Accuracy of counting by the eye bank correlated with image quality, while 35% of the cell counts determined by the eye banks were 10% higher or lower than the reading center.[206a] This study has prompted a need to develop new standards for cell counting among the eye banks in the United States.

It is fortunate that a surprisingly low endothelial cell density can maintain the cornea in a dehydrated, transparent state.[43],[207] In the SMAS, 40 (14%) of 277 subjects with a clear graft after 5 years had an ECD below 500 cells/mm².[43] This had been previously shown in other studies.[207],[208] Nonetheless, there is a critical endothelial cell density below which irreversible corneal edema occurs, explaining the sudden decompensation of some grafted corneas years after successful transplantation.[209]

Obata et al.[202] measured the endothelial cell density of 58 corneal grafts both before and after PKP. At the 2-week postoperative visit, endothelial cell density had decreased by only about 10%, indicating significant intraoperative endothelial loss had not occurred. Endothelial cell loss was most rapid during the early postoperative course but then gradually slowed. At the 3-month postoperative visit, endothelial cell loss averaged 33%. By 1 year, endothelial loss of all eyes averaged nearly 50%. Cell loss in the keratoconus subgroup, however, measured only 33% after 1 year. Langenbucher et al.[210] followed their patients beyond the initial postoperative period when cell loss is greatest and still noted a similar disparity among patients with keratoconus. They described an annual cell loss of only 2.9% in patients who underwent keratoplasty for keratoconus. On the other hand, patients who underwent PKP for FECD or bullous keratopathy had an annual cell loss of 11.2% and 19.3%, respectively. This was observed similarly in the SMAS.[48] Such findings have suggested that endothelial cells may migrate along a density gradient after penetrating keratoplasty.[211] This may explain the decreased endothelial cell loss seen in keratoconus, given the relatively normal peripheral host keratoconic endothelium. On the other hand, Ruusuvaara[212] has documented great endothelial disparities between the graft and the recipient, leading one to believe that migration may not occur in this fashion.

The presence or absence of a lens has also been shown to affect endothelial cell loss after PKP.[63],[204] Ing et al.[204] have shown that patients who are aphakic have the least cell loss after PKP, and patients who are phakic or pseudophakic have the greatest cell loss. Some of this cell loss may, however, be attributable to increased cell loss during the actual surgical procedure. Bourne has demonstrated that phakic patients lose significantly more endothelial cells during PKP than do aphakic patients.[213] This finding was attributed to the deeper anterior chamber and the absence of endothelial trauma from the lens–iris diaphragm in the aphakic eye. The difference in endothelial cell loss between phakic and aphakic eyes, however, has not been confirmed by other studies[63],[214] and needs further research.

Recent findings from the SMAS have shown that baseline donor ECD, at least following PKP for endothelial dysfunction disorders, does not predict graft failure 5 years postoperatively.[43] Interestingly, the 6-month ECD does predict failure. Among those that had not failed within the first 6 months, the 5-year cumulative incidence of failure was 13% in the 33 subjects with a 6-month ECD less than 1750 cells/mm² versus 2% in the 137 subjects with a 6-month ECD of 2500 cells/mm² or higher. These findings may not apply to endothelial keratoplasty (EK), which has an entirely different pattern of cell loss (see below) with usually twice as much loss in the first 6 months post EK, but a significantly lower rate of cell loss thereafter than PKP.

Although transplanted corneas recovered excellent deturgescence capabilities, Sato has demonstrated that, in comparison with normal corneas, a small but statistically significant increase in corneal thickness persisted.[219] The correlation between graft thickness and endothelial cell size or endothelial cell pleomorphism was not significant. The transfer coefficient of fluorescein from the aqueous humor to the cornea was, however, significantly greater in cases of uneventful PKP than in normal corneas, indicating a persistent increase in the endothelial permeability. Thus, endothelial function of the
clear graft is not completely normal. The correlation between graft thickness and endothelial permeability (as measured by the transfer coefficient of fluorescein) is statistically significant,[213,215,216] suggesting that endothelial permeability, rather than the variation in cell size (polymegathism) or cell shape (pleomorphism) of endothelial cells, is the major factor that determines the thickness of a clear corneal graft. Additional longitudinal morphometric studies are needed to correlate changes in parameters such as the coefficient of variation (variation in cell size, polymegathism) and percentage of hexagonal cell (variation in cell shape, pleomorphism) with subsequent increase in corneal thickness and subsequent graft failure.

Besides quantitative determination of changes in cell density and morphometric parameters (CV, percentage hexagonal cells), qualitative specular microscopy can detect early evidence of graft rejection not detectable by clinical examination and can thus be of value in following certain high-risk patients. During graft rejection episodes, one sees intercellular bright bodies, black inflammatory cells, and generally recognizable keratic precipitates on the endothelial surface (Fig. 14.16).[217–219]

**Endothelial keratoplasty**

Endothelial keratoplasty (EK) has rapidly become the procedure of choice for endothelial dysfunction since 2006. According to the Eye Bank Association of America (EBAA) 2008 statistics, EK increased from 1429 to 18,221 of the total keratoplasty cases (3% to 30%), over the past 3 years in the United States.[220] The procedure has been applied to all endothelial failure conditions, including FEDC, pseudophakic/aphakic corneal edema, the iridocorneal endothelial syndromes, posterior polymorphous corneal dystrophy, and failed graft.[221–233] The goal of EK is to remove only the diseased endothelial layer of the affected cornea. The first human cases were described by Melles[236] as posterior lamellar keratoplasty (PLK) in 1998, and refined by Terry using new instrumentation as deep lamellar endothelial keratoplasty (DLEK) in 2001.[238] Melles went on to develop a technique to excise Descemet’s membrane from the recipient cornea (descemetorhexis).[239] Price performed the first procedure using stripping Descemet’s membrane and named this form of EK, Descemet stripping endothelial keratoplasty (DSEK).[231] Gorovoy further refined the procedure utilizing a microkeratome to cut the donor, thus automated, and described the procedure as Descemet stripping automated endothelial keratoplasty (DSAEK).[222] Recently, Melles has developed a procedure solely transplanting Descemet’s with endothelium, Descemet membrane endothelial keratoplasty (DMEK).[223,240,241]

EK has grown rapidly in popularity because of its many benefits over PKP, including smaller wound size, less astigmatism, less wound dehiscence, and faster visual recovery. These advantages are tempered by greater initial potential donor trauma, a higher primary donor failure rate, and common dislocation of the donor necessitating repositioning in the early postoperative period. Initial studies with experienced surgeons have shown comparable success to PKP in regards to graft clarity at 3 years postoperatively, but longer-term 5-year graft success remains to be determined.[227] There is limited endothelial cell loss data utilizing either specular microscopy or confocal microscopy for the most recent methods for EK, DSEK, and DSAEK. Most authors have reported significantly greater cell loss in the first 6 months after EK compared to PKP. For example, one EK study showed 34% cell loss after 6 months, and 38% at 1 year, comparable to other studies.[224,227,228,232,233] Interestingly, although there is greater loss at 1 year when compared to PKP, the rate of cell loss begins to level off around 6 months, unlike PKP, as observed by several authors.[224,227,228,232,242] After 1 year, there is minimal loss to the second and third years, 7% between 6 months and 2 years, and 8% between 6 months and 3 years, compared with 42% in the eyes that underwent PKP in the Specular Microscopy Ancillary Study (SMAS) of the Cornea Donor Study (CDS).[49,227] Only one group in a small series has reported greater cell loss at 1 year in the PKP group than the EK group.[235]

No significant differences in cell loss have been shown between donor posterior lenticule prepared by the surgeon versus the eye bank.[229–231] The influences of variations in technique related to EK, including wound size and insertion methods, have not been well studied. In regards to wound size, studies have shown that both in cadaver eyes and humans, a smaller-sized incision for EK surgery results in greater acute endothelial area damage than larger size (5 mm) incisions.[229,229]

There have been no randomized trials comparing EK to PKP, but Price et al. reported in a prospective series of EK cases performed by two experienced surgeons compared to a series of PKP cases from the SMAS that at 6 months cell loss was 34% versus 11% and 38% versus 20% at 1 year in the EK versus PKP group, respectively.[232] This multicenter study with cell counts determined in a masked fashion by the same specular microscopy reading center for both studies confirmed single-site studies that the endothelial damage at the time of EK is greater as reflected in the greater loss at 6 months, but that the rate of loss thereafter is significantly less than PKP. This difference may relate to the larger donor (9 mm) with 26% more endothelial cells transplanted than with PKP, less damage to the peripheral endothelium that may ultimately be reflected in less central cell loss where specular microscopy is performed, and perhaps a lower rejection rate.[18,227] Long-term studies are needed to see if this difference in the rate of loss after 6 months compared to PKP is reflected in improved long-term graft cell survival associated with a more stable endothelial cell population.

**Donor corneas**

Specular microscopy is a useful method of screening tissue for keratoplasty. Evaluation of corneal tissue can be performed on whole globes[243,244] as well as corneas stored in tissue culture media.[104,245,246] In addition to historical and slit lamp information, most eye banks provide the ECD and sometimes morphologic information to help decide if tissue is accepted or rejected. At the discretion of the local medical director of each eye bank, the minimum donor ECD is established for keratoplasty. Generally, this minimum has been established for most eye banks at 2000 cells/mm². There are no good scientific data why the minimum was established at this level, except for the success of keratoplasty over the past 20 years utilizing this minimum criteria.[247] The Specular Microscopy Ancillary Study has reported that baseline donor ECD does not predict graft failure 5 years after PKP; however, the 6-month ECD was predictive of subsequent failure, as previously described in the PKP section of this chapter.[19] Among those that had not failed within the first 6 months, the 5-year cumulative incidence of failure was 13% ± 12% in the 33 subjects with a 6-month ECD less than 1700 cells/mm² versus 2% ± 3% in the 137 subjects with a 6-month ECD of 2500 cells/mm² or higher.[43]
Intraocular irrigating solutions

Closed intraocular surgery (e.g., phacoemulsification, vitrectomy) requires the introduction into the eye of a large volume of an irrigating solution over a relatively prolonged time. Numerous studies describe the effects of intraocular irrigation solutions on the corneal endothelium.[165,248–262] Endothelial structure and function are best maintained when solutions resemble aqueous humor in composition. Irrigating solutions not satisfying the basal metabolic requirements of the cells can produce adverse endothelial changes and in vitro perfusion experiments demonstrate that these endothelial changes and the resultant corneal swelling are caused by intraocular irrigating solutions that are deficient in certain essential components. As a result of these studies, intraocular irrigation solutions that more closely resemble aqueous humor are now commercially available.

Vitreocorneal contact

Persistent corneal edema, resulting from contact of formed vitreous humor with the corneal endothelium, can occur months to years after cataract extraction in which the posterior capsule is no longer intact, but the precise mechanism of corneal decompensation is not known.[283–296] Since corneal dehydration is controlled primarily by the endothelium, it is assumed that vitreous contact mechanically injures the endothelium and interferes with its physiologic function. Experimental evidence suggests that contact of solid, collagenous elements of the vitreous humor with the endothelium interferes with transport of fluid out of the cornea.[267] Contact between formed vitreous humor and the corneal endothelium, however, is not invariably followed by corneal edema. In those cases in which the phenomenon is encountered, the cornea may tolerate vitreous contact and remain clear and dehydrated for extended periods before edema is observed. Specular microscopic studies tend to support these concepts.[248] Several types of morphologic abnormalities are observed in the endothelium of corneas in the early stages of decompensation as a result of vitreous contact. Some endothelial cells are markedly enlarged and grossly abnormal in shape. Others contain abnormal bright or dark structures within their cell boundaries. Abnormal cell intersections and side length distribution are encountered, and the central guttate ex crescences of FECD are often seen.

Removal of vitreous humor from the anterior chamber by closed vitrectomy, with the elimination of vitreous contact, may result in substantial improvement in the appearance of corneal hydration and in some cases in the elimination of clinically significant corneal edema.[268–272] Surprisingly, those endothelial changes and the resultant corneal swelling are caused by intraocular irrigating solutions that are deficient in certain essential components. As a result of these studies, intraocular irrigation solutions that more closely resemble aqueous humor are now commercially available.

Epithelialization of the anterior chamber

The endothelial surface of the cornea has been photographed in vivo with the specular microscope during varying stages of epithelialization of the anterior chamber in an effort to define clinical signs that would permit a definite diagnosis to be made in the absence of histopathologic verification.[273–275] Evaluation of the corneal endothelium in this entity is laborious and demanding. Even in the most cooperative of patients, cellular structures may be difficult to detect, and often cannot be distinctly focused. A sharply defined border between normal corneal endothelial cells and the area of epithelial downgrowth is observed.[273] In contrast, in the region occupied by the clinically observed endothelial demarcation line, enlarged, abnormally shaped endothelial cells inferiorly blending into an acellular, structureless area superiorly have been noted.[274] By focusing more deeply in the seemingly structureless area superiorly, these investigators were able to visualize poorly defined structures that suggested multilayered epithelial cells. However, neither epithelial nor endothelial cells could be identified with certainty in the region above the demarcation line seen with the slit lamp biomicroscope; the diagnosis was later verified histopathologically. Improved resolution of the epithelial cells at the junction between epithelial downgrowth and the endothelial cells has been achieved.[276] When the endothelium is successfully visualized with the specular microscope, it is usually abnormal even though the advancing demarcation line is far away from the cells being examined.[274] Considerable cell loss seems to occur, as evidenced by the large size of the remaining cells. Whether this cell loss results from a traumatic insult prior to surgery and contributes to the subsequent epithelial invasion of the anterior chamber or, alternatively, whether it is produced by the advancing layer of epithelium, is not clear. When epithelialization is moderately advanced, cellular structures are seen that do not have the morphologic appearance of endothelial cells. Presumably, these are epithelial cells, but this is not certain. In the most advanced cases, no distinct cell boundaries can be seen. Only a disorganized, amorphous, membranous layer containing some formed structures is visualized. This is believed to represent a thickened, multilayered epithelial membrane whose structure does not permit satisfactory resolution by the specular microscope. The inability to see endothelial structures distinctly with the specular microscope suggests either that the endothelium is disorganized or that more than a single layer of cells is present. The invading epithelium also produces an irregular layer of fibular material along the epithelium-Descemet’s membrane interface[277] that may contribute to the gray appearance of the involved cornea and to the difficulty encountered in obtaining a clear image of the structures in the zone of specular reflection.

Blunt trauma

Blunt trauma to the cornea can damage the endothelium. Bourne and co-workers[278] have reported their findings on a 16-year-old boy who had suffered aullet gun injury of the right eye 2 years previously. Clinically, both corneas were clear and free of edema. Evidence of prior endothelial damage was revealed only by clinical specular microscopy. The endothelial cells on the right were enlarged and the central endothelial cell density was only 47% of that of the opposite, normal, left eye. Although it is suspected that in such instances the residual endothelial cells might be more susceptible to subsequent trauma than normal cells, no additional cell loss was documented following cataract extraction by phacoemulsification in this particular case.

The impact of small, nonpenetrating foreign bodies on the cornea may give rise to posterior annular keratopathy, clinically apparent gray rings on the corneal endothelium.[279–281] Specular microscopic studies demonstrate that posterior annular keratopathy occurring after blunt corneal trauma in humans represents a contusion injury and consists of disrupted and swollen endothelial cells. Reproduction of these rings in experimental animals also reveals that they consist of swollen or disrupted endothelial cells.[281] The center of each ring corresponds to the epithelial impact site of the foreign body, with the least disruption of the endothelium occurring here. The damaged cells may still be evident many days after the clinically visible endothelial rings disappear and, indeed, permanent cell loss may occur. It might be expected, the degree of endothelial cell loss appears to be related to the severity of the injury; a measurable decrease in cell density occurs only in the more severely injured corneas.

Contact lens wear

Both acute and chronic endothelial changes are seen with the specular microscope following contact lens wear. Within minutes of application of a contact lens, small dark endothelial blebs occur that disappear quickly if the lens is removed.[275,282–284] These endothelial blebs reach a maximum size in 20–30 minutes from the time the contact lens is placed on the cornea and then gradually decrease in size. Similar blebs occur during prolonged lid closure, such as during sleep,[285] and may represent the effects of hypoxia or lactate accumulation.

Long-term wear of either hard or soft contact lenses results in an increased polymegathism[286–301] that is not reversed upon cessation of lens wear.[296,297] although some recovery towards normal might occur.[301] The degree of polymegathism increases as the period of time the lenses are worn increases.[288,290,291,293,297,298] The degree of polymegathism also depends upon the type of lens worn.

Diabetes

Diabetes
In type 1 diabetes the cell density significantly decreases with age. However, no difference in corneal thickness or endothelial permeability to fluorescein has been described in two studies. Whereas another study showed increased permeability in subjects with type 1 diabetes and exacerbated by cystic fibrosis. Diabetic corneas also exhibit increased polymegathism and pleomorphism and a decreased percentage of hexagonality. However, Ohguro et al. have demonstrated that topical administration of aldose reductase inhibitor can reverse these morphologic changes, suggesting that aldose reductase may be involved in the etiology of corneal endothelial variations in diabetic patients.
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Chapter 15 – Confocal Microscopy

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Background

It is well established that confocal microscopy provides higher-resolution images with better rejection of out-of-focus information than conventional light microscopy. The optical sectioning ability of confocal microscopy allows images to be obtained from different depths within a thick tissue specimen, thereby eliminating the need for processing and sectioning procedures. Thus, confocal microscopy is uniquely suited to the study of intact tissue in living subjects. In vivo confocal microscopy has been used in a variety of corneal research applications on experimental animals since its development over 20 years ago. However, in recent years, the use of the confocal microscope on human patients has also expanded dramatically. In this article we review the latest developments and most current applications of clinical confocal microscopy of the cornea. In addition, we discuss an emerging confocal imaging technique, multiphoton-second harmonic generation imaging, which allows noninvasive assessment of collagen matrix organization in intact corneal tissue.

Historical overview

The optical design of confocal microscopy is based on the principle of Lukosz, which states that resolution may be improved at the expense of field of view.[1] In 1955, Marvin Minsky developed the first confocal microscope for studying neural networks in the living brain.[2] The Minsky microscope condenser focused the light source within a small area of tissue, with concomitant focusing of the microscope objective lens on the same area. Because both condenser and objective lenses had the same focal point, the microscope was termed 'confocal.' Since the introduction of Minsky's original microscope, the optical theory of confocal microscopy has been more formally developed and improved. In modern confocal microscopy, a point (i.e. diffraction limited) light source is focused onto a small volume within the specimen, and a confocal point detector is used to collect the resulting signal. This technique results in a reduction of the amount of out-of-focus signal from above and below the focal plane which contributes to the detected image, and produces a marked increase in both lateral (x, y) and axial (z) resolution.[3–5]

The use of a point source/detector in the confocal optical design trades field of view for enhanced resolution; therefore, a full field of view must be built up by scanning. The first scanning confocal microscope, developed by Petran et al.,[6,7] used a modified Nipkow disk containing thousands of optically conjugate (source/detector) pinholes arranged in Archimedean spirals (Fig. 15.1A). Light from a broadband source passes through the pinholes on one side of the disk, and is focused into the specimen. Detector pinholes on the opposite side of the disk prevent light from outside the optical volume, determined by the objective lens and pinhole diameter, from reaching a camera or eyepiece. Rotation of the disk results in even scanning of the tissue in real time. Because the illumination and detection of light through conjugate pinholes occurs in tandem, this microscope was named the tandem scanning confocal microscope (TSCM).
Current confocal systems in clinical use

There are three main confocal imaging systems used clinically: the TSCM, the HRT III (a scanning laser system), and the Confoscan 4 (a scanning slit system). Much of the in vivo imaging to date has been accomplished with the TSCM design. The TSCM is well suited for in vivo applications because it generates images in real time, and uses a broadband light source which causes less tissue damage than laser sources. Egger and Petran obtained the first images of cells from uncut and unstained tissue blocks including the brain, retina, and other organs.[8],[9] These initial observations were not repeated until 1985 when Boyde demonstrated the dramatic optical sectioning abilities of the TSCM by imaging osteocytes in intact bone without demineralization, grinding, or other destructive processing techniques.[10],[11] In 1986, Lemp et al.[12] were the first to apply confocal imaging techniques to the study of the cornea ex vivo. This work led to the design of a TSCM with a horizontally oriented objective (Tandem Scanning Corp., Reston, VA), which was more suited to use in ophthalmology (Fig. 15.1B).[13] Most TSCM systems currently in use employ a specially designed surface contact objective (24×, 0.6 NA, 1.5-mm working distance). The position of the focal plane relative to the objective tip is varied by moving the lenses within the objective casing (Fig. 15.1C). Thus, the depth of the focal plane within the tissue can be calibrated, and quantitative three-dimensional imaging is possible with this system.[14],[15] With this objective, the TSCM has an axial (z-axis) resolution of approximately 9 µm.[14] The TSCM system is no longer commercially available.

The HRT III with Rostock Corneal Module (Heidelberg Engineering, GmbH, Dossenheim, Germany) is a laser scanning confocal microscope (Fig. 15.2). It operates by scanning a 670-nm laser beam (<1 µm diameter) in a raster pattern over the field of view. This is accomplished using horizontally and vertically oriented scanning mirrors. The reflected light from the cornea is descanned using the same two mirrors, and directed to a photodetector using a beam splitter. The system typically uses a 63× objective lens (0.9 NA), and provides images that are 400 µm × 400 µm in size. The microscope produces images with excellent resolution and contrast, and has better axial resolution than the TSCM, due to the higher NA objective.[15a,b]
The Confoscan 4 is a variable-slit, real-time scanning confocal microscope, available commercially from Nidek, Inc. (Fig. 15.3). This general design was originally described and applied to corneal imaging by Masters and Thaer.\cite{16} Mounted on a slit lamp stand and using a 12 V halogen lamp for noncoherent illumination, the instrument can be used clinically in conjunction with a CCD video camera to examine the living eye. In this design, two independently adjustable slits are located in conjugate optical planes; a rapidly oscillating two-sided mirror is used to scan the image of the slit over the plane of the cornea to produce optical sectioning in real time.\cite{17} The system uses a 40× objective lens (0.75 NA) and digitized images are 460 µm × 345 µm in size. This is a user-friendly instrument that incorporates automated alignment and scanning software. In addition, the scanning slit design allows better light throughput and provides images with a higher signal to noise ratio than the TSCM. However, this is achieved at the expense of axial resolution, which has been measured at approximately 26 µm.\cite{18}
Fig. 15.3 The Confoscan 4 microscope
(Courtesy of Nidek Technologies).

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In Vivo Confocal Imaging Techniques

**Normal corneal structures**

Many aspects of the clinical imaging procedure are similar for all three confocal instruments. Before observation, a drop of topical anesthetic is placed on the patient's eye. Next, a drop of methylcellulose or other viscous solution is applied to the tip of the objective lens in order to optically couple it to the cornea (i.e. as an immersion fluid). The objective is then aligned perpendicular to the surface of the cornea in a manner identical to specular microscopy. The $x$, $y$ position of the image is controlled by moving the joystick on the microscope stand, and the $z$ position of the focal plane is changed using the objective drive control. However, the objective drive and scanning software differ significantly between systems. More detailed descriptions of the individual scanning procedures used for each instrument can be found elsewhere.[19],[20]

Normal corneal anatomy as observed with the TSCM is shown in Figure 15.4.[21] Confocal images are always taken en face: that is, the viewer sees thin slices of the cornea that are parallel to the epithelial surface. The borders of the surface epithelial cells are readily seen, as are the bright cell nuclei (Fig. 15.4A). Immediately beneath the basal epithelium, a fine nerve plexus can be detected (Fig. 15.4B).[22] In the corneal stroma, only cell nuclei are visible using TSCM under normal conditions, with a dark background in between (Fig. 15.4C, D). Interestingly, the interconnected cell processes of the keratocytes, which have been previously identified by Nishida et al.,[23] become visible under certain pathologic conditions, possibly due to tissue edema or cell activation. Large numbers of keratocytes are present in the anterior stroma as compared to the mid and deeper stroma, which show a lower cell density.[24] Prominent nerve fibers can be seen within the stroma, and tracked over long distances three-dimensionally. TSCM images of the normal endothelium appear similar to what is observed using specular microscopy (Fig. 15.4E).
Fig. 15.4 Corneal images digitized directly from a CMTF scan (A–E) and the corresponding CMTF intensity curve (G) in a human volunteer. (A) Epithelial image corresponding to peak A; (B) basal-epithelial nerve plexus image corresponding to peak B; (C) image of anterior layer of keratocyte nuclei corresponding to peak C; (D) stromal image corresponding to position D; (E) endothelial image corresponding to peak E; (F) 3-D reconstruction. (G) CMTF intensity curve. Horizontal field width (A–E) = 330 µm.


HRT III images of a normal human cornea are shown in Figure 15.5. Due to its higher signal-to-noise ratio and improved axial and lateral resolution, intraepithelial sectioning can be achieved, and wing cells (15.5A) and basal cells (15.5B) can be easily distinguished. The sub-basal nerve plexus can be clearly imaged (15.5C), and dramatic montages can be generated in order to map temporal changes in the overall organization and nerve branching patterns over large areas of the cornea.[25] Langerhans cells can also be distinguished and their density quantified using the HRT III microscope.[26] Images of stromal cells (15.5D, E) and corneal endothelium (15.5F) are generally similar to that observed with the TSCM, albeit with higher contrast.
The Confoscan 4 can also provide images of the basal epithelial cells and the sub-basal nerve plexus, but with less resolution than the HRT III.[20] High-contrast images throughout the corneal stroma can also be obtained. The Confoscan 4 is particularly well suited to imaging the corneal endothelial cells. Due to its thicker optical volume and high light throughput, high contrast, full-field images are easily obtained. The thinner optical section thickness of the TSCM and HRT III make endothelial imaging somewhat more difficult. Unlike specular microscopy, the optical sectioning capability of confocal microscopy allows imaging through edematous corneas. Software for endothelial cell analysis is included with both the Confoscan 4 and HRT III instruments. The Confoscan 4 can also be used with a 20× nonimmersion (dry) objective lens for endothelium imaging.

Confocal microscopy through-focusing

To collect and quantify 3-D information from the cornea, a technique termed confocal microscopy through-focusing (CMTF) is typically used. Wiegand et al. originally demonstrated that by rapidly focusing through the cornea at high speed, a z-axis intensity profile of the tissue can be obtained.[27] This technique is based on the observation that different corneal sublayers generate different reflective intensities when imaged using confocal microscopy; thus, the intensity profile can provide information about the depth and thickness of corneal cell layers.

In the TSCM system, CMTF scans are obtained by scanning through the cornea from the epithelium to endothelium at a constant lens speed. Images are detected using a video camera and digitized into computer memory. The CMTF intensity curve is then generated by calculating the average pixel intensity in a central region of each image, and plotting versus z depth (Fig. 15.4G). After a z series of CMTF images have been digitized, a cursor can be moved along the intensity curve as corresponding images are displayed. In this way, the user can identify images of interest and record their exact z axis depth.[28][29] Two major peaks corresponding to the superficial epithelium anteriorly (Fig 15.4A) and the corneal endothelium posteriorly (Fig 15.4E) are present in normal CMTF curves. CMTF intensity profiles
also showed smaller peaks corresponding to the basal corneal epithelial nerve plexus (Fig 15.4B) and the anterior layer of corneal keratocytes (Fig 15.4C). By measuring the distance between the various peaks, accurate and reproducible measurements of corneal, epithelial, and stromal thickness can be obtained.[28]

Unlike other more widely used methods for measuring corneal and epithelial thickness, confocal microscopy provides a series of high-resolution microscopic images which directly correspond to peaks in the intensity curve. Thus, the origin of the intensity peaks can be confirmed if necessary. CMTF scans can also be used to generate a 3-D image of the cornea by stacking the images and projecting them using surface or volume rendering (Fig 15.4F).

Cross-sections (x-z projection images) of CMTF scans from all three clinical confocal systems are shown in Figure 15.6. As mentioned previously, the TSCM uses an applanating objective which stabilizes the cornea, and the position of the focal plane within the tissue is changed by moving the lenses within the objective casing (Fig. 15.1C). A stack of evenly spaced images can thus be obtained and reconstructed (15.6A). The HRT III also uses an applanating tip to provide stability, and cross-sections with excellent resolution and contrast can be generated (Fig. 15.6B). Unfortunately, automated scans of only 60 µm can be generated at this time, and changing the focal plane over larger distances must be performed manually (by rotating the objective housing by hand). A modified prototype that overcomes this limitation has recently been described.[30] The Confoscan system uses a noncontact objective lens (for patient comfort), and movement of the entire objective lens is necessary to change the focal plane position within the cornea and generate CMTF scans. A trade-off with this design is that the cornea can move randomly with respect to the lens tip during a CMTF scan. A ‘Z-Ring’ which touches the corneal surface can be used to allow accurate calculation of z-axis position within the cornea during scanning,[31] but the distance between images in CMTF scans is generally not as uniform as that obtained using applanating objectives. As shown in Figure 15.6C, both the stability and axial resolution of the Confoscan 4 are reduced as compared to the TSCM and HRT III. There are clearly trade-offs in all designs that must be considered when purchasing an instrument.
Clinical Applications

Although confocal microscopy has been used extensively in research applications on experimental animals, its noninvasive nature makes it ideally suited for clinical use in ophthalmology. In recent years, the clinical application of in vivo confocal microscopy has expanded rapidly. Confocal microscopy has been used to monitor changes in keratocyte density during aging, in keratoconus patients, and following surgery. In addition, temporal changes in the density and organization of subepithelial nerves in response to surgery or disease can be assessed. The effects of contact lens wear on the morphology and thickness of the corneal epithelium has also been quantified, and such studies have provided important insight into how lens type and wear pattern influence bacterial binding and corneal epithelial homeostasis. There are numerous other applications of this technology in the literature which cannot be covered here due to space limitations; many of these are discussed in several recent review articles. Below, we discuss two of the most common applications of clinical confocal microscopy in more detail: (1) assessment of wound healing following refractive surgery, and (2) diagnosis of corneal infections.

Wound healing following refractive surgery

Because of its unique ability to image the cornea four-dimensionally at the cellular level (x, y, z, and t), confocal microscopy is ideally suited to monitoring the cellular events of epithelial and stromal wound healing, particularly following refractive surgical procedures (for reviews see references 48–50). For example, confocal microscopy allows measurement of wound gape, the depth of epithelial ingrowth, and the degree of corneal fibrosis in radial keratotomy wounds. Similar assessments can also be performed following penetrating keratoplasty (PK), or lacerating injury. As detailed below, confocal microscopy is especially well suited to assessing the corneal response to photorefractive keratectomy (PRK) and laser assisted in situ keratomeliosis (LASIK). It has also been applied following related procedures such as LASEK and epi-LASIK, as well as emerging surgical techniques such as Descemet stripping with automated endothelial keratoplasty (DSAEK).

PRK: CMTF has been used to assess multiple parameters associated with corneal wound healing following PRK. Changes in corneal, epithelial, and stromal thickness following surgery can be made from the CMTF curves. In addition, confocal microscopy can be used to assess the degree of subepithelial haze induced by the procedure. Confocal microscopy following PRK has demonstrated that the development of corneal haze is correlated with the activation of corneal keratocytes and transformation to a fibroblast or myofibroblast phenotype. These activated cells are more reflective than quiescent corneal keratocytes, and synthesize extracellular matrix components that also reduce corneal transparency. As shown in Figure 15.7, a 'haze peak' is observed in the CMTF curves after PRK, and the width and height of the haze peak indicate the thickness and reflectivity of the subepithelial tissue. An objective haze estimate can be obtained by calculating the area under the haze peak for each patient. Interestingly, increased subepithelial haze was detected using CMTF in patients who were graded clinically clear using the slit lamp, demonstrating the higher sensitivity of confocal microscopy. Nerve regeneration following PRK has also been assessed using confocal microscopy. Interestingly, regenerating subepithelial nerve fibers have been detected as early as 7 days after PRK, but require at least 6–12 months for complete recovery, perhaps due to the persistence of the
subepithelial scarring. Temporal changes in keratocyte density have also been documented following PRK.[32] Overall, in vivo confocal microscopy represents an important tool for quantitative assessment of initial photoablation depth, temporal changes in epithelial, stromal, and corneal thickness, cell loss and/or repopulation, and unbiased haze estimation after PRK.

Fig. 15.7 Three-dimensional reconstructions and corresponding confocal microscopy through-focusing (CMTF) scans of three human corneas 1 month after photorefractive keratectomy. (A) A clinically clear cornea (grade 0 haze). (B) Cornea with clinical grade 2 haze. (C) Cornea with clinical grade 4 haze. Note the increased subepithelial reflectivity in all three corneas. In the corresponding CMTF scans, a profound increase in both haze thickness (haze peak width) and haze intensity (haze peak height) is seen with increasing clinical grades. (From Möller-Pederson T, Vogel M, Li HF, Petroll WM, Cavanagh HD, Jester JV. Quantification of stromal thinning, epithelial thickness, and corneal haze after photorefractive keratectomy using in vivo confocal microscopy. Ophthalmology 1997, 104:360–8.)

LASIK: Confocal microscopy has also been used to assess numerous parameters following LASIK, such as epithelial thickness, flap thickness, interface particle density, keratocyte density, nerve damage and recovery, stromal cell activation, and interface haze.[33,38,62–74] Confocal microscopy has been used to assess the corneal response to both microkeratome-assisted LASIK, and LASIK with flap creation using IntraLase (IntraLASIK). Following traditional LASIK with a microkeratome, corneal haze is not generally observed clinically. Although regions of keratocyte activation and changes in keratocyte density have been observed by confocal microscopy, the overall stromal wound healing response is much less pronounced than that of PRK.[33,63–66,75–78]

Previous clinical studies have shown that IntraLase® provides fewer complications than mechanical microkeratomes, and results in better visual outcomes in most patients.[79–84] Consistent with these results, studies using confocal microscopy have demonstrated that IntraLASIK provides more accurate and reproducible flap thickness, as well as a significant reduction in the number of interface particles detected.[70,85–89] One concern regarding IntraLase® is that the procedure for flap creation can induce more damage than a microkeratome, and therefore appears to stimulate a more pronounced wound healing response. To address this issue, IntraLase® has evolved in recent years with the development of new lasers with higher pulse frequencies, which allow for faster procedure time, tighter spot placement, and the use of lower raster energies than first-generation lasers.
Confocal microscopy has confirmed that IntraLase® can induce significant keratocyte activation, particularly at higher raster energies, and that this may underlie clinical observations of haze in some patients. An example of a cornea with keratocyte activation and ECM haze is shown in Figure 15.8. In this patient, a raster energy of 2.6 µJ was used. A CMTF stack is shown in Figure 15.8A. Note the areas of increased reflectivity near the flap interface (15.8A, arrows). Examination of individual images revealed keratocyte activation near the interface, as indicated by highly reflective nuclei (Fig 15.8B–D, arrows). An increase in ECM reflectivity (ECM haze) was also observed surrounding the cells in many cases (Fig. 15.8B–D). In addition, cell processes were sometimes visible, suggesting local stromal edema and/or fibroblast transformation of corneal keratocytes. Normal keratocytes are observed above and below (Fig. 15.8E, arrowheads) the region of activation.

Fig. 15.8 (A) CMTF stack taken 3 months after LASIK with IntraLase®. Note the areas of increased reflectivity near the flap interface (arrows). (B) Single image taken from the CMTF stack in A, at the level of the flap interface. Note highly reflective keratocyte nuclei (arrows), indicating cell activation. An increase in ECM reflectivity (ECM haze) is also observed surrounding the cells (C) Single image from the CMTF stack taken 20 µm below the flap interface. Keratocyte activation and ECM haze is observed. (D) Single image from the CMTF stack taken 30 µm below the interface. A few activated keratocytes (arrow) and ECM haze is detected. (E) Single image taken 55 µm below the interface. Normal keratocytes are observed (arrowheads). Horizontal field width = 375 µm.

(From Petroll WM, Goldberg D, Lindsey SS, et al. Confocal assessment of the corneal response to...
In a recent paper, an analysis of confocal data from several studies was performed to determine the influence of different IntraLase® raster energies, pulse frequencies, and postoperative steroid treatments on the corneal response to IntraLASIK. In this study, for patients with the same steroid dosing regimen, a decrease in the percentage of eyes with keratocyte activation as well as amount of interface reflectivity was found as the raster energy was reduced (from 2.8 µJ to 1.2 µJ). However, when the steroid treatment regimen was shortened, activation and reflectivity increased even if the raster energy was low. Most eyes with keratocyte activation had little or no haze detected by clinical examination. However, at the highest raster energy used, two eyes were found to have significant clinical haze by slit lamp examination. Overall, the confocal data suggests that IntraLase® can induce significant keratocyte activation, which may underlie clinical observations of haze in some patients. However, activation can be avoided by using lower raster energies and an extended steroid treatment regimen.

**Infectious keratitis**

Because it provides much higher magnification than a slit lamp biomicroscope, confocal microscopy is ideally suited for early detection and diagnosis of a number of infectious organisms (for review see references 19 and 47). One important clinical application of the TSCM is the localization of *Acanthamoeba* cysts and trophozoites in the living eye for diagnosis and assessment of the efficacy of ongoing medical treatment.[21,91–96] Figure 15.9A shows the appearance of a pig cornea infected with *Acanthamoeba castellanii* with active trophozoite forms of the infectious organism present just beneath the epithelium (1). The cystic form is also seen (2); internal cyst detail can be noted with indentations suggestive of a double wall. The invading organism seems to ‘hollow’ the cornea in a series of internal tissue ridges, furrows, and cavities highly reminiscent of termites in solid wood. Figure 15.9B shows easily identifiable brightly reflective cysts (1) in a human patient who had a biopsy confirming *Acanthamoeba* infection. Figure 15.9C is from a patient who was being treated with Brolene™ (propamidine isetionate), and was showing symptoms of an active infection. Note the appearance of two types of highly reflective structures, presumably trophozoites (1) and cysts (2). The three-dimensional nature of this process can be assessed using confocal imaging; thus, the total spatial volume delineated by the infection can be quantitated, and response to antiamebal drug therapy monitored.
(B) Easily identifiable brightly reflective cysts (1) in a human patient who had a biopsy confirming *Acanthamoeba* infection. 

