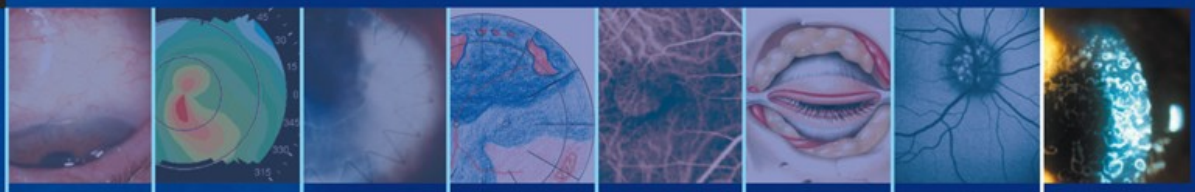
ESSENTIALS IN OPHTHALMOLOGY

G. K. KRIEGLSTEIN · R. N. WEINREB
Series Editors



Glaucoma | Cataract and Refractive Surgery | Uveitis and Immunological Disorders | Vitreo-retinal Surgery | Medical Retina | Oculoplastics and Orbit | Pediatric Ophthalmology, Neuro-Ophthalmology, Genetics | **Cornea and External Eye Disease**

Cornea and External Eye Disease

Edited by
T. REINHARD
F. LARKIN

Springer

Essentials in Ophthalmology

Cornea and External Eye Disease

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Cornea and External Eye Disease

Editors Thomas Reinhard
Frank Larkin

Cornea and External Eye Disease

With 103 Figures, Mostly in Colour
and 14 Tables

Springer

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Foreword

The series *Essentials in Ophthalmology* was initiated two years ago to expedite the timely transfer of new information in vision science and evidence-based medicine into clinical practice. We thought that this prospicient idea would be moved and guided by a resolute commitment to excellence. It is reasonable to now update our readers with what has been achieved.

The immediate goal was to transfer information through a high quality quarterly publication in which ophthalmology would be represented by eight subspecialties. In this regard, each issue has had a subspecialty theme and has been overseen by two internationally recognized volume editors, who in turn have invited a bevy of experts to discuss clinically relevant and appropriate topics. Summaries of clinically relevant information have been provided throughout each chapter.

Each subspecialty area now has been covered once, and the response to the first eight volumes in the series has been enthusiastically positive. With the start of the second cycle of subspecialty coverage, the dissemination of practical information will be continued as we learn more about the emerging advances in various ophthalmic subspecialties that can be applied to obtain the best possible care of our patients. Moreover, we will continue to highlight clinically relevant information and maintain our commitment to excellence.

G. K. Krieglstein
R. N. Weinreb
Series Editors

Preface

The second volume covers a broad range of conjunctival and corneal diseases, again with particular emphasis being placed on problem management.

Various new surgical approaches are currently being evaluated in the clinical setting, an example of which is posterior lamellar keratoplasty in Fuchs endothelial disease. While amniotic membrane transplantation has been in use for some years and for a range of indications, it is now becoming more and more popular for the treatment of ulceration in infectious keratitis. Tissue-engineered scaffolds as templates for corneal reconstruction are being investigated for possible future surgical approaches. Phototherapeutic keratectomy has been established for some years in the therapeutic repertoire for various phenotypes of corneal dystrophy: this intervention is now safe and effective in many patients with superficial dystrophic corneal opacities or recurrent erosion.

Molecular genetic evidence of corneal dystrophies is fascinating and has led to a completely new classification.

The chapter on corneal preservation shows the challenge for tissue banking behind the new surgical approaches. Inflammatory diseases of the cornea and conjunctiva remain a continuing challenge in every external eye disease clinic, described in the chapters on herpes simplex keratitis, ocular pemphigoid, adult inclusion conjunctivitis, and chronic blepharitis. Understanding of the biology of conjunctival melanoma is improving and confocal microscopy may become established as a new diagnostic aid and follow-up technique.

We hope you enjoy reading this book.

Thomas Reinhard
Frank Larkin

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Chapter 1

Fuchs Endothelial Dystrophy: Pathogenesis and Management

1

Leejee H. Suh, M. Vaughn Emerson, Albert S. Jun

Core Messages

- Fuchs endothelial dystrophy (FED) is a progressive disorder of the corneal endothelium with accumulation of focal excrescences called guttae and thickening of Descemet's membrane, leading to stromal edema and loss of vision
- The inheritance of FED is autosomal dominant, with modifiers such as increased prevalence in the elderly and in females
- Corneal endothelial cells are the major "pump" cells of the cornea that allow for stromal clarity
- Descemet's membrane is grossly thickened in FED, with accumulation of abnormal wide-spaced collagen and numerous guttae
- Corneal endothelial cells in end-stage FED are reduced in number and appear attenuated, causing progressive stromal edema
- Symptoms include visual blurring predominantly in the morning with stromal and epithelial edema from relatively low tear film osmolality
- FED can be classified into four stages, from early signs of guttae formation to end-stage subepithelial scarring
- Diagnosis is made by biomicroscopic examination; other modalities, such as corneal pachymetry, confocal microscopy, and specular microscopy can be used in conjunction
- Exact pathogenesis is unknown, but possible factors include endothelial cell apoptosis, sex hormones, inflammation, and aqueous humor flow and composition
- Mutations in collagen VIII, a major component of Descemet's membrane secreted by endothelial cells, have been linked to FED
- Medical management includes topical hypertonic saline, the use of a hairdryer to dehydrate the precorneal tear film, and therapeutic soft contact lenses
- Definitive treatment is surgical in the form of penetrating keratoplasty (PK)
- New surgical modalities such as various forms of endothelial keratoplasty are gaining popularity in the treatment of FED
- DLEK and DSEK avoid the surgical complications of PK, such as wound dehiscence, suture breakage/infection and high postoperative astigmatism
- Future directions in the treatment of FED include gene or cell therapy and continued advances in endothelial keratoplasty

1

1.1 Introduction

Fuchs endothelial dystrophy (FED) is a primary, progressive disorder of the corneal endothelium that results in corneal edema and loss of vision. The initial stages of FED typically begin in the fifth through seventh decades of life and are characterized by progressive accumulation of focal excrescences, termed "guttae," and thickening of Descemet's membrane, a collagen-rich layer secreted by endothelial cells. Eventually, there is loss of endothelial cell density and functionality as the "pump" of the cornea, causing vision-threatening corneal edema. Although corneal guttae are not pathognomonic for FED, the development of stromal edema defines this disorder.

1.2 Historical Perspective

In 1902 Ernst Fuchs initially described the disorder that would later bear his name, and he postulated that this disease of the elderly was related to changes in the posterior cornea that allowed for increased fluid movement from the aqueous into the corneal stroma [11]. He later published a case series of 13 patients with FED in which he suggested pathologic involvement of both the endothelial and epithelial corneal layers [12]. After the introduction of the slit-lamp biomicroscope in 1911, Vogt was the first to report detailed biomicroscopic observations of FED and coined the term "cornea guttata," in reference to focal excrescences on the endothelial surface, which when confluent, resembled beaten bronze [58]. The natural progression of FED from isolated, asymptomatic guttae to the formation of corneal edema with painful loss of vision was first noted in 1953 [53]. These and other important observations led to the understanding of FED as a primary disease of the corneal endothelium with secondary involvement of the other layers of the cornea.

1.3 Epidemiology and Inheritance

The prevalence of FED is difficult to estimate given its later onset, slow progression, and lack of symptoms in the early stages. Furthermore, mild guttae can occur in normal individuals in such conditions as aging, ocular trauma, ocular inflammation, and glaucoma. In a large study of 2002 normal individuals, Lorenzetti et al. found scattered central guttae in 0.18% of eyes in those between the ages of 20 and 39, and in 3.9% of eyes in those above 40 years of age [33]. Despite the lack of an accurate estimate of the prevalence of FED, it remains one of the most common indications for corneal transplantation, accounting for up to 29% of cases [1].

Fuchs endothelial dystrophy can be either sporadic or hereditary. In hereditary cases, the inheritance of FED has been demonstrated to be autosomal dominant, with penetrance as high as 100% [10, 35]. In a large study of 228 relatives from 64 pedigrees with FED, Krachmer et al. observed that 38% of first-degree relatives over 40 years of age were affected, suggesting autosomal dominant inheritance with possible genetic or environmental modifiers [30]. Some studies, including Fuchs' original case series, also report an increased prevalence and severity in female patients [12, 30, 49]. This may reflect a possible recruitment bias or a physiologic effect of sex hormones on corneal endothelial cell function and survival [1, 62]. The incidence of FED has been reported to be similar among white and black patients, and much lower in Japanese individuals [17]. Central corneal guttae have been reported in Japanese individuals and significant vision loss is rare in these patients [29].

Summary for the Clinician

- Corneal guttae can be present in non-affected individuals and are associated with conditions such as aging, inflammation, trauma, and glaucoma
- Fuchs endothelial dystrophy is defined as the accumulation of corneal guttae with stromal edema
- Inheritance of FED is autosomal dominant, but sporadic forms can occur

1.4 Pathology

The corneal endothelium is a neural crest-derived cellular monolayer that utilizes an ATP-dependent pump to maintain physiologic stromal hydration necessary for corneal clarity [13, 61]. Corneal endothelial cells in humans do not normally proliferate in vivo [25, 26]. Corneal endothelial cells are normally lost throughout life at an estimated rate of 0.6% per year, although higher rates of cell loss occur in the settings of trauma (both surgical and nonsurgical) and primary endotheliopathies [3, 7]. Corneal endothelial cell loss is compensated for through flattening and enlargement of remaining cells without cell division in order to maintain a continuous monolayer [61].

The corneal endothelial cells in end-stage FED are reduced in number and appear thinned with attenuated nuclei, as seen by light microscopy (Fig. 1.1) [17]. With scanning electron microscopy, corneal endothelial cells show evidence of degeneration with large vacuoles and swollen organelles with disrupted membranes [17]. Corneal endothelial cells also demonstrate dilated sacs of endoplasmic reticulum filled with a finely granular material along with a marked increase in cytoplasmic filaments and ribosomes, suggesting transformation to a fibroblastic cell type [17, 20, 62].

Normal corneal endothelial cells produce Descemet's membrane, beginning in utero and continuing throughout postnatal life [34]. Histologically and ultrastructurally, Descemet's membrane consists of an anterior "banded" zone subjacent to the corneal stroma and containing 110 nm of banded collagen and a posterior "nonbanded" zone that lies anterior to the corneal endothelium [62]. At birth, the thickness of the anterior banded zone is approximately 3 μm, and this varies little throughout life [62]. In contrast, the thickness of the posterior nonbanded zone increases from approximately 3 μm at age 20 to 10 μm at age 80 [9], reflecting the ongoing synthesis and deposition of Descemet's membrane by the corneal endothelium [22].

Normal Descemet's membrane contains collagen IV, collagen VIII, fibronectin, entactin, laminin, and perlecan [31, 32]. The supramolecular structure of Descemet's membrane resembles stacks of hexagonal lattices arranged parallel to the surface of the membrane [52]. Monoclonal antibody analysis has shown the lattice array of Descemet's membrane to be composed of collagen VIII, a nonfibrillar short chain collagen [50, 52].

The abnormalities of Descemet's membrane are a striking feature of FED. Descemet's membrane is invariably thickened in FED up to 20 μm or greater [62]. Thickened Descemet's membrane also contains numerous focal excrescences (guttae) along its posterior surface (Fig. 1.2a).

Descemet's membrane also differs strikingly from normal on electron microscopy. In addition to a relatively normal anterior banded zone produced in fetal life, the posterior nonbanded zone of Descemet's membrane is attenuated or absent in FED and is replaced by a markedly thickened posterior collagenous layer with an average thickness of 16.6 μm (Fig. 1.3a) [7, 20]. The posterior collagenous layer is characterized by a diffuse, granular banding pattern, focal posterior guttae, and the accumulation of spindle-shaped bundles with 110-nm collagen banding, known as wide-spaced collagen (Fig. 1.3b) [7]. The composition of wide-spaced collagen in the posterior collagenous layer of FED corneas was shown by immunoelectron microscopy to be collagen VIII [31].

Summary for the Clinician

- Corneal endothelium is a monolayer of cells that acts as the major pump to deturgesce the cornea and ensure clarity
- There is a normal attrition rate of endothelial cells of 0.6% per year; the rate is accelerated in FED
- Normal endothelial cells produce Descemet's membrane, made up of an anterior banded zone and posterior nonbanded zone, the latter of which expands with age
- In FED, Descemet's membrane is abnormally thickened, with attenuation or absence of the posterior nonbanded zone and replacement with abnormal collagen, known as wide-spaced collagen

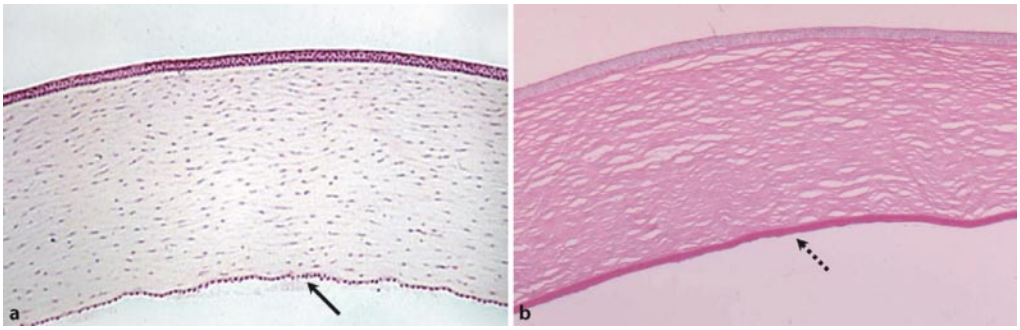

Fig. 1.1 **a** Light microscopy section of a normal human cornea. Note numerous endothelial cell nuclei lining the posterior surface (*arrow*). **b** Light microscopy section of FED cornea. Note the markedly thickened Descemet's membrane and the absence of endothelial cell nuclei on the posterior surface (*dashed arrow*). (Photos courtesy of W. Richard Green, M.D.)

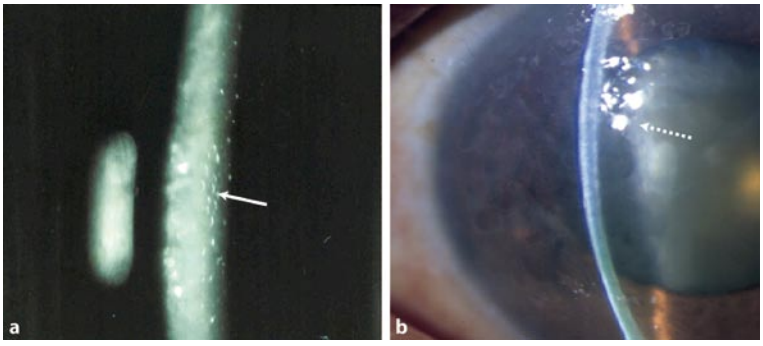

Fig. 1.2 **a** Slit-lamp biomicroscopy of stage I Fuchs endothelial dystrophy (FED; see Table 1.1). Note scattered, punctate, refractile endothelial guttae to the left of the *arrow*. **b** Stage III FED. Note thickening of the cornea, with the irregular surface and epithelial bullae indicated by scattered surface reflection (*dashed arrow*). (Photos courtesy of Walter J. Stark, M.D.)

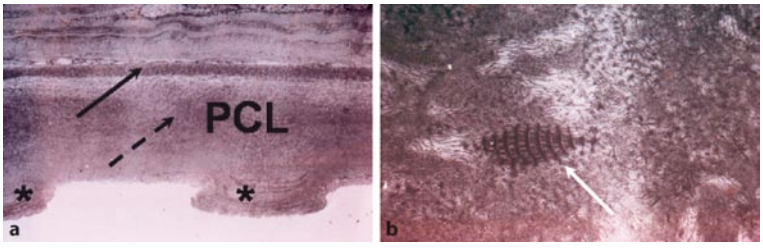

Fig. 1.3 **a** Low power electron micrograph of Descemet's membrane from a FED patient. Note the normal anterior banded zone (*arrow*), the markedly thickened and diffusely banded posterior collagenous zone (*PCL; dashed arrow*), and the focal posterior excrescences (guttae, *asterisks*). **b** High-power electron micrograph of PCL showing a spindle-shaped bundle with 110-nm collagen banding (wide-spaced collagen, *white arrow*). (Photos courtesy of W. Richard Green, M.D.)

1.5 Clinical Findings

The earliest clinical signs of FED include few (<10), central, focal excrescences (guttae) of Descemet's membrane (Fig. 1.2a). Over decades, accumulation of guttae coincides with the normal, gradual attrition of corneal endothelial cells occurring throughout postnatal life. Normal adult central corneal endothelial cell density is approximately 2,500 cells/mm², and a density of approximately 500–1,000 cells/mm² is the minimum threshold for physiologic corneal deturgescence. Once this threshold is crossed, corneal edema occurs, resulting in loss of vision and pain due to formation of epithelial bullae (Fig. 1.2b).

The clinical course of FED can be divided into four stages (Table 1.1) [1]. Stage I is characterized by biomicroscopic evidence of central corneal guttae, with a possibly thickened, grayish Descemet's membrane (Fig. 1.2a). At this stage, the patient is asymptomatic. In stage II disease, the vision may be predominantly blurred in the morning because of decreased tear evaporation, which lowers tear film osmolality when the eyes are closed [36]. Stromal and epithelial edema is notable on biomicroscopy. Stage III and IV disease are characterized by the presence of epithelial bullae, which cause pain upon rupture (Fig. 1.2b). Stage IV is distinguished by the presence of subepithelial scar tissue, resulting in further worsening of visual acuity, but relief from pain.

The diagnosis of FED is made principally on the basis of the biomicroscopic examination. Other modalities that have been used in conjunction with slit-lamp biomicroscopy include corneal pachymetry, confocal microscopy, and noncontact specular microscopy. Corneal pachymetry measures are of limited utility given the wide variation in corneal thickness of normal individuals. The greatest utility of pachymetry is in the consideration of penetrating keratoplasty (PK) in known or suspected FED patients being evaluated for cataract surgery (see Sect. 1.8).

Confocal microscopy and noncontact specular microscopy rely on slightly different methods of light emission and different patterns of light reflection at the interface between Descemet's membrane and corneal endothelial cells. The absence of corneal endothelial cells adjacent to and overlying guttae leads to transmission of light without reflection in these areas. Corneal endothelial cells (Fig. 1.4a) and corneal guttae (Fig. 1.4b) can be easily demonstrated with confocal microscopy. Both confocal and specular microscopy can aid in demonstrating corneal endothelial cell polymorphism and pleomorphism, as well as measuring endothelial cell density. These characteristics have potential clinical and research applications as markers of disease progression.

Confocal microscopy is superior to specular microscopy for evaluating the corneal endothelial layer in the setting of corneal stromal edema [8, 16]. However, the benefits of specular mi-

Table 1.1 Clinical stages of Fuchs endothelial dystrophy[a]

Stage	Symptoms	Clinical findings	Visual acuity
Stage I	No symptoms	Few to moderate corneal guttae	Normal (20/20)
Stage II	Mild to moderate loss of vision, no pain	Moderate to numerous corneal guttae, mild corneal edema	Mild to moderate reduction (20/20 to 20/80)
Stage III	Moderate to severe loss of vision and pain	Confluent corneal guttae, moderate to severe corneal edema, epithelial bullae	Moderate to severe reduction (20/100 to 20/400)
Stage IV	Severe loss of vision, reduced pain	Subepithelial scar, fewer epithelial bullae	Severe reduction (20/400 or worse)

[a]Adapted from [1].

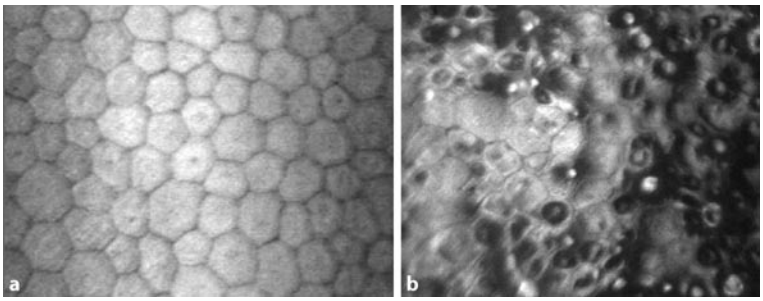

Fig. 1.4 **a** In vivo confocal microscopy image of normal corneal endothelial cells (CECs). Note ordered, hexagonal array of cells. **b** Confocal microscopy image of CECs in FED. Note the numerous excrescences (guttae) of Descemet's membrane as well as the irregular size and shape of the cells

croscopy over confocal microscopy include its relative cost-effectiveness and its ease of use [8]. Neither modality is effective in cases of extreme corneal edema or stromal opacity [16]. In addition, the utility of these auxiliary tests in the diagnosis of FED is primarily in unusual cases, as the diagnosis can usually be made on the basis of slit-lamp biomicroscopy.

Summary for the Clinician

- Diagnosis of FED is primarily made by the appearance of guttae with or without corneal edema on biomicroscopy
- Fuchs endothelial dystrophy can be classified into four stages: (I) presence of subclinical central guttae; (II) presence of stromal and epithelial edema; (III) presence of epithelial bullae; (IV) presence of subepithelial scarring

1.6 Pathophysiology and Genetics

Studies of FED have been predominantly limited to end-stage corneas because milder cases are asymptomatic and therefore less readily available for clinicopathologic correlation. Many of the observations likely reflect complex secondary changes occurring as a result of corneal endothelial cell decompensation. Furthermore, initiating events are largely unexplored, and virtually no information exists about early cellular and extracellular matrix changes leading to corneal endothelial cell loss.

Using scanning fluorophotometry, Wilson et al. demonstrated a decreased endothelial pump rate in corneas with advanced FED [63]. McCartney et al. demonstrated a decline in the density of ATPase pump sites in the basolateral corneal endothelial cell membranes [39]. Nucleus labeling, transmission electron microscopy, and TUNEL assays were used to demonstrate apoptosis in corneal endothelial cells of advanced stage FED corneas [6]. Serial analysis of gene expression studies of FED corneal endothelial cells demonstrated decreased transcripts related to apoptosis defense and mitochondrial energy production [14]. Whether corneal endothelial cell apoptosis is primary in the pathogenesis of FED or secondary to an abnormality of the basement membrane remains to be elucidated. Other proposed factors with unclear relevance include fibrinogen/fibrin, reduced sulfur content and increased calcium of Descemet's membrane, aqueous humor flow/composition, sex hormones, and inflammation [5].

To date, only mutations in the α2 collagen VIII (COL8A2) gene have been identified as causing FED [4, 15]. Biswas et al. performed genetic linkage analysis of a pedigree with three affected generations and identified an FED locus on chromosome 1p34.2-p32. DNA sequencing revealed a mutation in the COL8A2 gene resulting in a substitution of glutamine with lysine at amino acid 455 (Q455K). This

mutation cosegregated with FED in this pedigree and was absent in 244 ethnically matched control individuals [4]. The COL8A2 gene was sequenced in 115 additional unrelated FED patients, with a total of 8 individuals demonstrating mutations in the COL8A2 gene [4]. Gottsch et al. performed genetic linkage analysis in a large early-onset FED pedigree originally described by Magovern [35] and identified a second point mutation in COL8A2 at amino acid 450, resulting in a substitution of leucine with tryptophan (L450W) [15]. In contrast to common FED, members of this pedigree had an earlier onset of disease with children as young as 3 years of age affected and with distinct features such as a fine, patchy distribution of guttae.

Collagen VIII is a major component of normal Descemet's membrane and forms the abnormally thick posterior collagenous layer in FED corneas. Furthermore, the characteristic aggregates of wide-spaced collagen in Descemet's membrane of FED consist of collagen VIII [31]. Based on the implied functional effects of these mutations, one pathophysiologic hypothesis is that amino acid substitutions reduce the turnover of COL8A2, resulting in the abnormal accumulation of collagen VIII and abnormalities of Descemet's membrane. These abnormalities eventually become incompatible with endothelial cell function and survival, resulting in apoptosis. If this model proves accurate, additional candidate genes for FED could include other protein constituents of Descemet's membrane. To date, however, no published reports have associated mutations in these genes with FED.

Alternatively, the accumulation of collagen VIII in FED may occur as a secondary response to another primary insult to the endothelium. This possibility is consistent with the observation that a posterior collagenous layer of Descemet's membrane, presumably composed of collagen VIII, is present in other hereditary and acquired diseases of the corneal endothelium [23, 31, 38, 48]. Thus, a more complex relationship may exist between corneal endothelial cell dysfunction and abnormal accumulations of collagenous material in Descemet's membrane [15, 31].

Summary for the Clinician

- The pathogenesis of FED is unclear. Possible factors include sex hormones, inflammation, and endothelial cell apoptosis
- Collagen VIII is a major component of Descemet's membrane and is secreted by healthy and pathologic corneal endothelial cells
- Mutations in collagen VIII have been linked to FED

1.7 Differential Diagnosis

The diagnosis of FED is based on clinical findings, mainly slit-lamp biomicroscopy. Distinguishing FED from other entities is important because the diagnosis has implications for treatment and prognosis of both patients and their family members. Other entities that must be differentiated from FED include other posterior dystrophies, including posterior polymorphous dystrophy. In this autosomal dominant condition, groups of small round vesicles are found at the level of the endothelium, interspersed with sheets of gray material within Descemet's membrane [60]. This condition is not generally associated with stromal or epithelial edema or corneal guttae.

Another form of endothelial dystrophy, congenital hereditary endothelial dystrophy, is present at birth or early in postnatal life and is characterized by edema of the entire cornea and severe visual impairment [60]. Hassall-Henle bodies have the same appearance as guttae, but are located only in the peripheral cornea and are not associated with progressive visual loss or corneal edema [23]. Aphakic and pseudophakic bullous keratopathies are caused by endothelial cell dysfunction related to trauma during or after cataract extraction and presuppose a normal corneal endothelium prior to cataract extraction [23]. Inflammatory diseases, such as anterior uveitis or interstitial keratitis, may be mistaken for FED and can be differentiated by resolution of keratic precipitates with proper treatment in the case of anterior uveitis, or on the basis of se-

rologic testing for syphilis in the case of interstitial keratitis [59].

1.8 Management

1.8.1 Medical

Early treatment modalities are not specific for FED, but are commonly applied to all etiologies of corneal epithelial and stromal edema. These approaches involve artificially raising the osmolality of the tear film and include hypertonic saline solutions and ointments, as well as the use of a hairdryer in the morning to dehydrate the precorneal tear film [62]. The use of therapeutic soft contact lenses may help in relieving the pain from recurrent epithelial erosions, while decreasing irregular astigmatism in cases that have progressed to bullous keratopathy [62]. The use of cycloplegics and nonsteroidal anti-inflammatory agents may also aid in diminishing corneal pain from bullous keratopathy. The use of intraocular pressure-lowering medications may reduce corneal edema in patients with elevated or even normal intraocular pressure [1].

1.8.2 Surgical

If conservative management options do not provide adequate clarity of the visual axis or alleviation of discomfort or pain, surgical options may be considered [37]. PK has been regarded as the definitive procedure in patients with corneal decompensation due to FED. In one study of PK in patients with FED, the proportion of patients with visual acuity of 20/40 or better was 50% at 3 months postoperatively, and increased to 80% by 24 months [47]. The authors attribute this improvement over time to corneal healing, suture removal, and fitting of rigid contact lenses. A 10-year follow-up study of 908 patients who underwent PK for FED found a graft survival rate of 97% at 5 years and 90% at 10 years [56]. The most common cause of graft failure in these patients was endothelial rejection, followed by nonimmunologic endothelial failure. Uncorrectable irregular astigmatism was another leading cause of poor postoperative visual acuity. Others have reported graft survival rates of 89% at a mean follow-up of 8.4 years [44], and 81% after 10 years' follow-up [18] in patients with FED.

However, visual function after PK may not be dramatically improved, as one study found 42% of patients who had undergone corneal transplant for FED had visual acuities of worse than 20/200 at an average of 50 months after surgery [42]. The disparity between these results suggests that outcomes may be operator-dependent, and surgeons with more experience tend to have better results [57].

Because many patients with FED and corneal decompensation also have cataracts, attention has turned toward combined versus staged surgical management of the cataract and cornea. It has been suggested that combined procedures in the hands of an experienced surgeon have the same outcome as staged procedures with PK preceding cataract extraction [2]. The American Academy of Ophthalmology suggests that corneal thickness measurements greater than 600 μm portend a poor prognosis following cataract surgery and recommends consideration of combined cataract extraction and PK in these patients [21]. However, in the hands of a skilled surgeon, one study suggests that cataract extraction may be safely performed in patients with corneal thickness measurements up to 640 μm [51].

New posterior lamellar techniques to selectively replace diseased endothelium, as in FED, have been developed and are gaining popularity over traditional PK. In 1998, Melles described "posterior lamellar keratoplasty," or PLK, which consisted of manually dissecting both recipient and donor tissues at 80–90% stromal depth and transplanting the donor posterior lamellar disc through a scleral incision [40]. This technique was later modified and popularized as deep lamellar endothelial keratoplasty (DLEK) by Terry and Ousley [54].

Deep lamellar endothelial keratoplasty has the advantage over PK of being a "sutureless" technique, thereby avoiding the potential infectious and refractive complications associated with sutures. This technique also has the advantage of maintaining the tensile strength of the cornea, which is not possible with PK. The largest disadvantage of DLEK is its technical difficulty, even for highly-experienced anterior segment sur-

geons, and the potential media opacity created by irregularities in the lamellar dissection stage of the procedure. Early results of DLEK are encouraging, with mean best corrected visual acuities of 20/46 and 20/50 and mean average astigmatism of 1.34 and 2.3 D at 6 and 12 months respectively [43, 55].

More recently, Descemet's stripping with endothelial keratoplasty, or DSEK, has been developed, which eliminates the need to perform the recipient lamellar dissection and posterior button excision in DLEK. DSEK replaces the sometimes laborious lamellar dissection of the recipient cornea by simply stripping Descemet's membrane and endothelium, a maneuver first introduced in 2004 by Melles et al. [41]. The folded donor posterior lamellar button is then inserted into the recipient anterior chamber and allowed to unfold adjacent to the bare stromal surface, while maintaining the proper endothelial orientation. An intracameral air bubble is placed to promote attachment of the disc to the recipient stromal bed, and the patient is maintained in the supine position postoperatively. With attachment of the donor posterior lamellar disc, the donor endothelial cells deturgesce the recipient stroma and epithelium, allowing for a clear cornea (Fig. 1.5). Anterior chamber optical coherence tomography (AC-OCT) can be utilized to demonstrate attachment of the donor posterior disc (Fig. 1.6).

Initially, the donor lamellar button for DSEK was created with manual lamellar dissection. Recently, however, preparation of the donor endothelial button has been simplified by use of an automated microkeratome to cut the corneal button mounted on an artificial chamber. This variation has been termed Descemet's stripping with automated endothelial keratoplasty (DSAEK). However, the terms DSEK and DSAEK have generally become interchangeable, as use of the automated microkeratome for donor button preparation has gained popularity.

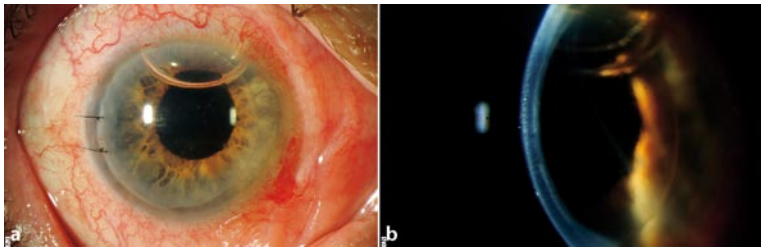

Fig. 1.5 **a** Slit-lamp examination of a cornea after Descemet's stripping with endothelial keratoplasty (DSEK) shows a clear stroma with a remaining air bubble that resolves postoperatively. **b** Slit-lamp beam shows thinning of the recipient stroma with an attached donor posterior disc. (Photos courtesy of William W. Culbertson, M.D.)

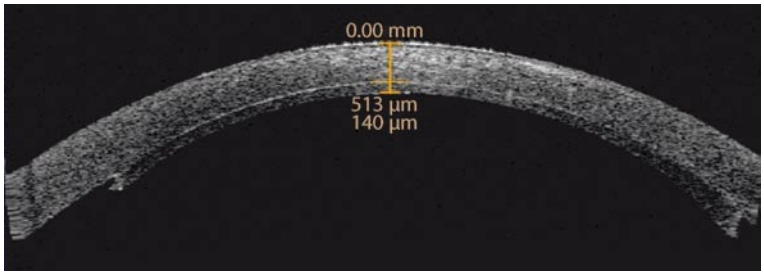

Fig. 1.6 Anterior chamber optical coherence tomography (AC-OCT) image shows a cross-section of a recipient cornea with an attached donor posterior disc. (Image courtesy of William W. Culbertson, M.D.)

The DSEK/DSAEK procedures have largely supplanted DLEK in the surgical treatment of FED. Advantages over DLEK include an easier technique of removing the diseased endothelium, less trauma to the recipient tissue, a more structurally sound recipient stroma, and a smoother corneal interface [43]. In a review of 50 eyes undergoing DSEK, Price et al. [45] showed that at 6 months, 31 eyes (62%) had best corrected visual acuities of ≥20/40 and 38 eyes (76%) had best corrected visual acuities of ≥20/50. At 6 months, the mean manifest cylinder was 1.5±0.94 D and the mean manifest spherical equivalent was 0.15±1.0 D. The most common complication encountered in DSEK is detachment of the lamellar disc, which has been reported in 15–30% of cases in the early postoperative period [46]. This complication, however, can be addressed in the immediate postoperative period by repositioning the button and/or injecting more air into the anterior chamber (called "rebubbling"). Figure 1.7 shows an AC-OCT image of a detached DSEK button (Fig. 1.7a) that was repositioned and rebubbled with subsequent reattachment and progressive resolution of stromal edema (Figs. 1.7b–f).

Summary for the Clinician

- Medical management of FED includes topical hypertonic saline, use of a hair dryer in the morning to dehydrate the precorneal tear film, and therapeutic contact lenses
- For several decades, penetrating keratoplasty (PK) has been the standard surgical treatment for FED
- Preoperative corneal pachymetry in FED patients can be helpful in assessing the risk of corneal decompensation after cataract surgery
- Cataract surgery alone should be considered in FED patients with corneal pachymetry less than 600–640 μm
- Various forms of endothelial keratoplasty are gaining popularity over traditional PK in the treatment of FED

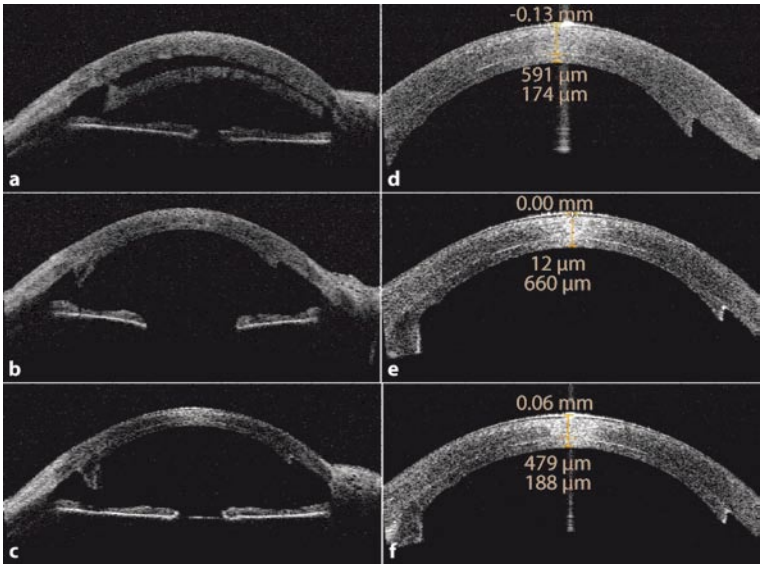

Fig. 1.7 **a** An AC-OCT image of a detached donor posterior disc. **b** Reattachment of the disc to the recipient stromal bed with repositioning and rebubbling within 1 week of the DSEK procedure. **c** The cornea at postoperative month 3. **d–f** Progressive deturgescence of the cornea after DSEK. (Images courtesy of William W. Culbertson, M.D.)

1.9 Future Directions

Future therapies for FED would ideally provide definitive treatment of the diseased endothelium early in the disease course, before vision loss or discomfort occurs. Improvements in endothelial transplantation will likely result in less invasive approaches with reduced complication rates and faster visual recovery. Although endothelial cells do not proliferate in vivo, several groups have demonstrated the proliferative capacity of human corneal endothelial cells in vitro [24]. Recent work demonstrating the ability to culture human corneal endothelial cells on several substrates, such as denuded Descemet's membrane and amniotic membrane [19], indicates promising areas of future research. Additional cell-based approaches could utilize our understanding of the mechanisms of cell cycle arrest in human corneal endothelium to stimulate cell division in vivo [27].

Fuchs endothelial dystrophy also represents an attractive disease for gene therapy-based approaches. Mutations in the COL8A2 gene have already been shown to cause FED, and additional causal mutations in different genes will probably be identified. Genetic modification of corneal endothelial cells has already been accomplished, and FED mutations could theoretically be corrected in corneal endothelial cells as a potential treatment for this disease [28].

1.10 Summary

Fuchs endothelial dystrophy is a primary disease of the corneal endothelium that is characterized by loss of endothelial cells and abnormalities of Descemet's membrane. These changes result in the decreased capacity of the endothelium to dehydrate and maintain the optical clarity of the corneal stroma and epithelium, resulting in pain and decreased visual acuity. Experimental studies on FED suggest endothelial cell apoptosis and abnormal basement membrane physiology as mediators in the pathogenesis of this disease. To date, PK has been largely successful in managing advanced FED. Newer techniques such as DLEK, DSEK, and DSAEK avoid the potential complications of PK and instead provide increased globe integrity, minimal refractive change, and faster recovery of vision. Future advances, including improvements in endothelial keratoplasty, endothelial cell transplantation/engineering, and gene therapy, represent promising new approaches to the management of this common corneal disorder.

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Chapter 2

Amniotic Membrane Transplantation for the Treatment of Corneal Ulceration in Infectious Keratitis

2

Arnd Heiligenhaus, Carsten Heinz, Klaus Schmitz, Christoph Tappeiner, Dirk Bauer, Daniel Meller

Core Messages

- Corneal ulceration may occur in diverse types of infectious keratitis, e.g., in herpetic, bacterial, or parasitic infections
- The diverse infectious entities can be distinguished by their clinical presentation
- Before treatment, corneal scrapings or biopsies should be obtained for proper microbiological evaluation
- The pathogenesis of infectious corneal ulcers includes micro-organism-related and immune-mediated factors
- Amniotic membrane may have anti-inflammatory, antiangiogenic, and antimicrobial effects, and may promote re-epithelialization
- An "onlay" (patch) or "inlay" (graft) technique can be used for amniotic membrane transplantation
- Patients must be followed up closely after surgery to detect any spread of infection and failure of surgery
- Appropriate antimicrobial medication should be given before and after surgery

2.1 Introduction

Severe infectious corneal ulceration commonly causes loss of vision. Management comprises antimicrobial medication and diverse approaches that aim to restore the integrity of the corneal surface. With this mind, amniotic membrane transplantation (AMT) has been recently introduced into the treatment regimen. In this chapter, clinical aspects and pathogenesis of the major groups of infectious corneal ulcerations and the basis and clinical application of AMT for the management of these corneal lesions are reviewed.

2.2 Clinical Aspects of Corneal Ulceration in Infectious Keratitis

2.2.1 Herpetic Corneal Ulceration

2.2.1.1 Clinical Features

Herpetic stromal keratitis (HSK) is a common infectious cause of blindness and is prevalent worldwide. Herpes simplex virus (HSV) establishes a latent infection in the neurons of the peripheral ganglia and in the eye, and may eventually become reactivated and cause lesions to recur. In a previous study, the prevalence of her-

petic eye disease was found to be 149 in 100,000 residents [61].

Herpes infections of the cornea present a broad range of clinical features. Infectious epithelial keratitis may appear as dendritic or geographic ulcerations (Fig. 2.1) that are caused by viral replication and epithelial cytolysis.

Stromal HSV keratitis has been classified as non-necrotizing or necrotizing. Immune-mediated stromal keratitis typically appears with infiltration, immune ring, scarring, and neovascularization. It often corresponds with a prior or current infectious epithelial lesion. The necrotizing ulcerating keratitis that occurs in about a third of patients with stromal keratitis shows dense, white, opaque inflammatory infiltration of the stroma (Fig. 2.2) and carries a high risk of keratolysis and corneal perforation occurring within only a few days.

The characteristic aspects of neurotrophic keratopathy are irregular epithelium, oval-shaped, persistent epithelial defects with smooth borders, and a lack of scalloped edges that are typical for infectious lesions. The corresponding ulceration has a gray–white opacification at the ulcer bed and overhanging epithelial border (Fig. 2.3). Ulceration and melting is a serious complication that can lead to perforation and loss of vision.

2.2.1.2 Treatment

As the infectious epithelial lesions are caused by viral replication, the lesions are treated with antiviral agents. In all patients with stromal ulceration and infiltration, other infective causes must be excluded, e.g., bacteria, fungi, *Acanthamoeba*.

The management of HSV-induced, immune-mediated keratitis with ulceration includes medical and surgical approaches. The immune-mediated inflammation is mostly treated with topical corticosteroids [75, 100]. However, corticosteroids may impair corneal re-epithelialization and wound-healing, and corneal melting and perforation have sometimes been the consequence. Topical application of cyclosporin A has been recommended, therefore, in order to avoid the potential side effects of corticosteroids [30].

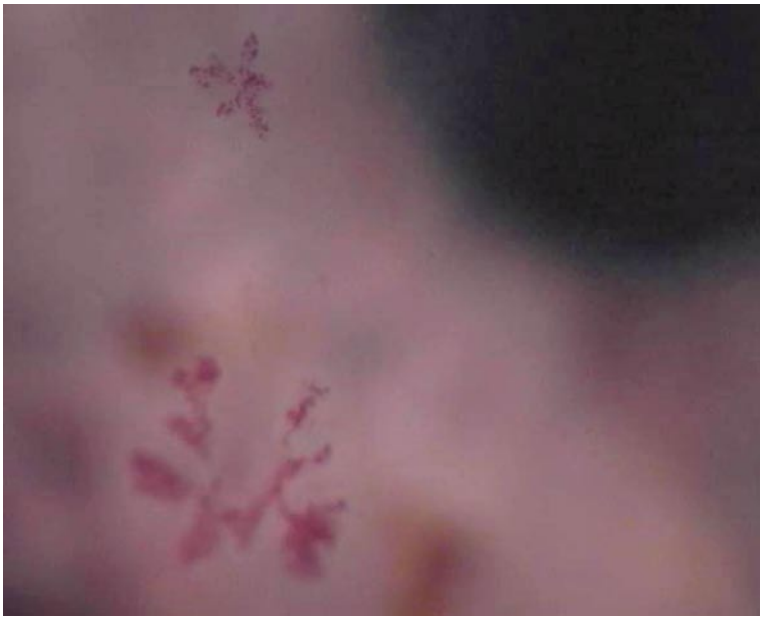

Fig. 2.1 Infectious dendritic ulcer in herpes simplex keratitis

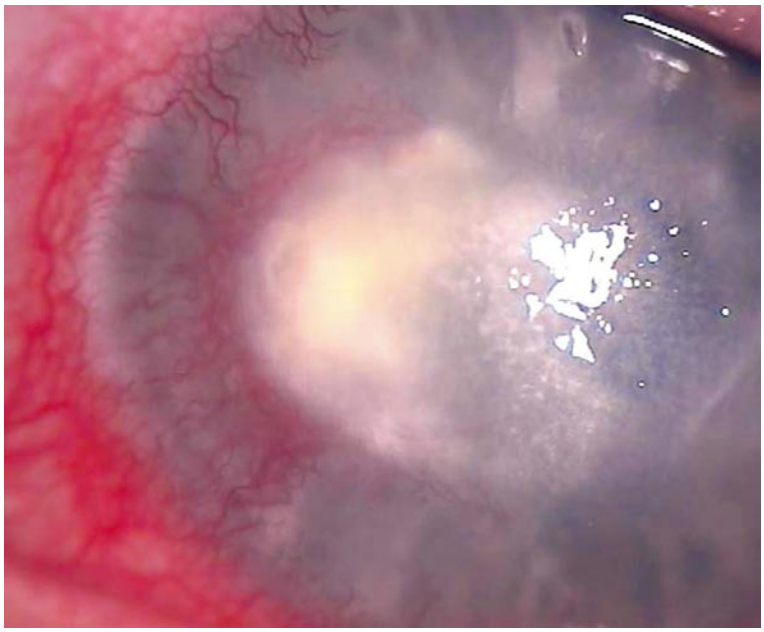

Fig. 2.2 Immune-mediated stromal necrotizing ulcerating herpes simplex keratitis

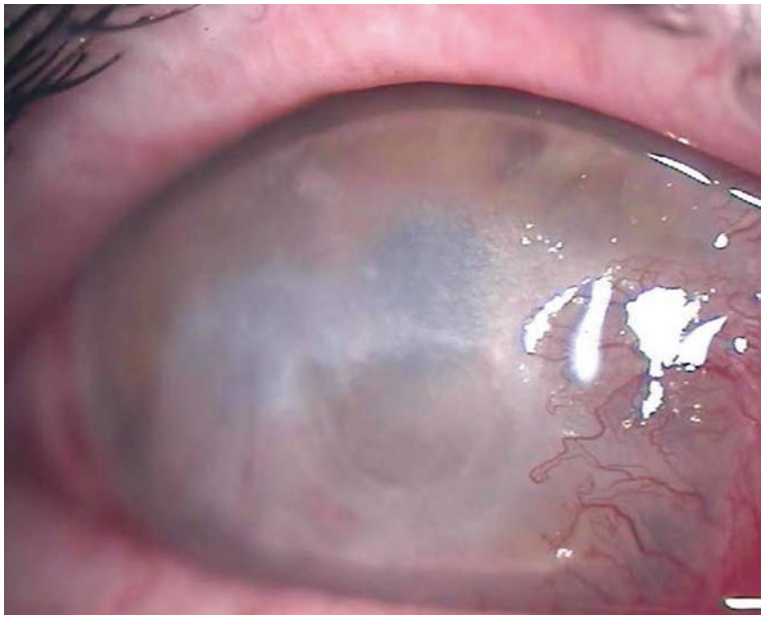

Fig. 2.3 Neurotrophic keratopathy in herpes simplex keratitis

Topical or oral aciclovir is required as an adjunct, for the treatment of actively replicating HSV or to avoid reactivation of latently persisting virus [2, 55].

Neurotrophic keratopathy should be treated with aggressive lubrication. As the toxicity of the topical antivirals may be a significant cause of keratopathy, it may be necessary to discontinue the drugs. If the ulcer does not heal, gentle debridement of the loose epithelium and bandage contact lenses represent an additional treatment option. Temporary tarsorrhaphy, either by botulinum toxin-induced ptosis or suturing may be indicated.

Whereas a conjunctival flap has historically been considered for treating nonhealing corneal ulcers, AMT is currently the preferred method. Lamellar or perforating keratoplasty is recommended for visual rehabilitation in patients with superficial or deep scar formation, when ulceration worsens, or when perforation is imminent.

Summary for the Clinician

- Infectious epithelial keratitis must be treated with antiviral drugs
- Immune-mediated stromal keratitis is mostly treated with corticosteroids
- Neurotrophic ulcers may be treated with aggressive lubrication

2.2.2 Bacterial Corneal Ulceration

2.2.2.1 Clinical Features

The majority of infectious corneal ulcerations are induced by bacterial infection. Among 88 patients with infectious keratitis in whom microbial testing was carried out, bacterial growth was observed in 66%, fungal growth in 15%, and a negative culture in 25% [96]. Bacterial ulcerative keratitis is a sight-threatening and potentially blinding disease. The extent of the disease varies greatly, depending on the underlying disease of the cornea and the pathogenicity of the infectious agent. The incidence also varies, depending on geographic and climatic factors and on urban or rural settings [5, 96]. Most corneal infections are restricted to corneal lesions as a result of ocular or surgical trauma, contact lens use, or other diseases that affect corneal integrity.

Most bacterial ulcers are caused by Gram-positive cocci, particularly coagulase-negative staphylococci [5, 96]. Other very frequently involved micro-organisms include Gram-negative *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The incidence of *Pseudomonas* is significantly higher in patients who wear contact lenses.

As clinical symptoms are not overt at the outset, these patients often do not present until the disease is advanced. Underlying corneal diseases such as bullous keratopathy reduce corneal sensitivity and delay diagnosis and initiation of therapy. Other general conditions such as diabetes mellitus also reduce corneal sensitivity due to generalized peripheral polyneuropathy [69].

The main clinical symptoms are commonly discomfort, with a watery eye, redness, and foreign body sensation. When the center of the cornea is affected, vision is disturbed. As a consequence of severe corneal ulceration (Fig. 2.4), anterior chamber inflammation with sterile or infectious hypopyon may be found.

2.2.2.2 Treatment

The initial clinical treatment approaches vary from empiric therapy to a cell culture-based approach, in which the antibiotic is often modified according to the culture and sensitivity results [9]. The need for a corneal culture is therefore the subject of controversial discussions [12]. When taking a microbial probe the proper technique should be used [3]. In some cases the use of a fine trephine achieves the best results [79].

The development of new antibiotics is becoming more and more restricted by resistance to the drugs. Irrespective of the class of the chosen antibiotic, treatment must start directly after the condition has been diagnosed. The goal of initial treatment with applications every 5 min for the first 2 h is to reach the minimal inhibitory concentrations quickly.

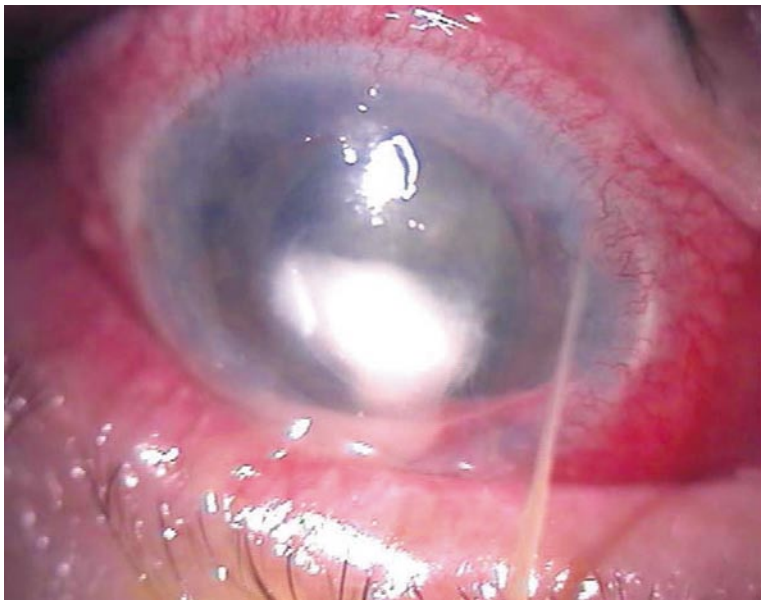

Fig. 2.4 Bacterial deep corneal ulceration with hypopyon

Previously, certain fortified eye drops (e.g., 5% cefazoline and 1% gentamicin) were recommended for treating bacterial ulcers. As these preparations were not commercially available, they were expensive and inconvenient to use. The development of the broad-spectrum antibiotics that are commercially available, such as the fluoroquinolones, have changed the general treatment patterns. As they are sensitive to the majority of Gram-positive and anaerobic Gram-negative bacteria, they currently represent the drugs of choice for treating bacterial ulcers. With the drugs that are commercially available at present, such as levofloxacin and the second-generation drugs ofloxacin und ciprofloxacin, there is a known gap in the coverage of the *Streptococcus* species. This gap can easily be bridged with topical penicillin or gentamicin.

Prior corticosteroid treatment can increase the risk of antimicrobial failure and corneal perforations [99]. The additional benefit of corticosteroid treatment in combination with antibiotics is not clear; however, experimental data suggest that there might be a benefit in combating the corresponding inflammation.

Oral tetracyclines may be beneficial and prevent corneal perforation [77]. After the acute phase of inflammation, epithelial debridement, intensive lubrication with artificial tears, temporary horizontal shortening of the lid fissure by tarsorrhaphy, or botulinum toxin injection may be helpful. Only under certain circumstances with progressive corneal melting should keratoplasty be considered in the acute phase of the disease.

Summary for the Clinician

- Bacterial ulcers are more common after corneal trauma or in association with contact lens use
- Cell culture-based antibacterial treatment should be attempted
- Treatment with topical fluoroquinolones should be started at a high dosage directly after diagnosis

2.2.3 Parasitic Corneal Ulceration

2.2.3.1 Clinical Features

The most common cause of parasitic corneal ulceration is *Acanthamoeba* infection, an ubiquitous living pathogenic organism. The incidence of *Acanthamoeba* keratitis is estimated at 1.1 to 1.3 per 1 million and increases up to 20.9 per 10,000 per year in individuals who wear soft contact lens for an extended period [74, 76].

Acanthamoeba keratitis patients present with severe ocular pain, tearing and photophobia, redness, and loss of vision. In the first few weeks, recurrent epithelial infiltration, pseudodendrites, haze, and epithelial defects can be seen. During the first month, characteristic radial perineural infiltrates may be noted, which are commonly associated with severe pain. Later, epithelial defects, nummular keratitis, or stromal infiltration develop. At more advanced stages, the typical ring infiltration, satellite lesions, stromal abscess, and breakdown of epithelium with corneal ulceration may follow [1, 78]. If infiltration progresses, the sclera and anterior chamber may be affected.

Acanthamoeba can be isolated from corneal scrapings or biopsy [1] or from contact lenses or their cases [44]. It can be detected by light microscopy or noninvasive confocal microscopy, PCR, cultivation on non-nutrient agar plates seeded with Gram-negative bacteria (e.g., *E. coli*), and using calcofluor white and acridine orange staining methods for cytological identification of cysts and trophozoites in tissue.

2.2.3.2 Treatment

In the early stages of *Acanthamoeba* keratitis, topical application of antimicrobials is effective. Early diagnosis and prompt institution of therapy are the best means of obtaining a good outcome [1].

In addition to aminoglycosides, propamidine isoethionate, hexamidine-diisethionate, diminazene, polyhexamethylene biguanide, chlorhexidine, miconazole, itraconazole, ketoconazole, and hexadecylphosphocholine are used for treatment [56]. Corticosteroids are contraindicated as monotherapy as they can activate *Acanthamoeba* cysts and trophozoites. Their concomitant use with the antimicrobial drugs is controversial.

Epithelial debridement may help reduce pathogens in the early phase, when only the epithelium is involved [38]. The timing of keratoplasty is also controversial. In general, keratoplasty is recommended for restoring vision after infection is brought under complete control. However, in the acute phase keratoplasty may be required if the cornea is already perforated or in cases of progressing disease that cannot be medically controlled [41]. Keratoplasty was only necessary in 1 out of 36 eyes when therapy was instituted at an early stage, while it was required in 23 out of 77 patients in whom the start of treatment was delayed [1].

Summary for the Clinician

- *Acanthamoeba* keratitis is especially common in individuals who wear contact lenses. Initially, epithelial keratitis is characteristic. Early diagnosis and prompt treatment are important

2.3 Pathogenesis of Corneal Ulceration

2.3.1 Pathogenesis of Corneal HSV-1 Ulceration

2.3.1.1 Necrotizing Stromal Keratitis

Herpetic stromal keratitis is an immunopathogenic disease, in which tissue injury usually results from immune responses to HSV antigens expressed in the inflamed tissue rather than from viral toxicity directly [86, 87]. Corneal destruction induced by herpes simplex virus includes rapid epithelial edema and necrosis, disintegration of the Bowman layer, necrosis and apoptosis of keratocytes, inflammatory cell infiltration, lysis of Descemet's membrane, and neovascularization. As the disease progresses, ulceration and corneal perforation may develop.

Various proteolytic enzymes are up-regulated in the cornea in HSV and participate in the development of corneal ulceration. There is sound evidence that resident corneal cells, infiltrating polymorphonuclear neutrophils (PMN), and macrophages produce tissue-degrading enzymes. The matrix metalloproteinases (MMPs) are a family of protein-cleaving enzymes that degrade extracellular matrix and basement membrane components. Tissue inhibitors of metalloproteinases (TIMPs) are the major endogenous regulators of MMP activity in tissues. The expression of MMP-2, -9 and -8 increases markedly in the region of corneal ulceration and necrosis after HSV-1 infection. By zymography, the active forms of MMP-2, -8, and -9 were largely increased in the corneas 14 days after infection [102]. This correlated with the subsequent heavy PMN infiltration in the cornea, which is a well-known cause of progressive tissue destruction in HSK [87, 88].

Increased levels of MMP also impeded re-epithelialization of the cornea after thermal injury in an animal model [17] and MMP was involved in initiating the epithelial defect that preceded stromal ulceration [18]. PMNs, upon activation, have the ability to release MMP-8 and MMP-9 from secondary granules. Since PMN collagenase can directly degrade stromal collagen and generate collagen peptide fragments that are chemotactic for PMN, the secretion of MMP-8 is probably responsible for the extensive stromal destruction and corneal ulceration after HSV-1 corneal infection.

Soon after HSV-1 infection of the cornea, macrophages are found in the epithelium and stroma. This is associated with a release of their catalytic enzymes, increased expression of IL-1 and TNF-α, increased MHC-II expression, and a T cell-mediated immune response. In addition, the expression of chemokines and ICAM-1 and the secretion of prostaglandin are dramatically increased. Subsequently, epithelium is lost, MMP expression increases in the epithelium and keratocytes, apoptosis is induced, and keratocyte migration is inhibited.

Down-regulation of TIMP-1 expression and sustained expression of TIMP-2 were found in the cornea in the late phase after HSV-1 infection [102]. The striking differences in their promoters and the feedback loops, and in the regulation of certain cytokines may explain the different patterns. In contrast to TIMP-1, TIMP-2 is frequently expressed constitutively and is not affected by cytokines.

The regulation of MMP activation and activity depends highly on the TIMP levels. Thus, a balance between proteases and inhibitors may determine the net enzymatic activity present in the cornea. Furthermore, many of the MMPs activate other MMPs, and this may also be relevant to the course of HSK. While the lymphocytic infiltrate is predominantly composed of CD4+ type-1 T cells expressing IL-2 and IFN-γ during the development of HSV-1 keratitis, the type-2 cytokine IL-4 participates in the late stage of inflammation [31, 35, 70]. As the expression and secretion of both MMP-activating enzymes and TIMPs are also influenced by cytokines, their composition presumably influences the MMP/TIMP balance during the development and healing of herpetic corneal ulcerations as well [102].

2.3.1.2 Neurotrophic Keratopathy

The mechanisms underlying neurotrophic keratopathy are impaired corneal innervation, reduced expression of neurotransmitters, damaged epithelial basement membrane, stromal inflammation, and toxicity from topical medications.

2.3.2 Pathogenesis of Corneal Bacterial Ulceration

The pathogenesis of bacterial ulcerative keratitis includes organism-related and host-derived factors. In several clinical studies, ocular trauma was the most common predisposing factor. Trauma related to contact lenses accounted for up to 50% of the cases in France [5], whereas wooden stick trauma was the predominating cause in India [96]. Other predisposing factors include ocular surface disease, lid abnormalities, and dry eye. The eyelid and the intact epithelial layer act as a natural protection against infection. Components of the tear film such as complement, lysozymes, lactoferrin, and ceruloplasmin aim to protect the host from infection.

Cell-mediated defense strategies include subepithelial mucosa-associated lymphoid tissue at the limbus and antigen-presenting cells (dendritic cells, Langerhans cells, and macrophages) that process bacterial antigens and present them to T-lymphocytes. Only if these specific or unspecific defense mechanisms are impaired can the micro-organism infect the cornea. Then, bacteria adhere to adhesion molecules on the traumatized surface. Only a few bacteria are able to penetrate an intact epithelial barrier, including *N. gonorrhea*, *N. meningitides*, *C. diphtheriae*, *Shigella*, and *Listeria*. The adherence of bacteria depends on the surface characteristics. For example, *Staphylococcus aureus* expressing fimbriae with several adhesions on it may contribute to the high rate of *Staphylococcus* infections. Additionally, bacteria produce enzymes that facilitate the invasion into the host.

After bacterial adherence, a complex response of the host's immune system is induced. Released cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF) of either the infiltrating inflammatory cells or the cornea promote the adhesion of leukocytes to the vascular endothelium. IL-1 is a potent mediator of inflammation and attracts PMN. TNF induces the release of other pro-inflammatory cytokines from inflammatory cells, such as PMN, macrophages, and T cells, and from the corneal epithelial and stromal cells.

Bacterial proteinases are able to invade and lyze the basement membrane and to induce progressive necrosis ("melting"). The destructive enzymes are also produced by corneal cells and invading inflammatory cells. Matrix metalloproteinases (MMP) are secreted and activated [17], subsequently causing epithelial defects and ulceration.

In a mouse model it was shown that higher levels of TIMP protected against perforation in *Pseudomonas aeruginosa* infections, while neutralizing TIMP also caused perforation. In this model, higher levels of TIMP reduced the influx of PMN and the destruction of the cornea [43]. The T cells were of particular importance for the development of the necrotizing keratitis, as corneal perforation occurred in mice with a predominantly Th1 response after infection with *Pseudomonas*, while in mice with a predominantly Th2 response the infection was less severe and perforation did not develop [27]. The perforation in the Th1 mice was induced by a sustained IL-12-driven production of IFN-γ, while IL-18-driven production of IFN-γ in the absence of IL-12 was associated with bacterial killing and less destruction [28, 40]. Several chemokines, such as MIP-1alpha and MIP-2, were up-regulated in susceptible versus resistant mice and were reduced in the absence of T-lymphocytes and PMN [25].

Even when bacterial amplification is stopped, the imbalance between MMP and their native inhibitor TIMP is maintained and corneal melting progresses. However, MMP also play an important role in tissue remodeling. Thus, the pathogenesis of bacterial corneal ulceration does not end when the infectious organism is eradicated, but also depends on the innate and adaptive immune response to bacterial inflammation [26].

2.3.3 Pathogenesis of Corneal Parasitic Ulceration

Acanthamoeba are ubiquitous in nature, e.g., in public water supplies, swimming pools, river water, ventilation ducts, air-conditioning units, beaches, surgical instruments, and contact lenses and their cases [44]. It is well known that contact lenses constitute a major risk factor for *Acanthamoeba* keratitis [74].

Two stages in the life cycle of *Acanthamoeba* can be distinguished. The vegetative trophozoite is a very active cell stage with a highly active metabolism that moves with amoeboid motion. The resistant cyst stage is a dormant cell form with minimal metabolic activity that can survive even under challenging conditions for months to years. As *Acanthamoeba* actively feed on microorganisms present on surfaces, bacteria are able to survive and multiply and may evade host defense and antibiotics [44].

Acanthamoeba trophozoites express mannose binding protein (MBP) on their surface, which binds to mannose-containing glycoproteins on the surface of host cells. This binding induces apoptosis in the host cell [44, 67, 82]. The adhesion also activates phagocytosis and toxin secretion. *Acanthamoeba* produce many hydro-

lytic enzymes, including proteases that degrade substrates for the feeding process and phospholipases that enable penetration into the host cells. Cell lysis and the generation of lipids then promote inflammation [44]. The collagenolytic enzymes are important for the evolution of ring infiltration and corneal ulceration [29].

The *Acanthamoeba*-induced corneal ulcers histopathologically appeared with some PMN infiltration, with epithelial and stromal necrosis and disruption of Bowman's layer. Only a few cysts and trophozoites were found in both the area of inflammation and the surrounding corneal tissue [47, 97].

Summary for the Clinician

- The pathogenesis of infectious keratitis involves microbial invasion and tissue damage, specific and unspecific immune responses, and enzymatic tissue destruction

2.4 Basics of Amniotic Membrane Transplantation

2.4.1 Anti-inflammatory Effects

The anti-inflammatory properties of the amniotic membrane (AM) are apparent when inflamed corneas are patched with AM. It has been shown that the AM stroma contains growth factors [51], natural inhibitors of various proteases [68], and antiangiogenic substances [24]. In addition, inhibitors of metalloproteinases and nitric oxide synthase and potent anti-inflammatory proteins were found, including the IL-10 and IL-1 receptor antagonists [16, 24].

The AM stroma decreased the TGF-β production and myofibroblast differentiation in human corneal and limbal fibroblast cultures [91, 92] and in the fibroblasts from conjunctiva and pterygia [58]. Corneal keratocytes produced fewer chemokines when cultured together with AM [8, 24]. Lymphocytes that were co-cultured with AM showed a reduced proliferative response and lower cytokine production [95]. In addition, after challenge with lipopolysaccharides (LPS), the expression of IL-1α and IL-1β is reduced in human limbal epithelial cells when co-cultured with AM stroma [83].

Previously, it was shown in an experimental mouse model that AMT can greatly improve HSK with deep ulceration within only 2 days [32]. Correspondingly, a rapid improvement has also been noted after AMT in patients with corneal herpes infections [33]. Subsequent experiments in the murine HSK model showed that the beneficial effect after AMT was predominantly due to local effects of the AM, as the systemic cellular and humoral immune responses directed against HSV-1 were not affected [33].

The HSK improvement after AMT was associated with markedly reduced expression and activity of MMP-2 and MMP-9 in the cornea. Expression of the pro-inflammatory cytokines TNF-α and IL-1α, which are well-known inducers of MMP-2 and MMP-9, was also decreased [34]. MHC-II expression in the HSV-infected corneas was greatly reduced after AMT.

HSV-1-specific T lymphocytes have been shown to be the principal inducers of HSK [15, 66]. Following AMT, infiltration with CD3+ and CD4+ T lymphocytes was greatly reduced in the cornea, and the concentration of T cell-specific cytokine IL-2 and IL-12 was lower, which is a sign of immunosuppression.

In agreement with this, cytokine expression was also reduced in splenocytes and lymph node cells that were obtained from HSK mice and co-cultured with AM. Antigen-specific and antigen-unspecific proliferation in these cells was also decreased as determined by 3H-thymidine assay. The flow cytometric analysis of the AM-treated cells disclosed a down-regulation of the cell-activation markers CD25, CD69, and MHC-II. Furthermore, these cells demonstrated nuclear fragmentation and condensation by DNA staining (Hoechst 33342) and increased amounts of the 200-kb DNA fragments on gel electrophoresis; using flow cytometry early signs of apoptosis (annexin V^{high}/$7AAD^{low}$) were detected after 2 h of co-cultivation with AM. As the addition of various recombinant pro-inflammatory cytokines (e.g., IL-1β, -2, -4, -6, or -10) did not prevent AM-induced T cell apoptosis, passive apoptosis

that is mediated due to a lack of cytokines is not the mediator of cell death.

Induction of activation-induced cell death (AICD) in T-lymphocytes requires that both the antigen/mitogen at the T cell receptor (TCR) and IL-2 are stimulated [19, 98]. The cell apoptosis is then mediated by the Fas receptor, and co-cultivation of T cells with cyclosporin A or rapamycin is thus able to block the cell death. However, this was not found with lymphocytes co-cultured with AM. The lymphocytes from FAS knock-out mice (lpr-/-) were not protected against AM-induced apoptosis either; therefore, AM induces lymphocyte apoptosis independently of AICD.

Taken together, the data suggest that AMT reduces the effector response of T-lymphocytes in the cornea with HSK. This effect is mediated, at least in part, by an activation- and cytokine-independent apoptosis of T cells in the cornea (Bauer et al., submitted).

Granulocytes (PMNs) represent the type of cell (90%) that predominantly infiltrates murine corneas with HSK. Our immunohistochemical studies of the AMT-treated murine corneas show that the number of PMN (CD11b+ or GR1+ cells) was significantly decreased in the AMT-treated corneas. This is associated with increased PMN apoptosis following AMT. Correspondingly, after incubation of PMN with AM, the cells were positively stained for annexin V+, were TUNEL-positive, and showed fragmentation markers typical of apoptosis.

Previously, inflammatory cells have been noted in AM tissue removed from inflamed corneas. The cells contained mainly CD14+ monocytes/macrophages, CD4+ T helper cells, and CD8+ cytotoxic T-lymphocytes. Many of the cells were TUNEL-positive [81]. No such inflammatory cells were found in the AM from mice with HSK, thus indicating that the trapping of PMN from the inflamed cornea into the AM is not the primary anti-inflammatory mechanism in the HSK model.

Using transmission electron microscopy, an increased number of macrophages with PMN apoptotic cell bodies was detected in HSK corneas 12 h after AMT. Depletion of macrophages by subconjunctival injection of Cl_2MDP liposomes induced accumulation of apoptotic cell bodies in the corneas. These data support the notion that macrophages in corneas with HSK play a pivotal role in the removal of apoptotic PMN and other cell debris (Bauer et al., submitted).

The strong decrease in PMN in the HSK model may also be caused by reduced levels of pro-inflammatory cytokines and chemokines. It has been shown that expression of IL-8, GRO-α and epithelial cell-derived neutrophil attractants (ENA) was decreased in keratocytes that were co-cultured on AM stroma [8, 83]. The chemokine CXCL1 participates in PMN chemotaxis and activation, and its expression is generally induced by TNF-α. Indeed, the HSV-infected corneas contained less CXCL-1 after AMT. However, CXCL-2, which is a chemokine that induces the migration of PMN into corneal tissue [101], was significantly increased in the corneas. It may be speculated that CXCL-2 was induced by macrophages after phagocytosis of the apoptotic PMN (Bauer et al. submitted). This is in agreement with the previous notion that murine peritoneal macrophages significantly increased the CXCL-2 expression after ingestion of apoptotic lymphocytes [94].

Macrophages of the cell line Raw 264.7 undergo apoptosis when cultured together with AM and with interferon (IFN)-γ, while apoptosis does not occur with IFN-γ or AM alone [60]. However, macrophage function was significantly impaired in activated and non-activated bone marrow macrophages (e.g., antigen presentation, cytokine production) after AM co-cultivation, independently of the presence of IFN-γ or apoptotic macrophages (our unpublished observation).

2.4.2 Anti-angiogenic Effects

Neovascularization is an unspecific response of the cornea to chronic inflammation. It may be caused by infectious or sterile corneal ulceration, immune reactions following keratoplasty, chemical or thermal burns, and by various other diseases. Although the pathomechanisms of corneal neovascularization are not completely defined, there is evidence that migration, proliferation, and differentiation of endothelial cells are up-regulated by various growth factors that are liberated during inflammation. The previously identified factors promoting inflammation

are vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF-α), basic fibroblast growth factor (FGF-2), transforming growth factors [22], and the very potent inflammatory mediator platelet-activating factor (PAF) [63]. The patterns of mediators of corneal neovascularization liberated by various stresses do not differ markedly [50].

There is sound experimental and clinical evidence that AM possesses antiangiogenic activity. Different mechanisms of antiangiogenic action are presumed to be involved. The AM contains large amounts of extracellular matrix, which possesses antiangiogenic activity by suppressing immunologic and inflammatory responses, and this was evident even in the absence of epithelial cells [81]. Additionally, soluble antiangiogenic factors are released by amniotic epithelial and mesenchymal cells, including interleukin-1 receptor antagonist, all four types of tissue inhibitors of metalloproteinase (TIMP), collagen 18, interleukin-10, thrombospondin-1, and pigment epithelium-derived factor (PEDF) [24, 50, 80]. As well as the inhibiting effect on vascular endothelial cell proliferation, a promoting effect of PEDF on corneal epithelial cell growth has been noted [80]. Thus, these two different effects after AMT may be synergistic in corneal injury repair and may amplify each other, since intact corneal epithelium also expresses PEDF [42].

The clinically observed antiangiogenic effect of AMT was already reported by Kim and Tseng in their original work on the treatment of experimental corneal disease in the rabbit [45]. The 10 eyes in the control group with stem cell deficiency demonstrated complete revascularization of the corneal surface after lamellar keratectomy within a follow-up of 3 months. Of the 13 eyes that were treated with lamellar keratectomy and combined with AMT, total vascularization was observed in only 3 eyes , while another 5 eyes had mid-peripheral and a further 5 had no or minimal vascularization.

In addition to controlling inflammation and reconstituting the corneal surface, which are commonly the major indications for AMT, reduced liberation of angiogenic factors and regression of neovascularization represent additional advantages of the procedure. Regression of corneal vessels has also been observed after AMT in patients with infectious keratitis [6, 33].

2.4.3 Promoting Re-epithelialization

Amniotic membrane has been widely applied for the treatment of various ocular surface diseases, including persistent epithelial defects, sterile corneal ulcerations, conjunctival defects, pterygia, stem cell deficiency, and chemical or thermal burns. AM, used as an inlay in these cases, functions primarily as a basement membrane substitute. Basement membrane, in general, serves to facilitate the migration of epithelial cells, reinforces the adhesion of basal epithelium, promotes cellular differentiation, and prevents cellular apoptosis. Most recently, AM has been used to provide a matrix for the ex vivo cultivation and expansion of limbal epithelial cells, which presumably contain limbal epithelial stem cells. Structural components and the presence of various growth factors have been considered as crucial action mechanisms of AM for promoting epithelialization in vivo and in vitro. Furthermore, AM protects the migrating epithelial cells against the frictional forces of eyelid movements.

