

Foundations of Corneal Disease

Past, Present and Future

Kathryn Colby
Reza Dana
Editors

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 Springer

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To Claes H. Dohlman, MD, PhD, in recognition of his many contributions to the field of Cornea and the many students of the field whose lives were made richer in every way by his mentoring and leadership.

Foreword

The art of teaching is the art of assisting discovery.

—Mark van Doren

To write the Foreword to a book dedicated to Claes Dohlman is a distinct honor. Every generation of our profession has been blessed with one or two brilliant exponents of our discipline whose persona and scientific contributions have put a stamp on the age and whose teaching, as in the words of poet Mark van Doren, has catalyzed discovery. Our generation has been gifted with the life and career of Professor Dohlman, certainly one of the preeminent exponents of contemporary ophthalmology.

Reading through this book with contributions by his eminent trainees is a testimony not only to the science that he has inspired but also to the great humanity of this modest giant. The 32 chapters in this important volume highlight Dr. Dohlman's contributions to research, illustrate the accomplishments of his students, and demonstrate the reverence felt for Claes Dohlman as a teacher, as a determined scientist, and as a kind and nurturing human being.

It is true that the legacy of Claes Dohlman is one that will reverberate through our specialty for many decades to come, as now the fifth generation of Dohlman trainees (direct or indirect) has emerged. I, for example, never trained at the Massachusetts Eye and Ear Infirmary. But my professional mentor was trained by one of Dr. Dohlman's fellows, and so, in essence, I am the professional counterpart of a "great grandson." This legacy is repeated again and again, such that almost every specialist in our field can trace his or her educational origins to the founder of the first Cornea Service in the United States.

Dr. Dohlman has been an "enabler," inspiring his students to couple excellence in patient care with determined and rigorous scientific inquiry and innovation. The contributors to this important volume exemplify the results of his efforts. And well into an age when most people are retired and are no longer engaged in the profession, Claes Dohlman continues tirelessly to inquire and inspire. We owe him a collective debt of gratitude for his numerous contributions to our profession and for his exemplary mentorship.

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Part I

Introduction & Historic Perspective

Reflections of a Dohlman Fellow

1

Kathryn Colby

“Call Me Claes”

I heard this phrase used to describe a first experience with Claes H Dohlman (CHD) numerous times as I sat in the audience of the Biennial Cornea Congress in Boston in October 2017. The second day of the congress was a celebration of Claes’ sixtieth anniversary at the Massachusetts Eye and Ear Infirmary (MEEI), with talks given by former Dohlman fellows, many of whom took an opportunity to weave their personal experiences with Dr. Dohlman into their speeches. Dr. Dohlman’s collegial disposition, especially with those lower on the academic totem pole, was apparent in many ways, but certainly none was more memorable than his insistence that his fellows address him by his first name. Of course, none of us did, at least for several years after our fellowships were completed. For me, even years after, it still felt uncomfortable to address the great man as anything other than Dr. Dohlman. As I sat in the audience that day, I was again struck by the tremendous contributions Claes had made to cornea, and to ophthalmology, over seven decades. What better way to honor the person who started the first corneal fellowship than a textbook on corneal disease, with chapters writ-

ten by his former fellows (and several close colleagues) on their areas of expertise.

The Harvard Medical School (HMS) Department of Ophthalmology’s website provides a succinct biography of Dr. Dohlman (<https://eye.hms.harvard.edu/claesdohlman>). Born in Uppsala, Sweden, and educated in Lund, Claes was recruited to Boston in 1958. The stint in Boston was originally planned to be 3 years, followed by a return to his homeland. This, of course, did not happen; instead Claes stayed in Boston, founded the Cornea Service at MEEI (the first of its kind), and started the first cornea fellowship (reviewed in Chap. 2 by James Aquavella, the first Dohlman fellow).

I had an opportunity to speak to Dr. Dohlman in the fall of 2018 about his career as part of the preparation for this chapter. Interested readers may wish to view the entire 30-minute interview online <https://youtu.be/zGmlURhzdVc>. Modest to a fault, Claes downplayed his contributions, but he did tell me that building the Cornea Service was one of his most treasured accomplishments. “Cornea was virtually a vacuum. There were some very good clinicians who did very good work on the cornea, but there was no structure to it. There was no teaching, no research. So I happened to fill in that gap. To my great surprise patients and referrals came by the hundreds and fellow applicants came.”

The Cornea Service at MEEI is proven to be a fertile training ground for fellows, producing a

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host of luminaries, many of whom have participated in this book. Early trainees who stayed on the Cornea Service as faculty to train subsequent generations of corneal fellows include herpes doyenne Deborah Pavan-Langston (Chap. 6), master corneal surgeon Kenneth Kenyon (Chap. 13), and immunologist extraordinaire C. Stephen Foster (Chap. 12).

Dr. Dohlman was promoted to HMS professor of ophthalmology in 1974, the same year he was appointed chairman of the department. After a 15-year tenure as chair, Dr. Dohlman returned to clinical practice and focused his research efforts on the refinement of the Boston KPro. Now, at the tender age of 96, Claes still works full-time, continuing research on his labor of love – the Boston KPro, and still providing advice and inspiration for our field. Included in this chapter

are photos of Dr. Dohlman throughout the years, submitted by chapter authors from this volume (Figs. 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 1.10, 1.11, 1.12, 1.13, 1.14, 1.15, 1.16, and 1.17).

“Do You Speak French?” In the Operating Room (OR) with Dr. Dohlman

Claes Dohlman, a generous, skillful and infinitely patient surgical mentor, instilled the importance of training the next generation of surgeons in all of us by his example. As a fellow, I remember only one case where Claes did an entire keratoplasty himself. The patient was an international VIP whose initial transplant had been done by Castroviejo, with a signature square graft. The



Fig. 1.1 (a) Claes Dohlman seeing patients in clinic 1 (late 1960s). (b) Claes Dohlman seeing patients in clinic 1 (late 1960s). (c) Claes Dohlman in the operating room (late 1960s). (Contributed by Deborah Pavan-Langston)

Fig. 1.2 Cornea Service group photo, 1980. (Contributed by Shigeru Kinoshita)



Fig. 1.3 Cornea Service group photo, 1986. (Contributed by Kazuo Tsubota)



patient was to return to his home country immediately after the surgery. Claes looked at me with some hesitation and said, “I had better do this one myself.” Of course, it was a treat for me to watch CHD operate – because in reality, he typically watched while the fellow did the case. I remember one instance during my fellowship when a semi-urgent penetrating keratoplasty (PK) on a teenager with severe hydrops was booked on a Saturday. I was on call and came late to the OR

after seeing other urgent patients. Claes had seen all the preliminaries – the patient was asleep, prepped, and draped. As I rushed into the OR expecting to see the case well under way, Claes said, “Welcome, doctor, your patient is ready for you.”

Dr. Dohlman also played an important role in the surgical education of the MEEI residents, generations of whom did their first intraocular surgeries (extracapsular cataract extraction –



Fig. 1.4 At the 1998 World Cornea Congress, Orlando, FL. (Back row, left to right) Claes Dohlman, Kenneth Kenyon, Eduardo Alfonso, Thomas John. (Front row, left to right) Scheffer Tseng, Kazuo Tsubota. (Contributed by Eduardo C. Alfonso)



Fig. 1.7 Kathryn Colby and Claes Dohlman with one of Dr. Colby's pediatric keratoprosthesis patients, MEEI Cornea Service, 2008. (Contributed by Kathryn Colby)



Fig. 1.5 Cornea Service faculty and fellows, 2007. (Contributed by Roberto Pineda)



Fig. 1.8 Reza Dana and Claes Dohlman, 2008. (Contributed by Reza Dana)



Fig. 1.6 Claes and Carin Dohlman with Kazuo Tsubota and his daughter Mika, 2007 Tear Film and Ocular Society meeting, Taormina, Italy. (Contributed by Kazuo Tsubota)



Fig. 1.9 Cornea Fellows with Claes Dohlman, MEEI Cornea Service, 2009. (Contributed by Jessica Ciralsky)



Fig. 1.10 Claes Dohlman, Jonathan H. Lass, Anthony Aldave, Gary N. Foulks and Shigeru Kinoshita, 2009. (Contributed by Jonathan H. Lass)



Fig. 1.13 Kathryn Colby, Claes Dohlman and Dimitri Azar at the American Academy of Ophthalmology meeting, 2013. (Contributed by Kathryn Colby)



Fig. 1.11 Claes Dohlman with Kathryn Colby and the MEEI Cornea Fellows at the 2010 World Cornea Congress in Boston. (Contributed by Kathryn Colby)



Fig. 1.14 Claes Dohlman and Hajirah Saeed, American Academy of Ophthalmology meeting, 2015. (Contributed by Hajirah N. Saeed)



Fig. 1.12 Claes Dohlman, Deborah Pavan-Langston, Kathryn Colby at the celebration for Dr. Langston's promotion to HMS Professor of Ophthalmology, 2012. (Contributed by Kathryn Colby)

ECCE) under his watchful eye, generally on Saturday mornings. He guided us through these early cases with kindness and respect – to this day, I think of Claes when I start to feel impatient with a trainee struggling with suture placement or steps of a case.