(C) TSCM image from a patient who was being treated with Brolene™, and was showing symptoms of an active infection. Note the appearance of two types of highly reflective structures, presumably trophozoites (1) and cysts (2). Horizontal field width = 350 µm.

**Fig. 15.9** *Acanthamoeba* keratitis. (A) 24 hours postinfection of a pig cornea, showing active trophozoites (1) and cysts (2) in the subepithelial region. Note the ridges and furrows (dark areas) within a markedly abnormal anterior corneal stroma. (From Cavanagh HD, Petroll WM, Alizadeh H, He Y-G, McCulley JP, Jester JV: Clinical and diagnostic use of in vivo confocal microscopy in patients with corneal disease, Ophthalmology 100:1444–1454, 1993.)

The detection of fungal keratitis has also become an important clinical application of confocal microscopy, due in part to the increased incidence of these infections in recent years.[97] Treatment of these infections is often slow and difficult, and early diagnosis is extremely important. Confocal microscopy has been shown to provide distinctive images of the filaments of *Fusarium solani* (Fig. 15.10)[98] and *Aspergillus* keratitis[64],[99] in human patients, and may provide a unique means for early detection of these infections. Elongated particles resembling *Candida* pseudofilaments have also been identified in infected corneas.[97] Other studies have demonstrated the potential usefulness of confocal microscopy in
diagnosing bacterial contact lens-related keratitis,[100] microsporidial keratitis,[101],[102] and Borrelia keratitis.[103] It should be noted that not all infectious agents can be detected and/or distinguished using confocal microscopy, and physical biopsy of the cornea is still often necessary to confirm diagnosis.[47,96,104] Bacterial and viral infections are difficult to image directly using current confocal systems, due to the small size of the infiltrates. Further studies are needed to clarify which infectious organisms can be reliably detected and distinguished using confocal microscopy.

Fig. 15.10  Fungal keratitis. *Fusarium* imaged using the HRT III confocal microscope. Horizontal field width = 300 µm. (Courtesy of Dr Brasnu and Dr Baudouin, Paris, France, and courtesy of Heidelberg Engineering, Inc. All rights reserved.)
Imaging Corneal Collagen Using Second Harmonic Generated Signals

The application of lasers to biology and medicine, while not new, has been greatly expanded by the development of ultrafast, femtosecond lasers that enable the focusing of very high-intensity light within small optical volumes to generate nonlinear optical effects including two photon excited fluorescence (TPEF), second harmonic generated signals (SHG), and laser-induced optical break down (LIOB).[105] SHG has particular relevance to studying the structure of the cornea, since collagen fibrils generate strong SHG signals that can be imaged using optical microscopic techniques. To generate an SHG signal, a material is excited by the simultaneous absorption of two photons of light of the same frequency and then the material emits a single photon of light that is double the frequency or half the wavelength of the excitation light. The ability to generate SHG signals is limited to materials that are highly ordered and noncentrosymmetric, such as collagen.[106] Hochheimer, in 1982, was the first to show that SHG signals could be generated from the rabbit cornea.[107] Past studies have also shown that imaging of SHG signals can be used to establish collagen fibril orientation, as well as study the three-dimensional collagen organization.[108],[109]

SHG imaging has more recently been used to study the supramolecular organization of collagen in the normal human and keratoconus cornea.[110],[111] In these studies a Zeiss 510 Meta confocal microscope attached to a Coherent Chameleon tunable Ti:Sapphire femtosecond laser was used. This system generated SHG signals from intact corneal tissue ex vivo using 800-nm infrared femtosecond pulses. Signals were then imaged using both the transmitted light detector and a 400-nm band pass filter (forward scattered signals) and the Meta detector collecting light in the 380–420-nm region (backscattered signals).[110]

**Normal human corneal stromal collagen organization**

Imaging of forwardscattered SHG signals from normal human cornea identifies distinct fiber-like structures, approximately 1 µm in diameter, that vary in length and orientation depending upon the depth within the cornea (Fig. 15.11A–C). Imaging of the backscattered signals, on the other hand, does not fully resolve individual collagen fibers as clearly as detected in the forwardscattered images taken from the same optical plane (Fig. 15.11D–F), although the general outline of the lamellar organization can still be detected. In the anterior cornea, small bands of five or fewer parallel fibers that run deeper into the cornea are seen as short segments aligned in random orientations (Fig. 15.11A). As in the forwardscattered image, backscattered images of the anterior cornea also show a highly interwoven lamellar organization in which narrow bands of collagen fibers run in random directions and cross over and under multiple lamellae (Fig. 15.11D). When both forward- and backscattered images are merged together, a more detailed view of the precise collagen organization can be detected (Fig. 15.12). Viewing of sequential images taken deeper within the cornea shows that these short collagen lamellae extend continuously deeper into the stroma, indicating that the short segments represented longer collagen fibers that run in and out of the plane of focus. This pattern indicates that the anterior collagen is organized into a highly interwoven lamellar structure with many lamellae running in a transverse, anterior–posterior direction and not parallel to the corneal surface. Deeper within the cornea, collagen bundles become wider, containing from five to ten fibers, but continue to pass through multiple optical planes, suggesting that lamellae continued to be highly interwoven (Fig. 15.11B). In the posterior cornea, large numbers of
fibers can be detected that are grouped together into orthogonally arranged lamellae that run parallel to the corneal surface and show markedly less interweaving than observed in the other regions (Fig. 15.11C). Imaging of backscattered SHG signals also detects interwoven yet broader lamellae within the middle layers of the stroma, similar to that detected in the forwardscattered image (Fig. 15.11E). In the posterior cornea the backscattered image is less distinct and does not show the same organizational pattern detected in the forwardscattered image (Fig. 15.11F).

Fig. 15.11 Forwardscattered (A–C) and backscattered (D–F) SHG images of human corneal stroma taken in the anterior (A and D), middle (B and E), and posterior (C and F) cornea. Bar = 50 µm. (From Morishinge N, Petroll WM, Nishida T, et al. Non-invasive imaging of corneal stromal collagen using two photon generated second harmonic signals. J Cataract Refract Surg. 2006;32:1784.)
Using digital image reconstruction, the anterior–posterior organizational pattern of collagen can also be evaluated using SHG imaging (Fig. 15.13). Such imaging shows that the anterior limiting lamina (ALL, Bowman's layer) generates a strong backscattered SHG signal that is detected between the corneal epithelium and the anterior stroma (asterisk). More strikingly, the transverse collagen lamellae detected in the anterior stroma can be seen inserting into the ALL (double arrowheads). The insertion of stromal collagen lamellae into the ALL has been previously detected by Komai and Ushiki using transmission electron microscopy.[112] Furthermore, as discussed by Bron, the anterior stromal collagen is known to have extensive anteroposterior interweaving with occasionally insertion into the ALL that may contribute to the anterior corneal mosaic.[113] The observations using SHG imaging complement these earlier observations and point to the extension of collagen lamellae from the ALL much deeper into the stroma than formerly appreciated. In this respect, studies by Muller et al.[113a] evaluating swollen human corneas have shown that the anterior 100–120 µm of stroma is resistant to swelling and maintains the corneal...
curvature. While it has been proposed that the interwoven nature of the anterior cornea is responsible for this mechanical property, this region also corresponds to the region containing transverse lamellae that extends to a depth of approximately 130 µm. Therefore, it is likely that the presence of transverse lamellae that insert into the ALL explains the rigidity of the anterior corneal stroma, particularly if these lamellae extend out to the limbus as suggested by Bron.[113] In this respect, these transverse lamellae may represent a similar structural feature to that of ‘sutural fibers’ identified in the dogfish,[114],[115] ‘Sutural fibers’ are thought to provide rigidity to the dogfish cornea and to play a role in the resistance to swelling following removal of the corneal endothelium, a similar function identified by Muller et al.[113a] for the anterior region of the human cornea.
Fig. 15.13 Reconstructed cross-sectional images of human cornea showing forward (A cyan), backward (B magenta) SHG signal co-localized with Phallloidin to detect actin and Syto-59 to detect nuclei (C green and red, respectively). High-resolution images show that SHG imaging can detect the anterior limiting lamina (Bowman’s layer, asterisk) and the insert of anterior collagen lamellae into the anterior limiting lamina (double arrowheads). Bar = 20 µm D. Four color overlay of A–C. (From Morishinge N, Petroll WM, Nishida T, et al. Non-invasive imaging of corneal stromal collagen using two photon generated second harmonic signals. J Cataract Refract Surg. 2006;32:1784.)

Collagen organization in keratoconus corneas

Keratoconus is an acquired or genetic disorder that leads to thinning of the paracentral cornea, resulting in corneal steepening and cone formation. Histologic examination of end-stage keratoconus reveals disruption of the ALL and corneal scarring. These findings suggest that keratoconus involves a defect in the mechanical strength of the paracentral cornea that leads to progressive thinning. Since the biomechanical strength of the cornea is generally thought to be associated with the axial strength of the collagen fibrils, understanding the 3-D collagen organization of the keratoconus cornea compared to the normal cornea may provide insight into the pathogenesis of this disorder.

Using SHG imaging, a recent study evaluating 13 corneal buttons obtained following corneal transplant surgery for keratoconus noted a marked loss or decrease in the presence of transverse stromal lamellae that inserted into the ALL.[111] Images from a representative subject from that report are shown in Figure 15.14. In regions where the anterior limiting lamina was intact (Fig. 15.14A, B), a distinct decrease in the number of transverse lamellae was detected (Fig. 15.14C) along with less lamellar interweaving (Fig. 15.14D) compared to normal. Furthermore, deeper into the anterior stroma (50 µm), the loss of lamellae detected by the forward detector was more dramatic (Fig. 15.14E) and lamellar interweaving appeared completely absent in the backward detector image (Fig. 15.14F). Three-dimensional reconstructions and evaluation of the cross-sectional organization confirmed these findings and indicated that the number of lamellae inserting into the ALL (asterisk) was remarkably reduced in some regions (Fig. 15.14G, arrow) and, when present, only extended a short distance into the anterior cornea. Reconstruction of a 10 µm × 230 µm slice through the three-dimensional data set taken from the backward detector also confirmed the marked lack of lamellar interweaving below the ALL (Fig. 15.14H), which was distinctly different from that of normal adult cornea.
Fig. 15.14  Second harmonic signals from keratoconus corneas detected with the forward (A, C, E, G) and backward (B, D, F, H) detector. (A, B) Bowman’s layer. (C, D) 10 µm below A. (E, F) 50 µm below A. (G) Three-dimensional reconstruction of the data set from forward detector showing a maximum intensity projection rotated 90° in the y axis through 230 µm of anterior stroma. Note that lamellae inserting into Bowman’s layer are absent (arrow) or shortened. (H) Three-dimensional reconstruction of a 10 µm thick slice through the data set from the backward detector showing a maximum intensity projection rotated 90° in the y axis through 10 µm of anterior stroma. Asterisk = Bowman’s layer. Bar = 50 µm.
Overall, based on SHG evaluation of 13 cases of keratoconus using maximum intensity projections to identify transversely oriented collagen lamellae, 12 of 13 showed marked abnormalities in the density and extent to which these 'sutural' lamellae extended deeper into the underlying corneal stroma.[111] These observations are in distinct contrast to SHG images obtained from similarly aged normal corneas which all show numerous transverse collagen lamellae that insert into the ALL. Overall, the findings based on SHG imaging suggest that there is an underlying defect in the organization of stromal collagen in keratoconus corneas that may explain the observed biomechanical differences between normal and keratoconus corneas.
Conclusions

This chapter has described the ability of confocal microscopy to resolve noninvasively structural and functional interrelationships, both temporally and spatially, in the corneas of human patients. Using confocal microscopy, the cellular details of fundamental biological processes such as inflammation, wound healing, toxicity, infection, and disease, which could previously be studied only under static or isolated conditions, can now be dynamically evaluated over time and the effectiveness of treatment modalities determined. The application of femtosecond laser-based nonlinear optical imaging of SHG signals from collagen is an important emerging technology which can be used to study noninvasively the structural organization of the human cornea with high spatial and lateral resolution. In the future, incorporation of multiphoton lasers into clinical confocal instruments may allow imaging of both cellular biology and interactions with extracellular matrix in human corneas, opening up a new world of potential applications.
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Chapter 16 – High-Resolution Ultrasound

Charles J. Pavlin,  
F. Stuart Foster

The use of high-frequency ultrasound as a diagnostic tool was pioneered at the University of Toronto.[1–7] We applied the term ultrasound biomicroscopy (UBM) to this method.[2] This method uses ultrasound in the frequency range of 25–100 MHz to produce cross-sectional subsurface images of the eye at an axial and lateral resolution ranging from 20 to 100 µm, depending on the choice of frequency. Ultrasound is increasingly attenuated at high frequencies, limiting penetration to the 4–15-mm range.

UBM has been used to examine a variety of pathologic conditions of the anterior segment and ocular adnexa including glaucoma,[8–10] anterior segment tumors,[11],[12] intraocular lens-related problems,[13],[14] trauma,[15] scleral disease,[16] and adnexal pathology. A non-specific imaging method, UBM can be used for any pathology that falls within its penetration limits. The superficial location of the cornea allows excellent penetration of this structure and the ability to image the majority of the underlying anterior segment. Ultrasound in the higher frequency ranges (60–80 MHz) can be used for corneal examination because of a lesser need for deep penetration and a greater need for high resolution. The cornea can usually be imaged by optical techniques, but in several conditions UBM can provide added information. Because sound is used instead of light, UBM provides a different type of information on internal structure and allows penetration of optical opacities. Accurate measurement of various corneal parameters can be performed.

This chapter reviews the use of UBM in corneal imaging and provides examples of clinical application.

Instrumentation

The instrumentation for UBM has been described extensively elsewhere.[1–3] A simplified block diagram of a UBM scanner is given in Figure 16.1. Signal processing in an ultrasound biomicroscope is identical to that in a conventional B-mode imaging system except that the operating frequency is approximately one order of magnitude higher. In this example, a 25–100-MHz transducer is moved linearly over the imaging field (typically 4–8 mm), collecting radiofrequency ultrasound data at each of 512 equally spaced lines (8 µm between lines for a 4-mm field of view). At each location, a 40–100-MHz ultrasound pulse is transmitted into the tissue, and the backscattered ultrasound is detected by the same transducer. The radiofrequency signal is received and amplified in proportion to the depth from which it originated, using time-gain compensation (TGC). By amplifying with the TGC signals from deeper structures more than superficial structures, it is possible to compensate for the attenuation of the ultrasound beam in the tissue. After the radiofrequency signal is processed nonlinearily to enhance the low-level signals, its envelope is ‘detected’ to produce the A-scan signal. This signal is then converted from analog to digital format and transferred to a special high-speed scan converter, stored, and displayed as B-scan data on a video monitor. The servo motion system and signal processing are controlled and synchronized by a computer. B-mode imaging is currently performed at 5–10 frames/second. This mode of ultrasonography has been shown to be safe for corneal use.[17] A variant of the instrumentation used is the arc scanner,[18] which moves the transducer in an arc that follows the curvature of the cornea. This instrument allows the
transducer to remain relatively perpendicular to the corneal surface over the entire corneal curvature. This allows a complete corneal image in one pass and also allows construction of three-dimensional corneal maps by using multiple passes in various meridians.
Image resolution

Ultrasound biomicroscopy image quality is determined by the choice of transducer frequency and focusing characteristics. Because of the frequency-dependent nature of losses in tissue, the choice of these parameters is a trade-off between resolution and depth of penetration. For anterior segment imaging to a depth of approximately 5 mm, it is convenient to use frequencies in the 50 MHz range, where resolutions of the order of 35 µm in the axial direction and 60 µm in the lateral direction are achieved. In the cornea, however, where penetration of less than 1 mm is required, it is feasible to use higher frequencies and consequently achieve higher resolution. When possible, we try to scan the cornea using frequencies between 60 and 80 MHz.

An example of an echo (radiofrequency signal) obtained from a normal cornea made with a strongly focused 60-MHz transducer is shown in Figure 16.2. Note the three prominent specular echoes received from (1) the fluid couplant–epithelium interface, (2) the epithelium–Bowman's membrane interface, and (3) the endothelium/Descemet's membrane–aqueous interface. The axial resolution and separation between these surfaces is difficult to interpret from the radiofrequency signal. By envelope detecting the radiofrequency signal using a Hilbert transform to generate the A-scan, it is possible to make estimates of the axial resolution and layer thicknesses based on the equation: $z = ct/2$ where $z$ is the thickness of the layer or resolved structure, $t$ is the time between echoes, and $c$ is the speed of sound in the medium. There is currently debate over the speed of sound in corneal tissue in vivo. Measurements by Ye et al.,[19] made at 50 MHz, suggest that an appropriate value for $c$ is 1575 m/s in this frequency range. Plots of radiofrequency and envelope amplitude versus $z$ for the epithelial region of Figure 16.2 are given in Figure 16.3 A and B, respectively. The width of the signal from the fluid couplant–epithelium interface measured at one-half maximum is a measure of the axial resolution of the system. For this particular transducer, the axial resolution, $z_{ax}$, measures 20 µm. The epithelial thickness, $z_{epi}$, in this case measures 49.46 µm in thickness, with a precision (standard deviation) of 1.13 µm for eight independent measurements. Note that the precision for measuring the thickness of resolved parallel layers is many times greater than the axial resolution. Reinstein et al.[20] have shown that the precision of corneal epithelial measurements can be further improved using deconvolution approaches. The above techniques make measurement of corneal epithelial thickness practical.
Fig. 16.2 Radiofrequency signal received from a normal cornea. Interfaces shown are (a) the fluid couplant–epithelium interface, (b) epithelium–Bowman's membrane interface, and (c) the endothelium–aqueous interface.
Fig. 16.3 Details of the radiofrequency signal (A) and envelope detected signal (B) received from the epithelial region of the cornea.

The curvature of corneal surfaces can also be measured with reasonable accuracy. Various curve-fitting algorithms may be used. Three-dimensional reconstructions of corneal surfaces are also possible.
Examination Techniques

Ultrasound biomicroscopic examination with more commonly available commercial instrumentation is performed using an eye cup and a fluid couplant such as methylcellulose or saline. The viscosity of methylcellulose decreases fluid loss during examination. The moving transducer is inserted in the fluid couplant and scanning is begun (Fig. 16.4). The examination is performed with an unshielded moving transducer in close proximity to the eye, and care must be taken to prevent corneal contact. A membrane over the transducer can simplify the procedure at the expense of some sound attenuation, but this is more suitable for deep structures as the membrane echo interferes with corneal echoes.
Fig. 16.4  An eye being examined using an eye cup with methylcellulose as a couplant. The moving transducer is placed in close proximity to the corneal surface. 
(From Pavlin CJ, Foster FS. Ultrasound biomicroscopy of the eye. New York: Springer Verlag; 1994. With kind permission of Springer Science & Business Media.)

The best image and most accurate measurement is achieved when the ultrasound beam is perpendicular to the structures being examined. Any obliquity will result in part of the backscattered signal not being detected. When the epithelial and endothelial reflections are maximized, one can be assured of reasonable perpendicularity to the cornea.
The Normal Cornea

The normal corneal surface on ultrasound biomicroscopy appears as a smooth, curved, specular reflection from the fluid couplant–epithelium interface (Fig. 16.5). Immediately below this interface is another smoothly reflective line that corresponds to the surface of Bowman’s membrane. The distance between these two lines represents the epithelial thickness. The stroma has a uniform, low reflectivity. At the posterior aspect of the cornea is another highly reflective line that is the interface between Descemet’s membrane/endothelium and the aqueous. Descemet’s membrane and endothelium cannot usually be differentiated from each other.

The corneoscleral junction is easily discerned (Fig. 16.6). The sclera is composed of irregular scleral collagen bundles which have a higher reflectivity than the regular corneal lamellae of the cornea. The junction shows a curved region of transition similar to that seen histologically. The scleral spur constitutes an easily identified landmark which is useful for measurements that require a fixed point of reference. The external limbus is about 1 mm medial to the scleral spur.
Fig. 16.6 Ultrasound biomicroscopic image through the corneoscleral junction (1). The sclera has a higher internal reflectivity than the cornea.
Corneal Disease

Corneal edema

Ultrasound biomicroscopy of an edematous cornea shows increased corneal thickness and higher reflectivity of the corneal stroma (Fig. 16.7). The epithelium is usually thickened, and the smooth, highly reflective surface line replaced by a more irregular, less reflective line. Epithelial bullae can be discerned as a slight separation of the corneal epithelium from the underlying Bowman’s membrane. Ultrasound biomicroscopy provides an accurate quantitative method of measuring corneal thickness and of following progressive changes in corneal disease.

![Ultrasound biomicroscopic image of an edematous cornea. The epithelium (1) and corneal stroma (2) are both thickened.](From Pavlin CJ, Foster FS. Ultrasound biomicroscopy of the eye. New York: Springer Verlag; 1994. With kind permission of Springer Science & Business Media.)

Fig. 16.7

Descemet’s membrane detachment

Descemet’s membrane detachment can occur as a complication of intraocular surgery.[21] This generally results in corneal edema in the area involved and may result in the entire cornea becoming edematous, decreasing visibility. Ultrasound biomicroscopy images Descemet’s membrane clearly (Fig. 16.8) when it is detached and can be used to map out the area of detachment prior to surgical repair.

![Ultrasound biomicroscopic image in a case of Descemet’s detachment following cataract surgery. The detached membrane is clearly imaged (arrow).](From Pavlin CJ, Foster FS. Ultrasound biomicroscopy of the eye. New York: Springer Verlag; 1994. With kind permission of Springer Science & Business Media.)

Fig. 16.8
Intraocular lens malposition

Intraocular lens displacement with corneal compromise can occur. This was more common with anterior chamber lenses, but can occur with posterior chamber lenses as well. The position of misplaced haptics is easily discernible by ultrasound biomicroscopy.

Imaging the anterior segment behind corneal opacities

Information on the status of the anterior segment behind corneal opacities can aid in planning pre-surgery. Ultrasound biomicroscopy allows imaging of the entire anterior segment unless extensive corneal calcification produces shadowing. The depth of the anterior chamber and state of angle opening can be evaluated. The status of the lens and the presence of pupillary membranes can be determined. Descemet's membrane detachments and irid adhesions to the posterior corneal surface can be imaged (Fig. 16.9).

![Ultrasound biomicroscopic image through an opaque cornea showing a closed angle and adhesion of the iris tip to the inferior corneal surface. (From Pavlin CJ, Foster FS. Ultrasound biomicroscopy of the eye. New York: Springer Verlag; 1994. With kind permission of Springer Science & Business Media.)](image1)

In pseudophakic corneal edema, the position of the intraocular lens can be determined. The position of haptics can be imaged and the amount of overlying tissue quantified if lens replacement is considered.

Corneal dystrophies

Intrastromal corneal pathology can be imaged in corneal dystrophies, congenital abnormalities, and systemic diseases. Abnormalities can be detected as either a disruption of the normal smooth corneal lamellae or a deposition of material of a different reflectivity from the normal cornea. Figure 16.10 shows a case of granular dystrophy.

![Fig. 16.10 Corneal dystrophies. (A) Clinical photograph of a patient with granular dystrophy. (B) Ultrasound biomicroscopic image shows the hyaline granules as highly reflective bodies in the superficial stroma, extending. (From Pavlin CJ et al. Ultrasound biomicroscopic assessment of the cornea following excimer laser photokeratectomy. J Cataract Refract Surg. 1994;20:206–211.)](image2)
**Peripheral corneal degenerations**

Peripheral corneal melting syndromes may be imaged by ultrasound biomicroscopy.[27] Areas of thinning can be quantitatively assessed. Figure 16.11 shows the ultrasound biomicroscopic appearance of an area of peripheral corneal thinning in a case of Terrien's marginal degeneration. The affected area shows corneal thinning, with an hourglass shape indicating thinning from both sides.

![Fig. 16.11 Peripheral corneal thinning in a case of Terrien's marginal degeneration (1).](image)

**Keratoconus**

Keratoconus can be assessed for variations in corneal curvature and corneal thickness.[28] Figure 16.12 shows the findings in a case of acute hydrops. The break in Descemet’s membrane is clearly demonstrated.

![Fig. 16.12 Keratoconus. (A) Acute hydrops in a patient with keratoconus. Descemet’s membrane and the endothelium have separated from the posterior corneal surface, showing internal fluid spaces in the stroma (1). (B) Acute hydrops shows a central defect due to separation between Descemet’s membrane and the endothelium (1).](image)
Corneal Tumors

Various tumors can involve the cornea either directly or as part of involvement of adjacent structures. Congenital tumors such as dermoids can involve the cornea.[29–31] It is frequently uncertain as to whether these tumors are superficial, or whether they invade the cornea. This differentiation can be important in planning therapy. Ultrasound biomicroscopy can image these lesions at depth, and determine whether they are superficial to Bowman's membrane or involve the corneal stroma. In the illustrated cases, the first case was a melanoma (Fig. 16.13A). Ultrasound biomicroscopy indicated that the lesion was superficial to Bowman's membrane (Fig. 16.13B), and surgery was performed with removal of the tumor, leaving the cornea intact. The second case was diagnosed as a mucoepidermoid carcinoma (Fig. 16.13C). Ultrasound biomicroscopy revealed full-thickness corneal involvement (Fig. 16.13D). Local removal was not possible. This case was treated by plaque radiotherapy.

Fig. 16.13 Corneal tumors. (A) Clinical photograph of melanoma involving the cornea. (B) Ultrasound biomicroscopy shows the lesion is superficial to Bowman's membrane with nor
Corneal Surgery

Keratoplasty

Penetrating

The junction between the host cornea and the graft is imaged clearly by ultrasound biomicroscopy. Frequently, the host cornea has a higher internal reflectivity, depending on the initial pathology. The graft itself has a low internal stromal reflectivity if it is clear. Graft failure will result in the signs of corneal edema, as outlined previously. Graft thickness can be measured.

The image of the junction varies, depending on the particular features of the graft and the postoperative interval. In the early postoperative period, a line indicating the junction can be discerned (Fig. 16.14). As healing takes place, the stromal junction becomes less clearly delineated. Discrepancy in thickness between the graft and host is frequently found, usually with a thicker host side, but occasionally, in a condition such as keratoconus, a thicker graft side can be noted. Irregularities of the inner wound can be discerned, with a step being present or an irregularity in Descemet’s membrane being noted. Other features such as iris adhesions to the wound can be imaged.

Fig. 16.14  Ultrasound biomicroscopic image of the margin of a penetrating corneal graft. The junction (1) is slanted. Epithelial and endothelial surfaces are reasonably well apposed.

Lamellar

The junction between the lamellar graft and the host graft bed can be imaged. Lamellar thickness and host cornea thickness can be measured independently. Irregularities such as elevated margins or epithelial ingrowth can be imaged.
Refractive surgery

Excimer laser keratectomy

Photorefractive and phototherapeutic keratectomy involve removing a thin layer of superficial cornea. The region of excimer laser photokeratectomy is imaged as a loss of the double line found superficially in normal corneas (Fig. 16.15). The interface between the stroma and the regenerated epithelium is generally less reflective than the interface between the epithelium and Bowman's membrane. The junction between treated and untreated cornea can be detected by the transition from the double reflection to a single reflection. The fine superficial stromal haze is usually below the resolution level of ultrasound biomicroscopy. More extensive scarring, however, can be detected as more highly reflective regions in the superficial stroma. Ultrasound biomicroscopy can provide some indication of the depth of corneal pathology before considering phototherapeutic keratectomy.

![Image](image-url)

**Fig. 16.15** Ultrasound biomicroscopic image of the junction between the treated and untreated region (inset) in a case of excimer laser photokeratoplasty. The double superficial echoes of the untreated region on the right give way to the single echo in the treated region where Bowman's membrane is missing. (From Pavlin CJ et al. Ultrasound biomicroscopic assessment of the cornea following excimer laser photokeratectomy. J Cataract Refract Surg. 1994;20:206–211.)

Laser-associated in situ keratomileusis

In laser-associated in situ keratomileusis (LASIK) the keratome incision line can be imaged, allowing one to assess depth of the incision and irregularities such as epithelial ingrowth. Figure 16.16 shows an example of the ability to define the incision interface. This patient had two separate keratome flaps performed. The inferior incision line is intact, but the more superficial flap was buttonholed. Ultrasound biomicroscopy clearly shows the edges of the superficial flap extending to Bowman's membrane centrally. Use of the arc scanner has also allowed construction of three-dimensional maps of various layers. Epithelial depth can be mapped using color coding (Fig. 16.17). This technique can shed light on the structural changes that are produced by this type of surgery, and follow variations such as epithelial and stromal thickness over time.

![Image](image-url)

**Fig. 16.16** Arc scan image of a cornea that has undergone LASIK with a buttonholed flap. Two separate flaps were created in this patient at different times. The first incision line is intact (1). The second more superficial incision line (2) extends to
Bowman’s membrane at the site where the flap was buttonholed.
(Courtesy of D. Reinstein, New York.)
**Fig. 16.17** Three-dimensional thickness maps of the corneal epithelium and stroma pre- and post-LASIK. The top two images show increased thickness and irregularity of the central epithelium. The bottom two images show decreased thickness of the central stroma.

Summary

High-frequency ultrasound has provided a method of imaging ocular structural detail previously below the resolution of conventional ultrasound. The cornea is a good candidate for imaging with this technique because of its superficial location, which allows use of higher-frequency transducers with the highest possible resolution. Light-based techniques such as anterior segment optical coherence tomography can also produce high-resolution images of the cornea. High-frequency ultrasound has superior penetration through opaque tissue and is preferable for determining the state of the underlying anterior segment.
Acknowledgments

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References


Chapter 17 – Anterior Segment Optical Coherence Tomography

Martin Heur, Yan Li, David Huang

Introduction

Optical coherence tomography (OCT) was initially developed by Fujimoto, Huang, and colleagues as a way to obtain near-histological resolution images of tissue without biopsy.[1] In OCT, a beam of light, typically in the infrared wavelength range, is scanned across a sample such as the eye. The optical delay of the reflected light is determined by interferometry to generate a ranging measurement called the axial scan (A-scan). The axial resolution of the A-scan depends on the coherence length of the light used, and is typically several microns (µm). Transverse scanning of the beam yields information to build cross-sectional or three-dimensional images. The imaging principle of OCT is similar to ultrasound and radar, but the spatial resolution of OCT is much finer due to light's shorter wavelength. Izatt and colleagues reported the first application of OCT in imaging of the cornea and anterior segment in 1994.[2] Since then, anterior segment OCT has undergone several iterations of refinement.

Optical coherence tomography relies on interferometry to measure distance. In the interferometer, a broad-spectrum light, typically from an infrared superluminescent diode (SLD), is split into sample and reference beams through a beam splitter. The reflections from the sample and reference arms are recombined at the beam splitter and their interference pattern is detected by a photodetector and converted into a digital signal. A computer processes the digital data to generate a cross-sectional image. The original OCT technology is now classified as time-domain OCT (TD-OCT), in which the reference mirror is moved through a range of delay, and the resulting interference patterns between the sample and reference beams are processed into an axial image. The scanning speed in TD-OCT is limited by having to physically cycle the reference mirror through the delay range. In order to speed up image acquisition, a new technology called Fourier-domain OCT (FD-OCT) has been developed. In FD-OCT, the reference mirror is stationary and the A-scan is generated by Fourier transformation of spectral interference patterns between the sample and reference reflections. Advantages of FD-OCT include improvements in scanning speed and signal-to-noise ratio that are achieved through elimination of reference mirror movement and
simultaneous detection of reflections from all layers of the target. The terms spectral OCT, spectral-domain OCT (SD-OCT), and frequency-domain OCT (FD-OCT) are synonymous with FD-OCT. Swept-source OCT (SS-OCT), also called optical frequency domain imaging (OFDI), is a subtype of FD-OCT. Table 17.1 lists the commercially available TD-OCT platforms for anterior segment imaging. An important consideration is the wavelength; longer wavelength light penetrates deeper into the sclera and iris, but has coarser resolution, and the best operating wavelength will depend on the intended application.

Table 17.1  -- Commercially available TD-OCT platforms

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>Type</th>
<th>Wavelength</th>
<th>Resolution</th>
<th>Scan speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visante</td>
<td>Carl Zeiss Meditec, Inc.</td>
<td>TD-OCT 1310 nm</td>
<td>18 µm</td>
<td>2 kHz</td>
<td></td>
</tr>
<tr>
<td>Heidelberg SL-OCT</td>
<td>Heidelberg Engineering</td>
<td>TD-OCT 1310 nm</td>
<td>25 µm</td>
<td>0.2 kHz</td>
<td></td>
</tr>
<tr>
<td>RTVue</td>
<td>Optovue Inc.</td>
<td>FD-OCT 830 nm</td>
<td>5 µm</td>
<td>26 kHz</td>
<td></td>
</tr>
<tr>
<td>Bioptigen SD-OCT</td>
<td>Bioptigen, Inc.</td>
<td>FD-OCT 840 nm</td>
<td>3–5 µm</td>
<td>17 kHz</td>
<td></td>
</tr>
<tr>
<td>Casia SS-1000</td>
<td>Tomey USA</td>
<td>FD-OCT 1310 nm</td>
<td>10 µm</td>
<td>30 kHz</td>
<td></td>
</tr>
</tbody>
</table>
Keratoconus Screening

Keratoconus is an ectatic disorder of the cornea characterized by progressive stromal thinning and steepening of the cornea, leading to a loss of visual acuity. Moderate to advanced keratoconus is easily recognizable by several distinctive clinical features, but the diagnosis of forme fruste keratoconus can be challenging. Keratoconus evaluations, done through corneal topography-based algorithms, can sometimes be ambiguous. When a patient presents with normal vision and shows only a slight inferior steepening on topography, the clinician is left to wonder whether subclinical keratoconus is present. In such situations, OCT-generated pachymetry maps (Fig. 17.1) could help confirm the diagnosis by detecting the eccentric focal thinning characteristic of keratoconus. Studies of eyes of keratoconus and unaffected patients showed that presence of the following pachymetric parameters in the central 5-mm area of an unoperated cornea should raise concerns for keratoconus:

1. The difference in the minimum thickness and median thickness in the central 5-mm area is less than $-63 \mu m$ (minimum − median $< -63 \mu m$).
2. The difference in average thickness of the inferior (I) octant and the superior (S) octant is less than $-31 \mu m$ (I − S $< -31 \mu m$).
3. The difference in average thickness of the inferotemporal (IT) octant and the superonasal (SN) octant is less than $-48 \mu m$ (IT − SN $< -48 \mu m$).
4. Minimum corneal thickness is less than 492 $\mu m$.
5. The thinnest region of the cornea is outside of the central 2-mm area.
Fig. 17.1 RTVue-CAM OCT pachymetry maps. (A) Normal cornea. (B) Keratoconic cornea. Three pachymetry indices (I−S = −55µm, IT−SN = −69 µm, minimum = 462 µm) met diagnostic criteria for keratoconus.

The above parameters evaluate for the presence of asymmetry in corneal thickness and thinning. One abnormal parameter is suggestive while two or more abnormal parameters are diagnostic of keratoconus.[3]
Refractive Surgery Evaluation

LASIK flap evaluation

Optical coherence tomography allows for the visualization of the LASIK flap in the immediate postoperative period. Li and colleagues have shown that LASIK flaps were detectable in all patients at 1 day and 1 week after surgery. Imaging of the flap in the immediate postoperative period allows for the evaluation of microkeratome and femtosecond laser performance (Fig. 17.2A). In addition to flap thickness evaluation, the flap morphology can also be analyzed. OCT analysis has shown that flaps created with the IntraLase femtosecond laser are more uniform in thickness, whereas those created with a microkeratome are thinner in the center.
Fig. 17.2 (A) RTVue-CAM OCT image 1 week after LASIK with flap creation by a femtosecond laser (IntraLase®). The image's quality was enhanced by registering and averaging 16 consecutively acquired frames. The flap thickness was measured with computer calipers to be 109–115 µm. (B) RTVue-CAM OCT taken 2 weeks after LASIK. A gap filled with epithelium was visualized between the temporal edge of the flap and the stromal bed edge. The width of the gap was measured with computer calipers.

Refractive enhancement

Corneal ectasia occurring after laser vision correction is a well-documented complication, and an important risk factor is residual stromal bed thickness less than 250 µm.[6] The capability to analyze flaps several years after LASIK using OCT becomes useful in refractive enhancement evaluations. Accurate measurement of the residual stromal bed thickness can help determine whether a LASIK enhancement can be safely performed. An attractive feature of OCT is that, unlike an ultrasound pachymeter, it can measure the residual stromal bed thickness without the need for a flap lift.