Amniotic membrane consists of three layers: a single epithelial layer, a thick basement membrane, and the avascular stroma. AM, corneal, and conjunctival basement membrane all contain collagen types IV, V, and VII, as well as fibronectin and laminin-1 and -5. However, analysis of the subchains of type IV collagen and laminin revealed that amniotic basement membrane more closely resembles the composition of conjunctival rather than that of the corneal basement membrane. Therefore, the AM may be useful as a replacement for the basement membrane of the conjunctiva. For instance, laminin-5, located in the amniotic basement membrane and also in cornea and conjunctiva, functions either as an adhesive substrate or may facilitate epithelial cell migration. This bi-functional behavior of laminin-5 helps us to understand how AMT promotes wound healing in ocular surface diseases [21, 53, 54].

Moreover, AM contains several growth factors, such as epidermal growth factor, keratocyte growth factor, and hepatocyte growth factor [51], which may support re-epithelialization after AMT. However, the levels of growth factors are reduced by the cryo-preservation process that is commonly used clinically.

In addition to promoting epithelial wound healing, recent advances in tissue engineering techniques have facilitated the ex vivo expansion of human limbal epithelial cells using different culture substrates or adjuncts such as AM, fibrin, or 3T3 feeder layer systems [73, 90]. Ex vivo expansion of autologous and allogeneic human lens epithelial cells on AM is considered to be an attractive and potent technology in a variety of clinical applications. Moreover, experimental studies have shown that outgrowth rate, cell-cycle kinetics, and cell phenotype characteristics of limbal and conjunctival epithelial progenitor cells are preserved during ex vivo expansion on AM, supporting the assumption that this tissue engineering technique might be useful for renewing the stem cell populations of the entire ocular surface [36, 64].

2.4.4 Anti-microbial Effects

There is evidence that AM has diverse properties against bacterial and viral infections. The observation that chorioamniotic membrane prevents the spread of bacteria from the maternal to the fetal site suggested that it might function as a physical barrier against infection [48, 49].

Various compounds that promote antimicrobial immunity can be induced in AM. The AM from patients with intrauterine infections contained inducible nitric oxide synthase [39]. In organ culture, the amnion contained a wide variety of cytokines, such as IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-11, IL-15, TNF-α, IFN-α, -β, and -γ, activin A, inhibin A, pre-B cell colony-enhancing factor (PBEF), and leukemia inhibitory factor (LIF) [93]. The production of cytokines in AM could also be induced by other cytokines and by autacoids (e.g., prostaglandin and oxytocin), steroid hormones (e.g., hydrocortisone and progesterone), mechanical stretching, LPS, and viral infections [93].

In addition, freshly isolated AM displayed a constitutive immunity against virus infections, e.g., herpes simplex virus type-1 (HSV-1), encephalomyocarditis virus (EMCV), and vesicular stomatitis virus (VSV). In addition, AM at term comprises constitutive and induced expression of TNF-α, IFN-α, IFN-γ, or IFN-β [71, 72].

Interestingly, AM contains its own interferon, which differs from IFN-α, β, and -γ or TNF. The AM-specific IFN revealed significant cross-species antiviral and anticellular activity that differed from that of human IFN-α and -β [20]. Finally, AM is also able to store antibiotics and release them over the course of a few days [46, 65].

Summary for the Clinician

- Amniotic membrane transplantation is associated with:
  - Topical anti-inflammatory effects, including apoptotic induction of immune cells and inhibition of metalloproteinases
  - Antiangiogenic effects
  - Promotion of re-epithelialization
  - Antiviral effects

2.5 Clinical Application of Amniotic Membrane Transplantation

2.5.1 Technique of Amniotic Membrane Transplantation

The AM is the innermost layer of the placenta and consists of a single layer of ectodermal amnion cells firmly attached to a basement membrane.

2.5.1.1 Human Amniotic Membrane Preparation

The preparation of human AM has been described previously [57]. Briefly, human AM is processed after elective cesarean delivery. In the donors, blood-borne microorganisms, such as human immunodeficiency virus types 1 and 2,

hepatitis virus types B and C, and syphilis, must be excluded by serologic tests. Additionally, hepatitis virus type C is excluded by means of polymerase chain reaction.

Under a laminar flow hood, the blood clots are removed by thorough washing with sterile saline solution containing 50 µg/ml of penicillin, 50 µg/ml of streptomycin, 100 µg/ml of neomycin, and 2.5 µg/ml of Amphotericin B. The amnion is separated from the chorion by blunt dissection. It is then flattened onto nitrocellulose paper with a pore size of 0.45 µm, with the epithelium/basement membrane surface facing away from the paper. The paper with the adherent AM is stored at –80°C in sterile vials containing Dulbecco-modified Eagle medium and glycerol.

2.5.1.2 Surgery

For the operation, a peri- or retrobulbar block or topical anesthesia is preferred. Depending on the depth of the corneal ulcer, a single or multilayer technique is chosen. Briefly, the base of the corneal ulcer is cleaned off the necrotic tissue. The poorly adherent epithelium adjacent to the ulcer and the blood is removed. Amniotic membrane may be used in an "onlay" (patch) or an "inlay" (graft) technique.

2.5.1.3 Onlay Technique

The onlay technique is recommended when epithelial wound healing must be supported, and good visual acuity can be expected in cases of superficial ulcerations. A large piece of AM is placed on top of the cornea as a temporary patch, extending over the entire limbus. It is anchored on the cornea with a running 10-0 nylon suture to the peripheral cornea or the perilimbal episclera (Fig. 2.5).

2.5.1.4 Inlay Technique

The inlay technique is recommended for patients with deep corneal ulceration. It aims to protect the remaining corneal tissue and prevent perforation. A piece of AM is trimmed to fit the shape and the size of the corneal ul-

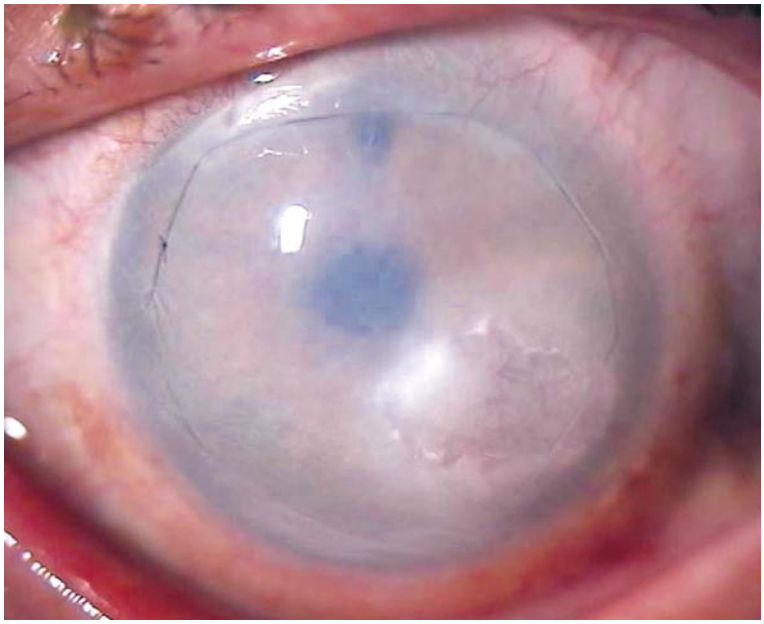

Fig. 2.5 Onlay technique of amniotic membrane transplantation in infectious corneal ulcers

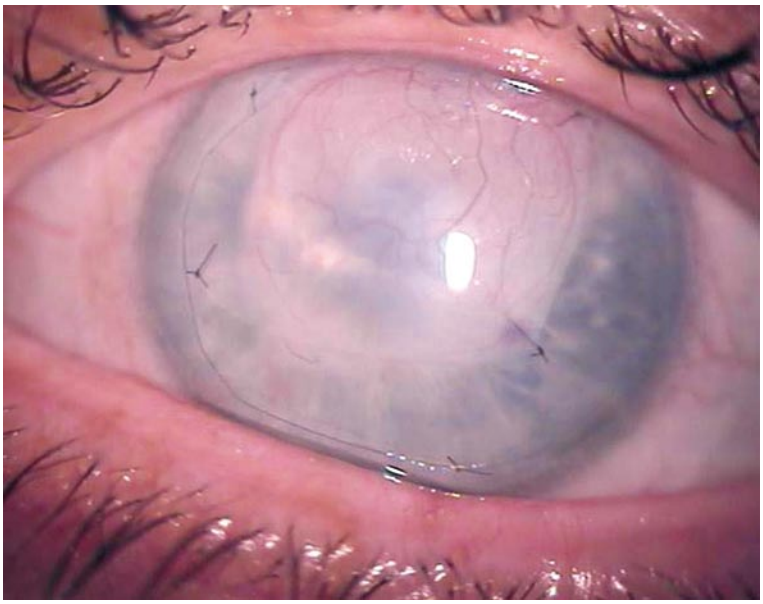

Fig. 2.6 Inlay technique of amniotic membrane transplantation in infectious corneal ulcers. Additional onlay amniotic membrane covering the entire cornea

cer and is then placed in the defect with the basement membrane side facing up. With this technique, AM functions as a basement membrane to guide the renewing epithelium. AM is then secured with interrupted 10-0 nylon sutures. When appropriate, the entire cornea is then covered with an overlay AMT (Fig. 2.6).

2.5.1.5 Multilayer Technique

For the treatment of deep corneal ulcerations, two or more layers of AM may be necessary, and all are fixed with multiple interrupted sutures [13, 23].

2.5.1.6 Postoperative Management

Commonly, a bandage contact lens is applied on the AM. The postoperative medication generally includes topical antibiotics, unpreserved phosphate-free artificial tears, and topical prednisolone.

Patients are followed up frequently with respect to visual acuity and slit-lamp appearance. Although visualization of the cornea is impaired by the whitish AM, the corneal stroma and anterior chamber can be adequately assessed. Epithelial wound healing can be judged by using fluorescent dye. The intraocular pressure can be measured reliably by applanation tonometry.

The most serious complication of AMT in treating infectious corneal ulceration is that the underlying infection spreads into the adjacent cornea, sclera, or anterior chamber. Therefore, it is mandatory that the antiviral or antibiotic treatment regimen be continued in sufficient dosages. Another serious complication is failure to obtain the desired effect of AMT. In highly inflamed eyes, the transplanted AM commonly dissolves and the procedure may need to be repeated.

Summary for the Clinician

- Human amniotic membrane is processed after elective cesarean delivery. Before use, various infectious diseases should be excluded in the donors
- The onlay technique is used when epithelial wound healing must be supported in patients with superficial ulcerations
- The inlay technique is used in deep corneal ulceration in order to protect the remaining corneal tissue
- Frequent postoperative follow-up is recommended

2.5.2 Use of Amniotic Membrane Transplantation in Infectious Corneal Ulceration

2.5.2.1 Herpetic Ulceration

Amniotic membrane transplantation has been applied as a basement membrane substitute for the management of persistent epithelial defects with and without ulcerations in herpetic keratitis patients. For the treatment of deep corneal ulcers, descemetoceles, and small corneal perforations, multilayered AMT has been recommended [23, 46, 52, 84]. Interestingly, AMT may lead to rapid improvement of corneal inflammation. A principal goal of infectious ulceration treatment is to prevent tissue destruction. Improvement in inflammation, neovascularization, and wound healing are major advantages of AMT.

Before using AM, a microbiological evaluation is necessary. An appropriate antiviral and antimicrobial medication should be instituted several days before AMT. Systemic acyclovir should be started at a dosage of 5×800 mg daily, and antibiotic eye drops and lubricants are given frequently. When ulcerative necrotizing keratitis progresses, AMT may be considered.

As the AM generally dissolves after a few weeks in these patients, a second AMT may be required. This may even occur when a multilayer AMT has been performed. Complete epithelial closure and healing of stromal inflammation can mostly be achieved within 2 to 4 weeks. Often, patients experience relief from discomfort immediately after surgery.

Generally, AMT is recommended as second-line therapy for the management of corneal ulcers that do not respond to medical treatment, bandage contact lens, or tarsorrhaphy [59]. However, AMT may represent the initial surgical approach to treating deep herpetic ulcerations [33].

In many of the patients, vision improved significantly after AMT. However, the final outcome may be limited by the severity of keratitis and the central localization of the ulceration, as dense scars and irregular, thick corneal tissue often develops during the healing process [33]. However, perforation of the globe can be avoided and inflammation arrested by the use of AMT.

Patients must be followed up postoperatively to detect any newly developing descemetocele, perforation, and hypopyon. Additionally, the ulcerative herpetic process may recur after the AM has dissolved. This is in agreement with observations that experimental HSK healed following AMT, but worsened immediately when the AM was removed [32]. Therefore, antiviral and anti-inflammatory drugs are required and must be continued for several months. In herpetic disease, systemic acyclovir at 5×400 mg daily is continued for 1 month and then 2×400 mg daily given as maintenance therapy for several months.

Whereas AMT for the management of acute infectious keratitis was presumed to be associated with a high risk of superinfection, this was not seen when adjunct antibiotics were used. AMT soaked with the antimicrobial drugs may function as a depot for drug delivery, similar to a collagen shield.

Summary for the Clinician

- Use of antivirals before and after surgery is recommended
- Amniotic membrane transplantation may represent the initial surgical approach to the treatment of deep herpetic ulcerations

2.5.2.2 Neurotrophic Ulceration

Neurotrophic keratopathy is a rare degenerative corneal disease that is commonly caused by impairment of trigeminal corneal innervation, thus leading to a decrease or absence of corneal sensitivity. Typically, corneal nerve damage is associated with an epithelial breakdown, such as punctuate defects that may potentially progress to corneal ulcers, melting, and perforation. Topical application of neuronal mediators such as the combination of substance P and insulin-like-growth factor-1 (IGF-1) or NGF alone has been shown to ameliorate neurotrophic keratopathy, and this was associated with a recovery of corneal innervation [4, 7].

Amniotic membrane has been successfully applied to promote corneal wound healing in neurotrophic conditions of different etiologies. Mono- to multilayered AM with or without an additional membrane as a patch is considered to be an effective surgical method for treating neurotrophic ulcers. AM is used as a patch when the stromal thinning is minimal and the ulcer bed appears to be non-necrotic. When used as a patch, AM dissolves after a short time period. The rationale for using AM as a patch in addition to using it as a graft is to prevent surface exposure and dryness, and in order to promote epithelial healing in patients with a poor blinking reflex.

Healing of the corneal surface after AMT correlated clinically with partial recovery of corneal sensitivity [14]. Moreover, AMT compared with conventional treatment strategies accelerated axonal sprouting in an experimental animal model of keratitis induced by herpesvirus [85].

It is still not known how the AM promotes axonal sprouting. There is general agreement that all forms of neuritic outgrowth are induced and guided by extrinsic signals from the local environment. Soluble, diffusible neurotrophins represent important factors that prevent apoptotic cell death and influence the growth patterns of distinct classes of neurons. Over the past few years, several studies have shown that AM expresses various other trophic factors, e.g., BDNF, NT-3, NGF, FGF-2, and EGF [89]. Interestingly, a high and therapeutic level of NGF was present in AM. Accordingly, topical NGF has been shown to promote epithelial healing and to recover corneal sensitivity in neurotrophic ulcers [4]. Thus, the high expression of TrkA (receptor of NGF) in the limbal basal epithelial cells suggests that NGF signaling favors limbal epithelial stem cell survival [89]. Further studies are needed to elucidate how AM might influence nerve regeneration and epithelial wound healing in neurotrophic keratopathy.

It is important to emphasize that AMT may only be considered as an option when intensive topical lubrication has failed to promote epithelial healing. In parallel, ocular defense mechanisms must be corrected, including correction of lid abnormality, tarsorrhaphy, botulinum toxin injection, and/or punctal occlusion for neurotrophic and evaporative alterations of the ocular surface.

Summary for the Clinician

- Try first to preserve or, if necessary, to restore ocular surface defense mechanisms before AMT
- Use AM as a patch and graft for the treatment of neurotrophic ulceration when intensive lubrication fails

2.5.2.3 Bacterial Ulceration

Only a small number of publications have reported on AMT in patients with bacterial ulcer. Here, AMT can be considered after sufficient antibiotic treatment for at least 2–3 days. Kim and co-workers used AM in 9 patients with bacterial corneal ulcer. Before application of the AM, the causative agent was identified (*Staphylococcus* spp., $n=4$, *Pseudomonas*, $n=5$). The AM was placed after the patient received antibiotic therapy and clinical improvement was observed. In 7 of the 9 patients, the epithelium healed completely. In another patient, a stable corneal surface was noted and in the last patient neovascularization was reduced. Visual acuity improved in 7 of the 9 patients. Three patients received a soft contact lens and two others underwent an additional debridement procedure [46].

Chen and colleagues describe their observa-

tion after AMT in 6 eyes with proven *Pseudomonas aeruginosa* infection. These patients received fortified eye drops for 1 week before the AM was placed. The authors report that the lesions became sterile 1 week after transplantation, and re-epithelialization and decreased inflammation were observed in 5 of the 6 cases. In the remaining patient, as the AM dissolved rapidly and the inflammation and the ulcer were still active, evisceration was ultimately performed [11].

Ma and co-workers (2002) reported similar results in 4 patients who presented with scleral melting and corneal perforation in recalcitrant infectious scleral and corneoscleral ulcers. All patients had *Pseudomonas* infections after pterygium surgery. Similar to the other groups these authors conclude that AMT is effective at promoting re-epithelialization and reducing corneal melting and inflammation.

Summary for the Clinician

- Use of appropriate antibiotics before and after surgery is recommended
- Amniotic membrane transplantation may be indicated in persistent epithelial defects and progressive corneal melting

2.5.2.4 Acanthamoeba Ulceration

Only very limited data have been published on the use of AMT as a treatment for *Acanthamoeba* corneal ulcers that are resistant to conventional treatment. Kim et al. (2001) treated 3 patients with *Acanthamoeba* corneal ulcers with AMT. In all of them, visual acuity improved and the corneal surface was stabilized. While complete re-epithelialization was obtained in 2 patients, in another 1 the epithelial defect did not heal completely, ultimately requiring penetrating keratoplasty [46].

Bourcier and colleagues (2004) described their experience with 6 patients suffering from *Acanthamoeba* corneal ulcers who were treated with AMT (mean follow-up 14 months). In 4 of them, complete re-epithelialization was achieved, and in another 2, partial healing. In all patients, ocular inflammation was reduced after AMT. Five out of six patients reported significant pain relief after AMT. The authors concluded that AMT is a safe and effective treatment approach for severe *Acanthamoeba* corneal ulcers, even during the acute phase of disease, and that it delays having to perform penetrating keratoplasty.

Hick and co-authors [37] described a patient with a severe *Acanthamoeba* corneal ulcer that was not stabilized with AMT. While re-epithelialization occurred 7 weeks after AMT, a recurrence was seen 2 months later. Ultimately, penetrating keratoplasty had to be performed.

2.5.2.5 Keratoplasty and Amniotic Membrane Transplantation

AMT has been suggested to be an effective approach to restoring corneal surface integrity in corneal ulcerations of various etiologies [10, 46, 52, 62]. The acute intervention with AMT may halt the inflammation and progression of corneal melting. Whereas deep corneal ulcers and corneal perforation historically were absolute indications for penetrating keratoplasty, these conditions can also be managed by using the inlay and multilayer AMT techniques. After healing and re-epithelialization are completed, a penetrating keratoplasty can then be performed for visual rehabilitation. By avoiding the need for a keratoplasty à chaud, the long-term outcome may be improved significantly [6, 10, 62].

To reduce corneal scarring and neovascularization, an additional AMT may be applied as a patch combined with the keratoplasty procedure. The anti-inflammatory factors liberated from the AM may decrease the risk of postoperative immune reactions [81]. In addition, the AM may prevent neovascularization of the transplant and promote rapid re-epithelialization of the corneal graft.

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Chapter 3

Corneal Regenerative Medicine: Corneal Substitutes for Transplantation

3

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Core Messages

- Corneal substitutes are needed to address the shortage of human donor tissues and the current disadvantages in some clinical indications, including immune rejection
- Substitutes have been designed to replace part of or the full thickness of damaged or diseased corneas. They range from prostheses, known as keratoprostheses (KPros), through naturally fabricated, cell-based, tissue equivalents, to tissue-engineered scaffolds that serve as templates for the regeneration of host tissues
- At present, widely accepted substitutes are not available although prostheses (KPros) have been in clinical testing or in limited clinical use
- The trends toward replacement of only damaged portions of the cornea and replacement of the epithelium by corneal limbal cell transplant has been gaining momentum
- Corneal substitutes that encourage regeneration of the host tissue may likely overcome the rejection problems and other postoperative complications of donor tissue transplantation and KPros
- There will probably not be a single "one-size-fits-all" corneal substitute for all indications. Instead, a small range of corneal substitutes that are tailored to different clusters of clinical indications will be available

3.1 Introduction

3.1.1 Key Corneal Properties and the Need for Substitutes for Donor Tissues

According to the World Health Organization, corneal diseases are a major cause of vision loss and blindness worldwide, in both adults and children [16, 57]. Estimates from the Vision Share Consortium of Eye Banks, USA, puts the number of affected individuals at over 10 million. It has also been estimated that ocular trauma and cornea ulceration results in 1.5 to 2 million new cases of corneal blindness annually [16]. In many cases, corneal blindness can be treated by transplantation of human donor tissue. Even though cornea transplants are the most successful organ transplants, graft survival rates are only moderate: 86% at 1 year postoperatively, 73% at 5 years, 62% at 10 years, and 55% at 15 years, with no

improvement in outcome seen over 15 years of grafting [58].

Apart from long-term graft survival issues, because of longer life expectancies, the increasing incidence of infectious diseases (HIV, hepatitis, CJD) [13, 24, 35] and the growing popularity of refractive surgery (surgically-treated corneas are significantly thinned and are therefore unacceptable as donor tissues), the demand for good quality donor corneas is expected to rise beyond the available supply. At the present time, the USA is the only country that is self-sufficient in the supply of donor corneal tissue and has a surplus; many other countries are experiencing a shortage.

In addition to donor cornea shortages, the success of corneal transplantation is dependent upon the clinical indication. For example, conditions such as inactive central scars or keratoconus are amenable to transplantation, while disorders such as autoimmune conditions, alkali burns or recurrent graft failures have poor prognoses [8]. An alternative for these patients is replacement of the damaged cornea with a tissue-engineered substitute.

Structurally, the cornea is avascular, comprising three main cellular layers: an outer stratified epithelial layer (about 50 μm thick), a stroma layer (central thickness about 500 μm), and an inner endothelial layer (5 μm thick). Sandwiched between the epithelium and stroma is the acellular Bowman's membrane, while the acellular Descemet's membrane separates the stroma from the endothelium. Biochemically, the cornea can be thought of as a hydrogel, a hydrated matrix composed of approximately 80% water [32, 53], 13.6% collagen (mainly type I), and 0.9% glycosaminoglycans [38].

In order to be clinically applicable, any fabricated corneal substitute would need to replicate the functions of the human cornea. The human cornea constitutes 75% of the refractive power for light aimed at the retina. Optical clarity (high light transmission, low backscatter) is therefore a major requirement for any successful corneal substitute. The cornea also functions as a tough protective barrier for the delicate internal eye tissues, and act as a first line of defense against infective pathogens [45].

The bioengineered corneal substitutes that have been designed to date are all aimed at minimally restoring light transmission and the protective function, replacing part or the full thickness of damaged or diseased corneas. They range from prostheses, known as keratoprostheses (KPros), through naturally fabricated, cell-based, tissue equivalents, to tissue-engineered scaffolds that serve as templates for the regeneration of host tissues. At present, widely accepted substitutes are not available [35], although there has been a lot of recent progress. This review provides a survey of the developments in the area of corneal substitutes, focusing in particular on important developments over the past few years.

3.2 Synthetic "Artificial Corneas" or Keratoprostheses

3.2.1 Development of Keratoprostheses

The development of keratoprostheses (KPros) has been on-going since the 18th century, with the earliest recorded insertion of a glass implant into a human patient being by Heusser in 1859 [20]. There have been four generations of keratoprostheses, as defined by the KPro Study group, which was created in 1990 to foster basic and clinical research on corneal substitutes [48].

First-generation KPros referred to the monoblocks or one-piece prostheses composed of synthetic plastics such as PMMA.

Second-generation KPros include the osteo-odonto keratoprosthesis (OOKP) with biologic skirts. This prosthesis, developed by Strampelli in 1964, consists of an optical cylinder of PMMA and an osteo-odontal biological support.

Third-generation KPros encompass a range of devices with synthetic plastic optics, with either metal parts to aid anchoring to host tissues, or attachments to donor tissue skirts for host integration.

Fourth-generation KPros include the optic-skirt model in which a solid optical core was surrounded by a porous skirt that encourages biointegration with the adjacent host tissues [9, 31, 54], thereby preventing implant extrusion.

The following is a synopsis of several examples of KPros that have either been tested clinically or that are currently in clinical use.

3.2.2 Keratoprostheses Tested Clinically or in Clinical Use

3.2.2.1 Boston Keratoprosthesis

The Dohlman-Doane KPro (or Boston KPro) is made of PMMA and there are two basic types. The single collar-button (type I) is the more frequently used. It consists of a front plate (5.5–7 mm diameter), a stem (3.5 mm diameter), and a back plate (7 mm diameter) with eight holes to facilitate hydration and nutrition of the graft. This KPro was approved by the US FDA and has been implanted into patients who have had repeated graft failures resulting from conditions that include herpetic keratitis, chemical burns and congenital glaucoma [2]. Complications from implantation of the Boston KPro include retroprosthetic membrane formation, glaucoma, retina detachment, and corneal melting. These complications have helped to narrow down the indications for use of the device. Studies on 133 patients between 2003 and 2005 demonstrated that the type I KPro is a good alternative today for patients encountering multiple graft failures and those in some cases with poor prognosis for primary penetrating keratoplasty.

3.2.2.2 Osteo-odonto Keratoprosthesis

The osteo-odonto keratoprosthesis (OOKP) has been used for patients with dry eyes and those with a minimal chance of graft survival. It consists of a central PMMA optic surrounded by a skirt that comprises an annular wafer taken from an autologous tooth. Prior to implantation into the eye, the OOKP is pre-implanted into the cheek of the patient to allow pre-colonization of the osteodental skirt with autologous fibroblasts. In general, this KPro, which has been in use since the 1960s, has had a lower extrusion rate than current other KPros, probably because the osteodental skirt material (mainly the mineral constituent, hydroxyapatite) provides a conducive initial environment for cell colonization [40] and these cells in turn facilitate graft–host integration and therefore stable anchorage of the prosthesis. Disadvantages of this KPro include the very complex surgical procedure and very limited visual field. In addition, decentration of the optic cylinder due to partial absorption of the osteodental lamina can occur, and the intraocular pressure cannot be measured accurately. Other complications that have been associated with this device include glaucoma, retroprosthetic membrane formation and inflammation. Smoking is a possible contraindication as an autologous tooth is used in the fabrication of the device and the teeth of smokers have not been effective as skirts.

3.2.2.3 AlphaCor™ Keratoprosthesis

The AlphaCor™ KPro, which is a newer iteration of the Chirila KPro [19], is made of poly (2-hydroxyethyl methacrylate (PHEMA). It is a one-piece device that comprises a transparent core and an opaque porous skirt, both of PHEMA, unified by interpenetration of the polymers, which differ only in water content [10]. There is therefore no glued or mechanical junction. The KPro has a diameter of 14 mm and a thickness of 0.5 mm. The optic has a radius of 3 mm. The porous skirt allows stromal cells to grow into it, thereby anchoring the device stably within the host eye. Implantation of the device involves a two-stage surgical procedure (Fig. 3.1). During the first stage, the device is implanted intra-stromally after removing a small area of the central posterior corneal lamella. The lamellar pocket is then sutured closed with a conjunctival flap over the anterior surface. The second procedure is usually performed 12 weeks later, and involves removal of the anterior corneal lamella and conjunctival flap to expose the optic (Fig. 3.1b).

This device has recently gained regulatory approval in North America, Australia, and Europe. The targeted clinical use is for patients with scarred, vascularized or diseased corneal tissues who are either not eligible for conventional donor tissue transplants or who have had multiple previous graft failures. Despite some promising results, complications still remain. These complications include the formation of retroprosthetic membranes, corneal melt, retained lenticular material, and optic depositions (Fig. 3.1c). Device extrusion has been observed (Fig. 3.1d), but is rare. Although integrated into the host tissue, the device is still a prosthesis, with no

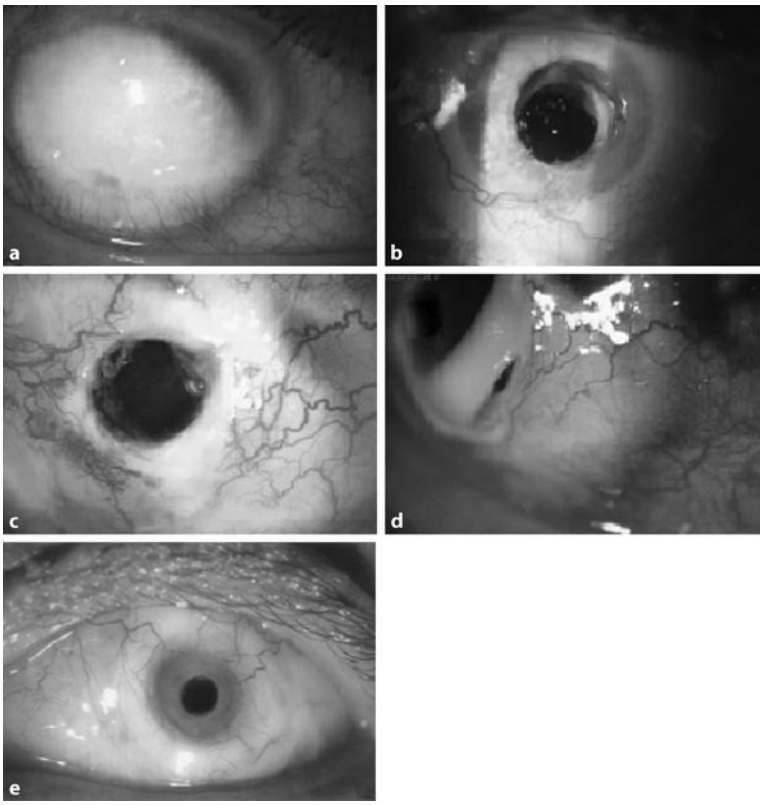


Fig. 3.1 The AlphaCor™ KPro is targeted at patients with corneal conditions not amenable to conventional grafting. For example, the preoperative cornea shown in **a** has had multiple graft rejections with severe vascularization and opacification. **b** The same cornea after implantation of the KPro and exposure of the optic. **c** The KPro is dislocated at 1 month post-exposure of the optic, and extrusion of the device (white rim is protruding) is seen **d** at 40 days after exposure of the optic. Photos reproduced from [7], with permission from Blackwell Publishing. **e** Successful implantation of the AlpharCor™ at 22 months postoperatively, with a clear optic. Photo reprinted from [8], with permission from Elsevier

epithelial overgrowth over the device after implantation. Contraindications for its use are in patients with a history of ocular herpes simplex virus-1 (HSV-1) infection, an abnormal tear film or uncontrolled high IOP. As for the OOKP, smoking is a possible contraindication, as brown deposits over the optic have been reported in smokers.

3.2.2.4 BioKPro III

The latest version of this colonizable KPro from the Legeais group, the BioKPro III, was recently clinically evaluated at Moorfields Eye Hospital, London, UK [22]. The BioKpro III consists of a central silicone optic (5 mm diameter, 500 mm thick) and a surrounding opaque skirt comprising a disc of porous fluorocarbon, PTFE. This skirt has an outer diameter of 10 mm, is 250 μm thick, with pores that are 80 μm in size. The device was inserted into 7 patients with severe corneal scarring due to ocular cicatricial pemphigoid, measles keratitis, thermal injury, Stevens-Johnson syndrome, aniridia, chemical injury, and congenital rubella. Implantation of this device, like the AlphaCor™, requires a two-stage procedure: initial device implantation step and subsequent optic exposure. As with the AlphaCor™, after implantation, the device was cov-

ered with either a conjunctival flap or a buccal mucous membrane graft, which was later opened to expose the optic. The follow-up was between 18–48 months. The results showed that the KPro failed in 6 patients due to extrusion occurring between 2 and 28 months postoperatively. Three patients developed retroprosthetic membranes and 1 developed endophthalmitis. Only 1 patient, who had a thermal burn, retained the KPro with vision improved from hand movements to 6/12. However, this patient also reported problems of mucus accumulation on the optic. Results from the clinical trial of this device were rather poor, indicating a need for further improvement to be clinically viable.

3.2.2.5 Seoul Type Keratoprosthesis

The Seoul-type KPro also has an optic and a skirt, but has additional haptics for increased post-implantation mechanical biostability [29]. Indeed, the distinguishing feature of this KPro is the double-fixation design, i.e., the device is anchored to the patient's eye both by suturing of the skirt to the cornea and by the fixation of the haptics to the sclera. The optic is made of PMMA. The skirt is made of either polyurethane or polypropylene, while the haptics comprise polypropylene monofilaments. Preliminary results of the first seven human cases indicated that like the AlphaCor™, resulting complications include retinal detachment, retroprosthetic membrane formation, and extrusion.

3.2.2.6 Pintucci Keratoprosthesis

The Pintucci KPro consists of an optical cylinder (3 mm thick and 5 mm long) made of PMMA with a refractive power of 60 D, to which a woven, circular and waterproof Dacron membrane (0.7 mm thick, 10 mm diameter) is fixed [21]. The Dacron membrane was designed to allow tissue integration. Like the OOKP, the device is pre-implanted into the patient for colonization of the skirt. This device, however, is implanted into the lower lid (and not cheek) for pre-colonization of the Dacron with connective tissue cells. Three months later, the prosthesis is removed from the skin bed, the Dacron felt is cleaned of more coarse tissue and then implanted in a manner similar to that of an OOKP.

The clinical results of 31 patients who had received implants between 1997 and 2004 were reported very recently [36]. The ages of the patients ranged from 7 to 65 years, with a mean age of 34 years. The indications for implantation in the patients were as follows: 11 cases of chemical burns, 11 cases of highly vascularized failed grafts, 6 cases of severe dry eyes with totally vascularized cornea, and 3 miscellaneous cases. All 31 of these eyes were unsuitable for conventional donor corneal transplants, or had had multiple failed keratoplasties. Twenty-eight bilaterally blind Asian patients, with vision not exceeding hand motion close to the face in the better eye, underwent the classical two-stage procedure to implant the PKPro. In another 3 patients, the PKPro was implanted as a one-stage procedure. The follow-up periods for all patients ranged from 6 months to 7 years. The results showed that unlike other currently used KPros with common complications, none of the eyes with implanted PKPros had infections or developed retroprosthetic membranes. Twenty-four of the 31 eyes improved to greater than finger counting at 1.5 m, enabling these patients to function independently. Four of the 31 eyes (13%) improved to 20/200 or better. However, 12 of the 31 eyes had significant complications, but only a few of these complications were vision-threatening.

3.2.3 Recent Developments in Keratoprosthesis Research

In recent years, artificial cornea researchers believe that re-growth of an intact corneal epithelial layer over the KPro may help to stabilize the tear film, and prevent extrusion and infection [54], which are complications observed with more traditional KPros. However, most of the KPro materials such as PMMA and PHEMA or PVA are non-cell adhesive. To improve cell adhesion and migration over the KPro, naturally occurring extracellular matrix proteins such as collagen, laminin, and fibronectin, or cell adhesive peptides derived from these proteins (e.g., RGD, YIGSR), have been grafted onto materials for prospective

KPros. The following are some of the most recent promising KPros and potential KPro biomaterials. Most of these are at the stage of in vitro development or animal trials.

3.2.3.1 Modification of Keratoprosthesis Biomaterials with Bioactive Factors

Over the past few years, there has been a flurry of KPro research in which biocompatible materials have been grafted with various cell adhesion peptide sequences from extracellular matrix molecules to enhance biological interaction with potential host cells [3, 14, 30, 39, 41] and even sheets of human amniotic membrane [56].

Sheardown and co-workers (cited in [3]) modified poly(dimethyl siloxane) (PDMS) surfaces by covalent attachment of combinations of cell adhesion peptides derived from laminin and fibronectin, and their synergistic peptides. The peptides studied included YIGSR and its synergistic peptide PDSGR from laminin and fibronectin-derived RGDS and PHSRN. Statistical analysis of the experimental adhesion results suggested that the concentrations of YIGSR, RGDS, and PHSRN used, as well as the synergistic effect of YIGSR and PDSGR, had significant effects on cell attachment and proliferation. Modification with multiple peptides resulted in greater adhesion and proliferation of corneal epithelial cells than modification with single peptides only, suggesting that surface modification with appropriate combinations of cell adhesion peptides and synergistic peptides may result in improved cell surface interactions.

Jacob and colleagues [26] investigated the corneal epithelial cell growth rate and adhesion to polymethacrylic acid-co-2-hydroxyethyl methacrylate (PHEMA/MAA) hydrogels that had been modified with combinations of bioactive factors including extracellular matrix proteins and cytokines (e.g., fibronectin, laminin, substance P, and insulin-like growth factor-1 [IGF-1]) and peptide sequences (e.g., RGD and fibronectin adhesion-promoting peptide [FAP]). These were either coated directly onto the surfaces or tethered through poly(ethylene glycol; PEG) spacers. They reported little or no epithelial cell growth occurring on the unmodified hydrogel surfaces at the end of a 15-day culture period. Only coated laminin showed an increase in epithelial growth, but only to about 20% confluence. It was found that only spacer tethered molecules provided the microenvironment for epithelial cells to reach 100% confluence. These studies show that biologically derived glycoproteins or peptide sequences can be used effectively to promote biointeraction with cells. However, the active motifs need to be accessible to the cells, as demonstrated by the PEG-tethered groups vs. coated surfaces. In addition, it is important to consider that the peptides or proteins are susceptible to biodegradation, and measures need to be taken to ensure that the long-term anchorage of overlying epithelial layers is maintained.

3.2.3 Stanford Keratoprosthesis

A very recent KPro that incorporates the grafting of bioactive factors with a change in bulk material design was developed by David Myung, Curtis Frank, and Christopher Ta at Stanford University (CA, USA). Their core-and-skirt KPro is based on a mechanically enhanced hydrogel material called Duoptix™. It consists of a "double network" of poly(ethylene glycol) and poly(acrylic acid) (PEG/PAA) in its central optic component that supports the growth of epithelial cells. Surrounding the optic is a microperforated rim designed to promote peripheral tissue integration with the host eye (Fig. 3.2). This design strategy integrates a number of polymer technologies that have been largely untapped in corneal tissue engineering.

This class of materials is distinguished from single network hydrogels by their high strength despite high levels of water (60–90%). The double network combination is particularly advantageous for an optical device due to the high strength, transparency, and permeability of the blend, as well as the intrinsic protein resistance and biocompatibility of its components, PEG and PAA [42]. The team has also used a versatile photochemical surface modification strategy to site-specifically tether cell adhesion-promoting biomolecules to these otherwise non-adhesive hydrogels. A further innovation is the application of photolithographic patterning to the fabrication of

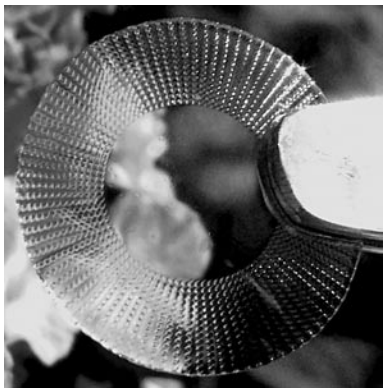


Fig. 3.2 Stanford KPro prototype showing the transparent, microperforated rim that is seamlessly joined to a transparent central optic. Photo courtesy of Dr. Christopher Ta, Stanford University, USA

the device, which provides a high level of control over the shape and structure of a hydrogel, and potentially over the growth and differentiation of cells. Longer trials are ongoing, but to date, this KPro has been shown to be tolerated well in an animal model for up to 6 weeks [5].

3.2.3.3 Collagen-based Keratoprosthesis

Sheardown and colleagues (cited in [12]) have developed a collagen dendrimer-based material for a keratoprosthesis. Residual dendrimer amine groups were modified to incorporate the laminin cell adhesion peptide, YIGSR, into the bulk of the matrix. The surface was also modified to incorporate YIGSR. The authors reported that YIGSR incorporation in the hydrogels promoted corneal epithelial adhesion and spread. In addition, the materials also allowed neurite extension from the NDC neuroprogenitor cell lines (Fig. 3.3).

Summary for the Clinician

- KPros that are designed to be biointeractive with the surrounding host cells and encourage biointegration appear to have solved many of the early problems of non-biocompatibility and extrusion. These later models have the promise to perform well in upcoming clinical testing
- However, it is very important to recognize that earlier prostheses and updated versions of these have now been tested clinically for decades and although not in widespread use, are still useful treatments for patients with corneal blindness who are unable to receive conventional corneal grafts or who have had repeatedly rejected grafts of donor corneas

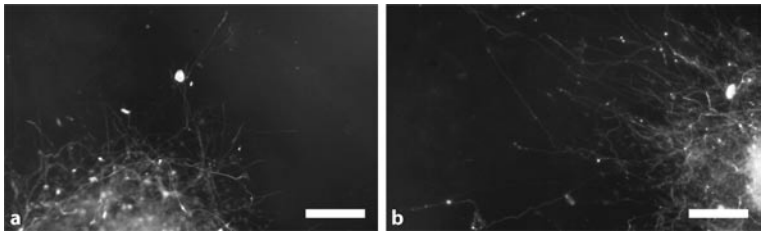


Fig. 3.3 Extension of longer neurites from **a** neuronal progenitor cell line, NDC, on a YIGSR-modified collagen-dendrimer hydrogels compared with **b** unmodified control hydrogels. *Bars*, 100 μm

3.3 Naturally Fabricated Corneal Replacements

3.3.1 Self-assembled Corneal Equivalents

Of the corneas developed as possible implantable corneal substitutes, the model developed by the Laboratoire d'Organogenese Experimentale (LOEX) uses a self-assembly approach [15]. Ascorbic acid is used to stimulate the production of collagen and other extracellular matrix molecules by stromal cells. These matrix glycoproteins are elaborated in sheets. These sheets are then stacked together, allowed further integration in culture and an epithelium is seeded on top of the stack. In their previous tissue-engineered blood vessels, they showed that high tensile strength could be achieved using this method [4] suggesting that this might be achieved in the corneal models as well. Structurally, these constructs show excellent corneal morphology and cells express appropriate tissue-specific markers. However, this very innovative approach necessitates the use of non-autologous cells to produce enough transplantable material rapidly for transplantation. It would take a much longer period of time for autologous biopsies to produce the amounts of collagen required than having a pre-synthesized matrix. In addition, no optical data from this corneal model was reported and, as with the prosthetic devices, these natural materials have not been assessed for the ability to regenerate nerves.

3.3.2 Corneal Layers Reconstructed on Pre-existing Natural Scaffolds

3.3.2.1 Corneal Epithelial Reconstruction on Amniotic Membranes

Some corneal disorders are caused solely by disease or damage of the ocular surface. For such conditions, current treatments involve the removal of a large piece of the cornea that includes the diseased or damaged region. However, more recent treatment approaches have targeted the replacement of the damaged area only, rather than the entire tissue through penetrating keratoplasty. Corneal epithelial stem cells have been successfully expanded in culture to create transplantable sheets [49]. Acellular or decellularized tissue sheets, such as small intestinal submucosa (SIS) or pericardium, have been extensively examined as naturally occurring scaffolds for tissue engineering. For corneal surface reconstruction, the human amniotic membrane (AM) has been widely investigated as a naturally occurring carrier. The AM has been used for the expansion of cells isolated from the limbal region in the creation of epithelial sheets for transplantation [50, 55] and has shown success in patients with severely depleted corneal limbal epithelial cells.

Corneal epithelial cells may also be cultured *ex vivo* and then transplanted without a carrier membrane [44]. This approach utilizes a 3T3 cell feeder layer upon which epithelial cells are seeded to produce epithelial sheets on temperature-responsive culture dishes. After allowing the sheets to form, they can be removed by lowering the temperature of the culture dish. These sheets created a stable epithelium that retained some of its stem cells upon transplantation onto the surface of rabbit corneas.

Several reviews on the topic of ocular surface reconstruction are available [43, 51, 52].

3.3.2.2 Reconstruction of Corneal Epithelial and Stromal Layers on Amniotic Membranes

Jang et al. [27] evaluated the efficacy of AMs as a scaffold for reconstructing the epithelial and stromal layers of the cornea for transplantation. Essentially, they seeded autologous corneal epithelial cells and fibroblasts on a lyophilized amniotic membrane (LAM) and transplanted the reconstructed corneal layers into a rabbit model of severely alkali-burned corneas. All rabbit eyes were treated with 1 N NaOH for 30 s to create an alkali burn with limbal deficiency and a deeply damaged corneal stroma. Their results indicated that wound healing and stabilization of the ocular surfaces occurred much faster in eyes that were grafted with the two reconstructed au-

tologous corneal layers than in eyes transplanted with autologous corneal epithelium alone or in untreated eyes. However, mild peripheral neovascularization was observed in the eyes that had received the reconstructed corneal epithelial and fibroblast layers. The control, untreated, alkali-burnt eyes exhibited typical limbal deficiencies with conjunctivalization and persistent corneal epithelial defects. These results indicated that transplantation of the corneal layer reconstructed from autologous corneal epithelial cells and fibroblasts on LAM could accelerate the recovery of the alkali-injured rabbit cornea and this result may be beneficial to the treatment of human corneas with similar problems.

3.3.2.3 Reconstruction of Corneal Endothelium on Denuded Corneal Stromas

Corneal endothelial layers have also been reconstructed on human corneal stromas that were denuded of all endothelial cells. These were used as scaffolds upon which human corneal endothelial cells were seeded [1]. The resulting stroma-endothelial constructs had endothelial cells that were morphologically similar to those of cells in vivo. The reported endothelial cell density was approximately 80% of that of normal human cornea. The pump function parameters were 55–75% of those of normal corneas. The advantage of this approach is that the strength of the reconstructed cornea is increased compared with that of previous versions constructed on soft collagen gels. However, the disadvantage of such constructs is the possibility of disease transmission from the cadaveric corneas.

3.3.3 Corneal Replacements with Noncorneal Cell Sources

Inatomi et al. [25] very recently reported the use of a two-step surgical combination of cultivated autologous oral mucosal epithelial transplantation (COMET) and penetrating keratoplasty to treat patients with severely depleted corneal limbal stem cells. Two patients with Stevens-Johnson syndrome and chemical eye injury were treated by COMET followed by penetrating keratoplasty performed 6 months later. After an average follow-up period of 22.5 months, the authors reported that the ocular surfaces were successfully reconstructed and the oral mucosal epithelial cells persisted over the follow-up period. No clinical complications, such as persistent epithelial defects, rejections, or recurrence of cicatrization, were encountered. Postoperative best-corrected visual acuity was 20/125 in one patient and 20/100 in the other. Although the reconstructed epithelium expressed keratin marker K3, but not K12, which is indicative of oral mucosal epithelium, the overall morphology and cell density of the basal, precursor cell layer in these grafts were similar to those of normal cornea. This suggests that oral mucosal epithelium can be an effective tissue substitute for corneal epithelia.

Summary for the Clinician

- To date, most methods utilizing "natural" approaches and materials appear to be directed at the replacement of specific corneal layers and in particular, for replenishing corneal stem or precursor cells where deficient
- Limbal transplants have had a good measure of success
- The possibility of using autologous tissues from a different but analogous source (e.g., oral mucosal) paves the way for the use of noncorneal precursor cells
- Other precursor cells being examined include embryonic stem cells [23] and bone marrow-derived stem cells [34]

3.4 Biomimetic Tissue-engineered Scaffolds as Templates for Corneal Reconstruction or Regeneration

3.4.1 Tissue-engineered Substrates as Cell Delivery Systems

As mentioned in the previous section, human amniotic membranes have gained popularity as

a substrate for delivering in vitro expanded corneal precursor or stem cells to patients with stem cell deficiencies. However, the membranes, being of human origin, carry the same risk of disease transmission as donor corneas. Hence, polymeric materials from both natural and synthetic sources have been studied as potential replacements for amniotic membranes.

3.4.1.1 Substrates for Corneal Epithelial Cells

An alternative to human amniotic membranes that has been used as a substrate for the delivery of the epithelial cells is a cross-linked fibronectin/fibrin gel. Han and co-workers [18] bioengineered an ocular surface tissue replacement comprising human corneal epithelial stem cells in a cross-linked, human, fibrin gel. The cells were suspended in a fibronectin/fibrin gel cross-linked by factor XIII (which was prepared from a fibrinogen-rich cryoprecipitate of human plasma). The suspended cells proliferated within the fibrin gel to near confluence over the 15 days. The presence of a protease inhibitor, aprotinin, in the incubation medium was essential to prevent gel degradation (fibrinolysis), which occurred within 24 h in its absence. This bioengineered corneal surface tissue was found to be a transportable, pliable, and stable tissue replacement. Because both the cells and the plasma components of the fibrin gel are of human origin (potentially derived from the patient), this tissue replacement represents a totally autologous, bioengineered replacement tissue.

The authors of the present review have examined the use of covalently crosslinked human recombinant collagen membranes as substrates for the expansion of human corneal limbal cells. In a collaborative study with Cellular Bioengineering Inc. (Hawaii, USA), we showed that these membranes support the adhesion and proliferation of both human corneal limbal cells and human endothelial cells (Fig. 3.4).

Ozturk and colleagues [47] fabricated a composite membrane using alginate sheets coated with chitosan and used the resulting composite membrane as base matrices for limbal epithelial cell cultivation, aiming to restore damaged human corneal surfaces with autologous corneal epithelial sheets generated by serial cultivation of the limbal epithelial cells over the different compositions of composite membranes in vitro studies. Cell attachment, spreading, and growth on chitosan-coated polymeric membranes were evaluated in vitro. The epithelial cell-cultured membranes were not studied in vivo with any animal models.

3.4.1.2 Substrates for Corneal Stromal Cells

Type I collagen is the dominant biopolymer in the human cornea (~70% of its dry weight). Because the cornea is immune-privileged, with modification, collagen may form the basis of scaffolds that allow for the reconstruction of corneas in vitro. Type I collagen sponges were used by Hubel and colleagues [6, 11] as scaffolds for culturing human corneal stromal fibroblasts. Changes resulting from cell–scaffold interactions include a change in mechanical properties such as Young's modulus over the 21-day culture period from 95 to 370 Pa. Permeability also changed from 5.3×10^{-8} – 4.2×10^{-7} m4 $N^{-1}s^{-1}$ and elaboration of fibronectin, decorin sulphate, and collagenase was observed. These data indicated a remodeling of the matrix by the fibroblasts, changing the properties toward those of a natural stroma. Indeed, the resulting tissue construct had a lamella-like microstructure rather than the porous, spongy structure of the collagen sponges. However, these stromas were only about 30% as efficient at transmitting light as excised rabbit corneas [46]. The addition of chondroitin sulphate increased the light transmission to about 50%.

3.4.2 Acellular Scaffolds as Bio-interactive Templates for Regeneration

As mentioned above, collagen, specifically type I, is the major component of natural human corneas. Type I collagen, which is a fibrillar collagen, is a molecule that contains various sites that are amenable to binding by various bioactive mol-

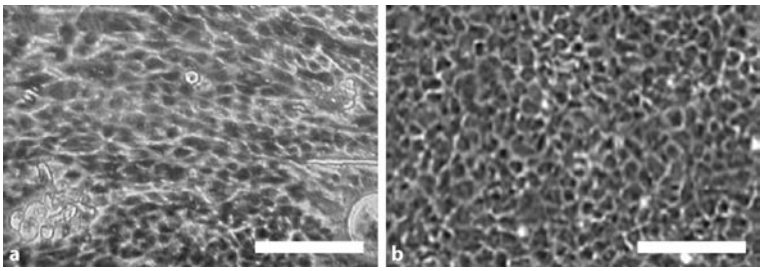


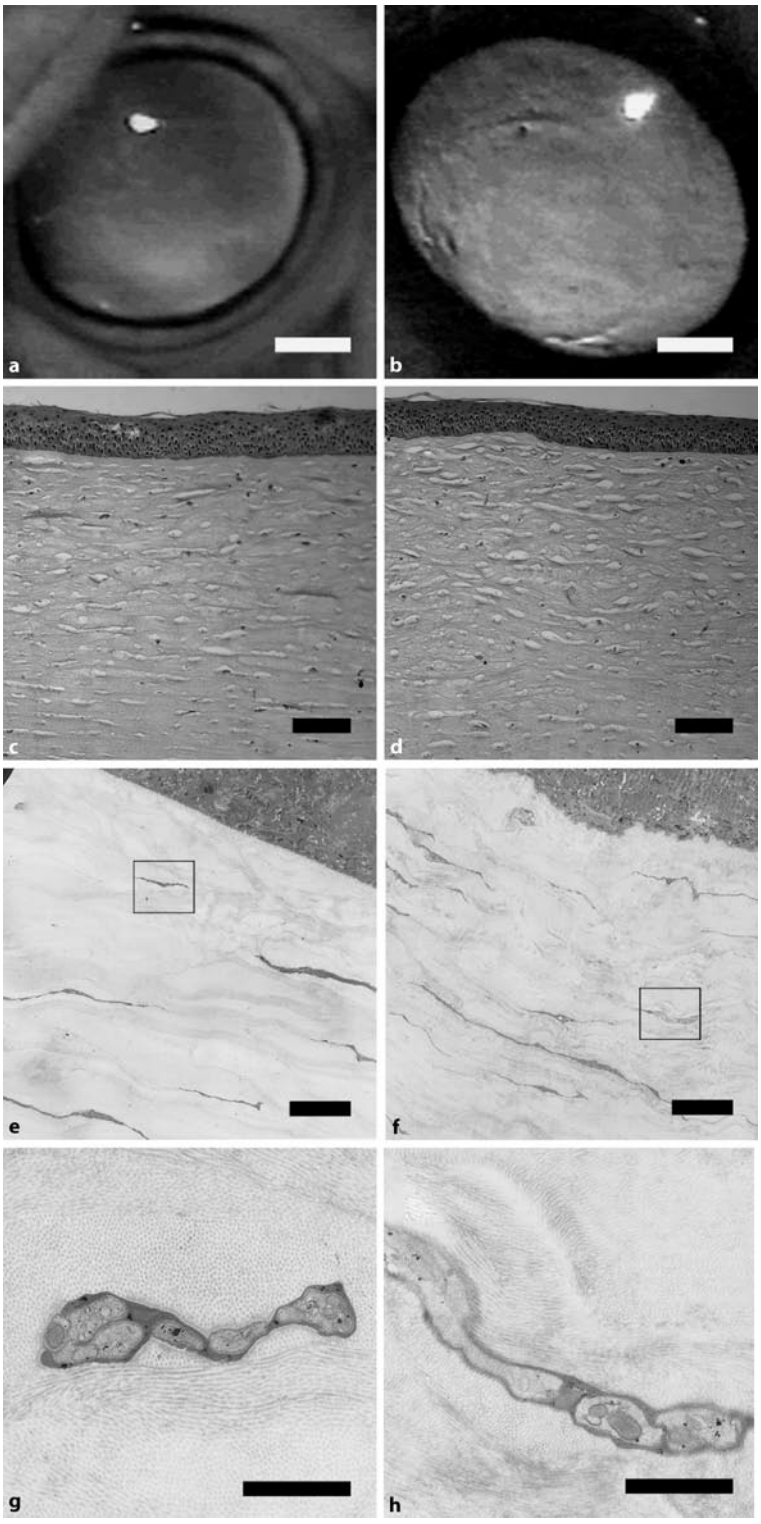
Fig. 3.4 Cross-linked recombinant human collagen hydrogel films supporting growth of confluent sheets of **a** corneal limbal epithelial cells and **b** corneal endothelial cells. Photo in b courtesy of Dr. Hank Wuh, Cellular Bioengineering Inc. *Bars*, 100 μm

ecules that modulate cellular behavior, and not just a mere structural protein. We have successfully fabricated a variety of collagen-based corneal substitutes that have been tested in rabbits, dogs and pigs as deep lamellar grafts. Hydrogels containing 90–95% water with white light transmission at 90% or higher were fabricated by molding to the dimensions desired. Both animal-extracted (porcine or bovine) collagens and recombinant human collagen were examined.

The simplest biodegradable substitutes that we fabricated comprised either medical grade porcine collagen or recombinant human collagen crosslinked with water-soluble carbodiimide,1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). EDC is from the family of protein crosslinking reagents that promotes the formation of zero-length crosslinks by facilitating the aqueous phase reaction between the amine of collagen and the carboxylic acid side-groups to form covalent, amide bonds. In contrast to other crosslinkers such as glutaraldehyde and diisocyanates, EDC does not itself become incorporated as part of the final crosslinks in these hydrogels. Furthermore, all unreacted reagents and by-products from the EDC crosslinking reaction are water-soluble and thus can be removed easily after gel formation. Hence, there is no possibility of toxic substance release into tissues from subsequent crosslink break down [17].

When implanted into animals without the use of postoperative steroids, the operated corneas remained relatively quiet, with no adverse inflammatory or rejection reactions observed. After 12 months' implantation in to mini-pigs, the implants remained integrated and optically clear. Detailed analysis at 6 months postoperatively showed restoration of the epithelium with intact barrier properties and tear film. Stromal cells had grown into the constructs, and, in addition, regeneration of corneal nerves as shown by the extension of neuritis into the implanted scaffolds was observed by in vivo confocal microscopy [33]. At 12 months postoperatively, we report that the porcine collagen implants have stably integrated into the host tissues and innervation can be observed at both light and transmission electron microscopic levels (Fig. 3.5). Early results from recombinant human collagen implants show similar results, but we are still completing a detailed analysis at this time.

We have also shown that synthetic materials can be stably combined with collagen to enhance specific properties. For example, we developed and implanted scaffolds comprising collagen-TERP5 [poly(N-isopropylacrylamide-coacrylic acid-coacryloxysuccinimide] that incorporated into the bulk, the laminin-derived pentapeptide, YIGSR. These were fabricated into the curvature and dimensions of a human cornea (Fig. 3.6a) and sutured into mini-pigs by deep lamellar keratoplasty. The incorporation of YIGSR in these implants significantly enhanced nerve regeneration, as seen by restoration of corneal touch sensitivity to almost preoperative levels within a 6-week test period, compared with allografts that showed little or no sensitivity at all (Fig. 3.6b). This observation in pigs corresponds to reports in which nerve in-growth and touch sensitivity recover slowly (>18 months after laser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◄ **Fig. 3.5** **a** Normal, untreated pig cornea and **b** contralateral cornea at 12 months postoperatively after implantation of a corneal substitute fabricated from crosslinked porcine collagen by deep lamellar keratoplasty. *Bars*, 2 mm. Histological sections, stained with H&E, through these corneas show an intact epithelium and full stroma in both **c** an unoperated control and **d** a region of the implant in the operated cornea. *Bars*, 100 μm. Transmission electron micrographs of the corneas show epithelial cells and stromal keratocytes, along with sections through sub-epithelial nerves in both **e** unoperated and **f** operated corneas. *Bars*, 5 μm. Higher magnification of the sub-epithelial region shows sections through nerves of the sub-epithelial plexus in both **g** control corneas and **h** implanted corneas. At this magnification, the collagen fibrils of the stroma in the region of the implant can also be seen to have been restored to a lamellar configuration. *Bars*, 1 μm

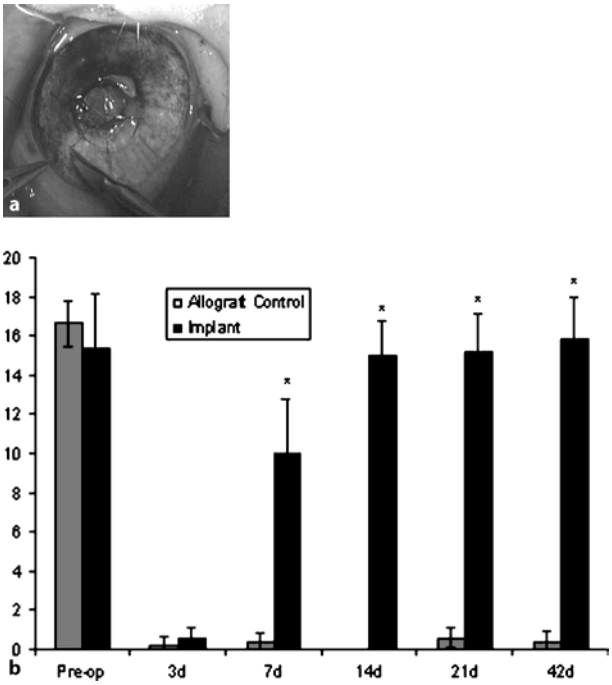

Fig. 3.6 **a** Collagen-TERP5 corneal substitute showing optical clarity and adequate robustness for handling. **b** Recovery of nerve touch sensitivity after implantation with corneal substitutes fabricated from collagen-TERP5 polymers bearing YIGSR, versus allograft controls. Regeneration is seen in the former, but not the latter over the 6-week postoperative test period. *Asterisks* indicate significant differences in sensitivity in experimental corneas from allograft controls by Student's *t* test, $p<0.05$

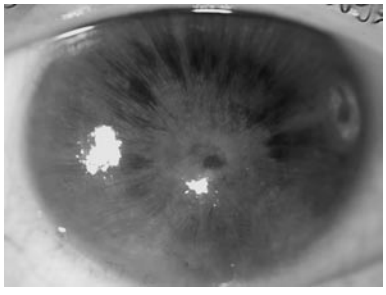

Fig. 3.7 Unsighted human cornea with implanted collagen hydrogel lenticule 7.5 mm in diameter and 50 μm thick, showing a quiet eye. Photo courtesy of Drs. Yuwen Liu and Christopher Marmo, Cooper Vision, USA

refractive eye surgery; >10 years after LKP) in humans [28, 37].

Very recently, in a collaborative study with CooperVision (Pleasanton, CA, USA), lenticules 7.5 mm in diameter and 50 μm thick fabricated from crosslinked medical grade collagen have been approved for very limited pre-clinical testing. The lenticules were implanted under the epithelium of three unsighted eyes, with informed consent. An example is shown in Fig. 3.7. In all three cases, there were no reports of discomfort, nor were any adverse inflammatory responses observed. This shows that collagen-based substitutes have the potential to be well tolerated as corneal substitutes.

3.4.3 Corneal Substitutes with Delivery of Drugs or Bioactive Factors

In order to stimulate or enhance regeneration, stem or precursor cells require an appropriate microenvironment that would include bioactive factors that modulate their behavior. In early experiments, we showed that corneal implants can also be modified to release bioactive factors such as drugs or growth factors without adversely affecting the optical or mechanical properties. Fig. 3.8b shows an example of an optically clear fabricated corneal substitute that was loaded with bovine serum albumin (BSA). The release of BSA is dependent upon initial loading (Fig. 3.8) and can be tracked by visualizing FITC-labeled BSA under fluorescence. We have been able to release growth factors in a similar manner over a minimum of several weeks (unpublished results).

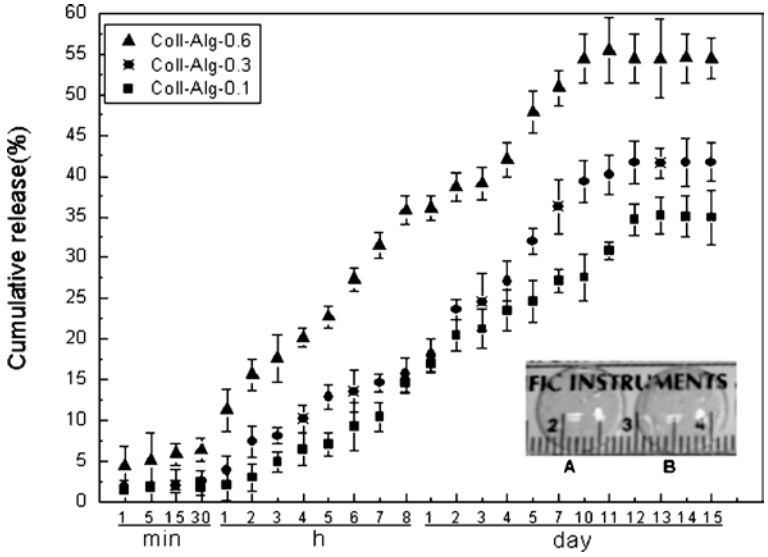

Fig. 3.8 Release kinetics of corneal constructs loaded with different concentrations of bovine serum albumen (BSA) as a model protein for drug or bioactive factor release, showing that higher loading levels (from 0.1 to 0.6 units) will release proportionally higher amounts. *Inset*: *A* corneal substitute and *B* similar substitutes loaded with BSA, showing that optical clarity was not affected

Summary for the Clinician

- Tissue-engineered scaffolds can be fabricated to replace as much of the cornea as needed
- They may be produced either fully furnished with living cells, ready for implantation, or as sterile, noncytotoxic matrices that become functional within a realistic timeframe by infiltration, ingrowth or overgrowth of cells and nerves from the adjacent, residual tissue
- Regeneration of the host cornea will (in theory) overcome the rejection problems and other postoperative complications of donor tissue transplantation and KPros
- In particular, corneal implants that allow nerve regeneration may also circumvent the potential problems resulting from the lack of nerve regeneration after surgery found both in human donor tissue and in synthetic KPros

3.5 Summary of Corneal Substitute Development and Future Clinical Use

- Significant advances have been made in the development of substitutes that are designed to replace part of or the full thickness of the damaged or diseased cornea. For example, diseases that affect only the epithelium, surface reconstruction by tissue engineering appears to be a viable alternative to more invasive full-thickness corneal replacements.
- Although corneal limbal epithelial cell expansion and transplantation are gaining momentum, there are no current corneal substitutes that are in widespread use to replace more significant tissue damage to more than one layer.
- The research to date would suggest that there is most likely not going to be a single "one-size-fits-all" corneal substitute. Instead, a small range of corneal substitutes, from prostheses to tissue-engineered substitutes that promote regeneration with or without pre-seeded stem cells will probably be available. These will be tailored to different groups of clinical indications.
- However, all the substitutes will most likely have common features such as:
  - A simple one-step implantation procedure
  - Some degree of bio-interaction to allow seamless integration into the hosts' tissues without any adverse immune or rejection problems for long-term viability
  - Stimulation of stable tissue regeneration (epithelial, stromal or endothelial ingrowth, regeneration of the corneal nerve plexus, or combinations of two or more of these) and tear film regeneration.
- With the pace of development and testing, it would appear that viable alternatives to donor corneas for transplantation are not far off.

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Chapter 4

Phototherapeutic Keratectomy in Corneal Dystrophies

4

Berthold Seitz and Achim Langenbucher

Core Messages

- Avoid "aiming and shooting" at all corneal pathologies to avoid dissatisfaction and frustration
- The best candidates for phototherapeutic keratectomy (PTK) are myopic eyes with diffuse, elevated lesions ("plus disease")
- Localized depressed (para-)central lesions (e.g., foreign body scars; "minus disease") are bad candidates for PTK, especially in cases of contact lens intolerance
- Remove as much of the diseased tissue mechanically with the blade/hockey knife
- Remove as little as possible with the laser to save tissue and allow for repeat PTK
- Use the laser predominantly for smoothing
- Use "masking agents" repeatedly during one session to smooth the surface
- Consider simultaneous refractive ablations, especially in cases of pre-existing hyperopia or after penetrating keratoplasty (PKP)
- Take into account potential subsequent PKP (save peripheral Bowman's layer for suture fixation)

4.1 Introduction

Anterior corneal pathology, such as corneal dystrophies, can be visually devastating. The development of novel surgical strategies and adjunctive technologies continues to improve the treatment of patients. Over the past decade, there has been a shift in the treatment of these conditions from corneal transplantation to excimer laser-assisted keratectomy, typically referred to today as "phototherapeutic keratectomy" (PTK) for visual restoration. PTK using the 193-nm excimer laser can produce significant visual improvement in these patients, and corneal transplantation or repeat transplantation can be delayed or even avoided. Besides superficial corneal dystrophies, degenerations, such as Salzmann's nodular degeneration, keratoconus nodules, and climatic droplet keratopathy can be treated successfully with PTK, too. Additionally, patients with anterior corneal scars from such etiologies as trauma, corneal ulcers, and prior refractive surgery can experience visual improvement with PTK.

The purpose of this chapter is to offer the clinician a practical approach based on the authors' 18 years' experience and personal perspectives concerning the treatment of corneal dystrophies, also drawing heavily upon the body of literature on this subject that has been growing immensely over the last 15 years.

4.2 Nonmechanical Corneal Surgery – Definition

"Nonmechanical" surgery of the cornea has been defined as minimally invasive, primary noncon-

tact corneal surgery using lasers of various types [27]. Typically, some amount of mechanical action is needed in the context of laser corneal surgery. Nonmechanical corneal surgery is classified as either primary "curative" or primary "refractive" applications.

4.2.1 Curative

4.2.1.1 Corneal Transplantation

Besides the classic full-thickness corneal transplantation (penetrating keratoplasty [PKP]) [16] some microsurgeons recommend anterior lamellar keratoplasty (ALKP) in cases of intact corneal endothelium (e.g., keratoconus) or posterior lamellar keratoplasty (PLKP) in cases of pure endothelial diseases (e.g., Fuchs' dystrophy). Over the last 18 years more than 2,300 excimer laser PKPs have been performed in Homburg/Saar and Erlangen with the advantage of less keratometric astigmatism after suture removal, higher topographic regularity of the corneal surface and, thus, better visual acuity with spectacles [33, 36]. Despite promising experimental results, the erbium: YAG laser has not yet been used for clinical application [45]. However, femtosecond lasers seem to be the next big thing for PKP, ALKP and also PLKP [39, 42], since intrastromal cuts are feasible without primarily affecting the corneal surface.

4.2.1.2 Phototherapeutic Keratectomy

The intended effects of PTK may be threefold:

- Removal of corneal opacities (o-PTK)
- Treatment of irregular corneal surface and astigmatism (a-PTK)
- Increase in and stabilization of epithelial adherence (e-PTK)

Such indications may overlap in a number of corneal diseases and may apply to variable degrees in a given pathology (Fig. 4.1) [20].

4.2.2 Refractive

The classic refractive surgical procedures on the cornea using the excimer laser have been photorefractive keratectomy (PRK), laser-assisted subepithelial keratectomy (LASEK), and laser-in-situ-keratomileusis (LASIK). In the latter procedure a stromal flap is created before the refractive correction with the laser takes place in the stromal bed. This flap creation can be performed with femtosecond lasers today (all-laser-LASIK = femto-LASIK).

Summary for the Clinician

- "Nonmechanical" corneal surgery has been classified in either primary curative or primary refractive applications
- Major curative applications are corneal transplantation (PKP, ALKP, PLKP) and phototherapeutic keratectomy (o-PTK, e-PTK, a-PTK)
- Major refractive applications are PRK, LASEK, and LASIK

4.3 Patient Counseling

The efficacy of PTK seems to be related to several factors, including:

- The nature of the corneal disorder
- The patient's subjective complaints
- The preoperative refractive error
- The treatment strategy, and
- The tissue ablation properties of the laser

Careful attention should be directed toward the specific patient complaints to better determine if PTK may be expected to achieve the desired clinical goals. Certainly, the patient should be completely informed of the diverse surgical steps. In particular, characteristic phenomena referred to as "sight," "sound," and "smell" have to be discussed with each patient in advance to avoid unexpected reactions intraoperatively.

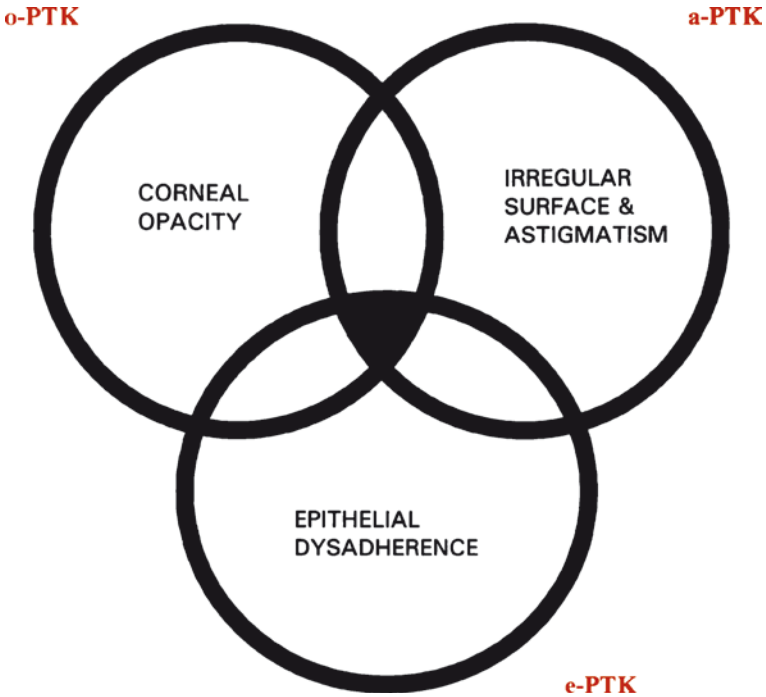

Fig. 4.1 Indications for phototherapeutic keratectomy: (1) corneal opacity (**o-PTK**), (2) irregular corneal surface and astigmatism (**a-PTK**), (3) and epithelial dysadherence (**e-PTK**). Such indications may overlap in a number of corneal disease processes and may apply to variable degrees in a given pathology (modified after [20]).