Even experienced surgeons with decades under their belts retain vivid memories of their early surgical cases. I imagine all of us who had the privilege of having CHD attend our cases still remember his characteristic clearing of his throat as a sign that the novice surgeon might be headed toward trouble. I remember especially clearly one of my early surgeries with Dr. Dohlman – it

Fig. 1.15 Group photo of the keratoprosthesis study group meeting in San Diego, 2015. (Contributed by Natalie A. Afshari)



Fig. 1.16 Photos from the 2017 Biennial Corneal Congress, Boston. (a) Kit Johnson, Claes Dohlman and Kenneth Kenyon (contributed by Roberto Pineda). (b) Claes Dohlman and Roberto Pineda (contributed by Roberto Pineda). (c) Claus Cursiefen and Claes Dohlman

(contributed by Claus Cursiefen). (d) Peter Laibson, Claes Dohlman and Reza Dana (contributed by Reza Dana). (e) Reza Dana, Claes Dohlman, Ilene Gipson and Shigeru Kinoshita (contributed by Reza Dana)



Fig. 1.16 (continued)



Fig. 1.17 Claes Dohlman and Kathryn Colby at the keratoprosthesis study group meeting, Barcelona, 2018. (Contributed by Kathryn Colby)

was October of my first year of residency. While I had grasped the basics of the ECCE, some of the subtleties, such as visualizing the mysterious posterior capsule, were still works in progress. Claes was pleased with my improvement over the month, opining that I was a quick learner – high praise that is still memorable some 25 years later. But, this particular patient was extremely nervous, and Claes thought it is best that we be surreptitious in our communications during the case, prompting him to ask me, “Do you speak French?” Enough to order dinner or purchase shoes in Paris, perhaps, but not enough to stay out of trouble in the OR. We made due with throat clearing and hand gestures. The case went fine without misadventures.

I performed my first PK with Claes by my side during my senior year of residency. These cases were generally reserved for fellows, but Dr. Dohlman’s fellow (and one of my other mentors), Roberto Pineda (Chap.25), was scheduled to be out of town. I was already headed into cornea after my upcoming year as the chief resident, and so I was allowed to cover the case.

Roberto coached me beforehand – I spent hours passing sutures in the wet lab and getting familiar with the trephines for corneal transplantation. The case was thrilling from my perspective – so precise and so delicate. The next day the graft was beautiful – crystal clear, with well-placed sutures. I remember thinking what a great corneal surgeon I was going to be! My delight was short-lived as Dr. Dohlman came in, took one quick look at the graft and said, “Oh, the pressure must have been very high overnight.” Of course, it was, necessitating oral and topical glaucoma medicines for the patient, and a lesson in humility for me (as well as a tangible demonstration of the importance of thorough viscoelastic removal).

“Your Patients...They Are Children”

Dr. Dohlman directly influenced my choice of cornea as a sub-specialty in a memorable way. In my second year of residency, I entertained the option of pediatric ophthalmology. Many things appealed to me about pediatric ophthalmology – the ability to care for the whole eye, the impact one could have on the life of a child, and the excellent job opportunities due to the undersupply of pediatric ophthalmologists. I sought Dr. Dohlman’s counsel to help me decide between cornea and pediatrics. After I prattled on for a bit, explaining my perceived advantages of pediatrics, Claes replied, in his characteristic Swedish accent, “yes, yes...that is all very true, but your patients...they are children.” Indeed, in typical CHD fashion, he summarized the entire situation in a few well-chosen words. I did choose cornea, but eventually found a way to combine it with pediatrics, starting a pediatric cornea service at the Boston Children’s Hospital.

I sought Claes’ input about another pediatric matter a few years later when I was managing a 4 year old with multiple non-healing grafts done for severe herpes simplex keratitis. Claes was getting ready to leave for his annual summer vacation to Sweden. I asked him if he thought a KPro might be a possible answer to this child’s problems. To say he was not enthusiastic about

the idea would be an understatement. I asked if he would at least think about it while he was in Sweden. He knew the right answer before the rest of us did and told me that he would “think about it in his nightmares.” I heeded his advice, and we stabilized the child’s eye with a permanent tarsorrhaphy, but sacrificed the vision. A few years later Esen Akpek at Wilmer published the first few cases of pediatric KPro [1]. Over the next decade or so, small numbers of these cases were done around the world, including my own cases at MEEI, but unfortunately the long-term outcomes of pediatric KPro proved to be the stuff of nightmares. Most recently, KPro surgeons from Canada have suggested that these cases should not be done until the field can get a better handle on KPro complications in children [2, 3]. Lesson learned – never doubt the wisdom of the father of cornea!

“Focus, Focus, Focus” Making an Impact on the Field Through Scholarship

Dr. Dohlman had a tremendous impact on the academic path of his fellows, again leading by example. His impact on me began almost from the very start of my residency. My first grand rounds was on one of his patients, a 17-year-old man with severe vernal keratoconjunctivitis (VKC). This was a textbook case of VKC, with classic findings. To this day, I still use images from this case when I give talks on this disease area. This case formed the basis of my “resident course paper,” a requirement for all of the MEEI residents that was published as a special issue of one of several journals [4]. While I learned a great deal working on this project with Claes, I learned much more by watching his approach to improving outcomes with the Boston KPro.

In the mid-1990s, the Boston KPro was fraught with vision-limiting complications including severe infection and device extrusion. I clearly remember Claes in the minor OR at MEEI wrapping pieces of donor cornea around the stem of a melting cornea to prevent device extrusion.

Endophthalmitis was not uncommon, and devastating when it occurred. Fast forward 20 plus years and now the Boston KPro is the most widely used artificial cornea in the world, with approximately 1000 new devices placed each year. How did this occur? The answer is focus, perseverance, and conviction. Innovation does not take a straight path and setbacks are common. During my interview, Claes stated, “My results in the beginning were just awful. But to have success in a field you just have to devote decades to it. ... You have to have a certain amount of inner security because of the great difficulties in the beginning in terms of not knowing who will do well and who will not do well.”

Claes devoted himself to the Boston KPro. What enabled him to make progress? In his words, “Little by little, I realized gradually the importance of nutrition and prophylaxis with antibiotics. And if you stick to it, things do get better.” Through the knowledge of the biology of the cornea, and of the literature in the field, Claes realized that the donor corneas were melting because the solid KPro backplate blocked nutrients from the aqueous. “It took me 10 years to realize that this (melting of the donor cornea) was nutritional in nature. And who had demonstrated that the corneal nutrition comes the aqueous exclusively and cannot be blocked? We did, twenty or thirty years prior.” It was his ability to understand the problem and synthesize prior knowledge that led to a design modification (holes in the KPro backplate) that improved corneal melting in noninflammatory diseases. Interested readers are encouraged to read Chap.15, which details more about the development and current state of the Boston KPro, truly one of Dr. Dohlman’s lasting gifts to our field.

These lessons – knowing the biology of the disease, knowing the relevant literature, being a careful observer of the disease, and having inner security to take a brave leap – played a key role in the development of Descemet stripping only (DSO) as a treatment for Fuchs dystrophy (reviewed in Chap.8). I learned these lessons from Claes while watching him focus and persevere to solve issues that impeded Boston KPro success in the 1990s.

Time to Walk Your Own Quarterdeck: Leadership Lessons from CHD

For many of us trained by Dr. Dohlman, the time comes when we are called to lead our own sections, departments, or medical schools. Included among the authors of this text are numerous cornea division chiefs, at least nine current or former department chairs, and two current or former medical school deans. Indeed, some consider MEEI and HMS as “chairman training school.” At present, approximately one quarter of seated ophthalmology department chairs have an historical tie to the Massachusetts Eye and Ear Infirmary or Harvard Medical School. In Chicago alone, four of the six ophthalmology chairs and one medical school dean are MEEI-trained.

What leadership lessons can we learn from CHD? Again, through his example, we see that leadership, like innovation, requires focus, perseverance, and a thick skin. One must not be so afraid to fail that one does not take bold steps when they are needed. Chairmanship is a service job, where the successful “captain” must put the needs of the department above his or her individual needs while walking the quarterdeck. Humility, generosity, and gratitude make the job easier. In Claes’ words, “You ask me what I have been proud of...I haven’t done anything particularly spectacular. But all the people that I was interacting with and learning from ... made my life very rich... So I certainly have a lot to be grateful for.” Indeed, so do those of us whom he trained.

Conclusions

Claes Dohlman has made numerous invaluable contributions to ophthalmology. Besides the Boston KPro, he has trained hundreds of cornea specialists who have gone on to make their own contributions to the field, and to train countless others. In this volume, we have collected a series of essays, written by former Dohlman trainees and close colleagues that reflect the state of the art in many important areas of cornea. It is our

hope that this text will be a lasting tribute to Dr. Dohlman – mentor, colleague, friend, father of the field of cornea and to us all.

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The Days of the Cornea Subspecialty: In the Beginning

2

James V. Aquavella

Background

In the late 1950s and early 1960s, there were no ophthalmic subspecialties. While some ophthalmologists expressed fields of greater interest, they all saw a variety of patients. No specialized postgraduate programs or formal clinical fellowships existed. Ophthalmology was not considered a discrete discipline but in conjunction with otolaryngology as a comprehensive eye, ear, nose, and throat specialty. Indeed, the American Academy of Ophthalmology and Otolaryngology represented both specialties and would conduct joint meetings. Not until 1979 did there occur a split into two separate entities, the American Academy of Ophthalmology and the American Academy of Otolaryngology [1]. Subsequently, otolaryngology added the discipline of head and neck to its title. It was in this climate that I first came to know Claes Dohlman.

In view of our long association, I was invited by the American Academy of Ophthalmology to contribute a chapter for this text to be published in honor of my mentor, good friend, and longtime colleague Claes Dohlman. The information presented herein is based on a talk presented at the Cornea Conference held in Boston in 2017 to

honor the many accomplishments of Claes Dohlman during his 60-year career. It is of note that over the past quarter century, I have attended several events dedicated to Claes' retirement, yet he continues to devote his full time to research, teaching, and patient care. There is no real prospect of Claes' retirement. Claes Henrik Dohlman will always continue to devote all his efforts to the benefit of his patients and profession.