LASIK complications

Optical coherence tomography can be valuable in the evaluation of LASIK cases with suboptimal outcomes. In a patient with decreased vision following LASIK, measuring the residual stromal bed thickness can aid in the evaluation for post-LASIK ectasia. The flap edge is usually well defined in OCT images, making it possible to visualize the position of the flap relative to the flap bed. In a case of decreased vision in the early post-LASIK period, flap striae were visualized at the slit lamp examination, but a gap between the flap and bed edges could not be appreciated. Optical coherence tomography was used to visualize the location of the gap and the direction of the flap shift (Fig. 17.2B). This was helpful for planning the flap lift and repositioning procedure.
Corneal Power Calculation

Fourier-domain optical coherence tomography is fast enough to calculate the central corneal power by averaging the measured instantaneous curvature of the central region. In postrefractive surgery patients, the usual relationship between anterior and posterior corneal curvatures no longer holds. Conventional keratometry can grossly overestimate the central corneal power after myopic LASIK and underestimate the central corneal power after hyperopic LASIK. A method that measures both anterior and posterior corneal curvatures is useful in calculating the true central corneal power in postrefractive surgery patients who are considering cataract surgery.
Corneal Opacities

Ultrasound has traditionally been used to provide imaging of and through an opacified cornea. In addition to higher resolution, OCT offers an additional advantage of being a non-contact modality. OCT can provide pachymetry mapping in opacified corneas more accurately than slit scanning devices.[7] OCT can also measure the depth of corneal opacity, thereby aiding in the preoperative planning (Fig. 17.3). Using the depth data, the surgeon can opt for an ablative approach such as phototherapeutic keratectomy if the superficial opacity can be removed while maintaining a residual stromal bed thickness of greater than 250 µm, or opt for anterior lamellar keratoplasty if replacement tissue is needed. If the opacity is too deep for ablation or anterior lamellar keratoplasty, then a penetrating keratoplasty can be considered. OCT can also be used to image anterior chamber structures such as the angle, iris and lens, iris and drainage tube through an opaque cornea, which can be helpful in assessing the mechanism of postoperative intraocular pressure elevation.[8]

Fig. 17.3 RTVue-CAM OCT image of a cornea with severe haze and scarring after photorefractive keratectomy enhancement, limiting best spectacle-corrected visual acuity (BSCVA) to 20/50. The central corneal thickness was determined to be 344 µm while depth of the opacity was determined to be 127 µm, indicating that there was not sufficient stromal bed thickness for a phototherapeutic keratectomy to remove the haze. The BSCVA was improved to 20/20–3 with topical corticosteroid treatment.
Cornea Transplant

**Posterior lamellar keratoplasty**

Since the inception of modern posterior lamellar keratoplasty in the late 1990s by Melles, the technique has undergone several cycles of refinement to Descemet's stripping automated endothelial keratoplasty (DSAEK).[9] The primary advantages of DSAEK over penetrating keratoplasty (PK) are a smaller and more secure wound, lower postoperative astigmatism, and faster visual rehabilitation.[10] Postoperative OCT scans can be used to monitor for lenticule detachment in patients with persistent postoperative corneal edema (Fig. 17.4).

![Montage of RTVue-CAM OCT sections showing detachment of the endothelial graft 1 week following Descemet's stripping automated endothelial keratoplasty.](image)

**Femtosecond-enabled keratoplasty**

Penetrating keratoplasty (PK) is evolving due to the ability to make complex-shaped incisions using a femtosecond laser such as the IntraLase® (AMO, Inc., Santa Ana, CA). A concern of traditional PK performed using trephine blades is the slow wound healing and the weak host–donor junction. Shaped incision designs such as the zigzag, top hat, and mushroom configurations created using the femtosecond laser result in greater resistance to wound leakage when compared to traditional PK.[11] The wound may be stronger and heal faster due to the interlocking host–donor junction and greater wound contact area. Proper apposition of the interlocking wound can be visualized with OCT (Fig. 17.5). OCT can also be very helpful in confirming proper matching of laser cuts and developing the proper suturing technique to obtain good wound apposition, especially in the early phases of adopting a new wound architecture.
Fig. 17.5 Montage of RTVue-CAM OCT sections taken 4 months after IntraLase®-enabled penetrating keratoplasty. The sutures had been removed. The zigzag wound junctions (red arrows) were well apposed.
Refractive Implants

Corneal implants

Keratophakia involves implanting a lens within the corneal stroma, under a lamellar flap created using a microkeratome or femtosecond laser. Use of earlier synthetic material resulted in tissue necrosis and extrusion secondary to impermeability of the implanted material to nutrients within the corneal stroma. A newer generation of synthetic material under development, such as silicone hydrogel, promises increased safety and refractive predictability. Keratophakic implants are optically clear on OCT and appear as dark spaces between the lamellar flap and stromal bed. OCT can be used to determine the position and depth of the implant within the cornea without the need of a flap lift.

The ACI 7000 (AcuFocus Inc., Irvine, CA and Bausch & Lomb, Rochester, NY) corneal implant is a ring made of an opaque biocompatible polymer that increases the depth of focus through the pinhole effect. It has shown promising results in an initial trial to treat presbyopia. The ACI 7000 corneal implant is placed under a corneal flap cut by a microkeratome or femtosecond laser. OCT can be used to evaluate the implant depth and centration in situ (Fig. 17.6A).
Intacs intrastromal ring segments (Addition Technology Inc., Fremont, CA) were initially developed to treat low myopia. The 150-degree segments function as spacers between collagen fibers, shortening the arc length of the fibers and flattening of the central cornea.[14] The degree of flattening is proportional to the thickness of the ring segments. Intacs have been shown to improve the best-corrected visual acuity and decrease myopia and astigmatism in keratoconic eyes.[15] Proper implant depth is critical for ensuring good visual outcome. Intacs segments appear as dark spaces within the corneal stroma on OCT (Fig. 17.6B). The implant depth, ideally at 70%, should be measured at the inner edge of the segment to avoid compression and optical artifacts above and below the implant.
Phakic Intraocular Lenses

Phakic intraocular lenses (PIOL) are designed to treat myopia by providing additional refractive power. Two PIOL, Visian Implantable Collamer Lens (ICL) (STAAR Surgical Co., Monrovia, CA) and Verisyse PIOL (Ophtec USA Inc., Boca Raton, FL), have received FDA approval for treatment of myopia ranging from −3 diopters to −20 diopters with astigmatism less than 2.5 diopters. Potential advantages of PIOL over corneal refractive surgery include a larger range of treatable ametropia, faster visual recovery, and better quality of vision.[16],[17] PIOL is also attractive for patients who are not candidates for corneal refractive surgery.

The refractive success and long-term safety of PIOL depend on preservation of the anterior segment structures such as the endothelium, trabecular meshwork, iris, and crystalline lens from mechanical trauma related to the PIOL. Currently used white-to-white angle measurement may not accurately reflect the internal anterior chamber width. OCT can provide accurate measurements such as anterior chamber widths, measured from angle recess to angle recess, along several meridians.[18] OCT can be used to determine the crystalline lens rise, the distance between the anterior pole of the lens and angle recess plane. Crystalline lens rise greater than 0.6 mm has been shown to be a risk factor for pigment dispersion in patients with Artisan PIOL.[19] OCT can also be used to simulate PIOL implantation to determine the virtual clearance of PIOL from the endothelium and crystalline lens.[20] OCT can provide the distances between the PIOL and the corneal endothelium, iris, and the crystalline lens postoperatively (Fig. 17.7). Long-term follow-up after PIOL implantation using OCT can help to detect eyes at risk for complications such as corneal decompensation, pupillary block, and cataract formation from PIOL-crystalline lens contact.
Fig. 17.7 RTVue-CAM OCT image showing a posterior phakic intraocular lens (Visian ICL) vaulting over the natural crystalline lens with good clearance of 471 µm as measured by computer calipers.

Financial interests

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References


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The term congenital refers to conditions that are present in the newborn. Congenital corneal opacities may result from hereditary, developmental, or infectious causes. They can be bilateral, and can be seen in isolation or in association with other ocular or systemic abnormalities.

The prevalence of congenital corneal opacity is approximately 3 : 100 000 newborns, and this figure increases to 6 : 100 000 if congenital glaucoma is included.[1] Although congenital clouding of the cornea is rare, the ophthalmologist must make an accurate diagnosis to predict the natural history of the disorder, to look for associated ocular and systemic abnormalities, to provide genetic counseling, and to begin appropriate medical or surgical therapy promptly.[2] This chapter will address the main features and differential diagnosis between the most common causes of corneal opacification seen at birth (Box 18.1).

Box 18.1

<table>
<thead>
<tr>
<th>Congenital opacities of the cornea – STUMPED classification[2]</th>
</tr>
</thead>
<tbody>
<tr>
<td>S Sclerocornea</td>
</tr>
<tr>
<td>Tears in Descemet's membrane</td>
</tr>
<tr>
<td>Letter</td>
</tr>
<tr>
<td>--------</td>
</tr>
</tbody>
</table>
| T      | Congenital glaucoma  
|        | Birth trauma |
| U      | Ulcer  
|        | Herpes simplex  
|        | viral  
|        | Bacterial  
|        | Neurotropic |
| M      | Metabolic (rarely present at birth)  
|        | Mucopolysaccharidoses  
|        | Mucolipidoses  
|        | Tyrosinosis |
| P      | Posterior corneal defect  
|        | Peters’ anomaly  
|        | Posterior keratoconus  
|        | Staphyloma |
| E      | Endothelial dystrophy  
|        | Congenital hereditary  
|        | Posterior polymorphous corneal dystrophy  
|        | Stromal: congenital stromal corneal dystrophy |
| D      | Dermoid |

History and Physical Examination

Diagnosing the condition causing congenital corneal clouding begins with the history and physical examination. A complete obstetric, maternal, paternal, and family history may be helpful in identifying many diseases. For example, the newborn's mother may have a history of a gestationally acquired rubella infection or previous vaginal or cervical herpes simplex infection. In addition there may be a relative with congenital hereditary endothelial dystrophy, making this diagnosis a strong possibility. After obtaining a detailed history, examination of the newborn is needed. This may be limited to a bedside or office evaluation. Often a careful slit lamp examination is possible using a handheld slit lamp. However, a complete examination is sometimes best accomplished under general anesthesia with the collaboration of a pediatric ophthalmologist, along with cornea and glaucoma specialists (Fig. 18.1). During the examination under anesthesia (EUA), A scan, B scan, and high-frequency ultrasound biomicroscopy (UBM) should be performed. UBM has been widely used for imaging the anterior segment structures of the eye with exceptional resolution (Fig. 18.2). Some studies have demonstrated that UBM is capable of delineating the corneal layers, with the exception of distinguishing between Descemet's membrane and the endothelium.[3]
Fig. 18.1 An infant with sclerocornea being examined under general anesthesia.
Biomicroscopic examination of the cornea and anterior segment can be performed with a handheld slit lamp at the bedside, in the office, or in the operating room. An infant lid speculum is sometimes used with topical anesthesia to provide exposure for a detailed examination, but it may be possible to examine the baby adequately by simply opening the lids. The portable slit lamp permits examination of the cornea with magnification and a slit beam. The slit beam makes this method superior to the operating microscope for corneal evaluation. Also, it is often easier to use than a regular slit lamp because the child can remain in the parent's lap during the examination and there is less movement of the patient's head.

When examining the corneal epithelium, a cobalt blue light with fluorescein can be used with the slit lamp or with a penlight to detect epithelial defects. Visualization through a cloudy cornea can be difficult, especially if the clouding is diffuse and dense. During an EUA, the view through the cornea may be improved by mechanically removing the epithelium. This can be accomplished with cellulose sponge debridement. If the baby is awake, a cotton fiber can be used to touch the cornea while movement of the other lid is used as an indicator of corneal sensation. This is especially important if herpes simplex keratitis or another disorder associated with decreased corneal sensitivity is suspected. If an infection is
present and corneal cultures are needed, general anesthesia is required and the operating microscope is used for the procedure.

The diameter of the normal infant cornea is 10.0–10.5 mm. Corneal enlargement, especially if unilateral, can be a sign of congenital glaucoma. Although corneal enlargement can often be detected by gross inspection, a measurement should be obtained. Corneal diameters should be measured both vertically and horizontally with a caliper. A photograph of the child with a ruler on his or her forehead can also be used to assess corneal diameters.

Intraocular pressure measurement is part of the essential examination in a newborn with a congenital cloudy cornea. Several devices are available for intraocular pressure testing, including portable applanation (Perkins, Draeger), indentation (Schiotz), electronic (TonoPen), pneumatic or noncontact (air puff) tonometers, and finger tension measurement. One study showed the pneumatonometer to be the most accurate in children and the TonoPen the least.[4]

General anesthesia typically lowers intraocular pressures, which in premature infants has a mean of 10.11 ± 2.21 mmHg.[5] One study in Korea found an average IOP of 11.85 ± 1.35 mmHg in children under the age of 2. During EUA this measurement should be taken as soon as possible after induction. The finger tension technique helps to detect elevated intraocular pressure while the infant is asleep.

Gonioscopy in the neonate may be best accomplished using a Koeppe lens, and the operating microscope rotated to the correct angle for viewing. In the office, a Koeppe lens and the indirect ophthalmoscope with a 20 diopter lens gives an adequate view.[6]

Dilation of the pupil can sometimes expose the pupillary opening to areas of clear cornea, allowing a view of the posterior structures even in the presence of corneal opacities (Fig. 18.3). The direct ophthalmoscope can be used with a Koeppe lens to obtain a good view of the optic nerve and the macula. An indirect ophthalmoscope with a 14 diopter lens can also be used to examine the disk, and with a 20 or 28 diopter lens for the macula and peripheral retina. Retinoscopy should be done if possible, but can be difficult through a cloudy cornea.

![Fig. 18.3 A](image1) Corneal opacity in a newborn with Peters' anomaly. B, Dilated fundus examination through the corneal opacity of the same patient.

When the corneal opacification is dense and visualization of ocular structures is impossible, there are
other ways to evaluate the eye. A-scan ultrasonography can determine the position of the iris and the lens, as well as the length of the eye. For a better evaluation of the anterior segment, UBM should be performed. UBM examination is not only very useful in evaluating the clinical diagnosis in congenital corneal opacification but also it acts as a preoperative guide in cases undergoing penetrating keratoplasty by detecting keratolenticular and iridocorneal adhesions and other ocular abnormalities, such as aniridia and congenital aphakia.[1] Awareness of anterior segment abnormalities can help in planning surgery and preventing complications.[3] B-scan ultrasonography provides imaging of the vitreous and retina when there is no view due to anterior segment pathology.

Examination of the patient's parents may also be helpful in establishing the diagnosis of various inherited conditions. In unilateral conditions the healthy eye must be thoroughly examined to look for any abnormalities. This may also be helpful in making the diagnosis in the fellow eye.

When examining an infant with a cloudy cornea, it helps to remember the STUMPED mnemonic developed by Waring and colleagues.[2] This can be used as a guide to the differential diagnosis of the neonatal cloudy cornea (Box 18.1).

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Sclerocornea (S TUMPED)

Sclerocornea is a primary anomaly in which scleralization of the peripheral part of the cornea, or of the entire tissue, occurs. It may occur alone or in association with other ocular defects. It generally appears sporadically, but can also be familial or autosomal dominant.[7] The embryogenesis and genetics are discussed in Chapter 57.

Sclerocornea is nonprogressive and is usually bilateral but commonly asymmetric (see Fig. 18.1).[8] The opacification of the cornea is smooth, white, and vascular; it appears to be an extension of the sclera without limbal landmarks, and is greater peripherally than centrally (Fig. 18.4). The vessels are fine continuations of conjunctival vessels, but deep vascularization can sometimes occur. Some patients have only the peripheral cornea involved (Fig. 18.4), whereas others have opacification of the entire cornea (Fig. 18.5).
**Fig. 18.4** Peripheral sclerocornea. Significant scleralization of the peripheral cornea sparing the central cornea.
Waring and Rodrigues have classified sclerocornea into four groups:[9],[10]

- Isolated peripheral sclerocornea: abrupt change from scleral-like tissue to clear cornea with no other ocular abnormalities (Fig. 18.4).
- Sclerocornea plana: flat corneas with keratometry readings of less than 38 diopters, leading to high hyperopia. The anterior chamber is usually shallow, but glaucoma is apparently not frequent. Pseudoptosis can be seen because the flat cornea supports the upper lid poorly. Sharkey et al.[11] reported it in association with epidermolysis bullosa dystrophica. Reduced corneal sensitivity has also been reported in cornea plana.[12]
- Sclerocornea associated with anterior chamber cleavage anomalies: commonly associated with Peters' anomaly, characterized by paracentral corneal adhesions.
- Total sclerocornea: the most common form causing congenital corneal opacity (Fig. 18.5). The corneas are totally opaque and vascularized, but the central cornea is not as densely opaque as the peripheral cornea. The opacification generally affects the full thickness of the stroma, and visualization of the endothelium, pupil, and the anterior chamber structures may be difficult or impossible. Histopathologically, the corneal stroma in sclerocornea resembles sclera. Precisely arranged stromal lamellae are absent and stromal vascularization is often present (Fig. 18.6). Electron microscopy has shown that the collagen fibrils of the stroma are arranged irregularly and are variable in caliber. Most notably, their diameter often is markedly increased (up to 1500 Å), resembling the diameter of the scleral fibrils (Box 18.2). Furthermore, in contrast to the normal human cornea, the collagen fiber diameter in sclerocornea reduces gradually in magnitude from anterior to posterior.[10]
### Box 18.2

**Histopathology in sclerocornea**

- Corneal stroma resembles sclera morphologically
- Precise arrangement of stromal lamellae absent
- Irregular arrangement of collagen fibers; variable in diameter
- Collagen fibrils thickened (up to 1500 Å in diameter); resemble scleral fibrils
- Diameter of collagen fibrils decreases in posterior stroma
- Changes in posterior cornea may resemble those seen in Peters’ anomaly
  (Descemet’s membrane attenuated or absent)

UBM has been reported to help the diagnosis of the sclerocornea itself (Fig. 18.7), to highlight potential associated structural abnormalities, and to facilitate surgical planning by identifying the pupil, which may or may not coincide with the clearest area of the cornea.\(^3\) The usefulness of anterior segment OCT has also been described.\(^{11}\) Patients with sclerocornea sometimes exhibit somatic abnormalities such as mental retardation, anomalies of the skin, facies, ears, cerebellum, and testes. A list of ocular and systemic abnormalities associated with sclerocornea is found in Chapter 57.
The differential diagnosis can be narrowed by history, clinical examination, ocular and systemic associations, and observations of the patient over time, and include arcus juvenilis, interstitial keratitis, Peters’ anomaly, and microcornea (Table 18.1).

### Table 18.1 -- Differential diagnosis of sclerocornea

<table>
<thead>
<tr>
<th>Sclerocornea</th>
<th>Arcus juvenilis</th>
<th>Interstitial keratitis</th>
<th>Peters’ anomaly</th>
<th>Microcornea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal opacification greater peripherally, smooth, white, and vascular extension of the sclera without limbal landmarks</td>
<td>Cornea is not vascularized Clear interval between the opacification</td>
<td>Later onset Associated</td>
<td>Central cornea narrower</td>
<td>Steep corneal curvature Narrow angles</td>
</tr>
<tr>
<td>Fine vessel continuations of conjunctival vessels, but deep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
conjunctival vessels, but deep vascularization can sometimes occur. Some patients have opacification of the entire cornea. Usually associated with other ocular abnormalities.

and the limbus may be associated with lipid abnormalities. With a red, inflamed eye, more opaque than periphery.

Small anterior segments. Different systemic associations.

Management is similar to that of other congenital corneal opacities. If the disorder is unilateral and the other eye has good visual acuity, the decision to operate becomes more difficult, and surgery can be performed only if other ocular structures are relatively normal. If the condition affects the central corneas bilaterally, causing a significant reduction in visual acuity, penetrating keratoplasty should be performed in an attempt to obtain useful vision. Awareness of other ocular abnormalities can be made by the use of UBM or OCT prior to surgery, reducing the risk of complications. However, the prognosis for a clear graft is still worse than in Peters’ anomaly, but better in recent years with the use of topical ciclosporin.
Congenital glaucoma

Congenital glaucoma is the most important disease in the differential diagnosis of congenital corneal clouding, because early diagnosis may allow adequate treatment and preservation of vision, and delayed diagnosis can result in irreversible visual loss. All infants with unilateral or bilateral cloudy corneas must be evaluated carefully for glaucoma. Primary congenital glaucoma is usually sporadic, but may be inherited as an autosomal recessive trait.

The first symptoms of primary congenital glaucoma are epiphora, photophobia, and blepharospasm. The first signs are elevated intraocular pressure, corneal enlargement and clouding, and optic nerve cupping.[15] The symptoms result from epithelial edema, caused by the elevated intraocular pressure. The cornea and sclera of infants and children are more elastic and distensible than those of adults. The infant's Descemet's membrane is also thinner (3–4 µm) than in the adult (10–12 µm). Therefore, the elevated intraocular pressure may lead to rapid enlargement of the eye (buphthalmos). As the eye enlarges, the cornea stretches, which causes Descemet's membrane to break. These ruptures disturb the endothelial barrier, and aqueous gains access to the corneal stroma and epithelium. The initial symptoms may worsen acutely with disruption of Descemet's membrane. In addition to the elevated intraocular pressure, breaks in Descemet's membrane contribute to diffuse corneal haziness. Early in the course of the disease, corneal clouding may be seen only intermittently and may precede breaks in Descemet's membrane.

Another corneal sign of congenital glaucoma is increased corneal diameter. The buphthalmic eye has a diameter greater than the normal infant corneal diameter of 10.0–10.5 mm. Parents sometimes bring these children after noticing slight haziness or enlargement of the corneas. The enlarged cornea and globe of buphthalmos generally are not present in the immediate postpartum period, but by 6 months of age approximately 75% of affected infants manifest corneal enlargement.[16]

The tears in Descemet's membrane can be single or multiple, and appear as elliptical, glassy, parallel ridges on the posterior cornea, either peripherally or across the visual axis. In congenital glaucoma these breaks have a random distribution, most commonly horizontal or concentric to the limbus, in contrast to the oblique and vertical orientation of the breaks in Descemet's membrane seen in birth trauma (Fig. 18.8).
The diagnosis of congenital glaucoma is not based on elevated intraocular pressure alone. Other signs, such as corneal haze, increased corneal diameter, increased cup to disk ratio, increased axial length, and gonioscopic abnormalities, support the diagnosis.

The management of congenital glaucoma is surgical. Procedures should be performed by a glaucoma specialist or a pediatric ophthalmologist, and initially include goniotomy and trabeculotomy. If these two procedures fail, trabeculectomy or aqueous shunt implantation are employed. Corneal clouding can complicate the treatment by making goniotomy and filtering trabeculotomy difficult owing to poor visualization of the anterior chamber angle.

**Birth trauma**

Injury to the cornea occurs from placement of the forceps blade across the globe and orbit during delivery, causing blunt trauma and rupture of Descemet's membrane.[17] Other soft tissue injuries such as unilateral periorbital edema and ecchymoses may accompany the trauma.[2] Left eyes seem to be affected more commonly than right eyes because neonates usually present in the left-occiput-anterior position.[18]

The elevation of intraocular pressure (IOP) is acute, distends the globe, exceeds the elasticity of Descemet's membrane, and produces tears that allow the cornea to imbibe aqueous with resultant stromal and epithelial edema.[2] The Descemet's tears in birth trauma are usually unilateral, central and, in contrast to congenital glaucoma, line up in a vertical or oblique pattern (Table 18.2; Fig. 18.9), presumably because the tip of the forceps has slipped over the rim of the orbit and compressed the globe vertically, stretching it horizontally to create the tears.[2]

<table>
<thead>
<tr>
<th>Birth trauma</th>
<th>Congenital glaucoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal IOP</td>
<td>High IOP</td>
</tr>
<tr>
<td>Normal corneal diameter</td>
<td>Large corneal diameter with buphthalmos (may get back to normal with decrease in IOP)</td>
</tr>
<tr>
<td>Corneal edema in the immediate postpartum period</td>
<td>Corneal edema weeks or months after birth</td>
</tr>
<tr>
<td>Corneal edema clears after weeks to months</td>
<td>Corneal edema clears after lowering IOP</td>
</tr>
</tbody>
</table>
Tears in Descemet's membrane are vertical or oblique

<table>
<thead>
<tr>
<th>Tears in Descemet's membrane are horizontal or concentric to the limbus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left eyes seem to be more frequently affected and other soft tissue injuries may accompany the trauma</td>
</tr>
<tr>
<td>No preference for either eye</td>
</tr>
<tr>
<td>Usually no photophobia</td>
</tr>
<tr>
<td>Photophobia</td>
</tr>
</tbody>
</table>

Birth trauma produces diffuse stromal and epithelial edema in the immediate postpartum period (Table 18.2). If the damage is not too severe, the corneal edema usually clears within weeks to months.

The young endothelium resurfaces the posterior cornea, and it synthesizes a new thick basement membrane that accentuates the edges of the tear and fills in the dehiscence. Once the corneal edema disappears, the edges of the break appear as rounded, glassy ridges that protrude from the posterior cornea and light up strikingly in retroillumination (Fig. 18.9B). Within the break, the new basement membrane has a beaten-metal, guttate appearance. Although the cornea remains clear, high residual corneal astigmatism, which may range from 4 to 9 diopters, requires urgent correction and amblyopia treatment. The steep meridian of the astigmatism parallels the Descemet's ruptures. Rigid gas-permeable contact lenses (Fig. 18.10) and patching are the first choice of treatment. The diagnosis is based on the history and clinical findings.

**Fig. 18.9** Vertical and oblique distribution of Descemet's tears in birth trauma. The tears are seen in (A) direct but sometimes are better seen in (B) retroillumination.

**Fig. 18.10** Birth trauma. A and B, Rigid gas-permeable lenses fitting in a 2-month-old child with high with-the-rule astigmatism due to forceps injury.
Later in life, the previously stressed endothelium may decompensate, with resulting corneal edema that requires penetrating keratoplasty to restore vision.[18],[19] The visual acuity may be limited by amblyopia from high astigmatism and anisometropia.

Refraction for glasses or contact lenses should be measured as soon as possible. Early fitting with rigid gas-permeable lenses has been reported in birth trauma (Fig. 18.10), showing excellent results in the prevention of amblyopia secondary to severe astigmatism.[20] Patching or atropine therapy is necessary to treat amblyopia. The corneal specialist should always try to work with a pediatric ophthalmologist in the treatment of amblyopia.

Another type of prenatal trauma that can produce a corneal opacity is an amniotic band that stretches across the face during development, often causing a cleft lip, sometimes associated with microphthalmia and a hazy cornea. The corneal haze probably represents direct trauma to the cornea and not tears in Descemet's membrane.[21]
Posterior Corneal Defect (STUMPED)

The term anterior segment dysgenesis replaced the original classification of anterior chamber cleavage syndrome and refers to a spectrum of congenital eye conditions that are associated with posterior defects of the cornea. They can be peripheral or central. Peripheral abnormalities are not covered in this chapter because they are associated with insignificant congenital opacity of the cornea. Unlike the peripheral abnormalities, in which the abnormal anatomy is visible, the central anomalies are characterized by a focal absence of the corneal endothelium and Descemet's membrane, which results in an overlying corneal opacity.[9] This leukoma often obscures other anterior segment abnormalities.

The central abnormalities are often classified into three groups: Peters' anomaly, posterior keratoconus, and congenital anterior staphyloma.[9] Peters' anomaly is the most common congenital opacity presenting to the tertiary cornea specialist, followed in frequency by sclerocornea, corneal dermoids, congenital glaucoma, microphthalmia, birth trauma, and metabolic disease.[89]

**Peters' anomaly (STUMPED)**

This congenital disorder is characterized by a central corneal opacity with corresponding defects in the posterior stroma, Descemet's membrane, and the endothelium.[9],[90] The peripheral cornea is usually relatively clear.[91] Synechiae frequently extend from the iris collarette to the edge of the posterior corneal defect (Fig. 18.13). The iris strands can appear as filaments, thicker bands, or broad sheets which form an arcuate iridocorneal adhesion. Lens abnormalities, including cataract and central cornelenticular adhesion, as well as corneal staphyloma, are variably present.[92],[93] The cornelenticular adhesion, when present, can be difficult to diagnose clinically because of the overlying leukoma. Incomplete development of the angle is common, helping to explain the high frequency of glaucoma (50–80%), which may present at birth or may develop later. The cornea is usually avascular.
Usually occurring bilaterally (80% of cases), but often asymmetrically (Fig. 18.14), Peters’ anomaly may vary morphologically from its most simple presentation, described as Peters’ anomaly type I (corneal opacity with iridocorneal adhesion),[94],[95] to more severe cases, Peters’ type II, with involvement of the lens and other ocular anomalies, in addition to the corneal opacity and iridocorneal synechiae (Table 18.5).[93] The corneal opacity (and the corresponding defect in Descemet’s membrane and endothelium) coincides with the area of the corneolenticular contact.
Table 18.5  -- Peters’ anomaly types I and II

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Corneal opacity + iridocorneal adhesions</strong></td>
<td><strong>Corneal opacity + iridocorneal adhesions + lens abnormality (position or transparency)</strong></td>
</tr>
<tr>
<td>– unilateral involvement predominates</td>
<td>– denser corneal opacity</td>
</tr>
<tr>
<td>– mild/dense central stromal nebular opacity bordered by iris strands that cross the anterior chamber from the iris collarette</td>
<td>– most frequently bilateral</td>
</tr>
<tr>
<td>– peripheral cornea is usually clear, but peripheral edema or scleralization may be present</td>
<td>– thought to be secondary in nature, rarely demonstrating a primary hereditary pattern[94]</td>
</tr>
<tr>
<td>– peripheral edema can regress (especially if associated with glaucoma successfully treated)</td>
<td>– keratolenticular adherence varies</td>
</tr>
<tr>
<td>– lens generally clear and in normal position</td>
<td><strong>Most characteristic:</strong> the lens directly adherent to the posterior corneal surface or firmly pressed against it</td>
</tr>
<tr>
<td>– usually isolated</td>
<td>Other cases: cortex or only lens fragments adhere to the corneal defect, the lens is in position but is cataractous</td>
</tr>
<tr>
<td>– associated ocular anomalies may be present (microcornea, sclerocornea, and infantile glaucoma)</td>
<td>– Severe ocular and systemic malformations</td>
</tr>
<tr>
<td>– vitreoretinal abnormalities rare</td>
<td><strong>Ocular abnormalities:</strong> microphthalmic with vitreoretinal abnormalities (PHPV*), microcornea, cornea plana, glaucoma, sclerocornea, colobomas, aniridia, and optic atrophy</td>
</tr>
<tr>
<td>– good visual acuity potential</td>
<td>– Systemic conditions: congenital cardiac defects, craniofacial dysplasia, skeletal abnormalities, and central nervous system and urogenital anomalies</td>
</tr>
<tr>
<td>– <strong>systemic abnormalities</strong> are uncommon</td>
<td>Other systemic conditions: external ear anomalies, pulmonary hypoplasia, syndactyly or polydactyly, camptodactyly, fetal transfusion syndrome, Wilms’ tumor[91]</td>
</tr>
</tbody>
</table>

Peters’-plus syndrome:[98] Peters’ anomaly + short stature, brachymorphy, mental retardation, abnormal ears, and, in some patients, cleft lip and palate[122] |

Krause-Kivlin syndrome:[99–100] Peters’ anomaly + facial abnormalities, disproportionate short stature, retarded skeletal maturation and developmental delay (probably inherited in an autosomal recessive manner)

* Persistent hyperplastic primary vitreous

Histopathologic findings used to be the only tool to help the diagnosis of Peters’ anomaly in cases in which the leukoma was severe, but UBM has been demonstrated to be very useful, clearly detecting central corneal edema and absence of Descemet’s membrane, as well as corneolenticular and iridocorneal adhesions when present (Fig. 18.15).[96] The histopathologic changes can be present in all layers of the central cornea (Fig. 18.16; Box 18.3).
Fig. 18.15 UBM image of Peters’ anomaly showing central posterior stromal defect with iridocorneal adhesions. (Courtesy of Elisabeth L. Affel, MS.)

Fig. 18.16 Histopathology of Peters’ anomaly (see also Box 18.3).

Box 18.3

**Histopathology of Peters’ anomaly**

- Central concave defect in the posterior corneal stroma (posterior ulcer)
- Disorderly stromal lamellae in ulcer bed
- Absence of corneal endothelium and Descemet's membrane in the posterior ulcer
- Corresponding area of central corneal edema and opacification
Keratolenticular adhesions to posterior cornea in some cases
Iridocorneal adhesions to margin of ulcer in some cases
Bowman's layer thickened or absent

Historically, the internal ulcer of Von Hippel has also been grouped with Peters' anomaly, but the former is probably an intrauterine inflammatory condition rather than a true developmental defect, and it has been considered in the differential diagnosis of Peters' anomaly.\[91\] However, some authorities believe the posterior ulcer of Von Hippel to be identical to Peters' anomaly without lens abnormalities. Other conditions in the differential diagnosis of Peters' anomaly include sclerocornea, dermoid, congenital hereditary endothelial dystrophy (CHED), and posterior polymorphous corneal dystrophy (PPCD) (Table 18.6).

Table 18.6  -- Peters' anomaly – differential diagnosis

<table>
<thead>
<tr>
<th>Peters' anomaly</th>
<th>Von Hippel's internal corneal ulcer</th>
<th>Sclerocornea</th>
<th>Dermoids</th>
<th>CHED</th>
<th>PPCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opacification</td>
<td>Central and localized</td>
<td>Inflammation characterizes Von Hippel's internal corneal ulcer, differentiating it from Peters' anomaly</td>
<td>Diffuse full-thickness</td>
<td>Diffuse edema</td>
<td>Less edema than CHED but also diffuse; usually normal pachymetry</td>
</tr>
<tr>
<td>Iris strands</td>
<td>Attached to leukoma</td>
<td>More pronounced peripherally</td>
<td>Usually unilateral</td>
<td>Can be central and often appear to have satellite lesions</td>
<td>Corneal changes in parents</td>
</tr>
<tr>
<td>Lens normal</td>
<td>or abnormal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Peripheral endothelium and</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>peripheral</td>
<td>Descemet's membrane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There are several proposed causes of Peters' anomaly (Box 18.4). The most often documented is the incomplete central migration of corneogenic mesenchyme (i.e., neural crest cells), accounting for posterior endothelial and stromal defects.\[97\] Goldenhar's syndrome is also due to the maldevelopment of neural crest cells and has been documented in association with Peters' anomaly.\[97\] Intrauterine infection, maternal alcoholism, and other teratogenic exposures can also be associated with Peters' anomaly (Box 18.4).\[98\]

Box 18.4

Proposed causes of Peters' anomaly

- Isolated Peters' anomaly
  - Autosomal recessive defect
  - Autosomal dominant defect
  - Chromosomal defect
Homeotic gene defect

Peters’ anomaly with other ocular malformations
  Monogenic defect
  Developmental field defect
  Homeotic gene defect*

Peters’ anomaly with systemic malformation
  Well-delineated single-gene disorders
  Developmental field defect
  Contiguous gene syndrome
  Homeotic gene defect*

Teratogenic effects of alcohol, rubella virus, or retinoic acid

* Genes that control differentiation of primordial cells and the development of different body segments.

Box 18.5

<table>
<thead>
<tr>
<th>CHED 1 vs CHED 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHED 1</strong></td>
</tr>
<tr>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Appears within the first 2 years of life</td>
</tr>
<tr>
<td>Slowly progressive</td>
</tr>
<tr>
<td>Pain, tearing, and photophobia</td>
</tr>
<tr>
<td>Parents may have PPMD changes</td>
</tr>
<tr>
<td><strong>CHED 2</strong></td>
</tr>
<tr>
<td>Autosomal recessive form</td>
</tr>
<tr>
<td>Present at birth</td>
</tr>
<tr>
<td>Nonprogressive nystagmus</td>
</tr>
</tbody>
</table>

Peters’ anomaly occurs most often as a sporadic disorder; however, both recessive and dominant inheritance patterns have been observed. Hereditary syndromes featuring Peters’ anomaly type II as the only anterior segment disturbance include Krause–Kivlin syndrome[99],[100] and Peters’-plus syndrome (see Table 18.5). The diagnosis of Krause–Kivlin syndrome should be considered in any child with anterior segment anomaly who is small for gestational age or who develops short stature.[98] Mutations in the eye development genes PAX6, PITX2, CYP1B1, and FOXC1 have been implicated in Peters’ anomaly.[101]

The clinician dealing with an infant affected by Peters’ anomaly should be aware of the possible associated malformations, obtain a thorough family history, and examine the eyes of the parents and the siblings. These patients should be screened for systemic malformations, especially those involving midline body structures such as the pituitary gland and the heart.[102] The management of these children should involve a multidisciplinary approach, including corneal and pediatric ophthalmologists, social workers, and – first and foremost – committed and informed parents.[103] Genetic counseling should take into consideration the fact that autosomal recessive inheritance is possible.

When glaucoma develops in infancy, it is treated surgically. In older children, medical therapy should be
used initially. If the Peters' anomaly is bilateral and visually disabling, corneal transplantation is often required to provide a clear visual axis and to try to restore vision. If a cataract is also present and has to be removed, the prognosis is worse.[91] Glaucoma represents the most common complication after the surgery. Penetrating keratoplasty and combined placement of a Molteno implant has been described with success,[104] although it may be preferable to control the pressure first.

Theoretically, surgery at the youngest age possible would be best to minimize the risk of deprivation amblyopia, but the decision to operate at a young age should be made by considering the risks of undergoing general anesthesia (for the initial surgery and multiple EUAs for suture removal over 2 months postoperatively), as well as associated systemic abnormalities and the general health of the patient.

The management of Peters’ and pediatric penetrating keratoplasty is discussed in detail in Chapter 57.

**Posterior keratoconus (STUM P ED)**

Posterior keratoconus is a very uncommon corneal abnormality characterized by a discrete local conical internal protrusion of the posterior corneal curvature with concomitant stromal thinning and variable haze. The lesion is usually circumscribed, crater-like, round or oval, and occurs centrally or eccentrically, singly or multiply. It may represent the mildest variant of Peters’ anomaly.[9] It has no relationship to anterior keratoconus.