4.3.1 Corneal Clarity

A crystal clear cornea is typically not the goal of a PTK to treat superficial corneal dystrophies. The goal is to postpone or even avoid lamellar or penetrating keratoplasty. Often, removal of major central opacities leads to a considerable increase in visual acuity [41]. Typically, some deposits are left in the midstroma (e.g., in granular dystrophy) without major disadvantages. The dystrophy may recur early after PTK, especially in macular dystrophy [17] and Reis-Bücklers' dystrophy [9, 23]. In contrast, map-dot-fingerprint dystrophy [29] and granular dystrophy [8, 26] will recur later. In the case of recurrence of the dystrophy, repeat PTK or PKP/ALKP may be necessary [3, 18, 47].

4.3.2 Visual Acuity and Refraction

Depending on the preoperative refraction PTK may lead to an increased best corrected visual acuity (BCVA), despite a decrease in the uncorrected visual acuity (UCVA). This may be due to a hyperopic shift after central PTK. A BCVA of 1.0 (20/20) is not the goal. Often, the patient is highly satisfied when the VA increases from 0.2–0.3 to 0.6–0.8. In some cases, even a hard contact lens may be necessary to achieve good vision. The patient must be aware of this potential problem in advance and should not be confronted with this issue for the first time after PTK in case a major hyperopic shift has occurred [44].

4.3.3 Recurrent Corneal Erosion Syndrome

Corneal dystrophies (especially anterior basement membrane dystrophy) are often associated with recurrent corneal erosion syndrome (RCES) [5]. Conservative treatment in cases of RCES may include:

- Gels and ointments
- Lubricants (to be applied, especially early in the morning and late at night)
- Nonpreserved artificial tears (in severe cases)
- Autologous serum [14]
- Therapeutic contact lenses or collagen shields may be required

Surgical alternatives include abrasion and thorough removal of basal membrane with a hockey knife, with or without anterior stromal puncture (ASP) [31].

Anterior stromal puncture should not be performed in the central optical zone to avoid glare, halos, and visual impairment postoperatively. After PTK, the success rate is not 100%, but somewhere between 85 and 90% [4, 38, 43]. Artificial tears and/or gels are still necessary after PTK. In particular, if PTK in corneal dystrophy is performed predominantly because of the pain caused by RCES, the patient must be aware of the potential loss of UCVA post-PTK.

Summary for the Clinician

- Careful attention should be directed toward the specific patient's subjective complaints and the preoperative refractive error to better determine if PTK may be expected to achieve the desired clinical goals
- Besides careful surgical planning, cautious patient selection and well-informed counseling are essential to achieve a satisfactory outcome for the individual patient

4.4 Diagnostic Approaches to Surgical Decision-making

Microsurgeons should realize that PTK is not the treatment of choice for all anterior corneal pathologies. "Aiming and shooting" at all corneal pathologies should be avoided to prevent from dissatisfaction and frustration. The primary diagnosis does not necessarily dictate the treatment of choice, since various clinical presentations of the same disorder may suggest different therapeutic approaches (Table 4.1). Appropriate recommendations regarding treatment of choice can be made only after analyzing the horizontal/

Table 4.1 Manual removal of superficial corneal pathology versus excimer laser phototherapeutic keratectomy (PTK)

Manual	PTK
Inexpensive	Expensive
Simple equipment	Complex equipment
Technically easy	Variable level of difficulty
May be repeated often	May be repeated, but risk of hyperopia
No change in corneal power	Hyperopic shift
Little risk of iatrogenic irregular astigmatism	May induce irregular astigmatism
Anterior stromal pathology not amenable to treatment	Effective ablation of Bowman's layer and anterior stroma

vertical distribution and the pattern of the pathology [41] and the applicability of manual vs. PTK techniques.

However, the ultimate determinant of the appropriate technique is the functional objective of the procedure:

- Visual objectives relate to final visual acuity. In assessing a patient's needs, not only is the final BCVA important, but any potential adverse alteration in UCVA that may result from undesirable hyperopic shift must be taken into account
- Nonvisual objectives include:
  - Reducing pain associated with RCES, which is a very typical complication of many progressed dystrophies [5]
  - Decreasing optical problems such as glare, halo, monocular diplopia or triplopia, and/or
  - Clearing the visual axis for subsequent cataract surgery

4.4.1 Patient Selection

Besides clearly defining the expectations of patients and re-checking whether these can be achieved with PTK, the individual corneal pathology itself is most critical for surgical decision-making. Contact lens tolerance should be an issue in the process of decision-making before surgery. In contrast to refractive surgery, or even laser PKP, the PTK procedure has to be planned on an individual basis. Therefore, it is imperative that the surgeon has a close look at the cornea at the slit-lamp immediately before surgery, even if he/she has seen the patient at an earlier visit.

4.4.2 Refractometry

Identical morphological presentations do not necessarily dictate the same treatment of choice, since functional requirements may suggest different therapeutic approaches. Reis-Bücklers' dystrophy in a myopic eye may be successfully treated with PTK. In contrast, the same pathology in a hyperopic eye may suggest only manual superficial keratectomy, at least if the patient is contact lens-intolerant. For this reason, objective and subjective refractometry of both eyes are mandatory in each patient before PTK to avoid anisometropia.

4.4.3 Biomicroscopy at the Slit-lamp

4.4.3.1 Pattern Assessment

In all instances of assessment of corneal dystrophies, the light at the slit-lamp must NOT be dimmed. For surgical decision-making the localization of the lesion is important:

- Whether it is multifocal (regular corneal tissue in between)
- Whether it is segmental (contiguous)
- Whether diffuse patterns of opacifications may be distinguished

In cases of epithelial basement dystrophy in particular the microsurgeon has to look carefully for subtle epithelial changes. In this case indirect illumination and retrograde illumination (with dilated pupils) may be required to detect the prevalent lesions.

4.4.3.2 Horizontal Extension

Modified after Hersh and Wagoner [20] corneal pathology may be categorized into the:

- Central (optically relevant) zone
- Paracentral zone
- Peripheral zone

The central zone is defined as the central 3–4 mm where the dystrophy may directly (through clouding of the visual axis) or indirectly (through the induction of irregular astigmatism) diminish visual function.

The paracentral zone is defined as the midperipheral area where the pathology may indirectly affect visual function by the induction of irregular astigmatism and light scattering/glare. More often, however, treatment for disorders in this region is considered for recurrent epithelial erosions or po-

tential extension into the visual axis. PTK in this region may produce alterations in visual function due to iatrogenic changes in the contour of adjacent corneal tissue in the visual axis.

The peripheral zone has little or no direct impact on visual function. However, in the case of high elevation, e.g., peripheral Salzmann's nodules (which some authorities consider a dystrophy) may induce irregular astigmatism due to tear film pooling. Multifocal (mid-)peripheral nodules may even induce considerable hyperopia and irregular astigmatism due to asymmetric tear film pooling, resulting in an "optical cornea plana" (Fig. 4.2) [7].

4.4.3.3 Sagittal Extension

To evaluate the sagittal extension, first a classification of the level of the corneal surface at the site of the lesion has to be made:

- "Plus disease" is defined as an elevated (nodular) lesion compared with the surrounding corneal surface
- "Zero disease" is defined as a lesion within the niveau of the surrounding corneal surface
- "Minus disease" is defined as a depressed lesion compared with the surrounding corneal surface

In addition, the sagittal extension of corneal disorders may be divided into:

- Pre-Bowman's layer
- Involving Bowman's layer
- Anterior stroma and mid-stroma

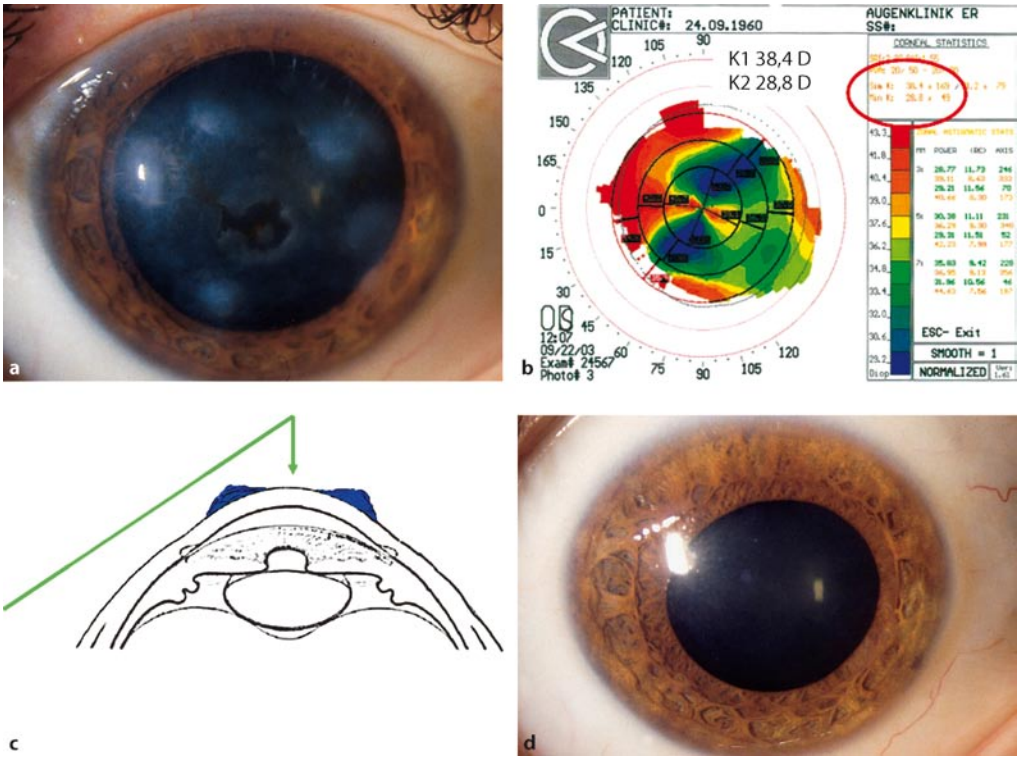

Fig. 4.2 **a–d** Induced high hyperopia and astigmatism due to circular multifocal (mid-)peripheral prominent corneal lesions (e.g., Salzmann's nodules) (**a**) slit lamp appearance before PTK, visual acuity **+13.5**-5.0/100°=0.4, (**b**) Corneal topography before PTK, (**c**) Explanation: Concept of "optical cornea plana" based on asymmetrical tear film pooling (exaggerated), (**d**) slit lamp appearance 3 month after excimer laser PTK, visual acuity uncorrected 0.8–1.0 p [Gottschalk K, Kruse FE, Seitz B; 102. Meeting of the DOG (oral presentation), Berlin, 23.–26. 09. 2004].

"Pre-Bowman's" refers to dystrophies that involve the epithelium and basement membrane, but completely spare Bowman's layer, e.g., map-dot-fingerprint dystrophy [29].

"Bowman's" refers to all pathologies incorporated into Bowman's layer with or without epithelial and epithelial basement membrane involvement, e.g., Reis-Bücklers' or Thiel-Behnke dystrophy. "Anterior stromal" refers to dystrophies that have extended beneath Bowman's layer into the anterior 100–150 µm of the cornea, e.g., anterior variants of granular or lattice dystrophy [8, 26, 41]. "Stromal" refers to disorders where excision of deep stromal lamellae (>150 µm) is required to achieve a satisfactory visual outcome, e.g., macular dystrophy [17, 51].

The depth of the lesion is defined as the most posterior extension of the opacification intended for resection. For example, granular or lattice dystrophy may involve the full thickness of the stroma, but if superficial keratectomy is performed for RCES and irregular astigmatism, the depth of involvement is classified as "anterior stromal." If the stromal pathology is the major contributor to decreased vision, and the stromal deposits must be addressed to improve VA, then the pathology is considered stromal [41]. Typically, in these cases PTK is not the appropriate therapeutic approach, but PKP or ALKP is required for satisfactory visual rehabilitation.

4.4.4 Enhanced Examinations

4.4.4.1 Keratometry

The cornea contributes about two-thirds to the refractive power of a human eye. Surgical procedures on the cornea may therefore influence the state of refraction considerably. Corneal astigmatism is an optical aberration, resulting from unequal refraction of entering light in different meridians of the corneal surface. Astigmatism before and after PTK is often irregular, i.e., two or more meridians are separated from each other by an angle unequal to 90°. Two or more steep hemimeridians are not located opposite to each other. The same may be true of the flat hemimeridians. In addition, the refractive power of corresponding hemimeridians may differ. Typically, patients accept much less subjective cylinder than is indicated by objective measures such as keratometry or topography analysis.

We suggested a semiquantitative classification of regularity of keratometry mires (Ophthalmometer, type H, 190071; Zeiss, Jena, Germany; 0 = regular, 1 = mildly irregular, 2 = moderately irregular, 3 = not measurable) [32, 37]. Before and after PTK it is recommended that the keratometric refractive power be documented separately in the steep and in the flat meridian with individual axis notation and assessment of degree of "keratometric irregularity" (Fig. 4.3). Instead of "41.0+4.0/0°" we suggest writing "41.0/0° (irreg. 1); 45.0/70° (irreg. 2)" [32, 37]. Thus, instead of measuring the power in orthogonal meridians, we attempt to assess the power in the flattest and the steepest meridians [32, 37].

4.4.4.2 Topography Analysis

Besides keratometry, topography analysis is indispensable to reflect the corneal power map over the central and midperipheral cornea. The effects of localized or diffuse lesions on the corneal curvature are best assessed with topography analysis. Refractive powers and individual axes of four or more hemimeridians are rounded up by system-specific indices, e.g. SRI (surface regularity index) und SAI (surface asymmetry index) of the TMS topography system [53].

4.4.4.3 Assessment of Corneal Thickness Profile

Ultrasound pachymetry can only supply the microsurgeon with the entire corneal thickness in one spot. In contrast, slit scanning tomography (Orbscan) or Scheimpflug analysis (Pentacam) provide a thickness profile of the cornea, including full information on the anterior and posterior corneal curvature. To assess the true sagittal involvement of the lesion anterior segment optical coherence tomography (OCT) may be used. To some degree, ultrasound biomicroscopy (UBM) allows for numeric documentation of the sagittal extension of the dystrophy into the cornea.

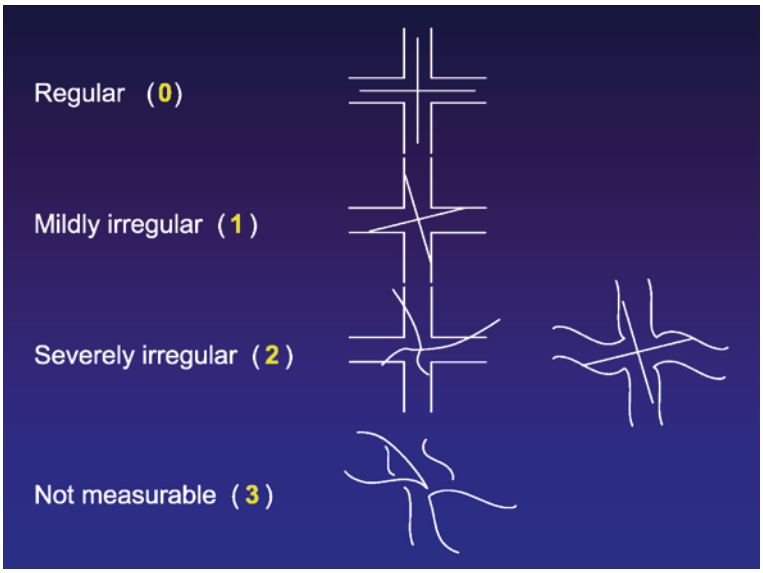

Fig. 4.3 Semiquantitative classification of regularity of keratometry mires (Ophthalmometer, type H, 190071, Zeiss, Jena, Germany; 0 = regular, 1 = mildly irregular, 2 = moderately irregular, 3 = not measurable) [32, 37]

Nevertheless, slit-lamp examination is still the most important qualitative examination technique for planning the surgical approach.

Summary for the Clinician

- The mindset of merely "aiming and shooting" at all pathology that can be physically reached by the laser may lead to disappointment for both the patient and the physician disappointment
- Beware of a philosophy best described as: "If you have a hammer everything looks like a nail"
- Slit-lamp examination is still the most important qualitative tool for planning the surgical approach

4.5 Strategic Planning and Surgical Techniques

Phototherapeutic keratectomy is not necessarily the treatment of choice for all anterior corneal pathology. While in many circumstances it represents a significant advance in our ability to excise pathology that was once difficult to remove manually, it does not always guarantee a superior result. In some situations it may produce an even less desirable outcome than manual superficial keratectomy (Table 4.2).

4.5.1 General Concepts

Corneal surgeons should look at PTK as a companion to manual superficial keratectomy that affords greater flexibility in tailoring the procedure to the specific clinical situation. Sound clinical evaluation and knowledge of the appropriate indications for both manual superficial keratectomy and PTK will maximize the opportunity for a favorable surgical outcome in a variety of

Table 4.2 Strategic planning and surgical techniques of excimer laser PTK

- Avoid "aiming and shooting" at all corneal pathologies to avoid dissatisfaction and frustration
- Remove as much of the diseased tissue as possible with the blade
- Remove as little tissue as possible with the laser (see Fig. 4.4)
- Use the laser predominantly for smoothing
- Use "masking agents" repeatedly during one session
- Consider simultaneous refractive ablation:
  - Prevention of hyperopic shift with deep ablation
  - High/irregular astigmatism + superficial opacities (topography-based ablation)
  - Asymmetric astigmatism after PKP + recurrence of dystrophies
  - Recentration of apex with medium stage keratoconus (in cases of contact lens intolerance)
- Take into account potential subsequent PKP (save peripheral Bowman's layer for suture fixation)

corneal disorders, especially corneal dystrophies [48].

In each individual patient, the actual ability of the procedure to accomplish the desired objective of removing the pathologic process AND to regularize the surface must be ascertained. Almost invariably, any dystrophy amenable to manual resection can be removed by PTK, although the converse is not true. Generally, the more posterior the pathology extends, the more likely manual keratectomy is to be technically less desirable than PTK. At Bowman's layer, disorders such as Reis-Bücklers' or Thiel-Behnke dystrophy may be easily resected manually. In contrast, anterior corneal dystrophies reaching into the stroma (e.g., granular or lattice dystrophy) are typically very difficult to remove manually with a blade, but are amenable to PTK [41].

In general, PTK should be performed on a "quiet eye." Possible confounding problems such as blepharitis and active infection must be controlled before proceeding with surgery. In addition, intraocular inflammation should be controlled (see Sect. 4.9 for contraindications). The intended effects of PTK may be threefold:

- Removal of corneal opacities (o-PTK)
- Treatment of irregular corneal surface and astigmatism (a-PTK)
- Increase and stabilization of epithelial adherence (e-PTK)

Such indications may overlap in a number of corneal diseases and may apply to variable degrees in a given pathology (Fig. 4.1).

4.5.2 Removal of Opacities

Generally, we recommend removing as much of the diseased tissue with the blade or hockey knife and removing as little tissue as possible with the laser. This is especially true for all "plus diseases" that are multifocally arranged. A cleavage plane is identified between abnormal tissue and Bowman's layer or stroma using the hockey knife to raise a tissue edge. To facilitate visualization and manipulation of the abnormal tissue, the corneal surface is kept dry. Traction may be applied with forceps to strip the abnormal material along its natural cleavage plane while the tip of a dry cellulose sponge may be used as an atraumatic dissection instrument. In the case of strong adherence, the blade may be carefully used to lyze adhesions or to scrape residual abnormal tissue. Care is taken to leave limbal stem cells intact [12, 20, 30, 43, 48]. Caution should be taken to remain in the cleavage plane, thus avoiding damage to Bowman's layer that may evoke further corneal scarring and irregularities. After mechanical scraping of residual tissue remnants with the hockey knife, laser ablation is performed. This technique may be called "sub-epithelial PTK" (Fig. 4.4).

In cases of regular corneal topography – which is very rare – a "transepithelial PTK" may be advisable. In this case the epithelium will mask the irregularities of the superficial stroma, acting as a biological "masking agent" and contributing to a smoother postoperative stromal surface. It has been suggested that transepithelial laser treatment will lead to less activation of corneal wound

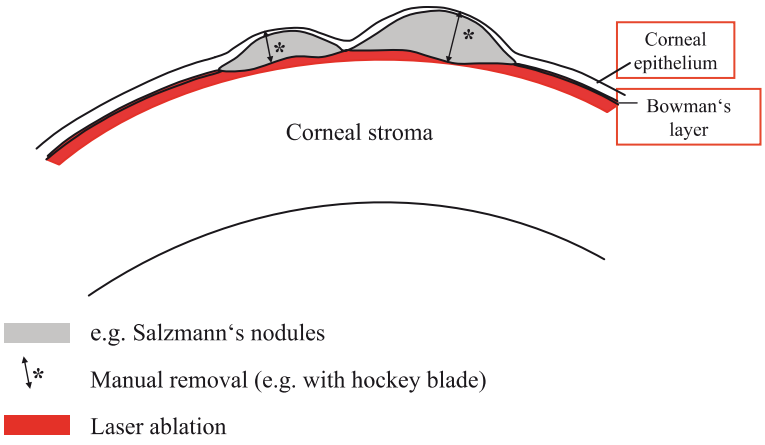

Fig. 4.4 Technical details: First major prominent subepithelial tissue is removed manually, e.g. with a hockey blade, before the excimer laser ablation is applied to remove intrastromal opacifications and especially smoothen the surface (by use of masking fluids) [7].

healing in the superficial stroma compared with mechanical removal [52]. However, different ablation rates in epithelium, stroma, and scar tissue have to be taken into account.

Microsurgeons should be aware of the fact that corneal dystrophies typically recur after some time. Thus, removing as little tissue as possible (although the cornea may not be completely cleared) and leaving enough tissue to enable repeat PTK is mandatory. In cases of stromal involvement of dystrophies in particular, corneal transplantation may be required later, if the outcome is not satisfactory for the patient. Therefore, peripheral Bowman's layer should be saved for suture fixation in case of subsequent PKP [47]. Typically, a treatment diameter of around 7.0 mm, with a small transition zone of around 0.5 mm, is adequate.

4.5.3 Smoothing of the Surface and Reducing Irregular Astigmatism

4.5.3.1 Repeated Application of "Masking Fluids"

It is important to note that, in general, the excimer laser will remove an equivalent amount of tissue over the entire area upon which it impinges (Fig. 4.5). Although opacities will be removed, irregularities of the surface will be maintained because tissue is removed parallel to the surface. Therefore, during most PTK procedures it is mandatory to use "masking fluids" repeatedly during one session. Methylcellulose (1%) has proven to fill in irregularities, thereby smoothing the surface to be ablated [20]. Hyaluronic acid (0.3%; e.g., Vismed®) may also be used successfully for this purpose [21]. The viscosity of these fluids is appropriate to fill in the "valleys" of an irregular surface while leaving the "peaks" exposed to the laser action [13]. This allows the surface to be smoothed with the laser while opacities are removed. The thickness of the masking fluid layer should be enough to smooth the valleys of the corneal surface, but not so much as to completely block the incoming laser beam. Collagen gels and other molding compounds have not been generally accepted by the community of corneal specialists.

During PTK, the microsurgeon must be aware of the rate and pattern of tissue ablation. Sensory feedback can serve as a guide. During the procedure, a blue fluorescence signals that epithelium is being ablated. This fluorescence disappears upon reaching the corneal stroma because of its lower water content. To best visu-

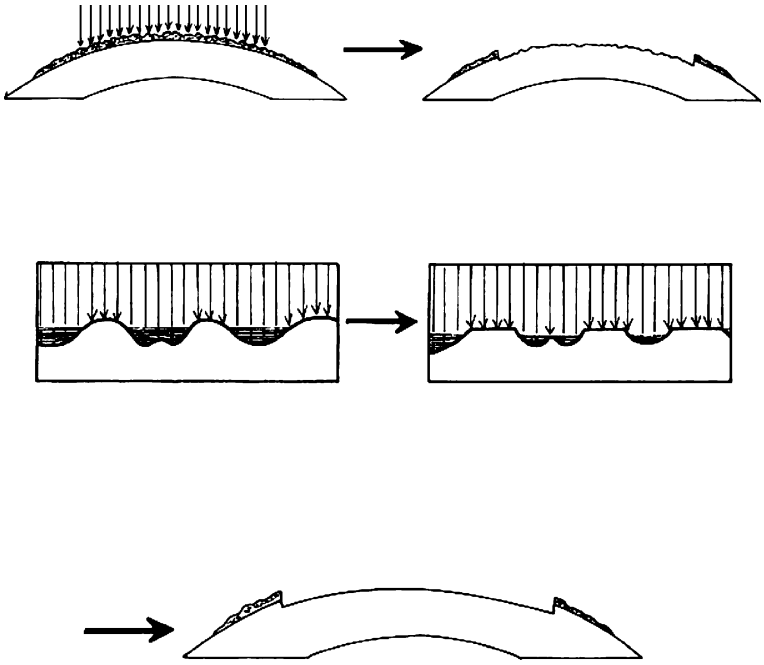

Fig. 4.5 Application of masking fluid to smooth the corneal surface. *Upper left/right*: Schematic drawing of an irregular corneal surface before/after excimer laser ablation. Note how the irregular corneal surface pattern is preserved even though the corneal substance is thinned. *Middle left/right*: Using a masking fluid to protect the "valleys" of the corneal surface by absorbing incoming laser energy will allow smoothing of the surface as the "peaks" of the irregular surface are removed by photoablation, leaving a smooth corneal surface (*Bottom*) (modified after [13, 20, 21]).

alize this epithelial fluorescence, the room light should be turned off and the illumination on the laser microscope should be dimmed. Methylcellulose, in contrast, tends to whiten and bubble upon ablation, and the normal loud snapping sound of laser-tissue interaction is muffled indicating partial masking of the incoming beam. If the sound is substantially muffled, removal of tissue will be minimal. An intermediate sound usually indicates satisfactory masking of tissue valleys, while peaks remain exposed to laser ablation [20].

The laser procedure is interrupted at frequent intervals and the patient may be examined at the slit-lamp to monitor progress of the procedure and to determine areas to be treated further. If no slit-lamp is available, typically two to three sessions with intended ablation depths of 40–50 µm over appropriate masking fluid are sufficient to regularize the corneal stromal surface after extensive mechanical debridement.

4.5.3.2 Simultaneous Refractive Ablation

Simultaneous refractive ablation may be considered in special cases as follows.

Besides introducing a transition zone instead of sharp ablation edges, a hyperopic correction may be added to prevent hyperopic shift with deep ablation or in cases of pre-existing hyperopia. In cases of concomitant high and/or irregular astigmatism and superficial opacities, topography-based ablation with a flying spot mode may be beneficial [22, 34]. In addition, asymmetric astigmatism after PKP with coexisting recurrence of a dystrophy may profit

from simultaneous topography-based (or wavefront-guided) excimer laser ablation. Furthermore, recentration of the corneal apex in cases of medium-stage keratoconus has been tried in the event of contact lens intolerance with quite limited success.

Generally, topography-based and/or wavefront-guided refractive surgery will not be covered extensively in this chapter. Nevertheless, years ago, irregular corneal surfaces after refractive surgery (e.g., decentered PRK or LASIK ablations, especially hyperopic, large central islands, or irregular astigmatism after PKP) were treated by various methods of a-PTK. Indeed, in such situations the term "therapeutic" or "curative" still seems to be more appropriate than the term "refractive."

4.5.4 Improvement in Epithelial Adhesion

In cases of nondystrophic RCES (e.g., of traumatic origin) some microsurgeons advocate transepithelial PTK with treatment scheduled in the pain-free interval. However, RCES in the context of progressive corneal dystrophies should preferably be treated with subepithelial PTK (= treatment of Bowman's layer after generous removal of loose and irregular epithelium).

We know from histologic and ultrastructural studies that excimer laser ablation results in a significant increase in hemidesmosomes (Fig. 4.6), thus improving epithelial adhesion to the underlying stroma [46]. For this reason even dystrophies such as the map-dot-fingerprint variant, which may be removed with mechanical corneal abrasion only, would be better treated with additional PTK to regularize the surface and improve epithelial adherence [29].

4.5.5 Laser Parameters

Excimer laser photoablation of corneal tissue takes place when the energy density per pulse (= fluence) exceeds 50 mJ/cm². Current excimer lasers in clinical use operate at energy levels rang-

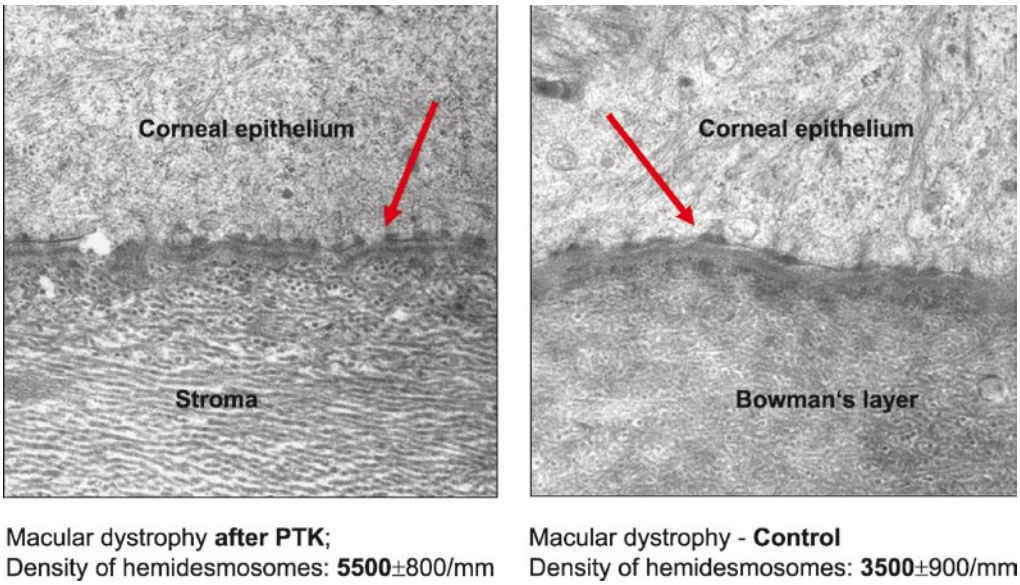

Fig.4.6 Increased number of hemidesmosomes *(arrow)* may be responsible for improved epithelial adherence after excimer laser PTK in macular corneal dystrophy. Without laser ablation 3,500±900 hemidesmosomes per mm of basal membrane length were measured. In contrast, after PTK 5,500±800 hemidesmosomes per mm of basal membrane length were measured [46].

ing from approximately 120 to 250 mJ/cm², pulse repetition rates of 5–200 Hz, and pulse duration of approximately 10–20 ns. The expected ablation rate of corneal tissue averages approximately 0.25 µm per pulse for an excimer laser operating at a fluence of 180 mJ/cm².

Before PTK the excimer laser must be calibrated. Appropriate laser energy and especially beam homogeneity should be ensured before any laser treatment. Preoperative laser calibration is necessary to ensure that the pilot laser beam is aligned with the excimer laser beam, that the cross-sectional beam homogeneity is acceptable, and that the ablation rate per pulse of the laser is properly adjusted. The different manufacturers of lasers provide detailed procedures for assessing laser calibration. If the laser performance does not conform to the guidelines, surgery should be postponed and the laser should be serviced.

Originally, so-called full spot (= wide field) lasers were in use. Today, computer-guided flying spot/slit mode lasers are the devices of choice. Typically, a central area with a diameter of 7–8 mm is treated with modern laser systems. The larger the treated zone, the smaller the risk of inadvertent refractive complications.

In rare instances (e.g., RCES [38] or localized pathology at the border of a corneal transplant), by use of a joy stick, manually guided spot lasers (~1 mm in diameter) may be effective.

4.5.6 Combination with Mitomycin-C

Combining PTK with temporary application of 0.02 mg% mitomycin-C on a Merocel sponge for 30 s after laser action may prevent scarring and recurrence of certain corneal pathologies, such as granular dystrophy type II and Salzmann's nodular degeneration [25]. Potential problems with this antimetabolite include hyperopic shift, epithelial healing problems, endothelial damage in thin corneas, and irreversible keratocyte damage with (late) melting. However, corneal toxicity of mitomycin-C is not yet well established.

Summary for the Clinician

- The 193-nm excimer laser has unique tissue ablation properties in corneal surgery as it removes corneal tissue micrometer by micrometer without concomitant damage to adjacent tissue or light damage to deeper structures of the eye
- A number of surgical strategies are available with the PTK procedure. Besides careful patient selection, the surgeon must choose an appropriate strategy tailored to the individual patient problem, and properly execute the procedure to optimize clinical outcome
- The best candidates for PTK are myopic eyes with diffuse elevated lesions ("plus disease")
- Localized depressed (para-)central lesions (e.g., foreign body scars; "minus disease") are bad candidates for PTK, especially in cases of contact lens intolerance
- In general, PTK should be performed on a "quiet eye"

4.6 Medical Treatment

4.6.1 Preoperative

Typically, nonsteroidal anti-inflammatory drugs (NSAID) are applied four times a day the day before and on the day of surgery to reduce corneal inflammatory reaction after PTK. There is no need to apply topical or systemic antibiotics before PTK. In some cases the operative eye may require pilocarpine (1%) to constrict the pupil and thereby facilitate centration of the procedure and improve visualization of the pilot beam on the cornea.

4.6.2 Intraoperative

Most procedures are done using only topical anesthetic drops. In cases of corneal dystrophies with intended subepithelial PTK we prefer co-

caine drops to break the hemidesmosomes, thus making mechanical epithelial removal easier. In cases of intended transepithelial PTK, cocaine should be avoided. Intraoperatively, masking fluids may be repeatedly used to achieve a regularization of the corneal surface depending on the degree of irregularity in a given eye.

At the end of surgery, we preferably apply cyclopentolate drops and ofloxacin ointment in conjunction with a pressure patch, to be changed daily until complete epithelial closure. Other microsurgeons prefer to apply ofloxacin eye drops and a therapeutic contact lens.

To reduce pain, we supply the patient with tramadol to be taken orally for 2 days.

4.6.3 Postoperative

We do NOT recommend administering NSAIDs to reduce pain after PTK because of their well-documented adverse effects on epithelial wound healing. After epithelial closure topical antibiotics are usually no longer necessary. However, lubricants or gels and nonpreserved artificial tears should be applied to promote epithelial remodeling without long-lasting superficial punctate keratopathy. Typically, topical steroids (e.g., fluorometholone [0.1%] or prednisolone acetate [1%] in the presence or likelihood of more profound stromal inflammation after deep ablation) are tapered slowly over some weeks or months. Depending on the depth of ablation, it might be started at four times a day and reduced by one drop a day every month.

Summary for the Clinician

- Preoperatively: nonsteroidal anti-inflammatory drugs and sometimes pilocarpine (1%)
- Intraoperatively: cocaine to improve epithelial removal in subepithelial PTK and masking fluids to regularize the surface
- Postoperatively: nonpreserved artificial tears and corticosteroids to be tapered slowly over weeks or months (depending on the depth of ablation)

4.7 Indications and Outcome

The potential indications for PTK are summarized in Table 4.3. In the following paragraph only a few specific details and/or recommendations concerning PTK in certain corneal dystrophies are to be given. It must be stressed in advance that not the class of dystrophy itself, but the individual distribution of opacification, the amount of surface irregularities, and the degree of RCES in combination with subjective symptoms determine the decision for or against an individual PTK modality in a given patient.

4.7.1 Criteria of Outcome

4.7.1.1 Morphology

At the conclusion of the PTK treatment the cornea typically has a ground-glass appearance. After epithelial healing, which is usually completed after 3–4 days, corneal luster is regained and visual acuity may be markedly increased despite residual opacities in the deep stroma.

Persisting focal opacifications (typically in the deeper stroma and in the corneal periphery) must be distinguished from "haze" early after laser ablation and from recurrences of the dystrophy, which typically appear in the subepithelial area after some years, depending on the type of dystrophy.

4.7.1.2 Function

Criteria to determine visual function after PTK include:

- Uncorrected visual acuity (UCVA)
- Best-corrected visual acuity (BCVA)
- Subjective refraction and spherical equivalent of refraction
- Astigmatism (refractive cylinder and keratometric/topographic astigmatism)
- Central corneal power (keratometric/topographic)
- Contrast sensitivity
- Patient's subjective assessment (photophobia, halo, glare, ocular surface discomfort)

Corneal dystrophies
Epithelial basement membrane dystrophies (map-dot-fingerprint dystrophy)
Meesmann-Wilke
Bowman's layer dystrophies
Reis-Bücklers
Thiel-Behnke
Granular
Lattice
Macular
Crystalline (Schnyder)
Recurrence on the graft after keratoplasty
(Bullous keratopathy in Fuchs dystrophy; in combination with amniotic membrane transplantation)
Other superficial corneal pathologies
Scars
Herpetic origin
Adenoviral keratoconjunctivitis
Scrophulous
Following other corneal infections
Traumatic
Salzmann's nodular degeneration
Pterygium
(with involvement of optical zone)
(without involvement of optical zone, no indication for PTK!)
Band-shaped keratopathy
Mechanical scraping
EDTA chelating
PTK just for smoothing
Keratoconus
Fibroblastic subepithelial nodules ("proud nebulae")
Irregular astigmatism (but: biomechanical stability of the cornea may be further decreased)
Persisting epithelial defects
Erosions
Ulcers
Recurrent corneal erosion syndrome
Trauma
Epithelial basal membrane dystrophies (especially Cogan, Guerry)
Idiopathic

The goal of PTK is to improve BCVA by 2–5 lines. BCVA of 1.0 (20/20) is not the goal! In emmetropic or even hyperopic eyes, UCVA may be worse after PTK due to a hyperopic shift. Typically, topographic regularity of the corneal surface will be improved after PTK. "Plus diseases" with prominent lesions are good candidates. In contrast, "minus diseases" (localized depressed lesions, e.g., foreign body scars) are bad candidates.

Although one of the important goals of PTK is to obviate the need for PKP, corneal transplantation may still be necessary in some cases. We performed a retrospective study on 21 eyes in 15 patients to examine the impact of previous PTK on the outcome of subsequent PKP in patients with stromal corneal dystrophies. We found that during PKP no technical obstacles appeared in the test group. No immunologic graft rejection occurred during follow-up. There were no significant differences in keratometric, topographic net astigmatism and refractive cylinder; keratometric and topographic central power. There was no difference between the test and control group with regard to BCVA, 6 months after PKP, after first and second suture removal. Surface regularity index (SRI) [53] tended to be superior after first suture removal following PTK. Thus, preceding PTK does not appear to impair the outcome of subsequent PKP in patients with macular and granular dystrophy [47].

4.7.1.3 Recurrent Erosions

To assess the effects of the e-PTK component, the patient's subjective report on ocular surface discomfort is valuable. Other important outcome measures are time period until complete epithelial closure after PTK and recurrence rate of corneal erosions. This should be given not only as a percentage, but also as Kaplan–Meier curves [5, 38].

◀ **Table 4.3** Indications for excimer laser phototherapeutic keratectomy. *EDTA* ethylenediamine tetraacetic acid

As yet, the exact mechanism by which interaction between UV light and corneal tissue during PTK results in better epithelial adherence is unclear. However, we were able to show that after PTK the number of hemidesmosomes is significantly increased (Fig. 4.6) [46].

4.7.2 Corneal Epithelium and/or Basement Membrane

4.7.2.1 Meesmann-Wilke Dystrophy

Meesmann-Wilke dystrophy is a rare bilateral autosomal dominant, exclusively epithelial dystrophy that usually appears very early in life. Tiny epithelial vesicles can be seen extending out to the limbus and may cause visual disturbance. Most patients are asymptomatic and hence require no treatment. Soft contact lens wear may help if patients show signs of RCES. Pure abrasion with a hockey knife should be able to remove all the pathologic epithelium. Nevertheless, PTK may be used to enhance the adhesion of the corneal epithelium, but results vary from good to medium, with the possibility of inducing major haze [12, 28, 30].

4.7.2.2 Epithelial Basement Membrane Dystrophy

Epithelial basement membrane dystrophy (EBMD) is the most common anterior corneal dystrophy [23]. Typically, Cogan's microcystic dystrophy may be distinguished from map-dot-fingerprint dystrophy (Guerry). For both variants of the entity EBMD, PTK using an excimer laser with low pulse energy and a low number of pulses can be considered an effective and minimally invasive treatment modality for achieving fast and durable epithelial closure, preventing recurrent corneal erosions, and increasing visual acuity in most patients (Fig. 4.7) [29]. In this study, no recurrence of corneal erosion was observed during a mean follow-up of 4.8 years. Asymptomatic dystrophy signs in the midperiphery became visible in 2 out of 15 eyes at 3 and 5 years after PTK.

4.7.2.3 Granular Dystrophy

According to genetic studies, granular dystrophy is an epithelial rather than a stromal dystrophy. The multifocal opacities are usually superficial, but can sometimes be located deeper in the stroma. In our department, granular dystrophy – besides RCES – was the type of dystrophy most frequently treated with PTK. The results are generally described as good (Fig. 4.8) [8, 12].

Histomorphometric analysis of deposits in the cornea suggest that granular dystrophy is a better candidate than lattice dystrophy for PTK, since the deposits are located more superficially and the central clear optical zone after removal of 100 µm of tissue is significantly larger in granular (from 484 ± 389 µm to 1,451 ± 1,954 µm) than in lattice (from 258 ± 183 µm to 846 ± 784 µm) dystrophy. Deposits were completely removed in 22% of the granular dystrophy samples. In both dystrophies, a clear central "pinhole" greater than 1 mm in diameter was achieved in around one-third of corneas [41].

To prevent a major hyperopic shift and to allow for repeat PTK in cases of recurrence, the primary ablation should be limited to less than 100 µm. Dinh et al. reported the average time to recurrence as 32 months and the average time to significant recurrence as 40 months (cited in [9]).

4.7.2.4 Lattice Dystrophy

Lattice dystrophy is characterized by the formation of branching filaments or bands in the corneal stroma. These are usually seen in the superficial part of the stroma, but it is not rare for them to penetrate deeper. The very center of the cornea is often opacified late in the disease, but it is eventually overtaken by a general superficial opacification that reduces vision. Ablation of the superficial stroma removes the diffuse central opacity as well as some of the stromal branching filaments. Nevertheless, the average outcome in terms of visual acuity is decent, but not impressive [12]. The dystrophy is often located too deeply to be completely removed. Spontaneous erosions are not unusual in lattice dystrophy, but healing time is often longer than for other spontaneous erosions. However, epithelial healing af-

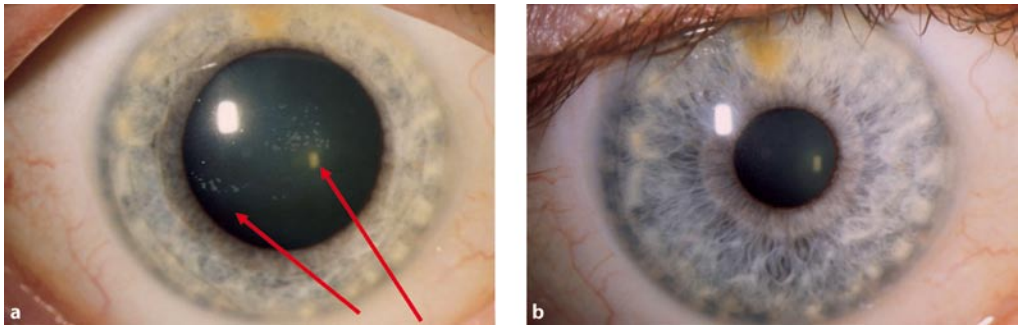

Fig. 4.7 Map-Dot-Fingerprint (epithelial basal membrane) corneal dystrophy: (**a**) slit lamp appearance before, (**b**) no recurrence 4 years after excimer laser PTK [29].

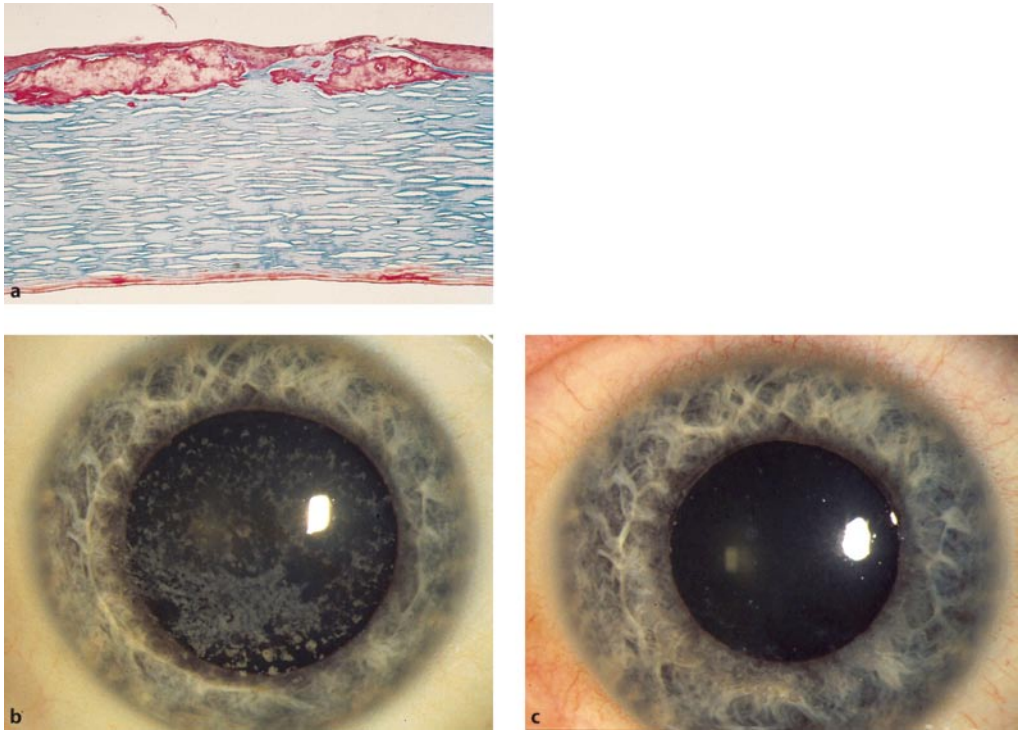

Fig. 4.8 Granular corneal dystrophy: (**a**) superficial deposits of hyaline material (Masson's trichome stain), (**b**) slit lamp appearance before, (**c**) 3 month after excimer laser PTK [8].

ter PTK is typically delayed, too. It may require up to 3 weeks [6]. Recurrence rates are steady, but fairly slow.

We performed a study to compare the visual and refractive outcome after PTK for superficial corneal opacities in granular and lattice dystrophy. PTK was performed in 62 eyes of 40 patients (granular dystrophy [n=50] and lattice dystrophy [n=12]) after epithelial debridement and pannus removal. Recurrence, if any, was noted during a

mean follow-up of 3.0±2.7 years. The BCVA improved in 79% and 62% of eyes in granular and lattice dystrophy respectively. Recurrences were observed in 10 eyes (20%) with granular and 2 eyes (17%) with lattice dystrophy. Our results suggest that PTK may be tried in patients with superficially accentuated opacities in lattice dystrophy, but the results are less favorable than those in granular dystrophy [8, 26].

4.7.3 Stroma

4.7.3.1 Bowman's Layer Dystrophies

The two types of Bowman's layer dystrophies have been confused for a long time. Both types are good candidates for PTK, allowing for increased visual acuity and reduction of RCES. Eyes with Reis-Bücklers' dystrophy seem to experience recurrence earlier and more severe than those with Thiel-Behnke dystrophy [9, 23].

4.7.3.1.1 Reis-Bücklers' Dystrophy

Reis-Bücklers' dystrophy is an autosomal dominant dystrophy where Bowman's layer is replaced with fibrocellular scar tissue that is classically described as a "saw tooth configuration." The opacifications resemble geographic maps, but deposits reach deeper than in EBMD. PTK for Reis-Bücklers' dystrophy is generally reported as being successful, but most recurrences appear within 1 year of PTK.

4.7.3.1.2 Thiel-Behnke Dystrophy

Thiel-Behnke dystrophy (autosomal dominant inheritance) typically shows honeycomb opacifications of the superficial central stroma. In contrast to Reis-Bücklers' dystrophy ("rod-shaped bodies") Thiel-Behnke dystrophy displays "curly fibers" in Bowman's layer on transmission electron microscopic evaluation. Significant visual improvement may be achieved with PTK. Recurrences seem more rare and later than with Reis-Bücklers' dystrophy [23].

4.7.3.2 Crystalline Dystrophy (Schnyder)

Two changes characterize this dystrophy. One is a diffuse general, but not very dense opacification of the corneal stroma. The other characteristic concerns the formation of subepithelial crystals in the center of the cornea that scatter light very effectively. Schnyder's dystrophy is not very common. The treatment is aimed at removing the central superficial crystals. It seems that general stromal cloudiness does reduce vision to some extent, as postoperative BCVA is usually about 20/40. However, visual acuity after PTK is maintained for a long time and recurrences seem to be very slow [9, 12, 28].

4.7.3.3 Macular Dystrophy

In the presence of superficial plaque-like opacities caused by macular corneal dystrophy PTK can moderately increase BCVA initially, although the diffusely scattered deep stromal opacities *cannot* be removed (Fig. 4.9) [17]. In all patients with a follow-up of more than 1.4 years a recurrence was observed, leading to PKP in 6 of the 10 eyes in this study. Despite possible complications primary PKP still seems to be the definite therapeutic option in patients with macular corneal dystrophy, because of the high recurrence rate after PTK in that study. PTK should be considered after detailed explanation of limited long-term prognosis with the typically young patients (end of second decade). In addition, RCES may also be treated successfully by PTK [12]. Maybe preoperative subtyping of the patient could help differentiate between PTK and PKP as initial treatment for macular corneal dystrophy in the future [17, 51].

4.7.4 Endothelium

Fuchs' dystrophy is by definition an inherited disease. With progressive endothelial dysfunction due to increasing cornea guttata it results in bullous keratopathy. There is no doubt that either PKP or PLKP are the treatment options of

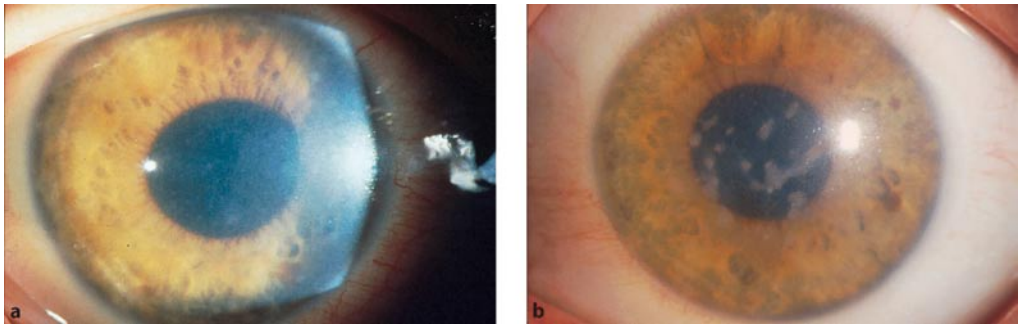

Fig. 4.9 Macular corneal dystrophy: (**a**) slit lamp appearance before, (**b**) 6 weeks after excimer laser PTK (Note: only the superficial plaque-like opacifications can be removed with PTK.) [17].

choice in eyes with good visual prognosis. However, in cases of low visual prognosis or patients with major non-compliance, superficial excimer laser ablation may be a palliative option. One of the rationales for PTK in bullous keratopathy is the chance to improve vision by ablating subepithelial scar tissue. However, exaggerated wound healing may lead to increased corneal scarring in those eyes [1]. Another reason for PTK concerns the alleviation of pain. For this purpose, deeper ablation up to 100 μm seems to be more effective than a more superficial approach. Combination with amniotic membrane transplantation (graft technique to ensure integration of amniotic membrane into the cornea) may even improve outcome. Postoperatively, a long-term therapeutic contact lens seems to be helpful.

4.7.5 Recurrences of Dystrophies on Grafts after Keratoplasty

Various dystrophies seem to recur after different periods of time post-keratoplasty. Whereas granular dystrophy recurs very often and early, macular dystrophy recurs very rarely and often not earlier than 10–15 years after PKP.

In corneal grafts, the recurrence of granular and lattice dystrophy changes often take the form of superficial diffuse opacification (Fig. 4.10). This type of opacification can be readily ablated and allows very acceptable vision for a few years. However, delayed epithelial healing has to be taken into account in lattice dystrophy [6].

On a corneal graft simultaneous correction of high astigmatism (in part) may be considered

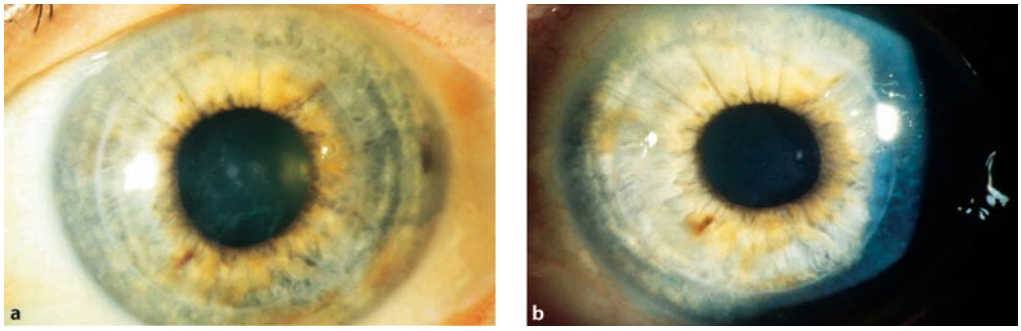

Fig. 4.10 Recurrence of lattice corneal dystrophy on the graft: (**a**) slit lamp appearance before, (**b**) 6 months after excimer laser PTK. (Be prepared for epithelial healing problems [6].)

[22, 34]. In addition, prophylactic systemic corticosteroids may be helpful in avoiding the induction of an immunologic graft rejection due to PTK [11, 19].

Summary for the Clinician

- Results of PTK may vary considerably depending on the goal of treatment and the type of disorder treated. Even individuals with a similar disease may have variable treatment results depending on the severity of the disease and the individual wound healing response
- Central corneal smoothing in a myopic patient may be rewarded with both a clearer cornea and reduced refractive error, while central scar removal in a hyperopic patient, although clearing the cornea, may result in unacceptable hyperopia and perturbation of the corneal topography
- Patients most suited to PTK appear to be those with surface excrescences in whom the procedure will both clear the cornea and smooth the corneal surface

4.8 Complications

Three postoperative healing stages may be distinguished following PTK:

- Re-epithelialization takes from 3–4 days (normal) to a few weeks (delayed) in some patients
- Stromal remodeling occurs over the subsequent weeks and months
- Stabilization of topography and refractive changes may take months

Consequently, general postoperative goals include encouragement of epithelialization, minimization of stromal scarring and optimization of refractive and topographic outcome [15].

Potential complications after PTK are listed in Table 4.4.

4.8.1 Delayed Epithelial Healing

Patients who have suffered previous ocular surface disease with loss of epithelial vitality may have problems with epithelialization following PTK. Eyes with severe ocular surface disease such as chemical burns, ocular cicatricial pemphigoid, atopic keratoconjunctivitis, and severe dysfunctional tear syndrome (DTS) should be treated with extreme caution – if at all (see Sect. 4.9). However, even in eyes with "pure corneal dystrophy" delayed epithelial healing may occur.

We performed a study to evaluate the time period necessary for complete epithelial healing after PTK carried out for various superficial corneal opacities [6]. One hundred and ninety-seven eyes were divided into nine groups:

- Group 1: EBMD including recurrences (n=15)
- Group 2: Reis-Bücklers' dystrophy including recurrences (n=12)
- Group 3: Granular dystrophy including recurrences (n=63)
- Group 4: Lattice dystrophy including recurrences (n=19)
- Group 5: Macular dystrophy including recurrences (n=10)
- Group 6: Herpetic scars (n=5)
- Group 7: Corneal scars of non-herpetic origin (n=31)
- Group 8: Salzmann's nodular degeneration (n=22)
- Group 9: Miscellaneous (such as bullous keratopathy, acute chemical burns, corneal degeneration; n=20)

After PTK, patients were examined daily at the slit-lamp using fluorescein and blue light. The time period necessary for complete healing of the epithelial defect was compared amongst these groups. Healing was considered delayed when the epithelium was not closed after 7 days. One hundred and sixty-one eyes (95%) healed within 7 days. Overall 63%, 80%, 85% of epithelial defects were closed within 3, 4 or 5 days respectively. Out of nine eyes that had delayed healing, 6 eyes (67%) belonged to the lattice dystrophy category. Mean time taken for healing in the lattice dystrophy group (8.6±8.4 days) was significantly longer

Table 4.4 Complications of excimer laser PTK. PKP penetrating keratoplasty, IOL intraocular lens

- Delayed epithelial healing
- Refractive and topographic changes:
  - "Hyperopic sift"
  - Paradoxical myopic shift
  - Irregular astigmatism
- Decentration
- Stromal "haze" and scarring
- Infectious ulcer/melting/perforation
- Immunologic allograft rejection after PKP
- Recurrence of disease
- Corneal ectasia
- Reactivation of herpes simplex keratitis
- IOL power calculation for cataract surgery in eyes after PTK
- (Subsequent PKP needed)

than in all other groups (means: 2.7 to 3.7 days). Besides adequate counseling, these patients with lattice dystrophy should be followed up closely until complete closure of the epithelium to avoid ulceration, scarring or even infection.

In general, potential reasons for delayed epithelial healing include:

- Systemic rheumatoid diseases (e.g., lupus erythematosus)
- Toxicity of topical medication (e.g., NSAIDs, steroids, gentamicin, preservatives)
- Presence of preoperative active ocular surface and lid inflammatory disease
- Dysfunctional tear syndrome (= DTS)
- Denervation after PKP

Such eyes, as well as eyes with lattice dystrophy, might need additional treatment (perhaps prophylactic) such as hyaluronic acid drops, autologous blood serum drops [14], a bandage soft contact lens, punctal plugs/occlusion, simultaneous amniotic membrane patching or even temporary lateral tarsorrhaphy or botulinum toxin injection to induce a temporary ptosis. Lid surgery to correct malpositioning should preferably be performed before PTK.

4.8.2 Refractive and Topographic Changes

Ablation of the corneal surface will lead to refractive and topographic changes after PTK. Such effects may be predicted by the type of corneal disorder treated and the surgical strategy employed. The anticipated refractive changes should be considered during surgical decision-making and strategic planning of technical details. Today, simultaneous hyperopic/astigmatic, or even topography-based refractive ablation, may be performed with acceptable results.

4.8.2.1 "Hyperopic Shift"

When performing PTK – at least with a broad-beam laser – the laser beam is of fixed diameter. Since the energy is optimally homogeneous over its face, the ablation rate would theoretically be similar over the treated area of the cornea. Thus, with a direct ablation without polishing, the surface profile would be expected to be preserved without change in corneal power or topography, and the refraction would similarly be expected to be unchanged. However, studies and clinical practice indicate that a "hyperopic shift" is frequently concomitant with PTK procedures [24]. There are several hypotheses to explain this phenomenon (Fig. 4.11):

- The full-spot (= wide-field) laser beam in practice may exhibit somewhat attenuated fluence ("beam inhomogeneity") at its peripheral aspect. Thus, the peripheral ablation rate may be reduced.
- The induced corneal flattening may be caused by an unequal postoperative epithelial thickness with creation of an epithelial lens power different from the curvature of the underlying treated stroma. Epithelial hyperplasia at the periphery of the treated area could be implicated as a cause, especially if a metal mask is used to protect peripheral Bowman's layer [8].
- The changing angle of incidence of the beam across the corneal dome might result in a lower fluence peripherally, with a decrease in effective tissue ablation and consequent cor-

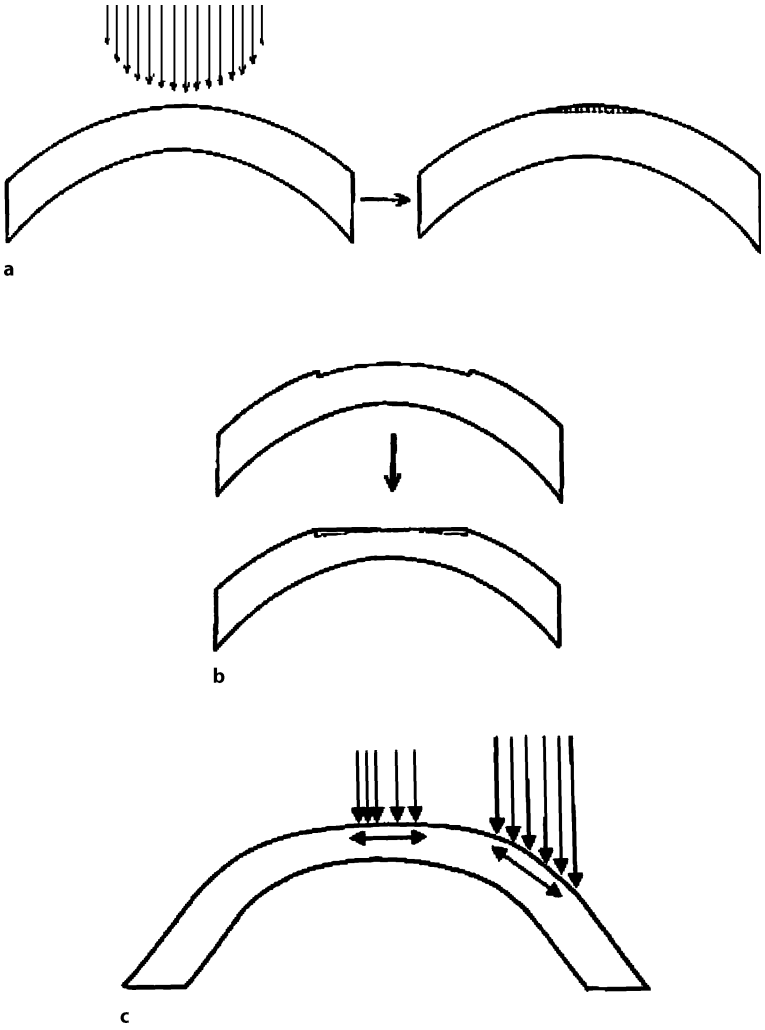

Fig. 4.11a–c Possible mechanisms of hyperopic shift after PTK. (**a**) Attenuation of laser fluence toward the periphery of the beam resulting in lower amount of tissue removal peripherally than centrally with focal flattening in the area of the treatment. Note the depicted flattening is exaggerated. (**b**) Relative epithelial hyperplasia at the peripheral aspect of the treatment zone leading to corneal surface flattening although the stromal curvature remains unchanged. (**c**) The changing angle of incidence of the laser beam across the corneal dome results in a lower fluence peripherally, decreasing the effective tissue ablation peripherally and leading to relative flattening (modified after [20]).

neal flattening. An analogous phenomenon may result when focusing the laser on the apex of the cornea with the consequence of a peripheral defocus with potentially less tissue ablation. Both aspects may be of questionable clinical impact.

- Other researchers suggest that removal of the central portion of corneal stroma lamellae may lead to centrifugal differential contraction of the remaining peripheral superficial lamellae with consequent central flattening. Dupps and Roberts favor the model of differential swelling of midperipheral collagen fibers after removal of central superficial stromal tissue [10].
- In addition, a peripheral meniscus of masking fluid (especially when using a metal mask)

may prevent the (mid-)peripheral corneal tissue from being ablated to the same extent as the central tissue.
- It may also be speculated that the laser plume might differentially block the periphery of the incoming beam, thus leading to less peripheral ablation [20].

4.8.2.2 Paradoxical Myopic Shift

Steepening of the central cornea may occur when more tissue is removed peripherally than centrally. Although this may flatten the focal area of cornea treated due to mechanisms discussed above, the overall optical contour of the cornea may steepen if peripheral tissue is removed. Typically, a paradoxical myopic shift occurs after removal of peripheral prominent pannus with the hockey knife before the laser is implemented.

4.8.2.3 Irregular Astigmatism (Focal Ablation)

e-PTK is supposed to smooth the surface and reduce pre-existing irregular astigmatism. However, irregular astigmatism may be induced inadvertently during the laser procedure. This may be caused by decentration of the ablation, which should be centered on the entrance pupil – not on the center of the pathology. If the pathology covers only half of the pupil it is indispensable to ablate nondiseased tissue to avoid irregular astigmatism. Uneven distribution of masking fluid with inadequately high viscosity may also result in focal hyperablation and irregular astigmatism. Typically, epithelial remodeling is able to compensate for some degree of irregular astigmatism by focal hyperplasia and focal hypoplasia. But this helpful mechanism may take some months.

4.8.3 "Haze"/Scars

In general, PTK has as one of its primary goals the amelioration of corneal opacity (o-PTK). Thus, postoperative stromal "haze" is of less concern in PTK than in PRK. Efforts promoting prompt closure of the epithelium should mitigate an adverse stromal wound healing response [52]. In addition, adjunctive use of topical corticosteroids (see Sect. 4.6.3) may also be helpful in avoiding excessive keratocyte activation and scar formation.

Early after PTK a trace to mild reticular subepithelial stromal haze seems to be quite common. The intensity seems to depend on the depth of ablation and it seems to fade away over a few months. In cases of pre-existing scars, the probability of renewed scar formation is higher. In these eyes the application of mitomycin-C should be considered [25].

4.8.4 Infectious Ulcer/ Melting/Perforation

The risk of microbial keratitis due to the iatrogenic introduction of an epithelial defect is very low, but this is a serious complication that can quite adversely affect the final visual outcome [2]. The greatest risk of microbial keratitis following PTK is either before re-epithelialization is complete or within the first few weeks of re-epithelialization, before the risk of recurrent erosion is virtually eliminated. Moreover, the previously diseased cornea is at greater risk of infection following surgery than the healthy cornea. Persisting epithelial defects in such eyes may afford an inviting substrate for microbial keratitis. Thus, infection will be discouraged with prompt re-epithelialization of the defect and strenuous efforts should be made to avoid persisting epithelial defects. We apply nonpreserved fluoroquinolone antibiotics as routine after PTK, because they are much less toxic to the epithelium than aminoglycosides. Following PTK, the patient should be closely followed in the face of a persistent epithelial defect, especially if a bandage soft contact lens is in place. Any infiltrate and infection should promptly be treated with broad-spectrum antibiotic coverage or so-called "fortified drops".

In analogy to refractive surgery, systemic vasculitis or collagenolytic disease (e.g., Wegener's granulomatosis, rheumatoid arthritis, systemic lupus erythematodes) are contraindications, because the cornea may melt, resulting in a perforated areactive ulcer.

4.8.5 Immunologic Allograft Rejection after PKP

Immunologic graft rejection after PKP may be prompted by any surgical procedure on the graft. PTK may be such a procedure, especially in cases of provoked inflammation due to prolonged time period until epithelial closure. There have been cases of immunologic graft rejection after PTK reported in the literature [11]. It may happen even years after PKP. Although it is unclear what precipitated the immune reaction (laser treatment itself, manual epithelial removal, alterations in the patient's medical regimen), it is clear, however, that immediate topical and systemic treatment with corticosteroids is indispensable to manage this rare complication after PTK. Administering a moderate dose of systemic corticosteroid (e.g., 80 mg prednisolone-acetate orally) for a few days after PTK on a corneal graft should be considered as a prophylaxis.

4.8.6 Recurrence of Disease

Patients with corneal dystrophies undergoing PTK may suffer recurrences following the procedure. Disorders such as lattice/granular/macular dystrophy, epithelial basement membrane dystrophy or Salzmann's nodular degeneration may recur at variable intervals post-PTK. It is important to advise the patient of this possibility. PTK or manual superficial keratectomy while clearing and smoothing the cornea does not cure the underlying disorder of the epithelium or keratocytes. In cases in which the disease recurs and becomes visually significant, PTK can be repeated as long as enough corneal tissue is available.

Our clinical impression based on scientific evaluation is that recurrences on the original cornea after PTK behave differently compared with recurrences of the same dystrophy on the graft after transplantation: While granular dystrophy recurs quite often and quickly on the graft [3] it recurs late after PTK [8]. While macular dystrophy recurs after decades on the graft, superficial plaque-like opacities do recur very quickly after PTK [17]. In contrast, map-dot-fingerprint dystrophy recurs very late. Some eyes did not show a morphologic recurrence of RCES after 9 years [29]. Present studies on recurrence of dystrophies after PTK should include cumulative recurrence rates according to Kaplan–Meier, not only the relative risk in percentage.

Reactivation of herpetic/adenoviral disease may be induced by UV light effects during PTK [49, 50]. Besides laser effects, manual trauma and postoperative use of corticosteroids may be factors in herpes reactivation. This may be true in cases of concomitant corneal scars after herpetic keratitis. Thus, active herpes keratitis is an absolute and herpetic scars a relative contraindication for PTK. If PTK is performed on a herpetic scar, a quiescent period of 6–12 months is preferred before PTK, and perioperative treatment with topical antiviral agents as well as oral acyclovir may act as prophylaxis against recurrent herpetic infection.

4.8.7 Corneal Ectasia

Corneas that are too thin (i.e., <400 µm) should not be treated with the laser, since additional tissue removal may destabilize the cornea or may further distort the corneal surface with the consequence of progressive myopia. In addition, deep laser ablation may damage corneal endothelium with concomitant shock waves. The analogous problem may appear after PRK or LASIK with too thin a residual stromal bed thickness [40].

4.8.8 Intraocular Lens Power Calculation for Cataract Surgery after PTK

For a decade it has been well known that intraocular lens (IOL) power calculation after myopic PRK/LASIK will result in postoperative hyperopia if no precautions are taken. The deviation from the intended spherical equivalent increases with the amount of myopic correction that preceded [35]. Since PTK inadvertently results in flattening of the cornea, this problem may also apply here – to some extent. Cataract surgeons should know about this potential risk of IOL miscalculation after PTK. Nevertheless, patients after PTK are typically much less demanding than patients

after PRK/LASIK in terms of achieving an optimal UCVA after phacoemulsification.

Summary for the Clinician

- The complications associated with PTK comprise those resulting from corneal surgery in general, in addition to topographic and refractive considerations. Patients should be carefully followed for persistent epithelial defects, and associated complications should be treated promptly
- A perfect removal of dystrophic deposits with a clear cornea may be complicated by unwanted refractive shifts. Patients, therefore, should be made aware of both the potential risk for refractive change (especially "hyperopic shift") and the alternatives, such as rigid contact lenses, available to optimize vision after PTK
- Autologous serum eye drops and amniotic membrane patch seem to be good treatment options in cases of persistent epithelial defects, especially with lattice corneal dystrophy

4.9 Contraindications

A vascularized pannus due to chronic blepharokeratoconjunctivitis (e.g., rosacea, atopia) should not be treated because after PTK, persistent epithelial defects and a quick recurrence of the pannus are to be expected (Table 4.5).

Likewise, limbal stem cell deficiency syndromes (e.g., after chemical burns, cicatricial pemphigoid, Fuchs-Stevens-Johnson syndrome) are absolute contraindications for PTK.

Recurrent or persistent epithelial defects due to acute epithelial herpetic keratitis should not be treated with PTK. Herpetic scars should only be treated with systemic/topical virustatic medication, e.g., acyclovir. Overall, acute infectious ulcers of any origin should not be treated with PTK.

Eyes with superficial corneal neoplasias (e.g., corneal intraepithelial neoplasia [CIN]) should not be treated with laser ablation since Bowman's layer serves as a protective shield against deeper expansion of the tumor. In these cases, blunt removal of tumor tissue from Bowman's layer is considered the method of choice.

As mentioned above, systemic vasculitis or collagenolytic disease (e.g., Wegener's granulomatosis, rheumatoid arthritis, systemic lupus erythematodes) are absolute contraindications, because the cornea may melt resulting in a perforated areactive ulcer.

Eyes with major quantitative keratoconjunctivitis sicca are considered to be relative contraindications. Concomitant lid disease (e.g., meibomitis) has to be treated first. Punctum plugs may be considered first and nonpreserved artificial tears must be substituted on a high frequency basis before PTK can be considered, with a limited chance of success.

Epithelial defects on the graft in the early course after corneal transplantation typically do not respond well to PTK. In these cases topical application of autologous serum eye drops [14] or the temporary surgical supply with an amniotic membrane patch not intended to be integrated into the cornea may be valid options.