Claes Dohlman has had an enormous impact on the practice and science of ophthalmology, not limited to the creation of the cornea subspecialty. A warm and friendly demeanor is the hallmark of his relationship with all, from the beginning undergraduate student to the most accomplished scientific minds and academic leaders.

Training

Following in the steps of his father, a chairman of ear, nose, and throat at Lund University in Sweden, Claes obtained his MD at Lund as well. He later received a PhD in medical research also from Lund University. He had trained earlier with Jonas Friedenwald at Johns Hopkins prior to returning to Sweden for PhD studies. What was proved to be the harbinger of an illustrious career occurred when Charles Schepens, having recently formed a research institute affiliated with Massachusetts Eye and Ear Infirmary, invited Claes to become one of the small numbers of

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research scientists. Edwin Dunphy then the Chair of Ophthalmology at Harvard and director of ophthalmology at Massachusetts Eye and Ear Infirmary provided Claes with a clinical appointment [2].

As I review the general circumstances surrounding Claes' achievements, it is clear that the environment which existed in Boston at the time, and in particular at the "Eye and Ear", constituted one of the enabling factors in his success. The juxtaposition of great clinical and scientific minds created a fertile environment in which he could ply his knowledge and expertise. Could the creation of a cornea subspecialty have been possible had Claes been offered a position in another institution in another city? I suspect his intellect and perseverance would have prevailed, achieving similar results even under diverse circumstances.

Historical Prospective

Massachusetts Eye and Ear Infirmary originated when doctors Edward Reynolds and John Jefferies established a charitable clinic (Fig. 2.1). Later in 1866, the first rotation of Harvard medical students occurred at the "Eye and Ear". This

subsequently evolved into a formal agreement for the teaching of Harvard students in ophthalmology and otolaryngology [3]. In 1932, Frederick Verhoeff was named to direct research and pathology at the institution, the Howe Laboratory having been formed in 1928.

Lucien Howe (1848–1928) received his medical degree from Harvard and practiced for 50 years in Buffalo, New York, prior to returning to Boston and establishing his laboratory. A number of Howe Medals are awarded annually, the most prestigious being that of the American Ophthalmological Society. Frederick Hermann Verhoeff (1874–1968) graduated from Yale in 1895 and received his MD from Johns Hopkins University in 1899. Verhoeff's impact was very significant. He was an individual with a management style perceived as being more dictatorial than collegiate. Over many years, he remained an active participant in patient care and research. I recall his presence at the weekly pathology conferences where he continued to forcefully express his opinions much to the chagrin of any junior physician presenting a case or defending a concept.

He frequently espoused his abhorrence of "the concealment of ignorance by the ostentation of seeming wisdom" [4]. His surgical techniques for

Fig. 2.1 A lithograph depicting the original Charitable Eye and Ear Infirmary. (https://commons.wikimedia.org/wiki/File:Massachusetts_Charitable_Eye_and_Ear_Infirmary,_Boston.jpg)



cataract surgery became so entrenched at his institution that the adoption of newer and more suitable techniques for cataract surgery were actually impeded. When asked if he was the best ophthalmologist in the country during a court proceeding his response was “there is no evidence to the contrary”.

While the mores of ophthalmic practice were relatively rigid everywhere at the time, Boston was fortunate to have been the venue for some of the great achievers in ophthalmology. In this crucible of great minds, Claes was able to interact with a number of other super achievers: Frederick Verhoeff, David Cogan, Charles Schepens, Morton Grant, Paul Chandler, Edwin Dunphy, and David Donaldson were among the many outstanding ophthalmic clinicians of the time.

Charles Schepens (1912–2006) lived in Belgium where he received his MD degree in 1935 from the University of Ghent. During the Second World War, he was an active participant in organized resistance to Nazi occupation. He escaped to England where he practiced at Moorfield’s Hospital, ultimately immigrating to the United States in 1947. He was a member of the Howe Laboratory, established the Retina Foundation and became a full professor of ophthalmology at Harvard in 1983 [5]. His Foundation soon became the source of numerous research and clinical developments, ultimately becoming an integral component of the Massachusetts Eye and Ear Infirmary’s research activities.

Edwin Dunphy (1896–1984) graduated from Princeton in 1918 and received his MD from Harvard in 1928. During the Second World War, he participated as a member of a special unit exploring the effects of poisonous warfare gases. He served as the Chairman of Harvard Ophthalmology from 1958 through the early 1960s [6]. He allocated the use of a small office in the rear of the main clinic floor as the first physical presence of what was to become the cornea service.

David Glendenning Cogan (1908–1993) was a 1929 Dartmouth graduate who received his MD from Harvard in 1932. His mother was an ophthalmologist as well. He became a member

of the Howe Laboratory, later serving as the Chairman of Harvard Ophthalmology (1955–1973) and Director of the Howe Laboratory from 1940 to 1974. He preceded Claes as the Chairman of Ophthalmology [7]. One of his early discoveries was the relationship of non-syphilitic interstitial keratitis with vestibular–auditory disease.

W. Morton Grant (1915–2001) established the glaucoma service at Massachusetts Eye and Ear infirmary and was an active researcher at the Howe Laboratory. He had graduated from Harvard and proceeded to acquire an MD from Harvard as well. He conducted research in ophthalmology and glaucoma without the benefit of formal residency training. His textbook “Toxicology of the Eye” was the first comprehensive study in the field [8]. Active collaboration with Paul Chandler later resulted in the creation of the Glaucoma Service.

Paul Chandler (1896–1987) received an MD from Harvard in 1924, and completed a residency at Mass Eye and Ear. While his individual accomplishments were numerous, the collaboration with resulted in the establishment of the Glaucoma Service in 1940. His textbook on glaucoma was authored with the assistance of Dr. Grant [9].

David Donaldson (?–1994) graduated from the University of Michigan in 1941 and was awarded an MD in 1945. He trained at the Henry Ford Hospital in Detroit where he worked with Henry Ford. Following residency in 1953, he became a member of the Howe Laboratory. His work with three-dimensional imaging photography of the eye resulted in the publication of two textbooks replete with slit lamp stereo photographs of anterior segment pathology [10].

The juxtaposition of individuals possessing strong accomplishments as well as strong egos created a scientific environment charged with political sentiment and noted for its lack of tranquility. While some institutions are organized in a group practice model, others have evolved into clusters of individual fiefdoms where there is a relative lack of allegiance to a common cause. Yet, Claes was able to survive multiple challenges during his tenure as Chairman.

Residents at the time included Richard Simmons, Herbert Kaufman, and Perry Rosenthal. And while the Schepens Foundation was in its infancy, the Howe Laboratory was in full operation so that a tradition of research was firmly in place at Massachusetts Eye and Ear Infirmary.

Initial Involvement

Following the completion of my residency in New York, I had the opportunity to participate in a few cornea surgery procedures performed by Ramon Castroviejo (1904–1987) and Benedict Rizzuti. Dr. Rizzuti worked at the Brooklyn Eye and Ear Hospital and Dr. Castroviejo at Columbia University as well as his private hospital. At the time, all ophthalmic surgery was performed with the surgeon standing. There were no stools or microscopes. My first introduction to an operating microscope was an awkward vertical Zeiss model originally designed as a laboratory device. Dr. Rizzuti had attempted to utilize a microscope for cornea surgery. Dr. Castroviejo had operating facilities in his 91st street home, a precursor to the ambulatory surgical centers of today. He wore magnifying loupes as did most eye surgeons at the time, and he too operated while standing. Some of his surgical procedures were filmed with commercial 35 mm film. He is credited for having performed the first successful cornea transplant at Columbia Presbyterian Hospital. He invented the technique of cutting the donor cornea transplant as a square rather than a circular piece of tissue. The main advantage of the technique was to enable the anchoring of the four corners in the peripheral recipient bed, thus limiting the formation of subsequent anterior chamber adhesions to the four corners of the graft (Fig. 2.2). Adhesions were common at the time, predisposing to angle complications and glaucoma [11].

I attempted to find an organized postgraduate teaching program in the field of cornea, but none existed. Edwin Dunphy suggested I travel to Boston and meet their new recruit, Claes Dohlman, who was interested in cornea disease.



Fig. 2.2 Photograph of Ramon Castroviejo circa 1930. (https://commons.wikimedia.org/wiki/File:Ramon_Castroviejo.jpg)

He had been joined by two local ophthalmologists also interested in the cornea, Arthur Boruchoff (1925–2013) and Edward Sweebe [12]. “Art” Boruchoff had graduated from Harvard in 1945 and received his MD from Boston University in 1951.

When I arrived in 1960, Dr. Sweebe had succumbed to a tragic illness, and his widow, Bobby Sweebe, became the receptionist/secretary for the unit. I was invited to participate in both the Foundation and the Clinic, and a modest amount of funding was procured. Claes has often described our initial meeting by my asking if I could be his fellow, and his now storied response was “what is a fellow?”

I was assigned to a small room on the top floor of the Charles Street Jail [13], notorious for having housed Sacco and Vanzetti, convicted terrorists, and later a former Boston mayor James



Fig. 2.3 Photograph of the Charles Street Jail as it appeared following its construction in 1851. (<https://www.flickr.com/photos/cityofbostonarchives/9321963420>)

Curley. The building was soon declared unfit for prisoners but obviously still considered suitable for fellows. The Charles Street Jail was first constructed in 1851 and subsequently purchased by Massachusetts General Hospital in 1991. The site was leased to developers who built the Liberty Hotel [14] (Fig. 2.3). The resulting structure maintained some of the original appellations such as the prison “yard” and “solitary confinement” rather than the traditional do not disturb notices appended to the room door.

And so I began to learn under Claes’ mentorship. In the clinic, I was exposed to the use of the newly developed topical steroids as well as the experimental use of idoxuridine for herpes simplex keratitis. Eeva-Liisa Martola and Stuart Brown made an appearance as unofficial and occasional members of the group.