The defect in posterior keratoconus is usually central. Sometimes pigment surrounds the edges of the posterior depression, suggesting previous contact with the iris.[9] Posterior synechiae may rarely be seen in the affected area. The condition is usually focal, but can rarely occur in a generalized form, with the concavity involving the entire posterior surface of the cornea.

The condition is most commonly unilateral but may be bilateral.[9] It is nonprogressive and usually sporadic. Familial and posttraumatic cases have been reported. The pathogenesis and causes of this condition are unclear. Descemet’s excrescences can also be present in or just outside of the area of involvement.[105] The corneal endothelium and Descemet’s membrane are present. On histologic examination Descemet’s membrane may be thinned, with a concomitant endothelial abnormality in the focally abnormal area.

Although the irregularity of the posterior cornea may affect vision to some extent, approximately 85% of the refractive power of the cornea is produced by the anterior surface, which is usually normal and uniform throughout the cornea. Keratometry and photokeratoscopy provide an incomplete picture of the surface geometry of posterior keratoconus, and more recent studies done with corneal topography evaluation detected changes on the anterior corneal curvature. Rao and Padmanabhan[106] demonstrated that posterior keratoconus manifests significant corneal surface alterations and that the changes in central and paracentral posterior keratoconus appear to progress with an increase in patient age. Mannis et al.[107] used topographic analysis to study the cornea of a patient with posterior keratoconus and demonstrated a central steepened ‘cone’ coincident with the area of circumscribed posterior keratoconus, as well as paracentral flattening.

Posterior keratoconus is commonly isolated but it can be associated with other ocular abnormalities, such as astigmatism, choroidal and/or retinal sclerosis, lens abnormalities, posterior polymorphous dystrophy, retinal coloboma, optic nerve hypoplasia, and ptosis. Systemic abnormalities can also be present, including mental retardation, hypertelorism, superiorly displaced lateral canthi, short stature, and genitourinary abnormalities.[108]

Visual acuity can be reduced due to amblyopia, refractive errors, or astigmatism, but vision is usually acceptable and keratoplasty is rarely indicated. Correction of refractive errors may be all that is needed to attain useful vision.

**Congenital anterior staphyloma (STUM P ED)**

Congenital anterior staphyloma (CAS) is characterized by a protuberant congenital corneal opacity.[109] The ectatic cornea often has a blue hue. It may extend beyond the plane of the eyelids, and secondary
epithelial metaplasia into keratinized, stratified, squamous epithelium occurs. The anterior segment frequently is disproportionately enlarged and extremely disorganized. There is virtually no possibility of attaining useful vision. One or both eyes may be involved.

The lesion can be vascularized and can transilluminate easily. The posterior portions of the thin cornea are usually lined by the remaining pigment epithelium of the atrophic iris.

As in Peters’ anomaly, the lens may be adherent to the posterior cornea. The pathogenesis of CAS is unknown, but the condition is thought to be secondary to an intrauterine infection or related to a developmental abnormality such as a severe type of Peters’ anomaly. In several cases one eye showed a frank staphyloma while the other demonstrated a Peters’ anomaly type II. In the CAS developmental abnormality there is failure of migration of mesenchymal tissues that ordinarily form the posterior corneal structures, iris, and angle. This maldevelopment, probably coupled with increased intraocular pressure caused by the angle abnormality, leads to corneal opacity and thinning, plus prominent buphthalmic enlargement of the entire anterior segment. Hereditary cases have been reported.

The diagnosis is made clinically and can be confirmed histopathologically if enucleation is performed. Descemet's membrane, Bowman's layer, and endothelium are typically absent.

The visual prognosis of CAS is very poor. Enucleation or evisceration may be considered in an attempt to improve cosmesis.
Three corneal dystrophies may exhibit diffuse corneal cloudiness at birth: congenital hereditary endothelial dystrophy, posterior polymorphous corneal dystrophy, and congenital stromal corneal dystrophy.

**Congenital hereditary endothelial dystrophy**

Congenital hereditary endothelial dystrophy (CHED), first documented in the English literature in 1960 by Maumenee, is a rare disease that has two forms (see Box 18.5): CHED 1 and CHED 2.[110],[111] The nomenclature for CHED and all corneal dystrophies has recently been standardized by the International Committee on the Classification of Corneal Dystrophies (IC3D).[111] CHED 1 is an autosomal dominant dystrophy, with its genetic locus at 20p11.2-q11. This has its onset in the first or second year of life, but is occasionally congenital. In these patients, corneal clouding ranges from a diffuse haze to a ground-glass, milky appearance. The corneal clouding is slowly progressive over 1–10 years. Patients usually present with photophobia and epiphora and the subsequent development of corneal clouding. The epiphora and photophobia resolve with the onset of clouding. Progressive diffuse corneal opacification develops, with an irregular edematous epithelial surface, scarring of Bowman's layer, and thickening of the stroma. The degree of clouding is usually symmetric, but occasionally one eye is more adversely affected.[112] The visual impairment in the dominant form is not initially as severe as in the recessive form, and patients therefore do not usually develop nystagmus.

CHED 2, previously referred to as Maumenee cornea dystrophy, is autosomal recessive and results from mutations in the solute carrier family, located on chromosome 20p13.[111] This form of CHED generally presents as bilateral corneal clouding at birth or shortly thereafter.[113] The corneal changes are stable and do not progress or regress. There are no associated symptoms, such as epiphora or photophobia, but patients often develop nystagmus because of the severity of the early visual loss. Occasionally, visual acuity is retained despite considerable corneal clouding. On slit lamp examination the epithelium appears roughened secondary to nonbullous epithelial edema. The stroma is often two to three times normal thickness and has a diffuse blue-gray, ground-glass haze (Fig. 18.17). The endothelium is generally difficult to observe because of the stromal haze. If seen, the endothelium is atrophic, irregular, or absent, and Descemet's membrane is thickened without guttae (Box 18.6; Fig. 18.18).
Fig. 18.17 Congenital hereditary endothelial dystrophy (CHED) with ground-glass diffuse stromal edema.

Box 18.6

Histopathology of CHED\textsuperscript{[125],[137]}

- The main characteristics are the severely degenerated corneal endothelial cells and abnormal thickening of Descemet's membrane.
- Changes in the epithelium, Bowman's layer, and the stroma are considered to be secondary to longstanding edema.
- Stromal lamellae irregular and separated by fluid pockets.
- Descemet's membrane: anterior banded zone normal, posterior nonbanded zone with aberrant collagen fibrils.
- Corneal endothelium atrophy with vacuolation, focal absence of cells and many multinucleated cells; melanin deposition can be present.

The differential diagnosis of CHED includes congenital glaucoma, posterior polymorphous corneal dystrophy (PPCD), Peters' anomaly, and inborn errors of metabolism, especially the mucopolysaccharidoses (Table 18.7). Both CHED and congenital glaucoma originate from defects in the neural crest cell contribution to the development of the anterior segment of the eye. Bahn et al.\[114\] state that congenital glaucoma is a result of abnormal crest cell migration, whereas CHED results from abnormal crest cell differentiation. Differentiating CHED from congenital glaucoma can be particularly difficult because measurement of the intraocular pressure may give unreliable results in the presence of stromal edema, and if severe corneal opacity has developed the optic disks cannot be inspected.\[114\],\[115\] These two entities may rarely coexist,\[116\] but inappropriate glaucoma surgery in CHED has been reported.\[117\]

<table>
<thead>
<tr>
<th>CHED</th>
<th>CSCD</th>
<th>PPCD</th>
<th>Congenital glaucoma</th>
<th>Birth trauma</th>
<th>Peters' anomaly</th>
<th>Mucopolysaccharidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated disorder</td>
<td>Corneal opacity: central and flaky, feathery appearance</td>
<td>Less edema</td>
<td>Enlarged corneal diameter</td>
<td>Patchy corneal edema from rupture of Descemet's membrane</td>
<td>Central and localized opacity</td>
<td>Corneal clouding is not usually seen at birth</td>
</tr>
<tr>
<td>Cornea avascular</td>
<td>Normal pachymetry</td>
<td>Usually normal pachymetry</td>
<td>Increased ultrasonic axial length measurements</td>
<td>Normal peripheral endothelium and Descemet's membrane</td>
<td>Conjunctival biopsy may be helpful</td>
<td></td>
</tr>
<tr>
<td>Eye is not inflamed</td>
<td>More common than CHED</td>
<td>Elevated IOP</td>
<td></td>
<td></td>
<td>Raised levels of mucopolysaccharide in the urine</td>
<td></td>
</tr>
</tbody>
</table>
Absence of iris abnormalities

It is important to distinguish between CHED and PPCD because the edema in posterior polymorphous corneal dystrophy may show slow clearing, so that penetrating keratoplasty may not be needed.[118]

Although the edema sometimes remains stationary, it usually progresses, and penetrating keratoplasty has been the recommended treatment of CHED to avoid amblyopia and restore vision.

**Posterior polymorphous corneal dystrophy (PPCD)**

Most reports on posterior polymorphous corneal dystrophy (PPCD) are based on adolescent or adult patients. PPCD is inherited as an autosomal dominant condition with variable expression in the vast majority of cases.[111]

Most patients with PPCD have bilateral, nonprogressive, asymptomatic disease that rarely requires penetrating keratoplasty. The pathogenesis of both CHED 1 and PPCD is considered to be due to a primary dysfunction of corneal endothelium.[110] These two entities also share clinical, histological, and embryological similarities, but significant differences between the phenotypic expression of these disorders exist (Table 18.7).

The severity and nature of the resulting endothelial dysfunction, which may be genetically controlled, ultimately determine the range of clinical signs that manifest in the form of CHED or PPCD.[110] PPCD is a milder disease characterized clinically by the presence of grouped vesicles or bands, geographic-shaped discrete gray lesions, and broad bands with scalloped edges and vesicles on the endothelial surface of the cornea (Fig. 18.19). Descemet’s membrane can be irregular and have a nodular or warty appearance.[119] These findings can be confirmed with specular microscopy.[119]
Although most patients with PPCD are asymptomatic, there is a form of PPCD characterized by congenital corneal edema which appears as a diffuse corneal haze at birth. In a review of eight families with PPCD, Cibis et al.\textsuperscript{[120]} found great variation ranging from mild endothelial defects to severe stromal and epithelial edema (Fig. 18.20). The largest published series of PPCD is that of Krachmer,\textsuperscript{[121]} who reported on clinical and pathological findings in 13 patients. Sometimes the cornea can be so opacified as to preclude detailed examination of the endothelium. The epithelium can be irregular, with a thickened stroma and deep feathery opacities (Fig. 18.20). If the endothelium is visible or the edema clears, findings are the same as those in adult PPCD described above. Nystagmus is present if the visual loss is significant.
It is important to differentiate PPCD from CHED, because the treatment is different. Some children with PPCD have experienced clearing of their corneal edema, eliminating the need for penetrating keratoplasty.[122] Examination of the child's parents may be helpful in making the diagnosis.[121] Despite the absence of significant symptoms, one of the parent's corneas will generally demonstrate the features of PPCD.

Several different loci have been identified in PPCD, including 20p11.2-q11.2, 1p34.3-p32.3, and 10p11.2. CHED 1 and PPCD share the 20p11.2-q11.2 locus. It has been proposed that CHED 1 may be allelic to PPCD.[123],[124]

If the cornea does not clear spontaneously, penetrating keratoplasty should be performed. One review of corneal transplantation in children suggests that patients with congenital corneal opacities from PPCD have a better visual prognosis after surgery than patients with other causes of congenital corneal clouding.

The histopathology of PPCD demonstrates epithelialization of endothelial cells (Fig. 18.21),[120] with multilayered cells linked by desmosomes with surface microvilli and intracytoplasmic filaments.
Histopathology of PPCD showing epithelialization of endothelial cells.

**Congenital stromal corneal dystrophy (CSCD)**

Previously referred to as congenital hereditary stromal dystrophy, this is a rare dystrophy caused by a mutation on the decorin gene on chromosome 12.[111] It was thought that all hereditary congenital corneal dystrophies were caused by endothelial dysfunction until a report by Witschel et al.[125] demonstrated a true CSCD in a small number of dominant pedigrees.

The anterior stroma demonstrates a diffuse, flaky-feathery opacification caused by corneal lamellar irregularities that are denser centrally and anteriorly than peripherally and posteriorly. The changes are stationary from birth. Nystagmus and esotropia are often present secondary to the profound visual loss. The corneal thickness is normal, and the epithelium, Descemet's membrane, and endothelium appear normal. There are no associated systemic abnormalities.

If the clinical diagnosis of CSCD is in doubt, the histopathologic features of the corneal button are typical and usually result in accurate diagnosis. The histologic abnormalities are confined to the stroma. The anterior banded layer of Descemet's membrane is missing. This abnormality is questionably caused by early dysfunction of the endothelium that becomes normal later. The epithelium, basement membrane, Bowman's layer, endothelium, and posterior banded layer of Descemet's membrane are normal.

As with CHED, no medical therapy is available for CSCD. Penetrating keratoplasty can be performed if the corneal opacification is severe.

**Other dystrophies**

Posterior amorphous corneal dystrophy (PACD) is a rare autosomal dominant dystrophy characterized by bilateral sheetlike opacification of the posterior stroma in association with corneal flattening and thinning.[126][127] Other ocular findings include hyperopia, marked corneal astigmatism, and progressive ectasia of the cornea.[128] Dunn et al.[129] recognized other features, such as anterior iris surface and stromal abnormalities, fine iris processes extending to Schwalbe's line for 360°, and extension of the opacity to the limbus. No abnormalities of the endothelium were detected, and visual acuity was only mildly affected. The condition appears to be nonprogressive.

Two forms of the disease were documented by Moshegov: a centroperipheral and a peripheral form. The first
is more severe and usually presents with keratometry readings below 41 diopters and central corneal thicknesses less than 0.5 mm, and the latter represents a less severe peripheral form with less hyperopia, some slight myopia, and keratometry readings above 41 diopters, but the central corneal thicknesses are similar to those with the centroperipheral form. Although the centroperipheral form of posterior amorphous corneal dystrophy is more likely to lead to presentation, most patients are asymptomatic. Castelo Branco et al.[130] recently reported two cases of PACD in the same family in which they studied the depth of stromal opacification using UBM.

This dystrophy can be very subtle in its appearance and easily overlooked. This led Moshegov et al.[127] to suspect that the prevalence of this condition is higher than the few reports in the ophthalmic literature suggest. Bowman's layer dysgenesis has been reported as a cause of congenital cloudy cornea.[131] It cannot be classified as a dystrophy because its inheritance is unknown. It presents as bilateral, noninflammatory, progressive corneal disease without associated systemic disease.[45]
Congenital Dermoids (STUMPE D)

Dermoids are solid benign congenital tumors that frequently arise at the inferotemporal corneoscleral junction. They are classified as choristomas because they contain cellular elements not normally present in that location: ectodermal derivatives, such as hair follicles, as well as sebaceous and sweat glands embedded in connective tissue and covered by squamous epithelium. They can also contain smooth and skeletal muscle, nerves, blood vessels, bone, cartilage, and teeth. In the eye they most often present as yellowish-white, solid, vascularized, elevated nodules straddling the corneal limbus. They vary greatly in size ranging from 2 to 15 mm in diameter. Corneal dermoids occur more commonly as single lesions but may be multiple, and they may be unilateral or bilateral, the former being the more common. Dermoids can be central and often appear to have satellite lesions.

These tumors usually occur in sporadic fashion, although their occurrence in cousins has been described. Corneal dermoids have been genetically mapped to chromosome Xq24-qter. They usually exhibit little or no growth but can occasionally enlarge. They typically extend into the deeper stroma without affecting Descemet’s membrane and the endothelium, but in some cases they replace all tissue anterior to the iris pigment epithelium.

Three different types of dermoid choristoma have been characterized according to the extent of involvement (Table 18.8). The grade 1 dermoid is the most frequent type. It is small, usually measuring 5 mm in diameter or less, single, limbal or epibulbar (Fig. 18.22). Already present at birth, it may enlarge, especially at puberty. In general they are superficial, but can rarely involve deeper ocular structures such as the ciliary body or the anterior chamber angle. In approximately one-third of cases these dermoids are associated with a broader syndrome complex such as Goldenhar’s syndrome. This syndrome is nonfamilial and consists of congenital abnormalities classically described by the triad of epibulbar dermoids, preauricular appendages, and pretragal fistulas. In one review of the literature, epibulbar dermoids were found in 76% of patients with Goldenhar’s syndrome and were almost always located straddling the inferotemporal limbus (Fig. 18.22A).

Table 18.8 -- Grading dermoids

<table>
<thead>
<tr>
<th>Grade 1 (limbal or epibulbar)</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>most frequent type</td>
<td>much larger</td>
<td>most severe type</td>
</tr>
<tr>
<td>small (5 mm in diameter)</td>
<td>covers part or entire central corneal surface</td>
<td>very rare</td>
</tr>
<tr>
<td>single</td>
<td>variable depth of stromal extension</td>
<td>entire anterior segment is involved</td>
</tr>
<tr>
<td>inferotemporal limbus</td>
<td>does not involve Descemet's membrane or</td>
<td>associated abnormalities: microphthalmos, posterior</td>
</tr>
</tbody>
</table>

Table 18.8 -- Grading dermoids

---

Krachmer > Volume 1 - Fundamentals and Medical Aspects of Cornea and External Disease > Part III - Differential Diagnosis of Selected Problems in Corneal and External Eye Disease > Chapter 18 - Congenital Corneal Opacities: Diagnosis and Management > //Congenital Dermoids (STUMPE D)
- it may enlarge (especially at puberty)
- superficial
- one-third of cases associated with Goldenhar's syndrome: nonfamilial; triad of epibulbar dermoids, preauricular appendages, and pretragal fistulas
- other abnormalities: coloboma of the lids, aniridia, microphthalmos, anophthalmos, neuroparalytic keratitis, lacrimal stenosis, Duane's syndrome, cardiovascular abnormalities, facial hemiatrophy, atresia of the external auditory meatus, accessory auricles, nevus flammeus, and neurofibromatosis.[135]

<table>
<thead>
<tr>
<th>the corneal endothelium</th>
<th>segment abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other abnormalities associated with dermoids include coloboma of the lids, aniridia, all grades of microphthalmos, anophthalmos, neuroparalytic keratitis, lacrimal stenosis, Duane's syndrome, cardiovascular abnormalities, facial hemiatrophy, atresia of the external auditory meatus, accessory auricles, nevus flammeus, and neurofibromatosis.[135]

The second type, grade 2 dermoid, is much larger, covering part of (Fig. 18.23) or the entire corneal surface, with variable depth of stromal extension.[134] This type generally does not involve Descemet's membrane or the corneal endothelium. It is the most important type in the differential of congenital corneal opacities.

**Fig. 18.22** Congenital dermoids. A, Limbal dermoid situated in the inferotemporal limbus. B, The same dermoid patient after lamellar keratectomy was successfully performed.
The third and most severe type, grade 3 dermoid, is fortunately the rarest. In this type, the entire anterior segment is involved. The tumor replaces the cornea, anterior chamber, and iris stroma, and is lined posteriorly by the pigment epithelium of the iris. Microphthalmos is common, and posterior segment abnormalities can also occur. Patients develop the different types of corneal dermoid depending on when during gestation the teratogenic effect took place. The earlier the onset, the more severe the malformation.

Gonioscopic examination of the angle beneath the tumor and UBM (Fig. 18.24) can indicate the depth of extension.
Fig. 18.24  Congenital dermoids. A, UBM shows a depth of a dermoid that reaches approximately 90% of the corneal stroma. Whenever excision is to be performed, a penetrating keratoplasty might be necessary. B, UBM image of another dermoid that leaves about 0.4 mm of cornea, indicating that in this case lamellar keratoplasty could be performed. (Courtesy of Elisabeth L. Affel, MS.)

The entities most likely to be confused diagnostically with corneal dermoids are corneal keloids, Peters’ anomaly, CHED, and sclerocornea (Table 18.9).[136]

Table 18.9  -- Differential diagnosis of dermoids

<table>
<thead>
<tr>
<th>Dermoids</th>
<th>Corneal keloids</th>
<th>CHED</th>
<th>Peters’ anomaly</th>
<th>Sclerocornea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellowish-white vascularized</td>
<td>Chalky white</td>
<td>Diffuse corneal edema bilaterally;</td>
<td>Corneal opacity +</td>
<td>Lost transition between cornea and</td>
</tr>
<tr>
<td>elevated nodules</td>
<td>solid masses</td>
<td>with glistening gelatinous texture</td>
<td>iridocorneal</td>
<td>sclera</td>
</tr>
<tr>
<td>– inferotemporal at the limbus</td>
<td>Inheritance may</td>
<td>Corneal opacity +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>junction</td>
<td>be recessive or</td>
<td>iridocorneal adhesions with or without</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>dominant</td>
<td>lens abnormality (position or transparency)</td>
<td></td>
<td></td>
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<tr>
<td>– may contain hair follicles,</td>
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<td>sebaceous and sweat glands,</td>
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<td>smooth and skeletal muscle,</td>
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<td>nerves, blood vessels, bone,</td>
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Limbal dermoids are usually a cosmetic rather than a visual problem; however, the vision may be impaired if there is encroachment into the pupillary area by either the tumor or the lipid infiltrate that is often present around the periphery. In some instances, irregular astigmatism appears. If astigmatism is the cause of visual loss in patients with corneal dermoid, correction of refractive errors can be attempted with spectacles, and patching may be necessary to treat amblyopia. They can also cause irritation (due to a hair or mass effect), or produce drying of the surrounding cornea by lifting of the lid during blinking.

The tumor should be cut flush with the corneal surface (see Fig. 18.24B), but it may recur. If an effort is made to excise the entire tumor, perforation may occur. Therefore, it is advisable to have corneal tissue available. A good but not perfect cosmetic result can be achieved with a lamellar graft. The dermoid tissue is often not solid enough to retain sutures, so grafts must encompass the entire tumor. Dermoids that are incompletely excised can recur.

Dermoids that involve or distort the central cornea (Fig. 18.23) can reduce the quality of the visual image and create amblyopia. If the patient has bilateral visually disabling dermoids, treatment is indicated. If the central cornea is involved (Fig. 18.23), penetrating and lamellar keratoplasty are vision-restoring procedures.
Penetrating keratoplasty can be performed for central dermoids if they are 7 mm or less in diameter. Larger central dermoids require a two-stage procedure: first the tumor is excised and a large lamellar graft is placed in the bed; once that is healed, a smaller central penetrating keratoplasty is performed. An alternative method can utilize a corneoscleral rim. Operating for cosmetic reasons or mild irritation can be unsuccessful if unsightly scarring occurs.

Histologic examination confirms the diagnosis of the corneal dermoid.
Corneal Keloids

Keloids are reactive fibrous tissue proliferations that represent the exuberant response of embryonic connective tissue to injury.[137] They are thought to be secondary to a vigorous fibrocytic response to corneal perforation or injury.[137] Corneal keloids are seen as white, sometimes protuberant, glistening, masses (Fig. 18.25). They often assume tumoral proportions and bear a superficial resemblance to dermoids. They may be found in either the central or the peripheral cornea, and also resemble the nodules in Salzmann's degeneration (Table 18.10). Like dermoids, keloids show variable degrees of extension and may replace the cornea or the entire anterior segment, and can involve the entire corneal surface.[137] Subtle differences between corneal keloids and dermoids include the glistening and jellylike quality of the keloids. The definitive diagnosis can be made by performing a corneal biopsy.[137] Immunohistochemical and electron microscopic studies have demonstrated the presence of myofibroblasts in these lesions, differentiating them from Salzmann's nodules.[138]
Corneal keloids can be associated with Lowe's syndrome. However, the etiology of keloids in Lowe's syndrome remains obscure. Considerations include excessive local delivery of amino acids and unknown noxious substances through the leaking corneal vessels, seepage of similar substances across the defective blood–aqueous barrier and the decompensated endothelium, repeated external trauma with associated inflammation, phenytoin (Dilantin) therapy, and congenital predisposition. No data are available on the management of progressive corneal keloids. Possible empirical regimens include local excision, pressure therapy, topical corticosteroids, and cromolyn sodium.[139–141]

Study of enucleation specimens has revealed associated findings including cataract, anterior staphyoma, ruptured lens capsule with lens fragments in the wound, buphthalmos, chronic glaucoma, and angle-closure glaucoma.[142]
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46. Rodrigues MM, Calhoun J, Harley RD: Corneal clouding with increased acid mucopolysaccharide accumulation in Bowman's membrane. *Am J*


Chapter 19 – Peripheral Corneal Disease

Stephen C. Kaufman

The Peripheral Cornea: Its Susceptibility and Response to Disease

The peripheral cornea is generally considered to be that portion located between the central 50% of the cornea and the limbus. This is the thickest region of the cornea, which is directly adjacent to the corneal limbus and internal angle structures. Although the peripheral cornea manifests some of the same disorders as the central cornea, its proximity to the limbus and conjunctiva results in a unique collection of abnormalities. These frequently stem from the distinctive architecture of the limbus, which includes a highly vascular zone with associated lymphatic tissue, scleral collagen, corneal collagen, and limbal stem cells. Thus, vascular inflammatory disorders, limbal infections, collagen vascular disorders, neoplastic disease, and local degenerations may affect the peripheral cornea in a distinctive way.

This chapter discusses peripheral corneal diseases by categorizing the disorders into five groups: (1) congenital/developmental/inherited; (2) inflammatory/autoimmune; (3) neoplastic; (4) environmental exposure/degenerative/iatrogenic; and (5) infectious (Table 19.1). A number of these items could be classified under more than one heading; however, a single heading was chosen to reduce redundancy. To reduce further redundancy, this chapter includes an abbreviated discussion of each topic. If further information is desired, please refer to the specific chapter for that disorder.

Table 19.1 -- Classification of peripheral corneal disorders

1) Congenital/Developmental/Inherited
   A) Lattice dystrophy type II
   B) Wilson's disease
   C) Cornea plana
   D) Sclerocornea
   E) Posterior embryotoxin
   F) Axenfeld–Rieger anomaly

2) Inflammatory/Autoimmune
A) Rheumatoid arthritis
B) Polyarteritis nodosa
C) Wegener's granulomatosis
D) Marginal keratitis
E) Phlyctenulosis
F) Mooren's ulcer
G) Vascular pannus

3) Neoplastic
   A) Pterygium
   B) Dermoid
   C) Benign squamous metaplasia
   D) Carcinoma in situ/intraepithelial neoplasia
   E) Squamous cell carcinoma
   F) Melanoma

4) Degenerative
   Degenerative disorders that are not associated with corneal thinning:
     A) Dry eye/tear film deficiency
     B) Corneal arcus
     C) Lipid keratopathy
     D) Calcific band keratopathy
     E) White limbal girdle of Vogt
     F) Furrow degeneration
     G) Limbal stem cell deficiencies

   Degenerative disorders that are associated with corneal thinning:
     H) Terrien's marginal degeneration
     I) Pellucid marginal degeneration
     J) Dellen

5) Infectious
   A) Bacterial
   B) Fungal
   C) Viral
   D) Miscellaneous
Congenital/Developmental/Inherited Disorders of the Peripheral Cornea

Corneal dystrophies are rare, inherited, primary corneal disorders. There are no corneal dystrophies that exclusively affect the peripheral cornea, although Lattice dystrophy type II is associated with systemic amyloidosis and primarily involves the peripheral cornea. A complete discussion of this disorder is found elsewhere in this book.

Certain congenital systemic disease manifest changes in the peripheral cornea. Wilson's disease is a genetic disorder which results in the accumulation of copper in a variety of tissues and affects the neurological system, liver and other organ systems. In the cornea Wilson's disease produces an orangey-brown ring in the periphery of the cornea. This Kayser–Fleischer ring consists of copper which is deposited in Descemet's membrane. Iron deposition can be seen in many situations and appears as a brown line or other shape. Intercellular deposits of iron have been seen in basal corneal epithelial cells as well as in Bowman's membrane. At first glance iron lines and rings can mimic the appearance of the copper deposits associated with Wilson's disease, but careful slit lamp examination will demonstrate that the brown iron deposits are located more superficially within the cornea, at the level of Bowman's membrane. The corneal copper deposition does not affect vision or require treatment. This autosomal recessive genetic disorder generally presents in the first two decades of life with liver dysfunction. Wilson's disease can be treated with systemic D-penicillamine, which prevents disease progression and reduces the amount of copper already deposited in tissues. Ocular examinations help to document reduction of copper in the tissues of the body.

There is a group of congenital corneal disorders that affect the appearance and structure of the cornea and are apparent at birth. Sclerocornea and cornea plana are congenital disorders generally associated with scleralization of the cornea and an extremely flat corneal curvature, with keratometry measurements as low as 20 diopters. Although the term cornea plana is generally used in Europe, sclerocornea refers to the same disorder in the United States. These disorders appear to be distinct, with overlapping features, but may represent a spectrum of interrelated clinical findings. Sclerocornea is associated with a spectrum of clinical findings, from peripheral cornea opacity to a completely opaque white cornea. Corneal vascularization may be seen in the opaque region, and the curvature of the affected area is typically flatter than normal. Cornea plana may resemble sclerocornea, as the
majority of cases also exhibit peripheral scleralization of the cornea. As the name denotes, cornea plana is a disorder that exhibits very flat corneal curvatures of 20–30 diopters and hyperopia. Additionally, because the corneal vault is reduced, these individuals also demonstrate shallow anterior chambers. Cornea plana can be associated with diffuse, deep stromal opacities, and so may be difficult to distinguish clinically from sclerocornea.[4] Both disorders can be associated with other ocular anatomic abnormalities.

A group of related congenital corneal localized opacities have been described. The first of these, posterior embryotoxon, produces a thickened, prominent Schwalbe's line, which is more anteriorly located than normal. It can be seen with gonioscopy as a fine ground-glass-like membrane and has been estimated to occur in 15% of normal eyes.[5] When posterior embryotoxon exists without additional pathology it does not require treatment. When it is associated with other peripheral corneal abnormalities, including multiple peripheral iris strands, it is termed Axenfeld–Rieger anomaly. This and other related congenital diseases are discussed in more detail in their respective chapters.
Inflammatory/Autoimmune Disorders of the Peripheral Cornea

Because of the proximity of the peripheral cornea to the limbal vasculature and conjunctival lymphoid tissue, this region of the cornea is especially susceptible to immunologic disorders. Furthermore, the peripheral cornea may be significantly involved in systemic collagen–vascular disease. Many systemic immune diseases cause a secondary ocular inflammatory response which results in disorders such as keratoconjunctivitis sicca, scleritis, episcleritis, peripheral corneal disorders and vasculitis. The ophthalmologist should remember that ocular disease can be the presenting sign of these systemic disorders. The most common inflammatory disorders that affect the peripheral cornea are discussed below.

Of the systemic vasculitides, rheumatoid arthritis is the most common. Rheumatoid arthritis is a multisystem disease which primarily involves the peripheral joints. Nonarticular vasculitis affects 25% of patients and is associated with cardiac disease, pulmonary disease, splenomegaly, and ocular disease.[6]

The most common ocular disorder associated with rheumatoid arthritis is keratoconjunctivitis sicca. This produces a dry eye, which may result in a mild punctate epithelial keratitis or stromal ulcerations. Dry eyes are discussed more extensively elsewhere in this book. A majority of patients with rheumatoid arthritis have little or no symptomatology; however, more severe immunologic complications include sclerosing keratitis, which manifests as a superficial and midstromal peripheral keratitis which is generally associated with a nonnecrotizing scleritis.[7] The associated infiltrates can enlarge or proliferate and produce a breakdown of the corneal epithelium, and a secondary stromal melt may ensue (Fig. 19.1). In addition, a peripheral corneal furrow may develop in an area adjacent to a sclerosing keratitis without the presence of an infiltrate. The area of stromal thinning typically has an intact overlying epithelium and can be differentiated from the more severe form of keratolysis because the furrow may progress but rarely results in perforation of the cornea (Fig. 19.2). Conversely, keratolysis is associated with an acute severe melting of the corneal stroma which can proceed to perforation. Keratolysis is most commonly seen in patients with rheumatoid-associated scleritis. Multiple studies have demonstrated the importance of documenting rheumatoid arthritis-related scleritis, as this heralds the presence of extensive systemic vasculitis. If the systemic vasculitis is untreated, the majority of these patients will die within 5 years.[8]
Fig. 19.1 Macroulcerative peripheral keratitis in a patient with rheumatoid arthritis. Observe the deep ulceration concentric with the limbus and the steep, undermined central border of the ulcer.
Like many vasculitis-associated disorders, which are related to the systemic disease, the ultimate treatment involves therapy directed toward control of the systemic vasculitis. This generally entails the use of systemic prednisone or other immunomodulator(s). The addition of topical corticosteroids can be beneficial in the treatment of episcleritis or scleritis; however, they should be used with extreme caution in patients with keratolysis as they can accelerate the ‘melting’ process. If the keratolysis or scleromalacia progresses, a patch graft may be needed. In severe cases multiple patch grafts will be necessary. Pretibial periosteum has been used for corneal and scleral patch grafts, with success in these severe cases, as it is not susceptible to melting.[9]

Polyarteritis nodosa is a systemic vasculitis which typically affects the eyes in approximately 20% of cases. A bilateral peripheral keratitis typically involves the peripheral cornea and begins with infiltrates located within the mid-stroma.[10] The infiltrates may remain isolated to one region or may coalesce circumferentially and eventually progress to melting of the corneal stroma. The treatment for this disorder requires systemic therapy directed toward control of the vasculitis.

Wegener's granulomatosis may present with corneal signs which are similar to those of the other vascular inflammatory diseases and is usually associated with involvement of the respiratory tract, nasal tissues, glomerulonephritis and other organ systems.[10] Two forms of the ocular disease have been described: a
severe progressive disease, which has a 1-year mortality of 82% if untreated; and a limited, less severe form.\[11\] Additionally, Wegener's granulomatosis may produce a concomitant orbital inflammation with associated proptosis and orbital pain. The diagnosis should include radiographic studies of the chest and sinuses, serum antineutrophil cytoplasmic antigens (ANCA), and possibly tissue biopsy. As in polyarteritis nodosa, ocular involvement may be the initial sign of the vasculitis, and therapy frequently requires systemic immunosuppression.

A group of diseases develop as the result of the subject's own exaggerated hypersensitivity reaction to an antigen. These immune reactions may only cause mild pain, but in severe cases can also result in significant corneal neovascularization and scarring. *Marginal keratitis* is a disorder which is thought to result from ocular hypersensitivity reactions to toxins produced by bacteria that commonly colonize the eyelids. This disorder can produce peripheral corneal infiltrates and ulcerations in severe cases. The peripheral corneal infiltrates generally occur adjacent to the limbus, with a clear intervening zone between the lesion and the limbus (Fig. 19.3). The patient typically complains of redness and pain. The lesions may first appear similar to an infectious infiltrate with an intact corneal epithelium over the infiltrate. These lesions can also be found in patients who wear soft contact lenses. Because the infiltrates associated with the marginal keratitis are not infectious in nature, culture of the lesions results in a diagnosis of a sterile infiltrate. The lesions can be treated effectively with a topical steroid; however, the treatment should also involve reducing the bacterial antigens and toxins that are the underlying cause of the marginal keratitis. Lid hygiene and other treatments for blepharitis can be very effective in reducing the recurrence of this disorder. If there is any question regarding the etiology of a peripheral corneal infiltrate, the initial treatment should include an antibiotic alone or in combination with a steroid, and must include close clinical follow-up.
Fig. 19.3  Catarrhal corneal ulcer in a patient with blepharitis. The inferior infiltrate is parallel to the limbus and separated from it by a clear zone.

*Phlyctenulosis* is an inflammatory disorder which is similar to marginal keratitis but involves a more severe reaction. The immune reaction associated with a phlyctenule can produce significant corneal scarring and significant vascularization of the cornea, but perforation is rare (Fig. 19.4). Current studies have commonly associated staphylococcal disease with phlyctenulosis; however, older studies demonstrated a strong association with tubercular disease.[12] Like marginal keratitis, treatment involves topical steroids or possibly other immunomodulating medications such as topical ciclosporin; however, if tuberculosis is suspected steroids should not be used until a TB test can be performed. Importantly, if the phlyctenular disease is thought to be related to staphylococcal bacteria, lid hygiene should be a part of any treatment regimen. If long-term treatment is necessary, the author has had success using a commercially available topical ciclosporin emulsion, frequently adding a topical corticosteroid during the initial month of therapy.

Fig. 19.4  Corneal phlyctenule. These lesions can ‘march’ across the cornea with progressive vascularization and scarring.
Mooren's ulcer produces a painful progressive peripheral ulceration of the cornea. The cause of Mooren's ulcer is unknown, but there are generally considered to be two clinical types. Patients with the limited type are typically over 40 years old, have unilateral disease, and respond well to medical therapy. A more 'malignant' type of Mooren's ulcer has been described that generally occurs in younger patients and has been seen more commonly in Nigerian men.\[13\],\[14\] The disorder typically starts with a peripheral corneal infiltrate which slowly progresses. There is generally no clear zone between the infiltrate and the limbus. As the disease progresses, an ulcer develops with a characteristic overhanging edge which has an intact epithelium (Fig. 19.5). If the progression of the disease can be halted, the surface of the ulcer may heal with conjunctivalization over the melted corneal stromal bed. Unfortunately, the severe form of the disease is very difficult to treat and perforation is common. A detailed discussion of the individual medical and surgical treatments for these disorders can be found in the chapters focusing on the specific disease entity.

Fig. 19.5 Mooren's ulcer in a young African man. A. There are 4 clock hours of dense peripheral infiltration. B. Despite treatment, 1 month later the previously infiltrated area is now deeply ulcerated and has extended further circumferentially.