Table 4.5 Contraindications for excimer laser PTK

- Vascularized pannus due to chronic blepharokeratoconjunctivitis (e.g., rosacea, atopia)
- Limbal stem cell deficiency syndromes (e.g., after chemical burns, cicatricial pemphigoid, Fuchs-Stevens-Johnson syndrome)
- Recurrent epithelial defects due to acute epithelial herpetic keratitis
  (Herpetic scars only with the aid of systemic/topical virustatic medication)
- Acute infectious ulcers
- Corneal epithelial neoplasia
- Systemic vasculitis or collagenolytic disease (e.g., Wegener's granulomatosis, rheumatoid arthritis)
- Major quantitative keratoconjunctivitis sicca
- Early postkeratoplasty epithelial defects
  (typically do not respond! Use autologous serum or amnion patch instead)

Summary for the Clinician

- Never treat actively inflamed eyes with PTK; in particular, avoid eyes with herpetic keratitis
- Avoid treating eyes with limbal stem cell deficiency of various origins
- Be very careful with eyes in patients with systemic vasculitis or collagenolytic disease
- Do not treat persisting epithelial defects on the graft early after keratoplasty with PTK (alternatives include autologous serum and amniotic membrane patch)

4.10 Closing Remarks

Excimer laser PTK is a powerful tool for the management of anterior corneal pathology, especially superficial corneal dystrophies. However, proper case selection is of paramount importance. Excimer laser-assisted superficial corneal surgery is – in many cases – an *art* based on science. Minor variations in technique may make the difference between success and failure. In a properly selected and well-counseled patient, PTK can significantly improve vision and quality of life, avoiding or at least postponing the need for corneal transplantation.

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Chapter 5

Classification of Corneal Dystrophies on a Molecular Genetic Basis

5

Francis L. Munier, Daniel F. Schorderet

Core Messages

- Corneal dystrophies represent a highly heterogeneous group of hereditary disorders, consisting of more than 30 distinct entities, of which only 16 have their molecular basis partially or completely elucidated. The genetic characterization of corneal dystrophies revealed both genetic heterogeneity, i.e. different genes (KRT3 and KRT12) causing one single dystrophic phenotype, as well as phenotypic heterogeneity with one single gene (TGFBI) causing different dystrophic phenotypes (see below 5.5 keratoepithelinopathies).

5.1 Introduction

Corneal dystrophies represent a heterogeneous group of hereditary disorders with a high variability in terms of age at onset, pattern of progression, and severity of visual impairment. The corneal dystrophies may be isolated or syndromic, i.e., associated with other ocular or systemic features. In this chapter we will focus on the well-established isolated corneal dystrophies for which a genomic locus and/or the disease-causing gene have been identified.

In the absence of epidemiologic studies, their frequency remains poorly defined. However, it is widely accepted that corneal dystrophies are ubiquitous conditions with possible inhomogeneous geographic distribution in certain populations, as a result of either founder effect or degree of consanguinity. The frequency of blinding dystrophic corneal conditions can be evaluated to 15–17% according to the reported indications for perforating keratoplasty in the Western world [12, 42].

Historically, the reports of the first two distinct corneal dystrophies were simultaneously published in 1890 by Groenouw [19] (granular type 1) and Biber [4] (lattice type 1). The hereditary nature of these conditions was documented by Groenouw in 1917 [20]. Since then, an increasing number of additional clinical entities have been reported with some 30 disorders so far. Upon request of the Nazi regime, promoting eugenics through sterilization of the disabled, Bücklers proposed the first genetic classification of corneal dystrophies in 1938 [9]. The sterilization law was applied in the case of recessive dystrophy. The first genomic localization was reported by Eiberg et al. in 1993 [14] for the granular type 1 corneal dystrophy, which led 4 years later to the discovery of the first causal gene defect [36].

5.2 Anterior Corneal Dystrophies (Epithelial, Basal Membrane, Bowman's Layer, Anterior Stroma)

5.2.1 Meesmann Corneal Dystrophy (MIM 122100) Including Stocker-Holt

The first description of this slowly progressive condition was published in 1935 [41].

First symptoms may manifest as early as infancy with photophobia, recurrent punctiform epithelial erosions, and lacrymation. Biomicroscopy reveals multiple tiny intra-epithelial vesicles surrounded by clear epithelium, affecting the en-

tire cornea (Fig. 5.1). The number of lesions, usually in excess in the interpalpebral area, increases with age and may be associated with a typically fluctuant visual impairment.

Light microscopy shows PAS-positive cysts throughout all epithelial layers together with the presence of slight acanthosis and thickening of the epithelium and basal membrane respectively. Upon electronic microscopy, the cysts appear as well-delineated, round lesions of 10–50 μm in diameter. Focal intracytoplasmic accumulation of fibrogranular material and abnormal aggregates of keratin filaments are present in the basal and suprabasal cells.

Therapeutic photokeratectomy is necessary only in the case of recurrent erosions. The use of soft contact lenses may improve visual acuity by decreasing the number of cysts as a consequence of the induced epithelial thinning.

Transmission is autosomal dominant with complete penetrance. Two loci have been identified on chromosome 12q13 and 17q12, corresponding to the genes coding for two constitutive intermediate filaments of the cornea, keratin 3 (KRT3) and 12 (KRT12) Keratins, also known as cytokeratins, belong to the water-insoluble cytoskeletal proteins forming 10-nm intermediate filaments in epithelial cells. Based on their relative charges, keratins are separated into two types: type 1, composed of 28 different acid forms within the range of 40 to 55 kD, and type 2, made of 26 neutral to basic forms with a molecular weight of 56 to 70 kD. In vivo, a basic and an acidic form pair to produce cell-specific

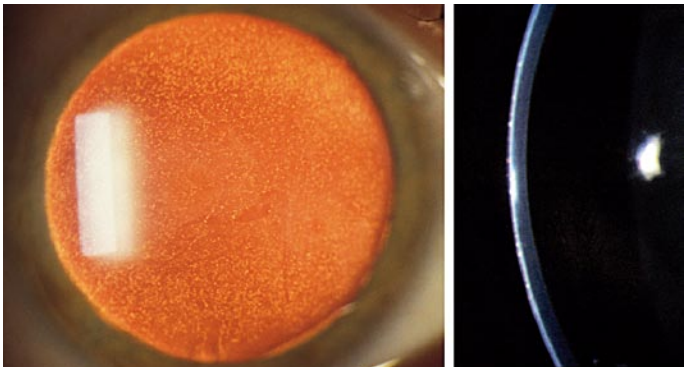
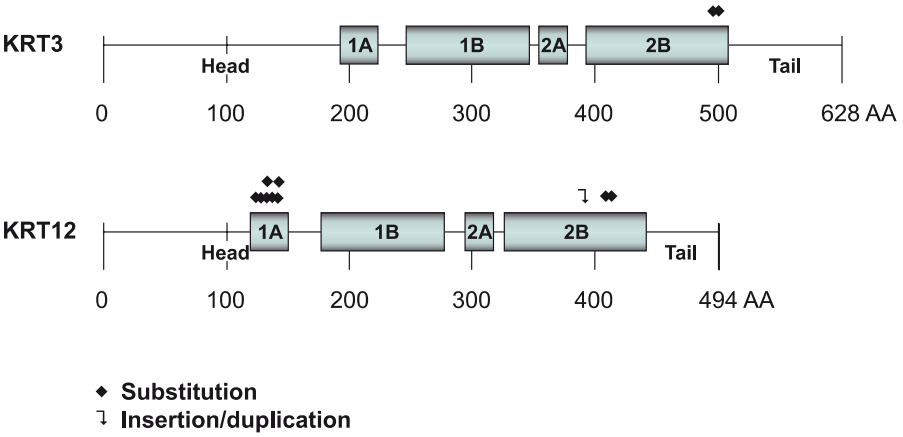


Fig. 5.1 Meesmann corneal dystrophy: retroillumination (*left*) and slit-lamp view (*right*)



Scheme 5.1

heterodimers. The heterodimer KRT3 (64 kD) and KRT12 (55 kD) is specifically expressed in the cornea and is important for the stability of keratocytes, as demonstrated by Kao et al. [25] in a KRT12 mouse knock-out model.

Seventeen mutations in exons 1 and 6 of KRT12 have been reported, as well as two mutations in exon 7 of KRT3 (Scheme 5.1). All of them are substitution mutations, but one is a duplication/insertion of 9 amino acids and they are all located in the keratin domains 1A or 2B.

5.2.2 Lisch Corneal Dystrophy

First described in 1992 [30], this slowly progressive condition is also named "band-shaped and whorled microcystic dystrophy of the corneal epithelium."

The patients remain asymptomatic as long as the visual axis is uninvolved, which may last until the third decade of life, when slight photophobia and ultimately blurred vision can occur. Biomicroscopy is characterized by the presence of well-delineated grey radial centripetal epithelial opacities, first sparing the center. These lesions begin in childhood and adopt various patterns, radial, band-shaped, and club-shaped, as they progress toward the center of the cornea with clear corneal epithelium between them (Fig. 5.2). There is no difference between males and females in terms of corneal opacity. Light microscopy demonstrates diffuse cytoplasmic vacuolization of the affected corneal epithelium across its whole thickness, containing osmiophilic, partly homogeneous, and partly lamellar material on electron microscopy.

Therapeutic use of contact lenses may improve corneal transparency.

The mode of inheritance is X-linked dominant with the locus of interest mapped to Xp22.3 [29]. The gene responsible is still unknown.

5.2.3 Epithelial Basement Membrane Dystrophy (MIM 121820)

The first description of this slowly progressive, early adult onset corneal dystrophy was given by Vogt in 1930 [57] and was further characterized by Cogan et al. [11] (dots = microcysts), Guerry [21] (maps), and Bron [8] (blebs). The disease is also known under three other eponyms: "map-dot-fingerprint dystrophy," "Cogan microcystic epithelial dystrophy," and "anterior basement membrane dystrophy." The condition is either asymptomatic or may cause recurrent erosions with pain, lacrymation, and blurred vision. Slit-lamp examination typically reveals the so-called maps, i.e., geographic lesions consisting of irregular islands of thickened hazy epithelium delimited by scalloped borders with or without dots, fingerprints, and blebs (Fig. 5.3, left). Cogan's dots are round, oval (50–500 μm in diameter) or comma-shaped grayish opacities clustered in an archipelago-like manner in the central cornea. Fingerprint lines correspond to parallel curvilinear lines best observed under retroillumination (Fig. 5.3, right). Bron's blebs are clumped sub-

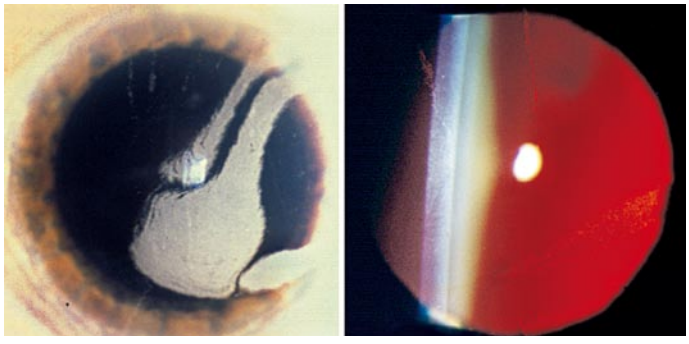


Fig. 5.2 Lisch corneal dystrophy: biomicroscopic view under diffuse light (*left*) and retroillumination (*right*). (Courtesy of Prof. W. Lisch)

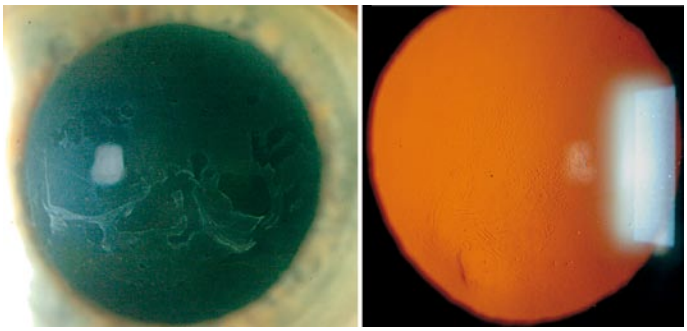


Fig. 5.3 Epithelial basement membrane dystrophy: biomicroscopic view under diffuse light (*left*) and retroillumination (right)

epithelial lesions (15–100 μm in diameter), also best seen under retroillumination. The light and electron microscopic equivalents of maps are aberrant multilamellar (sheets 2–6 nm thick) basal membrane with ectopic intraepithelial extensions. The fingerprint lines are rib-like intraepithelial extensions of basal laminar material and fibrillogranular substance (fibrils 17 nm in diameter and granular deposits 8 nm in diameter), while dots are intraepithelial pseudocysts containing pyknotic nuclei and cytoplasmic debris, and blebs subepithelial accumulation of fibrillogranular material.

Treatment varies according to the severity, from prescription of hypertonic NaCl with or without contact lens, sometimes following mechanical debridement. Therapeutic photokeratectomy is also an option in more advanced cases.

The vast majority of cases are sporadic with few reports of autosomal dominant transmission. The genomic localization is unknown. Two distinct TGFBI mutations (Scheme 5.2) have recently been reported in a subset of map-dot-fingerprint dystrophy patients representing less than 10% of the patients screened [6].

5.2.4 Gelatinous Drop-like Dystrophy (MIM 204870)

The *descriptio princeps* of this dystrophy was reported by Nakaizumi in 1914 [37] in a Japanese patient. This entity is also known under eponyms such as subepithelial amyloidosis or primary familial amyloidosis. An association with spheroidal degeneration has been observed by us (unpublished data) and others [3].

The initial manifestations start during the first decade of life with irritation, redness and tearing due to the appearance of sub-epithelial fluorescein-positive nodules of 500 μm in diameter with a band-shaped distribution (Fig. 5.4). These muriform nodules increase in diameter and number with age, leading to significant visual loss by coalescence and infiltration of the anterior stroma. Superficial neovascularization may occur. Light and electron microscopy identifies the deposits

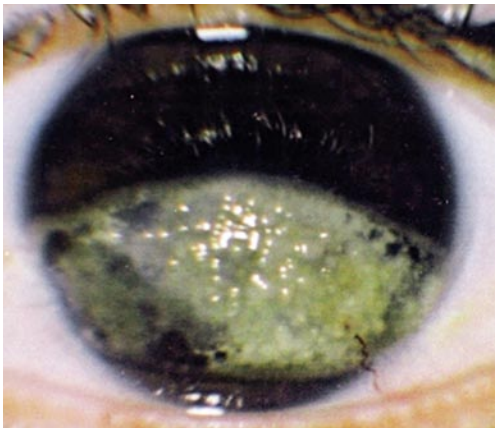
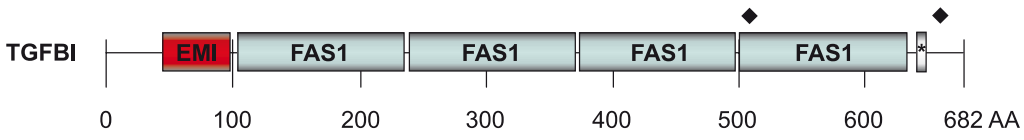


Fig. 5.4 Gelatinous drop-like dystrophy: biomicroscopic view under diffuse light (Courtesy of Prof. L. Chachoua)



◆ **Substitution**

* **RGD sequence**

Scheme 5.2

as amyloid material produced by basal epithelial layers, first accumulating between the epithelium and the Bowman's layer and then progressing within the superficial stroma. Amorphous globular deposits with positive staining for elastic tissue may also be present, corresponding to electrodense extracellular deposits under electron microscopy. Lamellar or perforating keratoplasty is usually performed by the third decade of life, but recurrence is the rule within 5 years.

Transmission is autosomal recessive and mutations have been identified in the tumor-associated calcium signal transducer 2 (TACSTD2, M1S1) gene on chromosome 1p32. The role of TACSTD2 is poorly understood. It encodes an antibody derived from the immunization of mice with a human stomach adenocarcinoma cell line. It contains two CD44 and one thyroglobulin domains and may play a role in cell proliferation and signaling.

Some 18 distinct mutations have been reported in this single exon gene, with Q118X present in the majority of Japanese patients.

5.2.5 Thiel-Behnke Corneal Dystrophy Type I (MIM 602082)

The initial report of this slowly progressive disorder was published in 1967 by Thiel and Behnke [53]. Other synonyms include: corneal dystrophy of Bowman's layer type II (CDB2), honeycomb corneal dystrophy, anterior limiting membrane dystrophy type II, curly fibers corneal dystrophy, and possibly Waardenburg-Jonkers corneal dystrophy. The childhood onset is characterized by recurrent erosions that are less frequent than in Reis-Bücklers' and may resolve with age. Vision deterioration occurs later than in Reis-Bücklers'. The biomicroscopic aspect is pathognomonic, with subepithelial reticular opacities producing a symmetric honeycomb appearance (Fig. 5.5). Histology features a destructed Bowman's layer replaced by fibrocellular tissue accumulating in a characteristic saw-toothed configuration. The electron microscopy signature is the presence of curly fibers with a diameter of 9–15 nm, which stain positively for keratoepithelin.

The mode of inheritance is autosomal dominant with complete penetrance, linked to the

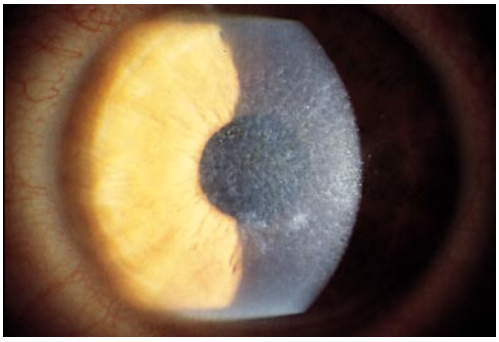


Fig. 5.5 Thiel-Behnke corneal dystrophy: biomicroscopic view under diffuse light (*left*) and retroillumination (*right*)

TGFBI (BIGH3) gene. The disease-causing mutation is invariably a substitution of arginine by glutamine at residue 555 (R555Q) (Scheme 5.3).

A single family with a similar phenotype (Thiel-Behnke corneal dystrophy type II) was reported by Yee et al. in 1997 [60]. Linkage data definitely excluded the TGFBI gene, and the initial 10q24 locus is being reassessed following reappraisal of the family (Yee, personal communication, 2007).

5.2.6 Reis-Bücklers' Corneal Dystrophy (MIM 608470)

Although first described by Reis in 1917 [44] and further delineated by Bücklers in 1949 [10], this clinical entity was frequently confused with Thiel-Behnke corneal dystrophy until the molecular basis of these closely related conditions was unambiguously identified by Okada et al. in 1998 [40]. Several eponyms exist in the literature including, corneal dystrophy of Bowman's layer type I, geographic corneal dystrophy, anterior limiting membrane dystrophy type I, granular dystrophy type III, superficial granular dystrophy, and possibly Grayson-Wilbrandt dystrophy.

Disease manifestations start in childhood with recurrent erosions and visual impairment occurring earlier than in Thiel-Behnke corneal dystrophy.

The slit-lamp examination shows confluent irregular (nonreticular) subepithelial geographic-like opacities (Fig. 5.6) giving rise to bilateral asymmetric status. Light microscopy typically displays a sheet-like connective tissue layer, replacing the Bowman's layer with granular Masson-positive deposits. Electron microscopy allows the identification of electron-dense rod-shaped bodies staining positively for keratoepithelin, the product of TGFBI (BIGH3).

Inheritance is autosomal dominant and linked to the TGFBI (BIGH3) gene. The geno-phenotypic correlation is absolute, with a substitution of arginine in position 124 by a leucine (R124L; Scheme 5.3).

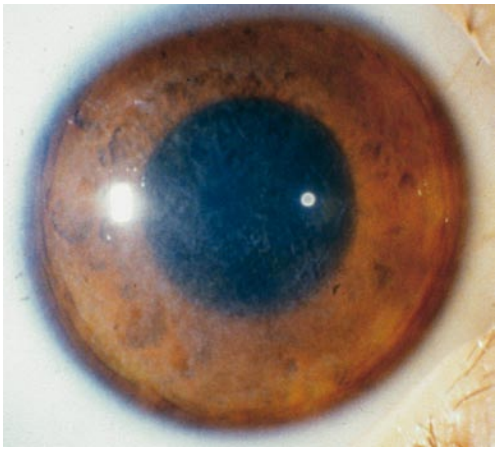


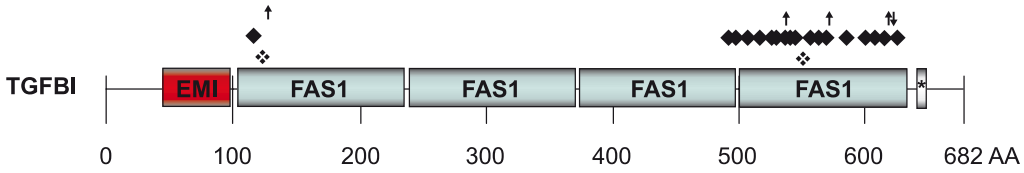
Fig. 5.6 Reis-Bücklers' corneal dystrophy: biomicroscopic view under diffuse light (Courtesy of Dr. R. Barraquer)

5.3 Stromal Corneal Dystrophies

5.3.1 Granular Dystrophy Type I (MIM 121900)

In the medical literature, granular dystrophy (also designated Groenouw type I corneal dystrophy) emerged as a new disease entity when first reported by Groenouw in 1890 [19]. This slowly progressive disorder may manifest at as early as 2 years of age with photophobia and pain accompanying recurrent erosions. Visual acuity decreases with age. Clinically, well-defined tiny whitish granules with bread crumbs (Fig. 5.7) or ring appearance (with a clear center) can be observed. In some cases, the deposits aggregate in a pseudoreticular (Fig. 5.7) or fractal pattern. The common denominator is the fact that the individual lesions are tiny and too numerous to be easily counted, unlike in granular dystrophy type II (Avellino phenotype). With age they tend to increase in number and to involve deeper layers of the stroma. Perforating keratoplasty is the treatment of choice. Recurrence in the graft can be seen 10 years after surgery. This may be delayed by the use of soft contact lenses.

In light microscopy, there are multiple stromal granular deposits extending from deep epithelium to Descemet's membrane and staining positive with Masson trichrome. Keratoepithe-



❖ Substitution at hot spot
◆ Substitution
↑ In frame deletion
↓ In frame insertion

* RGD sequence

Scheme 5.3

lin-positive rod-shaped bodies are seen upon electron microscopy.

Heredity is autosomal dominant with complete penetrance and incomplete dominance. In the vast majority of cases the disease-causing mutation is a substitution of arginine in position 555 by a tryptophane (R555W) [35, 36] in the TGFBI (BIGH3) gene on chromosome 5q31 (Scheme 5.3). In the homozygous state, the R555W mutation causes a more severe confluent variant of granular dystrophy, characterized by a diffuse placoid pattern of deposition [39]. Discrete mutants, such as R124S [50], R124L + delT125-E126 [13], and D123H [22] have been described in single families of Asian, French, and Vietnamese origin respectively.

5.3.2 Granular Dystrophy Type II (MIM 607541)

The initial description of this entity was independently recognized by Weidle (1988) [59] and Folberg (1988) [16]. Avellino corneal dystrophy, or combined granular-lattice corneal dystrophy, are other denominations found in the literature.

This slowly progressive dystrophy manifests during adolescence with photophobia, but can remain asymptomatic in less expressive cases. Recurrent erosions rarely occur. Vision decreases with age only if the central visual axis is affected. Biomicroscopy is pathognomonic with the presence of superficial whitish dots, visible as early as the end of the first decade of life, often displaying a firework pattern of distribution. During or after the second decade, the corneal deposits, typically larger than those of granular type I, present as snowflake-like, ring- or star-shaped lesions. Later, linear deposits (lattice lines) may be seen deeper in the stroma in some patients. Unlike in granular dystrophy type I (Fig. 5.7), the total number of lesions is easily identifiable, ranging from a single one to less than 50 per affected cornea (Fig. 5.8) and does not seem to increase with age. The opacities, upon light microscopy, extend from basal epithelium to deep stroma, and stain either with Masson Trichrome or with Congo red, indicating deposition of both hyaline and amyloid keratoepithelin-positive material. Rod-shaped bodies and fibrils are the corresponding EM findings. In the heterozygote form, perforating keratoplasty is performed around the 6th decade of life, while in the homozygote variant phototherapeutic keratectomy is indicated between the 2nd and 3rd decade. Recurrence occurs four times earlier in homozygotes than in heterozygotes [24]. Corneal electrolysis has been successfully applied to eliminate the recurrent deposits [31].

Inheritance is autosomal dominant with near complete penetrance and incomplete dominance. The disease-causing mutation is a heterozygote

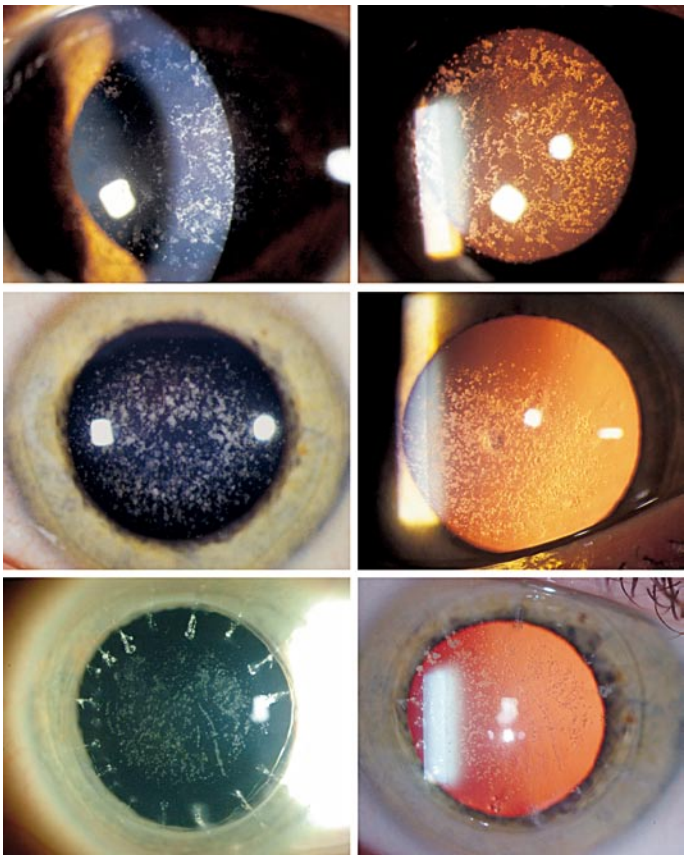


Fig. 5.7 Granular dystrophy type I: biomicroscopic view under diffuse light (*left column*) and retroillumination (*right column*) with classic bread crumbs phenotype (*top row*), pseudo reticular phenotype (*medium row*), and recurrence in the graft (*bottom row*)

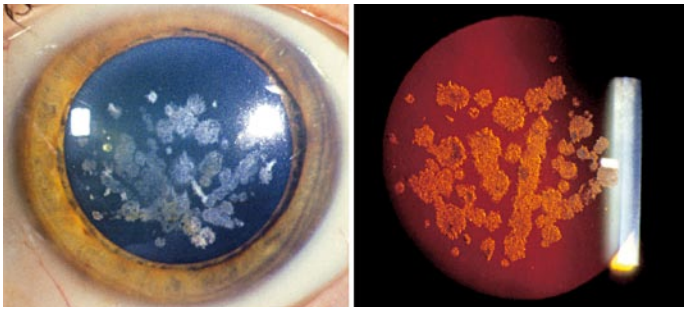


Fig. 5.8 Granular dystrophy type II (Avellino): biomicroscopic view under diffuse light (*left*) and retroillumination (*right*)

substitution of arginine to histidine at residue 124 in the TGFBI (BIG3) gene on chromosome 5q31 [36] (Scheme 5.3). R124H homozygotes develop the so-called "superficial variant of granular dystrophy," characterized by a much more severe course [32, 38] with two distinct anterior stromal phenotypes: discrete confluent round opacities or reticular opacities with round translucent space [58]. Unlike heterozygote corneas, homozygote ones demonstrate only hyaline deposits upon histopathologic examination.

5.3.3 Lattice TGFBI type Corneal Dystrophy (MIM 122200)

Biber reported the first description of lattice corneal dystrophy in his medical thesis in Zürich [4]. Since then, the classic form of lattice corneal dystrophy (LCD), or LCD type I is also known as Biber-Haab-Dimmer dystrophy. Lattice corneal dystrophy type II (Lattice Gelsolin type corneal dystrophy) is determined by mutations in the Gelsolin (GSN) on chromosome 9q34 and is part of systemic amyloidosis of the Finnish type (Meretoja syndrome). Numerous variants of TGFBI-related lattice dystrophy have been reported (types IIIA, intermediate I/IIIA, and IV).

The classic LCD type I is slowly progressive and becomes symptomatic in the first decade of life as a consequence of recurrent erosions. Superficial dots can be seen in the central cornea, which further accumulate along radial branching lines starting centrally and spreading both centrifugally and from anterior to posterior stroma. These deposits are not observed in Descemet's membrane, the endothelium, or in the 1-mm peripheral ring of the cornea. A diffuse stromal ground-glass haze develops later associated with visual impairment within the 4th decade of life (Fig. 5.9). Optic microscopy demonstrates the presence of deposits between epithelial basement membrane and Bowman's layer, as well as in the stroma where they distort the corneal lamellae. They stain positive with PAS, and Congo red. Green fluorescence is visible with a polarizing filter and red-green dichroism when a green filter is added. These deposits contain AA amyloid and stain positive with keratoepithelin antibodies, partly as a result of an aberrant 44 kDa aminoterminal fragment of the protein [26]. The accumulated material is made of extracellular, fine, electron-dense, highly aligned fibrils with a diameter of 80–100 angstroms.

Lattice corneal dystrophy variants (types IIIa, I/IIIa, and IV) have delayed onset with or without lattice lines (larger deposits than in classic type I with a limbus to limbus ropy appearance), with (types IIIa and I/IIIa) or without (type IV) corneal erosions, with an anterior to posterior (types IIIa and I/IIIa) or a posterior to anterior (type IV) progression. Deposits can be identified as AP and not AA amyloid.

Perforating keratoplasty is necessary by the 4th decade in LCD type I, and between the 4th and 7th decade in the LCD variants. Recurrence in the graft usually occurs 10 years following surgery.

Lattice corneal dystrophy type I and its variants have an autosomal dominant inheritance,

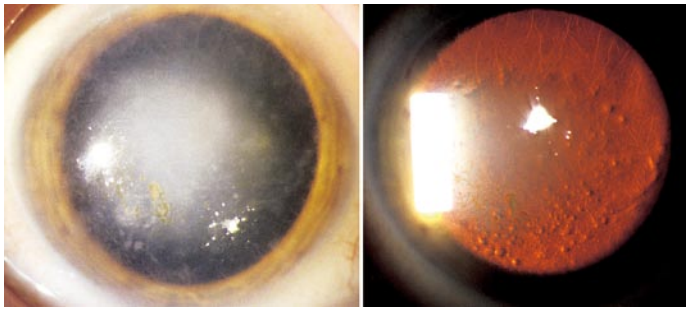


Fig. 5.9 TGFBI lattice dystrophy type I: biomicroscopic view under diffuse light (*left*) and retroillumination (*right*)

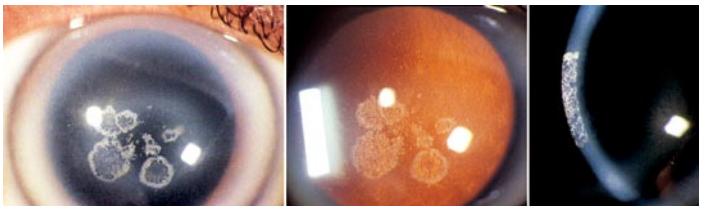


Fig. 5.10 Schnyder corneal dystrophy: biomicroscopic view under diffuse light (*left*) and retroillumination (*center*). Slit-lamp view of the superficial crystalline deposits and arcus lipoides (*right*)

and are caused by mutations in the TGFBI (BIGH3) gene on chromosome 5q31 [36]. LCD type I (classic) is the result of a substitution of arginine by cysteine at residue 124, while LCD variants are caused by at least 26 distinct mutants all targeting the fourth fasciclin-like domain of TGFBI (Scheme 5.3).

5.3.4 Schnyder Corneal Dystrophy (MIM 121800)

First mentioned by van Went and Wibaut in 1924 [55], a more complete description of the disease was published in 1929 by Schnyder [48]. The other denominations in the literature are Schnyder crystalline corneal dystrophy, hereditary crystalline stromal dystrophy of Schnyder, and crystalline stromal dystrophy. If onset takes place as early as childhood, most of the cases are diagnosed during the 2nd or 3rd decade. The disease course is slowly progressive. Vision decreases with age, as does the corneal sensation. Initially, the central cornea presents a superficial haze (anterior stroma) and/or subepithelial crystals (Fig. 5.10). Only 50% of cases will demonstrate the crystalline form. Later (in the 3rd to 4th decade) an arcus lipoides is noted, soon complicated (in the 5th decade) by a midperipheral panstromal haze, leading to haziness of the entire cornea.

Histologically, abnormal deposition consists of intra- and extracellular esterified and unesterified phospholipids and cholesterol in the basal epithelial cells, Bowman's layer, and stroma.

Phototherapeutic keratectomy, lamellar or perforating keratoplasty may be performed. The recurrence on the grafted cornea is seen only 20 years later.

Inheritance is autosomal dominant and the responsible gene has been mapped to chromosome 1p36 [49].

5.3.5 Macular Corneal Dystrophy (MIM 217800)

Groenouw was the first author to report this slowly progressive condition in 1890 [19]. It is also known as Groenouw corneal dystrophy type II or Fehr spotted dystrophy. Photophobia, impaired vision, and reduction of corneal sensitivity are the main symptoms. Recurrent erosions can also occur. Clinically, the disease is recognizable between the 1st and 3rd decade by a superficial stromal haze initially affecting the central cornea, then extending to the limbus. Later, poorly delineated whitish opacities (macules) appear (Fig. 5.11) within all stromal layers. The cornea is initially thinner than normal and guttate excrescences appear with age. Vision is significantly reduced between the 3rd and 4th de-

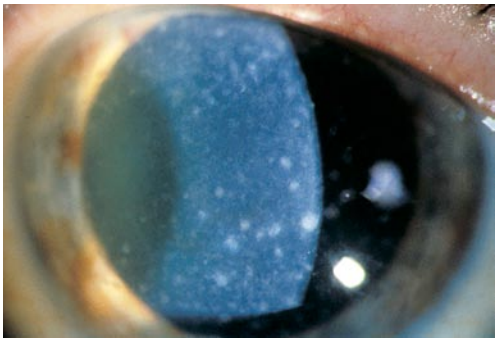


Fig. 5.11 Macular corneal dystrophy: biomicroscopic view under diffuse light (Courtesy of Dr. B. Frueh)

cade. Glycoaminoglycans, staining with Alcian blue, accumulate intra- and extracellularly in the corneal stroma, as well as in Descemet's membrane and the endothelial cells. The extracellular matrix observed in electron microscopy contains clumps of fibrillogranular material staining positively for glycosaminoglycans, while keratocytes display various stages of degeneration.

Perforating keratoplasty is usually indicated around the 4th decade. No significant recurrence in the corneal graft is observed.

Heredity is autosomal recessive and the disease is linked to the transmission of mutations in the N-acetylglucosamine-6-sulfotransferase (carbohydrate sulfotransferase 6) gene (CHST6) on chromosome 16q21 [2, 34].

Carbohydrate sulfotransferases are membrane-bound members of a group of enzymes that catalyze the sulfation of specific carbohydrates. As they share substrates, they also show sequence similarities. CHST6 has a short cytosolic tail at the N-terminus, a single transmembrane domain and a C-terminal region containing the sulphate donor binding site, the catalytic domain, and a carbohydrate specificity region. More than 120 distinct mutations have been identified so far. All of them occur within exon 3, the only transcribed exon of CHST6.

5.3.6 Congenital Stromal Dystrophy (MIM 610048)

The first description of this very rare form of dystrophy is given by Turpin in 1939 [54]. The diagnosis is made at birth in the presence of diffuse bilateral corneal clouding with flake-like, whitish opacities, both equally distributed throughout the stroma. The epithelium and endothelium are normal. Pachymetry documents normal or increased corneal thickness (670 μm). There are no signs of vascularization and no staining with fluorescein. Unlike congenital hereditary endothelial dystrophy, the affection is non-progressive or slowly progressive. However, the condition is amblyogenic and may be associated with nystagmus, requiring perforating keratoplasty at an early age. Recurrence in the corneal graft is rare. The histology demonstrates a fibrillar dissociation of corneal lamellae, which corresponds upon electron microscopy to abnormal layers of thin filaments randomly arranged in an electron lucent ground substance that separate lamellae of normal appearance. The diameter of collagen fibrils in all lamellae is approximately half that of normal collagen fibrils.

The heredity is autosomal dominant and is determined by mutations in the decorin gene (DCN) on chromosome 12q22 [7].

Decorin is a small leucine-rich proteoglycan expressed in many connective tissues where it binds mainly to collagen 1. In cellular models, decorin causes phosphorylation of the EGF receptor, thus activating ERK1/2 MAP kinases and inducing cell cycle arrest through activation of p21 [34].To date, only two distinct mutations have been reported in the literature among the three known affected families. Both mutations are small deletions affecting the reading frame of the mRNA and are expected to produce truncated proteins lacking the collagen binding site. Interestingly, decorin has been shown to bind keratoepithelin together with biglycan [43].

5.3.7 Fleck Corneal Dystrophy (MIM 121850)

François and Neetens reported in 1957 [17] a congenital, nonprogressive, asymptomatic form of dystrophy. The diagnosis is fortuitous in the presence of small discrete, discoid or ring-shaped, flat opacities (Fig. 5.12) equally scattered throughout the entire stroma and located from limbus to limbus. The epithelium, Bowman's layer, Descemet's membrane, and the endothelium are not involved. The expressivity is highly variable, ranging from a few lesions to hundreds, sometimes displaying an asymmetric distribution. The presence of a subset of abnormal swollen and vacuolated keratocytes containing mucopolysaccharides and complex lipids are revealed by a positive staining for Alcian blue or colloidal iron, and for Sudan black or oil red O respectively. Electron microscopy shows vacuoles containing membranes or fibrillogranular material.

Treatment is unnecessary. Perforating keratoplasty was performed in one patient suffering from fleck corneal dystrophy associated with keratoconus. No recurrence was observed after 10 years' follow-up.

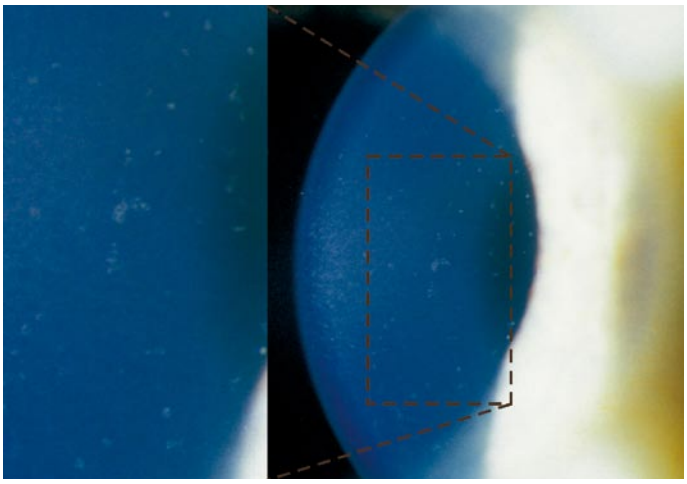
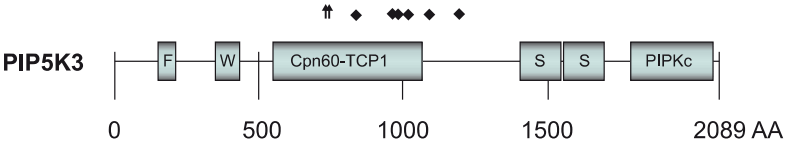


Fig. 5.12 Fleck corneal dystrophy: biomicroscopic view under diffuse light



◆ **Substitution**
† **Deletion**

Scheme 5.4

Heredity is autosomal dominant. The disease is genetically homogeneous with one single locus on chromosome 2q35, where at least eight pathogenic mutations of the phosphatidylinositol-3-phosphate 5-kinase type 3 (PIP5K3) gene have been found (Scheme 5.4) [28].

PIP5K3 is a member of the PIP5K family and catalyzes the phosphorylation at position 5 of phosphoinositol and phosphoinositol-3-phosphate to produce PI5P and PI3,5P. This kinase activity plays a role in intracellular membrane trafficking and regulates the endosome-to-trans-Golgi network retrograde transport. In vitro cellular experiments, in which PIP5K activity was abolished, induced intracellular vacuolations, an effect very comparable to the histopathology seen in affected cornea.

5.4 Posterior Corneal Dystrophies

5.4.1 Congenital Hereditary Endothelial Dystrophy (CHED1 MIM 121700; CHED2 MIM 217700)

The first report may have been published in 1887 by Saltini [45] in 3 out of 4 siblings from healthy parents, but it was only in 1960 that the disease was clinically and histologically characterized by Maumenee [33]. A bilateral diffuse corneal clouding, ranging from a hazy to a milky appearance (Fig. 5.13), is diagnosed at or shortly after birth in the recessive, nonprogressive form (CHED2), causing both nystagmus and amblyopia. The corneal thickness can be double or triple that of normal values. Secondary sub-

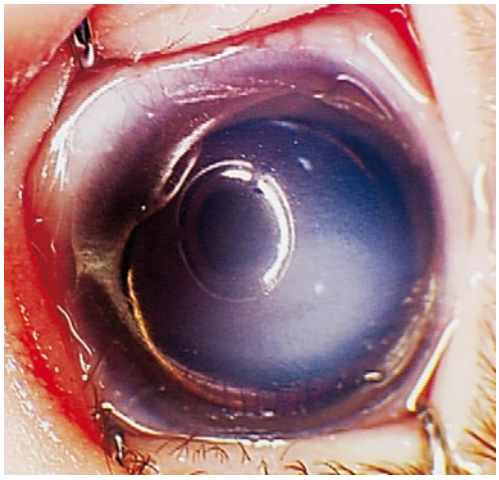


Fig. 5.13 Congenital hereditary endothelial dystrophy: anterior segment imaging with the Retcam

epithelial band-shaped keratopathy can occasionally be noted, as well as elevated intraocular pressure.

The clinical picture is similar in the slowly progressive dominant form (CHED1), whose onset is delayed until the first year(s) of life with an often asymmetric course. In this latter variant, photophobia and tearing, but not nystagmus, are present. Progression of corneal clouding takes place over 1–10 years.

Histologically, there is a diffuse thickening of Descemet's membrane as well as sparse and atrophic endothelial cells. In electron microscopy, multiple layers of basement membrane-like material can be seen within the posterior collagenous layer of Descemet's membrane, which contains collagen types I, III, V, and laminin, supporting a fibroblast-like change in the endothelium.

Perforating keratoplasty must be performed at diagnosis. There is no recurrence in the grafted cornea.

Autosomal recessive inheritance (CHED2) is more frequently observed than its autosomal dominant counterpart (CHED1). Linkage analyses mapped the disease loci to 20p11.2-q11.2 (CHED1) and to 20p13 (CHED2). The gene for CHED2 is a sodium-borate cotransporter SLC4A11, with more than 25 mutants identified so far [56]. The CHED1 gene has not yet been identified.

5.4.2 Posterior Polymorphous Corneal Dystrophy (MIM 122000)

In 1941, Schlichting [46] published the original description of this dystrophy, which is sometimes referred to as Schlichting corneal dystrophy. The condition is rarely congenital, with edematous clouding and secondary subepithelial band-shaped keratopathy. Otherwise, the disease has a slow to nonprogressive and often asymmetric course, with symptoms manifesting only in a subset of patients. Occasionally, corneal edema develops in adulthood necessitating keratoplasty. Recurrence may occur in the graft. Biomicroscopy detects isolated, confluent or clustered vesicular and blister-like endothelial lesions, as well as "railroad-tracks" (Fig. 5.14). The endothelium is replaced by multilayered epithelial-like cells with microvilli and desmosomes, which proliferating properties may cause secondary glaucoma due to trabeculum invasion. In addition, there is an extreme thinning or absence of the posterior non-banded layer of Descemet's membrane.

Heredity is autosomal dominant with incomplete penetrance. PPCD is genetically heterogeneous with at least three loci, namely PPCD1 on chromosome 20p11 [23] (but distinct from the VSX1 locus), PPCD2 on chromosome 1q34.2-p32.3 linked to Col8A2 [5] (Biswas et al. reported only two simplex cases with no other confirming reports [5]), and PPCD3 on chromosome 10p11.2 due to the gene ZEB1 (TCF8) [27]. ZEB1 mutations (more than 12 pathogenic allelic variants reported so far) appear to cause more than 25% of familial cases with a pleiotropic effect (abdominal and inguinal hernias).

5.4.3 Fuchs Endothelial Corneal Dystrophy (MIM 136800)

Fuchs gave the first clinical description in 1910 of FECD [18], the most common form of corneal dystrophy. This condition, also named familial corneal guttae, exists in a classic late-onset form (4th decade and later) and an early-onset variant (first decade). Impaired vision in the morning spontaneously improving during the day, with or without erosions from bursts of epithelial bullae,

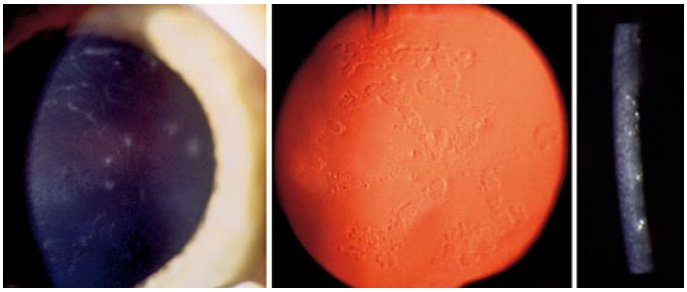


Fig. 5.14 Posterior polymorphous corneal dystrophy: biomicroscopic view under diffuse light (*left*) and retroillumination (*right*)

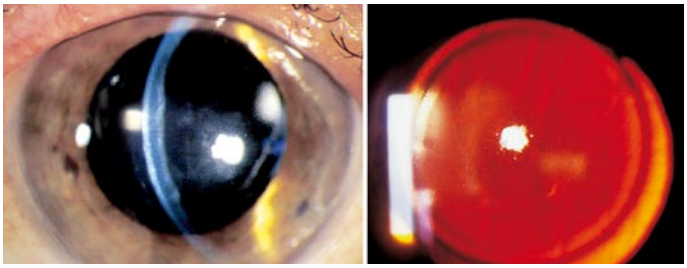


Fig. 5.15 Fuchs corneal dystrophy: biomicroscopic view under diffuse light (*left*) and retroillumination (*right*)

is typical of the symptomatology. Clinically, the endothelial face is characterized by the presence of guttata, i.e., centrally located excrescences and a beaten metal-like endothelial aspect spreading peripherally over time (Fig. 5.15). Endothelial decompensation causes a stromal edema with increased pachymetric values evolving toward bullous keratopathy with subepithelial fibrous scarring in the case of chronicity. Light microscopy reveals a diffuse laminar thickening of Descemet's membrane associated with hyaline excrescences and atrophic, enlarged endothelial cells. Electron microscopy demonstrates a newly produced layer of abnormal basement membrane material containing neocollagen, consisting of fibrils of various diameters (collagen VIII).

Perforating or deep lamellar keratoplasty is performed to restore vision.

Heredity is autosomal dominant with genetic heterogeneity. The classic FECD has already three loci, namely FECD1 on chromosome 13ptel-q12.13 [52], FECD2 on chromosome 15q [1], and FECD3 on chromosome 18q21.2-q21-32 [51]. The early-onset variant is linked to mutations in the Col8A2 gene on chromosome 1p34.4-p32 [5].

5.4.4 X-linked Endothelial Corneal Dystrophy

This dystrophy was described in 2006 by Schmid [47]. The disease course is progressive in males but not in females. In males the expressivity consists of:

- Congenital amblyogenic and possibly nystagmogenic clouding of the cornea, ranging from diffuse haze to a ground-glass, milky appearance
- Moon crater-like endothelial changes only
- Secondary subepithelial band-shaped keratopathy with moon crater-like endothelial changes

In females, asymptomatic moon crater-like endothelial changes are noted. Light microscopy highlights the irregular thinning of the epithelium and Bowman's layer, as well as irregularly arranged anterior stroma collagen lamellae. Descemet's membrane is irregularly thickened with small excavations and pits, associated with a loss of endothelial cells. Upon electron microscopy, an amorphous granular material accumulates where band-shaped keratopathy occurs. Bowman's layer is interrupted or irregularly thinner than normal. Descemet's membrane is thicker (20–35 μm) due to abnormal anterior and posterior banded zones. There is otherwise a complete absence of the posterior nonbanded zone. Endothelial cells are partly normal and partly degenerative. Unlike in posterior polymorphous corneal dystrophy, there are no desmosome-like adherent junctions between cells or tonofilament bundles within the cytoplasm. X-linked heredity has been proposed with a locus at Xq25.

5.5 Keratoepithelinopathies

Many corneal dystrophies described above are associated with mutations in TGFBI or TGFBI-interacting proteins, and thus, could be described as keratoepithelinopathies. KE, the protein encoded by TGFBI, is a member of the extracellular matrix present in large amounts in the cornea and also in many other tissues where it can be seen as a 68-kD protein. No or very reduced expression has been observed in the brain. This protein is composed of a signal peptide responsible for secretion, an EMI domain whose function is still unknown and four fasciclin-like domains. Fasciclin-like domains are regions homologous to the grasshopper fasciclin 1 gene, a gene involved in axon guidance. At the C-terminus, an RGD integrin binding site is present.

In mouse, KE is expressed in the first and second branchial mesenchymes as early as 11.5 dpc. During development, KE is concentrated in mesoderm-derived tissues: developing bones, cartilages, peribronchiolar structures, etc. It is also expressed in the heart, β-cells of the pancreas, bladder, and developing digits. Despite such a wide expression, abnormal KE only aggregates in the cornea. This was recently confirmed by the extensive necropsy of an affected member with TGFBI-related corneal dystrophy due to an R124C mutation [15].

It is tempting to hypothesize that all epithelial and stromal corneal dystrophies share a common metabolic origin that could arbitrarily be separated into two entities. The first group could represent pre-secretory dystrophies in which the main defect is located in the synthesis or intracellular transport, with concomitant clogging of the endoplasmic reticulum. The second group could be of the post-secretory type and would include defects in the catabolism of aberrant KE or KE-bound proteins (e.g., decorin) and would result in extracellular deposits or aberrant lysosomial processing. Different mutations in any of the structural proteins (e.g., TGFBI, KRT3, KRT12) of this pathway or in their functional components (e.g., PIP5K3) may result in either of the groups or both. Whether this is also true of endothelial dystrophies remains to be seen.

Progress in molecular genetics has allowed the characterization of most of these forms of corneal dystrophies and has paved the way to a new classification. In the future, clinical data will have to be substantiated with appropriate imaging, histology, biochemistry, and the exact molecular mutation.

Summary for the Clinician

- Recent molecular advances have simplified the categorization of corneal dystrophies. Among them, corneal dystrophies with mutations in the TGFBI/BIGH3 gene, now called keratoepithelinopathies, are characterized by unique genotype/phenotype correlations with hot spots at the FAS4 domain and at two arginine residues, namely R124 and R555. FAS4 mutations lead to a wide spectrum of amyloidogenic corneal dystrophies, while mutations at the R124 and R555 specifically cause granulogenic, mixte (granulogenic & amyloidogenic) or fibrocellular corneal dystrophies. With time, it is possible that such a correlation will also be possible for other corneal dystrophies.

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Chapter 6

Developments in Corneal Preservation

6

W. John Armitage

Core Messages

- Hypothermia and organ culture are the two main methods of corneal storage, the latter predominating in Europe
- Organ culture allows corneas to be stored routinely up to 4 weeks, although longer storage times up to 7 weeks have been reported
- Hypothermia allows up to 2 weeks of storage, but epithelium is not preserved as well as endothelium
- Some cryopreserved corneas have been successfully transplanted, but this method is now used only very occasionally for storing tissue for emergency transplants
- New surgical techniques and developments in gene therapy and tissue engineering are likely to increase the range and complexity of eye banking methodology

6.1 Introduction

The maintenance of viability of the cellular layers of the cornea is essential for the successful outcome of the great majority of corneal transplants. Reliable preservation of corneal viability eases the logistics of supply of tissue to transplant units and enhances the opportunities for ensuring the safety and efficacy of the tissue. Zirm's landmark transplantation of a cornea in 1905 used tissue from a living donor, an 11-year-old boy who had undergone a therapeutic enucleation [85]. The eye was kept in warm physiological saline solution and the transplant operation on the recipient started without delay. After the graft had been cut using a von Hippel trephine, it was held between two pieces of saline-moistened gauze over a container of hot, sterilized water. Zirm believed his careful handling of the tissue contributed to the successful outcome of the transplant. However, the impracticalities of routinely achieving such close proximity of the donor and recipient prompted Magitot [47] to suggest the use of corneas from deceased donors, and he also carried out some experiments on the refrigerated storage of whole eyes in serum. But it was Filatov in the 1930s [26] who should be credited for ultimately pioneering both the use of corneas from deceased eye donors and the storage of eyes in pots held in ice for several days (i.e., moist chamber storage).

It would be more than 30 years before there were any significant advances in corneal storage that improved on the hypothermic storage of whole eyes in moist chambers. In the mid 1960s, cryopreservation of corneas was achieved and a number of corneal transplants carried out with corneas that had been frozen [15, 36, 56, 59]. Owing to its perceived complexity, and despite its potential for truly long-term storage, cryopreservation never became widespread and today it is rarely used, except by a very few eye banks for storing corneas for emergency grafts or for storing nonviable corneal tissue. Two other techniques were developed in the early 1970s that have since underpinned routine eye banking practice, *viz.*, hypothermic storage of corneoscleral discs in medium [51] and organ culture [78].

With the increasing use of lamellar grafts (both anterior and endothelial keratoplasty) and limbal tissue and developments in tissue engineering and gene therapy, eye banks face new challenges in the processing and storage of ocular tissue.

Summary for the Clinician

- There are three main methods of preserving corneas: organ culture, hypothermia, and cryopreservation
- Cryopreservation is the only method that offers long-term storage, but is rarely used
- Storage of corneoscleral discs either in organ culture or in hypothermic storage medium are the predominant methods in use today
- Developing surgical techniques and new approaches to the treatment of corneal and ocular surface disease pose new challenges for eye banks

6.2 Hypothermic Storage

The maximum storage time for whole eyes in moist chambers was generally considered to be 48 h. Even so, some surgeons were unwilling to use tissue that had been stored for more than 12–24 h [76]. Clearly, with such short storage times the widespread distribution of tissue was impractical.

6.2.1 Storage of Corneoscleral Discs

In the 1960s, Stocker and colleagues [77] reported the successful transplantation of corneas stored in the recipient's own serum for up to 4 days. At around the same time in Japan, Kuwahara and colleagues [40, 41] investigated different ways of storing corneal grafts, both as whole eyes and corneoscleral discs. They measured lactic acid accumulation during storage and found a marked increase in whole eyes compared with corneoscleral discs, implying that storage of the latter would be advantageous. They devised a corneal storage solution with an electrolyte composition similar to human aqueous humor. This solution also contained chondroitin sulphate (1%) and ascorbic acid (0.1%) and corneas were successfully transplanted after 7 days of storage.

6.2.2 McCarey-Kaufman Medium

McCarey and Kaufman advocated the storage of corneoscleral discs in tissue culture medium 199 (M-199) containing 5% w/v dextran 40 to help limit stromal edema (M-K medium) [51, 52]. They also suggested, echoing Kuwahara's earlier observations, that removal of the cornea from the eye was advantageous in that it protected the corneal endothelium from deleterious post-mortem changes in the aqueous humor. The introduction of M-K medium was a major step that enabled corneas to be stored routinely in eye banks for several days. Although storage for up to 7 days was the aim, many eye banks typically stored corneas for only 4-5 days. Even so, the introduction of M-K medium had a substantial impact on the ability of eye banks to provide corneas for transplantation and it is still in use today, especially in developing countries, as it is inexpensive and straightforward to manufacture.

6.2.3 Further Development of Hypothermic Storage Media

Despite the encouraging results of Kuwahara and colleagues with chondroitin sulphate [41], it would be another 20 years before a corneal storage medium containing chondroitin sulphate (K-Sol) became commercially available [37]. K-Sol was widely used with good clinical results, but there were some problems with bacterial contamination of the medium [71]. Dexsol, based on Eagle's Minimum Essential Medium (MEM), contained a lower concentration of chondroitin sulphate (1.35%) than the 2.5% in K-Sol, but was supplemented with 1% dextran to help

control stromal hydration [44]. Currently, the most commonly used hypothermic storage solution is Optisol-GS [45, 72], which combines the main features of K-Sol and Dexsol. The basal medium is a mixture of M-199 and MEM and it contains 2.5% chondroitin sulphate, 1% dextran, and antibiotics (gentamycin and streptomycin). There are also other supplements including vitamins and ATP precursors. Growth factors have been added to both Dexsol and Optisol [42, 43]. While these appear to have an impact on corneal metabolic activity during storage, there is no apparent benefit in terms of clinical outcome after transplantation. Optisol-GS allows storage for up to 14 days; however, the endothelium is better preserved than the epithelium [53, 54], and the extent of epithelial defects after transplantation increases with storage time [38]. Most eye banks therefore limit corneal storage in Optisol-GS to 7-10 days.

Other media have since been formulated, such as Chen medium [13, 17, 18], to try to overcome these shortcomings. Damage from reactive oxygen species [65, 70] and generation of nitric oxide by corneas during hypothermic storage have also been reported, and the use of nitric oxide synthase (NOS) inhibitors advocated [35, 55]. Others have also focused on the macromolecular component used to control stromal swelling. Dextran and chondroitin sulphate are the two most widely used macromolecules in hypothermic storage media. Poloxamer 188 (Pluronic F-68), a nonionic surfactant, has been investigated as an alternative owing to its beneficial effects on cell membrane repair [50]. Poloxamer (1 mg/ml) in Optisol-GS reduced endothelial cell loss in bovine corneas to less than 1% after 12 days of storage, which was 10-fold less than after storage in Optisol-GS alone [75]. Despite these advances, there has been little further progress in extending hypothermic storage time.

6.2.4 Limitations of Hypothermic Storage

The perception of simplicity of hypothermic storage may be somewhat misleading. According to the Arrhenius relation, the rate of a chemical reaction is exponentially dependent on temperature. For many biological reactions, a 10°C fall in temperature reduces reaction rates 2 to 3-fold, which means that reaction rates at 0°C are between 13- and 58-fold lower than at 37°C. Cooling thus reduces metabolic demand and increases tolerance of anoxia, allowing cells to survive for longer in the absence of substrate renewal. There are, however, limitations that are cell-, tissue-, and organ-specific (see [28] for a review), resulting in maximum hypothermic storage times ranging from just a few hours (heart) to more than a month (erythrocytes). Cornea falls midway in this spectrum, although, as already mentioned, the epithelium appears to be less tolerant of hypothermic storage than the endothelium.

Cold reduces, but does not completely suppress metabolism and under hypothermic conditions the demand for energy is likely to exceed cellular capacity for generating high-energy compounds, resulting in a net loss of ATP. This is one of the areas that the Chen medium addresses [18]. This storage medium contains a nonlactate-generating substrate, ß-hydroxybutyrate, which helps maintain corneal metabolism and supports ATP generation during hypothermic storage. A paired in vitro comparison of human corneas stored in Chen medium or Optisol GS for up to 21 days found little difference in endothelial cell loss or rates of apoptosis in endothelium, keratocytes, and epithelium [58]. The only difference was that the corneas in Chen medium were thicker, which was at least in part a result of the difference in osmolality between the two media.

Active transport of ions across cell membranes will be suppressed by hypothermia to a greater extent than the passive movements of ions, leading to an increase in $[Na^+]_i$ and loss of $[K^+]_i$ as they diffuse down their respective chemical potential gradients. The negative charge on intracellular macromolecules causes an unequal distribution of diffusible ions across the plasma membrane according to the Gibbs-Donnan equilibrium, resulting in an influx of water and cellular edema. In an attempt to counter these ionic changes, the Na^+ and K^+ concentrations in organ preservation solutions are reversed in order to mimic intracellular ion concentrations and thus

lessen the transmembrane concentration gradients of these ions. This has also been investigated for cornea [80, 81], but has not been applied in routine corneal storage solutions.

Calcium homeostasis and proton exchange are also disrupted by the cold, the latter leading to cellular acidosis, altering the charge on proteins and hence their 3D structure and function. This acidosis may also release bound iron, which through the Fenton reaction with hydrogen peroxide could generate potentially harmful reactive oxygen species (ROS) [29]. Moreover, the reaction of superoxide with NO would produce peroxynitrite, which in turn could also have a number of damaging consequences, including membrane lipid peroxidation [55]. Consequently, iron chelators [65], NOS inhibitors [55], and other ROS scavengers [70] have all been considered as potentially useful supplements in corneal storage media.

Hypothermia and depletion of ATP cause the dissociation of tight junctions [6, 48]. This increases paracellular permeability and can lead to loss of the differential distribution of receptors between apical and basolateral membranes that is essential for the one-way movement of ions and water across transporting epithelial cell layers. In line with this, disruption of cytoskeleton and cell junctions has been demonstrated in corneal endothelium during hypothermic storage, leading to an increase in permeability of the endothelial barrier [34].

The possibility of phase transitions (liquid crystalline to gel) in membrane lipids could lead to segregation of transmembrane proteins into areas of low melting point lipids and disruption of membrane architecture [63]. Finally, protein metabolism and cellular repair mechanisms would also be suppressed.

The extent to which any of these changes occur in cornea, and their reversibility on warming, are important factors in determining the hypothermic storage time. Further improvements in hypothermic storage solutions are likely therefore to come from a better understanding of the physiology and biochemistry of hypothermic cornea.

Summary for the Clinician

- The efficacy of storage of whole eyes in moist chambers in ice was demonstrated by Filatov in the 1930s
- McCarey and Kaufman advocated hypothermic storage of corneoscleral discs in M-K medium and this became the method of choice for many years
- Further developments led to Optisol-GS, which contains both chondroitin sulphate and dextran as well as supplements such as vitamins and ATP precursors, and this is currently the predominant hypothermic storage medium for corneas
- Hypothermic storage, while technically simple, is limited to only 2 weeks in Optisol-GS. However, the endothelium is better preserved than the epithelium and many eye banks store corneas in Optisol-GS for no longer than 7-10 days
- Other supplements such as growth factors, NOS inhibitors, substrates to support metabolism and ATP generation, and alternative macromolecules to dextran and chondroitin sulphate for controlling stromal hydration may prove to be useful, but have yet to yield extended storage times
- Progress is likely to come from a better understanding of the physiology and biochemistry of corneas during hypothermic storage

6.3 Organ Culture

At around the same time that M-K medium was being developed [51], Summerlin and colleagues reported organ culture of cornea at 37°C for up to 1 month by applying a technique they had developed for skin [78]. This was shortly followed by the successful transplantation of organ-cultured corneas by Doughman and colleagues [21, 22]. There were earlier attempts to organ culture corneas [32, 33], but these pre-dated important advances in the development of tissue culture media [23].

6.3.1 Stromal Edema During Organ Culture

A clear advantage of organ culture over hypothermic storage was the substantial increase in storage time from just a few days to several weeks. However, the organ culture medium, Eagle's Minimum Essential Medium (MEM), containing 10% calf serum, did not contain dextran and corneas became edematous and thickened during prolonged storage. Attempts were made to reverse this swelling prior to transplantation by transferring the corneas to M-K medium at 4°C, but this resulted in an increased rate of postoperative endothelial cell loss [10]. Chondroitin sulphate (1.35%) was added to the organ culture medium, which reduced but did not prevent stromal edema. Even though thickened corneas were transplanted, the postoperative loss of endothelial cells was no worse than in corneal grafts where the corneas had been stored for far shorter periods in M-K medium [11]. The perceived complexities and disadvantages of organ culture, including an increased risk of fungal contamination [57], meant that hypothermic storage in M-K medium rather than organ culture became the method of choice for eye banks in North America and this trend continues today with the predominance of Optisol-GS.

6.3.2 Development of Organ Culture in Europe

Many European eye banks [46] and others elsewhere [60] now use organ culture routinely to store corneas for up to 4 weeks, and there have even been reports of successful transplants using corneas stored for up to 7 weeks [25, 27]. Sperling [73, 74] was one of the first to study corneal organ culture in Europe and he described the suspension of corneas, using a suture through the scleral rim, in medium in glass bottles. Kolstad transplanted organ cultured corneas without any attempt to thin them before use and reported a deleterious effect on outcome if corneas were stored for more than 1 week [39]. Sperling attempted to lessen the stromal edema by including dextran in the organ culture medium [74]; but it was subsequently demonstrated that dextran was taken up into endothelial cells, presumably by endocytosis, during prolonged organ culture [84]. Drawing on Doughman's [21] and Sperling's [74] work, Pels and Schuchard [61] described a method of corneal organ culture that contained the key elements of the methods now used routinely in many European eye banks. Corneas were suspended in HEPES-buffered MEM supplemented with 2% fetal bovine serum and containing antibiotics and an antimycotic. They were kept at 31°C for up to 40 days. Importantly, rather than trying to prevent stromal edema during organ culture, the corneas were thinned before transplantation in medium containing 5% dextran T500 for 24 h at 31°C. The corneas could be transported in this medium at ambient temperature and held for up to 4 days, which, as Sperling had already shown, was well-tolerated by the cornea [74]. The endothelium was examined both at the start and at the end of organ culture by transmitted light microscopy after staining with trypan blue to reveal dead or damaged cells and 1.8% sucrose to render cell borders visible. The endothelial cell loss varied between corneas but the majority lost fewer than 10–15%. The cell loss of 20-30% seen in a very few corneas was considered abnormal.

6.3.3 Microbial Contamination

Although the risk of bacterial and fungal contamination during organ culture was seen as a disadvantage by eye banks in the USA, organ culture, conversely, may be seen as more likely to help prevent the transplantation of corneas at risk of causing postoperative endophthalmitis. Not only are the antibiotics far more effective at the higher storage temperature, but there is also a greater opportunity for detecting contamination before the tissue is transplanted through routine microbiological screening of the medium during organ culture and monitoring of the medium for turbidity. Unlike hypothermically stored corneas, where up to 30% of corneoscleral rims have been shown to be culture-positive, the incidence of culture-positive rims from organ-cultured corneas is rare. Although there are no direct

comparisons, it has been estimated that the risk of postoperative endophthalmitis could be 10- to 20-fold lower with corneas stored by organ culture rather than hypothermia [16].

6.3.4 Integrity Corneal Cell Layers

Pels and Schuchard [61] reported a reduction in epithelial thickness during organ culture. This was also observed by Crewe and Armitage [20] who found that the epithelium was reduced from its normal 5-7 layers to just 3-4 layers of cells (Fig. 6.1). In spite of this, the stratified architecture of the epithelium was retained, as shown by rhodamine-phalloidin staining of actin filaments. Moreover, ZO-1, a tight junction protein, was localized to the intercellular borders of the flattened superficial cells, as in normal epithelium, with little ZO-1 evident in the lower cell layers. This suggested that the epithelial barrier was maintained, although this needs to be confirmed by transepithelial resistance or permeability measurements. An increase in the numbers of epithelial cells undergoing apoptosis was observed with time in organ culture [20]. This was shown by caspase-3 staining, an essential component of the apoptosis enzyme cascade. A similar increase in apoptotic cells was not observed in the endothelium, despite reports to the contrary using TUNEL staining, a rather less specific marker, to detect apoptosis [2]. Both apoptosis and necrosis have been observed during hypothermic storage, and increased incidence of endothelial apoptosis

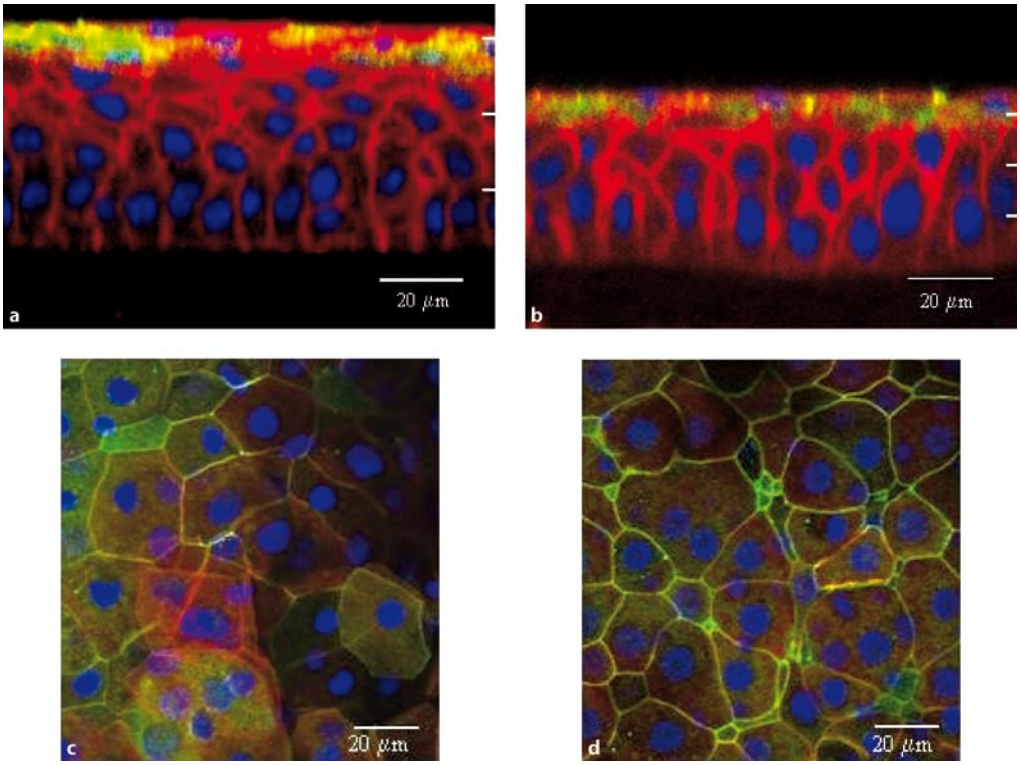

Fig. 6.1 Corneal epithelium stained for F-actin (*red*) and ZO-1 (*green/yellow*). Nuclei are counter stained by DAPI (*blue*). **a** Transverse XZ scans of cornea stored in a moist chamber for <24 h and **b** paired cornea after 14 days in organ culture showing reduction in number of cell layers and localization of ZO-1 to superficial cells. **c** XY scans of superficial cells in the same corneas, showing ZO-1 encircling cells at intercellular borders in moist chamber cornea and **d** after organ culture [20]. Reproduced with permission

has been reported in areas of endothelial damage and along Descemet's folds during organ culture. It still remains to be determined whether apoptosis or necrosis is the principal cause of endothelial cell loss during organ culture, and an answer to this may help to guide further development of organ culture media. Actin and ZO-1 staining of the endothelium during organ culture also revealed maintenance of typical cellular morphology, with ZO-1 localized to cell borders and bounded on either side by a distinct band of actin filaments (Fig. 6.2). Again, functional studies are needed to determine whether endothelial permeability is maintained as the mere localization of ZO-1 does not necessarily confirm intact tight junctions [20]. Other markers of tight junctions, such as occludin and JAM-A [49], would help provide a fuller evaluation of the integrity of endothelial intercellular junctions.

6.3.5 Toward a Defined Organ Culture Medium

The European Eye Bank Association Directory, an annual summary of activity of member eye banks in Europe, shows a range of different basal media, serum concentrations (2-8%), and storage temperatures (31-37°C) used for organ culture. Moreover, some eye banks do not change the medium during up to 4 weeks of organ culture, whereas others change the medium weekly. Despite these differences, the overall results and transplant outcome appear to be similar.

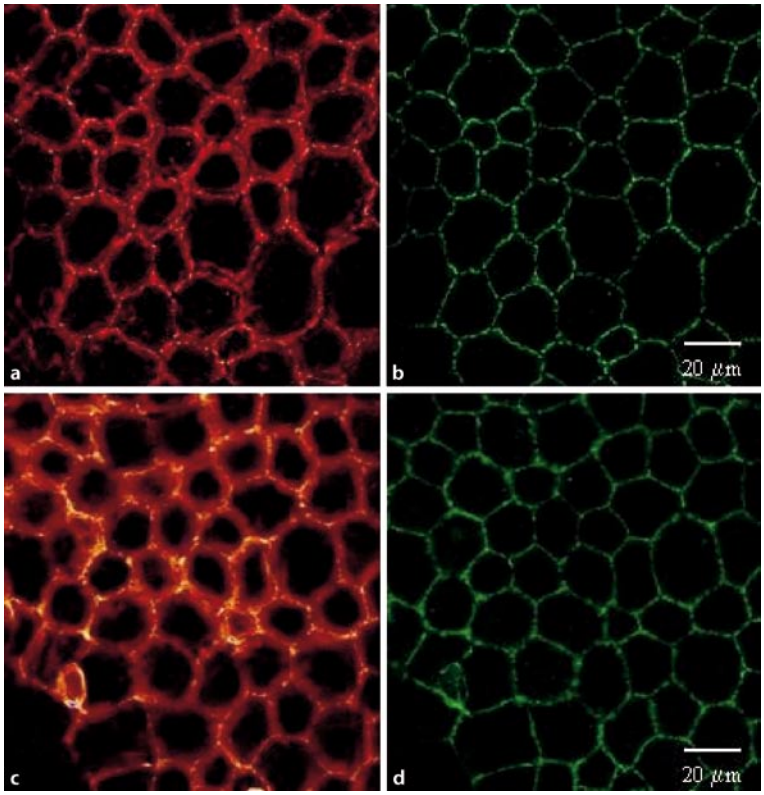

Fig. 6.2 Dual labeled XY scans of corneal endothelium stained for F-actin (*red*) and ZO-1 (*green*). **a**,**b** Moist chamber storage for <24 h and **c**, **d** after 14 days in organ culture [20]. Reproduced with permission

A desirable goal would be the avoidance of serum, and indeed any constituent of animal origin. As early as 1983, Adams and Lucas studied the use of a serum-free medium for the organ culture of cat and human corneas [1]. More recently, following the extensive work of Engelmann, Bednarz and colleagues, the efficacy of a number of serum-free media has been investigated, including a medium developed for the culture of human vascular endothelium (SFM) [9]. Very encouraging results using a completely defined medium free of all constituents of animal origin have also been reported by Thuret and colleagues [83]. Interestingly, this latter group also replaced the dextran used to reverse stromal edema following organ culture with Pluronic F-68 (Poloxamer 188), reporting reduced endothelial cell loss during this phase of storage immediately prior to transplantation. As mentioned previously, Poloxamer 188 has also been used during hypothermic storage [75]. Inclusion of hydroxyethyl starch (HES) during organ culture has also been studied as a way of controlling stromal edema while avoiding the deleterious effects of dextran [66].