In the Foundation, I was fortunate to meet the Director Andre Balazs and to work with Saiichi Mishima, Paul Payrau, Arvid Anseth, and Bengt Hedbys. David Maurice was a not infrequent visitor to our research facility. It is interesting to note that while these individuals pursued their

own independent projects, they were all involved in various aspects of cornea hydration.

Endre Alexander Balazs (1920–2015) devoted his productive life to the study of the structure and function of ocular connective tissues. He was asked by Charles Schepens to become the director of his newly established Retina Foundation and subsequently became an active member of the Howe Laboratory, while directing the work of the Foundation [15].

Jonas Friedenwald (1897–1955) was a 1916 graduate of Johns Hopkins University who received his MD also from Hopkins in 1920 [16]. He was an associate professor of ophthalmology when the young Claes Dohlman visited Hopkins participating in the work of his laboratory prior to returning to Sweden obtaining a PhD from Lund University.

David Maurice (1922–2002) trained with Sir Stuart Duke Elder at University College in London, receiving his PhD in 1951 [17]. He is well-known for his text on ocular physiology and having developed the principles of specular microscopy and confocal microscopy. He had

immigrated to the United States in 1968 and was on the faculty at Stanford University, later transferring to Columbia in 1993.

Saiichi Mishima (1927–2005) received his MD from Tokyo University and later studied with David Maurice in London [18]. He spent 2 years as a collaborator in Claes' laboratory at the Retina Foundation working with Arvid Anseth and Bengt Hedbys. His major work was in the area of fluid regulation in the cornea. He subsequently became the Chairman of Ophthalmology at the University of Tokyo.

Bengt O. Hedbys' working at the Retina Foundation was in the areas of cornea hydration, fluid flow, and cornea thickness changes. He collaborated with doctors Mishima, Arvid, and Payrau while in Claes' laboratory. He was a graduate of the University of Gothenburg [19, 20].

Arvid Anseth was born in Norway and obtained his MD from the University of Lund in 1961 prior to his tour at the Retina Foundation. His work at the Retina Foundation was based on the activity of cornea polysaccharides [21–24].

Paul Payrau also had a distinguished career as a member of Nazi resistance in France [25]. He along with his colleague Yves Pouliquen became a force in cornea research in France. He too is noted for his interest in cornea hydration, having proposed the transplantation of shark cornea tissue to ease the edema associated with Fuchs Dystrophy. He later became a member of the Rothschild Foundation research team.

When I arrived in Boston, the Schepens Retina Foundation was located in a converted west end tenement building with animal cages housed in the basement near the furnace unit. My technician was a Cuban expatriate, Antonio Gassett [26]. Claes later assisted Tony in gaining acceptance to medical school and he subsequently went on to become an ophthalmologist, working with Herbert Kaufman's department in Gainesville.

The Work Continues

From these meager beginnings, Claes brought forth and organized an entire cornea subspecialty. His dedication to both research and clinical

aspects was remarkable. He was a true patient advocate always searching for the truth, striving forward, and never allowing personal bias to intervene with research findings. His program involved uncovering and understanding basic biological and physiological principles, preliminary to developing appropriate and targeted medical and surgical therapeutic modalities.

This search for unvarnished truth, untainted by the prejudice of past or current ideas, is reminiscent of the tradition first espoused by Socrates with his admonition that we must not rely too heavily on the past. Words later recited by Plato in his dialogues of 360 B.C. and subsequently translated into Latin by Rodger Bacon as a part of his *Opus Magnus* in 1265 A.D.: “*Sed Magis Est Amicus Verus*”. The greatest friend is truth [27, 28].

In those early days, clear corneal transplants were the exception. I recall Claes showing me a clear graft in an eye subsequently blinded by glaucoma as the result of unrestricted use of the newly developed topical steroids. This concept of induced glaucoma he has carried on is evidenced by his insistence on the use of shunts in combination with keratoprosthesis devices.

Keratoprosthesis

The Columbia Presbyterian team of Arthur Devoe [29], Ramon Castroviejo (1904–1987), and Hernando Cardona [30, 31] implanted a number of early Cardona model devices in the late 1950s and early 1960s with generally unfavorable results. The device was individually manufactured in Columbia and shipped to the United States. It was available only in one aphakic power, but there were three models. The simplest had an iris design and either blue or brown coloration to its surface with the optical cylinder passing through a 3 mm opening in the subject's diseased cornea, secured by a threaded small posterior plate. It was termed the “Nut and Bolt” design (Fig. 2.4). The most common model involved a polymethyl methacrylate plate which was sutured to the cornea surface (Fig. 2.5). A central threaded 3 mm opening allowed the

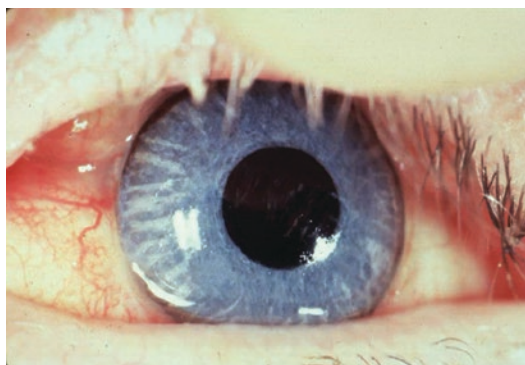


Fig. 2.4 The Cordona Nut and Bolt keratoprosthesis. Note the blue colored pattern of the front plate designated to match the fellow eye

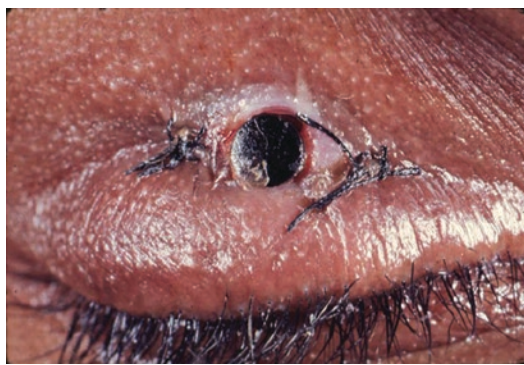


Fig. 2.6 The Cardona Through and Through model keratoprosthesis following surgery. Note the protrusion of the optical cylinder through the upper lid

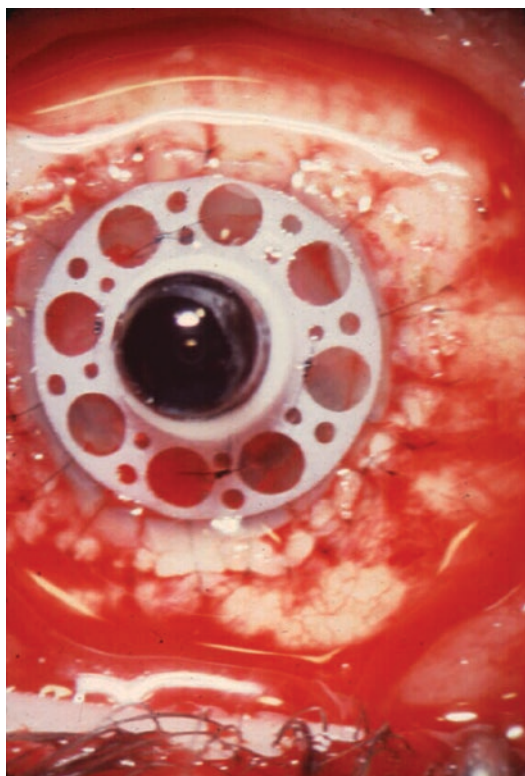


Fig. 2.5 The standard model Cardona keratoprosthesis in place covering the anterior cornea surface prior to placement of sutures

optical cylinder to pass through the cornea and protrude into the anterior chamber. It was often covered by buccal mucous membrane or perios-teum. The third variety was termed the “Through and Through” and was designed to pass through

the tarsus of the upper lid prior to a permanent tarsorrhaphy (Fig. 2.6). I had participated in a few of these procedures and noted that 100% were ultimate failures. Yet, over his 60-year career of dedicated KPRO research, Claes ultimately devised the Boston I device and success followed. The unit was first known as the Doane-Dohlman device. Claes had insisted on giving full credit to his collaborator.

Claes had become intrigued by the early work of Edward Stone [32], working in the Howe Laboratory to implant plastic materials in rabbit eyes, and then evaluating the Cardona device [30]. Thus began the Dohlman interest in bio-compatibility issues which must support device implantation. These early subjects all had end-stage disease, albeit originating from a variety and combination of disease entities.

The number of these complex cases was small, and many months of observation were necessary prior to attempting modifications in either surgical procedure or device construction which then again must be evaluated over time. The Food and Drug Administration was becoming interested in medical devices and new regulations were proposed. The Doane-Dohlman device was ultimately approved for human use in 1991. Numerous modifications followed prior to the introduction of what we now call the “Boston I” device. Others involved in keratoprosthesis work at the time were mostly interested only in the clinical applications rather than in basic research aspects. Aside from the original Columbia

University team in the United States, Louis Gerard in Texas, Peter Choice in England, the Barraquers in Columbia and Spain, the Russian Group in Odessa, and the Italians represented by Strampelli, Franceschetti, and Falcinelli were all active at the time. Unfortunately, there was no coordination, and the number and variations of devices and techniques proliferated with little overall improvement in results over time.

While formerly active in the field, I had become discouraged with the results, and had not implanted any devices for almost a decade. With the dawn of the twenty-first century, Claes [33] related to me the details of his new design and the concept of covering the implanted device with a bandage contact lens. Other innovations included the fenestrated back plate, the insistence on the use of prophylactic antibiotics, and the anticipation of elevated intraocular pressure. After only a few cases, I became a strong advocate and ultimately modified the surgical techniques to enable primary implantation in newborn infants [34]. A process encouraged by Claes while disparaged by a few other members of his Boston group. The use of the Boston type I device has proliferated worldwide. An early impediment was cost. But arrangements for manufacture in other sites, combined with some device manufacturing changes, now have the prospect of significantly increasing the availability of this technology even in impoverished countries.