When blood vessels and fibrous connective tissue from the limbus grow onto the peripheral cornea, secondary to inflammation, the result is a vascular pannus. A vascular pannus can occur in any location, depending upon the inciting inflammation. A pannus in an adult may extend from the peripheral cornea towards the central cornea and is generally flat. An exception to this occurs in infants and small children, who may develop a hyperplastic reaction to the inflammation, and the resulting pannus can become extremely exaggerated and elevated.\[15\]

Superior limbal keratoconjunctivitis (SLK) is an inflammatory disorder of unknown etiology which is associated with a peripheral corneal pannus, a punctuate keratopathy, a thickened superior conjunctiva which is chemotic and hyperemic, and a filamentary keratitis. As the name states, this is a disorder of the superior limbus and cornea. Patients complain of ocular irritation and a foreign body sensation. It maybe associated with keratitis sicca or thyroid disease. The etiology is unknown. Initial treatment should consist of preservative-free artificial tears. If this eliminates the discomfort, punctate keratopathy and filaments,
no further treatment is necessary, although the superior conjunctiva may remain thickened and hyperemic. The clinician can consider a bandage soft contact lens, conjunctival recession, or conjunctival resection with an amniotic membrane graft if further treatment is necessary. Please see the specific chapter concerning SLK if more information is desired.
Neoplastic Disorders of the Peripheral Cornea

Because the surface of the cornea is contiguous with the bulbar conjunctiva, tumors that affect the conjunctiva may also affect the cornea. The differences between the compositions of these tissues alter the range and frequency of neoplasias encountered clinically. Although malignant tumors of the peripheral cornea exist, benign neoplastic growths are far more common in this region.

One of the most common neoplastic growths of the peripheral cornea is a pterygium. Some clinicians consider a pterygium a degenerative growth because of the effect of the actinic damage on the substantia propria.[16] Pterygia typically occur in the interpalpebral area owing to its increased actinic exposure. Fibroblasts associated with the pterygium grow onto the surface of the peripheral cornea and eventually penetrate Bowman's layer, resulting over time in a corneal scar.

Because pterygia can recur, we do not remove those that minimally overlap the peripheral cornea. These small pterygia can be treated with artificial tears, and short-term NSAIDs or mild corticosteroids if inflamed. Some clinicians have advocated intralesional corticosteroid injections. When pterygia extend 1.5–2 mm onto the corneal surface or are extremely elevated and inducing astigmatism, we consider surgical excision. Although pterygia are not considered true neoplastic lesions, recent evidence suggests that a significant percentage are associated with squamous cell carcinoma, which is the most common neoplasm of the cornea.[17] Therefore, it is deemed prudent to send pterygia specimens for histopathology examination after their surgical excision. There are a number of methods to remove pterygia, but it is unclear which of the many techniques prevent recurrence with good cosmetic results.

Another fibrovascular growth which may be associated with the peripheral cornea is pyogenic granuloma. This reactive tissue response most commonly occurs after surgery, trauma, or infection. Although these lesions are much more common in the conjunctiva, there have been reports of pyogenic granuloma formation in the cornea.[18] Their growth is typically rapid and they are well circumscribed in their appearance. Pyogenic granulomas are composed of fibrovascular tissue and are not true granulomas as the name would imply. Therefore, they are easily excised.

Congenital lesions of the cornea are rare, but there are those that principally affect the peripheral cornea. Dermoid tumors are one of the most common congenital growths associated with the peripheral cornea. Dermoids are benign tumors that may grow with the eye but do not substantially enlarge. In addition, they may change in character slightly as the host progresses through puberty. They are most commonly found in the temporal limbal region and are characterized by a thick, firm surface which may contain hair, sebaceous glands, sweat glands, and fat. Dermolipomas are a type of dermoid tumor composed primarily of fat.[19] Dermoid tumors can be associated with other syndromes such as Goldenhar and Proteus syndrome; therefore, the presence of a dermoid should be correlated with other systemic findings.[20] Large dermoids can affect vision either by occluding the pupil or by inducing a significant amount of astigmatism. Dermoids may be excised by performing a superficial lamellar keratectomy; however, they can extend through the full thickness of the cornea and sclera, or peripherally beyond the equator of the globe, which may make their complete removal difficult. If a full thickness or deep dermoid tumor is suspected, careful surgical planning must be undertaken and imaging studies employed to determine its extent. If the surgical plan involves deep excision of the tumor, the surgeon should have sufficient tissue...
available for a patch graft.

The most common tumors of the limbus and peripheral cornea involve the squamous epithelium. Although the substantia propria of the conjunctiva is not directly continuous within the cornea, it abuts Bowman's layer. Additionally, the epithelium of the cornea and limbal conjunctiva represents a metabolically and mitotically active region. Thus, this location is predisposed to neoplastic transformation. Many types of benign squamous metaplasia have been reported, including benign hereditary dyskeratosis, squamous papilloma, pseudoepitheliomatous hyperplasia, and squamous dysplasia. Although these lesions may recur after excision, their potential for developing into a true malignant neoplasm is limited.

There are also malignant and dysplastic squamous epithelial changes that arise from this mitotically active limbal region of the eye. Most of these dysplastic lesions arise from the area of the interpalpebral region because of its increased solar exposure. Initially, most dysplastic lesions are gelatinous or clear, thickened, irregular, mildly elevated lesions. Jakobiec found that fewer than 10% of dysplastic lesions initially demonstrated leukoplakia. Interestingly, some studies have demonstrated an association between these lesions and human papilloma virus type 16.

Dysplasia of the corneal epithelium is typically meant to signify a partial epithelial layer involvement by the atypical epithelial cells versus a full-thickness epithelial layer involvement, which is termed carcinoma in situ or intraepithelial neoplasia (CIN). When these limbal lesions spread to the surface of the cornea they typically have a frosted-glass appearance which can be difficult to visualize at the time of surgical excision. Rose Bengal can be used to highlight the extent of the cellular irregularity. Limited areas of squamous dysplasia or carcinoma in situ may be removed mechanically, but larger lesions may require a limbal autograft to supply a new population of limbal corneal stem cells. A relatively new alternative to surgery employs topical interferon-alfa drops, which have been used successfully to treat a number of cases. These lesions have also been treated with topical 5FU and mitomycin C (MMC) with some success. However, treatment with interferon-alfa, 5FU or MMC is not always successful, and surgical excision of the tissue may still ultimately be necessary. Cryoablation of tissue at the time of surgical excision may be required to eliminate abnormal cells from the adherent limbal conjunctiva or to treat an associated fibrovascular pannus. Epithelial squamous carcinomas generally do not penetrate Bowman's layer; however, if this does occur, a wide excision of the affected tissue should be performed. Alcohol or cocaine can be used to facilitate the complete removal of the affected corneal epithelium, with an added excision of the surrounding normal tissue as part of the wide-excision procedure. Cryotherapy has also been employed and has significantly reduced the recurrence rate from 40% to less than 10%.
Fig. 19.6 Papillary form of CIN described histopathologically as severe dysplasia with focal areas of carcinoma in situ. The lesion straddles 180° of the corneoscleral limbus. The tumor was treated curatively with a combination of surgical excision and cryotherapy.

Other rare peripheral corneal tumors have been identified, such as limbal melanoma and basal cell carcinoma. These entities are discussed in detail in their respective chapters.
Degenerative Disorders of the Peripheral Cornea

This category of ocular disorders comprises a hodgepodge of entities that overlap other categories because they have many causes. Most are not commonly the direct result of other diseases but can be associated with many other disorders.

Several corneal degenerations are not associated with corneal thinning. These disorders are generally benign, but in some instances can become serious.

**Corneal arcus** is a benign condition that results from lipid deposition within the corneal stroma, Bowman’s layer and Descemet’s membrane. The corneal epithelium is intact. A white or gray circumferential deposit is seen with an intervening clear space between it and the limbus. Arcus occurs with increasing age and is not considered pathologic; however, it can also be associated with hyperlipoproteinemia, and a lipid profile should be obtained in any patient under 50 who exhibits a corneal arcus.[27]

**Lipid keratopathy** results in a characteristic dense, yellow-white infiltrate which is usually associated with the presence of an adjacent corneal blood vessel. The lipid deposition can occur suddenly or may progress very slowly over the course of many years. The infiltrate usually has a feathery edge and may or may not have a crystalline appearance. Lipid keratopathy occurs in both primary and secondary forms. The secondary form is associated with peripheral corneal vessels, which may be seen in association with a history of inflammation, infection, trauma, or previous corneal surgery. The primary form is not associated with increased serum lipid levels, inflammation, or adjacent vasculature. It appears as an arcus-like deposition but may be thicker and more prominent. In fact, the lipid is identical to the lipid found in arcus.[28] If this lesion extends more centrally and affects vision, a corneal transplant may be necessary to restore sight. Unfortunately, the lipid deposits may recur in the graft.

**White limbal girdle of Vogt** was initially described in 1930. Two forms of this degenerative disorder were described. Type I has a characteristic crescent-shaped white or gray opacity near the interpalpebral zones of the limbus. These opacities are located nasally and temporally and are separated from the limbus by a clear zone. Small Swiss cheese-like holes may be evident within the opacity. Vogt also described a second form of the disorder with no clear zone between the opacities and the limbus. The first form of the disease is probably related to early, minor calcific band keratopathy; however, these minor opacities are seen in older individuals and are not related to trauma, intraocular inflammation, or multiple eye surgeries.[29] They are asymptomatic and do not require treatment.[29]

**Calcific band keratopathy** can be present in the peripheral cornea, the central cornea, or both regions. The corneal calcium may appear as a gray-white haze, with or without a ‘Swiss cheese’ appearance in mild disease; in advanced disease it can also form dense white-gray plaques (Fig. 19.7). Once the calcium deposits become elevated, it is common for the patient to note a foreign body sensation. If the calcium deposits are centrally located, the patient will typically experience reduced vision. Calcific band keratopathy is frequently associated with intraocular inflammation, trauma, multiple eye surgeries, elevated serum calcium, or other systemic disorders.[30] The calcium can be removed by chelation with EDTA, mechanical debridement, and phototherapeutic keratectomy.[31] For a comprehensive discussion of the topic, please see the specific chapter for the disorder responsible for the calcific band keratopathy.
Fig. 19.7 Calcific degeneration. Calcium deposition in the cornea is associated with chronic vascularization or inflammation. Histopathologically, the calcium may be associated with a fibrovascular pannus or may occur deep in the corneal stroma, as opposed to calcific band keratopathy in which the calcium deposition is confined to the region of Bowman’s membrane.

Unlike other types of epithelium, the corneal epithelium is continuously replenished by new basal epithelial cells from corneal stem cells located at the limbus. The role of limbal stem cells in the turnover of the corneal epithelium was recognized as early as 1971.\cite{32} Our understanding of these unique corneal epithelial progenitor cells has evolved, and we have increased our understanding of corneal epithelial stem cells’ characteristics. Corneal epithelial stem cell deficiencies are seen in genetic as well as acquired disorders. It has been understood for some time that aniridia is frequently associated with limbal stem cell deficiencies. The severity of the deficiency varies from patient to patient, and not all those with aniridia require treatment for limbal stem cell failure. Similarly, the degree of limbal stem cell damage after trauma from a chemical injury or after an acquired disorder such as Stevens–Johnson disease varies from person to person and from eye to eye. Iatrogenic limbal stem cell deficiency may occur in some patients in association with contact lens wear.

In the mildest forms of limbal stem cell deficiency, limbal and peripheral corneal changes with vascularization and slight loss of transparency of the corneal epithelium may be the only signs. In more severe forms of the disorder the entire corneal epithelium may be replaced by a fibrovascular membrane with almost complete loss of transparency of the cornea.\cite{33} Many types of limbal stem cell transplant have been devised, but no single method is universally successful. Generally, autografts have the highest success rate if limbal stem cells can be harvested from the fellow eye, and patients who have small areas of limbal stem cell deficiency appear to have a better long-term prognosis. Patients who suffer from a complete loss of limbal stem cells and have suffered corneal scarring, epithelial defects, and a diffuse fibrovascular membrane covering the cornea are least likely to obtain a good long-term outcome after a limbal stem cell transplant. This may be partly due to acquiring a limbal allograft transplantation with the high potential for graft rejection because of the vascular nature of the limbal region, but also because this severe disorder is frequently associated with loss of accessory lacrimal glands and goblet cells from the
Thus, it is more difficult to maintain a healthy ocular surface, which adversely affects the survival of the newly grafted tissue.

Several disorders result in corneal thinning or apparent corneal thinning. The following entities are all associated with corneal thinning from differing etiologies.

Terrien’s marginal degeneration results in an idiopathic thinning of the peripheral cornea. This usually begins in the superior cornea but may occur in any peripheral region. The disorder typically begins as a fine stromal opacity which has a clear zone between it and the limbus. This area then begins to thin very slowly and becomes vascularized by the limbal vasculature. As the thinning progresses, corneal astigmatism may become evident and cause symptoms of blurry vision. Interestingly, this degeneration is usually not painful even during the active phase of the disorder. The epithelium may remain intact and the cornea may visibly bulge due to the ectasia. The affected area may extend circumferentially without signs of inflammation. This disorder can be associated with episcleritis or scleritis, which generally results in discomfort. The disorder is bilateral in the majority of cases, but can be quite asymmetrical. Peripheral grafts may be necessary if the cornea becomes too thin and perforation or fear of trauma-related perforation exists. Terrien's marginal degeneration can be differentiated from other disorders such as Mooren's ulcer by its characteristic lack of pain, lack of a central undermined edge to the thinning trough, and an intact epithelium (Fig. 19.8). Neither pellucid marginal degeneration nor furrow degeneration have lipid infiltrates associated with the central edge of the thinning region. No current medical treatment is curative.

![Fig. 19.8 Terrien's marginal degeneration, characterized by superior peripheral thinning, superficial vascularization (1), and](image-url)
Pellucid marginal degeneration is a bilateral, inferior corneal thinning. The normal cornea protrudes above an area of abrupt thinning inferiorly (Fig. 19.9). The affected area is clear (pellucid) and eventually results in an against-the-rule astigmatism. Because of the similarities between the entities that comprise the group of corneal ectatic diseases, it is believed that keratoconus, keratoglobus, and pellucid degeneration are related. Rigid gas-permeable (RGP) contact lenses can be used when spectacle correction is not sufficient. Otherwise, the surgeon may perform a tectonic lamellar graft over the thinned periphery, followed many months later by a central penetrating keratoplasty. During the placement of the tectonic graft, removal of a small amount of aqueous humor and reefing sutures may be useful to reduce the degree of steep corneal curvature.

Fig. 19.9 Thin slit-beam view of pellucid marginal degeneration. There is marked thinning of the cornea inferiorly, well below the area of maximal corneal protrusion. In contrast, the thinning in keratoconus is in the area of maximal corneal protrusion.

Furrow degeneration is seen in the elderly patient. Although a furrow may be apparent at the slit lamp,
careful examination reveals that this is not a true thinning but rather an optical illusion. Occasionally there is some minor thinning present, but this does not substantially progress nor is it associated with perforation of the cornea. No treatment is necessary.

*Dellen* appear as areas of thinning or excavation. The overlying epithelium is usually intact, but the base can be gray or hazy. Because dellen are the result of tissue thinning due to inadequate tear coverage and tissue drying, they are always associated with an adjacent elevation. Dellen can be seen in association with pingueculae, pterygia, dermoids, filtering blebs, and any other elevated bulbar structure. Treatment is directed toward rehydration, lubrication of the dellen, and elimination of the adjacent elevation. If the epithelium on the surface of the dellen is no longer intact, infection and perforation are critical concerns.

Surgery, surgical complications, and medication-associated (iatrogenic) peripheral corneal damage can mimic the appearance of certain corneal diseases. A careful review of the patient's history will help separate iatrogenic disorders from true corneal diseases.
Because the peripheral cornea is adjacent to the rich vascular supply of the limbus, the limbal lymphatic tissue, and inflammatory cells, microbial keratitis may be thought to be less frequent than in the central cornea. However, other factors increase the likelihood of a peripheral corneal infection.

Contact lens wear can be associated with peripheral corneal disease. Wearing contact lenses reduces the amount of oxygen available to the cellular components of the cornea, the tear flow under the contact lens is less than that which would otherwise pass over the cornea, and contact lenses may increase the temperature of the cornea. Additionally, the insertion and removal of lenses may produce regions of microtrauma to the corneal surface, limbus, and adjacent conjunctiva. Furthermore, if soft contact lenses are worn overnight, the general risk of a corneal infection is increased.[36]

Contact lenses require good maintenance and cleaning. Frequently, patients will not change or clean their contact lens storage cases, which can harbor bacteria and other microorganisms. Also, certain systemic diseases, such as diabetes and other diseases that slow tissue healing, can increase the risk of ocular infections.

In addition, other factors such as dry eye and lagophthalmos serve to compromise the ocular surface. When these conditions are present in an individual who also wears contact lenses, the risk of microbial keratitis increases significantly. Because microbial keratitis is covered extensively elsewhere in this book, only a few specific aspects of infectious peripheral keratitis will be discussed here.

Bacterial and fungal keratitis, which occurs in the inferior third of the cornea, could be secondary to corneal exposure. This should especially be considered in an individual who has suffered multiple ocular infections. Lateral, medial, or both types of tarsorrhaphy may be required to protect the ocular surface and prevent subsequent episodes of infectious keratitis.

Microbial keratitis from specific causes such as syphilis or tuberculosis can produce peripheral corneal changes. Syphilis is associated with a deep corneal inflammation with deep vessels in the peripheral cornea. Conversely, tuberculosis produces an acute inflammation that is more commonly unilateral and involves peripheral sectoral portions of the anterior cornea.

Herpes simplex and zoster can develop peripheral corneal ulcers that do not exhibit typical dendrite formation. Instead, they develop an irregular epithelium or epithelial defect over the affected area, followed by a region of stromal haze that assumes a crescent shape adjacent to the limbus. The associated limbus is typically hyperemic and the eye is painful.[37]

Herpes simplex and zoster may also produce a sterile or an infected neurotropic ulcer (Fig. 19.10). Therefore, corneal sensation should always be tested in patients who experience little pain in association with a corneal ulcer. Treatment is directed at healing the ulcer and protecting the ocular surface with a tarsorrhaphy. A scleral contact lens may protect the corneal surface once the ulcer is healed.
Peripheral corneal diseases are a diverse group of disorders. More information about each individual disorder can be obtained from the chapter on the specific topic.
References


Chapter 20 – The Corneal Ulcer

Carol L. Karp, Richard K. Forster

An ulcer (Latin ulcerus or sore) is defined as a lesion ‘caused by superficial loss of tissue, usually with inflammation.’ Ulcerative keratitis rarely occurs in the normal, healthy eye. A search for the causative agent as well as underlying alterations in corneal structure, immunity, innervation, or defense mechanisms should be considered. Several questions should be asked when managing a patient with a corneal ulcer:

• Is there an infectious agent causing the keratitis?
• What local host factors contribute to increased risk for ulceration?
• What exogenous risk factors exist?
• Are there endogenous factors such as an autoimmune disease, an inflammatory process, or an immunocompromised status?

Diagnosis

One of the first decisions in the diagnostic algorithm is to determine whether the lesion is infectious or sterile. Both historical and clinical features should be considered in this process. Important historical predisposing factors for infectious keratitis include microbial exposure by contact lens use, foreign bodies and trauma, previous ocular surgery including refractive procedures, or exposure to contaminated water. The importance of contact lens use cannot be overemphasized. The use and abuse of contact lenses have played a major role in the epidemiology of keratitis in the United States. In particular, the use of extended (overnight) wear lenses has been clearly associated with an increased risk of keratitis in comparison to daily wear lenses. A history of outdoor trauma, particularly from soil or vegetation, should raise a suspicion for fungi or other fastidious organisms causing the keratitis.

Impaired local host defenses also play a role in the pathogenesis of stromal keratitis. A history of ocular chemical injury, neurotrophic disease, exposure and lid or lash malposition, tear insufficiency, stem cell deficiency, bullous keratopathy, or previous herpetic disease should be elicited. Certain medications such as topical anesthetics and topical corticosteroids also can reduce the local defense mechanisms.

A careful medical history also can be helpful in identifying systemic abnormalities that may predispose to increased risk for keratitis. Such factors could include acquired immunodeficiency syndrome (AIDS), diabetes mellitus, malnutrition, alcoholism, and other chronic debilitating illnesses. Underlying autoimmune diseases such as rheumatoid arthritis, Wegener’s granulomatosis, and Sjögren’s syndrome, as well as subsequent immunosuppressive treatments are also important.

The clinical evaluation of the patient should be thorough, and an effort should be made not to confine the examination only to the corneal process. Consider why the patient developed a keratitis and whether it is more likely to be sterile or infectious. The cornea and adnexa may provide clues regarding the inciting agent or etiology.

As with all patients, the examination should assess both eyes. Examination of the lids, lashes, and conjunctiva should evaluate for associated skin and adnexal inflammation such as rosacea or seborrhea and mechanical lid dysfunction such as floppy lid syndrome (Fig. 20.1), entropion, or ectropion. Mucous membrane diseases such as ocular cicatrical pemphigoid, Stevens–Johnson syndrome, or chemical injury may lead to symblepharon and trichiasis. Dermatologic cicatrical diseases such as scleroderma or atopic dermatitis or previous surgery may also lead to a compromised ocular surface. The lacrimal system should be examined, in terms of assessing both adequacy of tear function and the possibility that an underlying dacryocystitis or canaliculitis is contributing to the corneal pathology.

Fig. 20.1 (A) Male, obese patient with floppy lids, rosacea, and seborrhea. (B) Floppy lid syndrome leading to infectious corneal ulceration secondary to Candida sp.
When examining the cornea, the epithelium, stroma, and endothelium should be evaluated with the recurring question of why the patient has a corneal ulcer. Most microbial keratitis is precipitated by an epithelial defect, and the epithelium and basement membrane of both eyes should be examined. The presence of reduplicated basement membrane, microcysts, or other findings of anterior basement membrane dystrophy may suggest recurrent erosion as the etiology for the initial epithelial insult. A rolled epithelial edge with an oval central lesion suggests neurotrophic pathology and a persistent epithelial defect (Fig. 20.2). Diffuse epitheliopathy should prompt consideration of a toxic reaction to topical medications such as antibiotics, antivirals, or anesthetics.

The appearance of the corneal stroma is the key to the underlying status of the eye. The classic features of an infectious stromal keratitis include coalescent stromal suppuration and infiltration (Fig. 20.3). The presence of a hypopyon, anterior chamber reaction, or endothelial plaques also may suggest infectious etiology. In contrast to the dense suppuration usually seen in infectious keratitis, sterile infiltrates may be characterized by the presence of visibly discrete inflammatory cells adjacent to the infiltrate, with minimal anterior chamber reaction (Fig. 20.4). Furthermore, indications of sterility in a postinfectious or treated keratitis include the presence of a resolved epithelial defect, or one in which the defect is healing over an area of previously dense infiltration (Fig. 20.5). In the setting of AIDS, the inflammatory response may be underwhelming.
Fig. 20.3 Early *Pseudomonas aeruginosa* keratitis in a soft contact lens user. Note focal infiltrate with overlying epithelial defect and stromal edema.

Fig. 20.4 Noninfectious keratitis secondary to a chemical injury. Note discrete midstromal inflammatory cell infiltrate (1), with no overlying epithelial defect.

Fig. 20.5 Infectious keratopathy with epithelial defect and infiltrate that was sterilized with 10 days of topical antibiotics. (A) Corneal epithelial defect with corneal infiltrate and mucus epitheliopathy.
Immune-related ulceration of the corneal stroma also may have varying degrees of stromal cellular infiltration. Immune-mediated infiltrates or ulceration may have an associated scleritis, episcleritis, or iritis. Vascularization or scleritis may be localized to the area of corneal pathology. A careful evaluation of the stroma of the other eye should be performed to rule out a possible peripheral immune-mediated marginal keratitis, such as seen in rheumatoid arthritis, Wegener's granulomatosis, and Mooren's ulcer. In general, the peripheral marginal melts, with or without epithelial defects, are rarely infectious. Keratitis associated with Gram-positive organisms is usually characterized by well-circumscribed lesions. Staphylococcus aureus usually progresses slowly, and the streptococci elicit an acute and highly suppurative reaction. In addition, the streptococci generally produce a deep central stromal ulceration with an advancing edge of infection, hence the term serpiginous ulceration, and are frequently associated with a sterile hypopyon. A unique presentation of streptococcal infection is the syndrome of infectious crystalline keratopathy (ICK), usually seen at the graft-host junction following keratoplasty and characterized by a paucity of inflammatory cells.

The Gram-negative bacteria usually produce soupy infiltrates, which are less discrete and have abundant conjunctival mucopurulent discharge. Features of less common keratitis include the stromal ring abscess and radial perineuritis of Acanthamoeba keratitis, often with complaints of extreme pain. The central 'cracked windshield' and the peripheral 'brush fire' appearance are features of Nocardia and Mycobacterium. Fungal infections usually have a hyphoid, feathery border and may develop satellite lesions (Fig. 20.6).

In the setting of infectious keratitis following laser-assisted in situ keratomileusis (LASIK), the clinical presentation may be atypical. Infections may initially be thought to be diffuse lamellar keratitis (DLK), delaying diagnosis and treatment. These patients may present without an epithelial defect, since the keratitis may be sequestered in the lamellar interface. In this scenario, the flaps may need to be lifted for culture and irrigation. Management of these cases can also be more difficult, as the antibiotics may not penetrate adequately through the anterior stromal flaps, necessitating flap amputation or penetrating keratoplasty for resolution (Fig. 20.7).

Fig. 20.6 (A) Aspergillus keratitis. Patient contracted infection while in refugee camp. (B) Penetrating keratoplasty for worsening Aspergillus keratitis.

Fig. 20.7 Patient with post-LASIK infection with Mycobacterium chelonae/abscessus. Flap was amputated to allow for better antibiotic penetration, but patient ultimately required a penetrating keratoplasty for cure.
Despite certain classic features, a specific etiologic diagnosis cannot be made by clinical appearance alone. Therefore, when an infectious agent is suspected from the examination, corneal scrapings for culture and cytology are indicated. When the infection is contact lens related, culture of the contact lenses and case may be helpful. In cases of patients presenting already on topical antibiotics, it may be necessary to discontinue all topical drops for 24 hours prior to culturing. In the setting of a repeatedly negative or equivocal corneal culture in a patient with progressive disease, a corneal biopsy should be performed. A corneal biopsy can also be invaluable in obtaining a microbiologic diagnosis in cases of infectious crystalline keratopathy. Corneal biopsies can be performed easily and rapidly at the slit lamp, are inexpensive, and may provide critical information in the evaluation of the pathologic process. We recommend that the biopsy be performed using a disposable 2-mm dermatologic punch (Fig. 20.8). Two sites should be selected at the active edges of ulceration. Biopsy specimens should be submitted to both microbiology and pathology. Corneal biopsies have also successfully been performed using the femtosecond laser.\[1\]

![Fig. 20.8 Process of obtaining a corneal biopsy for a worsening keratitis. (A) Unresponsive keratitis. (B) Using a lid speculum and topical anesthesia, a 2 or 3 mm sterile trephine is inserted.](image-url)

In addition to the usual smears and cultures, cases of possible Acanthamoeba should have calcofluor white stain and agar plating with an Escherichia coli overlay. If Mycobacterium is suspected, Lowenstein-Jensen plating should be used and an acid-fast stain performed. Cultures for fungi should be placed on Sabouraud's dextrose agar. Histopathology stains for fungi should include Gram, Giemsa, and Gomori methenamine silver. A new diagnostic tool is the confocal microscope, which may be especially helpful in cases of Acanthamoeba\[2\],\[3\] and possibly fungal keratitis.\[4\] Future improvements in microscopes and our increasing familiarity with interpreting the confocal images may make this tool increasingly useful.
Once the patient is receiving antibiotics, the treating physician must tailor the treatment to the host response. Parameters to judge clinical improvement include resolution of the epithelial defect, decreasing density and size of the infiltrate and inflammation, and decreasing pain. The clinical response should be assessed to modify the treatment. It is important to recognize that many topical antibiotics have toxic effects on the epithelium and may modify the corneal appearance, particularly noted as a punctate epitheliopathy and stromal edema. An important principle in the management of keratitis is that corneal healing takes time. Stabilization or lack of worsening should be interpreted as effective therapy because the reparative process of the cornea may be slow.

Depending on the inciting agent, treatment duration will vary. For example, most fungal infections and *Acanthamoeba* need prolonged treatment. Fungal keratitis seems to benefit from regular debridement. This helps to decrease the infectious load mechanically as well as to maintain an epithelial defect to permit better antifungal medication penetration. We use a sterile No. 69 Beaver blade for this purpose. Additional smears and cultures can be performed easily with the scraped material if necessary.

In infections following LASIK, the treatment of the keratitis can be very prolonged due to poor penetration of the antibiotics through the corneal flap. In several cases of severe keratitis, we have amputated the corneal flap in order to resolve the infection.[5]

In all cases of infectious keratitis, the goal is to sterilize the lesion and enhance corneal healing. In those cases with progressive keratitis or impending perforation, a tectonic or therapeutic penetrating keratoplasty may be needed. Another option for managing a worsening keratitis with failure to heal, especially when there is a component of exposure or lagophthalmos, is a conjunctival flap or amniotic membrane graft. We have seen many cases in which patients have been treated successfully with focal conjunctival flaps for peripheral corneal ulcers, effecting resolution of the corneal ulcer and maintaining a clear central visual axis.

Management of a corneal ulcer is challenging and must include careful history and clinical examination. This information will assist in the tailoring of the microbiologic testing, will direct optimal treatment for the patient, and can be modified as needed, based on the host response.
References


Chapter 21 – Corneal Edema

Vahid Feiz

Corneal transparency is an essential aspect of maintaining a clear retinal image. Transparency is dependent on a strict fluid and electrolyte balance within the corneal stroma. When this delicate process is disturbed, accumulation of fluid will lead to an edematous cornea with decreased transparency. The differential diagnosis of corneal edema is fairly extensive, but an understanding of normal physiology, a thorough history and clinical examination, as well as utilization of ancillary tests, will usually lead to the correct diagnosis and a specific plan of treatment.

Physiology

A number of mechanisms regulate corneal hydration. Not all of these are completely understood; however, a simple list, based on corneal anatomy, is discussed below.

Epithelial and endothelial barriers

Using electron microscopy as well as antibody staining against proteins such as ZO-1 and occludin,[1–3] tight junctions have been demonstrated at the epithelial and endothelial layers of the cornea. These tight junctions restrict the flow of electrolytes and fluid through the paracellular route. The integrity of the epithelial barrier can be clinically determined by application of dyes such as fluorescein. Phenomena such as microcystic corneal edema or bullous keratopathy occur when the epithelial barrier is intact, but fluid overload in the cornea leads to entrapment of fluid beneath the epithelium.

Tear evaporation

The role of tear evaporation in maintaining corneal dehydration is controversial. A commonly reported symptom by patients with Fuchs’ endothelial dystrophy is worse vision in the morning that may result from decreased tear evaporation and increased corneal edema during sleep.

Studies on the role of tear evaporation on corneal hydration, however, have yielded
conflicting results. In one study, the rate of recovery from hypoxic corneal edema was significantly faster with eyes open compared to eyes closed.\[4\] Another study investigating the effect of humidity on de-swelling of the cornea, however, failed to show any difference in the rate of resolution of edema at different levels of environmental humidity, which suggests a minimal role for tear evaporation on corneal dehydration.\[5\]

**Intraocular pressure**

The level of ocular tension can have a profound and variable effect on corneal stromal hydration. Chronic high pressure can lead to endothelial damage and subsequent edema. Acute rises in intraocular pressure (IOP), on the other hand, can lead to either increased or decreased hydration. A common observation in the early postoperative period after corneal transplantation with epithelial defect and high IOP is a clear, non-edematous cornea. High IOP after lamellar refractive surgery such as LASIK, however, can lead to accumulation of fluid in the interface leading to corneal edema, which can be mistaken as interface inflammation.\[6\]

**Metabolically active mechanisms**

Both epithelial and endothelial cells have specialized pumps at the cellular level that under normal physiological circumstances regulate the passage of fluid and ions across the cornea. These pumps provide net active transport of fluid out of the corneal stroma either to the ocular surface or into the aqueous. These active processes require oxygen and energy in the form of adenosine triphosphate (ATP). Depletion of either energy or oxygen, as in contact lens-induced hypoxia, can lead to edema at the level of epithelium or stroma. These active transport mechanisms have been studied extensively at both a cellular and biochemical level. For example, treatment of cornea endothelium with ouabain (a pump inhibitor) will result in stromal edema.\[7,8\]
Diagnosis

The diagnosis of corneal edema depends on obtaining a careful ocular history, clinical examination, and use of ancillary testing. Some of the causes of corneal edema are listed in Table 21.1.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Etiologies</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary endothelial failure</td>
<td>Congenital hereditary endothelial dystrophy (CHED), Fuchs’ dystrophy, iridocorneal endothelial syndrome (ICE), posterior polymorphous endothelial dystrophy (PPMD)</td>
<td>Primarily stromal, diffuse, progressive</td>
</tr>
<tr>
<td>Secondary endothelial failure</td>
<td>Acute or chronic trauma, chemical, inflammatory, hypoxia</td>
<td>Primarily stromal, focal or diffuse, acute or chronic</td>
</tr>
<tr>
<td>Normal endothelium</td>
<td>Elevated intraocular pressure</td>
<td>Primarily epithelial, microcystic, central or diffuse, acute</td>
</tr>
<tr>
<td>Epithelial failure</td>
<td>Epithelial defect</td>
<td>Stromal, surrounding defect, acute</td>
</tr>
</tbody>
</table>

Clinical history

Patients with corneal edema may have a range of presentations, from asymptomatic disease to severe pain and decreased vision. The location of the edema also influences the symptoms. For example, patients with stromal edema may not have discomfort, while epithelial edema with bulla formation can be very painful. Patients need to be questioned about the duration of symptoms, age of onset, unilaterality versus bilaterality, diurnal variation of the symptoms, use of topical medications, family history of corneal disease, previous ocular disease, and ocular surgeries. Information gathered at this stage can be very useful in determining a cause. For example, patients with corneal edema secondary to endothelial dysfunction often complain of worse vision upon awakening and gradual improvement during the course of the day. Similarly, dystrophies tend to be bilateral, so that unilateral symptoms are less suggestive of dystrophic causes. Box 21.1 has a summary of some of the relevant clinical history and its possible significance.

Box 21.1

<table>
<thead>
<tr>
<th>Important clinical history elements in diagnosis and management of corneal edema</th>
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<tbody>
<tr>
<td>Age at onset</td>
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<tr>
<td>Duration of symptoms</td>
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<tr>
<td>Unilateral versus bilateral symptoms</td>
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<tr>
<td>Family history of corneal disease</td>
</tr>
</tbody>
</table>
Examination

A systemic approach to slit lamp examination of the different layers of the cornea will ensure that relevant clinical findings are not missed. Corneal edema can manifest with clinical changes in the epithelium, stroma, and endothelium.

Normal epithelium should have a uniformly smooth surface free of staining with dyes such as fluorescein. This smooth surface turns gray with loss of luster in early, mild edema. As the condition progresses, microcystic changes occur, which can be either diffuse or sectoral. Later in the course, frank bullous keratopathy may develop. The visualization may be enhanced by the application of fluorescein (Fig. 21.1).
Fig. 21.1 Advanced corneal edema with bullae formation. Note the outline of the bullae highlighted by application of fluorescein.

Increased width of the slit beam, as well as presence of folds in the Descemet's membrane, are findings associated with stromal edema (Fig. 21.2). A clear stroma by itself does not rule out edema, since the cornea may stay optically clear with mild disease.
Using high magnification and a wide angle between the light source and the observer, endothelial abnormalities can be highlighted. Posterior irregularities and pigment may be suggestive of early Fuchs’ dystrophy characterized by guttae. These can also be highlighted using retroillumination through a dilated pupil (Fig. 21.3).
Ancillary tests

In addition to the clinical examination, the judicious use of ancillary testing can provide information to determine the stage and severity of the pathological condition and to provide diagnostic clues.

Pachymetry

As corneal hydration increases, the corneal thickness also increases. Pachymetry is the technique of measuring corneal thickness. The two common forms of pachymetry involve the use of ultrasound or optical slit beam measurement. Ultrasound is convenient, since the instruments are easy to use and are commercially available. Optical pachymetry can be performed manually with a slit lamp attachment. Alternatively, a topography unit employs slit beam technology automatically to measure corneal thickness. Although there is usually good agreement between the optical and ultrasound methods, when there is significant loss of cornea clarity, the optical methods become unreliable.

Specular microscopy

Specular microscopy can be used to determine the density and morphology of endothelial cells. The various methods of contact and non-contact specular microscopy as well as analysis of the morphology of endothelial cells provide valuable information that assists the clinician in determining not just the etiology but also the prognosis of corneal edema. In general, most agree that with an endothelial cell count of less than 700 cells/mm², corneal edema becomes increasingly likely.