Summary for the Clinician

- Organ culture at 31-37°C routinely provides up to 4 weeks' storage and successful grafts have been reported even after 7 weeks' storage
- A range of different basal media, serum concentrations and storage temperatures are used, all with similar clinical outcomes
- Serum-free, defined media are being developed with some encouraging results
- Stromal edema during organ culture is currently reversed by placing corneas in medium containing dextran for up to 4 days before surgery. Alternative polymers, such as Poloxamer, appear to be better tolerated by the endothelium, and HES has been included throughout the storage period to lessen the edema

6.4 Cryopreservation

Following the landmark discovery of the cryoprotective properties of glycerol in 1949 [62], early attempts were made to cryopreserve corneas by freezing [24, 67]. In the 1960s, two groups in the USA and UK independently reported methods of cryopreserving corneas for full-thickness transplants [15, 59]. Successful transplants were achieved, some surviving for many years [69], but the technique was considered to be too complex for routine application. Laboratory and clinical studies demonstrated that significant endothelial damage could result from freezing (see [79] for review) and now only very few eye banks keep cryopreserved corneas for emergency or nonviable grafts [14].

Research into corneal cryopreservation, in particular the impact on the endothelium, has nonetheless continued. Based on observations that cells in monolayers appear to be susceptible to damage through the spread of intracellular ice through gap junctions, the use of lower cooling rates than the typical 1–5°C/min has improved survival of rabbit corneas based on endothelial function and morphology [68]. The use of non-permeating cryoprotectants such as dextran has also been investigated, with encouraging results [31].

An alternative approach to avoiding the mechanisms of cryoinjury associated with freezing is ice-free cryopreservation by vitrification, which relies on a substantial increase in viscosity during cooling to prevent ice crystal formation [5]. The main barrier to this approach is the high concentration of solute needed to achieve vitrification at practicable cooling rates. Attempts have been made to vitrify both rabbit and human cornea, with retention of endothelial function being reported in the former [7, 12].

Rather than cryopreserving whole corneoscleral discs, these techniques may find an application for the storage of isolated corneal cells (e.g., limbal stem cells), cell sheets or tissue-engineered grafts.

Summary for the Clinician

- Cryopreservation currently is the only method that offers the prospect of truly long-term storage of corneas for PK
- Despite some successful transplants using cryopreserved corneas, cryopreservation is used only rarely for storing corneas for emergency grafts
- Cryopreservation may find greater utility in the storage of ocular cells, cell sheets or tissue-engineered grafts

6.5 Endothelial Cell Loss and Transplant Longevity

Evaluation of the endothelium by microscopy is important for determining the suitability of corneas for penetrating keratoplasty. Both quantitative and qualitative measures may be used, including cell density, pleomorphism, polymegathism, numbers of dead or damaged cells, areas of cell loss and extent of the folding of Descemet's membrane. Quite how these features relate to or can be taken as predictors of the outcome of PK is uncertain. So far as the impact of donor factors on quality of corneas is concerned, donor age clearly has an overriding influence [4], i.e., the older the donor, the less likely that the endothelial cell density will be acceptable for PK. This is not surprising given the normal loss of endothelial cells with increasing age; however, there is a wide variation in cell density within any given age group and many corneas from older donors, even above 80 years of age, are considered suitable for PK. While in North America an upper age limit of 60–65 years is the norm, age limits tend not to be set for corneas stored in organ culture. Instead, an assessment based on endothelial cell density is considered justified, and this appears to be borne out by clinical outcome data. There are studies investigating the efficacy of corneas from older donors and, in the short term at least, there appears to be little influence of donor age. Even if the endothelium is judged to be sufficient, it is thought by some that age-related stromal changes may influence visual outcome.

Eye banks typically set minimum endothelial cell densities for PK at between 2,000 and 2,500 cells/mm^2. Given the paucity of long-term endothelial cell density data from corneal transplants, the minimum cell density set by eye banks lacks a firm rational basis. Bourne has measured endothelial cell densities of grafts in patients up to 20 years after transplantation. Application of a mathematical model to describe the fall in endothelial cell density allows an estimation of graft longevity based on cell density at the time of transplantation (Fig. 6.3), providing a rationale for setting a minimum cell density suitable for PK [8].

Summary for the Clinician

- Examination of the corneal endothelium by light microscopy allows an estimate of endothelial cell density and exclusion of corneas for PK with damaged or otherwise insufficient endothelium
- Endothelial cell density and hence suitability for PK is most influenced by donor age, but many corneas from older donors over 70 years do have an adequate endothelium to be used for PK
- Modeling the long-term loss of endothelial cells in corneal transplants may help to provide a rational basis for setting minimum endothelial cell densities for donor corneas for PK

6.6 Future Developments

Eye banks have typically supplied tissue overwhelmingly for PK. Corneas unsuitable for PK owing to insufficient endothelium have been made available for other types of graft, including superficial anterior lamellar and cryolathed lenticules for epikeratoplasty. Sclera has also been provided for coating orbital implants and for reconstructive surgery. Over the past few years, surgeons have begun to require tissue for a range of more specialized grafts, including deep anterior lamellar keratoplasty, endothelial keratoplasty, and limbal tissue [3, 82]. The latter is to

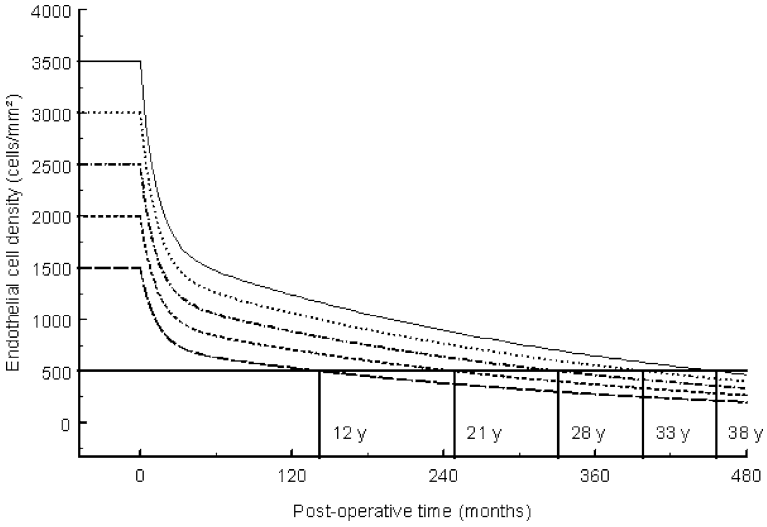

Fig. 6.3 Biexponential model of postoperative endothelial cell loss used to predict time for endothelial cell density to fall to 500 cells/mm² (the point where late endothelial failure and corneal decompensation are thought to become more likely) from various initial starting cell densities [8]. European eye banks typically set a minimum endothelial cell density for PK between 2,000 and2,500 cells/mm². This model suggests that late endothelial failure is unlikely to occur for at least 20 years with an initial cell density of 2,000 cells/mm². Reproduced with permission

treat limbal stem cell deficiency (LSCD), either by limbal tissue grafts or by transplantation of ex vivo expanded epithelial cell sheets [64]. Many of these are autografts, but allografts may be the only option for patients with bilateral LSCD. This raises the issue of how best to preserve the limbal stem cell population, as current techniques of corneal storage aimed at preserving the endothelium and epithelium may be suboptimal for the limbal stem cells.

Eye banks are already preparing grafts for endothelial keratoplasty using automated microkeratomes and preparation using the femtosecond laser may well become an alternative [19]. Again, issues of how best to preserve these grafts need to be addressed, given that these techniques are likely to become the treatments of choice for patients with endothelial disease, and it is clearly advantageous for the preparation, storage, and supply of these grafts to be centered on eye banks. Gene transfer to corneal cells, for example to reduce immunogenicity and the risk of allograft rejection [30], and tissue engineering to create and modify endothelial, stromal, and epithelial grafts are also on the horizon. These advances will undoubtedly have an impact on the way ocular tissue and cells are processed and stored in eye banks.

Summary for the Clinician

- The development of new surgical techniques for treating only those parts of the cornea that are defective are posing new challenges for eye banks in the processing and storage of tissue
- The prospects of gene therapy and tissue engineering will also create special demands, leading to increases in the range and complexity of eye banking methods

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Chapter 7

Herpes Simplex Keratitis and Related Syndromes

7

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Core Messages

- Herpes simplex keratitis (HSK), a result of infection predominantly with the Herpes Simplex Virus-1 (HSV-1) virus, is the most common infectious cause of unilateral blindness and one of the commonest indications for a penetrating keratoplasty
- The virus may reach the eye by one of two main routes: the "back door" route, that is, entry via the mouth with subsequent spread to the eye; or the "front door" route with direct entry onto the ocular surface with subsequent recurrence in the cornea
- Anterior segment disease caused by HSV-1 can affect all levels of the cornea, and also cause uveitis and iridocorneal endothelial syndrome
- Primary disease is usually asymptomatic and recurrent disease usually affects vision through scarring, thinning, and neovascularization. The outcome of infection depends on a number of factors, including viral genes encoded by each strain and the genetic make-up of the host and host immune system
- Recurrent corneal disease may be a result of anterograde axonal spread from the trigeminal ganglion, persistent or reactivated virus within the cornea. Usually, this is the result of re-activation of the initial strain, but super-infection with a new strain is also possible
- A combination of polymer chain reaction (PCR) and immunohistochemistry (IHC) offers high specificity and sensitivity for both active disease and diagnosis of corneal scars
- Topical antivirals are usually sufficient in epithelial disease. Treatment of stromal disease responds to initial topical antivirals followed by a combination of topical antivirals and steroids. Adequate levels of antivirals can be achieved in the anterior chamber following systemic administration in herpetic uveitis
- Orally administered systemic aciclovir reduces the recurrence of herpetic keratitis and keratouveitis
- Keratoplasty (penetrating, lamellar, and deep lamellar) is an effective treatment for HSV-1-induced corneal scarring, but rejection and recurrent HSK remain a cause for concern. Antiviral prophylaxis, however, leads to a significant reduction in post-transplant recurrence

7.1 Introduction

Herpes simplex keratitis (HSK) is a major cause of visual morbidity worldwide. In the USA, there are more than 500,000 cases of active herpes simplex ocular infection each year [99]. It is estimated that 60–70% of children under 5 years of age and 90% of adults are seropositive for the Herpes Simplex Virus HSV antigen. Despite the high prevalence of HSV infection, most individuals remain asymptomatic, with only 20–30% of patients developing symptomatic clinical disease, the majority of which relates to HSV labialis. Herpetic ocular disease has an estimated incidence of 5.9–20.7/10^5 and a prevalence of 149/10^5 patients per year in developed countries [45, 101, 128]. Although HSV-1 is primarily associated with disease of the ocular surface and external eye, iridocyclitis, uveitis and retinitis are equally devastating manifestations.

Primary HSV-related eye disease is usually asymptomatic and predominantly affects the external eye, conjunctiva, and periocular skin. In contrast, recurrent disease usually manifests as ulcerative and/or stromal keratitis. It is recurrent disease that leads to visual morbidity through corneal scarring, thinning, and neovascularization. Although HSK is usually unilateral, bilateral disease occurs in 1.3–12% of cases. Bilateral disease tends to occur in younger patients and to run a more protracted course [101, 167]. This may be particularly significant in the developing world, where younger patients may be affected with more severe disease, which may be compounded by the presence of malnutrition, and other diseases, as well as the lack of access to treatment [80]. HSK is the most common infectious cause of unilateral blindness [190] and amongst infective and inflammatory conditions, remains one of the commonest indications for a penetrating keratoplasty (PK) in the UK. In the period from 1987 to 1991, 10.5% of transplants were carried out to treat HSK [36], although this has reduced to 6% in the last 5 years (2000–2005; United Kingdom Transplant Service data).

Human simplex keratitis reflects the challenge between the host's ability to limit viral replication, spread and hasten its removal, while at the same time maintaining the integrity of the cornea and modulating wound healing to minimize scar formation and vascularization of this avascular tissue. Although there have been significant advances, with the development of different treatments that target the virus or modulate wound healing, it is the balance and timing of the introduction of these treatments during the course of HSK that is vital for a successful outcome. Detailed studies of the anatomy and life cycle of HSV-1 have been crucial to our understanding of the disease process, which together with advances in the understanding of wound healing, has allowed uncoupling of the host–viral interaction that governs HSK. During the course of this chapter, we will discuss those aspects of HSV virology that are important to our understanding of HSK, attempt to link this with aspects of corneal wound healing, and apply this knowledge in studies that address the diagnosis of and treatment of HSK.

7.2 Historical Observations

Herpes simplex virus infections of humans have been documented since ancient Greek times. Hippocrates described cutaneous spreading lesions that were presumably herpetic in origin [120]. Greek scholars defined the word *herpes* meaning to creep or crawl, in reference to the spreading nature of the herpetic skin lesions [14]. It was not until the beginning of the 20th century that the infectious nature of the herpetic lesion was recognized, while the host immune response was first described in the early 1930s [88, 103]. Although suggested by Lipschitz over 70 years ago, the antigenic and biologic differences between HSV-1 and HSV-2 were only demonstrated in 1968 by Nahmias and Dowdle [120].

The term "dendritic keratitis" was coined by Hansen Grut and then Emmeret who first defined the condition in 1885 [109]. They surmised that the origin of the disease was in nerve terminals in the epithelium and their theory was later supported by the concept that the agent causing corneal disease could be transmitted to the central nervous system [185]. Transmissibility of HSV from man to ani-

mal was first shown by Grüter (1912) and later by Lowenstein (1919), who inoculated rabbit corneas with material from human HSK [62, 103]. The potential self-limiting nature of HSK and the interaction of HSV-1 with the host immune system was illustrated by the frequency and severity of HSV infection in malaria and worsening of the disease with cortisone following its introduction in 1952 [21, 56, 186]. This underlies the current tenet of the way HSK is managed; antiviral treatment subsequently followed by manipulation of wound healing. In the 1970s, an improved understanding of the interaction between HSV and humoral and cellular immunity paved the way for new approaches in the management of ocular HSV infections [111–114]. Idoxuridine was introduced in 1962 as an anti-viral agent, but due to its toxicity began to be replaced in the 1970s by aciclovir and vidarabine [92, 140].

Our knowledge and understanding of the pathogenesis and management of HSK have come a long way since then, as will be discussed in the course of this chapter, and this may lead to the availability of newer treatment options for this complex condition.

7.3 Herpes Simplex Virus

7.3.1 Structure of HSV-1

Herpes simplex virus-1 belongs to the human herpes virus (HHV) family and is sometimes referred to as HHV type 1 or HHV-1. The virion is 120–300 nm in size and consists of an electron-opaque core containing the genome, an icosadeltahedron capsid, a tegument and an envelope. The capsid architecture is the most characteristic feature of the HHV family with an icosadeltahedron structure comprising 162 capsomeres. The capsid is surrounded by a tightly adherent tegument, containing proteins such as the important virion host shut-off protein. The envelope that surrounds the tegument consists of a lipid bilayer with embedded glycoproteins, which serve as attachment proteins (gB, gC, gD, gH), fusion proteins (gB), structural proteins, and immune escape proteins (gC, gE, gI). It is this envelope that makes HSV-1 sensitive to acid, solvents, detergents, and drying. The HSV-1 genome comprises 152 kb of double-stranded Deoxyribonucleic acid (DNA) wrapped as a toroid (Fig. 7.1). It is divided into long and short regions of unique

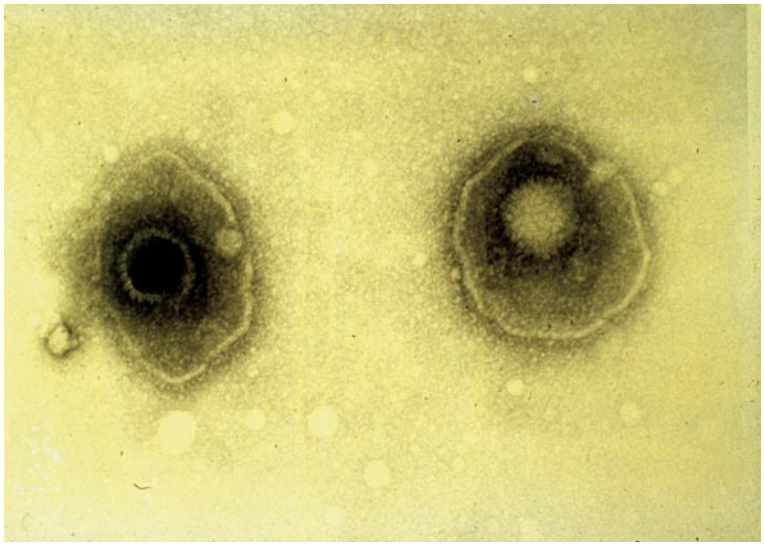

Fig. 7.1 Electron microscopy of herpes simplex virions. Note the central capsid containing the double-stranded DNA surrounded by the tegument and envelope (Reprinted with permission from [80])

sequences, termed U^L and U^S, and bounded by regions of internal and terminal repeats (Fig. 7.2) [80]. It is the variability in the number of these repeat regions that leads to the variability in the size of the genome [88, 119].

7.3.2 Viral Replication

Herpes simplex virus attaches to cell receptors with subsequent fusion of the envelope with the plasma membrane, leading to penetration of the virus into the cell, transport of the capsid to the nuclear pores with release of viral DNA into the nucleus. All of this is mediated by the embedded glycoproteins and tegument proteins. After entry into the cell, the virion components, specifically the virion host shut-off protein, are involved in the early shut-off of host macromolecular synthesis [80]. One of the earliest visible structural changes in the cell is enlargement of the nucleolus, and displacement toward the nuclear membrane where it fragments [161]. Transcription, replication of viral DNA, and assembly of new capsids takes place in the nucleus. [161]. Viral gene expression is coordinated as a sequential cascade and after packaging into preassembled capsids, the virus matures and acquires infectivity by budding through the inner lamellae of the nuclear membrane. The whole process takes approximately 18–20 h. Although local spread may occur from cell to cell, HSV-1 may also enter and be transported along sensory nerves to establish latent infections and potential disease at other sites [80].

Viral protein synthesis occurs in the cytoplasm with the sequential expression of approximately 80 viral proteins in three major kinetic classes: immediate early (IE), early (E), and late (L) genes. The first to be expressed are the products of the alpha (α) genes, the immediate-early (IE) proteins, the expression of which reaches peak rates of synthesis at 2–4 h post-infection and which continue to accumulate until late in the duration of the infection. The functions of these proteins are regulatory, that is, some of the products of the IE genes, also termed the infected cell polypeptides (ICP), such as ICP0, 4, 22, 27 are the primary mediators of viral gene expression. The beta (β) genes and their products, the early (E) proteins are dependent on the presence of α gene products and reach peak rates of synthesis at 5–7 h post-infection. They include DNA polymerase and viral thymidine kinase (TK), the substrate range of which is far greater than that of its host counterpart, such that it phosphorylates purine pentosides and a wide variety of nucleoside analogues that are not phosphorylated efficiently by cellular kinases. This is the basis for the effectiveness of various nucleoside analogues in the treatment of HSV. The early proteins are involved in viral DNA synthesis and are required for maximum expression of late (γ) genes, the products of which include virion structural proteins and viral glycoproteins [80].

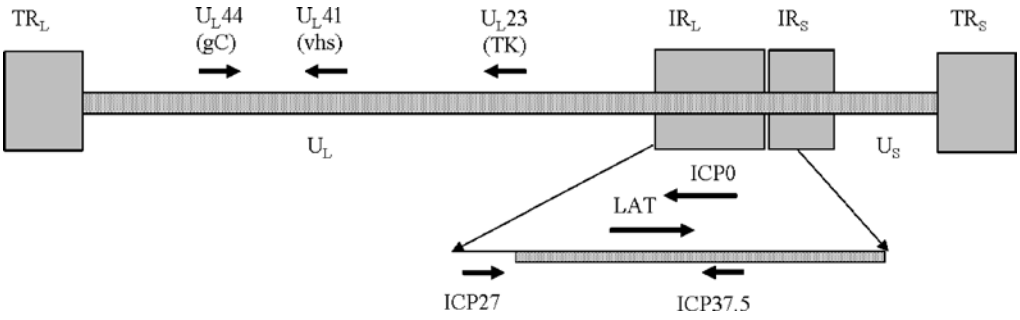

Fig. 7.2 Linear representation of the HSV-1 genome. *TRL* terminal long repeat, *UL* unique long region, *IRL* and *IRS* intermediate long and short repeats, *US* unique short segment, *TRS* terminal repeat short. Glycoprotein C (*gC*), thymidine kinase (*TK*), *UL41* (gene encoding virion host shut-off protein vhs), latency associated transcript (*LAT*), infected cell protein 0 (*ICP0*). Region coding for LAT overlaps, but is on the opposite strand to ICP0 (Reprinted with permission from [80])

7.3.3 Entry into the Host

Viral replication occurs at the site of inoculation, which increases the contact with and entry into sensory nerve endings. It is thought to be important for HSV-1 to multiply in the ganglion before the immune system has responded. Control of virus multiplication by CD8+ cytotoxic lymphocytes may decrease potential spread into the brainstem. HSV-1 appears to be transported by axoplasmic flow toward the Central Nervous System (CNS). Within the trigeminal ganglion (TG), infection is largely restricted to neurons, with little intraganglionic spread. Virus transported centrally may leave the nerve root to enter contiguous axons leading to zosteriform spread. Viral replication in or around the brainstem may still occur leading to neurological disease (meningitis, myelitis or encephalitis) [80].

The frequency of asymptomatic salivary compared with tear film shedding [79, 82] implicates the mouth as the main site for acquisition and spread of HSV-1 in the community. The virus may then reach the eye by one of two main routes: the "back-door" route [192] characterized by entry via the mouth with spread to the eye; or the "front-door" route [82], with direct entry into the ocular surface with subsequent recurrence in the cornea [80].

Following lower lip inoculation in the mouse, HSV has been recovered from all three divisions of the TG, supporting the theory of ocular disease having a "back-door" route. Asymptomatic primary herpetic eye disease may be equally important for the development of recurrent corneal disease. It is estimated that primary infections manifest clinically on only 1–6% of occasions [196]. Droplet spread to the mouse eye results in infection of the trigeminal ganglion, cornea, and iris, giving support to the concept of a "front-door" route (Fig. 7.3) [82]. This nontraumatic acquisition suggests that asymptomatic primary infection of the eye may lead to latency in trigeminal ganglion and the cornea. Reactivation of the virus at these sites may then lead to recurrent ocular disease.

7.3.4 Latency of HSV

7.3.4.1 Neuronal Latency

HSV-1 can establish both productive and latent infections. Productive infection results in epithelial lesions, and may lead to a latent infection in the trigeminal ganglion and possibly in the cornea itself. Although infection of non-neuronal cells usually leads to viral replication and subsequent death of the host cell, some non-neuronal cells may potentially harbor either persistent or latent virus. When HSV-1 infects sensory neurons, replication is limited, and the virus may be more easily maintained for the lifetime of the host [80].

The HSV-1 genome has been found to exist in three different states: linear, circular, and concatemeric. The preferred template for replication is a linear molecule. In the virion, genomes are linear, although during a latent infection the viral genome is in a circular form. An important discovery in the study of HSV was the finding of virus-specific transcripts in the central and peripheral nervous systems of mice and in human trigeminal ganglia during HSV-1 latency [171, 172]. These transcripts, the latency-associated transcripts (LATs), originate from the repeat regions, which are within the long internal (IRL) and terminal repeats (TRL), and are thus present in two copies per genome (Fig. 7.2) [80]. The LATs overlap with the terminus of the immediate early gene ICP0, but are transcribed in the opposite direction. During reactivation, the expression of the LATs decreases, but still remain at significant levels even after reactivated virus is detectable [170]. Because LAT partially overlaps the 3' end of the ICP0 gene on the opposite strand, it has been suggested that LAT suppresses the expression of ICP0 during latency by antisense repression, i.e., by RNA–RNA hybrid arrest of translation [172] and that establishment and reactivation from latency involves the products of both the ICP0 and LAT genes [97]. Although HSV-1 mutants with deletions in LAT are able to establish and maintain a latent infection [67], LATs appear to be important for reactivation from latency [96]. There is also evidence that during latency, expression of the latency-associ-

ated transcripts (LATs) prevent super-infection of the cell [104]. The HSV-1 immediate-early (IE) infected cell protein (ICP0) is crucial for reactivation and is proposed to promote lytic infections by destabilizing cellular proteins that inhibit the lytic viral life cycle, and by preventing circularization of the genome [75].

While the LAT region has not been definitively shown to encode any proteins, it has been implicated in a number of pathogenic functions, including neuronal survival and suppression of apoptosis, virulence, suppression of latent transcription, establishment of latency, and as previously mentioned, reactivation from latency. DNA cellular repair proteins are activated by HSV-1 and may be involved in controlling viral latency. Neurons are inefficient at DNA repair, in contrast to epithelial cell lines, where DNA repair proteins are induced in response to a variety of DNA-damaging agents or genotoxic stress. It

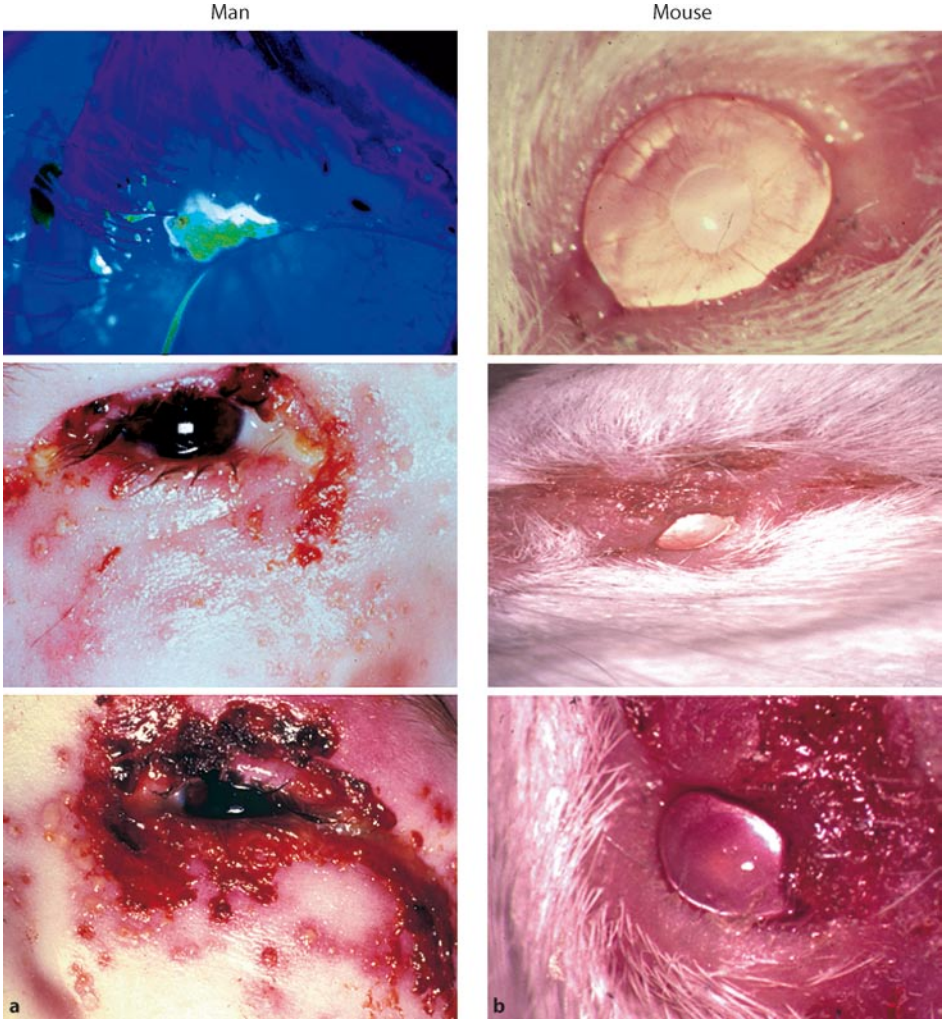

Fig. 7.3 Droplet spread of HSV-1. Note the similarity of the ocular surface disease (blepharitis and conjunctivitis) in the mouse and human eye. Younger mice developed more severe disease and a greater neutralizing antibody response (Reprinted with permission from [82])

would appear that activation of the DNA damage response is beneficial for viral replication, but that this cellular response is abrogated in neuronal cells. The delay in viral growth in the absence of DNA repair proteins may be due to a reduced ability to form stable replication structures leading to deficiencies in viral replication and contributing to the establishment of latency.

7.3.4.2 Non-neuronal Sites of Latency: HSV-1 in the Cornea

Although the TG is likely the main site harboring latent virus, there is good evidence that the cornea may also harbor latent virus. Initial studies reported the presence of viral antigens in the cornea. HSV-1 was then isolated following prolonged culture of the corneas of patients undergoing PK who had no clinical evidence of active herpetic disease raising the possibility of HSV-1 latency within the cornea [46]. Other studies confirmed the recovery of HSV-1 from the cornea following prolonged organ culture [37, 40, 81, 83]. Because of the difficulty in differentiating latent virus from a low-grade infection, molecular techniques – polymerase chain reaction (PCR) and in situ hybridization (ISH) – were subsequently used in an attempt to answer this question.

Abghari et al. [1] demonstrated LAT in the mouse cornea using ISH on dissociated corneal cells. In contrast, Gordon et al. [61], were unable to detect LATs in the corneas of latently infected mice and rabbits using ISH. The detection of LAT using PCR has been more informative. Cook et al. [38] found expression of LAT in 2 out of 9 corneas 41 days after inoculation in rabbits untreated with antiviral agents, while O'Brien et al. [130] found LAT expression in acutely infected, but not latently infected, rabbit corneas. Kaye et al. [82], using Ribonucleic acid (RNA)-PCR, found expression of LAT in 8 out of 10 human HSK corneas. In contrast, a study of 18 human patients with a history of HSK that was inactive at the time of surgery, and that had been treated with antivirals but not steroids, neither LAT nor transcripts coding for alpha proteins were detected by ISH. The difference between the results for PCR and ISH may be due to the difference in their relative sensitivities (particularly if there is a very low level expression) and sampling error. Although the presence of HSV-1 DNA may represent the persistence of defective viral genomes, most studies have reported intact regions in the majority of cases [82, 155]. Corneal and neuronal cells appear to regulate the LAT promoter in a similar manner and the finding of LAT in the cornea provides further evidence of corneal latency.

7.3.5 Transport of HSV-1 to and from the Cornea

Whether HSV-1 enters via the eye or the mouth, it has been found and recovered from the trigeminal ganglion, brainstem, and cornea. Virus is transported by retrograde movement of the nucleocapsid within the peripheral axons to the nuclear compartment of the sensory cell. After replication in the neuronal cell body, new components of the virion move by anterograde transport to both peripheral and central branches of the neuron [93]. It is probable that the mechanism of viral DNA transport involves microtubule motors [142, 187, 188]. It is essential that viral DNA replication occurs for HSV-1 to move to and be transported down the length of the axon [93]. Virus that is incapable of replication, fails to be delivered to the axonal compartment [93]. It is important therefore, for HSV-1 to multiply in the ganglion before the immune system has responded. Within the trigeminal ganglion, infection is largely restricted to neurons, with little intra-ganglionic spread. It is believed that cycles of HSV reactivation in latently infected neurons, accompanied by anterograde axonal spread to the cornea, lead to recurrent infections and scarring of the cornea [162, 163]. Importantly, however, recurrent ocular disease does not entirely depend on anterograde transport, with some disease recurring from local (corneal) reactivation.

As discussed in Sect. 7.3.4.2, there is evidence that the cornea may also harbor latent virus, which may therefore provide an alternative source of viral antigens that trigger HSK.

Polcicova et al. constructed an HSV mutant that replicated normally in the cornea, but was unable to return to the cornea from the sensory ganglia. They were able to cause recurrent HSK with this virus, which suggests that HSV-1 resident in the cornea can also initiate HSK [146].

Summary for the Clinician

- HSV-1 belongs to the human herpes virus (HHV) family consisting of an electron-opaque core containing the genome (152 Kb or double-stranded DNA, wrapped as a toroid), an icosadeltahedron capsid, a tegument, and an envelope
- HSV-1 virus can produce both productive and latent infections and may reach the eye by one of two main routes: the "back-door" route characterized by entry via the mouth with spread to the eye; or the "front-door" route with direct entry into the ocular surface with subsequent recurrent disease in the cornea
- On entry into the cell, the virion components (especially the host shut-off protein), are involved in the early shut-off of the host macromolecular synthesis. Viral protein synthesis involves sequential expression of three major classes of proteins: immediate-early or α, early or β, and late or γ proteins
- Latency-associated transcripts (LAT) are virus-specific transcripts that have been found in human and mouse trigeminal ganglia during HSV-1 latency. During reactivation expression of LATs decreases and it has been suggested that they suppress expression of the HSV-1 immediate early (IE) infected cell protein (ICP0), which is crucial for reactivation
- Although cycles of HSV reactivation in latently infected neurons accompanied by anterograde axonal spread to the cornea may lead to recurrent disease, similar disease may occur from reactivated or persistent virus in the cornea

7.4 Outcome of Infection

The pathogenesis of HSV disease is dependent on a number of factors, including the constellation of viral genes encoded by each strain working in concert to determine the virulence phenotype of that particular strain and the genetic make-up of the host and host immune system [80].

7.4.1 HSV-1 Strains

A strain refers to a single viral isolate obtained from an infected individual. Several hypervariable regions have been identified in the HSV-1 genome that encompass unique, tandemly repeated sequences, or reiterations that vary in copy number and nucleotide sequence [88, 152]. These give rise to the different strains of HSV-1 recovered from individuals. Studies have shown that different HSV-1 isolates cause corneal disease of varying severity, ranging from asymptomatic to very severe corneal damage [24]. Several viral genes in combination with host factors influence the virulence of the strain. For example, the SC16 strain has been found to produce more severe ocular disease in mice compared with the McKrae strain, a prototypic virulent HSV-1 strain [82]. In contrast, the KOS strain is much less virulent and differs in the ICP34.5 gene product [106, 144].

The initial infecting viral strain usually colonizes the sensory ganglia in most cases and its reactivation is thought to be the cause of recrudescent infections [6]. Super-infection with a new strain at the site of primary infection is also possible and PK is a particular risk factor, as discussed in Sect. 7.8.6 [149]. Overall mutation rates for HSV-1 have been estimated to be 3.5×10^{-8} mutations/site/year, which is less than that described for RNA viruses and does not explain intra-individual HSV-1 genotype differences [195]. This suggests the possible role of super-infection with other strains and generation of recombinant viruses with enhanced virulence. Norberg et al. [127] demonstrated both intra- and inter-genic recombinants and suggested that most full-length HSV-1 genomes consist of a mosaic of segments from different genetic groups. The link between genotype and site of infection has not been eluci-

dated [197] and strain differences alone are insufficient to explain differences in human response to infection. The strain of virus, together with the genetic make-up of the host and host immune system, has been suggested to determine the response to infection with HSV-1 [23].

7.4.2 Host Factors

Host genes may be involved in innate resistance and acquired immunity. A number of physical factors can affect the ability of the virus to establish infection. The first barrier to infection is the tear film, which acts to physically wash away infectious agents and also contains a number of antiviral agents such as lysozyme, immunoglobulin A, complement and Interferon (INF)-α, β, and γ. The outermost layer of corneal epithelial cells are post-mitotic cells, which are not capable of replication, and HSV-1 replicates best in metabolically active cells [26]. The intact epithelium thus forms an effective physical barrier to infection. Attempts have been made to map genes affecting innate resistance in animals. For example, in mice the *igh* locus on chromosome 12 confers some resistance to keratitis, the *Hrl* locus on chromosome 6 affects viral replication and reactivation [23] and loci on chromosomes 10 and 17 are thought to be specific for ocular disease [129]. Host immune response is further discussed under Sect. 7.4.1.

7.4.3 Viral Genes

Virulence genes such as those encoding infected cell proteins, ICP0, 4, 22, 27, and 47, play an important regulatory role and are predicted to be virulence determinants for corneal infection. ICP0 and ICP22 proteins affect the ability of the virus to replicate and the former also counteracts the anti-viral effect of INF, while ICP4 interferes with apoptosis of the infected cells [25, 77]. Mutations in DNA polymerase have been shown to induce drug resistance and alter neurovirulence. Gamma genes are expressed late in infection and those encoding structural proteins are likely to be virulence genes. For example gC and virion host shut-off protein-negative mutants (encoded by gene UL41), have reduced capacity to cause keratitis [22, 23] and periocular disease [165]. Many of the HSV-1 glycoproteins also act to blunt host defenses such as blocking the antibody-mediated destruction of the virus.

Summary for the Clinician

- The pathogenesis of HSV disease is dependent on a number of factors, including the constellation of viral genes encoded by each strain working in concert to determine the virulence phenotype of that particular strain and the genetic make-up of the host and host immune system
- A strain refers to a single viral isolate from an infected individual, which may differ in copy number or nucleotide sequence. Different strains cause different severities of corneal disease
- The initial infecting viral strain usually colonizes the sensory ganglia in most cases and its reactivation is thought to be the cause of recrudescent infections. However, super-infection with a new strain is also possible and PK is a particular risk factor. Super-infection may also result in the generation of recombinant viruses
- Host genes may be involved in innate resistance and acquired immunity
- Virulence genes such as those encoding infected cell proteins (ICP0, 4, 22, 27 and 47) play an important regulatory role. ICP0 and 22 affect the ability of the virus to replicate and ICP0 counteracts the anti-viral effect of INF, while ICP4 interferes with apoptosis of the infected cells

7.5 Clinical Manifestations

Herpes simplex keratitis is a complex ocular disease presenting with a variety of clinical manifes-

tations secondary to live viral infection, immune and inflammatory response, and their sequelae. All levels of the cornea can be affected and the keratitis can be broadly divided into epithelial, stromal, and endothelial disease, each with components of infectious and immune elements, which may be separated in time. Anterior segment disease also includes uveitis and iridocorneal endothelial syndrome. In addition, infection may be classified as primary or secondary, the latter including both initial and recurrent or recrudescent disease. The distinction between primary and secondary (based on serological conversion) becomes blurred when super-infection with other strains becomes involved. In addition, HSV-1 antibodies have been found in the tears of individuals who have no apparent serological evidence of infection [79]. Furthermore, the clinical manifestations of bilateral disease, which accounts for up to 10% of cases, tend to be more severe than those of unilateral disease. The disease may also affect children, which includes neonatal and congenital infection.

7.5.1 Primary Disease

Primary infection with HSV-1 usually occurs early in life after maternal antibodies have declined. The incidence of primary HSV-1 infection has two peaks: 1–5 years and again between 16 and 25 years. Overall, primary disease is probably sub-clinical in around 50% of cases, and even clinically recognizable disease is usually minimal, with few symptoms. Primary eye disease tends to involve vesicles or shallow ulcers on the eyelids and lid margins, a follicular conjunctivitis, and occasional corneal epithelial lesions which may coalesce to form dendritic ulcers. Stromal disease and iritis however, are rare in the primary infection [138]. HSV-1 conjunctivitis is usually self-limiting and resolves within 3–4 weeks. Antibodies to HSV appear during the second week [138]. In neonates, primary disease is usually accompanied by viremia, and eye disease may be just one manifestation of disseminated disease. The disease may manifest as conjunctivitis, keratitis or chorioretinitis (usually with disseminated or CNS disease).

7.5.2 Recurrent Infection

An important feature contributing to visual loss due to HSV infection is its recurring nature. Several factors have been suggested as potential triggers of recurrent ocular, orofacial, or genital HSV disease, including upper respiratory tract infection, fever, sunlight, seasonal conditions, emotional factors, psychological stress, trauma, and menstruation, although none of these were shown to be significant in the Herpetic Eye Disease Study (HEDS) [65].

The risk of recurrence after a single episode of dendritic keratitis has been reported to be 18–25%, and 43% in 2 years following two or more episodes [27, 71]. In another series by Wilhelmus et al. 40% of epithelial disease were recurrences and 25% of patients developed stromal keratitis over a 5-year period [206]. In the HEDS study 18% of patients developed epithelial keratitis and 18% stromal keratitis over an 18-month period. Although previous epithelial keratitis did not affect the risk of epithelial keratitis, previous stromal keratitis increased the risk of further stromal keratitis 10-fold, and was strongly correlated with the number of previous episodes. Patients who had suffered four or more episodes were 2.1 times more likely to have a recurrence in comparison to 1.4 in patients with two to three episodes [66]. This again points to the disease having a corneal source, and may relate to the quantity of virus in the cornea, as will be discussed below. A reasonable estimate of the recurrence rate of HSK after epithelial or stromal disease is approximately 10% per year [80].

The association between age, gender or race and the risk of recurrent HSV keratitis is conflicting, with some authors reporting no relationship, while others describe an increased risk in younger populations [209] and in males [206].

7.5.3 Bilateral Disease

Although predominantly unilateral, the incidence of bilateral disease has been reported as being 1.3–12% [101, 167]. It is more common in younger patients [207], in those with atopy [107] and in those with an altered immune system [143]. The incidence of recurrence is also higher in these pa-

tients, and is thought to be secondary to an altered T-cell function [107]. In one series the most common manifestation of bilateral disease was blepharoconjunctivitis (28.5%) and epithelial keratitis (71.4%). Most recurrences were unilateral, and 75% of patients with primary epithelial disease developed subsequent stromal disease. Recurrent stromal keratitis was seen in 64.3%, necrotizing stromal keratitis in 35.7% and progressive endothelitis in 14.2% of patients [167].

Bilateral disease follows a more prolonged course with more frequent recurrences and more severe complications compared with unilateral disease [167, 207]. For example, corneal vascularization has been reported to develop in 35.7% and a neurotrophic and lipid keratopathy in 28.55% [167]. Bilateral disease is also associated with a greater reduction in vision; up to 17% of patients have lost vision in the worst eye up to <6/60 secondary to corneal opacification [207].

7.5.4 Neonatal and Congenital Disease

Neonatal HSV can be secondary to intrauterine acquisition (4%) or infection at the time of birth (86%), while 10% present as postnatal infections [203]. Clinical manifestations vary with the time stage or trimester of HSV infection [76, 150]. The first case was reported by Batignani, in 1934 [121]. The incidence of neonatal HSV in the UK is around $1.65/10^5$ live births [189]. Neonatal HSV is a bilateral disease occurring in infants between 2 days and 2 weeks of age [204]. Thirty percent of mothers have signs of HSV genital infection at the time of delivery and 50% of infants are born prematurely [101]. Active ocular infection occurs in 20–25% of neonates with HSV-1 infection, with 5% developing HSV retinitis. Typical findings are corneal ulceration, anterior uveitis, cataract, vitritis, chorioretinitis, and optic atrophy [150]. The first case of congenital herpetic keratitis was reported by Hutchison et al. in 1975 [72]. When associated with encephalitis the prevalence of ocular disease is 60% with 20–25% developing retinitis [121]. Around 35% of disease is caused by HSV-1 and the remainder by HSV-2 [39].

7.5.5 HSK in Children

Although there are relatively few studies, herpetic keratitis in children appears to differ from that of adults. As previously alluded to, there is a higher incidence of bilateral disease in children than in adults, with reported rates of 10–26% (mean 16%) [12, 13, 29, 33, 145, 160]. The inflammatory response in children with stromal keratitis also appears to be greater, leading more commonly to scarring. Chong et al. found that 48% of their patients developed a residual corneal opacity, which resulted in amblyopia in 18% [29]. Children are also more susceptible to recurrences of ocular herpes. Reported studies indicate that approximately 50% (range 33–80%) of children with herpetic keratitis develop a keratitis recurrence within 1 to 2 years [12, 13, 29, 33, 145, 160].

7.5.6 Epithelial Keratitis

The earliest lesions in infectious epithelial keratitis are minute, raised, clear vesicles, that is, a punctate epithelial keratopathy similar to the vesicular skin/mucous membrane eruptions. Within 24 h the cell nuclei become laden with replicating virus and the infected cell swells up prior to releasing the virus into adjacent areas [47]. This is manifested clinically as a raised dendritic lesion that initially displaces fluorescein to produce "negative staining" [17, 69, 100] and progresses to a typical dendritic ulcer, the hallmark of HSK. It is a true ulcer extending through the basement membrane and its features include a branching linear shape with terminal bulbs and swollen epithelial borders that contain live virus. The base of the ulcer stains positively with fluorescein. Although the epithelial borders stain negatively with fluorescein, they can be demarcated with rose Bengal or lissamine green [169]. Enlargement of the ulcer may occur, leading to a geographic (amoeboid) ulcer (Fig. 7.4) [80]. Geographic ulcers account for 22% of all cases of initial infectious keratitis [100, 206]. Ulceration close to the limbus is associated with infiltration of leucocytes from the limbal blood vessels, limbal injection, vascularization, and frequently an underlying anterior stromal infiltrate.

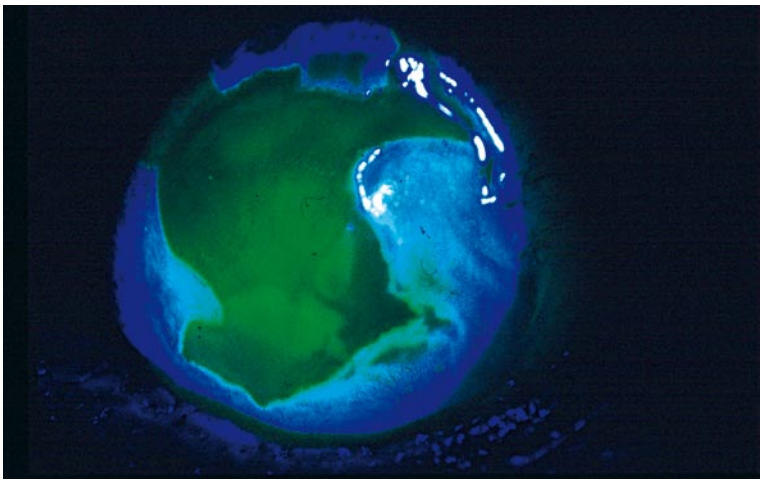

Fig. 7.4 Geographic ulcer. Note large corneal ulcer stained with fluorescein that is geographic in shape (Reprinted with permission from [80])

Patients with marginal or limbal herpetic ulcers are often more symptomatic and less responsive to treatment [139, 206]. Although epithelial disease usually resolves, sequelae may ensue such as a punctate epithelial keratopathy, recurrent corneal erosions or epithelial granularity. Some degree of anterior stromal reaction is common underlying the epithelial lesions and probably represents an immune response to the epithelial disease rather than stromal viral invasion. Herpetic ulcers may become secondarily infected with bacteria with associated features. A decrease in corneal sensation is more common than has previously been realized and neurotrophic keratopathy may occur as a result of impaired corneal innervation. This may manifest as loss of corneal luster and irregularity of the corneal surface. Punctate epithelial erosions may develop and progress to a persistent epithelial or metaherpetic ulcer, also known as an indolent or trophic ulcer. The ulcer is oval, shallow, with smooth borders of grey, elevated, thickened and rolled epithelium [17, 69, 100].

7.5.7 Stromal Keratitis

Herpetic stromal disease accounts for 2% of initial presentations and 20–48% of recurrent herpetic disease [42, 99, 128]. It results from viral invasion of the stroma, either from reactivation of latent virus in the supplying sensory nerves or from within the cornea itself.

The associated immune response contributes to the type of stromal disease seen. There may be no history of previous infectious epithelial keratitis and the epithelium is usually intact. The main characteristic is stromal inflammation, which may be focal, multifocal or diffuse and may be associated with anterior uveitis [205]. The inflammation may be chronic, recurrent or recrudescent leading to stromal scarring, thinning, neovascularization, and lipid deposition (Fig. 7.5).

Occasionally, a partial or complete immune ring, similar to a Wessely ring, is seen in the central or paracentral mid-stroma [115]. As with epithelial disease, marginal keratitis or limbitis tends to be associated with a greater inflammatory or immune response. Stromal neovascularization can be sectoral or diffuse and frequently occurs in several layers of the cornea, a condition described as "interstitial stromal HSV keratitis" [100]. Necrotizing stromal keratitis may occur. There is necrosis, ulceration, and dense infiltration of the stroma, usually with an overlying epithelial defect. Grayish white homogeneous abscesses with edema, keratic precipitates, and iridocyclitis are seen.

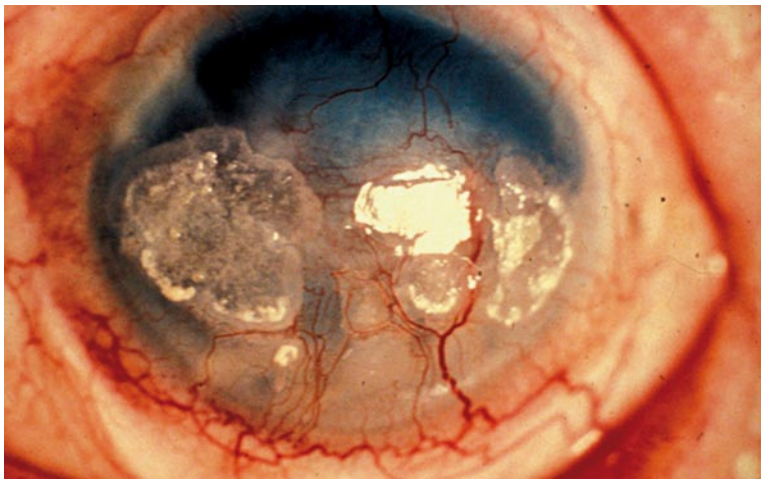

Fig. 7.5 Scarred and vascularized cornea secondary to human simplex keratitis (HSK)

Due to a combination of replicating virus and severe inflammatory response the lesion tends to be destructive and often refractory to treatment, especially where there is a superadded or associated bacterial infection [100].

7.5.8 Endotheliitis

There are conflicting views on whether HSV-1 endotheliitis is primarily due to infection or a herpetically induced abnormal immune response against the endothelium. Three phenotypic forms have been identified according to the pattern of endothelial disease: disciform, diffuse, and linear. The endothelial function may take many months to recover. It may be very difficult to distinguish between stromal inflammation and stromal edema secondary to endotheliitis.

A disciform type of endotheliitis is the most common form and is seen as a disc-shaped area of stromal edema in the central or paracentral cornea, usually involving the entire stromal thickness, giving a ground-glass appearance (Fig. 7.6). Keratic precipitates (KP) are present underlying the areas of stromal edema and mild to moderate iritis is usually present. A secondary rise in intraocular pressure may occur, which has been attributed to associated trabeculitis [4].

Less common are diffuse endotheliitis and linear endotheliitis. In the former there is diffuse stromal edema with underlying KP and mild to moderate iritis. A dense retrocorneal plaque of inflammatory cells accompanied by a hypopyon may be present. With linear endotheliitis, a serpiginous line of KP progresses centrally from the limbus accompanied by peripheral stromal and epithelial edema between the limbus and the KP [132].

7.5.9 Iridocorneal Endothelial Syndrome

Iridocorneal endothelial (ICE) syndrome is typically a unilateral condition characterized by a corneal endothelial abnormality often associated with corneal edema, angle anomalies, alteration in the iris structure (presenting as corectopia), and secondary glaucoma (Fig. 7.7).

Initially thought to be a developmental disorder, it is now thought to be of viral origin as a result of observations that disease onset occurs during the postnatal period and that the endotheliopathy resembles that seen in viral disorders with chronic inflammatory cells confined to the endothelial layer [3].

The disease manifestations are thought to be secondary to abnormal endothelial cells that can

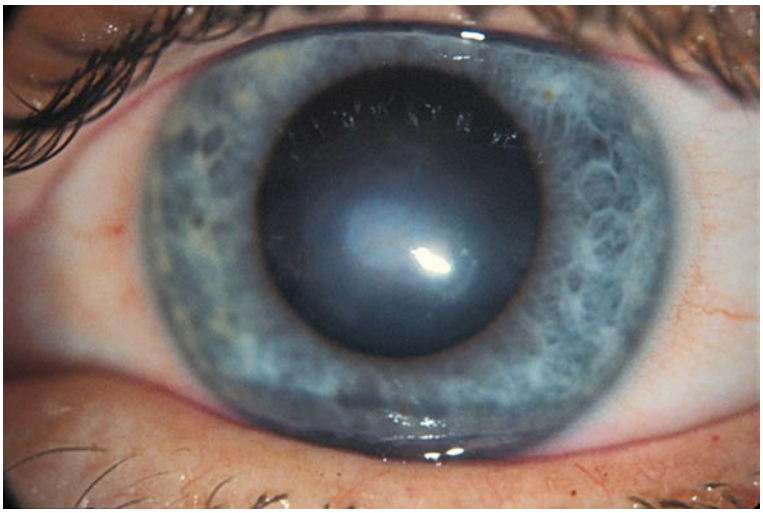

Fig. 7.6 Disciform keratitis. Note the disc-shaped area of stromal edema in the central cornea giving a ground-glass appearance

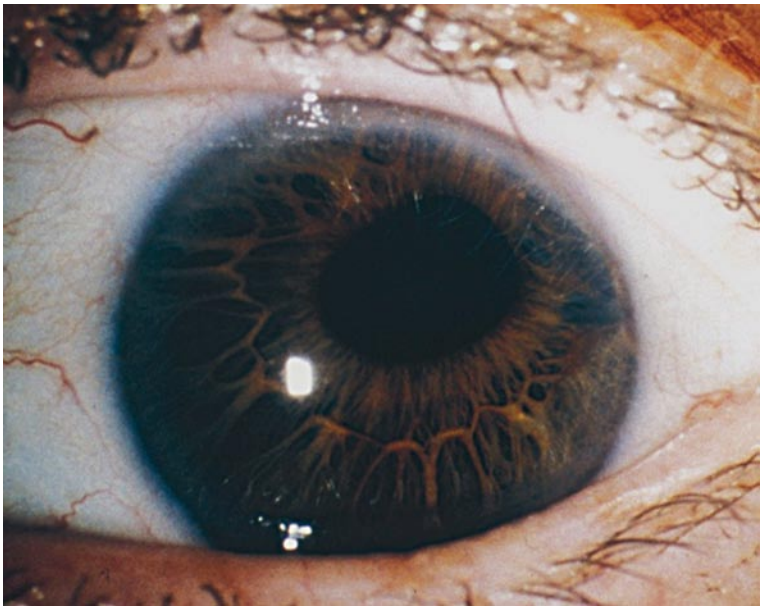

Fig. 7.7 Iridocorneal endothelial syndrome. Note the iris abnormality and corectopia

migrate across the trabecular meshwork and iris surface. It has been proposed that HSV-1 may play a role in altering these cells by transferring some unknown genes or gene fragments [69]. Indeed, HSV-1 DNA has been isolated from the aqueous and endothelium of some patients with idiopathic corneal endotheliopathy and iridocorneal endothelial syndrome. Some of these patients also respond to antiviral therapy [3].

7.5.10 Uveitis

HSV-1 uveitis reflects uveal inflammation related to the viral infection. The uveitis is usually associated with corneal stromal disease, but around 15% of cases of HSV-1 anterior uveitis may occur in the absence of corneal involvement [116]. Clues to a diagnosis of herpetic anterior uveitis include unexplained corneal scarring, decreased corneal sensation (50% of patients), focal or diffuse patchy iris atrophy, iris transillumination defects, anterior and/or vitreous cells, granulomatous or non-granulomatous keratic precipitates, posterior iris synechiae, and elevated intraocular pressure (Fig. 7.8). Sixty-five to ninety percent of patients with suspected HSV uveitis have been found to have a distorted or dilated pupil. It is usually unilateral (92%) and may follow a prolonged course with recurrent exacerbations [116].

Elevated intraocular pressure during intraocular inflammation is common, occurring in 30–90% of patients in various studies, compared with 18% for anterior uveitis in general [156, 181, 198]. HSV-1 has been suggested to be involved in the pathogenesis of Posner-Schlossman syndrome (also known as glaucomatous cyclitic crisis). Yamamoto and colleagues detected evidence of HSV-1 DNA from aqueous samples of 3 patients during acute attacks of the syndrome [210]. Some authors have therefore recommended an aqueous biopsy in all presumed cases of Posner-Schlossman syndrome [198].

Visual outcome is generally favorable with 94% (29 out of 31) in one study, retaining a visual acuity of 20/32 or more [198]. Primary herpetic uveitis (without a history of previous keratitis) seems to be more severe than uveitis in patients with previous corneal recurrences and the associated glaucoma may be a devastating complica-

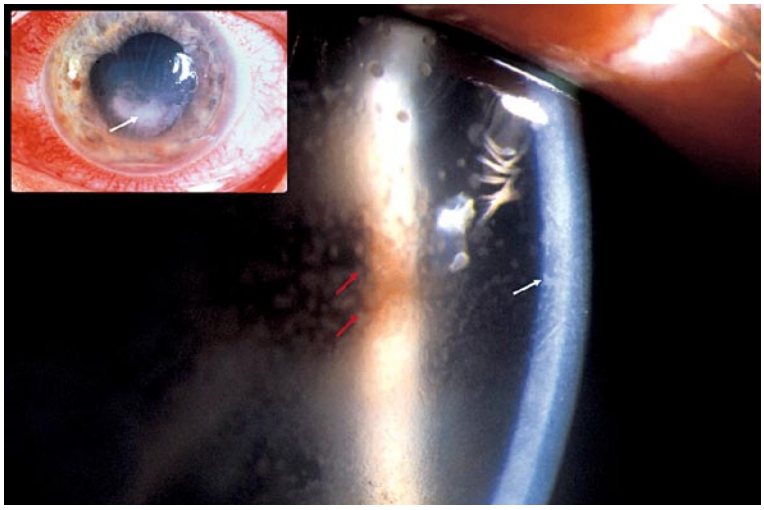

Fig. 7.8 Human simplex virus-1 uveitis. Note the stromal keratitis (*white arrow*), patchy iris atrophy, posterior iris synechiae (*inset*), and granulomatous keratic precipitates (*red arrows*)

tion. Absence of corneal involvement may delay diagnosis and therefore worsen visual outcome [156]. Cataract is also a common long-term complication, occurring in 20% of patients. Treatment of herpetic uveitis is discussed in Sect. 7.6.

Summary for the Clinician

- Human simplex keratitis can present with a variety of clinical manifestations secondary to live viral infection, immune and inflammatory response, and their sequelae
- Human simplex virus-1 induced keratitis can be broadly divided into epithelial, stromal, and endothelial disease each with infectious and immune elements. Anterior segment disease also includes uveitis and iridocorneal endothelial syndrome
- The incidence of primary HSV infection has two peaks: 1–5 years and again between 16 and 25 years. Primary disease is probably subclinical in approximately 50%. The disease is usually mild and stromal disease and uveitis are rare
- In neonates primary disease is usually accompanied by viremia and eye disease may be just one manifestation of disseminated disease. Active ocular infection occurs in 20–25% of neonates with HSV-1 infection
- A reasonable estimate of the recurrence rate of HSK after a single episode is 10% per year. The incidence of bilateral disease varies from 1.3 to 12% and is more common in younger patients
- In children, the incidence of bilateral disease is around 16%. They also tend to suffer more frequent and severe recurrences – approximately 50% within the first 2 years
- Herpetic stromal disease accounts for 2% of initial presentations and 20–48% of recurrent herpetic disease

7.6 Pathogenesis of HSK

7.6.1 Pathogenesis of Herpetic Keratitis

Corneal, conjunctival or lid epithelial disease is initiated by infectious virus that replicates in the epithelial cells and studies in animal models have shown the requirement of viral replication for this phase [9]. A threshold effect is observed for the inoculum size; in that once infection is initiated it proceeds to completion unless the viral load is reduced [87]. The severity of disease tends to be dependent on the age of the patient. Animal studies have shown that younger mice develop more severe herpetic eye disease [82]. As discussed in Sect. 7.4.1, disease severity is also dependent on the HSV-1 strain.

7.6.1.1 Viral Component

The persistence of HSV-1 in the cornea is important for understanding the pathogenesis of and treating HSK, in particular herpetic stromal disease. For example, it has been suggested that an epitope encoded by the UL6 gene of HSV-1 can mimic a corneal antigen ($IgG2^{ab}$ Ig isotype) and initiate an immune attack on corneal antigens [8, 105, 212], although there is evidence against this [48]. It is also possible that antigen expression may result from host transcription of viral DNA and class I MHC presentation of the viral protein [55, 194]. The presence, however, of an active inflammatory infiltrate in the corneas of patients with HSK, together with the finding of persistent HSV-1 DNA and antigen [193], makes either a low-grade productive infection or low-grade reactivation from a latent virus a more likely possibility. This association between long-term persistence of HSV-1 in ocular tissues and chronic inflammation has been demonstrated in several animal studies [9, 105, 117].

The likelihood of HSV-1 persistence either as a low-grade infection, following repeated reactivation from latency or from entry into the cornea from sensory nerves justifies the use of antivirals when treating all forms of herpetic keratitis.

7.6.1.2 Immune Component

Corneal epithelial disease induces an influx of innate immune cells such as polymorphonuclear leucocytes (PMN), macrophages, natural killer cells, and other mononuclear cells into the underlying stroma. Pro-inflammatory cytokines such as Interleukin (IL)-1α, IL-1β, IL-8, IL-6, Interferon (IFN)-α, Tumor Necrosis Factor (TNF^0)-α, macrophage inflammatory protein (MIP)-2, monocyte chemoattractant protein (MCP)-1, IL-12, and MIP1-α are released by the infected and neighboring cells as well as PMN [23, 176]. Langerhans cells present the HSV-1 antigens to T cells in draining lymph nodes, which are attracted by chemotactic cytokines. A transient corneal clouding may occur due to the toxic effect of cytokines on the endothelial cells. The viral reflux and PMN influx subside by days 4–5 post-infection and it is felt that PMN contribute to virus removal [184].

The importance of the immune response in stromal keratitis is apparent from the observations that in T-cell deficient mice, ocular infection with HSV-1 was not associated with the development of marked stromal disease [111, 175]. Immune response to HSV-1 infection can include T cell-mediated delayed-type hypersensitivity that may be critical to the elimination of the virus, cytotoxic T lymphocytes that may prevent viral spread, and IgG antibodies, which can neutralize the virus. The CD4+ Th1 cells are most commonly implicated and secrete IL-2, INFγ, and TNFα, which are mediators of inflammation. CD8+ cytotoxic T lymphocytes have also been linked to the increased incidence and severity of HSK.

Confusion still surrounds the role of CD4+ Th2 cells, which secrete IL-4, IL-5, IL-6, and IL-10, and have been shown to produce HSK in animal models. HSK can also result from an antibody-dependent, complement-mediated inflammatory insult. Natural killer cells display surface receptors for the Fc component of IgG and may mediate antibody-dependent-cell-mediated-cytotoxicity (ADCC). Further support for this mechanism comes from observations that immunization of mice with glycoprotein K results in more severe keratitis upon subsequent infection [23, 175]. HSV-1-specific T lymphocytes may also persist in corneas and memory T and B lymphocytes specific to HSV-1 circulate in the blood and lymph armed for the next encounter with the virus.

There is evidence that a chronic low-level viral infection persists in the stromal keratocytes, providing a continuous stimulus to antigen-specific T lymphocytes that kill the keratocytes by a cytotoxic mechanism [26].

Several hypotheses exist regarding the agents that drive the CD4+ T cells to orchestrate the inflammatory process. There is a role for the bystander activation of T cells, which are dependent on continuous viral replication with the release of cytokines [44].

7.6.2 Pathogenesis of Herpetic Uveitis

Eighty-five percent of cases of HSV intraocular inflammation present as iridocyclitis, while approximately 15% present as posterior segment inflammation including panuveitis and retinitis (acute retinal necrosis) [116]. Although not fully understood, the pathogenesis is thought to involve viral replication within intraocular nerves, ischemic vasculitis, and lymphocytic infiltration of the iris stroma. Epidemiological and experimental data suggest that oropharyngeal primary herpetic infection may lead to eye disease later in life.

7.6.2.1 Viral Component

As discussed above, HSV1 inoculation of the lower lip of mice has been shown to lead to latent infection of the entire trigeminal ganglion, including the ophthalmic division [191]. Recurrent disease may occur due to anterograde axonal spread of previously dormant HSV-1 virus from the trigeminal ganglion or from the cornea [80]. The presence of persistent viral infection may cause an inflammatory reaction manifested as uveitis, or can trigger the immune system itself against viral antigens, causing it to cross-react with uveal tissue and cause inflammation [98].

Herpetic uveitis may occur in the absence of keratitis, suggesting involvement of the autonomic nerves supplying the iris and ciliary body rather than the trigeminal nerve [179]. This theory is supported by post mortem studies, which have demonstrated latent herpetic infection of the central nervous system and autonomic nerves [52, 57, 201, 202].

A study by Labetoulle et al. [90] on mice demonstrated rapid propagation of HSV1 from oral mucosa to uveal tissues and cornea within 6–8 days post oral inoculation, which appeared to occur via the sympathetic nerves and superior cervical ganglion much more rapidly than via the trigeminal nerves or parasympathetic system. At 6 days post-inoculation, the entire ipsilateral superior cervical ganglion was infected, including neurons innervating the iris and ciliary body. The observation that the iris was always infected before the cornea supported the theory that eye disease resulted from the spread of virus from the nervous system [90].

7.6.2.2 Immune Component

Herpetic intraocular inflammation is characterized by the presence of predominantly mononuclear cells mainly composed of T lymphocytes with smaller numbers of macrophages and plasma cells also present. The immune privilege of the eye means that it constitutively expresses immunomodulatory cytokines such as transforming growth factor (TGF)-β and macrophage migration inhibitory factor, as well as other factors, which act to suppress T cell responses and those of other cells such as natural killer (NK) cells. These factors do not, however, affect the humoral immune responses.

A recent study has suggested that eyes with HSV uveitis contain antibodies with different virus protein targets from those found in the serum, thus exhibiting a compartmentalized B cell response to HSV ocular infection [141].

Another study found that NK cells were activated following intraocular inoculation of murine eyes with the KOS strain of HSV-1 [182]. It was suggested that NK cells play a role in preventing direct anterior to posterior spread of HSV in the eye, explaining why the ipsilateral eye did not develop experimental acute retinal necrosis. The NK response was thought to have both a local (eye, superficial cervical and submandibular lymph nodes) and systemic response (spleen).

The reason why the ocular immunomodulatory factors do not control NK activity is thought to be due to rapid viral replication outstripping the eye's ability to produce these factors.

7.6.2.3 Anterior Chamber-associated Immune Deviation

Ocular immune privilege results in part from induction of a deviant form of systemic immunity, termed anterior chamber-associated immune deviation (ACAID), which is another component that may modulate the ocular immune response to HSV infection. ACAID mitigates ocular autoimmune diseases and promotes corneal allograft survival. This is an animal model of immune tolerance, in which antigen injected into the anterior chamber of the eye is internalized by intraocular F4/80-positive antigen-presenting cells (APC) such as intraocular dendritic cells and macrophages [102], which process antigen, migrate across the trabecular meshwork and then to the thymus and spleen. In the spleen they induce the differentiation of antigen-specific B cells that act as ancillary APCs and are required for ACAID induction. These in turn induce the generation of CD4-positive and CD8-positive regulatory T cells resulting in tolerance to the antigen [7]. HSV-1 and HSV-2 have been shown to induce ACAID in animal models [2]. ACAID interferes with both Ag-specific delayed type hypersensitivity (DTH) and complement-fixing antibody production.

It is thought that DTH may play a role in reducing viral-induced anterior and posterior uveitis. Indeed, in the case of varicella zoster virus (VZV), it has been shown that patients with VZV-induced anterior uveitis and acute retinal necrosis (ARN) had lower skin DTH responses to VZV antigen compared with patients with VZV-induced skin disease alone (control group) [86].

7.6.3 Recurrence: Reactivation and Super-infection

As mentioned previously, a reasonable estimate of the recurrence rate of HSK after epithelial or stromal disease is approximately 10% per year. There is recurrence despite acquired immunity. A variety of stimuli may lead to viral reactivation, such as local injury, physical and emotional stress, fever, menstruation, exposure to ultraviolet light, and immunosuppression, although there is some disagreement whether these stimuli lead to recurrent ocular disease [17, 43, 65]. The molecular events leading to efficient reactivation center around ICP0 and LAT, as discussed in Sect. 7.3.4.2. Reactivated virus then either produces local disease if latent in the cornea, or travels down the sensory nerve to replicate in mucocutaneous epithelia, such as the conjunctiva, cornea or lid margin.

Super-infection with a different HSV strain has been noted for both genital and ocular disease. Remeijer et al. found that a third of corneas with recurrent herpetic keratitis (RHK) were super-infected with a different HSV-1 strain and 63% with the same strain of RHK [148, 149]. One patient in their study with bilateral HSK had different strains in each cornea. Although it would appear that super-infection occurs in the cornea, it is not clear whether this translates to super-infection within the trigeminal ganglion. Expression of LAT appears to interfere with super-infection by other HSV-1 strains [104] and latency in the trigeminal ganglion is accompanied by a chronic immune response, which may protect from super-infection [183].

7.6.4 Corneal Scarring and Vascularization

The pathogenesis of corneal scarring and vascularization in HSK is still uncertain.

7.6.4.1 Immune Component

There are conflicting reports in the literature as to the protective effects of CD4+, CD 8+ or both in corneal scarring [59]. It has also been suggested that Th1 (IL-2) responses may protect and Th2 (IL-4) responses may enhance scarring [58]. Similar controversies exist on the role of NK cells and macrophages [60]. One of the major events after HSV infection is the production of proinflammatory cytokines and chemokines, and an invasion of the cornea by PMN. This response helps to clear the virus, but at the same time lends entry to various cytokines and angiogenic factors secreted by the PMN.

IL-1 and -6 are important mediators of inflammation and a beneficial effect of IL-ra (interleukin-1 receptor antagonist) has been noted on disease severity and corneal scarring [18]. IL-10 and -12 can also suppress HSK lesions in animal models, possibly by the induction of antiviral cytokines such as interferon γ, and cellular defenses like NK cells and T cell apoptosis. IL-12 also has an antiangiogenic effect [94]. Co-ordinated phenotypic changes, extracellular matrix (ECM) deposition, and remodeling are the key elements in the process of tissue repair, as in corneal scarring.

7.6.4.2 Healing Response

Various cytokines and growth factors are involved in corneal wound healing and the most important of these are epidermal growth factor (EGF) and transforming growth factor β (TGFβ) [158]. TGFβ up-regulates fibroblast proliferation and ECM synthesis and reduces ECM degradation after injury.

One of the major challenges has been in understanding the differences between fetal (scar-free) and adult wound healing (with scarring). It is now known that "matricellular proteins," a group of disparate proteins expressed during development, but not in adults, are up-regulated in sites of tissue re-modeling and act temporally and spatially to provide regulatory signals in cell–cell and cell–matrix interactions [19].

One matricellular protein that has been extensively studied in the corneal in vivo models is the platelet-derived glycoprotein thrombospondin (TSP). Thrombospondins are a family of five glycoproteins, two of which, TSP 1 and TSP 2, are

involved in wound healing and are potent anti-angiogenic agents [5, 20]. Given that TSP 1 and 2 play an important role in corneal scarring and vascularization the next question is, what is their source in the cornea?

One mechanism could be by invading blood vessels in the cornea, which have been shown to appear as early as 24 h after infection in vivo [95]. Scarring also develops in an avascular cornea, where fibroblasts and not platelets are the predominant cells. This has led to the search for a local reservoir of TSP in the cornea. Hiscott et al. have shown this reservoir to consist of keratocytes that express TSP 1 and 2 in an in vitro stromal wound repair model [68]. TSP 1 acts by modulating cellular responses to ECM and can also bind and activate TGFβ [20].