Claes Dohlman's entire efforts were always focused upon delivering help to over 2 million individuals with irreversible cornea blindness not amenable to corneal transplantation and mostly located in third world countries. The resultant Boston I device has been implanted in over 15,000 eyes worldwide as of this writing.

The Research Continues

Clearly much work remains. There are complications to be addressed and avoided, as well as new techniques to be evaluated. The current keratoprosthesis laboratory at Massachusetts Eye and Ear Infirmary has been modeled into a research unit engaged in studying new materials, device modifications, related elevation of pressure and

intraocular inflammation, as well as many aspects of cost and distribution. From its inception, all of the funds generated were devoted to the laboratory and teaching. Claes has never received any remuneration from the sale of the device he invented.

While I may have been the first to be exposed to Claes' level of dedication, his impact on me was insignificant compared to impacting all of ophthalmology merging cornea research, education, and therapeutics into an integrated cornea subspecialty. Several hundred cornea fellows, post-doctorate PhDs, and colleagues throughout the world have been trained and become disciples. They in turn have established and led cornea programs in their own countries and institutions. His leadership does not allow for complacency. Claes Dohlman has taught us all to continually strive for the next level, to grasp Socrates' Magnus Verus.

From the fundamental work uncovering physiological concepts and the workings of cornea swelling pressure, stromal thickness changes, and the flow of aqueous into and out of the stroma under the control of the endothelial pump to Paul Payrau's attempt to implant elasmobranch cornea tissue to control Fuchs dystrophy, and now the Boston I device, his dedication to truth proliferates. We now have the proposed use of TNF alpha inhibitors not only in keratoprosthesis surgery but to mitigate pigment epithelial apothecosis and optic nerve damage following severe ocular injury [35].

In the final analysis, consolidation of a cornea subspecialty is truly a unique accomplishment. For over 60 years, generations of scientists and clinicians have gone forth to propagate Claes' concepts and techniques, now culminating in a means to correct otherwise irreversible cornea blindness.

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Dry Eye Disease: A Modern History

3

Michael A. Lemp and Gary N. Foulks

Introduction

The ocular condition in which dryness of the ocular surface is both a symptom and a clinical finding has been known for many centuries, but the nature of the normal functions of the ocular surface and the pathophysiology of the disease have only recently yielded new concepts, findings, and therapeutic approaches to this widespread problem. These discoveries have fundamentally altered our understanding of the disease and opened a new opportunity to diagnose the disease, and its subtypes, judge its severity, and approach the management of dry eye disease (DED) with new and more effective treatments. As we continue the expansion of our research, it is likely that analysis of the contents of tears may well reveal not only ocular diseases but systemic conditions as well.

The term *dry eye* was introduced by *de Roeth* in 1950 to supplant the use of *Sjogren syndrome*, which was in general use for eyes with evidence of lacrimal hyposecretion [1]. The latter term is now recognized as appropriate for a subset of patients with evidence of a systemic inflamma-

tory disease, one characteristic of which is dry eye. Several terms including keratoconjunctivitis sicca have been in use, but recently, there is general agreement that DED is a well-recognized designation familiar to scientists, clinicians, and the general public.

DED is by far the most common malady affecting the ocular surface and this story will be limited to DED; other conditions of the ocular surface will only be mentioned as they relate to DED. The principal themes which we think have had the greatest impact on our understanding of DED and new concepts of the approach to successful management of the disease will each be presented.

Theme I: Structure and Function of Components of the Tear Film and Ocular Surface

A major advance originating in the 1960s but coming to fruition in the mid-to-late 1970s was the delineation of the roles of the component ocular structures in the formation and maintenance of the precorneal tear film in health and disease. Most of the advances were related to clinical experiences and the relationship of dry eye to systemic illnesses such as Sjogren syndrome, rheumatoid arthritis, Steven Johnson syndrome, and other conditions affecting the ocular surface. It was noted that mucus secretion was decreased

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in patients with DED as opposed to other conditions. The roles for each of the components of the tear film and the ocular surface cells were beginning to be discerned in a series of experiments.

The ocular surface epithelium covering the cornea and conjunctiva are morphologically and functionally linked and it was long thought that healthy conjunctival epithelium could cover and replace severely damaged corneal epithelium. Thoft and Friend [2–4], however, demonstrated that there were significant biochemical differences between conjunctival and corneal epithelium and that transformation of conjunctival epithelium into corneal epithelium required considerable biochemical alteration including development of significant glycogen stores and hexose monophosphate shunt metabolism. Such transformation occurred in healthy conjunctiva but not in chemically damaged conjunctival epithelium. Further studies [5] confirmed the transition of conjunctival to corneal epithelium but with subtle changes in goblet cell populations. Subsequent studies identified the limbal epithelium as a critical source of corneal stem cells. It is now accepted that limbal cornea is the predominant site of corneal stem cells that are important in maintaining the integrity of the corneal epithelium [5–7].

Also important in recent research is the role of the mucin-containing glycocalyx which provides a protective covering for surface corneal epithelium [8]. Breakdown in the complete coverage allows for introduction of certain dyes, e.g., sodium fluorescein. This is a commonly employed diagnostic test for corneal damage in DED [9]. Although this is a useful test for identifying patients with positive staining and assumed disease, it is now recognized that small punctate staining in the corneal periphery is a common finding in normal subjects. This is thought to be due to an uncovering of new underlying epithelial cells as part of the normal cellular turnover prior to the generation of a new glycocalyx covering [10]. This is important particularly in qualifying subjects for inclusion in clinical trials and interpretation of possible effects of therapy.

Theme II: The Lacrimal Functional Unit

A basic understanding of the normal physiological roles played by elements of the ocular surface and their interrelations has represented a major step forward and has proved essential for further studies. The interrelated actions of the different components of the tears were first proposed by Stern, Pflugfelder, and Beuerman in 1998 [11] and subsequently validated by others [12]. The premise is that the cornea, conjunctiva, lacrimal glands, lids with the meibomian glands, and the drainage pathways are linked by a neural network which permits structures to react to changes in the environment or other components which compensate. The most obvious example of this is the compensation seen between the lacrimal glands and the meibomian glands of the lids. In early stage evaporative tear deficiency, there is an accompanying increase in aqueous tear production [13]. As DED develops, there is a breakdown in the stability of the tear film, and evidence of both subtypes of DED is seen, i.e., both aqueous tear deficiency and evaporative tear deficiency are characteristic.

Theme III: Tear Instability and Hyperosmolarity – Hallmarks of DED

The two most characteristic features of DED are tear film instability and tear hyperosmolarity. Both of these characteristic properties are seen as early stage events which lead to other damaging events seen most commonly with inflammation. Evidence of inflammation is seen in most cases of moderate to severe DED and leads to extensive damage to the ocular surface (see Theme IV).

The initial stimulus for upsetting the normal function and interactions between the components of the lacrimal functional unit remains unclear and may differ for each of the main subtypes of DED – aqueous tear deficient and evaporative (most commonly associated with meibomian gland dysfunction). The latter is

thought to be hormonally (androgen insufficiency) influenced and is by far the most common form of DED although with increasing severity of disease, as noted above, these two characteristics – instability of the tear film and tear hyperosmolarity – are uniformly present and act as precursors to the damage to the ocular surface and events such as inflammation.

The effects of instability of the tear film are seen in variability of a number of measures of disease, e.g., tear breakup time. The normal extent of continuous covering of the cornea between blinks is reduced. The normal interblink interval lasts at least 7 seconds, and in normal subjects the tear film should continue as an intact covering of the cornea [14]. Measurement of tear film breakup time with classical observation and timing has an inherent variability, but recent studies with ocular coherence tomography (OCT) of tear instability have demonstrated a very close relationship between elevated tear osmolarity levels and abnormal tear breakup [15].

Pioneers in the studies of tear film osmolarity in the 1980s and 1990s were Farris and Gilbard [16, 17]. Working with a laboratory methodology (freezing point depression), they demonstrated the centrality of hyperosmolarity in identifying DED. Subsequently, Tomlinson described the range and diagnostic reference levels of elevated tear osmolarity [18]. Although their technology was not suitable for routine in-office clinical use, they charted the path for the subsequent development of clinically useful instruments. Hyperosmolarity has been shown to lead to the development of inflammation of the ocular surface [19]. In addition, elevated tear osmolarity has been shown to have direct deleterious effects on the corneal and conjunctival epithelial cells [20, 21]. In a study of about 300 subjects with DED employing a new small volume methodology of electrical impedance (TearLab Corp), it has been shown that in normal subjects tear osmolarity as measured in the inferior lacrimal lake is highly stable between eyes and over time. Subjects with DED have elevated tear osmolarity (see diagnostic values in Table 3.1) with significant variability which responds to effective treatment by return-

Table 3.1 Referent values for the diagnosis of DED

Screening questionnaires	
OSDI [58].	>13
DEQ-5 [59]	>6
Tear breakup time [54]	<10 seconds
Tear osmolarity [22]	>308 mOsm/l or variance of >8 mOsm/l between eyes
Tear volume	
Tear meniscus height	<0.2 mm
Schirmer test [61]	<10 mm/5 mm [5]
(without anesthetic)	
Ocular surface staining [9, 31]	>2
(NEI scale 0–15)	

ing to normal levels [22–25]. This study differs from early reports of use of a 50 nl sample of tears (TearLab), wherein multiple readings were then averaged which failed to recognize the importance of the variability of osmolarity in DED subjects not only in tear osmolarity but also in other diagnostic tests, e.g., corneal staining, tear breakup time, and Schirmer testing [26].