In vivo confocal microscopy

In vivo confocal microscopy can be used to study the microstructural details of different levels of the cornea. The information collected using this modality can be helpful in determining the etiology of corneal edema based on the cellular morphology.[9]

Anterior segment optical coherence tomography

The principle of anterior segment optical coherence tomography (OCT) is analogous to ultrasound but with the emission and reflection of light instead of sound. The anterior segment OCT is an evolution of the retinal OCT. It provides images of anterior segment structures, including the cornea, iris, angle, and anterior lens. Some OCT units have an optical axial resolution of up to 18 µm and optical transverse resolution of up to 60 µm and can scan through an opaque cornea. The software on some units can automatically calculate the central corneal thickness (CCT), the central anterior chamber depth (ACD), the volume of the anterior chamber (AC) and the interspur distance.[10] Although not very useful diagnostically, these instruments can be extremely helpful in following patients after posterior lamellar keratoplasty for treatment of corneal edema (see below).
The treatment of corneal edema depends on the specific cause and the symptoms of the individual patient. This can vary from no treatment in an asymptomatic patient with early Fuchs’ dystrophy to keratoplasty in a patient with painful bullous keratopathy. A stepwise approach to the treatment of corneal edema is to address any associated ocular abnormality initially and, depending on the result, proceed with additional steps.

**Control of associated abnormalities**

**Inflammation**

Treatment of inflammation and the underlying cause of inflammation can be a very powerful tool in resolving corneal edema. Perhaps the most dramatic examples of this are the use of corticosteroids in corneal graft rejection and herpetic stromal keratitis. In the case of a corneal edema due to nonviral infections (bacterial, fungal, etc.), treatment of the underlying infection with appropriate agents will often lead to resolution of edema. In such cases, corticosteroids should be used with extreme caution and only when the infectious component is well under control. The use of corticosteroids in the absence of inflammation will have no effect on corneal edema.[11]

**Intraocular pressure**

In the setting of either acute or insidious IOP elevation, decreasing the pressure can improve or resolve corneal edema and prevent further damage to endothelial cells. In the past decade there has been a significant increase in the number of new pressure-lowering agents. The specifics of each class are beyond the scope of this text but one class, the carbonic anhydrase inhibitors (CAIs), deserves special attention. Inhibition of corneal carbonic anhydrase pumps may lead to decreased fluid flow from stroma to aqueous and progression to corneal edema. There are several case reports of irreversible corneal edema with the use of topical carbonic anhydrase inhibitors.[12],[13] This class of pressure-lowering agents should be used with caution in the setting of corneal edema or compromised endothelial cell function.
Management of epithelial and stromal edema

**Hypertonic agents**

Use of hypertonic solutions such as 5% sodium chloride drops and ointment facilitates the transition of fluid from epithelium. This in turn will improve microcystic and bullous epithelial keratopathy. Patients should be warned about the stinging associated with the use of these preparations. Glycerin is another hypertonic preparation that can have a dramatic but transient effect on corneal edema. This agent is useful for diagnostic purposes, as it allows better visualization of the corneal layers and the anterior chamber. It should be instilled after application of topical anesthetic, since it is too irritating for use on an unanesthetized eye. Other possibilities include corn syrup and honey, neither of which has practical applications.[14] The use of hypertonic preparations has minimal effect on stromal edema.

**Bandage contact lens**

Placement of an extended-wear bandage contact lens on the cornea can provide relief from the discomfort of bullous keratopathy and is used in the setting of poor visual potential or when surgical intervention is not recommended or is delayed. The contact lens chosen should have high oxygen transmissibility. The comfort provided by this modality must be weighed against the risk of contact lens-induced infectious corneal ulcer. Regular follow-up visits and the use of prophylactic topical antibiotics reduce the risk of complications.

**Anterior stromal cautery**

Application of light burns to Bowman's layer using a thermal cautery (Salleras procedure) leads to formation of scars and firm adhesion between the epithelium and the underlying stroma. This decreases the formation of bullae and microcystic edema. The technique is simple and can provide excellent pain relief. It should be reserved for eyes that have poor visual potential or are poor surgical candidates.

**Conjunctival flap**

Covering the cornea with vascular conjunctival tissue, after the epithelium has been
removed, provides coverage of corneal nerves. Vision is usually worse after the procedure and patients should be warned about this. This modality is usually reserved for eyes with poor visual potential or patients who are not candidates for corneal transplantation.

**Amniotic membrane**

In the past decade, application of amniotic membrane to rehabilitate the ocular surface has gained popularity. Short-term symptomatic relief of pain after application of amniotic membrane in the setting of corneal edema has been reported.[15],[16] Whether or not this method can provide long-term relief is not known.

**Excimer laser**

Phototherapeutic keratectomy of the anterior corneal stroma using excimer laser has been shown in several studies to provide pain relief for corneal edema with bullous keratopathy. Deeper stromal ablations tend to provide more relief than superficial treatments. Long-term data for this approach are not yet available but it is probably most valuable as a temporizing measure until a definitive treatment can be applied.[17]

**Penetrating keratoplasty**

Corneal transplantation is the definitive treatment for a range of corneal diseases, especially when the etiology is poor endothelial function. Transplantation provides the eye with a healthy functioning reserve of endothelial cells and new stroma. Adequate use of immunosuppressive agents, as well as modern surgical techniques, has resulted in very high success rates after keratoplasty. The goal of penetrating keratoplasty is both to rehabilitate the eye visually and to relieve symptoms of corneal edema.

**Endothelial keratoplasty**

Corneal transplant surgery for endothelial dysfunction has evolved over the last decade from penetrating keratoplasty (PKP) to posterior lamellar keratoplasty (PLK).[18] The technological advances have led to the development of a selective endothelial replacement surgery enabling rapid postoperative healing, early visual recovery, and minimal to moderate refractive change. The surgical techniques have further evolved from PLK to deep lamellar endothelial keratoplasty (DLEK), Descemet stripping endothelial keratoplasty (DSEK) and to Descemet stripping automated endothelial keratoplasty (DSAEK).[19],[20] At this stage, DSAEK is rapidly becoming the procedure of choice for endothelial dysfunction. The common feature of all these procedures is the transfer of donor tissue, including endothelium, Descemet's membrane, and a thin layer of posterior stroma.

Descemet's membrane endothelial keratoplasty (DMEK) is a new and evolving surgical technique in which only the Descemet's membrane and the endothelial cell layer are transplanted.[21] This procedure, while technically difficult, may theoretically result in better postoperative visual acuity compared to other endothelial keratoplasty techniques, since it may avoid an irregular interface. Long-term results of DMEK surgery are not yet available.
Collagen Cross-linking

Collagen cross-linking using riboflavin and ultraviolet A (UVA) is an experimental modality that has been utilized for the treatment of corneal ectasia. Some have employed collagen cross-linking for the treatment of cornea and have demonstrated decreased edema in the cross-linked portion of the cornea than in the untreated control area. A recent study evaluated the safety and efficacy of staged UVA cross-linking following intrastromal 0.1% riboflavin administration in eyes with advanced corneal edema.[22] The authors noted significant decrease in corneal edema and increased corneal clarity compared to the control eyes. Long-term follow-up and results from large studies on the use of this modality are not yet available.
References


Chapter 22 – Corneal Deposits

David A. Palay

Abnormal deposition of material in the cornea is easily discerned for two reasons. First, the cornea in its normal state is clear, and deposits of any type produce clouding. Second, the details of the cornea can be readily examined with a slit lamp, which magnifies the cornea under conditions of variable illumination.

This chapter presents a systematic approach to the evaluation of the patient with corneal deposits. The focus is on achieving the correct diagnosis by evaluating two major aspects of the deposits: the depth of the deposit in the corneal stroma, and the color of the deposits. Identifying the location and color of the deposits considerably shortens the differential diagnosis.

The cornea can be divided into three depths: superficial, stromal, and deep stromal. The superficial layer corresponds histologically to the epithelium, Bowman's layer, and the very anterior stroma. The stromal layer represents the bulk of the histologic, anatomic stroma. The deep stromal layer is defined as the deep posterior stroma, Descemet's membrane, and the endothelium.

The color of the deposits can be divided into three categories: pigmented, nonpigmented, and refractile/crystalline. Pigmented deposits can be any color, but typically are yellow or brown. Nonpigmented deposits are white or gray. Refractile or crystalline deposits are clear with indirect illumination, but may be white or gray with direct illumination. Occasionally, crystalline deposits may be polychromatic.

The reader is referred to the appropriate nine groupings in Table 22.1. The text highlights further specifics of the deposits.

Table 22.1 – Corneal deposits

<table>
<thead>
<tr>
<th>Category</th>
<th>Superficial</th>
<th>Stromal</th>
<th>Deep stromal</th>
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<tr>
<td>Pigmented</td>
<td>Cornea verticillata (Fabry's disease and drug effects)</td>
<td>Phenothiazines</td>
<td>Wilson's disease (copper)</td>
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<td>Striate melanokeratosis</td>
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<td>Chalcosis</td>
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<td>Epithelial iron lines</td>
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<td></td>
<td>Spheroidal degeneration</td>
<td>Siderosis (iron)</td>
<td>Mottled cyan opacification</td>
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<td></td>
<td>Adrenochrome</td>
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<tr>
<td>Nonpigmented</td>
<td>Subepithelial mucinous dystrophy</td>
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<td>Cornea farinata</td>
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<td></td>
<td>Coat's white ring</td>
<td>Macular dystrophy</td>
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<td>Calcific band keratopathy</td>
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<td>Mucin balls</td>
<td>Mucopolysaccharidoses</td>
<td>ichthyosis Argyrosis (silver)</td>
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</tr>
<tr>
<td>Refractile/crystalline</td>
<td>Meesmann's dystrophy Gelatinous droplike dystrophy Tyrosinemia II Intraepithelial ointment Gout (urate)</td>
<td>Lattice dystrophy Schnyder's dystrophy Bietti's dystrophy Immunoglobulin Cystinosis</td>
<td>Polymorphic amyloid degeneration</td>
</tr>
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**Superficial Deposits**

**Pigmented deposits**

**Cornea verticillata**

Cornea verticillata refers to linear opacities located within the corneal epithelium that assume a characteristic whorl-like pattern (Fig. 22.1). The opacities are located primarily in the inferior paracentral cornea and are not elevated. Their color may vary from white to brown. Cornea verticillata is seen in Fabry's disease and as a side effect from multiple systemic medications including amiodarone,[1] chloroquine, quinacrine, chlorpromazine,[2] indometacin,[3] clofazimine,[4] suramin,[5] naproxen,[6] and tilorone.[7] Fabry's disease is an X-linked lysosomal storage disease, which results in the accumulation of trihexosyl ceramide.[8],[9] Corneal changes are found in both the hemizygote male and heterozygote female carriers.[10]
Striate melanokeratosis

Striate melanokeratosis refers to pigment lines located in the epithelium, which extend from the limbus toward the central cornea (Fig. 22.2). These lines normally occur in darkly pigmented individuals but also can occur in lighter pigmented individuals after injury or inflammation. The deposits are probably the result of migration of pigmented limbal stem cells onto the cornea.
Fig. 22.2 Striate melanokeratosis.

Epithelial iron lines
Iron deposits in the epithelium have a yellow-brown coloration (Fig. 22.3). Pooling of tears in the region of topographic irregularities allows iron from the tear film to be deposited within the epithelium. Iron lines can be seen in the palpebral fissure (Hudson-Stahli), at the head of a pterygium (Stocker), surrounding the cone in keratoconus (Fleischer), at the head of a filtering bleb (Ferry), adjacent to areas of corneal elevation such as Salzmann's nodular degeneration, anterior to the sutures in keratoplasty (Mannis), and after keratorefractive surgery.[11],[12]
Spheroidal degeneration

Spheroidal degeneration produces golden-yellow globular deposits within the interpalpebral area (Fig. 22.4). The deposits are located within Bowman’s layer and the anterior stroma. In primary spheroidal degeneration, the deposits are bilateral and initially located in the nasal and temporal cornea; they can extend onto the conjunctiva. Secondary spheroidal degeneration is associated with ocular injury or inflammation. The deposits in secondary spheroidal degeneration will aggregate near the area of corneal scarring or vascularization.[13]
Adrenochrome deposition

Adrenochrome deposits are usually found within the conjunctiva, but rarely may occur on the corneal surface (Fig. 22.5). These brown-black deposits occur in patients treated with epinephrine eye drops for glaucoma.[14]
Subepithelial mucinous corneal dystrophy is an autosomal dominant condition, which results in the deposition of glycosaminoglycans in the subepithelial stroma (Fig. 22.6). Clinically, a diffuse homogeneous subepithelial haze is seen bilaterally. The haze is denser centrally and fades toward the periphery. Irregular gray-white opacities also may be seen centrally; these deposits may be raised.[15]
Coat's white ring

Coat's white ring is a superficial ring of iron deposition that remains after a metallic foreign body is removed (Fig. 22.7).[16] Small white opacities may be seen inside the ring. These rings develop when a rust ring from an iron foreign body is not entirely removed.
Calcific band keratopathy

Hypercalcemia and chronic ocular inflammation are the most common conditions associated with calcific band keratopathy (Fig. 22.8). The calcium is deposited within the epithelial basement membrane, Bowman's layer, and the anterior stroma. Clinically, subepithelial chalky white deposits are found in the interpalpebral zone. The deposition initially begins in the peripheral cornea, with a clear margin separating the deposit from the limbus. The clear interval is thought to represent the anatomic limit of Bowman's layer. Throughout the band are clear, small holes that give a 'Swiss cheese' appearance. The holes occur at sites where corneal nerves penetrate Bowman's layer.[17]
Fig. 22.8 Band keratopathy (image shown is calcific band keratopathy).

**Fluoroquinolone deposits**

Topical ciprofloxacin[18] and norfloxacin[19] therapy can result in the deposition of a chalky white precipitate within an epithelial defect (Fig. 22.9). Although they are predominantly white in appearance, a crystalline pattern also may be observed.
Mucin balls

Mucin balls are round white deposits that accumulate between the posterior surface of a contact lens and the corneal epithelium (Fig. 22.10). When the contact lens is removed, the mucin balls may be blinked away, but in some cases they remain adherent to the cornea for several hours. When they are removed they leave a depression in the epithelium. They are more common with rigid soft contact lens material such as high-Dk silicone lenses.[20]
Refractile/crystalline deposits

Meesmann’s dystrophy

Meesmann’s dystrophy is an autosomal dominant disorder that results in the accumulation of bilateral intraepithelial cysts throughout the cornea (Fig. 22.11). These cysts appear gray on direct illumination, but with retroillumination are transparent and are thus better viewed. The cysts are confined to the epithelium and extend to the limbus. The intervening cornea is clear.\cite{21}
Gelatinous droplike dystrophy

Gelatinous droplike dystrophy is an autosomal recessive disorder that results in the bilateral accumulation of amyloid deposits in the anterior corneal stroma (Fig. 22.12). This condition, as well as lattice dystrophy, is a form of primary localized corneal amyloidosis. Clinically, subepithelial raised, gelatinous deposits appear within the first or second decade. These deposits are refractile on indirect illumination, limited to the central cornea, and have been likened to the appearance of a mulberry.[22]
Tyrosinemia II (Richner-Hanhart syndrome)

Tyrosinemia II is an autosomal recessive inborn error of tyrosine metabolism caused by a deficiency of tyrosine aminotransferase. The deposits are located within the epithelium and subepithelial space of the central cornea (Fig 22.13). They are bilateral and appear as refractile branching linear opacities. The deposits can assume a dendritic pattern and can be confused with herpetic keratitis; however, they do not stain with fluorescein. The opacities may coalesce into a plaque-like configuration. The deposits may clear with appropriate dietary changes.[23]
Fig 22.13  Tyrosinemia II.
(Courtesy of Gary Foulks MD and Duke University, Durham, NC.)

**Intraepithelial ointment**

Rarely, ophthalmic ointment preparations can become entrapped in the epithelium after corneal abrasions have healed (Fig. 22.14). The clinical appearance is of clear globules within the epithelium.[24]
Gout (urate)

Patients with gout may have fine, yellow, scintillating crystals in the superficial cornea (Fig. 22.15). These crystals can become confluent in the interpapillary region and can form a pigmented band keratopathy.
Fig. 22.15  Gout (urate).
Stromal Deposits

Pigmented Deposits

Phenothiazines

Phenothiazines are synthetic antipsychotic drugs that can cause numerous toxic side effects when used in high doses. The corneal findings include a diffuse, granular, yellow-brown pigmentation located throughout the stroma, although usually more dense in the deep stroma (Fig. 22.16). Light exposure probably plays a role in the pathogenesis because the deposits are denser in the interpalpebral region. With very high doses, cornea verticillata or conjunctival pigmentation may be seen. [26]
Corneal blood staining

Corneal blood staining most commonly occurs in the presence of a hyphema and elevated intraocular pressure. Initially, there are small yellow granules within the posterior stroma. With continued presence of the hyphema, a rust-colored opacity of the stroma develops. With time, the blood in the cornea becomes yellow (Fig. 22.17). This opacity can involve the entire cornea and clears over several years, beginning at the limbus and progressing centrally.\textsuperscript{[27]}
Bilirubin

Elevated levels of bilirubin secondary to advanced liver diseases such as hepatitis, cirrhosis, or biliary obstruction can lead to a yellow staining in the peripheral cornea (Fig. 22.18). The staining is found throughout the corneal stroma but is more extensive in the deep stroma. The bilirubin is thought to diffuse from the limbal circulation. With severe elevations of bilirubin, the entire cornea may be stained.[28] It is almost always associated with conjunctival bilirubin staining.[29]
Siderosis

Ocular siderosis is iron deposition within intraocular structures (Fig. 22.19). It typically occurs in the setting of a metallic intraocular foreign body, although systemic causes of iron overload such as hemochromatosis can also lead to the condition. The cornea can occasionally be affected by siderosis if the foreign body is localized within the anterior chamber. Yellow-brown iron deposits can be seen within the posterior stroma.[30]
Nonpigmented deposits

Granular dystrophy

Granular dystrophy is an autosomal dominant condition that affects the central corneal stroma of both eyes (Fig. 22.20). Focal white ‘bread crumb’ deposits that assume irregular shapes are located throughout the stroma, but tend to be concentrated anteriorly. A 2–3 mm peripheral clear zone is free of deposits. The deposits initially appear within the first decade of life, and the intervening stroma is clear. Over time, the deposits tend to coalesce, and the intervening stroma assumes a ground-glass appearance. Visual impairment usually begins after the fifth decade.[21]
Macular dystrophy

Macular dystrophy is an autosomal recessive disorder that results in the accumulation of glycosaminoglycans (predominantly keratin sulfate) within stromal keratocytes and the intervening stroma (Fig. 22.21). Clinically, small, gray-white nodular deposits are seen within a diffuse stromal haze. The haze and deposits extend limbus to limbus and throughout the corneal stroma. Visual acuity is markedly decreased by the third to fourth decade.[21]
Fleck dystrophy

Fleck dystrophy is an autosomal dominant disorder that results in the bilateral accumulation of glycosaminoglycans and lipid within keratocytes. Clinically, discrete gray-white opacities are seen throughout the stroma (Fig. 22.22). The intervening stroma is clear. Bowman's layer, Descemet's membrane, and the endothelium are uninvolved. The opacities have sharp borders and may be round, oval, or wreathlike. Vision is not affected. Punctate lenticular opacities also may be present.[31]
Fig. 22.22 Fleck dystrophy. This autosomal dominant disorder is seen as an incidental finding. There are white, comma-shaped, stellate, circular, and wreathlike opacities at all levels of the corneal stroma. The opacities are white in direct light (1) and gray in indirect light (2). Histologically, the deposits are formed by distended keratocytes filled with complex lipids and glycosaminoglycans. Vision is not affected.

Lipid deposition

Arcus senilis is lipid deposition within the peripheral cornea, possibly secondary to increased permeability of the limbal vasculature. The lipid is initially deposited in the superior and inferior margins of the cornea. Clinically, the deposits appear as a hazy white circumferential band separated from the limbus by a lucid interval (Fig. 22.23). The outer margin of the deposit is sharply demarcated, and the inner margin is irregular secondary to an arterial diffusion gradient of lipid toward the central cornea and a venous clearing of lipid peripherally.[32]
Secondary lipid deposition occurs when corneal neovascularization is present. Associated conditions include trauma, interstitial keratitis, and corneal ulceration. Clinically, the lipid may assume either a fan-shaped pattern in front of active neovascularization or a disk-shaped pattern within chronic neovascularization.\[33\]

Systemic abnormalities in lipid metabolism including lecithin-cholesterol acyl transferase (LCAT) deficiency and fish eye disease, and Tangier disease can result in lipid deposition in the corneal stroma.\[34\]

**Mucopolysaccharidoses**

The mucopolysaccharidoses are a group of metabolic disorders that result in the accumulation of mucopolysaccharides secondary to deficiencies of lysosomal acid hydrolases. Corneal clouding is diffuse and composed of fine gray punctate opacities (Fig. 22.24). Hurler's, Scheie's, Morquio's, and Maroteaux-Lamy syndromes all demonstrate progressive corneal clouding. Hunter's and Sanfilippo's syndromes do not demonstrate clouding grossly, but may have slit lamp evidence of clouding at a later age.\[35\]
Refractile/crystalline deposits

Lattice dystrophy

Lattice dystrophy is an autosomal dominant disorder that results in the accumulation of amyloid within the corneal stroma (Fig. 22.25). There are refractile lines with nodular dilations. The lines are white in direct light and translucent or crystalline in indirect light. The deposits are more common in the anterior stroma. Usually there is a limbal clear zone. The dystrophy is progressive and the stromal haze may obscure the lines and dots. Recurrent epithelial erosions are a common finding in this disease and can lead to subepithelial opacification.[21]
Schnyder's central crystalline dystrophy

Schnyder's crystalline dystrophy is an autosomal dominant disorder that results in the accumulation of cholesterol and neutral fats within the central stroma (Fig. 22.26). Clinically, a diffuse gray stromal haze is seen with small crystals scattered throughout the haze. A significant number of patients also will have a dense corneal arcus. The haze and crystals usually assume a central disk or ring pattern. The clinical appearance of the dystrophy varies widely, but it typically presents during the first decade, with progressive opacification over time.\[21\]
Bietti’s crystalline dystrophy

Bietti’s crystalline dystrophy is a rare autosomal recessive disorder characterized by corneal and retinal crystals associated with retinal pigment epithelial atrophy and choroidal sclerosis (Fig. 22.27). The corneal crystals are small and located in the anterior stroma and subepithelial space in the peripheral cornea. The corneal crystals resemble cholesterol or other lipid deposits histologically, and the disorder may represent a systemic defect in lipid metabolism. This progressive condition usually presents in the third decade with symptoms of nyctalopia, poor dark adaptation, peripheral visual field loss, or central visual acuity loss.[36]
Systemic diseases with immunoglobulin deposition

Corneal crystals have been recognized in systemic diseases that result in excessive immunoglobulin production (Fig. 22.28). These diseases include primary amyloidosis, multiple myeloma, Waldenström's macroglobulinemia, lymphoma,[37] benign monoclonal gammopathy,[38] and cryoglobulinemia. The crystals can be seen as the initial clinical sign of these diseases. The crystals are seen throughout the stroma, but may be localized in the posterior stroma in some cases.[38] They are white in direct illumination and crystalline in indirect illumination. The crystals may be refractile or polychromatic. Histologically, the crystals are intracellular immunoglobulin deposition.[37]
Cystinosis

Cystinosis is an autosomal recessive disorder that results in the accumulation of nonprotein cystine in most body tissues. A defect in lysosomal cystine transport allows cystine to accumulate within the lysosomes of the cells. Cystinosis can present as a nephropathic or benign form. The nephropathic form is subdivided into infantile and late-onset groups.

Corneal manifestations are found in all three forms and consist of stromal deposition of iridescent crystals (Fig. 22.29). These crystals are deposited initially in the anterior peripheral stroma and, with time, the deposition proceeds posteriorly and centrally. Deposits of crystals in the cornea can cause severe photophobia and episodes of recurrent erosions. The crystals may also be deposited in the conjunctiva and retina.[39]
Fig. 22.29 Cystinosis.
Deep Stromal Deposits

Pigmented deposits

Copper deposition associated with Wilson's disease

Wilson's disease is an autosomal recessive disorder that results in the accumulation of copper in most body tissues. The Kayser-Fleischer ring is present in approximately 95% of patients with Wilson's disease. Clinically, it appears as a yellow-brown or green ring located at the level of Descemet's membrane in the peripheral cornea (Fig. 22.30). The copper deposition begins peripherally at Schwalbe's line and progresses centrally. There is no clear interval separating the ring from the limbus. Because of its peripheral location, gonioscopy may be required to locate the ring in its early stage.[40] Ocular copper deposition has also been reported with multiple myeloma.[41]
Ocular chalcosis refers to the deposition of copper within the eye (Fig. 22.31). Deposition typically occurs in the setting of an intraocular foreign body. Copper has an affinity for basement membranes and is preferentially deposited in Descemet's membrane, the lens capsule (characteristically forming a sunflower cataract), and the internal limiting membrane of the retina. The corneal deposition appears as a peripheral greenish discoloration of Descemet's membrane similar to the Kayser-Fleischer ring seen in Wilson's disease.\cite{42}
Ocular chrysiasis

Ocular chrysiasis occurs in the setting of oral or intramuscular gold therapy for rheumatoid arthritis. Gold deposits may be found in both the cornea and conjunctiva (Fig. 22.32). The deposits are located in the posterior stroma and Descemet's membrane and appear as yellow-brown granules, which may have a metallic sheen.[43] The deposits are visually asymptomatic.
Mottled cyan opacification in contact lens wearers

A mottled cyan-colored opacification at the level of Descemet’s membrane has been described in soft contact lens wearers. The opacity is seen in the peripheral and midperipheral cornea (Fig. 22.33). The cause is unclear, but it appears to be associated with long-term contact lens wear.[44]
Nonpigmented deposits

Cornea farinata

Cornea farinata is a degenerative disorder characterized by multiple, tan to white, punctate opacities located in the posterior stroma (Fig. 22.34). The opacities are usually bilateral. The deposits are visually asymptomatic.[45]
Pre-Descemet's corneal dystrophy

In pre-Descemet's corneal dystrophy, there are fine white punctate opacities within the posterior stroma just anterior to Descemet's membrane (Fig. 22.35). The disorder is familial, although the exact pattern of inheritance has not been established. The deposits are larger than those seen in cornea farinata. They may be located centrally, or in an annular, or diffuse pattern. They are visually asymptomatic.[46]
X-linked ichthyosis

X-linked ichthyosis is one of a group of hereditary skin disorders that results in hyperkeratosis and scaling of the skin. The disease is inherited in an X-linked recessive pattern, and corneal changes are found in both homozygote males and heterozygote females. These changes consist predominantly of discrete, gray-white, visually asymptomatic opacities located anterior to Descemet's membrane (Fig. 22.36). They are diffusely spread over the cornea and shaped as dots, commas, or filaments.\[47\]
Ocular argyrosis

Ocular argyrosis is the deposition of silver within the eye (Fig. 22.37). The deposition occurs in the conjunctiva and cornea and is the result of the topical medication Argyrol (a silver nitrate compound rarely used anymore) or industrial exposure to silver. Recent reports of ocular argyrosis have been due to self-application of eyelash tint.[48] The typical corneal change is a diffuse slate-gray discoloration of Descemet’s membrane. The deposits may be central or peripheral. Almost all cases of corneal deposition are associated with a grayish discoloration of the conjunctiva.[49]
Refractile/crystalline deposits

Polymorphic amyloid degeneration

Polymorphic amyloid degeneration is an age-related change of the cornea that is usually bilateral and does not affect vision. Patients are usually older than 50 years of age at the time of onset. In the deep corneal stroma are polygonal gray-white opacities and lines that are refractile in indirect illumination (Fig. 22.38). The opacities themselves appear similar to those seen in lattice dystrophy; however, they are usually less extensive, located in the deepest level of the stroma, and are not associated with the sequelae of lattice dystrophy. These deposits are not associated with any systemic disorder of amyloid deposition.[50]
Fig. 22.38 Polymorphic amyloid degeneration.
References


MY ORIGINAL WORK DRAMROO...
Chapter 23 – Corneal Infiltrates in the Contact Lens Patient

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An estimated 36 million Americans are contact lens wearers, comprising nearly 10% of the United States population. While the most feared complication of contact lens wear is infectious corneal ulceration, the ophthalmologist must distinguish several types of sterile contact lens-associated infiltrates from infected ulcers. Current variations in types of contact lenses, disinfection methods, and patterns of use all add to the complexity of managing corneal infiltrates in the contact lens wearer. In addition, contact lens users are subject to problems unrelated to contact lens use, such as staphylococcal hypersensitivity marginal keratitis.

History

A detailed history is the first important step in managing the acutely symptomatic contact lens patient. The onset, duration, and severity of symptoms aid in formulating a diagnosis. The history should include the type of contact lens worn, the pattern of lens usage, the cleaning and disinfection regimen, as well as the type and brand of cleaning solution. Breaks in standard contact lens care, such as exposure of the lenses or case to water, should be specifically sought, as this information is rarely volunteered. Any one of these pieces of information alone will certainly not make the diagnosis, but together, along with careful clinical examination, the information will guide initial diagnosis and management.

Pain

The patient's pain is an important distinguishing feature. As Stein et al. demonstrated, contact lens patients with moderate to severe pain are likely to have a positive corneal culture. Sterile corneal infiltrates tend to be associated with mild discomfort. Increasing pain is consistent with active infection, whereas decreasing pain after contact lens removal favors self-limited inflammation.

Type of contact lens and pattern of wear
It is necessary to determine whether the patient wears disposable (single use), frequent
replacement (discards after a few weeks), or conventional lenses, extended- or daily-wear soft contact lenses, or rigid gas-permeable lenses. Daily disposable lenses eliminate
standard contact lens hazards such as improper hygiene and storage, when used correctly.
Hyper-oxygen transmissible silicone hydrogel lenses were designed to reduce corneal
hypoxia, hypothesized to be a major risk factor for corneal infection. However, the relative
risk of microbial keratitis with silicone hydrogels was not significantly different compared to
planned replacement lenses in a studies by Dart et al.[3] and Stapleton et al.[4] Pure daily-
wear use of daily-wear disposable lenses was shown to have the lowest risk of severe
microbial keratitis. This was attributed to lack of exposure to pathogens in contact lens
cases. Both studies demonstrated that overnight use continues to be the main risk factor for
corneal infection. Dart et al. found that overnight wear, of any lens type, increased the risk of
corneal infection fivefold. Even occasional overnight wear (less than 1 day per week) was
associated with increased risk. Lens usage (daily vs extended wear), not lens type, was the
most important risk factor for corneal ulceration.

Designed specifically for overnight wear with lens removal during waking hours, reverse-
geometry rigid gas-permeable contact lenses are used to alter corneal shape to temporarily
reduce refractive error in orthokeratology. In a series of 123 cases, *Pseudomonas* accounted
for 37% of all cases, and *Acanthamoeba* was implicated in 33% of all cases.[5] Most of the
cases of microbial keratitis associated with orthokeratology occurred in a relatively short time
frame in East Asia, particularly China. In response, the Chinese government intervened to
regulate the orthokeratology market. It has also been suggested that the refractive effect was
associated with thinning of the central corneal epithelium in addition to the fitting relationship
of orthokeratology lenses and that this may compromise the epithelial barrier, thereby
increasing the risk of infectious keratitis.

**Contact lens solutions and hygiene**

Lens care history should include questions about solutions used and any recent changes in
solutions. Delayed-type hypersensitivity and toxic reactions to thimerosal were a problem in
the past.[6] Newer one-step disinfecting solutions are also associated with reactions including
multiple peripheral subepithelial sterile corneal infiltrates, as well as chronic or recurrent
follicular conjunctivitis and contact lens keratopathy, manifested by superior corneal
epitheliopathy and even superficial scarring due to localized stem cell deficiency.

Multipurpose solutions and no-rub formulas were introduced in recent years to improve
patient compliance. There have been two distinct outbreaks of nonbacterial contact lens-
related infectious keratitis: first, *Fusarium* keratitis, followed by *Acanthamoeba* keratitis, each
associated with the use of a particular contact lens solution – ReNu with MoistureLoc™ and
AMO Complete MoisturePlus™, respectively. Although both solutions met all FDA criteria for
safety and efficacy, this testing does not include evaluating efficacy against *Acanthamoeba*.
Further investigations did not reveal microbial contamination of either solution; however, both
solutions were ultimately removed from commercial markets. A study by Chang suggests
that exposure to *Fusarium* was likely from the sink area or shower water. Although
suboptimal contact lens hygiene practices appear unlikely as the major explanation for the
outbreak, one hygiene practice that was statistically significant on univariate analysis was
storing lenses by reusing contact lens solution already in the lens case.[7] Only
approximately half of *Acanthamoeba* cases were associated with AMO Complete, and the
resurgence of *Acanthamoeba* infections continues after its withdrawal. Joslin et al. proposed
that additional risk factors may be implicated in this resurgence of contact lens-related
Acanthamoeba keratitis.[8] Exposure through shower aerosolization may contribute to disease, as recent EPA regulations decreasing the allowable disinfection byproducts in the water supply has resulted in a higher microbial load. Discussions are underway at the FDA to change the standards for contact lens solutions.

Because inadequate lens care hygiene may increase the incidence of microbial keratitis, patients should be questioned about high-risk behaviors, which include topping off of old solutions in the case, infrequent replacement of the contact lens storage case, failure to wash hands before handling lenses, exposure of the lens or lens case to tap water, including swimming or showering while wearing lenses, and elimination of the digital rubbing step. The use of homemade saline and/or tap water to rinse or soak lenses was a problem in the past, associated with Acanthamoeba infection.[9] Acanthamoeba keratitis continues to be associated with infrequent and/or inadequate disinfection.[10]
Slit Lamp Examination

After taking the history and measuring visual acuity, a careful slit lamp examination is the next step. If the contact lens is in place, the fit should be noted and the lens removed. The presence of acute purulent discharge or evidence of chronic meibomian gland inflammation in the eyelids is noteworthy. The conjunctiva is evaluated for injection and follicles. Eyelid eversion is indicated to determine the presence of giant papillary conjunctivitis. The cornea should be examined carefully for epithelial irregularities, epithelial defects, infiltrates, and corneal edema. The size of the infiltrates and the overlying epithelial defects should be accurately measured and recorded. Abrasions in the acutely symptomatic contact lens wearer, even in the absence of an apparent corneal infiltrate, should be treated cautiously with frequent topical antibiotic ointment such as ciprofloxacin, tobramycin, or bacitracin/polymixin. Patching should be avoided, because severe Pseudomonas ulcers have developed overnight in this setting.[11] Topical steroids should not be used upon initial presentation. Anterior chamber reaction should be graded, as the presence of cells in the anterior chamber or a hypopyon are signs of infection.
Diagnosis

Distinguishing noninfectious corneal infiltrates from microbial keratitis is of crucial importance. Stein et al.[2] compared signs and symptoms in contact lens patients with infected and sterile infiltrates. Corneal infiltrates were cultured in all contact lens patients in this prospective study. Patients with positive corneal cultures had pain, anterior chamber reaction, a mucous discharge, and an overlying epithelial defect. It was concluded that patients with some or all of the clinical features associated with infection should be managed as infected cases. Notably, one-third of culture-positive infiltrates were smaller than 1 mm in diameter (Fig. 23.1). When corneal edema surrounds the infiltrate or when there is an anterior chamber reaction, even in the absence of an epithelial defect, infection requiring immediate intensive antibiotic treatment may be present. Small 1-mm peripheral infiltrates may, in fact, be infected (see Fig. 23.1). We agree with Donshik[12] who showed that a large number of patients with peripheral ‘sterile’ ulcers are in fact culture positive, and should be treated with antibiotics.
In the setting of extended wear of any soft contact lens or a tight lens, signs of acute or chronic hypoxia may be evident. Stromal and epithelial edema without an epithelial defect and a mild to severe anterior chamber reaction, with or without a hypopyon, may be present in acute hypoxia. Chronic hypoxia is often associated with conjunctival injection and superficial and deep corneal neovascularization. Sterile peripheral subepithelial infiltrates may be associated with both acute and chronic hypoxia (Figs 23.2, 23.3). With severe chronic hypoxia, deep neovascularization, scarring, and lipid keratopathy can develop.
Fig. 23.2 Culture-negative infiltrates (box) developed in a patient using disposable contact lenses for extended wear.
Fig. 23.3 These subepithelial infiltrates were most likely caused by a hypersensitivity to the contact lens solution.

To diagnose microbial keratitis definitively, cultures are necessary, but are performed less frequently.[13] Small infiltrates are not routinely cultured, but may be infectious (see Fig. 23.1). If the ulcer is getting worse, smears and cultures should be obtained if they were not performed initially, or they should be repeated, if they were negative. Smears and cultures are necessary for the diagnosis of fungal keratitis, which frequently presents as an unresponsive corneal ulcer. Cultures should be obtained if the infiltrate is more than 1 mm, if the keratitis is getting worse on treatment, or if an unusual organism (fungus, Acanthamoeba, or atypical mycobacterium) is suspected on the basis of the history or clinical appearance (Figs 23.4–23.6). We treat smaller infiltrates without cultures intensively with fluoroquinolone antibiotics. In contact lens patients, we prefer gatifloxacin or levofloxacin for their possible improved coverage of Pseudomonas.
Fig. 23.4 A severe *Pseudomonas* infection occurred in a daily-wear disposable lens user who mistook saline for disinfecting solution.
Fig. 23.5 This large, central ulcer initially presented as an abrasion and was treated with tobramycin–dexamethasone combination suspension.
Signs of delayed hypersensitivity or toxic reactions to contact lens solutions include peripheral, subepithelial opacities associated with conjunctival injection with or without follicles. Painful pseudodendrites have been recognized as an early sign of *Acanthamoeba* keratitis.[14] Diagnosis of *Acanthamoeba* keratitis at this early stage is easier to confirm on corneal scrapings and is associated with a better response to medical treatment.