Corneal vascularization in HSK requires active viral replication and procedures that minimize angiogenesis may diminish the severity of HSK [95, 213, 214]. HSV-1 differs from other viruses in that it does not encode molecular mimics of any known angiogenic factors. This suggests that HSV-1 infection may disrupt the normal equilibrium between angiogenic and anti-angiogenic stimuli, leading to an "angiogenic switch," initiating angiogenesis [166]. HSV-1 infection can induce the production of many angiogenic factors such as vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMP) 2 and 9, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), MIP-2 and MCP-1 [95, 211, 213].

Hypoxia due to corneal edema may also serve as an angiogenic stimulus. The source of these factors seems to be mainly PMN, but VEGF is also expressed in epithelial, endothelial, and stromal cells, as well as in macrophages [213]. These angiogenic responses are balanced by antiangiogenic factors such as transforming growth factor β, tissue inhibitors of metalloproteinase (TIMP), and TSP 1 and 2 [5, 211]. As discussed earlier TSP 1 and 2 are expressed by keratocytes and can bind and inhibit the activity of βFGF, VEGF, MMP2, and MMP9 [5]. HSV-1 infection can selectively suppress matrix protein synthesis and it has been

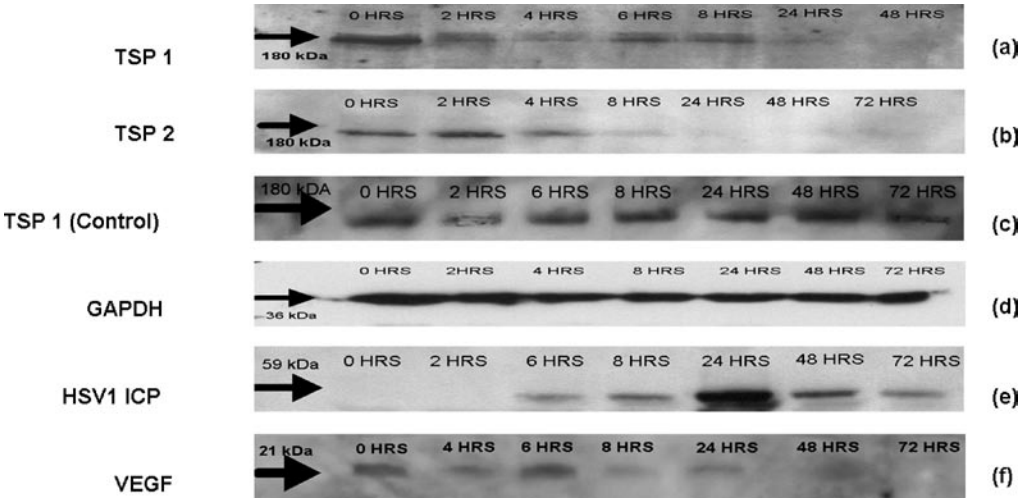

Fig. 7.9 Western blot analysis. Protein expressions of TSP 1, TSP 2, TSP 1 (control), GAPDH, HSV1:ICP27, and VEGF at different time intervals post-infection. **a, b** Densitometric analysis revealed a 50% reduction in the signal intensity of TSP 1 and 2 by 8 h, with a complete absence by 24 h post-infection. **c** There was no change in the expression of TSP 1 in sham-infected cultures. **d** There was no change in the expression of GAPDH at all times post-infection. **e** Immediate early viral proteins (HSV1:ICP27) were expressed by 6 h, reaching maximum intensity at 24 h post-infection. **f** VEGF expression continued till after TSP 1 and 2 expression had ceased (Reprinted with permission from [30])

shown that TSP 1 and 2 are selectively downregulated by infection in human keratocytes in vitro (Fig. 7.9) [30]. It has also been shown that keratocytes expressed VEGF after infection and that its production continued until after TSP 1 and 2 production had ceased (Choudhary et al., unpublished findings).

These findings support the hypothesis of alteration of the normal balance between angiogenic and antiangiogenic responses being the likely cause of corneal vascularization in HSK and may form the basis for the next generation of treatment options for this condition [80].

Summary for the Clinician

- It has been suggested that an epitope encoded by the UL6 gene of HSV-1 can mimic a corneal antigen and initiate an immune attack on corneal antigens, although low-grade infection/reactivation remains more likely
- Herpetic uveitis can occur in the absence of keratitis in approximately 15%, suggesting involvement of the autonomic nerves supplying the iris and ciliary body rather than the trigeminal nerve. Elevated IOP during herpetic anterior uveitis is common, occurring in 30–90% of patients compared with 18% for anterior uveitis in general
- Herpes simplex virus has been shown to induce anterior chamber-associated immune deviation (ACAID), which in turn inhibits both antigen-specific delayed type hypersensitivity and complement-fixing antibody production. It is thought that patients with lower DTH responses are more likely to develop herpetic anterior uveitis
- The pathogenesis of corneal scarring and vascularization are still uncertain, but are likely to involve various cytokines and growth factors such as epidermal growth factor and transforming growth factor β

Summary for the Clinician

- The matricellular proteins thrombospondin 1 and 2 are involved in wound-healing and are potent antiangiogenic agents thought to play an important role in modulating corneal scarring and vascularization. They have been demonstrated in keratocytes in an in vitro model of stromal wound repair
- Corneal vascularization requires active virus replication and procedures that minimize angiogenesis may diminish the severity of HSK. As HSV-I does not encode for any angiogenic factors it has been suggested that infection may disrupt the normal equilibrium between angiogenic and antiangiogenic stimuli, leading to an "angiogenic switch" initiating angiogenesis
- HSV-1 infection can induce the production of many angiogenic factors, primarily by neutrophils but also epithelial, stromal, and endothelial cells. Also, HSV-1 infection has been shown to selectively suppress matrix protein synthesis of TSP 1 and 2 in an in vitro model, thereby supporting the theory of an "angiogenic switch"

7.7 Diagnosis

7.7.1 Laboratory Diagnosis

An attempt should always be made to isolate or identify HSV-1. This may help avoid complications from misdiagnosis and inappropriate treatment. This is important not only because certain other conditions can mimic HSV-1 ocular disease, but also because it allows strain characterization and provides information regarding mutations and super-infection by other strains. Laboratory tests are aimed at cell cytology, viral antigen detection (immunoassays), viral DNA detection (polymerase chain reaction), and virus isolation (tissue culture).

Cytology is a simple method that can complement other, more specific tests. It can be carried out using cellular material obtained from corneal/conjunctival lesions smeared on a glass slide and fixed in ethanol. These can then be stained with Papanicolaou, Giemsa or Wright stains. Presumptive diagnosis is based on the presence of intranuclear inclusions and multinucleated giant cells. It has, however, a low sensitivity (57%) and should not be used on its own [177].

Herpes virus antigen can be detected by enzyme- or fluorescence-based immunohistochemical techniques. This is a quick technique with good sensitivity, but is prone to false-positive and -negative results. PCR is used for the detection of HSV-1 DNA and carries a sensitivity of up to 100%. Conventional PCR is a qualitative method, very useful for detecting evidence of HSV-1 in the tear film, conjunctival, cornea, and aqueous, but does not differentiate between latent and infectious virus. Competitive PCR on the other hand is quantitative, but it is complicated and time-consuming [49, 177]. Real-time PCR has now been developed, which is a quantitative method and is quicker and more sensitive. It can provide indirect evidence of virus replication by the number of DNA copies produced and hence can also be used for evaluating the efficacy of antiviral medications [110]. Fukuda et al. described PCR results from tear samples of patients with herpetic keratitis [54].

Viral DNA isolation was 100% in epithelial disease and persistent epithelial defect, 57% in disciform keratitis, and 0% in silent stromal and endothelial disease [54].

Isolation of HSV-1 in culture is the most reliable and specific method and is considered the gold standard for the diagnosis of HSK. Clinical specimens are inoculated into cell culture systems (e.g., corneal epithelial cells, foreskin fibroblasts, Vero cells), which are then observed for cytopathic effects characteristic of virus replication (Fig. 7.10). These effects develop within 24–48 h. Various techniques such as conventional tube culture and centrifugation enhancement of HSV replication (shell vial assay) can be used for isolation. Although viral culture carries a high specificity (100%), its sensitivity is very low. Virus isolation rates in one study were as low as 8.2% [177]. As each of the aforementioned tests detects a different component of the virus and is insufficient to confirm a diagnosis on its own, a combination of tests is recommended. PCR and

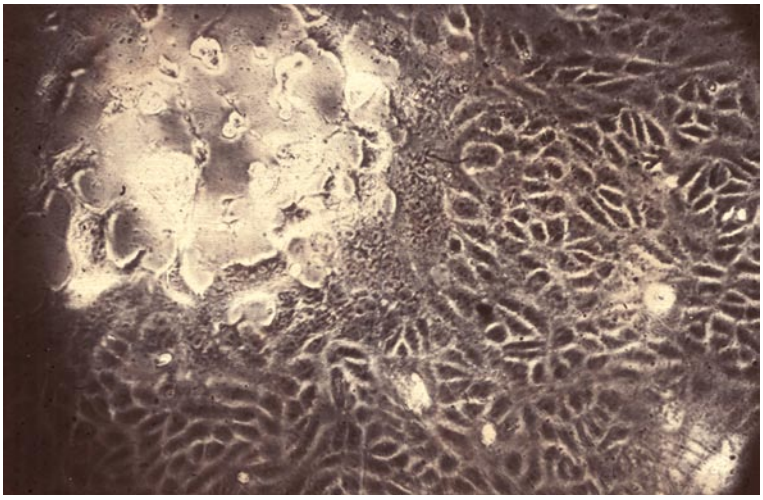

Fig. 7.10 Isolation of HSV-1 in culture. HSV-1 plaque in the monolayer of Vero cells (Reprinted with permission from [80])

IHC appear to be the most suitable combination, giving a specificity of up to 97%.

7.7.2 Aqueous Biopsy

A laboratory confirmation should be attempted in uveitis of unknown origin after exclusion of an underlying systemic disease because of the consequences of a diagnosis of viral anterior segment disease for treatment and prognosis. In one study of the results of aqueous biopsy in suspected HSV uveitis, the combination of antigen detection and DNA amplification by PCR confirmed the presence of virus in 29% of suspected cases (14 out of 45). Viral culture did not prove useful, being positive in only 2% (1 out of 45) [157]. Another study looked at the results of aqueous biopsy in 24 cases of suspected herpetic uveitis. Selection criteria were:

- Anterior uveitis
- Sectoral atrophy of the iris
- No associated keratitis or any other clinical involvement of the cornea before, during, or after the inflammatory process, with the exception of corneal edema caused by raised intraocular pressure

The study found that using PCR, 83% of samples were positive for HSV while 13% were positive for VZV. As might have been expected, the mean age was younger for HSV-related anterior uveitis at 34 years compared with 65 years for VZV [198].

7.7.3 Diagnosis of HSV-1 in Patients with a History of Inflammatory Corneal Scars

It is often difficult to determine whether a patient with a history of an inflammatory scar of the cornea or interstitial keratitis has had herpetic keratitis. The diagnosis of previous herpetic keratitis is particularly important for patients undergoing PK both for prognosis and because of the need for post-surgical antiviral therapy. Irrespective of whether HSV-1 can establish a latent infection in the cornea, it is necessary, therefore, to be able to establish a diagnosis of herpetic keratitis at the time of PK. Although culture remains the standard for the detection of a productive infection by HSV-1, it is an insensitive technique, particularly for the isolation of HSV-1 from the deeper layers of the cornea and may be incapable of detecting a latent infection. The aggressive use of antivirals may have had an effect on the ability to isolate virus from the cornea [80].

PCR is a sensitive method for detecting HSV-1 DNA in the cornea, with sensitivity of 82% and specificity of 78%. The longer the period between the last episode of HSK and surgery, the less the amount of virus and the less likely virus will be detected. For example, in the study by Kaye et al. [83], the median time in those patients with evidence of HSV-1 DNA was less than 2 years, while those patients with a negative result had a median interval of 7.5 years. This is in keeping with the results of O'Brien et al. [130], who found a decrease in the detection of HSV-1 DNA from 100 to 30% at 4 months post-infection of rabbit corneas. A similar trend has also been noted for the presence of HSV-1 antigen (Fig. 7.11), which suggests that both HSV-1 DNA and antigen have a limited life within the cornea [80]. This is compatible with the increased recurrence rate in patients who have had frequent episodes of HSK stromal keratitis, that is, a greater amount of HSV-1 in the cornea may be expected to lead to greater chances of reactivation and disease in the cornea.

Although HSV DNA has been found in the corneas of patients with no clinical or diagnosed history of herpetic keratitis, the amount of HSV-1 DNA is much less than in the corneas of patients with a diagnosis of herpetic keratitis. That is, HSV-1 DNA has been detected in an approximately 10–50 times greater amount in HSK than non-HSK corneas, particularly where the time interval between the last attack and surgery was relatively short. This supports the concept that a critical amount of HSV-1 is required before the infection becomes clinically evident.

Similar to PCR, IHC has good sensitivity and specificity for the detection of HSV-1, i.e., 74% and 85% respectively [83]. The combination of PCR followed by IHC therefore, is useful in obtaining a diagnosis in scarred corneas without

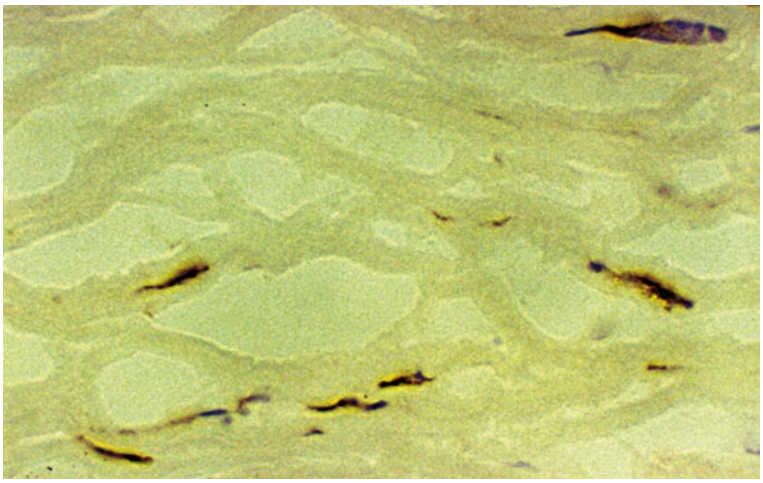

Fig. 7.11 Immunohistochemistry for HSV-1 antigen in the corneal scars. HSV-1 antibody, DAB, and hematoxylin counterstain showing HSV-1 immunoreactivity in the stromal keratocytes (Reprinted with permission from [80])

a history of HSK. Corneas should initially be screened for HSV-1 DNA by PCR, followed by IHC if HSV-1 DNA is present.

Summary for the Clinician

- Laboratory tests include cell cytology, viral antigen detection (immunoassays), viral DNA detection (PCR), and virus isolation (tissue culture). As each of these tests detects a different component of the virus and is insufficient to confirm a diagnosis on its own, a combination of tests is recommended. PCR and IHC appear to be the most suitable combination, giving specificity of up to 97%
- An aqueous biopsy with laboratory confirmation of HSV should be attempted in uveitis of unknown origin after exclusion of an underlying systemic disease, because of the consequences for treatment and prognosis

7.8 Treatment

7.8.1 Pharmacokinetics of Acyclovir

The bioavailability of aciclovir (ACV) as an oral suspension is around 12%. This tends to be less for higher doses due to saturation of absorption, for example, 20% for 200 mg compared with 12% for an 800-mg dose. Intravenous administration provides an 8 times greater bioavailability compared with oral administration. The efficacy of ACV depends on the levels over 12 h. Thus, 250 mg twice a day provides a similar maintenance level to 1,000 mg once a day. In terms of treatment dosage, ACV 400 mg five times per day provides therapeutic levels in the aqueous. ACV is eliminated in the kidney by glomerular filtration and tubular secretion.

Although drug-related toxicities are uncommon, ACV should be reduced in renal insufficiency. Adverse events have been reported with ACV, including gastrointestinal disturbances, development of a rash, weight gain, dizziness, headache, fatigue, sexual dysfunction, memory loss, tinnitus, anxiety, and hair loss.

In immunocompetent patients, treatment with ACV does not create resistant strains; however, ACV resistant isolates are more likely to be isolated from immunodeficient patients in whom ACV treatment has failed.

7.8.2 Herpetic Epithelial Disease

Although most cases of herpetic ulceration will eventually resolve, the use of topical antiviral agents significantly improves outcome. Numerous studies have shown the benefit of using topical antivirals in the treatment of herpetic epithelial or ulcerative keratitis [63]. Improved resolution of bilateral disease has also been found using both topical and systemic antivirals [80, 107].

7.8.3 Herpetic Stromal Disease

An important issue in the treatment of HSK centers on the use of topical steroids in patients with stromal disease. The HEDS studies showed a benefit in the treatment of herpetic stromal disease using a combination of steroid and antivirals, with a reduced duration of HSK [11, 208]. Faster recovery and improved outcome have been reported with ACV and dilute steroid compared with ACV alone [34, 133]. Topical steroids and trifluridine shortened the course and prevented progression [208], although there was a questionable increase in recurrence in those patients receiving topical steroids.

Not all studies have shown the benefit of topical steroids in the treatment of HSK. Protracted tapering when on steroids is a problem, with frequent recurrences during the period during which the steroids are being reduced [41]. Steroids may aggravate the severity of the keratitis [180] and there are reports concerning the development of glaucoma in patients on topical steroids.

Some studies have shown the benefit of topical antivirals in patients with stromal disease [16, 159, 178], showing a reduction in both number (0.14 to 0.029) and duration (3.08 to 7.8 days) of recurrences in patients on antiviral treatment [164]. Of note, topical ACV without steroids has been shown to suppress stromal inflammation [108].

What has not been answered, however, is whether initial treatment with antivirals prior to the commencement of topical steroids has an influence or benefit on recurrence. The HEDS study showed no deleterious effect when the introduction of steroids was delayed [208]. This is an important issue, as the amount of HSV in the cornea may have a bearing on recurrence. A reasonable option would be to use topical antivirals alone in the first week of treatment followed by the addition of topical steroids in the second or third week. This may have the potential benefit of leading to a reduced amount of functional virus in the cornea and thereby reducing recurrence rates and more protracted disease [80].

7.8.4 Herpetic Uveitis

While either topical or systemic antivirals alone are likely to be inadequate in the treatment of herpetic uveitis, they are of benefit in reducing recurrence and may shorten the length of the disease process. As with most anterior uveitides, topical steroids are necessary for the treatment of the condition [35]. Although the evidence is outstanding, it may be reasonable to initiate treatment with topical and systemic antivirals (aciclovir 800 mg five times per day), subsequently followed by the introduction of topical corticosteroids titrated according to clinical response.

A randomized, placebo-controlled trial of oral acyclovir (10-week course of oral acyclovir, 400 mg, five times daily) for iridocyclitis caused by HSV was attempted by the Herpes Epithelial Disease Study Group, but was abandoned due to insufficient patient recruitment after only 50 of the 104 patients who were originally planned for were enrolled in more than 4 years. While the number of patients recruited in this trial was too small to achieve statistically conclusive results, the trend in the results suggested the benefit of oral acyclovir in the treatment of HSV iridocyclitis in patients receiving topical corticosteroids and trifluridine prophylaxis [63].

These results were supported by another nonplacebo-controlled study that compared 13 patients on prophylactic acyclovir (600–800 mg/

day) with 7 patients on no prophylactic therapy and found much lower rates of recurrence among patients on acyclovir [153].

7.8.5 Prevention of HSK Recurrence

Barney and Foster [10] reported no recurrence of HSK on ACV for 12–15 months compared with 44% recurrence in patients not taking ACV. In addition, the number and duration of recurrences of epithelial disease decreased with long-term ACV [164]. Continued oral ACV reduces the recurrence rate of all forms of HSV eye disease. Oral administration of 400 mg twice daily over 12 months, followed by 6 months of observation, leads to a 45% reduction in risk of recurrence (32% placebo vs. 19% ACV) in patients with a history of HSK.

The HEDS [64] reported a 43% reduction in HSK in patients treated with ACV (28% for placebo vs. 14% for ACV). In addition, a benefit of long-term ACV (mean of 34 months) has also been shown for patients with recurrent epithelial herpes simplex infection [164].

Recurrence is more common after recent ocular surgery and it would be appropriate to prophylactically treat patients with a history of HSK who have undergone corneal surgery.

As mentioned in Sect. 7.8.1, long-term treatment in immunocompromised individuals may increase the risk of ACV-resistant mutants. The frequency of ACV-resistant mutants in the natural virus population has been estimated to be approximately 10^{-4} to 10^{-3} [32, 70]. Okuda et al. [131] found in a murine model, however, that ACV-sensitive virus tended to replace and suppress the generation of ACV-resistant virus during treatment with ACV. This would suggest that conventional ACV therapy in immunocompromised hosts may not induce ACV-resistant HSV [80].

7.8.6 Recurrence after Penetrating Keratoplasty

Once a visually disabling corneal scar occurs, options for improving vision are limited to surgery, in the form of either penetrating or lamellar keratoplasty, or rarely photokeratectomy.

One of the major problems following PK for HSK, is the development of recurrent disease in the new cornea, and secondly, corneal transplant rejection following or concurrent with recurrent herpetic disease (Fig. 7.12) [80]. Van Rooij et al. [199, 200] reported a recurrence rate of 27% in first year, Cobo et al. [31] 18% by 2 years, Fine and Cignetti [50] 12% after 3 years, Langston and Pavan-Langston [91] 15% after 2 years, and Moyes et al. [118] 24% within 1 year. The recurrence rates of HSK following PK therefore vary from 12 to 27% compared with 10% per year in non-PK patients off treatment. Conversely, the incidence of newly acquired HSV keratitis after PK is 14-fold higher than in the population [147]. This increase may be due to reactivation of latent virus, transmission via the tear film, or transmission from virus either latent in the donor cornea or carried via the donor cornea following reactivation in the trigeminal ganglion [147–149].

HSV-1 DNA has been detected more frequently in the corneas of patients undergoing repeat corneal transplantation, and has been suggested as a possible factor for HSV-1 in graft failure. Importantly, however, Nicholls et al. [126] and Openshaw et al. [134] were unable to elicit disease in uninfected animals by transplanting corneas containing HSV-1. It is probable, therefore, that HSK post-transplantation arises from host HSV-1 and is unlikely to be accounted for by transplanting an infected cornea [80].

The use of both topical and systemic antivirals has lead to a significant reduction in post-transplant recurrence of HSK. Several studies have supported the use of antivirals in the postoperative period and during episodes of transplant rejection. Most episodes of HSK will occur within the first year following PK and so it would appear reasonable to treat patients prophylactically for at least 1 year postoperatively. Van Rooij et al. [200] found that recurrent disease while on a 1-year course of aciclovir occurred in 9% of patients compared with 27% in the placebo group. Similarly, Foster and Duncan [51] noted 6% recurrence after 2 years using topical antivirals and Moyes et al. [118] showed an 11% rate of recurrence on topical antivirals vs. 24% recurrence without treatment. This effect has also been documented in animal stud-

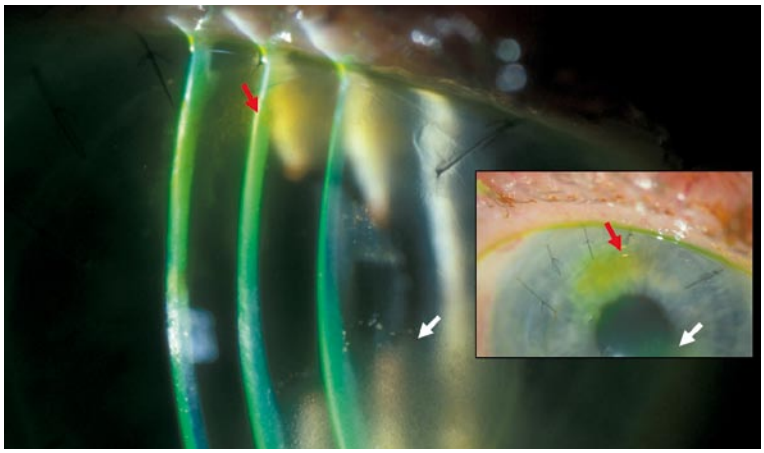

Fig. 7.12 Recurrence of HSK in a corneal transplant. Note the recurrent corneal ulcer at the interface (*red arrow*) and line of keratic precipitates with inferior corneal edema (*white arrow*) in a patient who had a corneal transplant for HSK (Reprinted with permission from [80])

ies, with Beyer et al. [15] showing a reduction in the shedding of HSV in the tear film from 82 to 0%, ulcerations from 82 to 10%, and stromal keratitis from 56 to 12% in rabbits after PK [80].

7.8.7 HSV Vaccines

Although antiviral medications can suppress symptomatic disease, asymptomatic shedding, and transmission, they neither cure nor alter the natural history of HSV infections.

Manipulation of the immune response is one potential method of decreasing disease burden. Considering the impact of HSV-1 ocular infections on visual morbidity, the prospect of developing an effective vaccine against HSV-1 ocular infection is encouraging. The ability of the virus, however, to establish latency, the periodic recurrences and the role of immune responses and host factors in the disease pathogenesis add to the complexity of developing an effective HSV-1 vaccine. As recurrent disease is primarily responsible for visual morbidity, a prophylactic vaccine should be able to target the establishment of latent infection.

It has been suggested that following colonization of the neural ganglia with a particular HSV-1 strain, subsequent infection with a highly virulent strain may still cause local disease, but rarely does the second virus colonize the ganglia and establish latency [28, 78]. Although it was suggested that infection with a "good virus" early in life may provide life-long protection, there is now evidence against this. As discussed above (Sect. 7.6.3), super-infection with a different strain of HSV-1 even in the same tissue, for example, the cornea, is now recognized [148]. This has also been described in the genital tract [154]. Nevertheless, it is not known when super-infection occurs and it may be that early exposure or immunization to one strain of HSV-1 may be partially protective. It is also not clear how different HSV-1 strains differ antigenically at the DNA level. This may also account for super-infection. If one accepts the protective nature of antigenically different strains, then clearly young hosts who have never been exposed to HSV-1 infection would be targets for prophylactic HSV-1 vaccines.

Therapeutic vaccination to prevent recurrence in a person who has already been exposed to HSV-1 virus is a daunting task [78]. Therapeutic vaccines for genital HSV-2 infection have reached phase II clinical trials with some success, but are still in the preliminary stages for

HSV-1 infection [89, 173, 174]. The candidates for HSV-1 vaccine are based on protein subunits, live recombinant or attenuated viruses, killed or inactivated viruses, and viral DNA preparations.

7.8.7.1 Subunit Vaccines

Subunit vaccines have been developed against specific viral glycoproteins. The technical difficulty of isolating large-scale preparations has been overcome by using recombinant DNA techniques. Most commonly targeted are glycoproteins gB and gD as they are involved in viral binding and entry [168].

Koelle and Ghiasi studied the effect of subunit vaccines for each of the 11 HSV-1 glycoproteins in BALB/c mice. They found that gB, gC, gD, gE, and gI have a beneficial effect on eye disease and protect mice against lethal challenge whereas gG, gH, gJ, gL and gM provide no protection against death or eye disease. Glycoprotein gK vaccine, on the other hand, exacerbated eye disease [89].

The drawback of peptide vaccines is that they can induce immunity only against limited regions of viral protein. Moreover, although severity of primary HSV-1 disease and long-term stromal scarring are reduced, there is little or no effect on the establishment of latency and recurrent disease [84, 122].

7.8.7.2 Live and Killed Virus Vaccines

Genetically modified mutants of HSV, which are less virulent or less likely to re-activate, have been tried in animal models with some success. The KOS strain is the most efficacious naturally occurring avirulent HSV-1 strain. Other targets have been HSV-1 viruses lacking virulence factors such as γ34.5 gene, vhs- mutant [85]. Genetically modified mutants are, however, weak inducers of humoral and cell-mediated immunities and inclusion of immunostimulatory genes such as cytokine genes (IL-2-, IL-4-, IL-12p35-, IL-12p40-) has been used to improve their efficacy [89, 135, 136]. However, there is the risk of a live virus vaccine reverting to some other form. Killed vaccines have therefore been tried, but are not as efficacious [89].

7.8.7.3 DNA Vaccines

Subunit vaccines cannot induce innate or CD8+ cytotoxic T lymphocyte responses and hence DNA vaccines have been introduced for a "complete response." Osorio et al. have shown that DNA vaccines work better with regard to the clinical severity of eye diseases, virus titers, and establishment of latency [137]. A cocktail of five or more HSV-1 glycoprotein vaccines works better at eliminating eye disease and establishment of latency [89]. Although DNA vaccines are more effective at decreasing the severity of acute ocular infection, and reduce the viral load in the trigeminal ganglion, they have little on no effect on viral latency and recurrent disease [53, 73].

7.8.7.4 Periocular Versus Systemic Vaccination

Periocular vaccination has been suggested, as local immunity plays an important role in HSK, and allows administration of sub-optimal vaccine dosage in comparison to systemic vaccination. Nesburn et al. compared the efficacy of periocular and systemic gB2/gD2 and live HSV-1 KOS vaccines. Periocular vaccination provided better protection against eye disease, although systemic vaccines produced higher neutralizing antibody titers [125]. Interestingly, periocular glycoprotein vaccines seem to affect the severity of stromal keratitis, but not epithelial keratitis [74, 125].

7.8.7.5 Therapeutic Vaccines

A wide range of vaccines have been tested, with encouraging results for protection against primary ocular disease (as discussed above), but their results in recurrent disease have been disappointing. Live HSV-1 vhs-deficient mutants have been tested as therapeutic vaccines, but these only reduced the severity and not the incidence and duration of latent disease. The results of sub-unit vaccines have been similarly disappointing [84, 124]. It has been suggested that a successful therapeutic vaccine should be able to modulate the nature of immune response, providing a higher degree of protection at the

mucosal surface of the eye while limiting the pro-inflammatory effects of virally induced Th1 response [151]. Richards et al. vaccinated latently infected mice intranasally with a mixture of HSV-1 glycoproteins, and found a reduction in viral shedding and the incidence and duration of corneal disease following reactivation of HSV-1. Similar results were obtained by periocular vaccination with HSV-1 and HSV-2 glycoprotein D vaccines [123].

Summary for the Clinician

- For herpetic stromal disease, initial treatment with a topical antiviral to control viral reactivation and productive infection with the subsequent addition of a topical steroid is reasonable
- Prophylactic oral aciclovir reduces the recurrence of all forms of herpetic eye disease
- The recurrence rates of HSK following PK vary from 12 to 27% per year, compared with approximately 10% per year for non-PK patients off treatment. The use of both topical and systemic antivirals has led to a significant reduction in post-transplant recurrence of HSK
- Manipulation of the immune response in the form of an HSV-1 vaccine is still in its developmental stage, but may have the potential of decreasing the disease burden in the future

7.9 Conclusion

Herpes simplex keratitis, a result of infection predominantly with the HSV-1 virus, is the most common infectious cause of unilateral blindness and one of the commonest indications for a PK [36, 190]. The virus may reach the eye by one of two main routes: the back-door route [192], that is, entry via the mouth with spread to the eye; or the front-door route [82], that is, direct entry into the ocular surface with subsequent recurrence in the cornea [80]. Subsequently, it can establish both productive infection and latent infection in the trigeminal ganglion or cornea itself.

Primary disease is usually asymptomatic and recurrent disease usually affects vision through scarring, thinning and neovascularization. Anterior segment disease can affect all levels of the cornea and also cause uveitis. HSV-1 is also implicated in the ICE syndrome.

The recurrence rate is approximately 10% per year [80]. The mechanisms controlling establishment and reactivation from latency are not well understood, but HSV-1 ICP0 is said to be crucial for reactivation [75]. Virus-specific transcripts (LAT) are detected during HSV-1 latency. They are implicated in neuronal survival, anti-apoptosis, virulence, suppression of transcription, and establishment of and reactivation from latency [80]. Recurrent corneal infection may arise from anterograde axonal spread from latently infected trigeminal ganglion, but now there is evidence that it may also result from virus that becomes latent or persists in an infectious state within the cornea [146, 162]. Although recrudescent infections are usually the result of re-activation of the initial infecting viral strain, super-infection with a new strain is also possible [6, 149].

The outcome of infection depends on a number of factors, including constellation of viral genes encoded by each strain and the genetic make-up of the host and host immune system [80]. Ocular immune response to HSV-1 may also be modulated by ACAID, a deviant form of systemic immunity [2]. Diagnostic tests are aimed at cell cytology, viral antigen or DNA detection, and virus isolation. Virus isolation offers specificity of 100%, but has low sensitivity. A combination of PCR and IHC offers high specificity and sensitivity for both active disease and diagnosis of corneal scars.

While topical acyclovir is sufficient for epithelial disease, a combination of topical and/or oral acyclovir and topical steroids is required for the treatment of stromal keratitis and uveitis. Penetrating or lamellar keratoplasty is the only definitive treatment for HSK-induced corneal scarring and vascularization, but carries a high risk of development of recurrent disease in the new cornea and corneal transplant rejection. The recurrence of HSK in PK varies from 12 to 27% [50, 199, 200]. Antiviral prophylaxis, both systemic and topical, can lead to a significant reduction in the post-transplant recurrence of HSK [51, 118, 200].

The pathogenesis of corneal vascularization in HSK is uncertain, but it is an important and early event in the development of stromal disease and the ability of a strain to cause stromal disease correlates with its ability to induce corneal vascularization [80]. Evidence now suggests that HSV-1 infection disrupts the normal equilibrium between angiogenic and antiangiogenic stimuli leading to an angiogenic switch, and thrombospondin 1 and 2, potent antiangiogenic factors, appear to be two of the key players [30, 80]. The addition therefore, of TSP1 and TSP2, or their angioactive elements, in the early stage of the disease, may enhance existing or provide alternative antiangiogenic therapy in HSK where stromal vascularization is related to disease severity [80].

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Chapter 8

Management of Ocular Mucous Membrane Pemphigoid

8

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Core Messages

- Early diagnosis and appropriate treatment of ocular mucous membrane pemphigoid (MMP) will prevent its severe sight-threatening complications. After excluding other causes of conjunctival scarring, a clinical diagnosis of ocular MMP, based on a history of progressive scarring and typical clinical signs, is sufficient. A negative immunofluorescence biopsy does not exclude the diagnosis of ocular MMP
- Once the diagnosis has been made, it is important to first eliminate and treat local ocular surface disease causing inflammation such as blepharitis, dry eye or exposure, microbial infection, and toxicity
- Persisting inflammation, following treatment of surface disease, is due to the underlying immune disorder and usually requires systemic immunosuppression
- By using a stepladder strategy for immunosuppressive therapy, the risk of a poor initial response in mild to moderate disease is justified by less toxicity and the undiminished prospects of further therapeutic success with more toxic agents, whilst in aggressive disease initial treatment with toxic agents is justified
- Providing the ocular surface inflammation is controlled, clear corneal incision cataract surgery is safe and does not require increased perioperative immunosuppression. Preoperative lid and conjunctival cultures and treatment of pathogenic colonization is recommended
- Lid split and lamellar repositioning, or retractor plication surgery, for mild to moderate cicatricial entropion does not involve incising conjunctiva and increased immunosuppression is not necessary, but close follow-up to detect and treat any disease exacerbation is recommended
- Fornix reconstruction surgery, and other surgery involving operating directly on the conjunctival fornices carries a high risk of a severe disease exacerbation and requires increased systemic immunosuppression for at least 2 months prior to embarking on surgery, along with a short perioperative course of oral corticosteroids
- Keratoplasty and ocular surface reconstructive surgery to rehabilitate vision have a very guarded prognosis in ocular MMP, due to the problems of poor epithelialization, melt, infection, corneal vascularization, and disease reactivation in a hostile environment, and are contraindicated in dry eyes
- Keratoprosthesis surgery is high risk and complications are frequent, but good visual outcomes can be achieved

8.1 Introduction

Mucous membrane pemphigoid (MMP) is an immune-mediated, subepithelial blistering disease where lesions involving mucous membranes and the skin typically heal with scarring. Ocular involvement occurs in 70% of patients. Up to 30% may go blind without appropriate treatment [25], although with immunosuppression the prognosis has improved and this figure has halved. Progressive scarring of the conjunctiva disrupts the protective action of the eyelids and tears, and the associated episodes of inflammation lead to vascularization and opacity of the cornea and surface failure (see Table 8.1). Tear deficiency and ocular surface inflammation are responsible for the majority of symptoms, and affect both comfort and the progression of disease. Loss of vision is due to both surface failure and dry eye.

Management of ocular mucous membrane pemphigoid involves firstly treatment of local ocular surface disease, following which a more accurate assessment of the activity of immune-mediated inflammation can be made and immunosuppressive therapy given where appropriate. Providing the ocular surface inflammation is controlled, contact lens wear or cataract surgery can recover some vision, whilst vigilance for microbial infections is maintained.

In this chapter, a strategy for the management of ocular mucous membrane pemphigoid, also referred to as ocular cicatricial pemphigoid (OCP), is presented, including recent developments in immunomodulatory therapy for recalcitrant disease.

8.2 Diagnosis

Early diagnosis and commencement of appropriate treatment are essential to prevent the sight-threatening complications of MMP. The earliest clinical sign is scarring at the medial canthus, with loss of the plica and later the caruncle. Other signs, in order of progression, are subepithelial reticular fibrosis of the tarsal conjunctiva, conjunctival infiltrate due to increased cellularity and collagen formation, shortening of the fornices, symblepharon, and cicatricial entropion.

Progression of conjunctival scarring, or the development of cicatricial entropion, should

Table 8.1 Ocular surface failure in mucous membrane pemphigoid (MMP)

Ocular surface failure has two principal causes in MMP, and distinguishing these is essential to successful management. The functional problems associated with ocular surface failure are reduced vision and comfort.		
Cause of Ocular Surface Failure	**Clinical Features**	**Impression Cytology Features**
Metaplasia may be idiopathic, or more usually results from severe dryness	Opaque corneal epithelium which may be keratinized and vascularised, but stable	Epithelial cell pyknosis. No signs consistent with dysplasia or stem cell failure
Stem cell failure where the capacity of the peripheral corneal epithelial "stem cells" that maintain a normal clear corneal epithelium, has been affected	An opaque and unstable corneal epithelium, that may be associated with anterior stromal vascularisation and stromal opacification	Presence of goblet cells (although not in cases where there has been severe loss of goblet cells due to MMP), and/or the presence of cytokeratin 19 (a conjunctival epithelial cell cytoskeletal marker) and the absence of cytokeratin 3 or 12 (corneal epithelial cell cytoskeletal markers)

alert the clinician to the possibility of mucous membrane pemphigoid. However, all types of chronic conjunctival inflammation can induce fibrosis, including ocular rosacea, infective conjunctivitis (endemic trachoma, membranous streptococcal and adenoviral conjunctivitis), systemic diseases (atopic keratoconjunctivitis, sarcoidosis, scleroderma, and Sjögren's syndrome), drug-induced pseudopemphigoid, trauma, chemical or thermal injury, and artefacta (self-induced conjunctival trauma) [45]. Relevant history, examination, and laboratory investigations will identify whether these alternative causes of fibrosis are involved.

The conjunctival signs in MMP may be identical to those observed in other muco-cutaneous disorders (graft versus host disease, Stevens-Johnson syndrome, and toxic epidermal necrolysis, cutaneous or discoid lupus erythematosus, lichen planus), and in those produced by other immunobullous disorders (pemphigus vulgaris, bullous pemphigoid, linear IgA disease, epidermolysis bullosa acquisita, dermatitis herpetiformis) although usually the conjunctival signs in the latter disorders are very mild with minimal morbidity, if any. Furthermore, in many of the mucocutaneous and immunobullous disorders, the skin or oral disease precedes the eye disease so that there is rarely any confusion. Subgroups of patients with predominantly mucosal linear IgA disease and epidermolysis bullosa acquisita are recognized as a having a form of MMP [6]. In Stevens-Johnson syndrome, major exacerbations of conjunctival inflammation can occur many years after the acute disease, leading to a condition indistinguishable from MMP, both in terms of the clinical signs and immunopathology [5].

For practical purposes, a clinical diagnosis of ocular mucous membrane pemphigoid, based on a history of progressive conjunctival scarring and the presence of typical clinical signs, after exclusion of other causes, is adequate in most cases outside a research setting. Laboratory investigations require specialized immunopathology services and conjunctival biopsies are positive in ocular MMP in only 60–80% of cases [45]. However, laboratory investigations for MMP can be useful, if positive, in cases in which there is doubt about the diagnosis, and when distinguishing MMP from diseases such as lichen planus, lupus erythematosus or pemphigus vulgaris, which have characteristic immunopathological features of their own [45]. If the biopsy is negative for linear basement membrane staining, this does not exclude the diagnosis of ocular MMP [1, 3]. Bulbar conjunctival biopsy is easy and safe providing the fornix is avoided [29].

Direct immunofluorescence for IgA, IgG, IgM, and/or complement, showing linear staining along the conjunctival basement membrane is characteristic of MMP [6]. Biopsies are often negative in acute disease and may revert to normal in time or after treatment [4, 17]. The yield of direct immunofluorescence may be increased by ensuring that biopsies are perilesional (in the rare cases in which conjunctival ulceration is present) and by taking biopsies from involved extraocular sites, which appear to have a higher rate of positive results compared with conjunctival biopsies [45].

Routine histopathology is of little value in the diagnosis of MMP because the conjunctiva is fragile and detection of basement membrane zone cleavage unreliable. However, it can be helpful to exclude atopic disease and sarcoidosis. Unusual features, such as conjunctival thickening in unilateral disease, merit conjunctival biopsy to exclude conjunctival neoplasia, which can produce fornix shortening and mimics unilateral MMP.

In current clinical practice, there is no sensitive or specific laboratory test, either to establish the diagnosis of MMP, or to monitor the response to therapy. Indirect immunofluorescence to identify circulating autoantibodies plays a limited role, as patients with ocular MMP rarely have detectable circulating autoantibodies. Even when using salt split skin techniques to increase the detection rate, in pure ocular disease, in contrast to disease affecting other mucous membranes and skin, antibodies are only detectable in 29% [28]. Research is underway to develop more sensitive and specific ELISA assays for detection of these autoantibodies [2].

Summary for the Clinician

- A negative immunofluorescence biopsy does not exclude the diagnosis of ocular MMP. After excluding other causes of conjunctival scarring, a clinical diagnosis of ocular mucous membrane pemphigoid is sufficient, based on a history of progressive scarring and typical clinical signs
- Routine histopathology is usually of little value in the diagnosis of ocular MMP, but can exclude conjunctival neoplasia
- Although patients with ocular MMP rarely have detectable circulating autoantibodies, testing can be helpful in establishing the diagnosis in some patients

8.3 Principles of Management

Whilst 10% of patients with ocular MMP present with acute conjunctivitis, limbitis, and rapidly progressive disease, the majority present with subacute or low-grade chronic inflammation and gradually progressive scarring. Successful management of ocular MMP involves identification and prompt treatment of each of the components of the disorder leading to progressive disease. Progression in MMP is due to:

- Immune-mediated inflammation
- Inflammation associated with secondary ocular surface disease
- Infections
- Treatment toxicity (Fig. 8.1)

These problems lead to a dry eye, surface failure, and corneal blindness. Prompt treatment is required to prevent progression to surface failure and corneal blindness because of the poor prognosis for rehabilitation, by corneal graft surgery and/or surface reconstruction.

Patients with MMP require lifelong therapy. Fundamental principles to assist the long-term relationship between the patient and clinician include:

- Placing realistic limits on patient expectations. It is important to help patients come to terms with the reality that their symptoms can be ameliorated, and progression of disease may

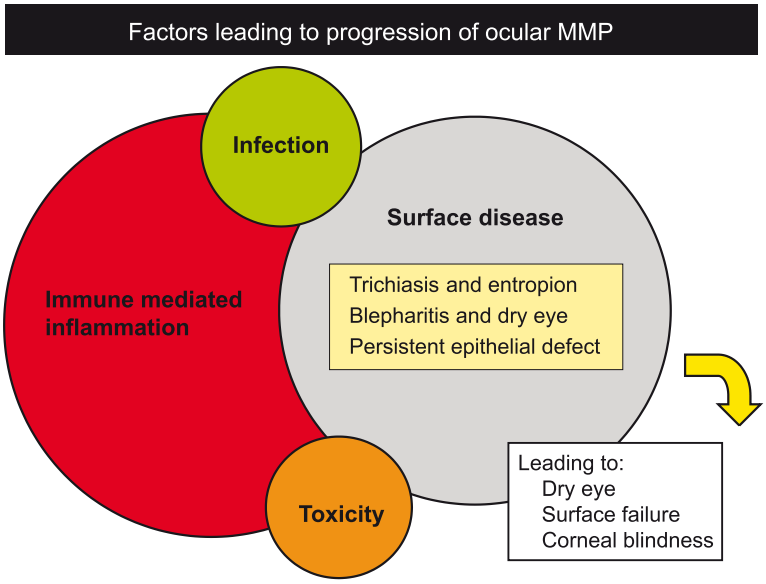


Fig. 8.1 Factors associated with progression in ocular mucous membrane pemphigoid (MMP)

be prevented, but that they will not be cured by treatment.
- Limit iatrogenic damage – "less is more." Surgical treatments to improve vision, particularly keratoplasty, may have catastrophic consequences in patients with MMP. It is often better, in the long term, for patients to maintain some vision with their existing cornea and use supportive measures, than to risk blindness from surgery. Iatrogenic damage can also result from toxic keratoconjunctivitis, and from the side effects of topical steroids (masking and promoting infection, glaucoma, and cataract), the use of which requires a risk-benefit analysis.
- Agree on a treatment strategy with patients, and their other physicians and general practitioner. It helps both the clinicians involved and the patient if a treatment strategy is outlined at an early consultation.
- Educate the patient and provide social support in terms of blind registration, magnification aids, and mobility training.
- Facilitate clinic access. MMP patients may require treatment in emergency situations at short notice, as they frequently develop sight-threatening corneal ulceration and/or infection.

8.4 Inflammation Associated with Ocular Surface Disease

Untreated inflammation associated with blepharitis, dry eye and filamentary keratitis, trichiasis and entropion, and persistent epithelial defects, impairs vision and comfort as well as affecting the progression of disease. This ocular surface disease is secondary to previous or current lid and conjunctival scarring and inflammation, and is responsible for damage to the cornea.

8.4.1 Blepharitis

The type of blepharitis present, either anterior or posterior lid margin disease or both, should be established, as the treatment differs (see Table 8.2). Scarring of the meibomian gland orifices due to MMP contributes to posterior lid margin disease. Efforts to improve meibomian gland dysfunction will limit the disturbance to tear film wetting, which degrades the already compromised ocular surface. The surface of the eye becomes unwettable when toxic-free fatty acids, produced by bacterial lipases associated with accumulated meibum, contaminate the tear film.

Blepharitis also contributes to colonization with bacterial pathogens, to which these patients with conjunctival scarring and keratinization are already susceptible. The presence of bacterial pathogens in MMP is one of multiple factors contributing to the constant threat of microbial keratitis. Potential pathogens are recovered from the lids and/or conjunctiva in 85% of MMP patients compared with 49% of controls [33]. Although the majority of pathogens are Gram-positive staphylococci, a greater proportion of Gram-negative organisms are found in MMP (see Table 8.3). Regular bacterial cultures of the eyelids and conjunctiva are an important part of preventive management in MMP. This is particularly crucial prior to any intraocular or periocular surgery.

Lid scrubs, lid massage and low-dose systemic antibiotics take 4–6 weeks to have an effect. This regime should be continued for a minimum of 2–3 months, if benefit is shown. Following this, a maintenance regime of lid scrubs (for anterior lid disease), hot compresses, and tarsal massage (for posterior lid disease) should be instituted. In the case of relapse, a repeat 3-month course of oral antibiotic treatment can be given. An additional advantage in using oral tetracyclines is that they can also improve inflammation related to the underlying immune disorder in MMP [37]. Topical ciclosporin has been shown to improve meibomian gland function, and is used to treat inflammation associated with dry eye [35]. Providing it does not cause toxicity, it may be useful in MMP.

8.4.2 Dry Eye

Dysfunction of several components of the ocular surface–lacrimal gland reflex unit are responsible for dry eye and tear film instability in MMP. The pathogenesis is summarized in Table 8.4.

Table 8.2 Treatment of blepharitis in ocular cicatricial pemphigoid

Aims of treatment	Therapeutic guidelines
For anterior lid margin disease (staphylococcal blepharitis, seborrheic blepharitis)	
Treat infection	Staphylococcal and mixed staphylococcal/seborrheic groups Topical antibiotics – chloramphenicol ointment or bacitracin/polymyxin or appropriate agent according to sensitivities – 2–4× daily to lid margins Oral doxycycline (100 mg daily) or erythromycin (500 mg bd) for 10 days, or azithromycin (500 mg daily) for 3 days
Clean lid margins	"Lid scrubs" – 1–2× daily with cotton wool bud dampened in boiled water to remove debris or with proprietary lid cleaning pads
Lid hyperemia and exudate	Topical chloramphenicol ointment and hydrocortisone (0.5–1%) to lid margins 2–4× daily for 1 month
For posterior lid margin disease (meibomianitis, meibomian dysfunction, meibomian seborrhea)	
Mechanically unblock meibomian glands	Apply hot compresses for 3–5 min to liquefy meibomian secretions, followed by massage of tarsal plate with cotton wool bud (or finger), to express lipid from glands, 1–2× daily
Alter meibomian secretions	Oral doxycycline 100 mg daily or minocycline 100 mg daily or erythromycin 500 mg bd for 12 weeks minimum
Pharmacological improvement of meibomian gland function	G. ciclosporin 2× times daily or ciclosporin ointment
For the tear film	
Restore tear film	Artificial tears – drops 2–4 hourly, or unpreserved gel 3–4× daily
For associated conjunctivitis (papillary or mixed follicular and papillary)	
Reduce inflammation	See Sect. 8.4, Inflammation associated with ocular surface disease. Fluorometholone (0.1%) or unpreserved prednisolone 0.3–0.5% 4× daily for 1 week, progressively reducing to ×1 daily over a further 4 weeks
Treat associated skin disease	Seborrhea – medicated soap and shampoo (ideally containing glycolic acid [10–15%]) Acne rosacea – oral doxycycline 100 mg daily or erythromycin 250 mg bd for 12 weeks. If severe or refractory, refer to a dermatologist

Table 8.3 Results of lid and conjunctival cultures in patients with ocular cicatricial pemphigoid (*OCP*) and controls (adapted from [33])

	Controls, n=57 (%)	OCP, n=78 (%)
No pathogens recovered	29 (51)	15 (19)*
Pathogens recovered	28 (49)	63 (81)*
Type of pathogen		
Mannitol-positive staphylococci		
Conjunctiva	11 (39)	42 (67)
Lids	25 (89)	55 (87)
Gram-negative organisms[a]		
Conjunctiva	3 (11)	7 (25)
Lids	3 (11)	7 (25)
Gram-positive organisms excluding staphylococcus[b]		
Conjunctiva	0 (0)	2 (3)
Lids	0 (0)	2 (3)

* Difference was statistically significant, $p<0.001$

[a] *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Alcaligenes faecalis, Serratia marcescens, Haemophilus influenzae*

[b] *Streptococcus pneumoniae, group A beta-hemolytic streptococci*

Table 8.4 Pathogenesis of dry eye in mucous membrane pemphigoid

Component of Dry Eye	Mechanism
Disruption Of The Tear-Secreting Apparatus	
Lipid layer dysfunction	Posterior lid margin disease Disrupted meibomian lipid expression and spreading due to distorted lid closure
Mucin deficiency	Reduced conjunctival surface area Loss of goblet cells
Aqueous deficency	Cicatrisation of lacrimal ductules Coexistent Sjogren's syndrome
Tear Film Instability	
Altered protein component of tears	Toxic inflammatory products in tears
Ocular surface epithelium	Conjunctival and corneal squamous metaplasia
Efferent Pathway Dysfunction	
Compromised tear spread and renewal	Cicatricial entropion and eyelash-globe contact
Corneal exposure and lagophthalmos	Lid-globe adhesions impairing lid closure Impaired Bell's phenomenon due to conjunctival shrinkage restricting ocular motility

A 5-fold increased rate of tear evaporation has been observed in MMP, attributed to meibomian gland dysfunction [33]. Tear film break-up times and Schirmer's tests may be normal until late in the disease, when keratoconjunctivitis sicca results from obliteration of the lacrimal gland ductules [18].

Dry eye predisposes to recurrent epithelial breakdown, to which these eyes with surface failure are already susceptible, and the resultant complications of delayed epithelialization, microbial keratitis, and corneal perforation. Increased tear osmolarity decreases concentrations of nutritional factors that support the normal function of ocular surface tissues, and increases inflammatory mediators and proteases.

A step-wise approach to the management of dry eye helps to rationalize therapy:

- Tear conservation: simple conservation methods, such as the use of wrap-around glasses, for example those available from sports stores, and a humidified environment, should be implemented.
- Punctal occlusion has often occurred spontaneously as part of the disease, and is helpful in conserving tears, providing blepharitis and its associated conjunctival inflammation have been controlled.
- Supplementation with pharmaceutical tear substitutes is often needed for symptomatic relief of ocular surface symptoms. If preserved lubricants are used more than 4–6 times daily in eyes with aqueous tear deficiency, there is a risk of ocular surface toxicity developing (see Sect. 8.6). A few patients will react to excipients other than preservatives, such as EDTA, in topical preparations, but this is uncommon. There are few guidelines on the choice of an optimal unpreserved lubricant for any individual patient. For patients with surface symptoms and minimal aqueous tear deficiency, hypromellose, polyvinyl alcohol, carmellose, carbomer gels, and hyaluronic acid are available unpreserved in different countries. Individual preferences for these amongst patients vary widely. For those with severe aqueous tear deficiency isotonic saline, or balanced salt solution (for intraocular use), may be preferred by patients despite their short contact time, as they leave no residue and cause no blurring of vision. Patients with corneal keratinization may prefer ointment for comfort. Soft paraffin may be preferred to the more widely available lanolin and paraffin mixtures because of the reduced viscosity.
- Topical ciclosporin and steroids to address ocular surface inflammation related to keratoconjunctivitis sicca. Topical cyclosporin or weak topical steroids such as fluorometholone or prednisolone (0.5%) can be used for short periods if the inflammation appears to be related to ocular surface desiccation.
- Mucolytics such as acetylcysteine (5% or 10% unpreserved) can clear debris in the tear film.
- Address exposure keratopathy due to lagophthalmos and poor Bell's phenomenon by lid surgery (see Sect. 8.4.5).
- Autologous serum drops in 20–100% concentrations can be very valuable for individual patients and result in both subjective and objective improvements in the ocular surface. This is probably due to their effect as a physiological, rather than as a pharmaceutical tear replacement, as serum contains many of the components of lacrimal tears [36].
- Contact lenses: large gas permeable corneal lenses, fitted to the limbus, or gas permeable scleral lenses may improve surface hydration and vision in some patients. Hydrogel lenses are not recommended in dry eyes, as they lose a percentage of their water content in all eyes and increase tear evaporation. Low water content silicone hydrogels can be effective in relatively dry eyes, when used with frequent unpreserved lubricating tears. The increased risk of infection associated with soft lenses compared with rigid lenses must be considered, along with the frequent colonization of these eyes by pathogenic bacteria.
- Assess for concomitant Sjögren's syndrome: Sjögren's syndrome and rheumatoid arthritis can accompany MMP, and require treatment specific to these conditions.
- Oral pilocarpine: the parasympathomimetic pilocarpine can be useful in some MMP patients with severe dry eye. This stimulates tear secretion through the unoccluded lac-

rimal ductules. The side effects of sweating, flushing and nausea are minimized when the dose is increased gradually from 5 mg daily for 1 week, then 5 mg bd for 1 week, then 5 mg tid for 1 week, to a maximum dose of 5 mg qid.

- Salivary gland transplantation has been performed in patients with absolute tear deficiency (Schirmer's I test <2 mm). Salivary tears, however, do not possess the same nutritional and visual properties as lacrimal tears, and despite having a wetter eye, the ocular surface may not be improved and outcomes of keratoplasty may be no different [22]. We have discontinued using this procedure for these reasons.

Summary for the Clinician

- Meticulous treatment of blepharitis is important in ocular MMP, as it is can be responsible for ongoing conjunctival inflammation, poor surface wetting, and colonization with pathogenic bacteria
- A step-wise approach to the treatment of dry eye, beginning with simple measures and escalating to more complicated therapies, helps to rationalize treatment
- Aqueous tear production (measured by the Schirmer's test) may be normal until late in the disease, when it indicates a poor prognosis

8.4.3 Filamentary Keratitis and Punctate Epithelial Keratitis

Filamentary keratitis is largely due to tear deficiency and blepharitis. If it does not improve with treatment of the dry eye and lid margin disease, it often responds to the mucolytic acetylcysteine 5–10% used 1–4 times daily. Therapeutic silicone hydrogel contact lenses can improve the symptoms of filamentary keratitis. These are safe in eyes with at least 5 mm of wetting on a Schirmer's I test without anaesthetic, combined with frequent use of unpreserved lubricating tears. With appropriate treatment, this form of keratitis usually resolves after a few months.

Punctate epithelial keratitis is often secondary to lid margin disease, conjunctival inflammation, topical medication toxicity, and tear deficiency, which need management. Lubricating ointment at night is often helpful.

8.4.4 Keratinization

Keratin occurring on the lid margin or tarsal conjunctiva may cause repetitive trauma to the ocular surface with blinking and may contribute to corneal scarring and vascularization. Keratinization of the cornea can obstruct vision and cause discomfort. Topical vitamin A as 0.05% retinoic acid drops (Moorfields Pharmaceuticals) is effective in about 30% of patients, given daily or on alternate days. Its availability is limited. It may irritate the ocular surface. We have no experience of the effect of retinoic acid ointment. If vitamin A is not available or does not work, excess amounts of keratin causing discomfort can be lightly scraped off, but recurs. Therapeutic contact lenses can also be used to protect the cornea from tarsal conjunctival keratin (see sect. 8.4.6).

8.4.5 Trichiasis, Entropion, and Lagophthalmos

Several methods of lash ablation exist to treat trichiasis. For odd lashes or metaplastic lashes, epilation is useful in the short term, but regrowth occurs in 4–6 weeks. Electrolysis or laser thermo-ablation has a more prolonged effect. Although electrolysis alone has a relatively low success rate of 29% [12], greater success is achieved when additional cut-down to the lash root is carried out so that electrolysis can be applied to the lash follicle. Argon laser thermo-ablation has a reported 50% success rate at 12 months, with further success following retreatment [12], but we do not use this frequently. It is important to take care not to cause scarring with excessive use of either electrolysis or laser ablation.

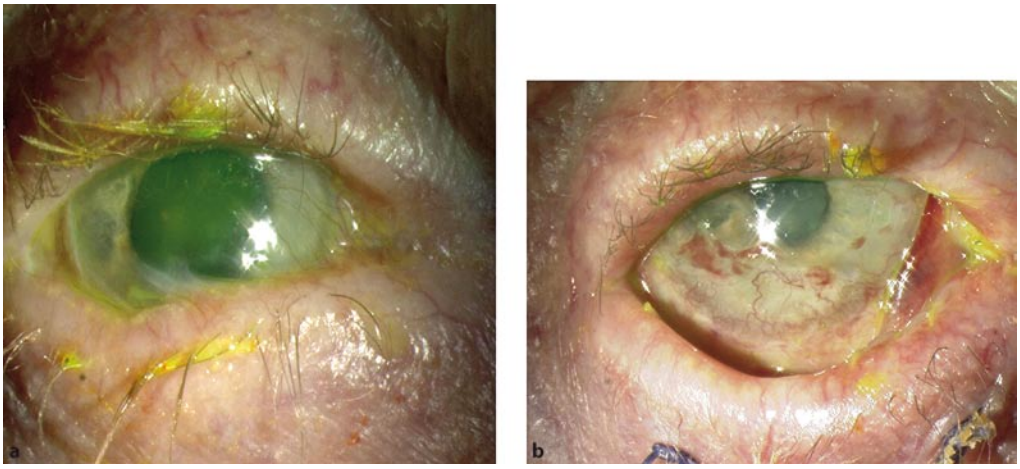


Fig. 8.2 Fornix reconstruction surgery to treat corneal exposure. **a** Symblepharon between the cornea and lower eyelid obstructing closure and resulting in lagophthalmos in a patient's only eye. **b** Two weeks following buccal mucous membrane graft surgery to reconstruct the lower fornix and amniotic membrane transplantation to the inferior corneal defect

For misdirected lashes, gray line lid split and anterior lamellar excision or cryotherapy are effective. We prefer anterior lamellar excision as it causes less inflammation and less damage to adjacent meibomian glands compared with cryotherapy. Cryotherapy may also cause reactivation of disease. Care should be taken to avoid overtreatment with cryotherapy that may result in distortion of eyelid architecture and can contribute to lid margin keratinization.

When there is adequate lid closure and no lagophthalmos, repair of mild to moderate cicatricial entropion of the upper lid can be carried out by a gray line lid split and anterior lamellar repositioning, with a success rate of 61%. Similiary, a lid split and Jones retractor plication can be carried out for the lower lid, which has a success rate of 54%. Tarsal rotation or excision procedures may sometimes be necessary [12].

Indications for fornix reconstruction using mucous membrane grafts or other grafts include:

- The presence of lagophthalmos and a restricted Bell's phenomenon, to prevent exposure keratopathy and corneal perforation (Fig. 8.2)
- Cicatricial entropion with severe posterior lamellar shortening where anterior lamellar surgery alone would result in lagophthalmos and corneal exposure
- When access is necessary in preparation for contact lens wear, cataract surgery or ocular surface reconstructive surgery with limbal stem cell grafts
- To correct ptosis

Fornix foreshortening per se does not require surgery. Labial or buccal mucous membrane, hard palate or nasal mucosa can be used for posterior lamellar grafting. Nasal mucosal grafts are reported to have the advantage over buccal mucosa of providing a source of goblet cells with the potential for improving the tear film. However, in practice we do not use nasal mucosal grafts because the mucosa is very thick, the mucus produced is very viscous, and, in our experience, does not improve the tear film. Auricular cartilage can also be used as a scaffold for a posterior lamellar mucosal graft when the lid needs to be lengthened.

Careful case selection for mucous membrane grafting is imperative. Patients with a favorable

response to immunosuppression are more likely to show sustained improvement after surgery. Fornix reconstruction surgery should be performed before the development of advanced ankyloblepharon or terminal dryness, as results are poor in this group of patients. Heiligenhaus et al. [27] reviewed the 2-year results of buccal mucous membrane grafting in 26 eyes. They found that although an improvement was maintained in 35% of eyes, 62% developed serious postoperative corneal complications, sometimes requiring permanent tarsorrhaphy. Our own experience of this technique has been more favorable. Amniotic membrane transplantation, with or without intraoperative mitomycin C, has been used for conjunctival fornix reconstruction in MMP. Although in our center the outcomes of amniotic membrane for fornix reconstruction have not been as promising, good results with a long follow-up of between 16 and 72 months have been reported by Tseng et al. [43, 46].

Lid surgery that does not involve directly operating on the conjunctiva is less likely to reactivate the underlying disease, and can be carried out whilst the conjunctiva is still inflamed. This includes electrolysis and lid split with anterior lamellar repositioning or inferior retractor plication. Surgery of this nature can be carried out without the need for perioperative steroids or an increase in immunosuppression. However, close postoperative follow-up should be maintained, as inflammation can be induced in a proportion of cases, which must be treated aggressively. When incisions of the conjunctiva are necessary, for example in tarsal rotation surgery and mucous membrane grafting for fornix reconstruction, the disease must be completely controlled by appropriate immunosuppression for at least 2 months prior to surgery, and immunosuppression continued during and after the surgery, to prevent failure and redevelopment of fornit contracture and entropion, due to uncontrolled inflammation or reactivation of disease. In addition, at our center, we use a perioperative 6-week tapering course of oral prednisolone 1 mg/kg/day when conjunctival incision surgery is carried out, to prevent or treat an acute reactivation [27].

Summary for the Clinician

- Odd lashes are managed with regular epilation or electrolysis, and misdirected lashes with a lid split and anterior lamellar excision
- Providing there is no lagophthalmos, a lid split and anterior lamellar repositioning (upper lid) or lid split and Jones retractor plication (lower lid) are frequently successful in treating cicatricial entropion. As these procedures do not involve incising the conjunctiva, perioperative steroids or increased immunosuppression are not necessary, but close follow-up should be maintained in the event of postoperative disease reactivation.
- Fornix reconstruction surgery is indicated when there is significant lagophthalmos and corneal exposure, or when repair of entropion would result in lagophthalmos. Labial or buccal mucous membrane is most frequently used for fornix reconstruction. Careful case selection is critical. Fornix surgery can be carried out only after immunosuppression has successfully controlled inflammation for at least 2 months, and a short course of perioperative steroids is advisable

8.4.6 Persistent Epithelial Defects and Corneal Perforation

Recurrent or persistent corneal epithelial defects are responsible for additional ocular surface inflammation and can be a stimulus to the onset of corneal vascularization and blindness. Stepwise management starts with the simplest therapy and escalates to more complex and invasive treatment as required:

- Exclude infection or alternative causes such as herpes simplex keratitis at the outset.
- Correct the precipitating problem: remove ingrowing lashes, prevent corneal exposure, treat dry eye with punctal occlusion, provid-

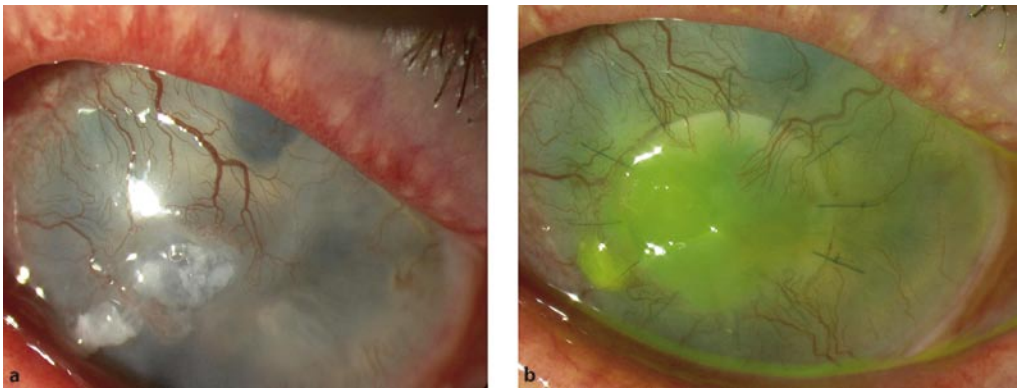


Fig. 8.3 **a** Central corneal melt associated with a focal persistent epithelial defect, filled with mucus, just prior to perforation. **b** Perforation repaired with a lamellar patch tectonic corneal graft. Note the progression of surface failure

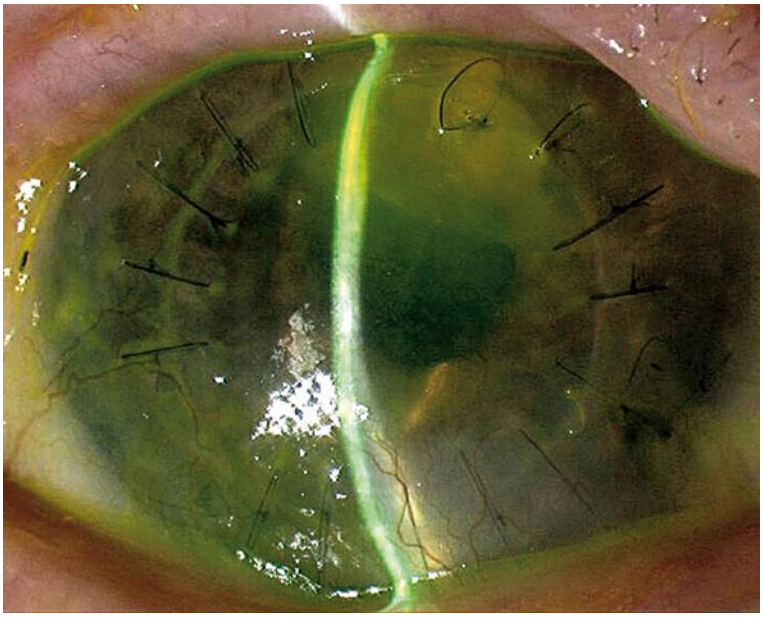


Fig. 8.4 Penetrating keratoplasty in MMP. There is a persistent epithelial defect superiorly and two loose sutures at 1–2 o'clock. The corneal graft is beginning to vascularize inferiorly

ing blepharitis is controlled. Improve epithelial stability by using frequent nonpreserved ointment to reduce shearing forces. If these measures are unsuccessful, try therapeutic soft lenses if the eye is wet, silicone hydrogel lenses for drier eyes, and limbal fit rigid corneal or scleral lenses in dry eyes. Limbal fit contact lenses and scleral lenses provide greater corneal coverage than conventional rigid lenses, thus protecting the cornea from trichiasis and lid scarring and keratin. An alternative to therapeutic lens use, or for use when lenses are unsuccessful, is closure with a botulinum toxin ptosis (although this is not always effective because short fornices prevent full closure), or sutured temporary central tarsorrhaphy.

- Stimulate epithelialization by controlling inflammation, thus reducing toxic inflammatory mediators, and consider autologous serum drops, which can be effective if previous measures fail [36].
- Improve the basement membrane substrate over which the epithelium heals with either an amniotic membrane graft, within the confines of the persistent defect, combined with a temporary amniotic membrane patch over the whole surface, or a lamellar corneal graft. A lamellar graft may be necessary when there is substantial loss of stroma due to melt associated with the persistent defect.
- If the above procedures are unsuccessful in establishing a stable epithelium, renewal of the epithelium by placing a conjunctival flap or free buccal mucosal graft may be necessary.

If corneal perforation occurs, temporize with therapeutic contact lenses and/or corneal glue followed by either a patch lamellar tectonic cornea graft (Fig. 8.3), or penetrating keratoplasty. Penetrating keratoplasty should be carried out only if absolutely necessary, as the outcome is usually very poor due to impaired epithelialization, infection and surface failure (Fig. 8.4). Macleod et al. [31] reported the results of tectonic keratoplasty in 6 eyes with MMP. Progressive surface failure and vascularization of the graft, melt leading to desmetocele formation, and development of glaucoma were the main complications. Although 3 patients gained visual improvement from the procedure, this was not maintained. More recently, we have had better results in 3 carefully selected patients with penetrating keratoplasty (see Sect. 8.8.3).

Summary for the Clinician

- Ensuring healing of epithelial defects is critical, as they can provide a stimulus for corneal vascularization and irreversible blindness. In a stepwise approach:
  - Identify and treat any viral or bacterial infection
  - Correct the precipitating problem, e.g., trichiasis
  - Improve epithelial stability with ointment, contact lenses or lid closure
  - Stimulate epithelialization by reducing inflammation and using serum drops
  - Improve the basement membrane substrate by amniotic membrane transplantation or a lamellar corneal graft
  - Use a conjunctival flap or buccal mucosal graft if the above measures fail

8.5 Infections

Inflammation due to microbial keratitis and bacterial conjunctivitis can cause progression of disease. Patients with MMP have multiple risk factors for developing microbial keratitis. The lids and conjunctiva are colonized by bacterial pathogens, and recurrent or persistent epithelial defects are frequent due to exposure, trichiasis, lid margin disease, and ocular surface failure. In patients on topical steroids or taking immunosuppression, microbial keratitis often manifests without an infiltrate, as a rapidly progressive melt within an epithelial defect. A limited corneal scrape on a single slide and blood agar plate is recommended, to determine the causative organism prior to commencing empirical therapy. Intensive topical fluoroquinolone (ofloxacin, levofloxacin, ciprofloxacin) therapy is usually combined with an intensive topical cephalospo-

rin (the latter to target resistant streptococcal and staphylococcal species that frequently colonize eyes with chronic conjunctival disease). After 3 days of intensive therapy, there are likely to be few residual microbes and the antibiotic frequency can be reduced to 4 times daily, to limit toxicity. At the same time, topical steroids can be commenced, initially 4 times daily, to limit the damaging effects of the host inflammatory response to infection. Persistent colonization with, and recurrent infection of epithelial defects by, methicillin-resistant *Staphylococcus aureus* may warrant admission for intensive nasal mupirocin therapy, chlorhexidine body washes [20] and long-term topical chloramphenicol, which is effective against MRSA [21].

8.6 Toxicity

Treatment toxicity results principally from the preservative benzalkonium chloride, a component of most reusable bottles of eye drop preparations as well as glaucoma medications and the aminoglycoside antibiotics. The effects of topical treatment toxicity cannot be distinguished from those of the ocular surface disease, and include punctate keratopathy, papillary and follicular conjunctivitis, poor surface wetting, and drug-associated pemphigoid. After the withdrawal of toxic topical therapy, the mean recovery period is 2 weeks, but may extend to 3 months [7]. Management of this component requires that the use of unnecessary topical treatment is avoided, unpreserved topical medications are used as far as possible, and alternatives to aminoglycoside antibiotics, which are very toxic, are prescribed.