Normal subjects have similar results in both eyes, but the differences seen between dry eyes are a clinically useful measure of the diagnostic variability with a positive predictive average of 90% for correctly identifying DED. In addition, tear osmolarity was the only diagnostic test that reflected DED severity with increasing values [24, 25]. A comprehensive review of the literature published in 2014 identified over 160 papers evaluating tear hyperosmolarity of which 72% were positive, 21% neutral, and 7% negative [27]. Of the last category, major flaws in correctly identifying subjects for inclusion as normal versus disease, and failure to follow published study design accounted for the negative findings. This comprehensive review concluded that “the literature broadly supports the use of tear film osmolarity as an objective numerical measure for diagnosing, grading severity, and managing treatment of DED.”

Of the two major subtypes of DED, evaporative DED is the most common; as disease severity increases, evidences of both types of DED are seen presumably as a compensatory response [28, 29] (see above).

Theme IV: The Role of Inflammation in the Pathogenesis of DED

It is intriguing to note that one of the earliest descriptions of DED was of keratoconjunctivitis sicca which has a clinical presentation of marked inflammation as part of the disease process [30]. The subsequent emphasis on inadequate aqueous tear production as the underlying cause of DED was probably due to the much larger number of patients with DED who did not express prominent clinical signs of inflammation. As knowledge of the extent and importance of DED advanced, it was clear that guidelines were needed for the classification and study of the disease. This was provided in the 1995 Report of the NEI/Industry Workshop on Dry Eye which highlighted the major categories of aqueous deficient versus evaporative dry eye and proposed guidelines for evaluation of both categories of aqueous deficiency and excess evaporation [31]. With more intense study of the ocular surface, particularly the identification of inflammatory cells and cytokines in the conjunctiva and the lacrimal glands, both primary and secondary, attention turned to the role of inflammation in the pathogenesis of DED [32, 33]. A comprehensive review of the role of inflammation in DED is available from the Reza Dana team at the Massachusetts Eye and Ear Infirmary which have been leaders in this field of inquiry [34].

Numerous studies have identified contributing inflammatory cells, cytokines, and chemokines in the inflammation cascade that is part of the pathogenesis of DED [35, 36]. Systemic immune disease such as Sjogren disease can be a cause of inflammation, but elevated tear osmolarity has also been identified as a stimulating factor [20, 37]. Determination of levels of most of the inflammatory molecules in the tears or in the ocular surface requires special laboratory measurement, but a point-of-care test for MMP9 is available for use in the clinic (InflammaDry®, Quidel, San Diego, CA) [38]. This assay provides a dichotomous outcome, with levels above 40 ng/ml producing a positive result, but is not specific to any source of ocular surface inflammation.

Theme V: The Important Role of Neurobiologic Aspects of the Ocular Surface and DED

The complexity of the neurobiology of the ocular surface has been expertly reviewed in the TFOS DEWS II Pain and Sensation Report [39]. The cornea is the most highly innervated portion of the eye and has highly specialized nerve fibers and receptors to detect mechanical, chemical, and thermal heat sensations (polymodal and mechanical nociceptors), while thermal cold sensors detect changes in cold temperature and high osmolarity [39]. The polymodal and mechanonociceptors perceive potential discomfort and cold receptors control blinking and tear secretion via sensory pathways in the trigeminal ganglion. Many of the environmental exposures which the ocular surface encounters produce no overt discomfort, but in ocular surface disease, such as DED, nociceptive receptors can signal discomfort severe enough to qualify as pain, which is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” [40]. Therefore the TFOS DEWS II Pain and Sensation Report advocates that the “dryness” described by patients with DED should be considered a form of pain [39].

As noted previously, inflammation of the ocular surface is an integral part of the pathophysiology of DED and such inflammation can cause both sensitization of corneal nerves and damage to those nerves [41, 42]. The peripheral neural sensitization can further induce central neural sensitization that may lead to chronic pain [39]. Neurological changes in the ocular surface could explain the dissociation of signs and symptoms that has long been recognized in DED and which complicates clinical trial results [43]. The early hypersensitivity that has been described in early dry eye disease (and the loss of sensation with advancing disease) may lead to this discrepancy in signs and symptoms [44–46].

A further complicating feature of neurosensory dysfunction affecting the eye is neuropathic pain that occurs in the absence of clinical signs of DED. This condition can be disabling and

difficult to treat but it is not caused by and should not be confused with DED [39, 47, 48]. A questionnaire developed specifically for neurologic aspects of ocular pain has been validated as reliable [49]. Future studies evaluating specific anatomical features of the corneal nerves such as in vivo confocal microscopy [49] or the presence of nerve growth factors in the tears or tissue may help define the differences between the two conditions [50–52].

Theme VI: The Vicious Cycle of DED

In the arena of many newly described aspects and manifestations of DED, perhaps one of the most important advances is that of linking the key elements of DED and their interactions. This concept was introduced by Baudouin and associates in 2007 [53, 54]. This is presented also in the DEWS I Report [55]. The design identifies the *Core Mechanisms* of tear instability and tear hyperosmolarity. In addition to direct damage to the ocular surface from these, the latter has been shown to induce inflammation of the ocular surface. In a subset of systemic inflammatory disease, e.g., Sjogren syndrome and graft-versus-host disease, an early involvement in inflammation in the lacrimal glands is seen [56].

Theme VII: Recent Advances in the Diagnosis and Treatment of DED and Novel Concepts

The challenge of establishing a diagnosis in a disease with great variability in the correlation of signs and symptoms, such as DED, has recently been discussed in the TFOS DEWS II Workshop Report and a sequence of screening and clinical testing to establish the diagnosis and categorization of DED has been recommended [48]. These recommendations include identification of symptoms of ocular surface disturbance through validated questionnaires (OSDI, DEQ, IDEEL, SANDE) prior to further testing. If screening questionnaire confirms that a patient might have DED, it triggers diagnostic testing of

noninvasive tear breakup time, tear osmolarity [measured prior to breakup time if FBUT used], and ocular surface staining with fluorescein and lissamine green [observing the cornea, conjunctiva, and eyelid margin]. Categorization of the subtype of DED is made based upon features of MGD [57], tear lipid thickness or dynamics, and tear volume assessment, as predominantly evaporative or predominantly aqueous deficient. Referent values for diagnosis are listed in Table 3.1 [58–61]. It is important to note that other conditions that provoke ocular surface disease need to be excluded and that discomfort in the absence of disturbed homeostasis of the tear film, such as neuropathic cornea, must also be excluded.

In addition to the traditional management approach to DED of educating the patient in ways to minimize the environmental activities that stress the tear film and use of topical artificial tear preparations, there are new options for therapy, including topical anti-inflammatory agents. Topical cyclosporin was FDA approved in 2003 to treat reduced tear production thought due to inflammation [62]. In 2017, topical lifitegrast was approved for the treatment of signs and symptoms of DED [63]. Although not all patients tolerate the stinging upon instillation, those who can use the drugs often see improvement in symptoms and signs of DED [64]. Recent clinical trials demonstrate that topical KPI-121 lotoprednol preparation also reduces signs and symptoms of DED [65].

Novelty in non-anti-inflammatory therapy is also apparent in some approaches to improved lubrication of the ocular surface with LubricinTM, a lubricant naturally occurring in joint articular surfaces [66] and with another biologic, LacripepTM, a peptide derived from the lacritin molecule that is present in normal tears but deficient in DED [67].

Novel devices to treat other aspects of DED have been FDA-cleared and commercially marketed. The LipiFlowTM system is available to treat refractory MGD by controlled application of heat and pressure to the eyelids [68]. TruTearTM has recently been introduced as a stimulant to tear secretion by intranasal application of

an electrical current with evidence that such stimulation increases tear production, meibomian gland secretion, and goblet cell activation [69, 70]. There remains intense interest in these areas of research which augurs well for further understanding of the processes operative in DED and newer forms of management.

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Herpetic Keratitis: The Genesis of a Career in the Early Days of HSV Keratitis Research

Peter R. Laibson

I met Claes Dohlman for the first time at an antiviral conference in 1963 sponsored by Allergan Pharmaceuticals in Irvine, California. We had both been invited to discuss the first antiviral drug effective against a human virus, herpes simplex. The medication was 5-iodo-2'-deoxyuridine, or IDU. It had initially been reported by Kaufman, Nesburn, and Maloney in 1962 [1], while Herbert Kaufman was an ophthalmology resident at Massachusetts Eye and Ear Infirmary.

Herpes simplex viral keratitis was particularly difficult to treat, as there was no specific antiviral medication effective at that time against herpes or any other viral disease in man. Gunderson in 1936 [2] had reported on the use of iodine solution to treat dendritic keratitis and other forms of herpetic keratitis, and this was one of the more popular treatment options prior to the discovery of IDU. The strong iodine solution, acting as a chemical debriding agent, destroyed the epithelium infected with HSV by dislodging this tissue layer. Iodine also acted as a mild to moderate antiviral agent. Sery had looked at many different chemicals for HSV treatment and had suggested silver nitrate solution [3].

I had become interested in herpetic keratitis in 1961 as a first-year resident at Wills Eye Hospital working with Ted Sery and Irving Leopold, using

weak silver nitrate to treat HSV dendritic keratitis. When IDU became available, we treated rabbits infected with herpes simplex virus with IDU drops, and published our results reaffirming Kaufman's earlier observations [4]. Even though a number of publications had shown efficacy of IDU for human herpetic dendritic keratitis, there were very few prospective double-blind studies testing this new therapy. Smith, Kline and French Pharmaceutical Company, the manufacturer of IDU, was located across the street from Wills Eye, and Irving Leopold, our ophthalmologist-in-chief, was able to procure IDU drops and its vehicle (placebo) for such a double-blind study. We found, as did Burns [5], that IDU was significantly more effective than the vehicle. In our study, one physician examined and treated all 100 affected patients, while in the Burns study, 41 physicians treated and evaluated 81 cases.