Ring-shaped infiltrates in contact lens wearers can be a diagnostic challenge. Sterile stromal rings are thought to be similar to Wessely rings associated with bacterial endotoxin developing within 7 to 10 days.[15] Rings of acute corneal suppuration occur in the setting of frank microbial keratitis within 1 to 2 days (see Fig. 23.4). The central area is relatively clear due to corneal melting. Ring infiltrates, which are the hallmark of late *Acanthamoeba* keratitis, typically develop weeks after the onset of symptoms.[16][17] These ring infiltrates are usually associated with the intense pain and severe inflammation characteristic of this devastating infection. Anesthetic abuse is also associated with ring infiltrates similar to those seen in *Acanthamoeba* keratitis. While *Acanthamoeba* keratitis is often misdiagnosed as herpes simplex, HSV stromal keratitis is typically associated with relatively mild discomfort and is responsive to medical therapy.

Ocular conditions unrelated to contact lens use may cause infiltrates in contact lens patients. Patients with blepharitis may present with perilimbal infiltrates related to staphylococcal hypersensitivity. Patients with chronic follicular conjunctivitis may have chlamydial conjunctivitis. Staphylococcal hypersensitivity reactions and chlamydia can be difficult to distinguish from reactions to chemicals in contact lens solutions, but they do not recur with resumption of lens use.
Treatment

Treatment is dictated by the suspected underlying cause determined from the history and clinical examination. Regardless of the presumptive diagnosis, patients must be instructed to return immediately if they have new or increasing pain, a decrease in vision, or if they develop a white spot in their cornea. If a reaction to preservatives in lens solutions is suspected, discontinuing lens wear until symptoms and signs resolve, replacing the contact lenses, and switching to a hydrogen peroxide disinfection system or preferably single-use daily disposable lenses are recommended. Refitting tight lenses and avoiding extended-wear lenses are warranted if hypoxia is thought to be the underlying cause. Topical steroids are best avoided as the initial treatment of infiltrates in contact lens wearers, although there are differences of opinions on their use later. Hypoxic infiltrates and solution reactions often resolve without corticosteroids (see Fig. 23.1). Inappropriate treatment of early infectious infiltrates with topical corticosteroids can have serious adverse effects, especially if the infiltrate proves to be caused by fungal infection. We recommend low-dose antibiotics for suspected sterile infiltrates, although they are not always necessary.

Infectious corneal infiltrates associated with contact lens wear must be treated immediately. The most frequent organisms isolated are *Pseudomonas* and *Staphylococcus aureus*. Standard care for suspected microbial keratitis is intensive broad-spectrum antibiotic therapy. Small infections are treated with fluoroquinolones such as gatifloxacin every 30 minutes after a loading dose every 5 minutes for five doses. For more serious infections, over 1–2 mm in size, broad-spectrum topical fortified tobramycin and cefazolin or vancomycin are given every 30 minutes around the clock. Newer fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin, provide enhanced coverage of both Gram-positive and Gram-negative organisms, respectively. It is unproven that the lower minimal inhibitory concentration (MIC) of gatifloxacin for *Pseudomonas* and *Serratia* is clinically relevant, but we prefer it in contact lens patients. Levofloxacin 1.5% has been shown to reach corneal tissue levels well above the mean for the most common ocular pathogens.[18]

There is controversy regarding the efficacy of fortified drops versus fluoroquinolones. Some data suggest they are equivalent.[19] In our experience, some patients' infections with sensitive organisms progress despite intensive fluoroquinolone therapy but respond to fortified antibiotic drops (see Fig. 23.4). There is increasing resistance to *Staphylococcus* and some recent reports of *Pseudomonas* resistance to fourth-generation fluoroquinolones.[20],[21] Patients should be hospitalized if possible if the ulcers are 2 mm or greater in diameter or are worsening on outpatient treatment (see Figs 23.4–23.6). The
decision for hospitalization also depends on the patient's or his or her family's ability to comply with frequent medications and follow-up care, and the availability of a hospital that can administer drops at a frequent rate.
Follow-up

After resolution of the corneal ulcer, many patients are anxious to resume contact lens wear. We recommend the use of daily disposable lenses worn for 1 day and then discarded, to avoid the risks of inadequate disinfection and extended wear. If compliance is in doubt, contact lens use should be avoided.
Case Examples

Case 1

A disposable extended-wear lens user presented with three infiltrates measuring 0.2–0.5 mm in diameter (see Fig. 23.1). Because of surrounding corneal edema and moderate anterior chamber reaction, infection was suspected. Cultures were taken, and intensive treatment was begun with fortified tobramycin and cefazolin drops every 30 minutes as an outpatient. Cultures were positive for heavy growth of *Pseudomonas*. The patient responded well to treatment.

Comment: Small infiltrates may be infected. Because small infiltrates may be caused by virulent organisms such as *Pseudomonas*, one should treat them with intensive topical antibiotics and not topical corticosteroids.

Case 2

An extended-wear disposable lens user presented to the emergency room with two peripheral infiltrates (see Fig. 23.2). Cultures were done, and treatment was begun with intensive topical ciprofloxacin. She responded well. Cultures were negative.

Comment: In the absence of anterior chamber reaction, or surrounding corneal edema, these infiltrates were likely to be sterile. Sterile infiltrates improve without topical corticosteroids.

Case 3

A daily-wear frequent replacement lens wearer presented with bilateral redness, pain, foreign body sensation, and tearing (see Fig. 23.3). She used Opti-Free (Alcon Labs, Fort Worth, TX) cleaning solution. Her slit lamp examination revealed central and mid-peripheral subepithelial infiltrates (SEIs) bilaterally, and she had no history of acute conjunctivitis. The SEIs resolved over 1 month with the use of a moderate-strength corticosteroids.

Comment: Patients can manifest a hypersensitivity to their contact lens solutions. If there is no epithelial staining, mild to moderate steroids can carefully be used short term with good effect. Once the reaction has resolved, the patient can resume contact lens use with new
lenses and preservative-free contact lens solutions, or switch to daily disposable lenses.

**Case 4**

A frequent replacement daily-wear lens wearer who used saline instead of disinfecting solution by mistake developed a severe *Pseudomonas* infection (see Fig. 23.4). She was treated initially with intensive topical ciprofloxacin and referred because of worsening. She was hospitalized and treated with fortified tobramycin and mezlocillin. She did well and recovered most of her vision.

Comment: In our experience, intensive fortified antibiotics are more effective than intensive ciprofloxacin, even in *Pseudomonas* infections sensitive to both. Patient education about the types and purposes of various contact lens solutions is critical in preventing infections.

**Case 5**

A daily-wear frequent replacement lens wearer presented to her local ophthalmologist with a foreign body sensation and pain (see Fig. 23.5). She was diagnosed with a small corneal abrasion and was started on tobramycin–dexamethasone combination suspension twice a day. Two days later she presented to our service with a large, central ulcer and a hypopyon. The ulcer was cultured, and the patient was admitted for frequent fortified topical tobramycin, piperacillin, and vancomycin antibiotics. After 1 month, the ulcer had healed, but her vision only improved to counting fingers at 1 foot due to scarring.

Comment: A small central abrasion in a contact lens wearer should be treated with frequent (every 2 hours) antibiotic ointment with good Gram-negative coverage and watched carefully. Steroids should not be used in the initial management of a contact lens abrasion or ulcer, and patching is contraindicated.

**Case 6**

A 12-year-old girl presented with a large paracentral ulcer with a radial keratoneuritis (see Fig. 23.6). She was a daily-wear frequent replacement lens wearer who used a multipurpose solution. The patient had been on frequent fluoroquinolone antibiotics for 2 days before being referred. She was cultured and admitted for around-the-clock fortified antibiotics. No organisms grew in the cultures. Two years later, her vision was 20/25 with spectacle correction and she had a mild paracentral stromal scar.

Comment: Presumed infectious keratitis in contact lens wearers can present even in patients who ‘do everything right.’ Aggressive fortified antibiotics started early in the disease course often result in good outcomes. It should also be noted that radial keratoneuritis is not specific for *Acanthamoeba* keratitis.
References


Chapter 24 – The Red Eye

Christopher R. Croasdale, Michael B. Shapiro

The goal of this chapter is to aid the clinician in using a logical framework for diagnosing a red eye. The target audience is primarily non-cornea-specialized ophthalmologists and optometrists. In-depth discussion of specific conditions can be found elsewhere within The Cornea, and other appropriate reference texts.

Defining a Red Eye

Redness is not a symptom, but a nonspecific sign. A red eye from the patient perspective signifies the visible appearance of abnormal redness of the globe, lids, or adnexal structures. The three major processes responsible for the majority of cases are subconjunctival hemorrhages, inflammation, and vascular abnormalities. Of these, conditions with inflammation account for the majority of red eye presentations. Diseases where vascular congestion may give the appearance of the redness of inflammation are the least common. In some instances, two or all mechanisms can occur simultaneously.
Successful diagnosis requires being knowledgeable of the possibilities. One cannot diagnose what one does not know. In training, diseases are studied and organized according to various classification schemes, such as: infectious diseases, inflammatory diseases, diseases of the retina, or diseases of cornea, etc. In practice, the clinician usually determines a number of probable diagnoses based on the initial history. One does not think, ‘This is a red eye patient and here is every diagnostic possibility.’ Instead, one enters the examination room with specific diagnoses in mind and seeks the additional historical and examination findings which either support the diagnosis or lead to other possibilities. When the diagnosis is not readily forthcoming, one may need to sit back and review the various diagnostic categories (Table 24.1).

Table 24.1 -- Causes of a red eye

<table>
<thead>
<tr>
<th>DISORDERS PRIMARILY OF THE GLOBE</th>
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</thead>
<tbody>
<tr>
<td>Extraocular</td>
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<tr>
<td>Conjunctivitis (and keratitis, when mechanism is the same)</td>
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<tr>
<td>Infectious</td>
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<tr>
<td>Viral (adenoviral, HSV, VZV, etc.)</td>
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<tr>
<td>Bacterial (Chlamydia)</td>
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<td>Fungal, parasitic</td>
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<tr>
<td>Inflammatory</td>
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<tr>
<td>Idiopathic</td>
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<tr>
<td>Superior limbic</td>
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<tr>
<td>keratoconjunctivitis</td>
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<tr>
<td>Allergic and hypersensitivity reactions</td>
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<tr>
<td>Atopic blepharoconjunctivitis</td>
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<tr>
<td>Phylectenular (Staphylococcus, tuberculosis)</td>
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<tr>
<td>Environmental/seasonal allergies</td>
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<tr>
<td>Vernal</td>
</tr>
<tr>
<td>conjunctivitis</td>
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</table>
Medications (brimonidine, apraclonidine, dorzolamide, trifluridine, etc.)
Contact lens solutions
Contact dermatitis/conjunctivitis
(Atropine solution, poison ivy, etc.)

Cosmetic products

Toxic reactions
Chemical exposures (industrial and home cleaning products, etc.)
Topical medications (aminoglycosides, neomycin, etc.) and preservatives (e.g., benzalkonium chloride)
Molluscum contagiosum (lesion usually on lid)

Mechanical/irritant
Contact lens related
Factitious
Foreign body (insect parts, plants debris)
Exposed sutures, glaucoma drainage devices, scleral buckle elements
Mucus fishing syndrome
Any eyelid position/function abnormality
Floppy eyelid syndrome
Imbrication
Trichiasis,
lagophthalmos

Trauma

Systemic immune-mediated
Stevens-Johnson syndrome
Ocular cicatricial pemphigoid
Graft versus host disease
Ligneous conjunctivitis

Neoplastic lesions causing inflammation and/or increased vascularity of conjunctiva
Benign lesions
Pinguecula, pterygia, nevi (amelanotic)

Malignant lesions
Limbal in origin (conjunctival intraepithelial neoplasia, squamous cell carcinoma)
Nonlimbal in origin (primary lesion elsewhere, usually the lid: squamous,
basal and sebaceous cell carcinoma)
Melanoma

**Noninflammatory conjunctival redness**
Subconjunctival hemorrhage
Abnormal vascular engorgement
   - Polycythemia vera

**Cornea and/or conjunctiva (dry eye conditions)**
Dry eye syndrome (deficient tear syndrome)
   - All combinations of aqueous, mucin or lipid deficiency
Evaporative/exposure keratoconjunctivitis
   - Paralytic (Bell's palsy, etc.)
   - Nocturnal
Abnormal lid anatomy with inadequate closure
   - Congenital
   - Postsurgical
      - Trauma repair
      - Cosmetic/functional eyelid surgery
      - Reconstruction after tumor excision

Proptosis
   - Graves' disease or other orbital process

Abnormal blink reflex/frequency (often multifactorial, e.g. Parkinson's disease, systemic medication side effects, especially anti-Parkinson's medications, antipsychotic medications, etc.)

Filamentary keratitis
Neurotrophic keratoconjunctivitis
   - Postviral (HSV, VZV)
   - Idiopathic
      - Topical medications (anesthetic abuse, excessive nonsteroidal antiinflammatory drugs)
      - Postsurgical (trigeminal nerve ablation)

**Cornea**
Recurrent corneal erosion/traumatic abrasions
Endothelial decompensation of any cause with resultant bullous
#### Epidemioses of the Eyelids and/or Adnexal Structures

**Episclera/sclera**
- Infectious episcleritis/scleritis
- Inflammatory episcleritis/scleritis

**Intraocular**
- Infectious or inflammatory
  - Endogenous/exogenous endophthalmitis
  - Chorioretinitis, retinitis
  - Neovascular glaucoma
  - Acute-angle-closure glaucoma
  - Ocular ischemic syndrome
  - Postsurgical (e.g. retained nucleus, toxic)
  - Postintravitreal injection (toxic)
  - Uveitis (anterior, intermediate, posterior)

**Neoplastic**
- Any primary or metastatic malignant tumor
- Masquerade syndromes

**Disorders of the Eyelids and/or Adnexal Structures**

**Eyelids**
- Blepharitis
  - Infectious (viral, bacterial, parasitic – lice, *Demodex*)
  - Inflammatory
    - Meibomitis, hordeola, chalazia
    - Rosacea
    - Seborrheic

- Abnormal anatomy/function
  - Ectropion, entropion, trichiasis/distichiasis
  - Floppy eyelid syndrome
  - Imbrication

- Neoplasms
  - Benign (keratoacanthoma)
  - Malignant (primarily basal, squamous and sebaceous cell carcinomas)

**Nasolacrimal system**
<table>
<thead>
<tr>
<th>Lacrimal gland</th>
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<tr>
<td>Infectious/inflammatory (dacryoadenitis)</td>
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<td>Malignancy</td>
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<tr>
<td>Canaliculitis</td>
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<td>Dacryocystitis</td>
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<td>Nasolacrimal duct obstruction with secondary conjunctivitis</td>
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**Orbit/periorbital structures**

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<th>Infectious</th>
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<td>Preseptal and orbital cellulitis</td>
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<th>Inflammatory</th>
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<td>Sarcoïdosis, Wegener's granulomatosis</td>
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<td>Thyroid-related orbitopathy</td>
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<td>Myositis</td>
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<td>Vasculitis</td>
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<td>Ruptured dermoid cyst</td>
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<td>Sinus mucocele</td>
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<th>Abnormal vascular engorgement</th>
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<td>Arteriovenous malformations</td>
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<th>Carotid-cavernous fistula</th>
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<td>Dural shunts</td>
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<th>Hemangiopericytoma</th>
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<td>Orbital varix</td>
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Subconjunctival Hemorrhages and Telephone Triage of a Red Eye

This diagnosis is one of the easiest to determine among causes for a red eye, but deserves discussion due to its frequency of occurrence, healthcare utilization costs, and implications when misdiagnosis occurs. This cause usually takes but a moment to diagnose once the clinician sees the patient. Although sometimes dramatic in appearance, the occurrence of a spontaneous subconjunctival hemorrhage alone rarely signifies a risk to the health of the eye or patient. The greater clinical significance of subconjunctival hemorrhages is in the challenge they present to ancillary clinic staff performing telephone triage, and the risk of delaying appropriate treatment for other causes of a red eye if a misdiagnosis is made.

Types of patients for whom misdiagnosis over the phone is most critical include patients who have had recent surgery, particularly any type of intraocular surgery where endophthalmitis is a possibility. Additionally, eyes post glaucoma filtering surgeries have a long-term risk of bleb-related infection. Corneal transplant recipients have long-term risk of allograft rejection. A misdiagnosis of ‘probable subconjunctival hemorrhage’ in such patients can have potentially severe consequences.

The skills and knowledge of the persons likely to be responsible for performing telephone triage will vary widely. In addition to obtaining a history of the present problem, the patient should be asked about prior similar occurrences, other past ocular history, including specifically any recent or past eye surgery, and current medical conditions and medications (specifically anticoagulants and other related agents). Two key symptoms to clarify are whether there is an associated change to or loss of vision, or any significant discomfort or pain of the involved eye. A simple conjunctival hemorrhage alone should not produce an affirmative response to either of these questions. If the response is affirmative, the triage person must strongly consider that another condition may exist.

Even if ‘some other condition’ is the assessment of the person performing the telephone triage, this does not necessarily indicate the need for emergent or urgent evaluation. The final decision of when or whether the patient needs to be seen will be determined by many factors, chief among which include the knowledge, experience, and self-confidence of the triage person. There is no substitute for experience and knowledge in this process, and this is an area in which most of us can provide helpful training to those in our clinics and healthcare facilities providing such service.
Redness due to Inflammation

The primary process that produces inflammation and hyperemia can originate with the globe (intra- or extracocular), the orbit, or the lids and adnexal structures. Successful treatment usually requires correctly identifying the etiology, although some conditions are self-limited and will resolve regardless of whether the correct diagnosis is determined or the correct treatment initiated. In other cases, despite the etiology remaining unknown, successful resolution of the inflammation with antiinflammatory medication may occur.

It is important to determine whether inflammation is the primary process, or a secondary reaction, in order to successfully treat the problem. An example of the former is a patient who presents with ocular cicatricial pemphigoid and cicatrizing conjunctivitis. The conjunctivitis may temporarily appear better with topical steroids, but ultimately will worsen unless the correct diagnosis is made and systemic immunosuppression is used to control the systemic disease. An example of inflammation as a secondary reaction can be seen with a missed retained intraocular foreign body. Topical steroids may suppress or temporarily eliminate the inflammatory reaction, but until the primary problem of the foreign body is diagnosed and it is removed, the inflammation will recur with discontinuation of the steroids. The goal is to determine and treat the underlying cause, and not just the symptoms or signs.
Red Eyes due to Vascular Abnormalities

This category includes all noninflammatory conditions that can result in an eye appearing red. In addition to commonly occurring subconjunctival hemorrhages, discussed above, are a heterogeneous number of less common conditions which either increase or reduce the normal vascular pattern of the globe and adnexal structures. An example of the former can occur when a carotid cavernous sinus fistula results in increased venous pressure and dilation of the episcleral and conjunctival vessels. An unusual case of the latter was seen in a patient who received more than a dozen unilateral intravitreal injections of Avastin (bevacizumab) for macular degeneration. The patient wondered why the contralateral eye was red. That eye did not symptomatically feel irritated, or reveal signs of such, but the vessels of the sclera, conjunctiva, and lids were more prominent than the other 'youthful-appearing' eye of this elderly woman. The conclusion was that the Avastin was having an extraocular effect on the vessels of the globe and lids, resulting in a reduction of the caliber of vessels and the asymmetric appearance of the eye redness.
In clinical practice, the greatest barrier to obtaining an adequate history is the decreasing amount of time most spent between most clinicians and their patients. Typically, the history will be obtained by a technician, and often be but a few sentences or less. Depending on the condition, this may suffice. For the auto mechanic with a sore, red eye which started while he was working under a car without protective eyewear, a probable corneal foreign body is a reasonable presumptive diagnosis. One might move quickly to the slit lamp examination. If an offending foreign body is present, the diagnosis is made and treatment rendered. If not, this may be the point when the clinician sits back and begins to ask additional questions.

In general, acute (hours to days) and subacute (days to a few weeks) causes of a red eye are more likely to have a single identifiable cause. Examples include foreign bodies in the cornea or conjunctiva, corneal abrasions and erosions, acute conjunctivitis, and many contact lens-associated problems. Some examples of intraocular conditions include angle-closure glaucoma and uveitis. Most of these acute and subacute conditions do not pose significant diagnostic challenge; with an appropriate history and examination the cause is usually determined.

Chronic (greater than several weeks duration) and recurrent red eye conditions can require greater experience and skill to diagnose. A careful history by the clinician is often necessary, particularly when a patient has seen multiple providers and received multiple treatments over many months or longer. Such an example is the patient with a 6-month history of recurrent, unilateral conjunctivitis who has been treated with multiple courses of topical antibiotics by various providers without improvement. Eventually, a combination of antibiotic and steroid solution is tried for presumed nonspecific viral conjunctivitis, and the redness and irritation improve. But upon discontinuation, the conjunctivitis recurs. Finally, the patient is referred to your office, where a prominent follicular conjunctivitis is found, along with a small umbilicated lesion hidden amongst the upper lid lashes. Molluscum contagiosum is correctly diagnosed, and after curettage of the lesion there is resolution of the follicular conjunctivitis, without recurrence.

Other cases may be far more complex. Take the 76-year-old woman with long-standing glaucoma who has had bilateral filtration surgery, twice in the right eye and once in the left. The initial trabeculectomy in the right eye was 10 years prior, without mitomycin, and failed after 4 years. She was then back on four glaucoma medications for another 4 years until a repeat trabeculectomy with mitomycin-C was done 2 years prior to presentation, with
resultant overfiltration and borderline hypotony. The left eye trabeculectomy was done 7 years prior, with partial bleb failure despite needling after 5 years, leading to reinitiation of brimonidine 0.15% three times daily. Both eyes are pseudophakic, and the left also has a history of a retinal detachment with scleral buckle repair before the trabeculectomy. She states that for the last year or longer, both eyes are always red, and painful, with discharge mainly from the right eye. Both eyes itch, and the left eye has had recurrent subconjunctival hemorrhages (per the referring glaucoma specialist). She states that use of artificial tears only brings relief for a few minutes. Her general medical conditions include hypothyroidism, hypertension, depression, osteoarthritis, chronic allergies, and atrial fibrillation. Medications include Synthroid (levothyroxine), hydrochlorothiazide, atenolol, amitryptiline, acetaminophen, Coumadin (warfarin), and Allegra (fexofenadine).

External examination reveals an obese woman with prominent eyes due to a combination of shallow orbits, asymmetric lateral flare, lower lid retraction with several millimeters of scleral show, and mild inferior punctal ectropion. Both upper lids seem moderately floppy, but there is no lash ptosis. The right upper lid is mildly ptotic. On slit lamp examination, all four lids have mild scurf on the lashes, with moderate atrophy and inspissation of the meibomian glands, and prominent telangiectasia of margins. The bulbar conjunctiva of the right eye reveals a large, diffuse bleb at 12 o’clock, with 360 degrees of chemosis from overfiltration. There is mild diffuse injection. An exposed 10-0 nylon suture tail is visible at the medial aspect of the bleb. There is 2+ clear stringy mucus in the inferior cul-de-sac, and a mixed papillary and follicular reaction of the inferior palpebral conjunctiva. Eversion of the right upper lid shows a 4+ papillary reaction of the palpebral conjunctiva. The right cornea has a moderate superficial punctate epitheliopathy of the inferior 30%. Fluoroscein instillation confirms the exposure of the suture tail, and also reveals a moderate amount of fine, diffuse punctate staining of the elevated, chemotic conjunctiva. The tear lake volume seems average.

Additional aspects of the left eye include the following. A mild dull pink appearance of the bulbar conjunctiva, with a small to moderate vascularized and encapsulated bleb at 11 o’clock. Eversion of the upper lid reveals a 1+ papillary reaction. The lower lid palpebral conjunctiva has a 2+ follicular reaction and 1+ papillary reaction. The cornea has diffuse 1+ superficial punctate epitheliopathy. Fluoroscein application does not reveal any additional abnormality of the conjunctiva. The tear lake volume is mildly less than the right eye.

This is not an unusual example in today’s practice of a patient referred for chronic red eyes. Obtaining and concisely organizing the pertinent history, which covers many years, would exceed the capabilities of most technicians. The multitude of findings reveals inflammatory changes due to multifactorial processes in each eye. There are the long-term toxic effects of glaucoma medications and their preservatives on the conjunctiva of both eyes, with the additional hypersensitivity reaction to brimonidine in the left eye. Multiple ocular surgeries, including two glaucoma filtering operations on the right eye, and the scleral buckle and filtration surgery on the left eye, cause significant periods of conjunctival inflammation, subsequent fibrosis, and long-term adverse effects on the cells and structures involved in the production and maintenance of a healthy tear film and ocular surface. In the right eye, two additional direct effects of the glaucoma surgery include the abnormal anatomic elevation of the conjunctiva due to overfiltration and chemosis, causing abnormal tear film dynamics with relative exposure and abnormal wetting, as well as the papillary conjunctivitis due to the exposed nylon suture irritating the upper palpebral conjunctiva. Both eyes have mild anterior blepharitis, as well as posterior lid disease, further contributing to the inflammatory milieu of the ocular surface. Amongst the systemic medical conditions was the history of Graves’ disease with mild lid signs of flaring of the lateral canthi, and mild lower lid retraction, adding
another potential evaporative dry eye component. Obesity is associated with floppy eyelid syndrome. Several of the systemic medications are known contributors to dry eye disease, including the diuretic, the antidepressant, and the antihistamine. The anticoagulant increases the likelihood of subconjunctival hemorrhages.

This example of the complex interaction of numerous conditions with the resultant nonspecific sign of conjunctival injection underscores why one cannot try to follow an algorithmic flowchart to arrive at the correct diagnosis. There are often multiple conditions that must be identified and considered in terms of potential contribution to the overall end result of redness.
Physical Examination

The physical evaluation begins as soon as one encounters the patient. Characteristics such as personal hygiene, body weight, and habitus can provide useful information, especially in patients who are poor historians. Body structures should be observed for structural changes secondary to systemic diseases, such as the hands and skin in rheumatoid arthritis or scleroderma. A light source is used to inspect the external facial features. Many causes of red eye can be overlooked if one immediately ‘zooms in’ with the high-power view of the slit lamp. The skin of the lids and ocular adnexa is examined for signs of inflammation, past or recent trauma, scarring, unusual pigmentation, scaling, oil content, and texture.

The presence of exophthalmos, either bilateral or unilateral, may be accompanied by injected conjunctival vessels and give definitive clues to the etiology. In addition to the more frequently occurring problem of thyroid-related eye disease, a number of uncommon orbital and intracranial conditions may present as a red eye because of either congestion or exposure of vessels of the globe. A partial list includes hemangiomas, arteriovenous malformations, lacrimal gland tumors, metastatic tumors, and mucoceles.

Palpation for masses as well as for areas of tenderness along the lid margins, lacrimal gland, and lacrimal sac can be performed if entities such as dacryoadenitis, canaliculitis, and dacryocystitis, in addition to tumors of these structures, are suspected. Enlarged preauricular nodes may be present in cases of viral or chlamydial infection or, rarely, in cases of ocular or orbital malignancy.

Examination of the facial features is followed by evaluation of the eyelids for abnormalities in structure or function. Particularly in cases of long-standing unilateral or asymmetric red eye problems it is not rare to detect a problem of the lids or adnexa that has been overlooked by others. Structural abnormalities can be due to past trauma, or prior oculoplastic surgeries (cosmetic or functional), or frequently from age-related involutional changes of the eyelids and adnexal structures.

Lid position is evaluated for ectropion and entropion. The presence of upper eyelid lash ptosis, often accompanied by mild upper lid ptosis, are frequently missed signs of floppy eyelid syndrome. Instructing the patient to squeeze the lids tightly closed can reveal an intermittent, covert spastic entropion or, less commonly, imbrication. Imbrication is the abnormal sliding of the upper lid over the external margin of the lower lid, resulting in mechanical irritation of the palpebral conjunctiva of the upper lid. It is most likely to occur
after horizontal lid tightening procedures, particularly if the lower lid tightness is much greater than that of the upper lid. The chronic irritation produces a papillary reaction, increased mucus formation, and ocular surface inflammation.

Another less common, and usually asymmetric condition, is mucus fishing syndrome. The history frequently is of a chronic conjunctivitis with production of stringy white mucus. It can be quite helpful to ask the patient to describe or demonstrate exactly how he removes the mucus. Typically, the patient is mechanically wiping or touching the inferior fornix and palpebral conjunctiva with a cloth, tissue, or cotton-tipped applicator, etc., in order to remove mucus, thereby creating a chronic cycle of irritation and inflammation, which causes continued increased abnormal mucus production. Findings may include greater distractability of the lower lid of the affected eye, along with a papillary reaction of the inferior palpebral conjunctiva.

Observing for asymmetry of the lower lid tear menisci during the initial general inspection can reveal a clue of underlying nasolacrimal outflow dysfunction. The patient may have a history of recurrent unilateral conjunctivitis, which improves with topical antibiotic treatment. Epiphora may or may not be significant, and sometimes will not be appreciated or mentioned by the patient during episodes because the other signs and symptoms of redness and discharge are of more prominence and concern. If the patient is referred, the episode of conjunctivitis may be resolved by the time of appointment, and the tear meniscus asymmetry may provide one of the few clues that leads to further evaluation of the nasolacrimal drainage system. Although acquired stenosis or obstruction anywhere along the pathway is a more common etiology, a large number of other causes is possible, ranging from infectious canaliculitis, to benign or malignant tumors intrinsic or extrinsic to the nasolacrimal system.

Once the face and ocular adnexa have been examined, one continues without magnification by looking at the globes, fornices, and palpebral conjunctivae. The lower lids are first gently retracted manually, then the upper eyelids are lifted. Abnormal lid laxity should be noted. If floppy eyelid syndrome is suspected, it is easier to demonstrate asymmetric laxity by instructing the patient to gaze inferiorly while the examiner uses the thumb of each hand to lift the upper lids simultaneously. Significantly floppy eyelids will evert rather easily, and usually reveal a marked papillary reaction. Another oft-missed condition that is relatively easily diagnosed with this step is superior limbic keratoconjunctivitis. The classic superior quadrantic bulbar conjunctival injection is much easier to detect without magnification. This is true also for assessing other conjunctival, episcleral, and scleral processes. Areas of scleral thinning may appear bluish-black due to the visibility of the underlying choroid, indicating possible past episodes of scleritis. Signs of ocular cicatricial pemphigoid, such as subconjunctival fibrosis, fornical shortening, and symblephara are also less easily missed with unaided visual inspection.

Functional eyelid problems refers to conditions where the eyelid anatomy is normal, but function is abnormal. Examples include seventh nerve pareses from any cause (postviral, postsurgical, trauma, stroke, etc.), with a number of possible sequelae: incomplete blink, lagophthalmos, and various degrees of exposure keratoconjunctivitis. Nonparalytic states of dysfunctional blinking and/or closure can also result from use of certain medications or diseases, such as: long-term antipsychotic or other psychiatric medications, advanced Parkinson's disease, or any number of severe chronic medical or end-of-life situations, where the mental state may be declining or subdued, and where frequent narcotic pain relievers may be in usage. Such patients often have multiple possible contributing factors for a red eye or eyes, chief among which include evaporative dry eye and exposure keratoconjunctivitis. One should observe the frequency, symmetry, and completeness of blinking. This is best
done unobtrusively while talking with the patient, or in situations where the patient is not
communicative, with the attendant family or caregiver. If the patient is conscious that this
behavior is being observed, he or she may unintentionally alter the pattern of blinking.
Complete closure, without lagophthalmos, should occur with involuntary blinking every 5 to 8
seconds, and there should be good apposition of the lids to the globe, with the punctal
openings normally aligned.

Slit lamp biomicroscopy is performed, beginning with low-power magnification and a broad,
oblue beam. Blepharitis, with secondary or concomitant ocular surface disease and
inflammation, is a common cause of both acute and chronic red eye. Lash cleanliness and
quantity are noted. Madarosis can occur with the more common forms of anterior blepharitis,
but should also prompt consideration of uncommon eyelid conditions, such as basal cell,
sebaceous gland, or squamous cell carcinomas. Infestation of the lashes with parasites such
as *Phthirius pubis* may also be the source of red eyes. The umbilicated lid lesions of
molluscum contagiosum can be hidden in the lash line.

The tear film is examined, both qualitatively and quantitatively. Dry eye syndrome is
commonly either the primary or a contributing cause of red eye. Qualitative abnormalities
include the presence of excess mucus or debris. Quantitative assessments can include the
height of the tear meniscus along the inferior lid margin and tear breakup time.

Next, a cotton-tipped applicator is used to retract the lower eyelid. This technique is
suggested for both patient comfort as well as the examiner's safety. The inferior fornix is
examined for discharge. A watery or mucoid discharge suggests a viral process; a more
viscid discharge is seen in allergic reactions; and a thick, purulent exudate is commonly seen
in bacterial conjunctivitis. Biomicroscopy may also reveal inflammatory membranes. True
membranes, when removed, detach the underlying adherent epithelium, leaving a raw and
bleeding surface. Pseudomembranes, when removed, cause no epithelial disruption and
thus no bleeding. Distinction of the two types can be difficult, and is not highly diagnostic.
Membranes in general are uncommon. In the acute setting, viral or bacterial conjunctivitis is
the likely cause. Noninfectious conditions include Stevens-Johnson syndrome, alkali injuries
and, rarely, ligneous conjunctivitis.

Conjunctival concretions rarely cause inflammation. A careful search for any foreign material,
from recent trauma to a more remote injury often forgotten in the history by the patient,
should be undertaken. The tear drainage system is also inspected. By rolling the cotton-
tipped applicator from the nose outward along the canalicular system, purulent material may
be milked from the canaliculi, or a stone may be palpated. Signs of acute dacryocystitis
would likely already have been noted on external examination, with redness and swelling
over the lacrimal sac, though in a situation where perhaps a prior episode had partially
resolved one might illicit pain upon compression of the sac, and sometimes a dramatic reflux
of mucopurulent material.

Next is assessment of the inferior palpebral conjunctival surface. The understanding of
follicles and papillae in ocular disease can present a challenge but provides major clues in
diagnosing the diseases with which they are associated. A follicular reaction consists of
aggregates of lymphocytes that appear as whitish bumps surrounded by a red base. Follicles
are normally absent in infants, fairly prominent in children, less commonly seen in adults, and
bring to mind a specific differential diagnosis. Acute follicular reactions are common with viral
infections such as adenovirus and the herpes family. Chronic follicular reactions (present for
longer than 3 weeks) can develop with certain infections, such as trachoma, inclusion
conjunctivitis, Parinaud's ocuologlandular syndrome, and molluscum contagiosum, and as a
toxic response to topical medications.

A papillary reaction, characterized by a red velvety appearance in which a central, elevated tuft of vessels is surrounded by a pale base, is seen with nonspecific inflammation and is less specific. Often, however, the palpebral conjunctiva has a ‘mixed’ papillary and follicular reaction. Prominent papillary hypertrophy may suggest allergic reaction. The superior palpebral conjunctiva is inspected by everting the upper eyelid. Once again, it is important to look for foreign bodies and to assess the palpebral conjunctiva. The appearance of ‘cobblestoning’ is indicative of giant papillae, which is seen in vernal disease as well as contact lens-induced giant papillary conjunctivitis. A retained suture from previous eye surgery also can cause focal giant papillary conjunctivitis by mechanical irritation of the superior tarsal conjunctiva. Scarring of the superior tarsal conjunctiva also should be documented and may be diagnostic, as in the case of the linear white scarring known as Arlt’s lines, which are seen in trachoma.

The bulbar conjunctiva is examined next. Inflamed pingueculae and pterygia are a common cause of red eye visits. Less common are phlyctenules, raised whitish to yellow nodules with surrounding dilated blood vessels that represent a type IV delayed hypersensitivity to a foreign antigen. These lesions occur near the limbus and can invade the cornea. Often, they will partially stain with fluorescein due to breakdown of the overlying epithelium. Conditions that can cause phlyctenular keratoconjunctivitis include staphylococcal disease, tuberculosis, fungal antigens, lymphogranuloma venereum, and nematodes. Conjunctival tumors, including benign and malignant lesions, can all present with ocular inflammation.

Distinguishing which layer of the ocular surface is inflamed is important but not always easy. Engorgement of the episcleral vessels can be sectorial or diffuse. Episcleral vessels are distinguished from conjunctival and scleral vessels by being thicker, running in a radial direction from the cornea, blanching with the application of topical phenylephrine 2.5%, and being immobile when the overlying conjunctiva is moved. The most common cause of episcleritis is idiopathic inflammation, although it also can be seen with systemic collagen vascular diseases such as rheumatoid arthritis, polyarteritis nodosa, systemic lupus erythematosus, and Wegener's granulomatosis. Scleral vessels are deeper and have a characteristic violaceous hue that does not blanch with phenylephrine.

The cornea often provides critical diagnostic information because clinically significant redness and keratitis often coexist. A broad oblique beam is used to scan the overall appearance. Epithelial punctate keratitis often can be noted even without fluorescein dye. An inferior pattern suggests dryness, exposure, entropion, ectropion, or blepharitis. A more diffuse distribution can be consistent with acute conjunctivitis or keratitis medicamentosa. Filaments are most commonly seen in dry eye states such as keratitis sicca, superior limbic keratoconjunctivitis, or situations where there is limited upper lid excursion due to any cause. Micropannus suggests inclusion conjunctivitis, trachoma, ocular rosacea, contact lens abuse, staphylococcal hypersensitivity, vernal keratoconjunctivitis, superior limbic keratoconjunctivitis, and herpes simplex infection. Subepithelial opacities can be seen in epidemic keratoconjunctivitis, chlamydia, herpes simplex, staphylococcal disease, and nummular keratitis.