Summary for the Clinician

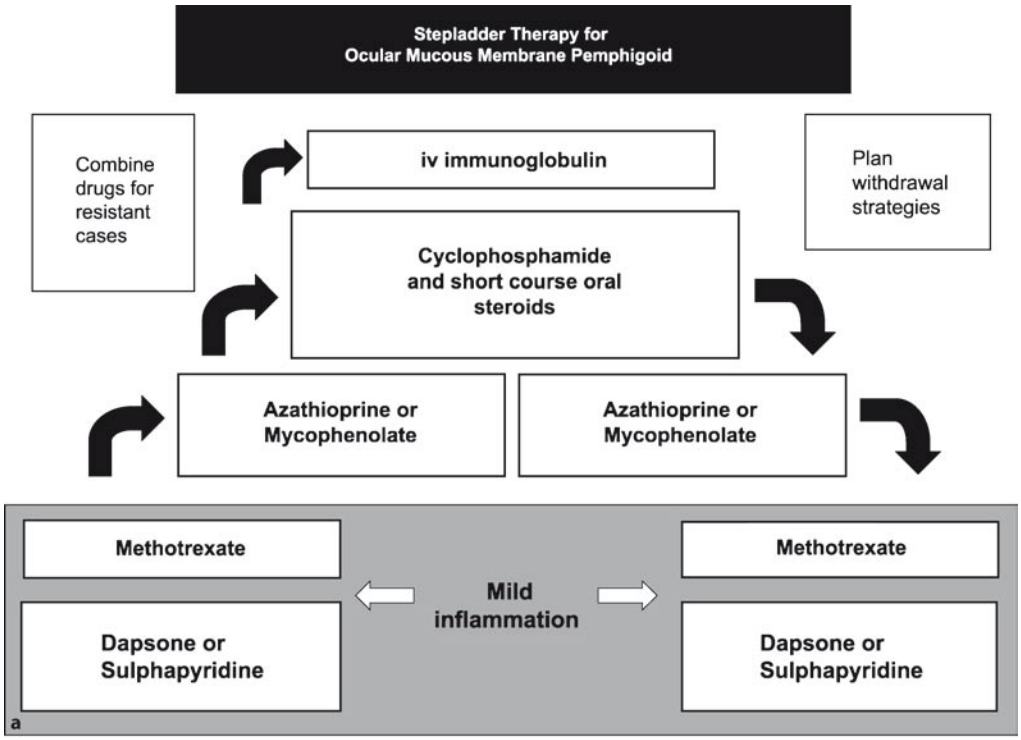
- Patients with MMP have multiple risk factors for microbial keratitis, which can manifest as a rapidly progressive melt alone, in the absence of any infiltrate. This can be a stimulus for the onset of corneal vascularisation and blindness.
- Preservative toxicity can be responsible for ocular surface inflammation and can take up to 3 months to resolve

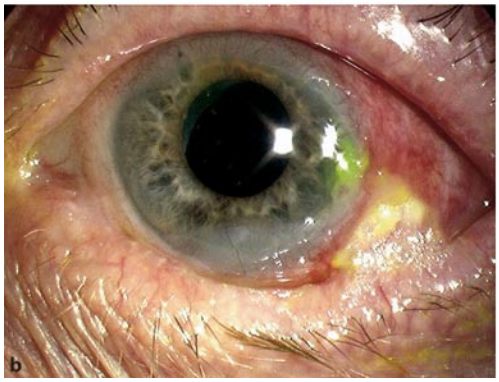
8.7 Systemic Immunosuppression for Immune-mediated Inflammation

Systemic immunosuppression is necessary to target inflammation due to the underlying immunological disorder, after treating inflammation related to ocular surface disease. Whether the underlying autoimmune disease is active and requires systemic immunosuppression is best evaluated by examining the upper bulbar conjunctiva, which is least likely to be affected by exposure, trichiasis, and lid margin disease. There is no evidence that topical therapy alters the natural history of the disease [18] and without systemic treatment, conjunctival cicatrization progresses in 64% of patients over 10–53 months [33]. With current immunosuppressive regimens, cicatrization can still progress in 10–43% of patients [14].

About 25% of OCP patients do not require immunosuppression [14], as they have mild disease, or due to the patient's age the expected deterioration in vision due to slowly progressive disease is limited. Furthermore, in end-stage "burned out" disease, immunosuppression is not necessary as thus treatment can only arrest scarring, not reverse it. For mild hyperemia and edema, low-dose topical steroid can be used for comfort if the patient is aware of symptomatic improvement, but there is no evidence of its efficacy in MMP, and the adverse effects of cataract and glaucoma generally outweigh the benefits. The role of topical ciclosporin in reducing surface inflammation in MMP is unclear. A few case reports describe improvement of MMP affecting the eyes and skin following use of topical tacrolimus [24]. Theoretically, both cyclosporin and tacrolimus can inhibit local T lymphocyte activation, which has been shown to be present in active mucous membrane pemphigoid.

We use a stepladder approach to immunosuppressive therapy, depending on the activity of the disease (Fig. 8.5a). Briefly, for "step-up" therapy, dapsone (diaminodiphenylsulfone, 25–50 mg bd) or sulphapyridine (500 mg od to 500 mg bd, given as sulfasalazine [500 mg bd to 1,000 mg bd] when sulphapyridine is unavailable), or methotrexate is prescribed for mild or moderate inflammation. For disease that does not respond after 2–3 months of first-line therapy, azathio-





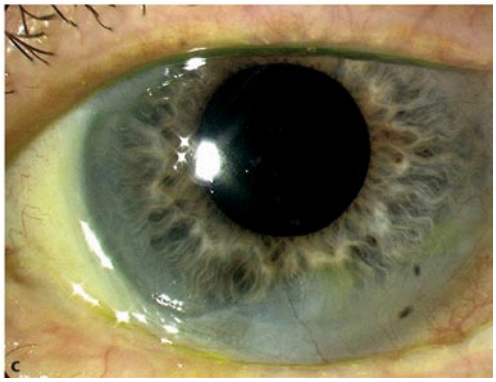


Fig. 8.5 **a** Stepladder immunosuppressive therapy for ocular MMP. Please see text for an explanation. **b** Inflamed eye prior to commencing immunosuppression. Note the patch of keratinization inferotemporally. **c** After 4 months of immunosuppressive therapy, the eye is white and quiet. The superficial corneal vascularization inferiorly due to partial stem cell deficiency has not progressed

prine (1–2.5 mg/kg/day) is added (when there is some response) or substituted (if there is no response) to these first-line agents. For those intolerant of azathioprine, mycophenolate mofetil (500 mg–1 g bd) is used. Severe inflammatory disease is treated at the top of the stepladder with cyclophosphamide (1–2 mg/kg/day). As optimal effects with cyclophosphamide are not achieved until 8–12 weeks following the initiation of therapy, an adjunctive reducing regimen of oral corticosteroids (prednisolone commencing at 1–2 mg/kg/day) is employed, sometimes in conjunction with intravenous pulses of methylprednisolone (500 mg–1 g, up to three doses over 3 days). Due to an increased risk of bladder carcinoma, the safe duration of treatment with cyclophosphamide is limited to 12 months, so immunosuppression is "stepped-down" to the less toxic medications azathioprine, mycophenolate mofetil, methotrexate, or dapsone at the end of this period. Combination therapy is used for resistant cases, and includes the combination of a sulpha (dapsone, sulphapyridine) and myelosuppressive agent (azathioprine, mycophenolate, cyclophosphamide), and/or the addition of prednisolone, either as a maintenance dose of ≤7.5 mg/day or as a brief tapering 6- to 8-week course. High-dose oral corticosteroids do not play any role in long-term management, as the high doses required to control disease cannot be sustained due to adverse side effects [25], and the inflammation quickly recurs when the dose is reduced.

Once complete control of inflammation has been achieved (Fig. 8.5b,c), immunosuppression is continued for at least 12 months. Following this, the dose is slowly tapered and can be stopped if the patient wishes, providing they understand that it must be recommenced if disease activity recurs. Life-long follow-up is necessary, because disease recurs in up to one-third of patients. If the disease has been severe and resulted

Table 8.5 Results of studies reporting the efficacy of systemic immunosuppressive agents used in ocular mucous membrane pemphigoid (MMP)

Immunosuppressive agent	Study	Disease severity	Percentage of patients responding to treatment
Nicotinamide and tetracycline	[37]	Mild to moderate	50
Dapsone	[38]	Mild to moderate	83
	[44]	Mild to moderate	45
	[18]	Mild to severe	70
Sulfasalazine	[15]	Mild to moderate	50
	[9]	Mild to moderate	45
Azathioprine	[44]	Mild to moderate	45
Methotrexate	[32]	Mild to moderate	89
Cyclophosphamide and steroids	[13]	Severe	79
	[18]	Severe	12/12 patients
Cyclophosphamide	[44]	Severe	89
Tacrolimus	[30]	Severe	33
Cyclosporin	[18, 19][a]	Mild to moderate	2/22 patients (9)
Mycophenolate	[50]	Severe	9/10 eyes
Intravenous immunoglobulin	[40]	Severe	8/8 patients

[a]Combined results of two case series

in loss of useful vision in one eye, lifelong therapy is recommended.

Intravenous immunoglobulin appears to be useful for recalcitrant disease [41] or as an alternative to conventional immunosuppression; however, it is too expensive and impractical for use as routine therapy. In our center it has been used as adjunctive therapy in patients unresponsive to other drugs. Methotrexate controls inflammation in 89% of mild to moderate disease [32], but we have concerns about irreversible hepatic and pulmonary fibrosis occurring with prolonged therapy and therefore do not use it routinely. Monoclonal antibody therapies are potential new treatments for severe refractory MMP. There are case reports of success using the anti-tumour necrosis factor α (TNF-α) agents etanercept [39] and infliximab [26] in patients with severe refractory MMP. Other monoclonal antibody therapies that have been reported to be effective in case reports include rituximab, the anti-CD20 antibody, which depletes B cells [10], and the interleukin-2 antagonist daclizumab [34].

The results of studies reporting the efficacy of each of the different immunosuppressive agents used in ocular MMP are summarized in Table 8.4. In our center, we have recently reviewed the results of treatment with mycophenolate in 34 patients and have found that it is very well tolerated and effective in ocular MMP, with only 15% of patients developing adverse effects and a response to treatment evident in 81% of treatment episodes. In comparison, both dapsone and cyclophosphamide were associated with adverse effects in 31% of our patients (see Table 8.6).

Blood pressure, weight, urinalysis, and blood tests must be evaluated weekly upon the initiation of therapy, and thereafter 2-weekly or monthly in all patients on immunosuppression, to screen for drug-related toxic side effects. These agents should be used either by physicians experienced in administering immunosuppressive agents, or in conjunction with a medical physician. In patients taking cyclophosphamide, lymphopenia is universal and used to titrate treatment; the target lymphocyte count is $0.5–1.0\times10^9$ cells/ml [13]. The proportion of patients developing toxicity associated with each of the different immunosuppressive agents, in a recent review at our center, is shown in Table 8.5.

Summary for the Clinician

- No topical treatment is effective at controlling the activity of OCP or altering the natural history of the disease. Only systemic immunosuppression alters the natural history
- Using a stepladder strategy for immunosuppressive therapy, the risk of a poor initial response in mild to moderate disease is justified by less toxicity and the undiminished prospects of further therapeutic success with more toxic agents, whilst in aggressive disease initial treatment with toxic agents is justified. These established therapies are successful in controlling inflammation in over 90% of patients with MMP
- Intravenous immunoglobulin and monoclonal antibody therapies are potential new treatments for recalcitrant disease

8.8 Improving Vision

Once ocular surface inflammation and progression have been controlled, attention can be turned to other means of improving vision. Moderately advanced conjunctival cicatrization can still be compatible with good vision in ocular MMP. Elder et al. found no statistical association between forniceal shortening and visual impairment due to corneal disease [14]. However, when there is extreme loss of the inferior fornix down to less than 3 mm, corneal complications become more frequent and severe.

8.8.1 Contact Lenses

Rigid gas permeable contact lenses are usually necessary to correct irregular astigmatism associated with corneal vascularisation and scarring in MMP. If fitted to the limbus, these have the additional advantage of protecting against trichiasis and dry eye (Fig. 8.6). Unfortunately, elderly patients with poor vision may have difficulty with contact lenses. Providing there is adequate forni-

Table 8.6 Proportion of patients developing toxicity with immunosuppressive therapy in ocular MMP[a]

Drug (n= number of patients)	Status	n (%)	Adverse effect (n= number of patients with each adverse effect)
Dapsone (n=90; 2852 patient months)	Discontinued therapy	28 (31%)	Anaemia (8), rash (9), malaise (6), headache (2), diarrhoea (2), elevated LFTs (2), thrombocytopenia (1), dysplastic blood film (1), paraesthesia (2), back pain (1), stomach cramps (1), worsening of tinnitus (1)
	Continued therapy	5 (5%)	Anaemia (5)
	Total	**33 (37%)**	
Sulfapyridine or sulfasalazine (n=48; 514 patient months)	Discontinued therapy	12 (25%)	Rash (5), nausea & vomiting (3), malaise (1), depression (1), elevated LFTs & indigestion (1), lymphopenia (1), worsening of tinnitus (1), dizziness (1)
	Continued therapy	3 (6%)	Nausea (2), anaemia (2)
	Total	**15 (31%)**	
Ciclosporin (n=7; 65 patient months)	Discontinued therapy	2 (29%)	Elevated creatinine (2), hypertension (1)
	Continued therapy	2 (29%)	Elevated creatinine (2), hypertension (1)
	Total	**4 (57%)**	
Methotrexate (n=4; 58 patient months)	Discontinued therapy	1 (25%)	Elevated LFTs (1)
Prednisolone (n=55; 143 patient months short tapering course, 988 patient months <20mg/day low dose)	Discontinued therapy	4 (7%)	Osteoporosis (2), hallucinations (1), malaise (1)
	Continued therapy	1 (1%)	Peptic ulcer (1)
	Total	**5 (9%)**	

n = number of patients (% of patients receiving drug). Anaemia, haemoglobin <13.3 g/dl (males) and <12.0 g/dl (females). Lymphopenia, <0.5x109/l. LFTs liver function tests.
No secondary malignancies detected
[a]From a recent review at our centre.

Table 8.6 *(continued)* Proportion of patients developing toxicity with immunosuppressive therapy in ocular MMP[a]

Drug (n= number of patients)	Status	n (%)	Adverse effect (n= number of patients with each adverse effect)
Azathioprine (n=60; 1230 patient months)	Discontinued therapy	24 (40%)	Nausea & vomiting (8), headaches (4), elevated LFTs (3), malaise (3), dizziness (2), tinnitus (1), depression (1), diarrhoea (1), myelosuppression (1), itch (1), arthralgia (1), myalgia (1), chest & back pain (1), numbness (1), shivering spells (1)
	Continued therapy	2 (3%)	Dizziness (1), abnormal blood results (1)
	Total	**26 (43%)**	
Mycophenolate (n=34; 506 patient months)	Discontinued therapy	5 (15%)	Malaise (2), diarrhoea (1), rash (1), headache (1), shortness of breath & tremor (1)
	Continued therapy	4 (12%)	Elevated LFTs (1), anaemia (1), myelosuppression (1), muscle cramps & insomnia (1)
	Total	**9 (26%)**	
Cyclophosphamide (n= 55; 512 patient months)	Discontinued therapy	17 (31%)	Lymphopenia (5), lethargy & malaise (3), nausea & vomiting (2), diarrhoea (1), abdominal discomfort (2), anorexia & weight loss (2), severe pancytopenia (2), anaemia (1), elevated LFTs (1), rash (2), headache (1), insomnia (1), myalgia (1), unsteady on feet (1), shortness of breath (1), dysuria (1)
	Continued therapy	10 (18%)	Lymphopenia (5)
	Total	**27 (49%)**	

n = number of patients (% of patients receiving drug). Anaemia, haemoglobin <13.3 g/dl (males) and <12.0 g/dl (females). Lymphopenia, <0.5x109/l. LFTs liver function tests.
No secondary malignancies detected
[a]From a recent review at our centre.

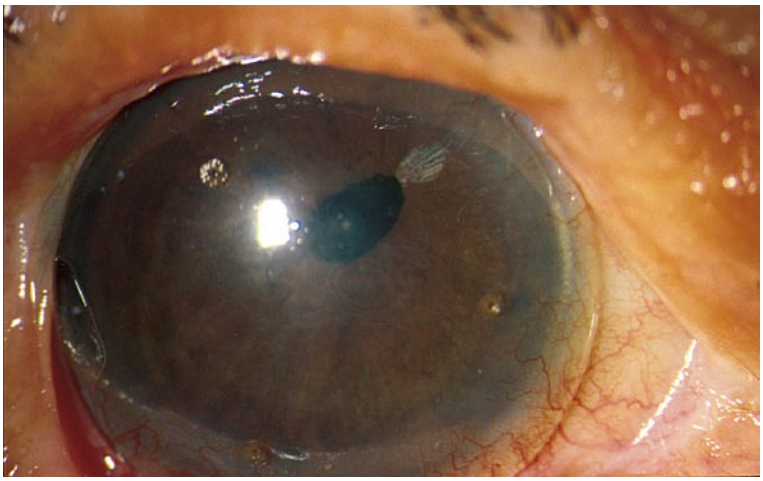


Fig. 8.6 Limbal fit rigid gas permeable contact lens protecting the cornea against exposure and trichiasis, as well as correcting irregular astigmatism

ceal depth, scleral gas permeable contact lenses can also correct irregular astigmatism and protect against surface drying, but development of hypoxia and vascularization must be monitored.

8.8.2 Cataract Surgery

Cataract surgery is commonly required in MMP. Reasons for this include the elderly patient population, the use of topical and systemic steroids to control inflammation, and the development of cataract following severe keratitis or corneal perforation. Geerling et al. [23] and Sainz de la Maza et al. [40] have reported the results of cataract surgery in ocular MMP. Visual acuity improved by two or more lines in both series. All conjunctival inflammation must be controlled before embarking on surgery. If surgery is limited to corneal incisions only, no additional immunosuppressive therapy is necessary during the perioperative period. If there is active blepharitis, it is recommended that lid cultures be taken 2 weeks prior to surgery and appropriate topical antibiotic therapy be commenced 7 days prior to surgery. General anesthesia is preferable for cases with short fornices, as 4/0 silk sutures placed through the gray line to retract the lids and canthotomies may be necessary. In these cases, conventional lid specula cannot be retained in the contracted palpebral apertures and may cause increased vitreous pressure due to taut symblephara and adhesions.

Two percent hydroxypropylmethylcellulose (HPMC) spread over the cornea prevents drying during surgery and improves the view in scarred and irregular corneas. Paraxial rather than coaxial illumination by the operating microscope can dramatically improve the view for phacoemulsification, in the presence of irregular astigmatism and opacity [31]. Use of a wick and appropriate head positioning minimizes pooling of fluid that occurs when silk sutures rather than speculae are used to retract the lids. Temporal corneal incisions are used for ease of access when superior and inferior symblephara are present. Avoid creating cataract surgical wounds in areas of peripheral corneal thinning, which may have occurred following keratitis. In these cases, providing additional perioperative immunosuppression is given, a scleral tunnel wound can be used. When central corneal scarring is too advanced to permit phacoemulsification, then a corneal section extracapsular cataract extraction can be carried

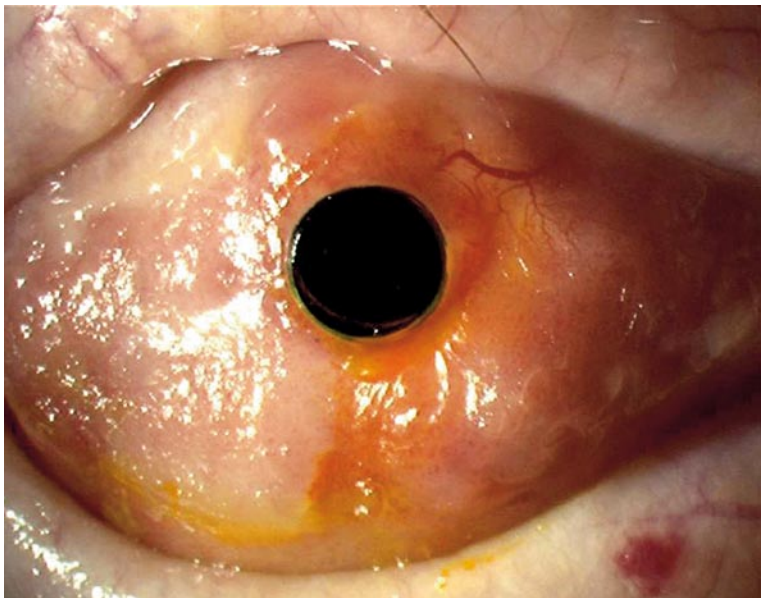


Fig. 8.7 Osteo-odonto keratoprosthesis for endstage ocular MMP

out, with the wound enlarged to allow "open sky" surgery if needed, followed by closure with 10/0 nylon. Trypan blue staining of the anterior capsule assists in visualization of the capsulorrhexis through scarred and irregular corneas.

Extensive canthotomies are sometimes necessary, in order to gain access to the globe for surgery. The extent of the canthotomy required may not be discovered until surgery is commenced, but if anticipated, increased immunosuppression for the conjunctival surgery is recommended. Large conjunctival defects resulting after canthotomy may be closed with amniotic membrane, combined with topical mitomycin C administered at the base of the defect. Intracameral antibiotics and intensive topical fluoroquinolone (for example, one drop every 2 min for 12 min), can be used instead of subconjunctival antibiotic injections as prophylaxis against endophthalmitis. Subconjunctival injections are avoided as these may reactivate conjunctival inflammation. Careful postoperative follow-up is necessary, to identify and treat indolent perioperative epithelial defects and any excessive conjunctival inflammation.

8.8.3 Corneal Graft Surgery

Keratoplasty, either penetrating or lamellar, is seldom successful for visual rehabilitation because of the formidable problems of corneal epithelialization in the poor ocular environment that accompanies MMP. Dry eyes have a very poor prognosis for success following keratoplasty. Failure to epithelialize predisposes to corneal vascularization, melt, desmetocele formation, and perforation. Microbial infection is frequent. Invasion of the corneal surface by vascularized conjunctival epithelium, due to corneal epithelial stem cell deficiency, also results in vascularization and opacification of the corneal graft.

Tugal-Tutkun et al. reported the results of keratoplasty in 9 eyes with MMP [49]. Despite aggressive preoperative treatment including systemic immunosuppression, mucous membrane grafts, and lash cryotherapy, there was a high rate of complications with generally poor visual results. Five eyes developed persistent epithelial defects, leading to ulceration in 4 cases. One required a keratoprosthesis and 5 required a fur-

ther tectonic regraft. Vision was improved in 3 eyes, unchanged in 3, and worse in 3.

The results of tectonic penetrating keratoplasty in 6 eyes have been reported by Macleod et al. [31]. Although 3 patients gained visual improvement from the procedure, this was not maintained due to progressive vascularization of the graft, melt leading to desmetocele formation, and development of glaucoma.

Case selection for keratoplasty is critical. In our center we have recently carried out three penetrating keratoplasties for visual rehabilitation in eyes with MMP and adequate tear production indicated by a Schirmer's I test (without anaesthetic) result of >5 mm. In all 3 eyes, fornix reconstruction surgery was performed preoperatively to ensure good lid closure, and the keratoplasty surgery was carried out under increased systemic immunosuppression. With a follow-up of 14–25 months, vision has improved by 3 to 6 lines in all 3 eyes. Two of the patients are younger than 60 years of age and in these patients the surgery has been successful. One of the patients is frail and elderly and was colonized with methicillin-resistant *Staphylococcus aureus*, which caused microbial keratitis and melt 4 months postoperatively in the initial corneal graft. 15 months ago this was replaced by a new graft, and there has been a stable ephitelium on this graft since the introduction of antologous serum drops.

8.8.4 Ocular Surface Reconstructive Surgery

Chronic inflammatory insult to the limbal epithelial stem cells is a cause of surface failure, and an important reason for the failure of keratoplasty surgery in MMP. Reconstruction of the ocular surface by removal of the fibrovascular pannus, which obstructs vision, transplantation of limbal stem cells, and lamellar or penetrating keratoplasty for stromal corneal vascularization and scarring have been reported to improve the outcome. Tsubota et al. [47] reported successful visual improvement in 9/9 eyes with OCP using limbal allografts and amniotic membrane transplantation, with systemic immunosuppression using dapsone and ciclosporin. Penetrating keratoplasty was also performed in 5 of the 9 eyes. However, these promising results at 5 months were not sustained with prolonged follow-up. The same authors reported that surface reconstruction using this technique was successful in 41% of a group of both Stevens-Johnson syndrome and OCP patients, at an average of 3 years' follow-up [48]. Other reports of the outcomes of surface reconstructive surgery using living-related limbal allografts [8], or cultivated epithelial stem cells [42] describe results for only 2 or 3 OCP eyes and it is not possible to draw conclusions from these reports.

8.8.5 Keratoprosthesis

For dry eyes with ocular MMP, keratoprosthesis surgery can restore vision. Usually, by this stage, the disease has burned out. If not, it is important that any disease activity is controlled with adequate immunosuppression before commencing surgery. A satisfactory keratoprosthesis for patients with MMP is the osteo-odonto keratoprosthesis (OOKP) (Fig. 8.7), originally described by Strampelli. It is a heterotopic autograft, where the cornea is replaced by a polymethylmethacrylate (PMMA) optical cylinder glued to a biological support (haptic) made by human living tissue (autologous osteodental lamina). Recent long-term results reported by Falcinelli et al. [16], including 39 eyes with OCP, indicate an 85% probability of retaining the OOKP 18 years after surgery, with a mean best corrected acuity of 0.8 at 10 years in OCP patients. Compared with other keratoprostheses, there is a low risk of infection and extrusion, and it is particularly resilient to the hostile environment of a dry keratinized eye. Its main disadvantages are that the surgery involved is high risk and extensive, cosmesis is poor, and the field of vision is limited. Successful long-term visual outcome in OCP patients using the Dohlman keratoprosthesis has also been reported [11]; however, complications are not uncommon. These include necrosis of surrounding tissue, aqueous leak and endophthalmitis, retinal detachment, and prosthesis extrusion.

Summary for the Clinician

- Hard contact lenses are useful to correct irregular astigmatism associated with corneal scarring. If fitted to the limbus or sclera, they can in addition protect against trichiasis and surface drying. Regular review for microbial infections is mandatory
- Clear corneal cataract surgery is frequently successful, using silk suture lid retraction and canthotomies to gain access to the globe. No increase in immunosuppression is necessary unless conjunctival incisions are made
- Corneal graft surgery and ocular surface reconstruction for visual rehabilitation have a guarded prognosis in OCP, and should not be embarked upon in dry eyes
- Keratoprosthesis surgery, with an osteo-odonto keratoprosthesis can improve vision successfully, but the surgery is high risk, and complications both during and after surgery are frequent

8.9 Recommended Clinical Practice

The following recommendations can be made regarding management of ocular mucous membrane pemphigoid:

- Blepharitis, dry eye, microbial infection, and toxicity are responsible for local ocular surface inflammation and must first be excluded or treated. Persistent inflammation following treatment of surface disease is due to the underlying immune disorder and requires systemic immunosuppression using a stepladder strategy. For recalcitrant disease, use of intravenous immunoglobulin or monoclonal antibody therapy may be necessary.
- Clear corneal cataract surgery and entropion surgery not involving the conjunctiva are safe and do not require increased perioperative immunosuppression, whilst increased systemic immunosuppression is necessary for fornix reconstruction and other surgery in which the conjunctiva is incised.
- Keratoplasty and ocular surface reconstructive surgery to rehabilitate vision have a very guarded prognosis in OCP, particularly in dry eyes.
- Keratoprosthesis surgery carries a high risk, but can successfully restore vision.

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Chapter 9

Adult Inclusion Conjunctivitis

9

Philippe Kestelyn

Core Messages

- Urogenital chlamydiosis due to *C. trachomatis* serovars D-K is the most frequent sexually transmitted disease in the industrialized world
- Although often asymptomatic, it is responsible for significant morbidity
- Adult inclusion conjunctivitis arises from transfer of bacteria from the genitalia to the eye (autoinoculation)
- The prevailing clinical presentation is that of a chronic red eye with a moderate amount of mucopurulent secretions
- The differential diagnosis includes the different causes of follicular and chronic conjunctivitis, uni- or bilateral
- The clinical suspicion is confirmed by laboratory methods
- Nucleic acid amplification tests have the highest sensitivity and specificity and have supplanted culture methods as the gold standard
- Systemic administration of doxycycline, erythromycin, azythromycin or fluoroquinolones is the treatment of choice
- Sexual partners of the patient should be screened as well

9.1 Introduction

The Chlamydiae are nonmotile, Gram-negative bacteria, that are metabolically deficient in their ability to synthesize ATP. Their dependency on an exogenous source of energy explains their obligate intracellular life cycle. Chlamydiae undergo a biphasic development cycle, forming distinctive intracellular inclusions that permit identification by light or fluorescence microscopy [18].

The genus *Chlamydia* is composed of four species: *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, and *Chlamydia pecorum*. The different serovariants or "serovars" of *Chlamydia trachomatis* are responsible for the following diseases: serovars A, B, Ba, and C cause trachoma, a chronic and potentially blinding keratoconjunctivitis endemic in many developing countries where poverty goes hand in hand with deficient sanitation and poor hygiene; serovars D to K are the cause of one of the most common sexually transmitted diseases worldwide and both adult and neonatal inclusion conjunctivitis arise from transfer of bacteria from the genitalia to the eye; and serovars L1, L2, and L3 are the etiological agent in venereal lymphogranulomatosis. The observation under the microscope of inclusion bodies in conjunctival cells infected with Chlamydiae explains the term inclusion conjunctivitis [19].

9.2 Epidemiology

Adult inclusion conjunctivitis is due to *Chlamydia trachomatis* serovars D, E, F, G, H, I, J, and K. The bacterial reservoir is the genital tract of the sexually active adult. Urogenital chlamydiosis is the most common STD in the developed world and accounts for approximately 3 million new cases each year in the USA [4]. The highest prevalence is found in young, sexually active adults. The disease accounts for 40–50% of all cases of nongonococcal urethritis in men [10].

According to the WHO, chlamydial prevalence rates in pregnant women range from 2.7 to 8% in Europe [24].

A big problem in the control of chlamydial disease is the fact that 50% of men and 70–80% of women with sexually transmitted chlamydial infection are asymptomatic. Less than 10% of prevalent cases are diagnosed [20, 21]. The genital infection causes urethritis, cervicitis, endometritis, salpingitis, and perihepatitis in women and is a major cause of sterility. In men it causes balanitis, urethritis, prostatitis, and epididymitis [3]. The rising prevalence of chlamydial infection and its association with an increased risk of cervical cancer, sterility, and acquisition of HIV raises the question of whether a comprehensive European-wide screening policy is needed [16].

Transmission to the eye occurs in the vast majority of cases by autoinoculation with infected genital secretions from either the patient or his partner. Direct infection from the eye of one patient to the eye of another is possible but uncommon, and could account for the small number of affected patients without concomitant genital disease. Indirect infection from inadequately disinfected swimming pools and from contact with contaminated inert surfaces has been described, but is rare [11, 13, 23].

9.3 Clinical Picture

The incubation period of adult inclusion conjunctivitis varies from 2 days to 3 weeks. It starts as unilateral, papillary conjunctivitis with mucopurulent secretions and swelling of the ipsilateral premandibular lymph glands. Later, typical follicles will develop on the upper and lower tarsal plates. Swelling and infiltration of the subconjunctival tissue may obscure the vertical vessels of the upper tarsal plate, mimicking the classic picture of inflammatory trachoma at this stage (Fig. 9.1) [1, 6]. Pseudomembrane formation seen in neonatal inclusion conjunctivitis is not seen in the adult form. Involvement of the second eye may ensue, but is not always present.

Corneal involvement includes discrete pannus formation, superficial punctate keratopathy,

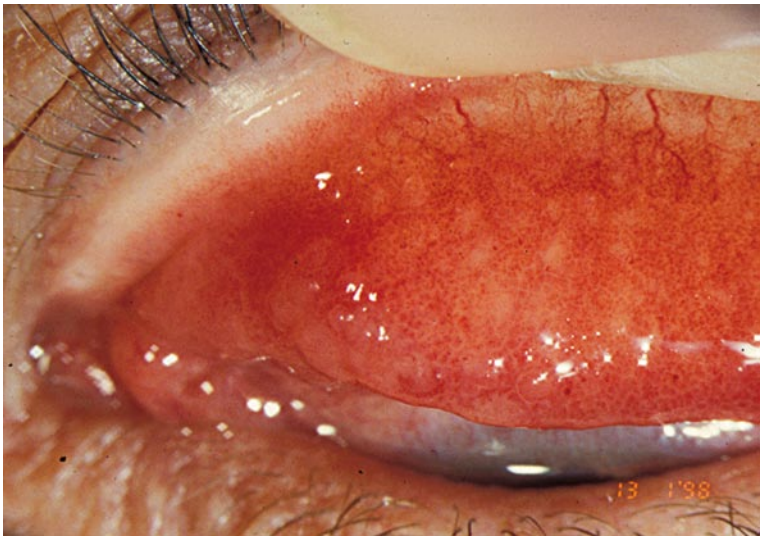

Fig. 9.1 Typical findings on the upper tarsal plate of an adult patient with inclusion conjunctivitis. Inflammatory swelling of the tarsal conjunctiva with papillary reaction and the presence of numerous typical follicles. The underlying tarsal vessels are obscured by the inflammatory reaction

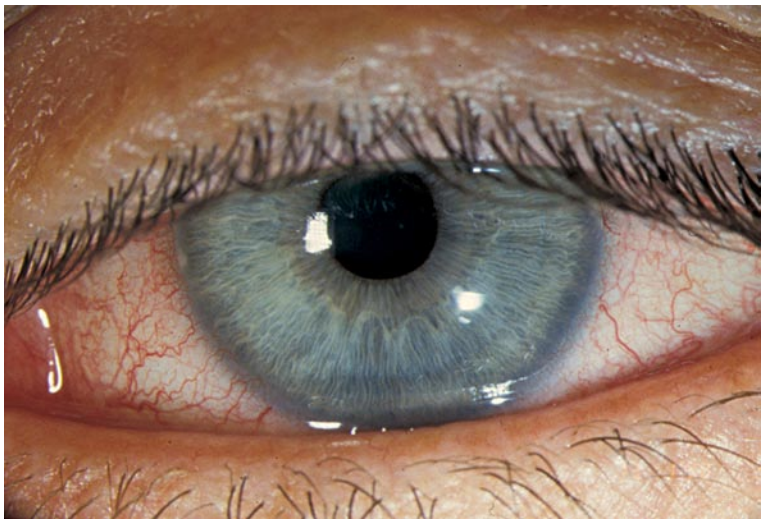

Fig. 9.2 Conjunctival redness in the chronic phase of inclusion conjunctivitis

and more seldom, marginal infiltrates [7]. Frank corneal neovascularization and conjunctival scarring typical of trachomatous keratoconjunctivitis are not often observed in adult inclusion conjunctivitis.

The initial phase of acute infection is often missed and most patients will present with a chronic red eye (Fig. 9.2). They will complain of mucopurulent secretions and sticky eyes in the morning, foreign body sensation, and photophobia. Inspection at this stage will show a papillary and follicular conjunctival reaction; eventually there will be discrete corneal changes, but the inflammatory swelling of the subconjunctival tissue will no longer be present [8].

The differential diagnosis of adult inclusion conjunctivitis is in the first place the differential diagnosis of chronic follicular conjunctivitis and should include the following entities: classic trachoma, adenoviral epidemic keratoconjunctivitis, herpes, Newcastle disease virus conjunctivitis, chronic allergic conjunctivitis, acne rosacea, chronic blepharitis, and even floppy eyelid syndrome [15].

9.4 Laboratory Diagnosis

The clinical diagnosis of adult inclusion conjunctivitis may be difficult and therefore identifying Chlamydiae in conjunctival scrapings or direct diagnosis may be very useful. Because Chlamydiae are obligate intracellular pathogens, the scraping should include infected cells. Therefore, specimens that contain only exudate or secretions, but no cells, are unsatisfactory. For conjunctival specimens any purulent exudate should be removed before collecting epithelial cells by vigorously rubbing a dry swab over the everted palpebral conjunctiva. The specimen is then transferred to a transport medium that makes it possible to perform both culture and DNA amplification techniques from a single swab. The likelihood of isolation is optimized if specimens are refrigerated immediately after collection at 2–8° Celsius and kept at this temperature during transport. The delay between collection and laboratory processing should be less than 48 h. Specimens that cannot be processed within 48 h may be frozen at -70° Celsius, but this is likely to result in a 20% loss of viability. Freezing at -20° Celsius should be avoided altogether [2].

The following laboratory methods are available to identify chlamydial infection:

- Culture methods
- Nonculture methods:
  - Direct cytologic examination to identify inclusion bodies using staining methods
  - Identification of the chlamydial antigen
  - Nucleic acid amplification techniques (NAATs)
- Serologic tests

9.4.1 Culture Methods

Culture methods on viable cells used to be the gold standard for the diagnosis of chlamydial infection. They have lost this status with the advent of nuclear acid amplification techniques, or NAATs, over the last decade because of their relative insensitivity: culture methods have a specificity that approaches 100%, but their sensitivity is only 70–85% in comparison with NAATs (see below). Other disadvantages of culture techniques include the requirement for a stringent cold chain for the transportation of specimens, high cost, high level of technical expertise required, and a time delay before obtaining results of 3–7 days.

9.4.2 Nonculture Methods

- Staining of conjunctival scrapings with Giemsa to demonstrate typical chlamydial inclusion bodies is not recommended for the diagnosis of adult inclusion conjunctivitis due to its lack of sensitivity [17]. Moreover, recognition of chlamydial inclusions requires considerable expertise.
- Antigen detection methods include the direct fluorescent antibody (DFA) test, based on direct visualization of the chlamydial organism by staining with fluorescein-labeled specific antibody; the enzyme immunoassay (EIA) test, based on the immunochemical detection of antigen; and the DNA hybridization probe based on the detection of chlamydial rRNA. All these tests are commercially available and commonly used (Microtrak DFA, Behring Diagnostics; Chlamydiazyme, Abbott Diagnostics; Microtrak EIA, Behring; PACE 2, Gen-Probe). Since these tests have reduced sensitivities and specificities relative to culture methods, they are more prone to error, especially in populations with low prevalence of the disease [2].
- The development of NAATs has been the major advance in the field of chlamydial diagnosis in the last decade. A number of commercial tests are available: polymerase chain reaction, or PCR, tests (Amplicor, Roche Diagnostics), strand displacement amplification (SDA, Becton Dickinson), and ligase chain reaction (LCR, Abbott Laboratories). They all combine exquisite sensitivity with very high specificity and are considered the new gold standard in the diagnosis of chlamydial disease [12].

9.4.3 Serologic Tests

Serologic tests are in general not useful in the diagnosis of genital tract infection caused by *Chlamydia trachomatis*. Antibodies elicited by infection are long-lived and a positive titer will not distinguish a previous from a current infection. The presence of IgM is an unreliable marker of acute infection since it is often not present. The presence of antichlamydial IgG in tears might be helpful for diagnosis in patients with suspected chlamydial conjunctivitis, since IgG seems to be absent in tears from patients with only urethritis [9].

9.5 Treatment

Since adult inclusion conjunctivitis results from autoinfection in patients with genital disease in the vast majority of cases, systemic treatment is mandatory to prevent extraocular morbidity and ocular reinfection. The classic treatment includes oral doxycycline, 100 mg twice a day for 1 week. In pregnant women erythromycin is given, 500 mg four times a day for 1 week. Azythromycin 1 g as a single dose is a safe and effective alternative for doxycycline and should be considered if compliance is an issue. Pregnancy is no contraindication to the use of azythromycin, but cost may be a consideration [14]. Oral

fluoroquinolones are also effective agents against *C. trachomatis,* either ofloxacin (300 mg orally twice a day) or levofloxacin (500 mg orally once daily) for 1 week. Screening and treatment of infected sexual partners of the patients, as well as counseling about safe sex, should be part of the comprehensive care [5].

The Centers for Disease Control and Prevention (CDC) does not recommend routine test-of-cure visits during the post-treatment period. If for some reason a test-of-cure seems indicated, only culture methods should be used. NAATs are less useful for this indication, as they may pick up residual DNA in the early post-treatment period in patients whose infection has been cured [2].

Summary for the Clinician

- Adult inclusion conjunctivitis should be suspected in patients with uni- or bilateral chronic conjunctivitis, especially in young adults, and clinical suspicion should be confirmed by laboratory testing
- The clinician should be familiar with the correct sampling and transport methods for conjunctival specimens and understand the strengths and limitations of the particular laboratory tests for chlamydia infection available at his local lab facility
- The clinician should take into account cost, compliance issues, and potential side effects when selecting an effective antibiotic agent for systemic use
- Sexual partners of the patients should be screened as well
- Counseling on safe sex should be provided for the patient and his partners

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Chapter 10

Chronic Blepharitis: Diagnosis, Pathogenesis, and New Treatment Options

10

Claudia Auw-Haedrich, Thomas Reinhard

Core Messages

- Chronic blepharitis can cause severe corneal changes, but is surprisingly often not diagnosed
- Pathogenesis often includes skin diseases, but also bacterial involvement, meibomian gland dysfunction with altered meibomian gland lipids, hormonal imbalance
- Important sequelae of chronic blepharitis are dry eye syndrome, corneal involvement like keratitis, phlyctenules, pannus, vascularization, corneal ulcers, and lid changes like trichiasis and madarosis
- The treatment includes mechanical measures, preservative-free artificial tears, steroids, antibiotics, immunomodulatory eye drops (e.g., steroids, cyclosporin) or ointment (e.g., FK506, Pimecrolimus), and occasionally surgical treatment

10.1 Introduction

Chronic blepharitis is one of the most common diseases of the eyelids, which is surprisingly not often recognized and thus not treated or not properly treated. Since corneal changes are not uncommon, sometimes effecting severe visual disturbance, we emphasize the importance of recognizing and treating this disease, which is not always easy.

10.1.1 Clinical Course and Pathogenesis of Chronic Blepharitis

Chronic blepharitis may cause irritative symptoms such as burning, itching, tearing, photophobia, foreign-body sensation or pain, symptoms that are associated with dry eye. Erythematous eyelids with accumulation of debris along the eyelid margin disturb some of those patients. Not only adults but also children may be affected. Blepharokeratoconjunctivitis is common in children with the more severe manifestations in the Asian and Middle Eastern populations. Therapy is effective and loss of sight can be prevented in most cases [66].

On slit-lamp examination the following pathologic changes of the lid margin might be seen [10, 44].

10.1.1.1 Anterior Blepharitis

10.1.1.1.1 Staphylococcal

Collarettes (fibrinous material around the base of the eyelid lashes), erythematous lid margins (Figs. 10.1, 10.2), madarosis, trichiasis (Fig. 10.3) [11].

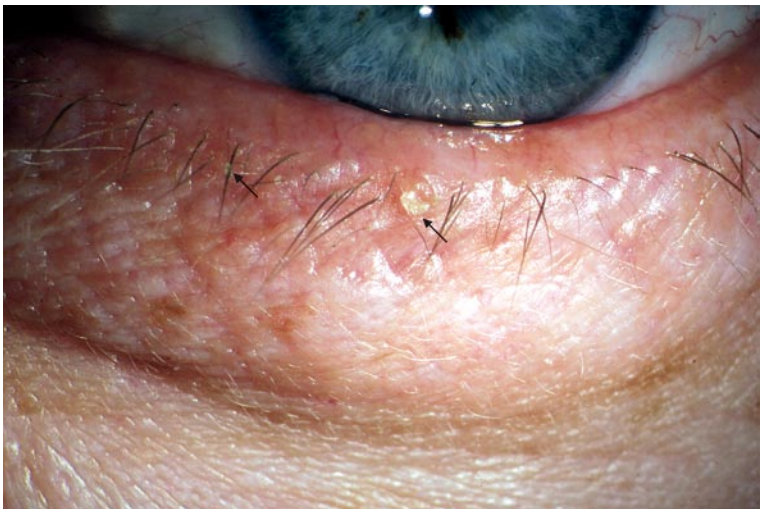

Fig. 10.1 Collarettes (fibrinous material, *arrows*) and erythematous lid margin in anterior blepharitis

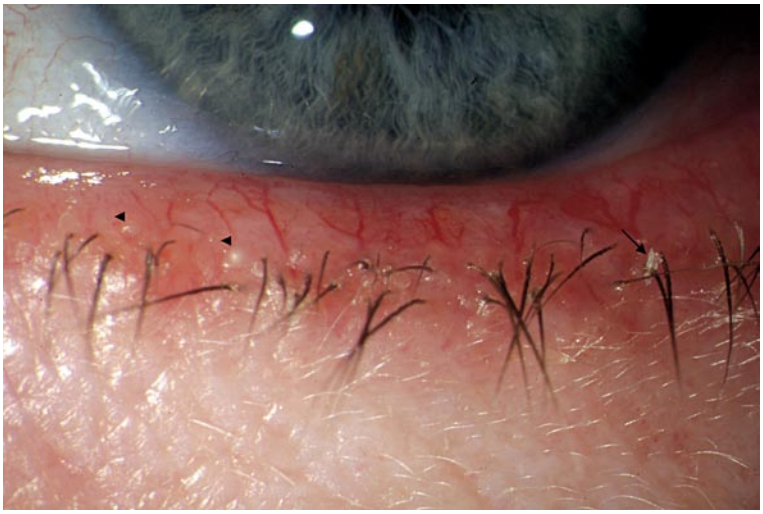

Fig. 10.2 Increased vascularization of the lid margin, a few collarettes (*arrow*), and small cysts of the Zeis gland ducts (*arrowheads*)

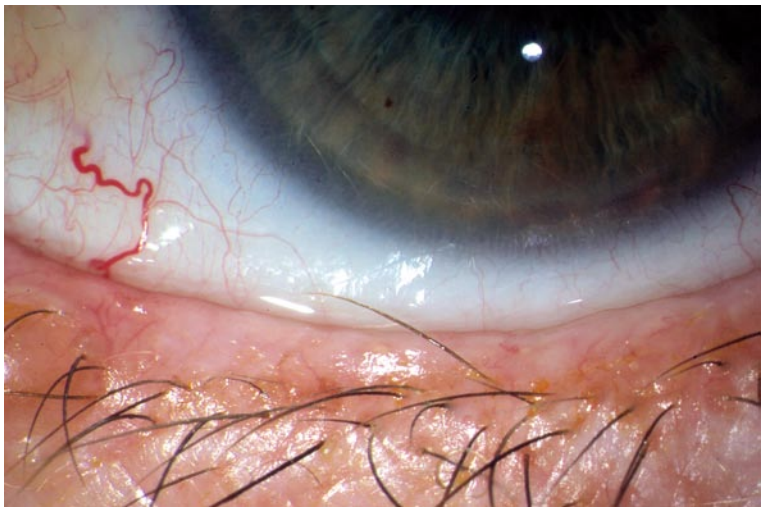

Fig. 10.3 Trichiasis in chronic blepharitis with irregular lid margin

10.1.1.1.2 Seborrheic

An excess of secretion (or accumulated secretion due to obstruction) contained in the dilated ductules may be expressed in normal liquid form. The lid scaling and crusting is typically more oily or greasy than that seen in staphylococcal blepharitis and the inflammation less marked.

10.1.1.1.3 Mixed Seborrheic/Staphylococcal

More inflammation signs are found.

10.1.1.2 Anterior-posterior Blepharitis

Seborrheic blepharitis with secondary meibomitis is usually found.

10.1.1.3 Posterior Blepharitis

It causes meibomian gland loss and distichiasis due to metaplasia of the ductules. Posterior blepharitis is often associated with meibomian gland dysfunction, the latter leading to chronic blepharitis [10].

10.1.1.3.1 Changes in the Lid Margin

- Swelling and rounding of the posterior lid margin (Fig. 10.4)
- Increased vascularity of the lid margin (Fig. 10.2)
- Hyperkeratinization of the cutaneous part of the lid margin
- Irregularity of the posterior lid margin especially in cicatrizing and ulcerating blepharitis

10.1.1.3.2 Changes in the Mucocutaneous Junction

Anteroplacement

The mucosa may spread forwards, so that the orifices appear to lie in the mucosal tissue.

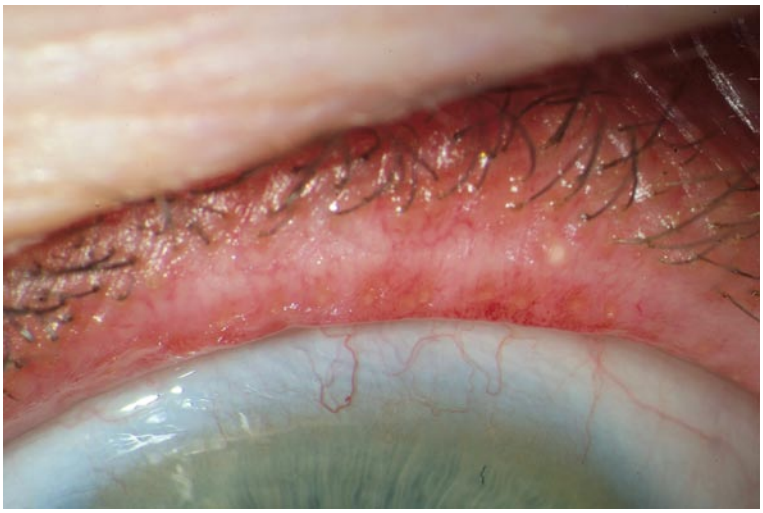

Fig. 10.4 Swelling and rounding of the posterior lid margin, plugging with inspissated secretion. The punctum of the orifices are not clearly visible and the lipid is less expressible

Retroplacement

Posterior movement of the mucocutaneous junction (MCJ), with spreading, keratinizing squamous metaplasia of the posterior lid margin that extends to the tarsal plate.

Mucosal Absorption

The MCJ and the orifices are in the same position, but come to lie at a new posterior lid margin.

Ridging

Ridge-like elevation of the MCJ or of the tissue running between the orifices.

10.1.1.3.3 Orifices

The number may be reduced.

"Pouting"

An early sign of meibomian gland disease (MGD) is elevation or pouting of the orifice, which may be dilated, expression may demonstrate plugging or blocking with inspissated secretion (Fig. 10.4). One of the reasons might be the elevation of the meibomian lipids' melting point with increased viscosity, stagnation, and plugging of glands [16, 65]. Pouting is also considered to be an age-related change in persons without blepharitis [26].

Retroplacement

The orifices become ovally elongated in the plane of the ducts and posterior movement may be accompanied by duct exposure. This occurs in cicatricial changes.

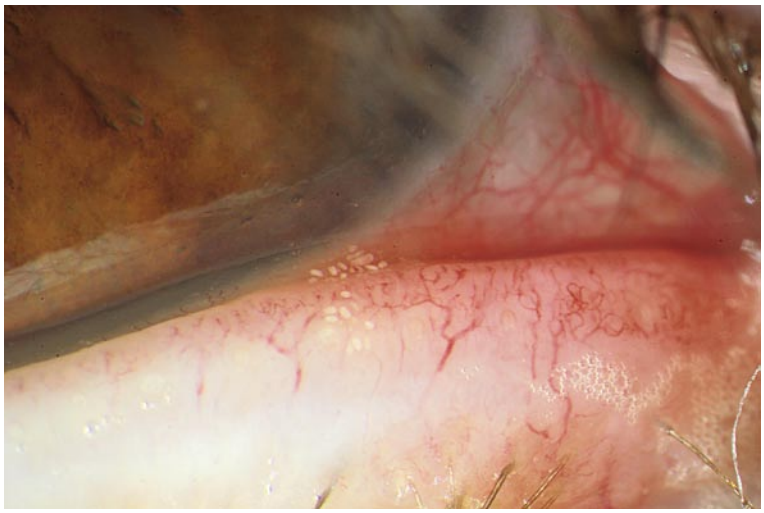

Fig. 10.5 Granular secretion in chronic blepharitis, foamy tear fluid, and increased vascularization of the lid margin

Obliteration

The punctum of the orifice may not be visible and the lipid is not or is less expressible (Fig. 10.4).

10.1.1.3.4 Acini

Due to the obstruction, concretions or chalazia may be visible. A comparison of melting points of normal meibomian fluid and that contained in chalazia found that meibomian secretion had a melting point range of 32–33.8°C whereas in chalazia, a melting point range of 35.4–37.4°C was found [65].

10.1.1.3.5 Secretion

- Clear
- Turbid
- Granular (Fig. 10.5)
- Inspissated: the tooth-paste like material (Fig. 10.6) contains keratinized epithelial cells [23]

10.1.1.3.6 Tear Film

Tear film may contain foam as a sign of an excess of unsaturated fatty acids with the formation of soap [48] (Fig. 10.5).

Summary for the Clinician

- Chronic blepharitis can be categorized into anterior and posterior blepharitis
- Anterior blepharitis can be associated with seborrhea and bacterial infection
- Posterior blepharitis affects mainly the meibomian glands and is commonly preceded by meibomian gland dysfunction

10.1.1.4 Pathogenesis

10.1.1.4.1 Skin Diseases

Often, skin diseases are the underlying cause of chronic blepharitis. One third of those patients suffer from seborrheic dermatitis. Seborrheic dermatitis is characterized by hyperkera-

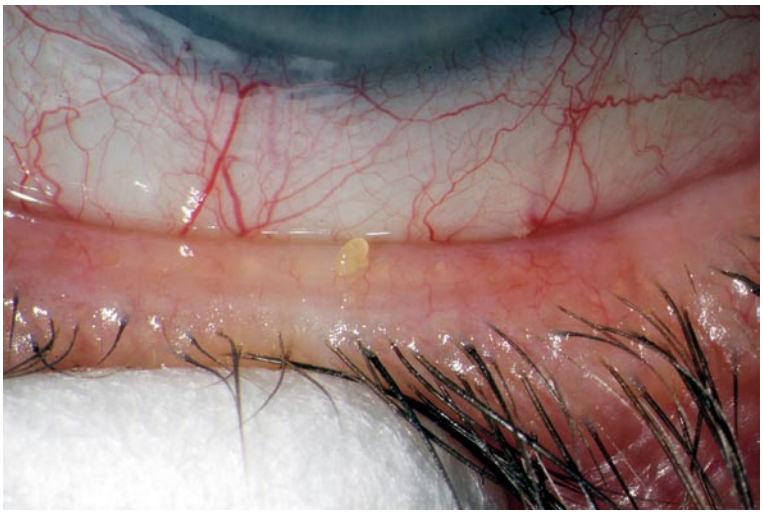

Fig. 10.6 Tooth-paste-like material in posterior chronic blepharitis

tinization of the skin in areas rich in sebaceous glands. No evidence of sebaceous gland hypersecretion has been reported. Seborrheic dermatitis was found to be present in 100% of patients with seborrheic blepharitis plus secondary meibomitis, and was present in 82% of patients with seborrheic blepharitis and meibomian seborrhea [44]. Another third of patients with associated skin disease suffer from acne rosacea [25]. Nearly 100% of patients with acne rosacea had blepharitis affecting the meibomian glands, and ocular disease preceded cutaneous manifestation in 20% [8]. Chalazia are frequent in patients with rosacea [35]. Ten percent of the patients with chronic blepharitis associated with skin disease suffer from atopic dermatitis [25]. Ectodermal dysplasia, with the absence of meibomian glands, also leads to chronic blepharitis [6, 24, 29, 46].

Abnormal ductal keratinization, which appears to be important in the pathogenesis of meibomian gland dysfunction, was observed in patients who had inspissation of the meibomian gland duct orifices and stagnations of secretions, but minimal bacterial involvement [19, 27, 30]. Histologically, keratinocytes are increased in the meibomian gland ducts and orifices [19].

Summary for the Clinician

- Often, chronic blepharitis is associated with skin diseases like seborrheic dermatitis, acne rosacea, atopic dermatitis, and seldom with ectodermal dysplasia with a common pathogenesis

10.1.1.4.2 Bacterial Infections

Earlier, most cases of chronic blepharitis were thought to be caused by bacterial infections [64]. McCulley showed that this is the case in a subgroup of chronic blepharitis, namely, staphylococcal blepharitis and combined seborrheic staphylococcal blepharitis [44]. In these cases, coagulase-negative *Staphylococcus aureus*, *Propionibacterium acnes* [13, 43] or *Pityrosporum ovale* grow in cultures from the lid margin and lead to alteration of the meibomian lipids due to bacterial lipase [13]. *S. aureus* and *P. acnes* produce fatty wax esterase and triglyceride lipase; in addition to these enzymes *S. aureus* produces cholesterol esterase [13]. All these bacteria are also found in normal lid margins with-

out blepharitis [12], but in chronic blepharitis they may become more numerous and possibly specific strains are responsible for the observed lid disease [16]. These bacterial lipases change the meibomian lipids, i.e., elevation of cholesterol after cholesterol esters have been digested by cholesterol esterase. Cholesterol esterase, on the other hand, increases bacterial growth and duplication, especially of *S. aureus* [58]. It is possible that in the presence of cholesterol esters and bacteria able to hydrolyze them, the resultant cholesterol might provide a substrate for other bacteria to flourish [16]. These reports suggest that extraocular bacterial populations and their corresponding lipase/esterase activity might be more important than is generally realized in promoting the development of chronic blepharitis. The presence of cholesterol esters appears to be necessary for the development of meibomian gland dysfunction.

Bacterial lipopolysaccharides induce the production of cytokines like TNFα by phagocytic leucocytes or normal cells like keratinocytes [33]. Cytokines induce the elevation of ROS, which show toxic effects that also promote chronic blepharitis and keratoconjunctivitis.

Blepharitis may be secondary to changes in the meibomian lipid composition caused by local bacterial flora, direct infection, a cell-mediated hypersensitivity response, or exotoxin. The former appears to be the most important in meibomian gland dysfunction [16].

Summary for the Clinician

- Bacterial infections are only responsible for chronic blepharitis in a small number of cases
- Here, mainly *S. aureus* produces cholesterol esterase, which transforms cholesterol ester into cholesterol, changing the lipid composition of the meibomian glands and supporting further bacterial growth. Bacterial liposaccharides induce cytokine production, with subsequent toxic effects, supporting chronic blepharitis

10.1.1.4.3 Altered Meibomian Lipids

From the most extensive quantitative study by Nicolaides the following data concerning the normal composition of meibomian lipids are available: wax esters make up 35% of the secretions, sterol esters 29.5%, polar lipids 16%, diesters 8.4%, triglycerides 4%, free fatty acids 2.1%, and free sterols 1.8% [47].

Besides the above-mentioned change in cholesterol esters, further pathologic changes associated with chronic blepharitis are described:

Polar lipids serve as a surfactant between the aqueous layer of the tear film and the nonpolar lipid layer; its thickness is most likely one to three molecules [56]. The polar lipid composition and polar lipids' normal fatty acids, which were highly unsaturated in meibomianitis, differed not only from those of controls, but also those from other types of chronic blepharitis [56, 57].

In meibomian gland secretions from blepharitis patients secretory phospholipase A2 (sPLA2) was twice as high as in normal patients [60]. This is significant because sPLA2 activity results in release of the proinflammatory lipid precursor, arachidonic acid, an unsaturated fatty acid. It is a precursor for PgE2 and leukotriene B4; the latter and the arachidonic acid itself activate the inflammatory TNFα [36]. The chemoattractant lipid aldehyde HNE (4-hydroxynonenal) is formed from unsaturated fatty acids as linoleic acid in the presence of ROS [52].

The loss of polar lipids, including the transitional triglycerides, may destabilize the tear film by either changing the dynamics of the nonpolar lipid phase involved in blocking water evaporation or altering the interface between the polar and nonpolar phases that involve triglycerides [43].

Dougherty et al. found that monounsaturated fatty acids from wax/sterol esters (nonpolar component) *in*creased in patients with chronic blepharitis compared with normal participants [15]. On the other hand a *de*crease in unsaturated non-polar fatty acids [43] and oleic acid [55] in chronic blepharitis, especially meibomianitis, lowers the melting point, resulting in so-

lidification at normal surface eye temperatures and explains the changes in consistency and color of the secretion, with subsequent plugging of the meibomian gland orifices. Under normal conditions the melting point of the meibomian gland secretion lies between 19 and 32°C. Production of irritative moieties such as fatty acids, lipid layer destabilization secondary to an increase in polar content, and an environment more suited to bacterial growth support chronic blepharitis [16].

Conjunctival impression cytology demonstrates epithelial impairment, rupture of intercellular junctions, loss of goblet cells, and deficient mucin secretion [34]. These abnormalities constitute both the source and consequence of chronic inflammatory reactions at the ocular surface [5].

Summary for the Clinician

- Altered composition of the meibomian gland secretion influences the clinical appearance of chronic blepharitis
- An *in*crease in monounsaturated fatty acids from wax/sterol esters and a *de*crease in the unsaturated fatty acids of nonpolar lipids also occur, while the latter condition increases the melting point and leads to stagnation and plugging of the meibomian ducts
- An increase in secretory phospholipase A2 (sPLA2) results in the release of arachidonic acid, an unsaturated fatty acid and proinflammatory lipid precursor
- The chemoattractant lipid aldehyde HNE (4-hydroxynonenal) is formed from unsaturated fatty acids as linoleic acid in the presence of reactive oxygen species (ROS)

10.1.1.4.4 Meibomian Gland Loss

Seventy-five percent of the blepharitis patients had meibomian gland loss and only 20% of controls experienced gland drop-out. This feature is examined best by meibography using an infrared video camera or infrared photography [38].

10.1.1.4.5 Hormonal Influence

Hormones probably also play a role in the pathogenesis of chronic blepharitis. Androgen deficiency [61] or androgen insensitivity syndrome in women [62] seems to be associated with chronic blepharitis. Androgen as well as estrogen receptors could be confirmed immunohistochemically in the basal cells of meibomian glands [2, 17] and the expression of estrogen receptors in meibomian glands does not seem to correlate with dry eye parameters such as break-up time or Schirmer test [2].

Summary for the Clinician

- Androgen deficiency seems to be associated with chronic blepharitis

10.1.2 Sequelae of Chronic Blepharitis

10.1.2.1 Dry Eye Syndrome

It is envisioned that there is no mucin layer, as such, separate from any aqueous layer, but that an aqueous–mucin layer is in intimate contact with the polar lipid phase, a phase with both surfactant and structural properties [42]. The nonpolar phase depends on this polar phase for the structural integrity necessary to establish an effective water vapor barrier at the lipid–air (anterior) interface. Thus, the polar and nonpolar lipid phases together form a functional tear film lipid layer adjacent to the aqueous–mucin layer [41]. The composition of the aqueous layer, on the other hand, could also influence the stability of the adjacent polar lipid phase [56].

Dry eye is found in 25–56% of patients suffering from chronic blepharitis (in 48% of

patients with obstructive MGD and 79% of those with seborrheic blepharitis) [9, 37, 38, 44]. Thirty-five percent of the patients show a very unstable tear film with low break-up time and a low Schirmer test result [40]. Shimazaki et al. found that patients with meibomian gland drop-out have a higher rate of tear production than controls, possibly due to reflex tearing [53]. Conjunctival injection, papillary hyperplasia, and punctate staining of the ocular surface are common. Corneal pannus, ulcerative keratitis [11], limbal stem cell deficiency, with subsequent scar formation or lid ectropion, occur less commonly, but emphasize the importance of making the diagnosis of MGD [16]. On the other hand, 78% of patients with dry eye suffer from chronic blepharitis [22]. As already mentioned above, the tear film is often foamy, probably due to the presence of soap [48]. Low levels of the polar lipids phosphatidylethanolamine and sphingomyelin in meibomian secretion have been reported to be associated with signs of keratoconjunctivitis sicca (e.g., evaporative dry eye) in blepharitis patients [54].

Summary for the Clinician

- Dry eye is the most common sequela of chronic blepharitis due to changes in the meibomian secretion and its impact on the two lipid layers of the tear film: the polar and the nonpolar phases
- Surprisingly, often hyposecretion of the watery phase also plays a role

10.1.2.2 Corneal Involvement

Corneal findings can include punctate epithelial erosions, marginal infiltrates, marginal ulcers, pannus (Fig. 10.7), and phlyctenule formation, not only in adults but also in children [20]. Corneal involvement occurs most commonly in the positions where the limbus is crossed by the upper and lower lid margins, at the 2-, 4-, 8-, and 10-o' clock positions. Corneal infiltrates can progress to infection and even perforation. Healed infiltrates often leave scars [11]. The explanation for the more peripheral position of infiltrates lies in the preponderance

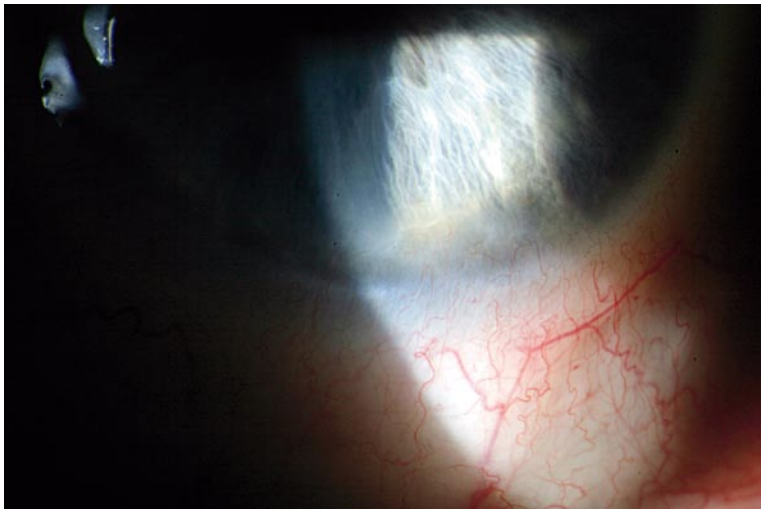

Fig. 10.7 Peripheral pannus and increased corneal vascularization due to chronic blepharitis

of antigen-presenting Langerhans cells in the cornea periphery. C1, the recognition unit of the classical pathway of the complement, is present in a larger amount in the peripheral compared with the central cornea. This may explain why the antigen–antibody complexes activate the complement more effectively in the peripheral cornea [11]. The development of pannus and limbal stem cell deficiency – at least in some cases with no bacterial involvement – is thought to be based on hypersensitivity.

Corneal disease is most common with the staphylococcal variant of anterior lid disease, but can also result from posterior blepharitis.

Summary for the Clinician

- Corneal involvement in chronic blepharitis is a serious sequela that can lead to severe visual impairment and limbal stem cell deficiency
- Due to the peripheral location of the Langerhans cells and complement 1, most changes are initially seen in the peripheral cornea

10.1.2.3 Changes in the Cilia and Lid Position

The disease process can result in damage to the lids with trichiasis with or without lid malpositioning (Fig. 10.3), poliosis, madarosis, notching, entropion, and ectropion.

10.2 Treatment

Treatment of an underlying dermatologic disease is essential. Nevertheless, topical basic treatment measures are quite uniform for all blepharitis subtypes.

10.2.1 Mechanical Measures

Warm compresses for 5–10 min heat the debris and crust on the lid margin and the meibomian secretion to or above melting point so that they are easily removed with the lid scrubs. In patients with meibomian gland dysfunction an eyelid massage to express the pathologic, now more fluid meibomian secretion is very useful.

Treatment is the same for all causes and has the goal of removing offending irritants. Lid scrubs may be performed using a soap bar, diluted baby shampoo or a commercially available lid scrub [3, 32]. It has been suggested that the alkalinity of some soaps may be beneficial [67]. We recommend lid scrub without using any additional agent. Wash cloths, cotton buds or commercially available pads may be used for the lid scrub. In the acute phase, the whole procedure, including warm compresses, eyelid massage, and lid scrubs, should be performed twice daily.

Summary for the Clinician

- Mechanical measures like warming the lids, lid massage, and lid hygiene are the most important treatment in all kinds of chronic blepharitis and should be performed at least once a day

10.2.2 Treatment of Accompanying Dry Eye Syndrome

Differentiating the disturbances of the three tear film layers in "sicca syndrome" and stabilizing each component is more effective than artificial tears alone [21].

In patients with meibomian gland dysfunction in particular, the symptoms of dry eyes are due to hyperevaporation in association with a pathologic tear film lipid layer. Here we recommend lipid-containing gels, for instance carbomer gel with triglycerides as fat components. Recently, liposome sprays were introduced that are to be sprayed on the lids to support the tear film lipid layer. They are supposed to lower the surface temperature and reduce inflammation. Liposome sprays contain phospholipid vesicles, essential fatty acids, linol and linolen acid as well as vitamin E, which may be a substitute for meibomian gland secretion. It is important to use tear

film substitutes without preservatives since these affect corneal interepithelial tight junctions and cause severe changes in the conjunctiva with loss of goblet cells and deterioration of the initial condition [5]. Other alternatives would be dexpanthenol or hyaluronic acid-containing eye drops.

Summary for the Clinician

- Depending on the kind of impairment of the tear film (lipid phase, watery phase) suitable preservative-free artificial tears should be applied at least five times a day

10.2.3 Immunomodulatory Treatment

10.2.3.1 Steroids

Severe courses need topical steroids to achieve improvement, especially in allergical forms. In other subtypes steroids become necessary if the cornea shows marginal infiltrates or phlyctenules [11, 20]. Also, preservative-free eye drops should be used, e.g., dexamethasone three times daily for 2 weeks. Due to possible side effects like secondary glaucoma or cataract this medication should only be applied at a low dosage for a limited period of time [11, 29].

10.2.3.2 Cyclosporin

Rubin and Rao found in their prospective randomized study of 30 patients that posterior blepharitis improved significantly from the initial study visit with both cyclosporin (0.05%) treatment and tobramycin/dexamethasone, with cyclosporin showing more improvement in secretion quality. A higher percentage of patients in the cyclosporin treatment group showed improvement in their symptoms of blurred vision, burning, and itching and more cyclosporin-treated patients experienced resolution of lid telangiectasia [51]. In another prospective randomized study of 22 patients with severe steroid-resistant *atopic* blepharitis, Akpek et al. observed significant improvement in signs and symptoms using cyclosporin A (CsA; 0.05%) compared with the placebo group [1]. In contrast to these studies, the prospective randomized investigation of Perry et al., including 33 patients with posterior blepharitis, did not reveal any statistically significant difference in ocular symptoms, lid margin vascular injection, tarsal telangiectasis, and fluorescein staining, but instead a statistically significant decrease in the number of meibomian gland inclusions in the CsA group compared with the placebo group [49]. Therefore, CsA seems to be more advantageous in patients with an underlying abnormality in their immune response. In our experience, CsA 1 or even 2% two times daily is often necessary to obtain alleviation of symptoms and signs.

10.2.3.3 FK506 and Pimecrolimus

Mayer et al. demonstrated in 14 consecutive patients that topical FK506 (0.1%) ointment turns out to be an excellent therapeutic option for the treatment of severe atopic blepharitis [39]. The ointment should be used twice daily at the lids and lid margin. In a lower concentration of 0.03%, also used twice daily, tacrolimus proved effective in 2 patients with blepharokeratoconjunctivitis [28]. A possible side effect might be a higher vulnerability to herpes simplex keratitis [28]. In selected cases we successfully applied an alternative medication, pimecrolimus (Elidel®), twice daily, to the lids.

Summary for the Clinician

- Steroids (always preservative-free) are important in the acute phase; in the chronic phase with fewer or improved signs of chronic blepharitis immunomodulatory eye drops like cyclosporin should be used
- In addition, FK506 or pimecrolimus ointment for the lids, including the lid margin, may be useful

10.2.4 Antibiotic Treatment

10.2.4.1 Topical Antibiotic Treatment

Antibiotics should be used only in cases of staphylococcic, mixed staphylococcic, and seborrheic blepharitis and MGD associated with acne rosacea. The identification of the infectious agents is important and treatment should be adjusted to the antibiogram. The most commonly used topical antibiotics are aminoglycosides or quinolone, applied at the lid margin. Tetracyclines are the mainstay of therapy in MGD associated with acne rosacea. The effect is not only related to its antibiotic effect, but to its modulating effect on sebaceous glands, in this case meibomian glands. Topical metronidazole in combination with lid hygiene also proved useful in patients with rosacea-associated blepharitis with less effect on the ocular surface signs compared with those of the lids [4]. Antibiotic treatment should not last for more than 14 days if possible [25], but in some cases longer treatment may be necessary. The topical antibiotics should be applied 2–3 times daily after lid hygiene. Topical application of salicylic acid could be useful via its inhibiting influence on prostaglandin synthesis and its mild antibiotic effect [29].

10.2.4.2 Systemic Antibiotic Treatment

Lipase production – but not growth of *S. epidermidis* – was found to be inhibited by low levels of tetracycline [14]. Inhibition of lipase production could result in lowered levels of toxic hydrolysis products (free fatty acids), which may exacerbate the disease process. Meibum thickness and tear break-up time both improved greatly after tetracycline treatment in patients with ocular rosacea [68].

Minocycline normalizes the function of diseased sebaceous glands and also diminishes the bacterial load of lids and conjunctiva in patients with chronic blepharitis associated with acne rosacea and seborrheic dermatitis. The most common isolated bacteria, including coagulase-negative *Staphylococcus* (CNS), *S. aureus* and *P. acne*, but not *Corynebacterium*, had a significantly decreased bacterial count under minocycline therapy compared with baseline ($p<0.05$). Thus, it was concluded that one of the mechanisms of newer generation tetracycline analogues may be a decrease in or elimination of bacterial flora from the eyelids [63]. Free fatty acids in meibum from acne rosacea and seborrheic blepharitis patients were also decreased by oral minocycline treatment suggesting a lipase inhibition effect and/or a direct effect on ocular flora [42, 59]. In children, erythromycin produced positive results [18, 45, 66], with steroid-sparing effect [20]. Hammersmith et al. found in their retrospective study that recurrences are common and may be successfully managed with low-potency steroid therapy [20].