As a thank-you for attending its conference, Allergan arranged for Claes and me the Sunday after the meeting to visit Disneyland in Anaheim. Claes was not only interested in Disneyland, but particularly what I was going to do after my residency in ophthalmology. Our informal chat while touring that Sunday was my fellowship interview. On July 1, 1964, Jules Baum, John Stanley, Jerry Goldman, and I arrived at the Cornea Service of the Massachusetts Eye and Ear Infirmary. We joined Stuart Brown, already a fellow there for a year, who was working on intralamellar stromal implants with Miguel Refojo and Claes. This was

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the early research on the artificial cornea, or keratoprosthesis (K-Pro). Steve Klyce also joined the group that year to work with Sai Mishima.

My corneal fellowship was extraordinary, making friends and meeting new colleagues who have remained in my life for over 50 years, and participating in science that was always interesting and at times groundbreaking. Sai Mishima and Shu Kitano were doing their research with Claes at the Retina Foundation, while Arthur Boruchoff, Mike Wiedman, and Eva-Lisa Martola were in the clinic with Claes. Bobbie Sweebe, the secretary, was our mother hen. Joining this stellar group for weeks at a time were Bengt Hedbys from Sweden and David Maurice from Stanford. What a remarkable combination of inspired researchers, motivators, and friends! Fortunately for the Massachusetts Eye and Ear Infirmary, this ability to attract unique individuals continues to this day under Reza Dana and his colleagues, including Claes Dohlman at 96.

As a corneal fellow, I began several research projects on the corneal unit of the Retina Foundation, now known as the Schepens Eye Research Institute. My interests however, which had started as a resident at Wills Eye, continued to be with herpetic keratitis. Recurrent herpetic keratitis remained one of the main problems with HSV ocular herpes, even as the discovery of IDU had significantly aided the treatment of superficial ocular herpes [6]. Recurrent HSV ocular disease was the cause of most of the scarring and deep corneal herpetic involvement. How to prevent the recurrence of this disease after the initial corneal infection was the major challenge, as there was no experimental animal model for recurrent herpetic keratitis.

Many similarities were evident between naturally recurring herpetic keratitis in humans and the experimentally induced herpetic keratitis in rabbits. In both rabbits and humans, dendritic epithelial keratitis and corneal anesthesia were seen. Favorable response to antimetabolite drugs such as IDU and deleterious effects to corticosteroids occurred. Disciform keratitis was encountered in both species and circulating antibodies formed after infection with herpes. Most importantly, there was persistence of her-

pes simplex virus and recurrence of infection with or without keratitis.

I had read about recurrent herpetic encephalitis in mice and rabbits induced by systemic epinephrine and thought we could replicate these findings for herpetic keratitis in rabbits. I was introduced to Sidney Kibrick MD at Boston University, who had collaborated with a prior Dohlman fellow, Bernard Zucker. The encouragement of Dr. Dohlman to work with Dr. Kibrick, even though it required driving daily from the Retina Foundation, where the infected rabbits were housed, to Boston University, where the viral cultures were performed, led to the creation of an experimental animal model for recurrent herpetic keratitis [7]. It was also shown by slit lamp observation and positive cultures that these herpetic recurrences could be repeatedly induced in the same rabbit with epinephrine parenteral stimulation over long periods, up to 8 months [8].

After my corneal fellowship at the Massachusetts Eye and Ear Infirmary, I returned to Wills Eye Hospital in November 1965 to work on the Cornea Service as one of the first geographic full-time physicians employed by the hospital, where Wilfred Fry MD was the head. Herpes simplex viral keratitis at that time was a significant problem and almost five percent of our corneal practice was related to herpetic viruses. Three percent of the corneal transplants done at Wills Eye Hospital were done for the ravages of herpes simplex virus infection.

Despite the proven efficacy of IDU for the treatment of superficial herpetic keratitis, it was not effective in all cases of dendritic keratitis. Toxicity to the IDU drops also became a problem. The dosage regimen for IDU therapy was difficult, requiring drops hourly while awake and every 2 hours during sleep for up to 2 weeks. Many patients could not fulfill these rigid requirements. Often after the first 7–10 days of this therapeutic regimen, ocular injection and conjunctival swelling occurred. In some patients, conjunctival edema caused blockage of lacrimal drainage, resulting in excessive tearing, which secondarily rendered the IDU applications ineffective. In addition, viral resistance to IDU drops occurred.

It was difficult to determine if failure of response to IDU was caused by viral resistance or improper application such as multiple skipped doses, with patients unable to consistently use drops hourly while awake and two-hourly while asleep, even after the introduction of IDU ointment for bed-time use. Because of these challenges with IDU therapy, alternate antimetabolite drugs were investigated.

Adenine arabinoside (ara-A) was the next antiviral medication to be investigated. Unlike IDU, ara-A was not incorporated into the DNA molecule. It displaced thymidine with iodine in that molecule. Ara-A was a nonspecific intracellular inhibitor of viral replication. It was available as an ointment, thereby requiring fewer applications. In multiple studies, ara-A ointment applied five times a day was as efficacious as IDU in treating dendritic superficial herpes simplex keratitis [9]. Patients using ara-A ointment had significantly less injection, burning and tearing, and the five times daily application was readily tolerated. It was also shown to be efficacious in cases of herpetic keratitis where IDU was less effective and/or intolerant [10].

Two different antiviral medications for treating herpetic keratitis were officially available for use in the United States by the 1970s, IDU and ara-A ointment. Neither of these was the ideal antiviral for treating this disease. Such an antiviral should be nontoxic and neither was. Ideally, a topical antiviral against herpes simplex keratitis should eliminate the viral growth in the corneal epithelium within the first two virus cycles or 48 hours after treatment initiation. Neither of the two available antivirals reached that efficacy. Therefore, the search for an ideal antiviral proceeded.

Kaufman and Heidelberger in 1964 [11] described a new antiviral, trifluorothymidine (F3T). Numerous studies in the 1970s concluded that this antiviral was the best choice of the three drugs approved by the United States FDA to treat herpetic keratitis. It still took an average of a little over 5 days for an epithelial HSV lesion, usually a dendritic ulcer, to completely re-epithelialize using trifluridine. F3T drops were prescribed nine times a day while awake. As with ara-A and

IDU, F3T seemed to have little effect on stromal or disciform keratitis, metaherpetic keratitis, or herpetic uveitis, all corneal diseases which may not be directly caused by replicating herpes simplex virus.

Many ophthalmologists, particularly those practicing before the era of antiviral herpetic drugs, felt that rapidly removing herpes virus in the corneal epithelium, either in the form of dendritic or geographic keratitis, allowed for rapid healthy corneal re-epithelialization and reduced the incidence of stromal disease or deeper forms of herpetic keratitis. Before the use of antiviral therapy, therefore, Gunderson's epithelial debridement with strong iodine solution or just gentle mechanical debridement of the epithelial lesion hastened healing of epithelial herpes.

Even after the introduction of antiviral therapy for epithelial HSV, there were many ophthalmologists who preferred initial debridement which quickly rid the epithelium of replicating virus, followed by patching until reepithelialization. We had seen patients at Wills who after such treatment quickly developed recurrent dendritic or geographic keratitis. Therefore, a study was designed to treat patients with epithelial HSV with either trifluridine (F3T) drops or debridement, followed immediately by a similar course of trifluridine or debridement alone and patching [12].

It soon became apparent that this third arm of the study, debridement and patching, led to herpetic epithelial recurrences in 4 to 10 days in almost 2/3 of the patients. In this study, debridement combined with trifluridine offered no advantage over the antiviral treatment alone. Until the era of oral antivirals and the even newer topical antivirals such as gancyclovir ointment, topical trifluridine drops continued to be the treatment of choice for epithelial keratitis.

Every corneal fellow and research associate who had the opportunity over a 60-year period to work and study with Claes Dohlman and his associates at the Massachusetts Eye and Ear Infirmary and the Schepens Eye Research Institute understand profoundly the unique qualities of this experience. The thoughtful genesis of ideas for projects and the meticulous

attention to details along every step of the work were the hallmarks of his relationship with those of us lucky enough to be a part of the Corneal Unit. All of the facets of Claes's leadership in my fellowship prepared me for my future 50-year career at Wills Eye Hospital. I am forever grateful to him for his friendship, guidance, and support.

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Part II

Perspectives on Important Corneal and External Diseases

Fungal Keratitis

5

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Worldwide, fungal keratitis (FK) constitutes up to 50% of all microbial keratitis. In the United States, fungi are responsible for 6–20% of all corneal infections; this varies widely by geographic location [1]. Filamentous (*Aspergillus*, *Fusarium*, and *Curvularia*) and yeastlike fungi, such as *Candida*, are most commonly associated with FK. FK is a serious ophthalmic condition, which is more likely to perforate and require therapeutic keratoplasty than bacterial keratitis [2]. This can be attributed to a combination of the pathogenicity of the organism, delays in diagnosis, and uncertainty regarding optimal treatment strategies. Treatment of FK is further limited by a lack of commercially available antifungal ophthalmic treatment options. Currently, natamycin ophthalmic suspension (Natacyn®) is the only commercially available antifungal for topical use approved by the US Food and Drug Administration [3]. In this chapter, advancements in research on the pathogenesis, diagnosis, and treatment of fungal keratitis will be detailed.