Signs of anterior uveitis, such as keratic precipitates, cells in the anterior chamber or anterior vitreous, miosis, posterior synchiae, and injection of the perilimbal blood vessels, usually allow the proper diagnosis to be made with little difficulty. The remainder of the eye examination must not be overlooked. Measurement of the intraocular pressure and a retinal evaluation are crucial to the diagnosis of a variety of other causes of red eye.
Occasionally, ancillary tests are required. Schirmer testing, probing, and irrigation of the lacrimal system, transillumination, exophthalmometry, corneal sensitivity measurements, keratometry, gonioscopy, ultrasonography, fluorescein angiography, and radiology can be considered and may give the extra information needed to make the correct diagnosis. In cases of suspected infection or in cases that have not responded to routine therapy, standard culture techniques and conjunctival scrapings for microscopic examination are indicated. The topical anesthetic test is often useful. If a patient is complaining of ocular irritation or pain accompanying a red eye that is not consistent with the findings, two drops of proparacaine are instilled without the patient being aware of the anesthetic effect. If the patient reports that the pain is not relieved or is actually worse, consideration must be given to factitious disease, hysteria, or a condition that has a more posterior origin. A systemic work-up is sometimes mandatory, especially when malignancies, immunocompromised states, and systemic inflammatory or infectious conditions are contemplated.
Conclusion

It is always gratifying to diagnose and treat a medical condition correctly, especially in a patient referred by another practitioner. Determining the correct diagnosis involves three components. First, one needs an appropriate knowledge base. Second, an accurate and thorough history is usually critical to solve the more complicated or chronic cases of red eye. And finally, avoid rushing to the slit lamp to make the quick diagnosis.
Chapter 25 – Minimal Visual Loss : Determining the Role of the Cornea

David Litoff

Evaluating minimal visual loss is a frequent problem for the ophthalmologist. A complete history and ocular examination are essential in determining the cause of any reduction in visual function. Small alterations on the surface of the cornea can have profound effects on visual acuity. In addition, small internal structural changes in the cornea may result in visual loss secondary to light scattering and decreased transparency. In this chapter, we review various techniques for evaluating the role of the cornea in different conditions that may present with minimal visual loss.

There are several categories of corneal abnormalities which can produce minimal visual loss. The first, irregular astigmatism, causes decreased vision because of an irregular optical refracting surface. (Box 25.1 summarizes common causes of irregular astigmatism.) The second, corneal opacities, causes decreased vision from decreased transparency and increased light scattering of the cornea. (Box 25.2 summarizes common causes of corneal opacities.) A third category is irregularity of the posterior corneal surface. Often, these different corneal abnormalities coexist, making evaluation more difficult.

Box 25.1

**Irregular astigmatism**

- Keratoconus
- Postrefractive ectasia
- Post-trauma
- Corneal warpage
- Keratoconjunctivitis sicca
- Recurrent erosion
- Toxic epitheliopathy
- Corneal dystrophies
  - Anterior corneal dystrophies
    - Map-dot-fingerprint
dystrophy
  - Meesmann's dystrophy
Reis-Bücklers' dystrophy

Stromal dystrophies
  Granular dystrophy
  Lattice dystrophy
  Macular dystrophy

Corneal degenerations
  Pterygium
  Pellucid marginal degeneration
  Band keratopathy
  Salzmann's degeneration

Box 25.2

Corneal opacities

Traumatic corneal scars
Postsurgical corneal scars
Postinfectious corneal scarring
Corneal dystrophies
  Anterior corneal dystrophies
    Reis-Bücklers' dystrophy

Stromal dystrophies
  Granular dystrophy
  Lattice dystrophy
  Macular dystrophy

Posterior corneal dystrophies
  Fuchs’ endothelial dystrophy
  Congenital hereditary endothelial dystrophy
  Posterior polymorphous dystrophy

Corneal degenerations
  Pterygium
  Pellucid marginal degeneration
  Band keratopathy
  Salzmann's degeneration
Keratoconus
Atopic keratoconjunctivitis
Ocular cicatricial pemphigoid
Corneal infiltrates
Corneal edema

History

A complete history is essential in evaluating patients with minimal visual loss. Family history, recent trauma, or any past ocular surgery can be important in determining the etiology of visual loss. Determining if patterns of visual loss exist can help in the evaluation of these patients. In addition, the ophthalmologist must determine if the visual loss is transient, permanent, or progressive. Table 25.1 outlines ocular symptoms with the corresponding corneal conditions.

Table 25.1 -- Symptoms that often correlate with corneal abnormalities

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Corneal abnormalities</th>
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</thead>
<tbody>
<tr>
<td>Haze and halos</td>
<td>Epithelial edema</td>
</tr>
<tr>
<td>Monocular diplopia</td>
<td>Irregular astigmatism</td>
</tr>
<tr>
<td>Decreased morning vision</td>
<td>Stromal edema</td>
</tr>
<tr>
<td>Glare</td>
<td>Corneal scars</td>
</tr>
<tr>
<td>Multiple changes of glasses</td>
<td>Keratoconus</td>
</tr>
<tr>
<td>Pain</td>
<td>Epithelial defect</td>
</tr>
<tr>
<td>Family history of decreased vision</td>
<td>Corneal dystrophies</td>
</tr>
<tr>
<td>Awakening with foreign body sensation</td>
<td>Recurrent erosion</td>
</tr>
</tbody>
</table>
Examination Techniques

Visual acuity
When examining patients with minimal visual loss, the ophthalmologist must determine the best corrected visual acuity. One of the most common causes of minimal visual loss is an unrecognized refractive error. The standard method of determining best-corrected visual acuity utilizes a Snellen chart in a dimly lit room and asking the patient to read the letters without pausing.

- Snellen visual acuity measures the patient's ability to resolve fine spatial details on a high-contrast target.[1],[2]
- Best-corrected visual acuity only reveals limited information about visual function.

Pinhole aperture
- Increases depth of focus and corrects small amounts of refractive error.
- Reduces irregular astigmatism and light scattering in the cornea.
- Further improvement with pinhole aperture after best refraction suggests irregular astigmatism.

Contrast sensitivity function (CSF)
- Contrast sensitivity is a very important aspect of visual function.
- It is measured with a contrast sensitivity chart such as the CSV-1000E by Vector Vision.
- The method of evaluating visual function is based on sinusoidal patterns of varying spatial frequencies.[3]
- CSF provides a more complete assessment of visual function than Snellen visual acuity.[1]
- CSF can show abnormalities prior to any changes in Snellen visual acuity in patients with corneal edema and early keratoconus.[4],[5]

Diagnostic rigid contact lens refraction
- Useful technique in evaluating patients with suspected irregular astigmatism.
- Corrects irregular stigmatism and indicates amount of decrease in vision attributed to this mechanism.

Technique
- Topical anesthesia is placed in eye.
- Contact is fit on the average keratology reading.
- A large-diameter RGP lens is chosen, within 3 diopters of patient's refraction.
- Over-refraction is performed and compared to the patient's best-corrected spectacle correction.
- If improvement is noted with rigid contact lens, irregular astigmatism is inferred.

Potential acuity meter
- The potential acuity meter (PAM) has some use in predicting potential acuity with small corneal scars.
- Small corneal scars can result in scattering of the PAM beam and underestimating the potential visual acuity.
- Although sometimes helpful in evaluating patients with minimal visual loss, the PAM can produce misleading results.[6]

Retinoscopy
- Useful in identifying stromal opacities and irregular astigmatism.[7]
- Careful attention to the quality of the reflex can often demonstrate subtle corneal abnormalities.
- With experience, estimating the degree that the cornea affects vision is possible.

Slit lamp examination
- A complete slit lamp examination is essential in examining patients with minimal visual loss.
- A systematic slit lamp examination of the cornea with low and high magnification, and with various types of illumination, should be performed.

Fluorescein evaluation
- Fluorescein dye forms a thin film over the cornea, visible with a cobalt blue light.
- Fluorescein dye is very useful in evaluating the smoothness of the epithelial surface.
- Unevenness in the distribution of the fluorescein dye is suggestive of irregular astigmatism (Fig. 25.1).
Fig. 25.1 Epithelial surface irregularity demonstrated with topical fluorescein dye in a patient with a recurrent erosion.

**Keratometry**

- A keratometer is a useful instrument for measuring the curvature of the central region of the cornea.
- Additional information is obtained from the quality of the reflected mires.
- If the keratometry mires are irregular, irregular astigmatism is present (Fig. 25.2).
Computerized corneal topography

Computerized corneal topography is useful in evaluating patients with minimal visual loss. There are two basic systems currently available: elevation-based topography systems and Placido-based videokeratoscopes. The elevation-based topography systems include the Pentacam, which utilizes Scheimpflug images to determine anterior and posterior corneal topography, corneal pachymetry, and Zernike analysis, which describes wavefronts.[8] The Orbscan employs stereo-triangulation to measure the anterior and posterior corneal surface as well as pachymetry. The Placido-based systems work by projecting a Placido disk onto the cornea. The image is photographed and digitized, resulting in curvature maps of the anterior corneal surface. Topography can be used to rule out certain corneal abnormalities which contribute to minimal visual loss. Early keratoconus, pellucid marginal degeneration, or postrefractive ectasia can be extremely difficult to diagnosis without accurate topography. In addition, most of the topography systems can measure corneal irregularity and help confirm etiology of minimal visual loss. The Pentacam has multiple indices to determine the irregularity of the cornea, including Q-Val (asphericity quotient) and ISV (index of surface variance). The TMS™, made by Tomey, calculates the surface asymmetry index (SAI) and the surface regularity index (SRI) with each topographic evaluation. These measures calculate the degree of asymmetry and the amount of local irregularity in the cornea. The SRI can be useful in predicting optical performance of the anterior corneal surface and has been shown to provide a good correlation with best-spectacle-corrected visual acuity.[9] The Oculus Pentacam topography system utilizes Scheimpflug images to determine information about the cornea (Fig. 25.3). One program, Belin/Ambrosio Enhanced Ectasia, is helpful diagnosing keratoconus and other ectatic corneal abnormalities by looking at front and back elevation and correlating them with corneal thickness maps. Corneal topography can be useful in predicting the best-corrected visual acuity in patients. With the Placido-based videokeratoscopes such as the Humphrey Topography System, high values of corneal irregularity measurement (CIM) suggest significant irregular astigmatism and correlate with visual loss. Computerized corneal topography is one of the most sensitive methods of diagnosing early keratoconus, postrefractive ectasia, pellucid marginal degeneration, and contact lens-induced corneal warpage, even in the absence of any slit lamp findings.[10]
Wavefront analysis

Wavefront analyzers are able to measure lower-order errors (sphere, astigmatism, and axis), as well as higher-order aberrations or irregular astigmatism. Wavefront analysis, a relatively new technology, allows for measurement of wavefront errors.[11] There are several methods to measure aberrations of the eye. The most common is the Hartmann-Shack wavefront sensor. In the ideal eye, parallel rays of light are refracted by the eye and form wavefronts that focus exactly on the retina. Aberrations result in wavefronts that do not focus perfectly on the retina. The Hartmann-Shack sensor detects the wavefront leaving the eye though a lenslet array on a charge-coupled device (CCD) camera. By calculating the displacement of the images on the CCD camera, the wavefront error is calculated. Irregular astigmatism or higher-order aberrations can be determined quantitatively as a set of coefficients of the Zernike polynomials.[12] Zernike coefficients of the higher-order aberrations can be derived from corneal topography. In a normal ametropic eye, defocus (myopia or hyperopia) is the largest aberration followed by astigmatism. The higher-order aberrations are typically small, comprising only about 10–20% of the aberrations of the eye. However, in eyes with significant irregular astigmatism, these higher-order aberrations comprise a much larger percentage of the total aberrations of the eye. Wavefront analysis is an additional tool that can be very powerful in determining the etiology of minimal visual loss.[13] Although wavefront analysis is a relatively new technology, with future developments it may become more common in evaluating patients.
Common causes of punctate epithelial keratitis

Selected Conditions Causing Minimal Visual Loss

**Dry eyes**

- Dry eyes are a frequent cause of minimal visual loss.
- The surface dryness results in irregular astigmatism, increased wavefront aberation, and subsequent decreased vision.[14],[15]
- Dry eyes often results in a decreased tear film thickness and stability, which can exacerbate any irregularities of the underlying cornea.

**Ectatic disorders**

**Keratoconus**

Keratoconus is one of the more common causes of 'unexplained' minimal visual loss that the ophthalmologist encounters. Keratoconus is a noninflammatory corneal thinning disorder characterized by thinning of the central cornea and anterior protrusion of a conical cornea, usually inferiorly.[16],[17] This abnormal topography results in irregular corneal astigmatism. In addition, keratoconus results in large amounts of high-order aberrations. Typically, the patient is a young adult with minimal visual loss who has often seen several different medical specialists. The patient has often had frequent spectacle changes, and may be convinced that a serious medical condition is present.

- Symptoms include blurry vision, photophobia, glare, and ocular irritation.
- Findings include prominent corneal nerves, Fleischer ring, stromal thinning, corneal stress lines, and apical scarring.
- Myopic astigmatism is usually present and, with retinoscopy, a scissoring reflex suggestive of irregular astigmatism is commonly seen.[18]

With modern topography, early keratoconus is relatively easy to diagnose.[19–21] Several automated detection programs are currently in use to detect and quantify keratoconus.[21–24] Wavefront analysis in patients with keratoconus has revealed an increase in higher-order aberrations.[12]
Ectasia following refractive surgery

- Corneal ectasia is another cause of minimal visual loss in patients who have had refractive surgery.
- Corneal ectasia is shown by topography: progressive corneal thinning with protrusion and irregular astigmatism.[25]
- Most of these cases probably had unidentified forme fruste keratoconus prior to their refractive surgery.

Pellucid marginal degeneration

- Uncommon cause of minimal visual loss seen in patients 20–40 years of age.
- Keratometry exhibits marked against-the-rule astigmatism.
- Slit lamp examination reveals inferior thinning between 4 and 8 o’clock.[16]
- Corneal topography usually shows a characteristic pattern of marked flattening of the central cornea with inferior peripheral steeping.
- A diagnostic rigid contact lens refraction produces an excellent optical correction and is useful in demonstrating the cause of the decreased vision.

Terrien's marginal degeneration

- A rare cause of minimal visual loss.
- Slit lamp examination reveals peripheral corneal thinning with opacification and superficial vascularization, often superiorly.
- Progression leads to against-the-rule astigmatism and irregular astigmatism.[26]

Punctate epithelial keratitis

Multiple conditions cause punctate epithelial keratitis (PEK) and minimal visual loss. Box 25.3 summarizes common causes of punctate epithelial keratitis.

- PEK results in minimal visual loss secondary to irregular astigmatism.
- Slit lamp examination with fluorescein dye helps confirm the diagnosis.
- Retinoscopy, keratometry, corneal topography, and the use of rigid contact lens refraction are useful in documenting irregular astigmatism as the cause of visual loss.
- Placing a single lubricating drop on the cornea often improves vision in patients with PEK.

Box 25.3

<table>
<thead>
<tr>
<th>Common causes of punctate epithelial keratitis</th>
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<tbody>
<tr>
<td>Infectious keratitis</td>
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<tr>
<td>Viral keratitis</td>
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<tr>
<td>Herpes simplex keratitis</td>
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<tr>
<td>Epidemic</td>
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<tr>
<td>keratoconjunctivitis</td>
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<tr>
<td>Bacterial keratitis</td>
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</tbody>
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Chlamydia
Blepharitis
Neurotrophic keratitis
Dry eye condition
  Exposure
  Keratoconjunctivitis
  sicca

Xerosis
Toxic keratitis
Chemical injury
Allergic reaction
Trauma
Trichiasis
Entropion
Superior limbic keratoconjunctivitis
Thygeson’s superficial punctate keratopathy

**Corneal scars**

- Mild corneal scars can result in minimal visual loss.
- Corneal scars can cause decreased vision due to irregular astigmatism, light scattering, and decreased transparency of the cornea.
- Careful slit lamp examination, retinoscopy, corneal topography, and rigid contact lens refraction are all useful in confirming the cause of the decreased vision.

**Corneal dystrophies**

**Anterior corneal dystrophies**

Map-dot-fingerprint dystrophy is a common bilateral epithelial corneal dystrophy. Many patients are asymptomatic. However, minimal visual loss secondary to epithelial irregularity is common. Recurrent erosions can further contribute to irregular astigmatism in these patients.

- Slit lamp examination with a broad oblique beam demonstrates maplike opacities.
- Retroillumination can highlight dots and fingerprints.
- Fluorescein dye can be used to delineate microcysts and areas of elevated epithelium.
- Keratometry is useful in demonstrating irregular mires (see Fig. 25.2).
- Corneal topography can demonstrate areas of irregularity that correspond to the location of the corneal dystrophy.
- Rigid contact lens refraction confirms the visual loss is secondary to irregular astigmatism.

**Meesmann’s dystrophy**
• Meesmann's dystrophy is another anterior corneal dystrophy that can present with minimal visual loss.
• Symptoms include tearing, photophobia, and irritation.
• Slit lamp examination with retroillumination reveals multiple tiny, regular, clear intraepithelial cysts diffusely spread across the entire cornea.[27]
• Tiny vesicles contribute to irregular epithelium, resulting in visual loss.
• Rigid contact lens refraction can be helpful in identifying the amount of irregular astigmatism.

Reis-Bücklers' dystrophy

• Often presents early in life with recurrent erosions.
• Slit lamp examination reveals diffuse reticular opacification in Bowman's membrane.[28]
• With recurrent episodes, subepithelial scarring occurs, which results in irregular astigmatism, decreased corneal transparency, and light scattering.
• Retinoscopy, slit lamp examination, and keratometry are useful in estimating degrees of corneal opacification and irregular astigmatism.

Stromal corneal dystrophies

Stromal corneal dystrophies are relatively uncommon. They can result in visual loss secondary to stromal opacities, causing decreased corneal transparency. If the opacities extend anteriorly, epithelial erosions and irregular astigmatism may occur.

Granular dystrophy

• Generally does not cause a decrease in vision until middle age.
• Slit lamp examination reveals discrete, focal, white granular deposits occurring in the axial portion of the corneal (Fig. 25.4).[29]
• Retinoscopy will overestimate the degree of decreased vision.
Fig. 25.4 Slit lamp photography using broad oblique illumination and indirect illumination in a patient with granular dystrophy and minimal visual loss.

**Lattice dystrophy**

- Presents with decreased vision by late adolescence.
- Slit lamp examination reveals refractile lines in a lattice pattern over the central cornea, best seen by retroillumination. [29]
- Recurrent erosions result in subepithelial scarring and irregular astigmatism.
**Macular dystrophy**

- Macular dystrophy affects visual acuity much earlier than other stromal dystrophies.
- Slit lamp findings include diffuse central stromal clouding with a ground-glass appearance best visualized with diffuse illumination.[29]
- Frequent recurrent erosions result in episodes of pain, photophobia, and visual loss.
- The visual loss is secondary to irregular astigmatism and corneal opacities.

**Endothelial disorders**

Posterior corneal disorders are common causes of minimal visual loss. The mechanism of decreased visual acuity includes corneal edema, which reduces corneal transparency and increases light scattering. Visual loss depends on the severity of the disease.

**Fuchs’ endothelial dystrophy**

- Bilateral, slowly progressive endothelial dysfunction.
- With slit lamp examination and specular reflection, the endothelial cells are noted to be larger and polymorphic with multiple guttae.[30]
- Early disease is characterized by minimal edema, resulting in minimal visual loss.
- The visual loss is often worse in the morning and improves during the day due to surface evaporation and stromal deturgescence.
- Pachymetry can be useful in detecting early corneal edema.
- Specular microscopy can demonstrate a decreased number of endothelial cells and guttae.
- With progression, epithelial edema results, with marked decrease in vision.

**Posterior polymorphous dystrophy (PPMD)**

- Bilateral dominant inherited corneal disorder.
- Slit lamp examination reveals scallop-edged endothelial bands or vesicles in Descemet's membrane, best visualized by indirect lateral illumination.[31]
- Progressive stromal and later epithelial edema develop.
- Visual loss worse on awakening and improves during the day.

**Iridocorneal endothelial syndrome**

- Unilateral condition occurs more commonly in women than men.
- Patients are usually between the ages of 30 and 50.
- Slit lamp findings include corneal endothelial abnormalities, peripheral anterior synechiae, iris atrophy, and iris nodules.[32]
- Visual loss occurs because of progressive corneal edema.

**Corneal degenerations**

Corneal degenerations can present with minimal visual loss. The mechanisms include
decreased corneal transparency due to central corneal opacities and irregular astigmatism. Historical data and careful examination can usually determine when the visual loss is the result of corneal pathology.

**Pterygium**

- A pterygium can result in minimal visual loss secondary to irregular astigmatism or by growing across the visual axis with decreased corneal transparency.
- The amount of irregular astigmatism can be determined using retinoscopy, keratometry, or corneal topography analysis.
- With-the-rule astigmatism is common, while corneal topography may reveal localized flattening central to the pterygium apex.

**Corneal warpage**

Contact lens wear can induce changes in the shape of the cornea that result in irregular astigmatism.[33],[34] Hard contacts are the most common type; however, both rigid gas permeable contacts lenses and soft contact lenses can induce corneal warpage.
- Typically, visual acuity is normal with contacts but decreased with spectacles.
- Keratometry reveals distortion of mires.
- Corneal topography is a very sensitive aid for the diagnosis of corneal warpage. A typical pattern of central irregular astigmatism, loss of radial symmetry, and loss of the normal pattern of peripheral corneal flattening is often seen.[35]

**Keratorefractive surgery**

One of the more common causes of minimal visual loss is seen in patients following keratorefractive surgery. Many times, a careful refraction can detect a refractive error that was not previously documented as the cause of the visual loss. A slit lamp evaluation should be performed noting any unusual corneal haze, scarring, or epithelial ingrowth.[36] Irregular astigmatism is one of the most serious and frequent complications of keratorefractive surgery.[37],[38] Corneal topography and wavefront analysis can aid in the diagnosis of the irregular astigmatism and variance of the corneal power in the optical zone following keratorefractive surgery.[38] Corneal topography may provide important information on the optical quality of the treated zone, the stability of the procedure over time, and the accuracy of the centration of the optical zone over the pupil.[39],[40] Contrast sensitivity function has been shown to be depressed following LASIK.[41] A decrease in contrast sensitivity function after refractive surgery can often explain why the patient's perceived vision is worse than the measured Snellen acuity. A rigid contact lens refraction can aid in the etiology of decreased vision in keratoreactive patients, although some patients have central corneas that are so flat that an inadequate contact lens fitting can result in misleading information.
References


Patients commonly present with ocular symptoms of itching and burning. Due to their typical self-limiting, non-sight-threatening origin, it is tempting to dismiss these symptoms until the patient has either failed conventional therapy or the condition becomes chronic. Occasionally, more advanced ophthalmic or systemic diseases are hidden in these presenting ocular symptoms. Finding and treating the source of burning and itching is essential to providing excellent care to the patient.

The intent of this chapter is to provide an overview of the clinical approach to a patient referred with burning or itching eyes. Many different causes of burning and itching are listed in Table 26.1. Burning and itching can cohabitate, thereby making it difficult to discern which is the primary symptom associated with the initial cause. Isolating the precipitating factor in a patient with burning and itching eyes starts with listening to the patient's primary symptom.

Table 26.1 -- Differential diagnosis of possible causes of dry eyes

<table>
<thead>
<tr>
<th>Dry eye syndrome</th>
<th></th>
</tr>
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<tbody>
<tr>
<td><strong>1</strong> Aqueous tear deficiency</td>
<td></td>
</tr>
<tr>
<td>a Sjögren's syndrome</td>
<td></td>
</tr>
<tr>
<td>i Primary Sjögren's syndrome</td>
<td></td>
</tr>
<tr>
<td>ii Secondary Sjögren's syndrome</td>
<td></td>
</tr>
<tr>
<td>1 Associated with collagen vascular diseases like rheumatoid arthritis, systemic lupus erythematosus, polyarteritis nodosa, scleroderma or Wegener’s granulomatosis</td>
<td></td>
</tr>
<tr>
<td>b Non-Sjögren's aqueous tear deficiency</td>
<td></td>
</tr>
<tr>
<td>i Congenital</td>
<td></td>
</tr>
<tr>
<td>1 Aplasia or hypoplasia of the lacrimal gland</td>
<td></td>
</tr>
<tr>
<td>2 Anhidrotic ectodermal dysplasia</td>
<td></td>
</tr>
<tr>
<td>3 Aplasia of the lacrimal nerve nucleus</td>
<td></td>
</tr>
<tr>
<td>4 Congenital familial sensory neuropathy with anhidrosis</td>
<td></td>
</tr>
<tr>
<td>5 Cystic fibrosis</td>
<td></td>
</tr>
</tbody>
</table>
6 Familial autonomic dysfunction (Riley-Day syndrome)
7 Holmes-Adie syndrome
8 Multiple endocrine neoplasia

ii Acquired
1 Senile or idiopathic atrophy of lacrimal gland
2 Associated with systemic illness
   a Hematopoietic disorders
   b HIV/AIDS
   c Graft-versus-host disease
   d Malignant lymphoma
   e Lymphosarcoma
   f Thrombocytopenic purpura
   g Lymphoid leukemia
   h Hemolytic anemia
   i Hypergammaglobulinemia
   j Waldenström's macroglobulinemia
   k Chronic hepatitis
   l Primary biliary cirrhosis
   m Felty’s syndrome

3 Endocrine dysfunction
   a Hashimoto’s disease
   b Menopause

4 Renal disorders
   a Renal tubular acidosis
   b Diabetes insipidus

iii Obstructive (cicatricial conjunctivitis)
1 Stevens–Johnson syndrome
2 Epidermolysis bullosa
3 Chemical/radiation burns
4 Ocular cicatricial pemphigoid
5 Trachoma
6 Acne rosacea
7 Chlamydia
8 Diphtheritic keratoconjunctivitis
9 Congenital syphilis
10 Dermatitis herpetiformis
11 Epidemic keratoconjunctivitis
12 Syphilis (congenital and acquired)
13 Exfoliative dermatitis
14 Impetigo
15 Scleroderma
16 Vaccinia
17 Reiter’s syndrome
18  Linear IgA
19  Medications
20  Infiltrative lesions of lacrimal gland
   a  Sarcoidosis
   b  Lymphoma
   c  Hemochromatosis
   d  Amyloidosis

21  Neuroparalytic
   a  Cranial nerve VII and geniculate ganglion
   b  Greater superficial petrosal nerve
   c  Sphenopalatine ganglion and lacrimal branch
   d  Cranial nerve V and gasserian ganglion

22  Nutritional/debilitating disorders
   a  Typhus
   b  Cholera
   c  Starvation
   d  Ascorbic acid and vitamin B12 deficiency

23  Postsurgical: partial or total dacryoadenectomies
24  Medications
   a  Antimuscarinics
   b  Antihistamines
   c  Beta blockers
   d  Phenothiazines
   e  Psychotropics

2  Increased evaporative loss
   a  Exposure
      i  Facial nerve (Bell's) palsy
      ii  Ectropion
      iii  Decreased blink
         1  Altered mental status
         2  Progressive supranuclear palsy
      iv  Proptosis (thyroid eye disease)
      v  Postsurgical (blepharoplasty)
      vi  Arid, warmer climates
      vii  Ptosis
   b  Mucin tear abnormalities
      i  Vitamin A deficiency
         1  Malnutrition
         2  Digestive tract disorders
The Primary Symptom

It is commonly understood that burning is associated with a dry eye condition,[1] whereas itching is linked to allergies.[2] Although these generalizations are not mutually exclusive, they can help direct a concentrated dialogue toward a particular etiology.

Onset

Establishing an onset of symptoms can help identify a potential cause. The introduction of allergens (such as soap, shampoo, perfume, pets, contact lens solution) or a change in environment (employment, housing, or seasons) can precipitate itching and burning. It is important to establish the frequency, duration, and daily occurrence of the primary symptom when investigating a possible cause. Dry eyes upon waking are typically associated with nocturnal exposure or immune-related lacrimal dysfunction, whereas patients with evening symptoms are more likely to have meibomian gland dysfunction.[3] Ocular burning that occurs only while staring at a computer at work[4] on a desk situated below a heating vent is a common clinical scenario with multiple avenues to aggravate a dry eye.[5] Identifying exacerbating or alleviating factors is also helpful in finding a cause for the primary symptom. For example, dry eyes improve with rainy, humid weather and deteriorate in arid, windy conditions.[6] Listening to patients not only is an effective mechanism for identifying aggravating stimuli but also increases patient awareness for future prevention.

Past medical history
Systemic prescription medications (antidepressants, diuretics, contraceptive pills) and over-the-counter medications (nasal decongestants and antihistamines) can exacerbate aqueous deficiency. Hormone levels in postmenopausal women, men treated for prostate conditions, or women taking estrogen replacement therapy can play a role in chronic dry eye conditions. Previous systemic illness can exacerbate tear production and affect the ocular surface, as is commonly seen in patients following bone marrow transplantation, external beam radiotherapy to the head or neck, or as a sequelae of chronic cicatrising conjunctivits like Stevens–Johnson syndrome. Active medical diseases such as rheumatoid arthritis can cause lacrimal gland dysfunction producing a dry eye (Sjögren's) syndrome.

A review of positive or negative responses to previous ocular therapies is also valuable information which will assist in diagnosing the cause of the primary symptom. For instance, knowing that a patient's symptom briefly responds to supplemental tear therapy allows the provider to focus additional therapies such as increasing tear volume, retention, adherence, and composition. On the contrary, should symptoms not be relieved at all with previous therapy, one should reconsider the initial diagnosis.

**Contact lens wear**

Whereas new technologies in contact lens manufacturing have improved water content and permeability, contact lenses still significantly affect the ocular surface. The amount of contact lens wear, in addition to hygiene and cleaning solutions, are important historical factors which can contribute to possible causes of burning or itching eyes.

**Review of systems**

Review of systems is an important reminder that many systemic conditions can affect the ocular surface. Rheumatology, dermatology, endocrinology, infectious diseases, and oncology patient can all have conditions which affect the ocular surface and contribute to the primary symptom. Advanced age is also a risk factor for dry eyes, as natural hormonal changes and advancing lacrimal dysfunction can affect tear production.
The Examination

External examination

When examining a patient with burning or itching eyes, it is essential to start by stepping away from the slit lamp microscope. Dermatologic nasal telangiectasias, rhinophyma and inflamed posterior lamellae are diagnostic of acne rosacea. The presence of Dennie–Morgan folds or ‘allergic shiners’ is a classic sign of atopic disease. Proptosis, inferior scleral show, increased palpebral aperture or inflamed muscle insertions are important signs for thyroid eye disease. Masked facies, shuffled gait, and decreased blink rate are seen in patients with Parkinson’s syndrome. Many of these conditions can be observed as the patient walks into the examination room.

Lids and lashes

The upper and lower eyelids should be examined for malposition (entropion, ectropion, or ptosis) which could cause exposure keratopathy, tear irregularity or cornea irritation. The unconscious blink effort and rate should be observed at the slit lamp to assess for complete closure and adequate Bell’s phenomenon. Chronic exposure and irritation seen in floppy eyelid syndrome can be diagnosed via upper lid double eversion with simple lid elevation. Examining the meibomian glands for inspissation, foamy accumulations, or expression of waxy sebum can help target therapies to improve the lipid tear layer. Inflammation of the posterior lamella can be visualized in dermatologic diseases such as rosacea and localized infections or chalazions. Examination for eyelash misdirection and infestation can be a source of burning or itching. Performing epilation of lashes with cylindrical dandruff has a high incidence of demonstrating Demodex mite infestation (Fig. 26.1) when examined under a compound microscope.

Fig. 26.1 (A) Slit lamp photograph of upper eyelid with cylindrical dandruff surrounding lashes. (B) Compound microscope demonstrates multiple Demodex folliculorum surrounding...
The Ocular Surface

Conjunctiva

Slit lamp examination of the type of conjunctival reaction (papillary or follicular) can assist in developing a differential diagnosis (see Table 26.2). Patients with follicular conjunctivitis, numerous nits (eggs), and active lice infestation of the lashes present with extreme itching (Fig. 26.2). The presence of giant papillary conjunctivitis (GPC) is typically associated with allergic or irritant causes.[22] Seasonally associated Horner-Trantas dots, shield ulcers, and perilimbal pigmentation in the setting of GPC are suggestive of vernal keratoconjunctivitis. Examining the conjunctival fornix for the presence of inflammation, foreshortening, symblepharon, or membranes can reveal systemic immune-mediated cicatrizing illnesses such as ocular cicatricial pemphigoid, linear IgA deficiency, or Stevens–Johnson syndrome. Varying stages of bulbar conjunctival hyperemia can be associated with systemic graft-versus-host disease in bone marrow transplant patients. Common conjunctival staining (rose Bengal, fluorescein, or lissamine green) patterns can be helpful when searching for a cause of burning or itching. Nasal conjunctival vital dye staining can suggest a mucus fishing behavior. Alternatively, an edematous superior bulbar conjunctiva stains positive with lissamine green, indicating a loss of goblet cells typically associated with superior limbic keratoconjunctivitis. Staining the rugae of redundant conjunctival tissue seen in conjunctivochalasis is an often overlooked diagnosis commonly presenting with symptoms similar to dry eye syndrome. On the contrary, conjunctivochalasis can also obstruct the punctal opening, thereby causing epiphora.[23] Patients with vitamin A deficiency from malabsorption syndromes or specific dietary restriction can present with burning and areas of conjunctival keratinization (Bitot spot), xerosis, and nyctalopia.[24]

Table 26.2 -- Differential diagnosis of follicular and papillary conjunctivitis

<table>
<thead>
<tr>
<th>A</th>
<th>Acute follicular conjunctivitis</th>
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<tbody>
<tr>
<td>1</td>
<td>Inclusion conjunctivitis</td>
</tr>
<tr>
<td>2</td>
<td>Adenoviral conjunctivitis</td>
</tr>
<tr>
<td>a</td>
<td>Pharyngoconjunctival fever</td>
</tr>
<tr>
<td>b</td>
<td>Epidemic keratoconjunctivitis</td>
</tr>
<tr>
<td>3</td>
<td>Newcastle disease</td>
</tr>
<tr>
<td>4</td>
<td>Influenza</td>
</tr>
<tr>
<td>5</td>
<td>Herpes zoster</td>
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<tr>
<td>6</td>
<td>Herpes simplex</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>B</th>
<th>Chronic follicular conjunctivitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Medications/toxins</td>
</tr>
<tr>
<td>2</td>
<td>Inclusion conjunctivitis</td>
</tr>
<tr>
<td>3</td>
<td>Moraxella</td>
</tr>
<tr>
<td>4</td>
<td>Folliculosis</td>
</tr>
<tr>
<td>5</td>
<td>Molluscum contagiosum</td>
</tr>
<tr>
<td>6</td>
<td>Lyme disease</td>
</tr>
<tr>
<td>7</td>
<td>Pediculosis (lice)</td>
</tr>
<tr>
<td>8</td>
<td>Cat-scratch fever (Parinaud's oculoglandular syndrome)</td>
</tr>
<tr>
<td>9</td>
<td>Trachoma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>Papillary conjunctivitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacterial conjunctivitis</td>
</tr>
<tr>
<td>2</td>
<td>Allergic conjunctivitis</td>
</tr>
<tr>
<td>a</td>
<td>Atopic keratoconjunctivitis (GPC)</td>
</tr>
<tr>
<td>b</td>
<td>Vernal keratoconjunctivitis (GPC)</td>
</tr>
<tr>
<td>3</td>
<td>Daerocystitis</td>
</tr>
<tr>
<td>4</td>
<td>Caniculitis</td>
</tr>
<tr>
<td>5</td>
<td>Superior limbic conjunctivitis</td>
</tr>
<tr>
<td>6</td>
<td>Floppy eyelid syndrome (GPC)</td>
</tr>
<tr>
<td>7</td>
<td>Foreign body/toxin (contact lens) (GPC)</td>
</tr>
<tr>
<td>8</td>
<td>Mucous fishing syndrome</td>
</tr>
<tr>
<td>9</td>
<td>Keratoconjunctivitis sicca</td>
</tr>
<tr>
<td>10</td>
<td>Blepharoconjunctivitis</td>
</tr>
</tbody>
</table>
GPC: Giant papillary conjunctivitis.

**Fig. 26.2** (A) Everted upper eyelid demonstrating severe follicular conjunctivitis. (B) Note eggs (nits) on lashes and louse with human blood filling its digestive system.

**Tear film analysis**

Investigating the tear film is important when examining a patient with burning or itching eyes. Evaluating the tear meniscus, grading the ocular surface fluorescein stain, establishing a tear film break-up time, and performing a Schirmer’s test are all important as clinical investigations in dry eye patients.[25]

**Cornea**

Slit lamp examination of the ocular surface is pivotal in the work-up of a patient with burning and itching eyes. Extended contact lens wearers may present with limbal neovascularization or central/peripheral cornea edema.[26] Exposure to chronic preservative-based contact lens cleaning solutions and chemical injuries can present with a whirl keratopathy suggestive of limbal stem cell deficiency. Fluorescein staining of discrete white superficial Thygeson granules typically seen in young boys without conjunctival injection is another cause of extreme photophobia and burning. The presence of filaments, dry patches, and punctate erosions indicates keratoconjunctivitis sicca syndrome. Conjunctival or cornea lesions such as pterygia, intraepithelial neoplasia, or squamous cell carcinomas can also cause tear film instability and burning.[27]

While there are many possible causes for burning and itching eyes, the purpose of this chapter is to help clinicians review their approach to these patients. The most important initial step is to listen to each patient’s primary symptom and historical events up to
presentation. After taking a detailed patient history and reviewing symptoms, narrowing a differential diagnosis can be achieved by performing a focused external and anterior slit lamp examination.
References


14. Nichols JJ, Sinnott LT: Tear film, contact lens and patient-related factors associated with