Summary for the Clinician

- Topical antibiotics according to an antibiogram should be used in patients with anterior blepharitis associated with bacterial infection for a limited period of time
- Posterior blepharitis in acne rosacea may also require topical antibiotic treatment
- Systemic use of antibiotics (erythromycin) is useful in children due to the steroid-sparing effect, and in patients with acne rosacea (e.g., minocycline) with its regulating effect on the meibomian glands

10.2.5 Surgical/Invasive Treatment

Intralesional triamcinolone acetonide injections (0.1 to 0.2 ml triamcinolone acetonide [40 mg/ml]) were reported to reduce chalazia, but patients with chronic blepharitis required more injections before resolution [7].

Misdirection of only a few eye lashes would be well treated by epilating measures like electro- or laser-epilation. Nevertheless, the underlying, often missed, cause of trichiasis in chronic blepharitis is eyelid entropion with relative shortening of posterior lamellar tissue compared with the anterior. This condition is often missed since the malpositioning can be discrete and the patients undergo unsuccessful epilating measures [50]. In these cases anterior lamellar repositioning with

mucosa transplantation or tarsal fracture and rotation of the distal fragment [31] lead to satisfying results.

Summary for the Clinician

- Surgical treatment in chronic blepharitis includes epilation, repositioning of the anterior lamella with mucosa transplantation and tarsal fracture, and rotation of the distal fragment

10.3 Conclusion and Outlook

Many unclear aspects of chronic blepharitis have been unraveled over the last few years, especially the association among skin diseases, bacteria, changed lipid composition, and hormonal imbalance. Specific proinflammatory pathways supporting chronic blepharitis have become clearer and offer new therapeutic options. The influence of innervation, vascularization, and other proinflammatory proteins in chronic blepharitis are topics that need further investigation.

10.4 Current Clinical Practice and Recommendations

Mechanical measures remain the most important part of therapy in all types of chronic blepharitis. Topical antibiotics should be applied in cases associated with bacterial infection and those related to acne rosacea. Systemic antibiotics are useful in children and in patients with acne rosacea. Topical steroids and immunomodulatory substances are especially important in patients with conjunctival and corneal involvement.

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Chapter 11

New Aspects on the Pathogenesis of Conjunctival Melanoma

11

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Core Messages

- Conjunctival melanoma is very rare, but occurs more frequently in white than in black individuals
- Conjunctival melanoma often occurs in the setting of primary acquired melanosis (PAM) with atypia and is less often associated with a conjunctival nevus
- Conjunctival melanoma shares many clinical, epidemiological and molecular features with cutaneous but not uveal melanoma
- It is plausible that the presence of clinically atypical nevus of the skin (particularly when in a familial setting and in association with cutaneous melanoma) is etiologically linked to conjunctival melanoma
- There is substantial evidence implicating ultraviolet radiation (UVR) as inducing conjunctival melanoma, probably through the mitogen-activated protein kinase pathway
- Because conjunctival melanoma occasionally arises in parts of the conjunctiva protected from UVR exposure, alternative pathways may exist

11.1 Introduction

11.1.1 Background

Conjunctival melanoma is distinctly uncommon and published data still often appear in the form of case reports or small case series. This melanocytic neoplasm causes death in 30% or more of the patients and the prognosis of the individual patient is often very difficult to predict. Although radiotherapy, and more recently topical chemotherapy, have emerged as successful adjunctive treatments, surgical excision remains the treatment of choice for most patients [77].

The phenotypic differences between conjunctival and uveal melanoma have long suggested that there are significant differences in the pathogenesis of these tumors. Given recent advances in the epidemiology and molecular biology of conjunctival melanoma, it now seems likely that the molecular mechanisms driving conjunctival melanoma tumorigenesis and tumor spread are more akin to cutaneous and mucous membrane melanoma than to melanoma arising in the uveal tract.

11.1.2 Conjunctival Melanocyte

The conjunctiva is a mucous membrane covering the pericorneal surface of the anterior part of the eye and the posterior surface of the eyelids. Except at the limbus and palpebral margin (where stratified squamous epithelium is present) the conjunctiva is lined by stratified columnar epithelium. Neural crest-derived melanocytes closely

resembling dermal melanocytes are lodged in the basal layers of the conjunctival epithelium. Each melanocyte contains an abundance of insoluble brown or black pigment called melanin that is formed by the enzymatic oxidation of tyrosine. The melanin pigment is confined to granules within the cytoplasm of each melanocyte. These melanin granules may be released by incontinent melanocytes to be engulfed by melanophages or even epithelial cells, causing such cells to appear pigmented, a defensive mechanism inducing tanning to protect against sun exposure [41, 59]. This event is more common in dermal melanocytes than in the less pigmented conjunctival melanocytes, as the conjunctiva (or uvea) typically does not tan. Interestingly, in vitro data suggest that uveal melanocytes lack expression of the alpha-melanocyte-stimulating hormone (alpha-MSH) and the alpha-MSH ligand, both of which are abundantly expressed in dermal melanocytes [51]. Although the alpha-MSH status of the conjunctival melanocyte is presently unclear, this may explain why most ocular tissues fail to increase their pigmentation in response to ultraviolet radiation (UVR).

In black people, the limbal pigmentation (clinically often referred to as ethnic or racial melanosis) is primarily due to melanin granules transferred from basal melanocytes to epithelial cells. Similarly, the squamous cell neoplasia presenting in black people may appear pigmented due to an increased uptake of melanin by epithelial cells. In the conjunctiva, similar to the skin, the number of melanocytes is probably constant and the heavier pigmentation of black individuals is probably caused by increased metabolic activity in the melanocytes [39, 77].

11.1.3 Historical Setting

The first case report of a melanoma presenting in the bulbar conjunctiva is attributed to Benjamin Travers, who described the clinical features and surgical management in a treatise published in 1820 [91]. However, 15 more years elapsed before the close resemblance to that of cutaneous melanoma was recognized [52] and 32 more years until conjunctival melanoma was found to be composed of pigmented cells [21]. In the 1930s, Algernon Reese recognized the importance of what is currently termed primary acquired melanosis (PAM) of the conjunctiva as a frequent setting for conjunctival melanoma [69]. The confusing (and sometimes conflicting) nomenclature used for PAM over the decades to come probably contributed to the sometimes overzealous surgical management of PAM and conjunctival melanoma in the past [99]. The historical background of conjunctival melanoma and PAM has been covered in more detail elsewhere [77].

Summary for the Clinician

- Conjunctival melanoma is distinctly uncommon
- The preferred management is complete surgical excision
- The melanocyte is neural crest-derived and contains melanin granules
- The protective tanning phenomenon of the skin when exposed to sunlight does not occur in the conjunctiva

11.2 Precursor Lesions

11.2.1 Acquired Conjunctival Nevus

The common acquired nevus of the conjunctiva is much akin to the common acquired nevus of the skin. It is typically detected in early childhood or adolescence as a junctional nevus, later acquires a stromal component, loses the epithelial connection, and eventually involutes. Clinically, the conjunctival nevus is seen as a well-defined lesion, usually presenting in the juxtalimbal area, but sometimes appears in the semilunar fold or caruncle. The color often ranges from light tan to deep chocolate brown, but some 30% of lesions are nonpigmented. The bland-appearing nevus cells are arranged in cellular nests and typically lack cytological atypia. If an epithelial component is present, the nevus cells are characteristically present at the basal layer, the so-called junctional zone, only. Invasion of more superficial parts of the epithelium may herald an onset of malignant

transformation. Furthermore, conjunctival nevi occurring in the palpebral or forniceal conjunctiva are exceedingly rare and suggestive of malignant disease [14]. Clinically, cyst formation usually signifies a benign nevus, but epithelial inclusion cysts may occasionally occur associated with melanoma. Some rare nevus variants, which, even by histopathological examination may be confused with melanoma, include the spindle and epithelioid cell Spitz nevus [29, 45], the spindle cell nevus of Reed [78], and the deep, so-called blue nevus [45]. The features of the conjunctival dysplastic nevus have not yet been clearly defined [29]. Because these nevus variants are themselves very rare, malignant transformation is only reported in a few cases [24].

The natural history of conjunctival nevi includes an initial increase in size followed by regression and involution. A clinical series of 149 patients with a conjunctival nevus observed for a mean period of 11 years without intervention showed a change in color in 13% and a change in size in 8%, with malignant transformation occurring in only 3 patients. There were no apparent clinical features of conjunctival nevi that predicted later evolution into melanoma [82].

National surveys indicate that nests of nevus cells may appear within or adjacent to conjunctival melanoma in 17% of tumors [79]. Also, several well-documented cases suggest that malignant transformation of a clinically diagnosed conjunctival nevus might occur, even after periods of dormancy for 20–60 years [11, 23, 38, 87]. While compelling evidence clearly demonstrates that nevi may sometimes progress to melanoma, this seems to be a very rare event. In one series based on a histopathological national registry only 1 out of 343 (0.3%) conjunctival nevi showed malignant transformation [34]. In a series from a tertiary referral center, progression to melanoma occurred in 3 out of 410 patients with conjunctival nevi (0.7%) [82]. Because of the frequency of conjunctival nevi in the general population (not known, but probably within the range 1–3%) and the referral bias of lesions either submitted to a tertiary referral center or for surgical excision followed by histopathological examination, the true frequency of progression to melanoma is probably even lower (Fig. 11.1).

11.2.2 Primary Acquired Melanosis

The intriguing entity known as PAM is defined clinically as golden to chocolate-brown patches of pigmented conjunctiva. These patches may cover all aspects of the conjunctiva or be confined to one or more of the quadrants (Fig. 11.2). Typically, PAM features a "waxing and waning" course over time. Sometimes, PAM (in particular if treated by cryotherapy) may appear "*sine pigmento*." This unpigmented subtype tends to blend imperceptibly with the normal conjunctiva and usually requires histopathological examination to be fully appreciated.

To completely assess the extent of PAM all parts of the conjunctival sac need to be examined and this requires upper eyelid eversion to

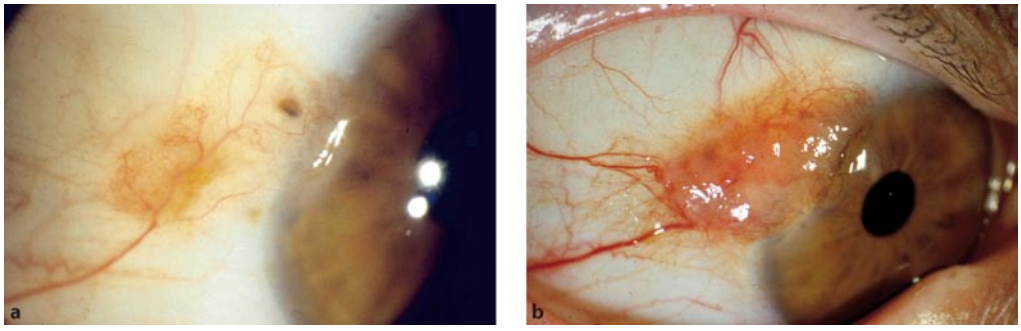

Fig. 11.1 Nevus-like lesion of the bulbar conjunctiva of a 40-year old woman. **a** Note clinically atypical features with lesion extending onto the corneal surface. **b** The patient returned 13 years later with progression of the lesion. Excisional biopsy then showed conjunctival melanoma in association with a nevus

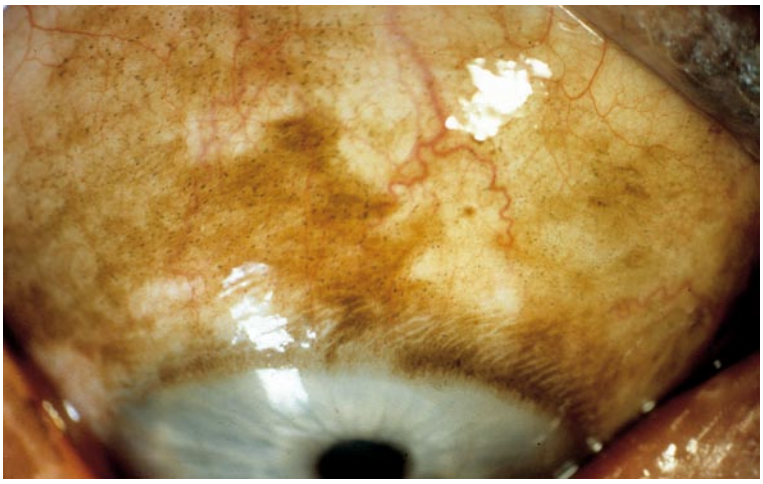

Fig. 11.2 Extensive primary acquired melanosis (PAM) of the conjunctiva in a 67-year-old man. Incisional biopsy showed PAM with atypia. The patient was followed up for 3 years and then developed a thickened nodular lesion. Biopsy confirmed progression to invasive melanoma

study the upper fornix. Once termed "precancerous melanosis" and believed to almost invariably progress to melanoma, PAM is now recognized to include two subtypes with very different risks of malignant transformation: PAM with and without atypia [28]. Both subtypes include melanocytic hyperplasia of the basal layer of the conjunctival epithelium. The atypia, when present, may be further characterized as cytological atypia, structural atypia (disorderly appearance of melanocytes in the superficial epithelium) or, more frequently, a combination of both (Fig. 11.3). The distinction between PAM with and without atypia by definition requires a biopsy followed by histopathological examination. A cytological examination will fail to detect structural atypia and cytological atypia confined to the deeper parts of the epithelium is unlikely to appear in a cytological sample. Histopathologic studies have shown PAM with atypia to occur in association with conjunctival melanoma in 71% of tumors (Fig. 11.4) [79].

Primary acquired melanosis with atypia progresses to melanoma in approximately half of patients, with a median interval of 2.5 years to the formation of an invasive tumor [28]. Lesions with high tumor cell cycling activity are more likely to progress to melanoma than PAM with atypia with a low proliferative rate [75]. It is conceivable that lesions featuring extensive atypia are more likely to progress than lesions with minimal atypia confined to the basal epithelial layer [28]. The proportion of PAM without atypia that eventually develops atypical features is not known, but this is probably an infrequent event as PAM has been reported in proportions as high as 10–36% of controls [35, 80]. It seems highly unlikely that PAM without atypia will progress to melanoma without entering an intermediate phase of atypical melanocytes appearing within the epithelium (PAM with atypia).

11.2.3 Other Potential Precursor Lesions or Entities

Dysplastic nevus syndrome (DNS) is usually defined as clinically atypical nevus of the skin occurring in a sporadic or familial setting, and is sometimes associated with hereditary cutaneous melanoma. In the latter form it is often referred to as the familial atypical mole melanoma (FAMM) syndrome. Individuals with DNS in a setting in which two or more family members have dysplastic nevi and cutaneous melanoma are at a life-time risk of developing skin melanoma ap-

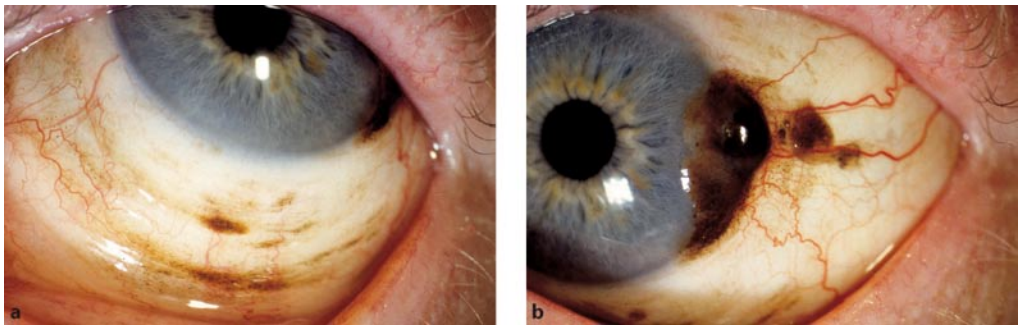

Fig. 11.3 **a** Primary acquired melanosis (PAM) of the lower bulbar conjunctiva in a 57-year old man. **b** A nodular pigmented lesion was present in the temporal part of the bulbar conjunctiva. Histopathological examination after surgical excision showed invasive melanoma

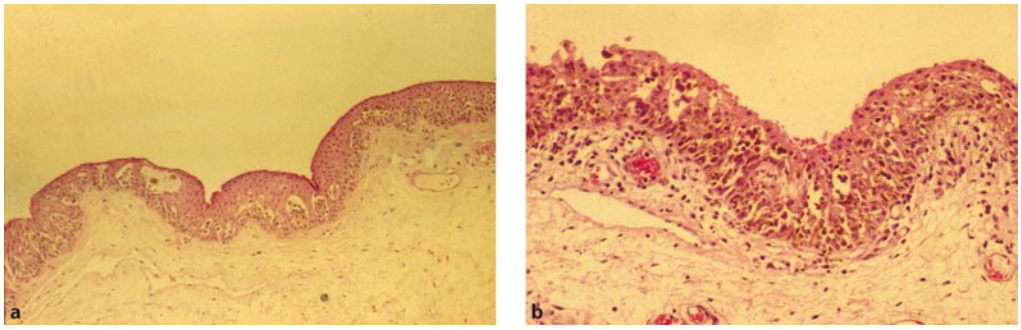

Fig. 11.4 Microphotographs of conjunctiva showing PAM with atypia. **a** Pagetoid intraepithelial growth is present in the left part and basal melanocytic hyperplasia of atypical melanocytes in the right part of the lesion. **b** The other lesion features extensive intraepithelial full-thickness growth of atypical melanocytes corresponding to melanoma in situ

proaching 100% [47]. Conversely, individuals featuring one or more sporadically occurring dysplastic (clinically atypical) cutaneous nevi are at a much lower risk of developing skin melanoma as some 18% of the population is expected to feature dysplastic nevi of the skin [5].

The first case of sporadic DNS (then referred to as the B-K mole syndrome) associated with conjunctival melanoma dates back to 1961 [48]. Since then, a few case reports and small case series of conjunctival melanoma or PAM occurring in multiple family members or in the setting of DNS/FAMM have been published [6, 7, 32, 63, 95]. One case-control study using hospital-based controls suggested that clinically atypical skin nevi occur more frequently in patients with either uveal or conjunctival melanoma [8]. Another case-control study compared individuals with DNS/FAMM and an estimated life-time risk of developing skin melanoma approaching 100% with population-based controls, but failed to find more conjunctival pigmented lesions in individuals with DNS/FAMM [80]. In this study, all detected conjunctival lesions were biopsied, but no histopathologically atypical lesions were present. Two case-control studies reported that conjunctival nevi are more prevalent in patients with sporadic DNS, but the clinical implications of this are unclear as the overwhelming majority of conjunctival nevi will never progress to melanoma [71, 90].

Thus, no conclusive evidence exists of an association between the presence of clinically atypical skin nevi, either in a sporadic or familial setting,

and conjunctival melanoma. The several case reports make an etiological link between DNS/FAMM and conjunctival melanoma plausible. Clearly, conjunctival melanoma is so rare that a prospective study designed to detect a casual relationship between DNS/FAMM and conjunctival melanoma would not be practical.

Patients with neurofibromatosis are prone to developing neural crest-derived tumors and a single case report documents conjunctival melanoma presenting in a patient with neurofibromatosis [89]. Again, conclusive evidence of a casual relationship is lacking and the finding may be co-incidental. Importantly, oculo(dermal) melanocytosis, a congenital disorder characterized by proliferation of episcleral and uveal melanocytes (and an increased risk of developing uveal melanoma) is not associated with conjunctival melanoma.

Summary for the Clinician

- The conjunctival nevus only very rarely transforms into melanoma
- The most common precursor of conjunctival melanoma is primary acquired melanosis (PAM) with atypia
- Half conjunctival lesions featuring PAM with atypia will transform into melanoma
- The distinction between PAM with and without atypia requires a histopathological examination
- Case reports but no conclusive evidence link the dysplastic nevus syndrome (DNS) and familial atypical mole melanoma (FAMM) syndrome to conjunctival melanoma

11.3 Epidemiology of Conjunctival Melanoma

11.3.1 Incidence of Conjunctival Melanoma

Population-based studies indicate that the incidence of conjunctival melanoma is within the range 0.024–0.08 per 100,000 [43, 62, 79, 93, 97]. While data derived from a central registry generally tend to be more reliable, data based on questionnaires or a local registry (assuming all conjunctival melanoma are filed with the registry) are more prone to bias. However, current data not derived solely from a central registry give an approximately similar incidence (Table 11.1) [60].

11.3.2 Age and Gender Incidence

There are no data indicating a significant gender difference in the incidence of conjunctival melanoma. Like cutaneous and uveal melanoma, conjunctival melanoma very rarely presents in children and young adolescents [2, 13, 55]. There is an approximately 2-fold rise in the average annual age-specific incidence from individuals 30–49 years of age to individuals 70 years of age or older at diagnosis [77].

11.3.3 Ethnic and Regional Incidence Rates

Skin melanoma is very much less frequent in black people than in white. Correspondingly, case reports have long underlined the rarity of a conjunctival melanoma occurring in a black individual [16, 19]. Details of the few reported cases with conjunctival melanoma occurring in black people have been summarized elsewhere [83]. Data now more than 30 years old estimate the annual incidence of conjunctival melanoma in black people as being 0.012 per 100,000 in the USA [74]; half or less than the incidence rate reported from largely white populations. Registry data indicate that in Singapore the number of patients with conjunctival melanoma roughly equals that of people with uveal melanoma (in an ethnically mixed population of Chinese, Malays, and Indians) suggesting that the regional and/or ethnical variation in incidence rate is even greater for uveal than for conjunctival melanoma [49]. Also, data from heterogeneous populations, including diverse ethnicity suggest that atypical features in conjunctival melanocytic hyperplasia typically occur in the white population only [17].

Table 11.1 Annual incidence of conjunctival melanoma per 100,000

Incidence	Period of time	Country	Reference
0.024	1969–1991	Sweden	[79]
0.04/0.03	1943–1997	Denmark	[43]
0.051	1967–2000	Finland	[93]
0.03/0.01–0.02/0.05	1974–1998	United States	[42]
0.04/0.04	1986–2000	United States	[57]
0.05*	1950–2002	The Netherlands	[60]

*Based on the assumption of 70% inclusion

Data based on regional differences (correcting for ethnic origin) are scarce. Recent population-based data indicate that combined eyelid and conjunctival melanoma incidence increases in non-Hispanic whites with increasing latitude, i.e., the further away from the equator, the higher the incidence. This would correlate with increasing sun light (and therefore UVR) exposure. However, when excluding eyelid melanoma (which are largely skin melanoma correlating with exposure to UVR) no statistical significant correlation with latitude remained [98].

11.3.4 Environmental Factors

Studies are very scarce and have usually focused on environmental factors associated with UVR exposure. Also, such studies are usually based on interviews and prone to recall bias. One recent case-control study failed to associate conjunctival melanoma with welding or the use of sunlamps [94].

11.3.5 Incidence Trends

Overwhelming evidence indicates that the incidence of skin melanoma is rising, with some 4–5% annually in the Western world, the increase largely being attributed to increased exposure to UVR. Studies recently published, essentially based on the same populations, show that the incidence of uveal melanoma is stable or perhaps even declining slightly [10], arguing against a causal relationship between UVR exposure and the development of uveal melanoma [84]. Similarly, the incidence of melanoma arising in the non-UVR exposed female genital tract is declining over the same period of time [67]. Interestingly, evidence is now accumulating that the incidence trend over time for conjunctival melanoma does not parallel that of uveal melanoma (or vulvar/vaginal melanoma), but appears to mimic the rising incidence of cutaneous melanoma. However, the rarity of conjunctival melanoma makes detection of time trends difficult and large populations will have to be observed over a significant period of time.

There has been a tendency for population-based studies from Scandinavia, derived from essentially similar white and ethnically homogeneous populations, to report lower incidence rates when based on earlier periods and higher incidence rates when including data based on later periods [43, 62, 79, 93]. Data from earlier periods may underestimate the true incidence because of ascertainment bias being more likely during the early years of cancer registries. Alternatively, the incidence of conjunctival melanoma remained essentially stable in the 1960s and 1970s, followed by a rise in later years [92].

The incidence rise in cutaneous melanoma in Western populations is paralleled by a similar increase in the incidence of conjunctival melanoma [93]. Other data suggest that conjunctival

melanoma incidence may have increased from 0.01–0.03 per 100,000 per year during 1974–1983 to 0.05 per 100,000 per year during the period 1989–1998 [42]. These studies have been supported by a large population-based survey identifying a statistically significant 2-fold increase in conjunctival melanoma appearing in white men over a period of 27 years. This rise was largely driven by an increase in the incidence among those over 60 years of age. For reasons unknown, no similar rise was seen in white women from the same population [97].

Moreover, registry data confirmed by histopatheological reevaluation of specimens now indicate a dramatic 7-fold increase in the annual age-standardized incidence of conjunctival melanoma in Sweden during the period 1960-2005 (Triay E. et al, upublished data).

Summary for the Clinician

- Conjunctival melanoma is very rare in children and adolescents
- There is no gender difference in conjunctival melanoma
- The incidence of conjunctival melanoma is higher in whites than in blacks
- The incidence of conjunctival melanoma (unlike uveal melanoma) appears to be increasing

11.4 Relationship to Melanoma Occurring in Other Species or Sites

11.4.1 Conjunctival Melanoma in Other Species

Conjunctival melanoma and atypical PAM have occasionally been reported to arise in animals like dogs [9, 25, 50], cats [12, 20], horses [54], and deer [73]. The few published case reports suggest that melanoma is in most species (as in humans) most frequently present in the bulbar conjunctiva, but that melanoma of the palpebral conjunctiva does occur [73].

11.4.2 Mucous Membrane Melanoma

Many mucous membranes harbor epithelial melanocytes, which are usually non-pigmented (similar to conjunctival melanocytes), but often increase their pigmentation in pathological conditions such as nevi, melanosis, and melanoma. Population-based data on approximately 62% of the US population and including patients diagnosed with melanoma during the period 1996–2000 indicate that melanoma occurring in a mucous membrane (including the conjunctiva) is very rare (Table 11.2) [57]. Data suggest that conjunctival melanoma is about as rare as anorectal melanoma or melanoma of the nasal cavity, but twice as common as melanoma of the accessory sinuses. Melanoma occurring in the genital tract is some eight times more common in women (i.e., melanoma of the vulva and vagina) than in men (i.e., melanoma of the penis). The trend of age-specific incidence is similar for cutaneous, ocular (largely uveal), and mucosal melanoma, with melanoma being more common in the elderly. Interestingly, the white/black incidence ratio is largest for cutaneous melanoma (melanoma being 16 times more common in white people), intermediate for ocular (largely uveal) melanoma (melanoma being 8–10 times more common in white people), and lowest for

Table 11.2 Proportion of melanoma occurring in various sites in 1996–2000 in the US population

Site	Proportion of all melanoma (%)
Skin	95.0
Uvea	3.4
Conjunctiva	0.24
Nasal cavity	0.19
Accessory sinuses	0.10
Oral cavity	0.12
Anorectal	0.22
Genital tract	0.58

Modified according to [57]

mucosal melanoma (melanoma being twice as common in white people) [57].

Histopathology reports indicate that most melanoma derived from mucous membranes lack a clear association with a nevus. Rather these tumors arise from a pigmented (or occasionally nonpigmented) spot (macula) typically featuring a radial growth phase lentiginous-like pattern [72]. Apparently, this pattern includes the proliferation of atypical melanocytes along the basal layers of an atrophic, epithelium. Although the different terminology used for atypical melanocytic proliferation arising in diverse mucous membranes is confusing, the overall histopathological pattern closely mimics the features of PAM with atypia seen in the conjunctiva. Oral mucosal melanoma and the lentiginous precursor premalignant melanocytic dysplasia arising from the oral mucosa share many histopathological features with melanoma and atypical PAM of the conjunctiva.

11.4.3 Cutaneous Melanoma

Cutaneous melanoma is usually divided into the lentigo maligna, superficial spreading, nodular, and acral lentiginous subtypes according to histopathologic criteria. The lentigo maligna melanoma subtype constitutes some 10% of all skin melanoma [15] and is the most common skin melanoma subtype on the face [61]. It typically occurs on chronically sun-exposed skin (often in the head and neck region) arising from lentigo maligna; a slowly progressive, variably pigmented macula. The rate of progression of lentigo maligna to invasive melanoma (lentigo maligna melanoma) is approximately 5% [96], but some lentigo maligna lesions probably enter an intermediate phase (referred to as melanoma in situ, lentigo maligna type) with a higher risk of transformation into invasive melanoma [88]. Interestingly, reports now claim that melanoma in situ, lentigo maligna type and lentigo maligna melanoma are the two most rapidly increasing subtypes of in situ melanoma and invasive melanoma in California. The reason for this is unclear, but this trend will eventually make the head and neck region (with a UVR exposure pattern similar to the bulbar conjunctiva) a more common site for cutaneous melanoma [86].

Lentigo maligna may be histopathologically defined as atypical melanocytic hyperplasia at the dermal–epithelial junction, typically appearing in atrophic epithelium and malignant melanoma in situ of the lentigo maligna type by in addition at least two of the following three criteria: pagetoid spread of atypical melanocytes, confluence of melanocytes replacing the epithelial basilar layers, and nesting of atypical melanocytes [88]. One or more of the above features is typically also seen in conjunctival PAM with atypia. The close association between lentigo maligna and PAM with atypia is further underlined by reports of conjunctival melanoma occurring in close proximity to previously excised lentigo maligna melanoma of the eyelid skin [40], by conjunctival melanoma being accompanied by pigmentation of the eyelid margin [70], and by spread of lentigo maligna onto the conjunctiva. It is important to recognize that conjunctival PAM without atypia does not carry a significant risk of malignant transformation and as such should be clearly distinguished from PAM with atypia [1, 30]. It is conceivable that PAM with atypia featuring several of the above histopathologic patterns carries a higher risk of subsequent progression to melanoma than PAM with atypia characterized by atypical melanocytic hyperplasia confined to the junctional zone, similar to lentigo maligna. Conjunctival PAM with atypia with high proliferative activity is at a higher risk of progression than lesions with lower cell cycling [76].

11.4.4 Uveal Melanoma

Uveal and conjunctival melanoma are often referred to as "ocular melanoma." This may erroneously suggest that melanoma of these two sites constitutes one single entity. In fact, there now appears to be a multitude of differences clearly separating uveal from conjunctival melanoma. Whereas uveal, conjunctival, and cutaneous melanoma largely share a similar protein expression pattern, the phenotype of conjunctival melanoma is closest to that of epithelioid cell cutaneous melanoma [44].

In the USA, uveal melanoma is approximately 12 times more common than conjunctival melanoma [42, 57] and the term "ocular melanoma" tends to act as a surrogate for uveal melanoma, as the comparatively low frequency of conjunctival melanoma will not significantly influence the findings. Whereas both uveal and conjunctival melanocytes are neural crest-derived, melanocytes of the uvea, but not the conjunctiva will have to migrate to the deeper layers of the mesoderm. Nevi are known to arise in both sites, but are probably more frequent in the uvea. Conversely, PAM, or a histopathologically similar lesion, does not occur in the uvea. In-transit metastases of primary melanoma have been reported from the conjunctiva, but not the uvea. The current concept holds that uveal melanoma exclusively spreads hematogenously and that metastatic spread is preferentially to the liver. Conversely, lymphatics are abundant in the conjunctiva, and melanoma dissemination from this site typically first presents in the draining regional lymph nodes and parotid gland [77].

Summary for the Clinician

- Conjunctival melanoma occurs in a number of species
- Equivalents of PAM are present in the extraocular mucous membranes
- The lentigo maligna melanoma is the most common skin melanoma subtype in the face and resembles conjunctival melanoma
- Lentigo maligna of the skin may extend onto the conjunctiva and appear as PAM
- Uveal and conjunctival melanoma are two separate entities

11.5 Molecular Events and Protein Expression in Conjunctival Melanoma

11.5.1 Mitogen-Activated Protein Kinase Pathway

Mutations in the serine/threonine kinase BRAF within the mitogen-activating kinase pathway may induce significantly elevated BRAF activity compared with wild-type BRAF. Postulated as largely induced by UVR, activating mutations of BRAF and N-RAS have also been reported in melanomas arising in tissues with minimal or no previous UVR exposure.

While mutations in the BRAF (and N-RAS) gene are present in a high proportion of skin melanoma [22], these mutations appear to be more frequent in skin melanoma occurring in sites with intermittent sunlight exposure compared with sites with chronic or no sun light exposure [66]. The most common activating BRAF mutation in skin melanoma is T1796A (T1799A) leading to substitution of valine with glutamic acid at codon 600 (V600E, formerly V599E) [66]. This BRAF mutation is notably absent in melanoma derived from UVR-sheltered mucosa [26].

Mutations of N-RAS have not been found in conjunctival melanoma [27], but BRAF mutations have recently been detected in a proportion of conjunctival melanoma, but not in uveal melanoma [33, 85]. It seems that the V600E substitution is present in approximately half of conjunctival nevi, but not in PAM with or without atypia and only in conjunctival melanoma occurring in association with nevi [36]. This is largely paralleled by a high frequency of activating BRAF mutations found in cutaneous nevi, a much lower prevalence of BRAF mutations in skin melanoma, and BRAF mutations in melanoma in situ being very rarely found [66], suggesting that skin nevi (and possibly conjunctival nevi) might require more genetic events than simply BRAF (or N-RAS) mutations to progress to melanoma. Also, it could be suggested that conjunctival nevi and PAM with atypia might progress to melanoma using different pathways. The molecular events driving PAM with atypia to progress to conjunctival melanoma remain unclear.

11.5.2 Mutation of the p53 Gene and p53 Protein Overexpression

It was once hypothesized that p53 protein overexpression could be used as a surrogate for p53 gene mutation based on the assumption that p53 gene mutation would result in p53 protein overexpression. Vulvar melanoma typically presents in minimally or non-UVR-exposed tissue, but the p53 protein expression pattern is similar to that in facial skin melanoma, which originates in chronically UVR-exposed tissue. More importantly, there is no concordance between detected mutations of the p53 gene and p53 protein expression in samples from melanoma originating from either of these two sites [68]. It is now clear that p53 protein overexpression occurring without p53 mutation is a common event in cutaneous and mucous membrane melanoma [37]. This suggests that the high p53 protein overexpression associated with progression of PAM with atypia to invasive melanoma and the increased expression of p53 protein after local recurrence of conjunctival melanoma reflect an aggressive phenotype rather than a unique molecular event [76].

11.5.3 Other Molecular Studies

The nucleoside diphosphate kinase (NPDK) is a ubiquitous enzyme produced as several isoforms (largely NPDK A and NPDK B) by expression of the NM23 genes. While NPDK A is involved in tumor metastasis, NPDK B acts as a transcription factor for c-myc. Loss of NM23 protein expression has been correlated with adverse prognosis in cutaneous melanoma and is potentially implicated in skin melanoma pathogenesis. However, the apparent lack of correlation with disease progression and prognosis in conjunctival melanoma suggests that NM23 protein is not directly involved in the pathogenesis of conjunctival melanoma [81].

Molecular studies have also attempted to explore other pathogenetic avenues. However, there is presently no evidence implicating hormonal influence [18, 31] or papillomavirus infection [56] in the pathogenesis of conjunctival melanoma.

Summary for the Clinician

- Activating BRAF mutations are present in conjunctival melanoma (but not uveal melanoma)
- No other molecular data indicate an alternative pathogenesis

11.6 Potential Pathogenetic Pathways

11.6.1 Sunlight and UVR Exposure

The UVR is a part of the electromagnetic spectrum, including radiation, with a wavelength of 100–400 nm, typically subdivided into UVR type A (315–400 nm), UVR type B (280–315 nm), and UVR type C (100–280 nm). Exposure to solar UVR, in particular UVR type B, has been postulated to cause as many as 90% of new cases of cutaneous melanoma in Western populations [4]. Much epidemiologic data suggest that intermittent exposure to solar UVR during childhood is probably more important than chronic exposure as an inducer of skin melanoma. There is also convincing evidence that skin melanoma incidence is significantly higher in white people of high-sunlight regions than in those of low-sunlight regions [65].

Patients with xeroderma pigmentosum are prone to developing UVR-inducible tumors and case reports have documented atypical PAM [64] or conjunctival melanoma [3, 46, 58] in patients with xeroderma pigmentosum. This implicates UVR as causing not only cutaneous but also conjunctival melanoma. As outlined above, recent epidemiologic data indicate that conjunctival melanoma and cutaneous (but not uveal or vulvar) melanoma share the same incidence trend and are becoming more frequent. Also, much data suggest that conjunctival and cutaneous melanoma might have many of the molecular events indicating disease progression in common

and therefore might arise via similar pathways, in particular the mitogen-activated kinase pathway. This is supported by consistent clinical reports that conjunctival melanoma most frequently arises from the bulbar portion of the conjunctiva, the part of the conjunctiva that receives the highest UVR exposure.

Conjunctival melanoma is of particular interest as a model for melanoma induced by UVR exposure because the conjunctiva includes parts with significant exposure to ambient UVR (most of the bulbar conjunctiva) as well as parts that by definition receive virtually no UVR (tarsal and forniceal conjunctiva). This dual feature makes the conjunctiva unique among tissues known to give rise to melanoma. Even the iris will have some UVR filtered by the cornea and melanoma occurring in other mucous membranes (e.g., those lining the vulva and vagina) originate in tissues with exclusively minimal (if any) previous UVR exposure.

11.6.2 Alternative Pathways

Whereas BRAF mutations are frequent in skin melanoma, arising in sites with intermittent sun exposure, such mutations also appear in melanoma from cutaneous and mucous membrane sites relatively (or sometimes completely) protected from exposure to UVR [53]. This is evidence indicating that activating BRAF mutations may occur through mechanisms other than (intermittent) UVR exposure.

The similar histopathologic features of PAM with atypia and the radial growth phase, the lentiginous-like pattern seen in melanoma derived from other mucosal membranes, suggest that the pathogenetic mechanisms might be similar. Because nearly all melanoma of a mucosal membrane (except melanoma of the bulbar conjunctiva and the lip) originate from a tissue that is UVR protected, other mechanisms of tumor induction are probably important for a subset of conjunctival melanoma. Such mechanisms may or may not include activating BRAF mutations.

Summary for the Clinician

- Epidemiologic and molecular data suggest that conjunctival and cutaneous melanoma share similar pathogenetic pathways implicating ultraviolet radiation (UVR) as the causative agent
- Because part of the conjunctiva is exposed to UVR and part is protected from UVR, alternative pathways should exist for a subset of conjunctival melanoma

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Chapter 12

In Vivo Confocal Microscopy in Healthy Conjunctiva, Conjunctivitis, and Conjunctival Tumors

12

Elisabeth M. Messmer

Core Messages

- In vivo confocal microscopy allows the visualization of the ocular surface at the cellular level
- With near infra-red laser scanning in vivo confocal microscopy, opaque structures such as the conjunctiva and the lid margin can be scanned in addition to the transparent cornea
- This new technique does not only permit imaging of the known anatomy, but is also able to disclose specific signs in conjunctival inflammation. It allows the differentiation and documentation of papillary and follicular conjunctivitis, acute and chronic inflammation, cicatrization as well as granuloma formation
- Benign and malignant tumors of the conjunctiva show typical features under in vivo confocal microscopy, which may facilitate decision-making and biopsy in these patients
- Atypical epithelial cells may be demonstrated in conjunctival intraepithelial neoplasia
- Melanocytic lesions of the conjunctiva can be clearly differentiated, and the diagnosis of melanoma can be established with high sensitivity and specificity by an experienced examiner
- However, due to limited knowledge in the field and a small scanning area, in vivo confocal microscopy is no substitute for the gold standard, histology, and a biopsy is required in clinically or in vivo microscopically suspect lesions of the conjunctiva

12.1 Introduction

Microscopic evaluation of ocular structures has always been a challenge for ophthalmic clinicians and researchers. Confocal microscopy, first described by Minsky in 1957, allows in vivo examination of the human ocular surface at the cellular level [17, 18, 20]. The fact that both the illumination (condenser) and observation (objective) systems are focused on a single point (are "confocal"), dramatically improved axial and lateral resolution.

Several principles are realized in confocal microscopes: tandem scanning, scanning slit, and near-infrared laser scanning confocal microscopy. Although it has high axial and transverse resolution, tandem scanning confocal microscopy is not able to visualize specific structures in the cornea such as basal epithelial cells due to its low light throughput [1]. Scanning slit confocal

microscopy allows high quality imaging of the cornea, but is mainly limited to scanning transparent tissues due to its halogen light source. In near-infrared in vivo confocal microscopy a pinpointed laser with consistent light intensity is used to scan the tissue, directly through an objective microscope lens. The reflected light is collected through a confocal receiver and used to construct a digital image in an area of 400×400 µm. In addition to high resolution imaging of the transparent cornea, laser scanning confocal microscopy allows the visualization of opaque structures such as the tongue, the conjunctiva, and the lid margin [10, 16–18, 25].

In contrast to conventional light microscopes for histological analysis where transverse sections are the norm, confocal microscopy provides a coronal optical section through the structure examined. However, slightly oblique sections may also be obtained and may add valuable information.

12.2 In Vivo Confocal Microscopy of the Ocular Surface

12.2.1 In Vivo Confocal Microscopy of the Cornea

In the 1990s, the first confocal images showed optical sections of the corneal epithelium with superficial, wing, and basal epithelial cells, Bowman's layer, the corneal stromal keratocytes, and the endothelial cell layer of excised human eyes and human eyes in situ [2, 3, 9, 13, 14]. Besides the study of normal corneal anatomy, an important clinical application of confocal microscopy has been the early detection and diagnosis of infectious conditions, including *Acanthamoeba* [15, 22] and fungal keratitis [4, 26]. Moreover, confocal microscopy was used to evaluate corneal wound healing following refractive surgery and penetrating keratoplasty [21].

12.2.2 In Vivo Confocal Microscopy of the Conjunctiva

In vivo confocal images of the healthy bulbar and tarsal conjunctiva, the lid margin and of conjunctival disease were only published recently[12, 17]. For scans of the bulbar and tarsal conjunctiva, and the lid margin, the patient's head has to be positioned in the head rest according to the area of examination. A fixation tool should be offered for steady gaze. Near-infrared in vivo confocal microscopy allows visualization of the conjunctiva up to 200 µm in depth from the epithelial surface of the conjunctiva [17].

In vivo confocal microscopy is able to aid in the differential diagnosis of conjunctival inflammation and has proved to be helpful in the differential diagnosis of conjunctival tumors.

12.2.2.1 Normal Bulbar Conjunctiva

The anatomy of normal conjunctival epithelium composed of superficial, intermediate, and basal cells can be demonstrated by in vivo confocal microscopy. Large to giant hyporeflective round- to oval-shaped cells with a nucleus displaced peripherally or a central pore, sometimes crowded in groups, are visible throughout the epithelium. These cells are probably goblet cells (Fig. 12.1). The fact that goblet cells may be observed with in vivo confocal microscopy is of utmost impor-

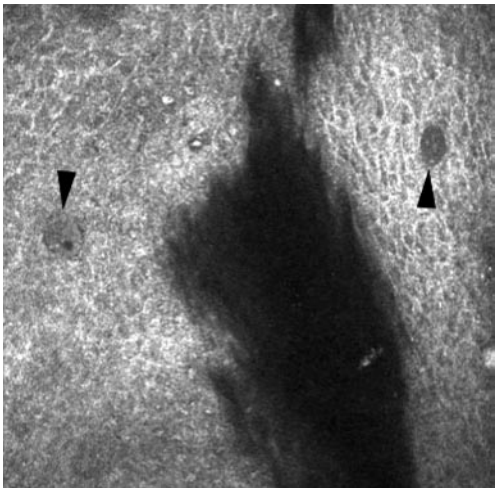

Fig. 12.1 Conjunctival epithelium with presumed goblet cells (*arrowheads*)

tance for the in vivo diagnosis of conjunctivalization associated with corneal stem cell deficiency.

In the basal epithelial layer hyper-reflective dendritic cells are to be seen.

Subepithelially, the basement membrane appears as a prominent hyper-reflective band with multiple inserting fibers. In contrast to in vivo confocal microscopy of the cornea, where stromal collagen fibers are not visible under normal conditions, the dense irregular collagen network of the conjunctival stroma appears hyper-reflective and clearly visible in healthy and diseased tissue [17]. Single fibroblasts may not, however, be detected in healthy conjunctival stroma, but are visible on scans of filtering blebs after glaucoma surgery [19]. The stroma contains perfused vessels, cystic spaces often in close association with vessels –probably lymphatic channels – and single inflammatory cells. The episclera and sclera are too deep to be visualized with in vivo confocal microscopy.

At the corneal limbus the conjunctival epithelium and stroma form the well-known palisades of Vogt with associated vessels and presumed lymphatics in vivo microscopically (Fig. 12.2) [11, 17]. Small pigmented granules and delicate dendritic cells are often visible in the basal epithelial cells.

12.2.2.2 Normal Tarsal Conjunctiva

The normal tarsal conjunctiva shows a thin epithelium composed of 2–3 layers of small, cuboidal epithelial cells under in vivo confocal microscopy. The epithelium is moderately infiltrated by inflammatory cells with a round hyper-reflective nucleus, probably lymphocytes. In contrast, the normal tarsal conjunctival stroma is heavily infiltrated by a round cell infiltrate (Fig. 12.3). Tiny subepithelial microcysts, often containing inflammatory cells, are a frequent finding [17].

12.2.2.3 Normal Lid Margin

At the lid margin, meibomian gland orifices, lashes, and adjacent glands of Zeis are visible by in vivo confocal microscopy (Fig. 12.4). Adenoid structures including meibomian gland ducts lined with a multilayered epithelium as well as gland acini appear at approximately 30 μm in depth in the upper and at about 80 μm depth in the lower tarsus. Sometimes hair follicles may be observed at a depth of 150–200 μm [17].

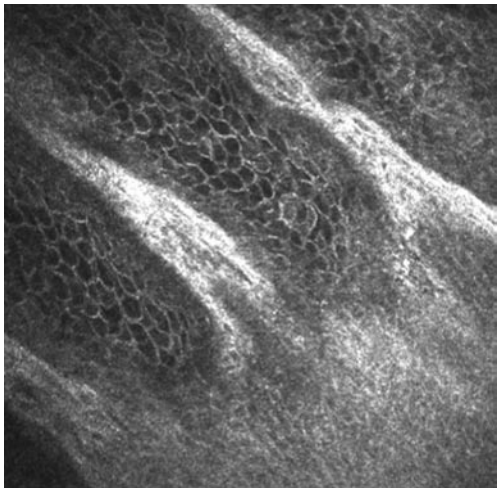

Fig. 12.2 In vivo confocal images of a normal limbus. Rete pegs of the limbus (palisades of Vogt)

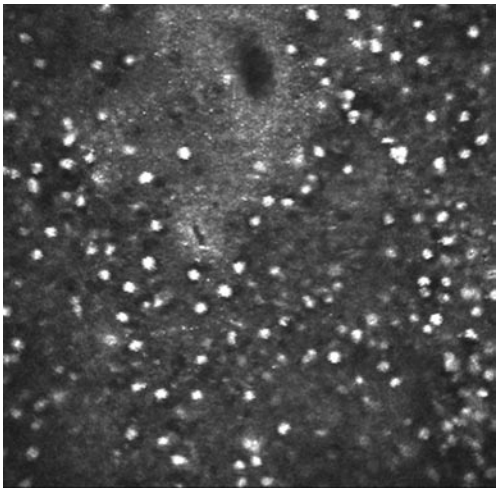

Fig. 12.3 In vivo confocal microscopy of the tarsal conjunctiva with subepithelial round cell infiltrate

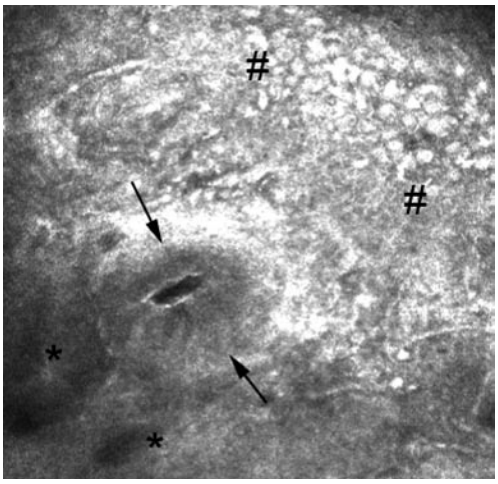

Fig. 12.4 Lid margin with skin , (*asteriks*), meibomian gland orifice (*arrows*) and tarsal conjunctiva (*hash*)

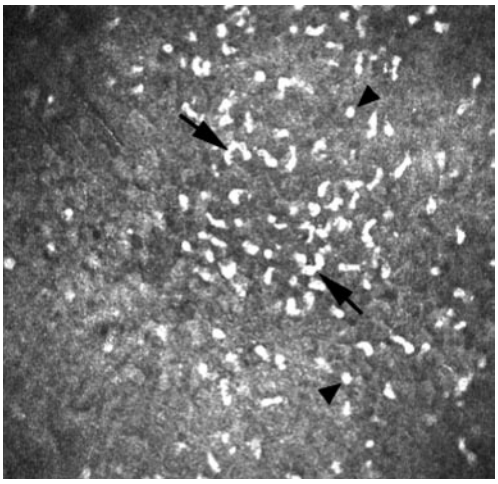

Fig. 12.5 In vivo confocal microscopy in acute conjunctivitis. Mixed cell infiltrate composed of round cells (*arrowheads*) and cells with multi-lobate nucleus compatible with neutrophils (*arrows*)

Summary for the Clinician

- Principles realized in confocal microscopes include tandem scanning, scanning slit, and near infrared laser scanning confocal microscopy
- All in vivo confocal microscopes permit high quality imaging of the cornea at the cellular level
- In vivo confocal microscopy using near-infrared laser light is, in addition, able to demonstrate the normal anatomy of the bulbar and tarsal conjunctiva as well as the lid margin

12.2.3 In Vivo Confocal Microscopy in Ocular Surface Inflammation

12.2.3.1 Acute and Chronic Conjunctivitis

In conjunctivitis the inflammatory infiltrate is significantly increased in the epithelium and stroma as observed by in vivo confocal microscopy. In acute conjunctivitis a mixed cellular infiltrate consisting of cells with round, hyper-reflective nuclei and cells with multi-lobate nuclei is present in the bulbar conjunctiva (Fig. 12.5). In chronic conjunctivitis the epithelial and stromal infiltrate is mainly composed of round cells and large cells with hyper-reflective nuclei and hyporeflective perinuclear halos. A rare dendritic cell can be found in between the inflammatory infiltrate. Small hyper-reflective round cells are observed to roll and adhere to vessel walls in the video mode images obtained from inflamed conjunctival stroma.

12.2.3.2 Papillary Conjunctivitis

Tarsal papillae can be seen in confocal images in contact lens wearers without clinically manifest giant papillary conjunctivitis and patients with atopic keratoconjunctivitis. They consist of a fibrotic core with a central vessel surrounded by inflammatory cells (Fig. 12.6).

12.2.3.3 Follicular Conjunctivitis

In follicular conjunctivitis a hyporeflective core containing rather hyporeflective round cells is surrounded by a hyper-reflective capsule and vessels (Fig. 12.7).

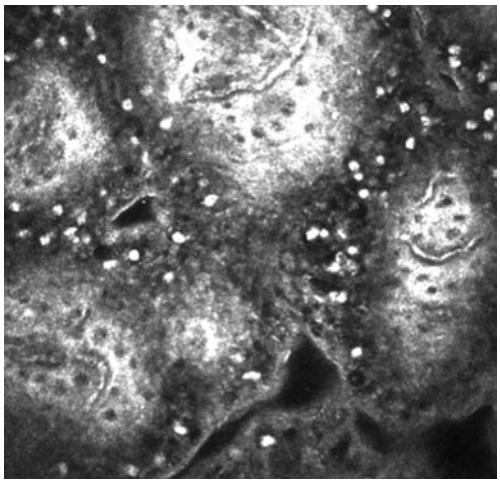

Fig. 12.6 Confocal images of papillary conjunctivitis demonstrating a fibrotic core with a central vessel surrounded by inflammatory cells

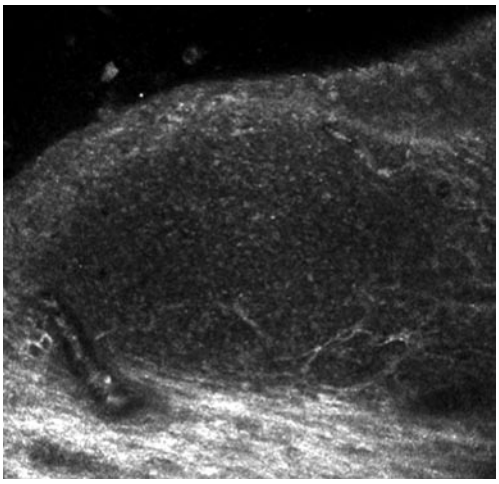

Fig. 12.7 In vivo confocal microscopy in follicular conjunctivitis. Follicle-containing hyporeflective round cells surrounded by a hyper-reflective capsule and vessels

12.2.3.4 Cicatrizing Conjunctivitis

In cicatrizing conjunctivitis, including trachoma, Stevens-Johnson syndrome, conjunctival lichen planus or severe atopic keratoconjunctivitis, a hyper-reflective fibrotic network is observed subepithelially (Fig. 12.8). In active inflammation fibrosis is associated with infiltration of rather monomorph, hyper-reflective round cells, whereas in atopic keratoconjunctivitis tarsal papillae are observed in addition to subepithelial scarring by in vivo confocal microscopy.

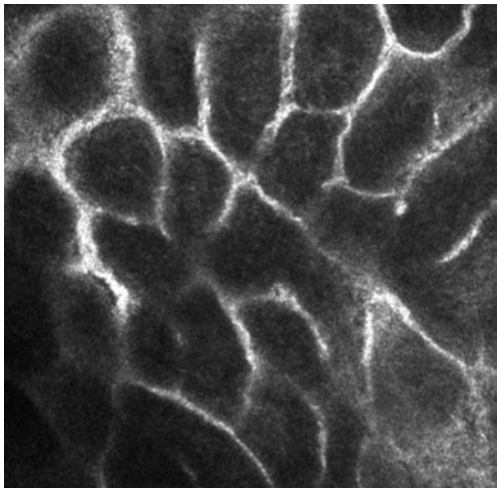

Fig. 12.8 In vivo confocal microscopy in cicatrizing conjunctivitis exhibiting subepithelial hyper-reflective nets of fibrosis

12.2.3.5 Conjunctival Granuloma

A conjunctival granuloma following pars plana vitrectomy exhibited surprisingly few inflammatory cells and moderate vascularization by in vivo confocal microscopy. A vermiform structure, most probably a Vicryl suture, was embedded in hyper-reflective, fibrotic tissue [17].

12.2.3.6 Blepharitis

Dysfunction of the meibomian glands with inflammation and obstruction has been suggested to be an important factor in the pathogenesis of chronic blepharitis. However, few objective tests are available to examine meibomian glands directly. Patients with anterior blepharitis, meibomitis, meibomian gland dysfunction or severe keratoconjunctivitis sicca associated with blepharitis were examined with near-infrared laser in

vivo confocal microscopy. Scans of the tear film, the tarsal conjunctiva, the hair follicles, and of the meibomian glands were analyzed. In patients with posterior blepharitis/meibomitis profound pathology was visible with dilatation and obstruction of meibomian gland ducts (Fig. 12.9). In 15 out of 19 patients with blepharitis/meibomitis, but not in meibomian gland dysfunction, intense inflammation was observed in the tarsal conjunctival epithelium and stroma. In all patients with blepharitis hair follicles appeared within normal limits indicating that changes in the lash follicles do not seem to play an important role in the disease process [16].

Summary for the Clinician

- The following inflammatory reactions of the conjunctiva and lid margin are clearly discernible by in vivo confocal microscopy:
  - Acute and chronic conjunctivitis
  - Papillary and follicular conjunctivitis
  - Cicatrizing conjunctivitis
  - Blepharitis/meibomitis and meibomian gland dysfunction

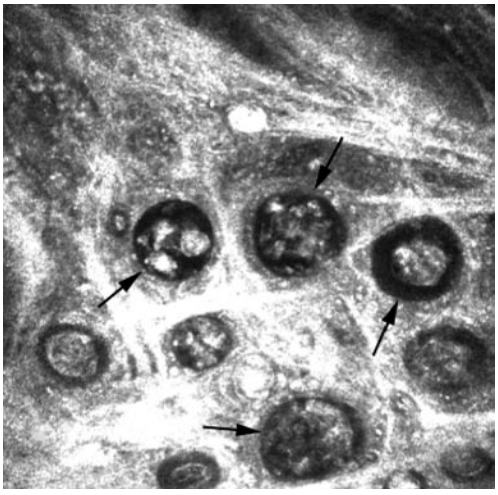

Fig. 12.9 In vivo confocal microscopy of blepharitis/meibomitis with obstruction of meibomian gland ducts (*arrow*)

12.2.4 In Vivo Confocal Microscopy in Epithelial Tumors of the Ocular Surface

12.2.4.1 Benign Epithelial Tumors

12.2.4.1.1 Pterygium/Pinguecula

Pterygia and pinguecula are clinically diagnosed correctly in the majority of cases [8]. Thus, additional clinical examinations are rarely necessary. The histopathologic hallmark of pterygia and pinguecula is elastotic degeneration of the collagen in the substantia propria. Often, there are associated secondary epithelial changes on the surface, including acanthosis, parakeratosis, or hyperkeratosis [24].

In vivo confocal microscopy reveals an irregular, partly acanthotic epithelium with goblet cells, thickened collagen fibers in the conjunctival stroma, prominent vascularization, and the deposition of amorphous, partly hyper-reflective stromal debris resembling calcium deposits.

12.2.4.1.2 Papilloma

Conjunctival papillomas are quite common acquired epithelial lesions. They have a relatively characteristic clinical appearance, are pedunculated or sessile, and may be located in the inferior fornix, the palpebral conjunctiva, the caruncle or the limbus. Histopathologically, the lesions are composed of papillary fronds of fibrovascular tissue covered with variably thick epithelium [24]. On in vivo confocal microscopy, papillomas show a papillary surface in oblique sections. The markedly thickened epithelium is infiltrated mainly by round cells and surrounds a fibrovascular stromal core (Fig. 12.10).

12.2.4.2 Malignant Epithelial Tumors – Conjunctival Intraepithelial Neoplasia and Squamous Cell Carcinoma

Conjunctival intraepithelial neoplasia (CIN) and squamous cell carcinoma typically oc-

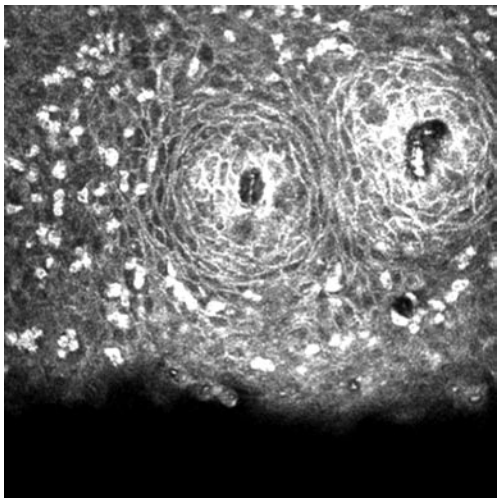

Fig. 12.10 In vivo confocal microscopy of conjunctival papilloma. Fibrovascular stromal core surrounded by a thickened epithelium and round cells

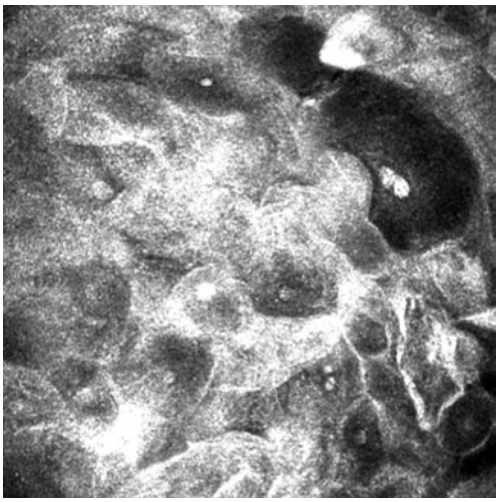

Fig. 12.11 In vivo confocal microscopy of conjunctival intraepithelial neoplasia demonstrating large dysplastic epithelial cells

cur in older individuals and are more common in men than in women. CIN most often arises in the interpalpebral fissure and appears as an elevated plaque or a gelatinous mass with fronds of blood vessels near its surface. Keratinization may be present. When the dysplastic cells break through the basement membrane of the basal epithelial layer and invade the substantia propria, the lesion is classified as a squamous cell carcinoma [8, 24]. The clinical diagnosis of squamous cell carcinoma may be difficult, as it has a spectrum of presentations, from subtle white dots corresponding to parakeratosis to a process that may mimic chronic conjunctivitis.

In CIN, in vivo confocal microscopy may demonstrate a keratinized hyper-reflective surface and an acanthotic epithelium with abnormally large, dysplastic epithelial cells featuring a prominent nucleus and nucleolus (Fig. 12.11). CIN is especially easy to evaluate in the area of the limbus and on the corneal surface. As the penetration of in vivo confocal microscopy in the conjunctiva is limited to about 200 μm, and keratinization may further restrict scanning, the demonstration of invasive growth in squamous cell carcinoma is difficult.

12.2.5 In Vivo Confocal Microscopy in Melanocytic Tumors of the Ocular Surface

Pigmented lesions of the conjunctiva such as nevi, primary and secondary acquired melanoses, and malignant melanomas present a challenge to both the ophthalmologist and the pathologist. The difficulty in clinical diagnosis is overwhelming. Whereas nevi were clinically diagnosed correctly only 49% of the time, melanoma was clinically suspected in 56% of the cases [8]. In addition, diagnosis is hampered by the fact that almost 30% of conjunctival nevi are clinically almost entirely non-pigmented [7]. Other pigmented conjunctival lesions such as primary acquired melanosis with and without atypia as well as secondary acquired melanosis of the conjunctiva may be clinically indiscernible as well. This may be deleterious given the fact that progression from PAM with atypia to malignant melanoma occurs in 75% of patients with epithelioid cells and in 90% of patients whose predominant growth pattern is not basilar hyperplasia [5–7, 23]. Laser confocal microscopy is able to differentiate between distinct melanocytic conjunctival lesions in vivo. Although in comparison to histology the magnification and the window of imaging of in vivo

confocal microscopy are limited, the examination of several different areas of interest provides a satisfying overall picture.

12.2.5.1 Benign Melanocytic Tumors

12.2.5.1.1 Nevus

In vivo confocal microscopy of conjunctival nevi demonstrates typical histological features such as stromal nests and/or diffuse collections of stromal, medium-sized, uniform cells that may be hyper-reflective (corresponding to pigmented nevus cells) or hyporeflective (representing amelanotic nevus cells). In compound nevi, these nests may be observed in conjunctival epithelium and stroma. Moreover, stromal pseudocysts with multilayered nonkeratinized epithelium are routinely present. They may be filled with amorphous substance or cellular debris (Fig. 12.12). Small dendritic cells are a rare feature seen in conjunctival nevi [18].

12.2.5.1.2 Primary Acquired Melanosis with/without Atypia

Hyper-reflective cells, granules and patches confined to the basal epithelium are a typical sign of PAM without atypia or secondary acquired melanosis (complexion-associated melanosis) as seen on in vivo confocal microscopy. This is especially evident in oblique images. Small hyper-reflective dendritic cells are often observed in the basal epithelium (Fig. 12.13). In contrast, in PAM with atypia hyper-reflective cells, granules and patches are typically observed throughout the entire epithelium. Most of these lesions extend into the corneal epithelium with highly reflective granules visible in basal corneal epithelial cells. However, the hallmark of PAM with atypia is networks of giant, hyper-reflective, dendritic cells (Fig. 12.14). These large dendrites were not observed in any benign melanocytic lesion of the conjunctiva, but were associated with melanomas arising from PAM with atypia [18].

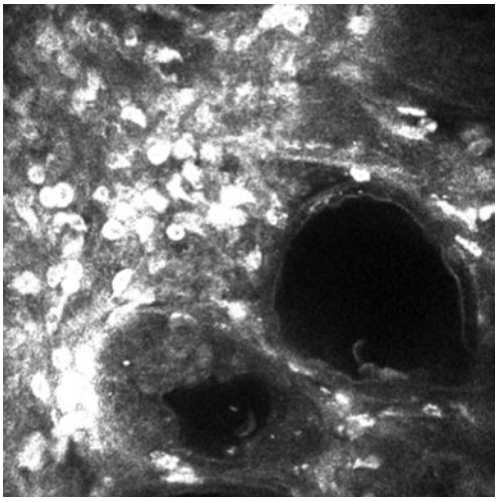

Fig. 12.12 In vivo confocal microscopy of conjunctival nevus demonstrating epithelial pseudocysts and nests of hyper-reflective and hyporeflective medium-sized, monomorph cells compatible with nevus cells

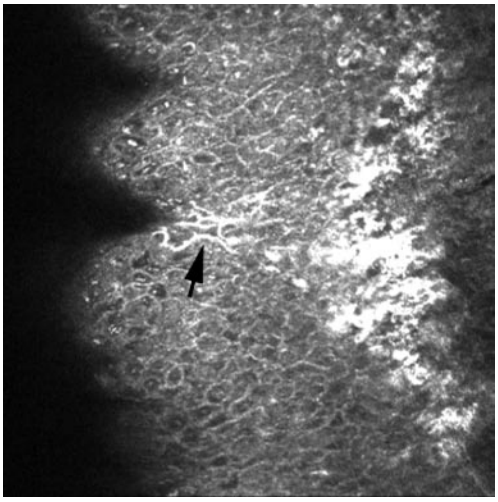

Fig. 12.13 In vivo confocal microscopy of primary acquired melanosis without atypia. Hyperreflective granules in basal limbal epithelium associated with small dendritic cells (*arrow*)

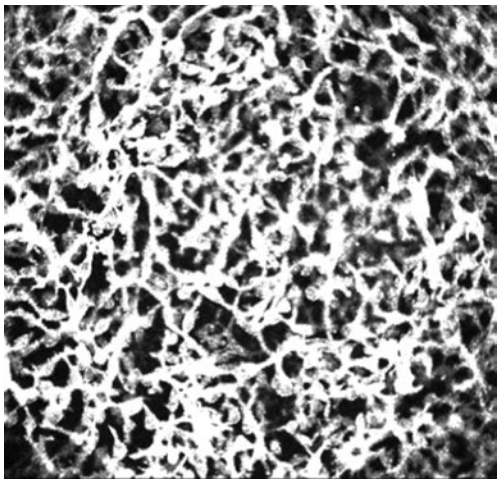

Fig. 12.14 In vivo confocal microscopy of primary acquired melanosis with atypia showing arborizing networks of giant dendritic cells

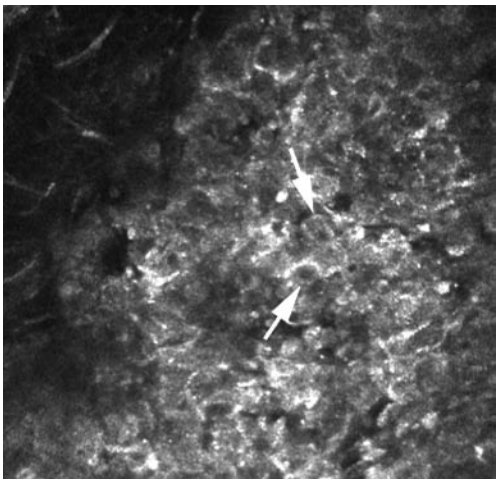

Fig. 12.15 In vivo confocal microscopy of malignant conjunctival melanoma demonstrating cells with large and atypical hyper-reflective nuclei and prominent hyporeflective nucleoli (*arrows*)

12.2.5.2 Malignant Melanocytic Tumors – Malignant Melanoma

Malignant melanomas of the conjunctiva are typically covered by a thin epithelial layer as seen by in vivo confocal microscopy. In pigmented lesions, highly reflective cells are visible directly subepithelially. The visibility of detailed cellular structures, however, is improved in deeper, less reflective scans. In amelanotic lesions the identification of tumor cells is facilitated by overall lower reflective images. The lesions contain accumulations of cells with large and atypical hyper-reflective nuclei and prominent hyporeflective nucleoli (Fig. 12.15). However, typical spindle or epithelioid cells, and/or a fascicular tumor formation, cannot be discriminated by in vivo confocal microscopy. Tumor cells are often surrounded by small, highly reflective round cells, most probably inflammatory cells. Multiple large vessels are evident in the lesion. In vivo confocal microscopy showed a sensitivity of 89% and a specificity of 100% to diagnose malignant melanoma of the conjunctiva correctly. The positive and negative predictive values for diagnosing conjunctival melanoma were 100% and 5% respectively. The area under the receiver operating characteristic (ROC) curve was 96% [18].

Typical features of malignant melanoma could also be observed in patients with extrascleral growth of uveal melanoma by in vivo confocal microscopy [18].

12.2.6 In Vivo Confocal Microscopy in Other Lesions of the Conjunctiva

12.2.6.1 Conjunctival Amyloidosis

Conjunctival amyloidosis most often occurs sporadically as a primary localized deposition in the absence of antecedent or coexisting adnexal disease [24]. In vivo confocal microscopy of a patient with conjunctival amyloidosis disclosed large hyper-reflective areas and bands with net-like appearance as well as hyporeflective amorphous substance in the conjunctival stroma (Fig. 12.16). These observations are compatible with the diffusely distributed, eosinophilic, homogeneous, birefringent material seen in the conjunctival stroma on histology.

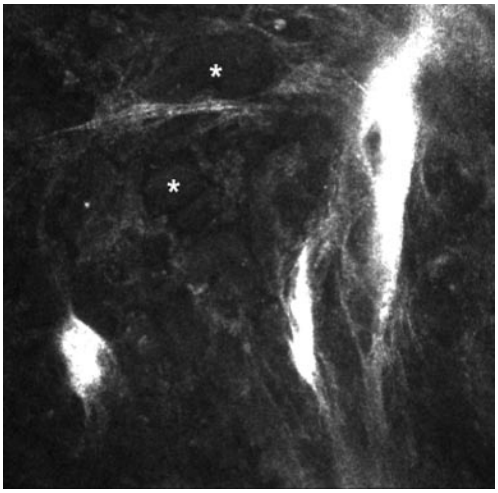

Fig. 12.16 In vivo confocal microscopy of conjunctival amyloidosis with hyper-reflective band-shaped and hypo-reflective amorphous (*asterisks*) stromal substance

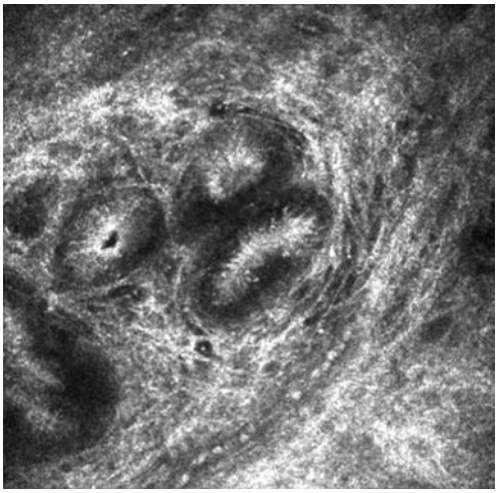

Fig. 12.17 In vivo confocal microscopy of a limbal dermoid demonstrating tightly packed collagenous tissue and adenoid structures

12.2.6.2 Limbal Dermoid

Epibulbar dermoids arising within the limbal and canthal portions of the conjunctiva are classified as choristomas because they contain displaced epithelial and dermis-like elements normally not found in these areas. On histology, these lesions may be either solid, well-defined limbal dermoids, more diffuse dermolipomas or complex choristomas with variable contents. Limbal dermoids typically demonstrate a dense collagenous stroma with hair follicles, sweat, and sebaceous glands [24]. In vivo confocal scans may show tightly packed collagenous tissue with vascularization, and adenoid structures (Fig. 12.17). In dermolipoma, hyporeflective spaces, most probably adipose tissue, are a prominent feature seen by in vivo confocal microscopy.

Summary for the Clinician

- In vivo confocal microscopy is a valuable new tool in the differential diagnosis and follow-up of conjunctival tumors
- Benign and malignant epithelial and melanocytic lesions of the conjunctiva show typical in vivo confocal microscopic features similar to histopathology, and can be clearly differentiated
- In vivo confocal microscopy exhibits a high sensitivity and specificity to diagnose melanocytic tumors of the conjunctiva compared with the gold standard histology
- The use of the in vivo confocal microscope is encouraged as an important diagnostic advice; yet, clinically and/or in vivo microscopically suspect lesions require biopsy. However, biopsy may be guided by the added information gained by in vivo confocal microscopy

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