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Pathogenesis

Microorganism invasion frequently occurs secondary to alterations of the corneal surface that affect the integrity of the corneal epithelial layer, creating an opportunity for organisms to invade the underlying layers [1]. This invasion leads to a mostly innate immune-mediated inflammation, potentially resulting in subsequent tissue necrosis and corneal perforation [4]. Over the last decade, research has focused predominantly on studying the innate immune response which acts as the first line of defense to microorganism's invasion.

The dynamic balance between host defenses and the virulence of invasive fungi is critical in the development of FK. Receptors that play a critical role in the innate immune system include C-type lectin-like receptors (CLRs), toll-like receptors (TLRs), nucleotide binding oligomerization domain-like receptors (leucine-rich repeat-containing receptors, NLRs), and receptors belonging to the scavenger receptor (SR) family. These pattern recognition receptors (PRRs) mediate the secretion of cytokines and chemokines, promoting the infiltration of neutrophils, macrophages, and T cells [5]. In the process, reactive oxygen species (ROS) can be generated. ROS are a double-edged sword that can help the host eliminate exogenous pathogens but also cause damage to mitochondria, inducing cellular dysfunction and inflammation [6]. Hua et al. explored the mechanism by which

ROS-induced oxidative stress contributed to the pathogenesis of fungal keratitis using an in vivo experimental keratitis mouse model and an in vitro culture of human corneal epithelial cells (HCECs). ROS production was markedly increased in fungal corneas and HCECs exposed to *Candida albicans*, accompanied by p38 mitogen-activated protein kinases (MAPK) activation [7].

As part of the innate immune response, macrophages also play a significant role as a first line of defense against fungal infections. They promote the production of cytokines and chemokines to increase polymorphonuclear infiltration. IL-6, IL-8, and MCP-1 are key chemokine orchestrators of the inflammatory response against fungal infection [5]. Taylor et al. have identified in mice models an essential role of IL-17 producing neutrophils and Th17 cells in regulating the growth of fungal hyphae and the severity of corneal disease [8]. IL-17 was also studied by Karthikeyan et al. where production by peripheral blood neutrophils was examined in patients with fungal keratitis and in uninfected individuals in southern India. IL-17 gene expression and intracellular IL-17 were detected in all groups, but levels were significantly elevated in neutrophils from patients with keratitis [9].

To counter the innate immune response, fungi produce enzymes that destroy corneal tissue and antimicrobial proteins to survive and evade host defense mechanisms [10]. Gharamah et al. reported in vitro screening of enzymes from fungal isolates of patients with fungal keratitis. Out of 25 fungal isolates, 80% were positive for protease, 84% for lipase and urease, 28% for blood hemolysis, and 100% for phosphatase and catalase enzymes [10]. Similarly, Selvam et al. studied the extracellular enzymes produced in human fungal keratitis from a total of 68 *Fusarium* spp. and 86 *Aspergillus* spp. *Aspergillus* spp. had more protease (82.5% of 86) and amylase (88.3% of 86) activity than that of *Fusarium* spp. (protease: 70.5% of 68; amylase: 57.3% of 68). However, *Fusarium* spp. were comparatively active with regard to cellulase and lipase than *Aspergillus* spp. (cellulase, 72% of 86; lipase, 68.6% of 86) [11].

Another important virulence factor produced by fungi are mycotoxins. Mycotoxins have been implicated in acute systemic disease in cases where no evidence of infection with known etiological agents exist or no improvement in the clinical evolution has occurred following medical treatment. The disease, which can be fatal, has been termed mycotoxicosis [12, 13]. Only a few studies have investigated the role of fungal toxins as contributing factors in human corneal infections [10, 11, 14, 15]. Gharamah et al. reported on a total of 25 fungal isolates from patients with fungal keratitis and found 13 positives for 7 types of mycotoxins in culture (aflatoxins B1, B2, G1, and G2, sterigmatocystin, fumagillin, diacetoxyscirpenol, zearalenone, T-2 toxin, and trichodermin) [10]. Naiker and Odhav et al. studied *Fusarium* isolates from 29 patients with mycotic keratitis *Fusarium* species and found 76% produced fusaric acid, moniliformin or fumonisin B₁. Naiker and Odhav et al. determined a linear relationship between toxin concentration and cell death in vitro [15]. Given 124 *Aspergillus* spp. isolates from FK patients, Selvam et al. screened for aflatoxin-producing ability, of which 41 (33%) isolates were detected with aflatoxin activity. Aflatoxin B2, G1, and G2 were more common compared to aflatoxin B1. Interestingly, no mycotoxin was detected from *Fusarium* spp. This is important since the toxic effects of aflatoxins in the cornea involve haziness of the cornea and separation of corneal lamellae in addition to infiltration by polymorphonuclear leukocytes [11]. Finally, Raza et al. found that isolates of *Fusarium* species from 18 patients with keratomycosis could produce mycotoxins in vitro. Of these isolates, 13 produced nivalenol, 6 produced deoxynivalenol, 9 produced T-2 toxin, and 2 showed the presence of diacetoxyscirpenol. Raza et al. did not find any correlation between the presence of toxins and the severity of the disease [14].

Finally, fungi are also capable of generating biofilms, which have been implicated in contact lens outbreaks. In particular, *F. solani* forms biofilms more often than other species complexes [16]. The ability to form biofilms makes an isolate more resistant to antifungal agents than their

counterparts. *F. solani* biofilms have demonstrated reduced susceptibility, mainly for amphotericin B, which could in part explain the worse clinical outcomes for *F. solani* compared with other *Fusarium* species [17]. N,N-Hexyl,methylpolyethylenimine (HMPEI) has been shown to be potential broad antifungal and antibacterial for biofilm. Over the last decade, it has been brought to interest for the use of HMPEI for devices-related biofilm infections such as keratoprosthesis [18].

Diagnosis

Even though the clinical presentation of fungal keratitis has some key clinical characteristic features on the slit lamp exam, such as deep infiltrates with feathery margins and satellite lesions,

the clinical presentation of fungal keratitis can mimic a bacterial or parasitic infection making the clinical diagnosis very challenging (Figs. 5.1 and 5.2). In one study, clinical examiners correctly predicted the presence or absence of microbial recovery in 79 out of 104 (76%) ulcerative keratitis cases, but only 31 (42%) were subcategorized properly. This may be due to overlapping clinical features of keratitis making it difficult to distinguish one from another [19].

A rapid way to differentiate fungal versus bacterial cause of keratitis is the use of Gram staining and KOH wet mounts. When combined with Giemsa or KOH stains, sensitivity has been shown to be 96.6%–98.3%, respectively [20]. Corneal cultures and/or biopsies are needed for diagnosis of FK although fungal isolation via conventional cultures may take 5–15 days to become positive. Prompt diagnosis of fungal

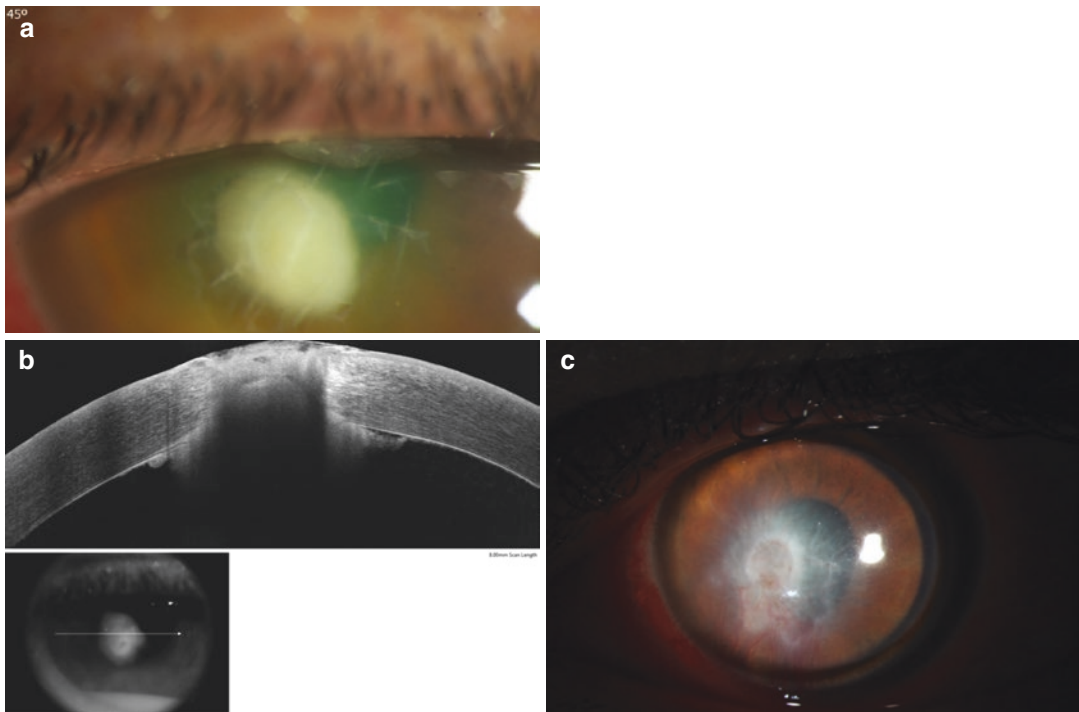


Fig. 5.1 Case 1. Photos represent a patient with fungal keratitis secondary to *Paecilomyces lilacinus*. Photo (a) shows the clinical presentation as a fungal ball and deep infiltrates with feathery margins and satellite lesions type of keratitis. This photo demonstrates how the patient presented to our institution. Photo (b) demonstrates the results of the anterior segment optical coherence tomogra-

phy that are compatible with a deep corneal infiltrate and endothelial plaque. The patient was treated with amphotericin topical, voriconazole topical/oral, and corneal glue. Photo (c) shows the patient's cornea after corneal infection healed, with neovascularization going to the area of the glue

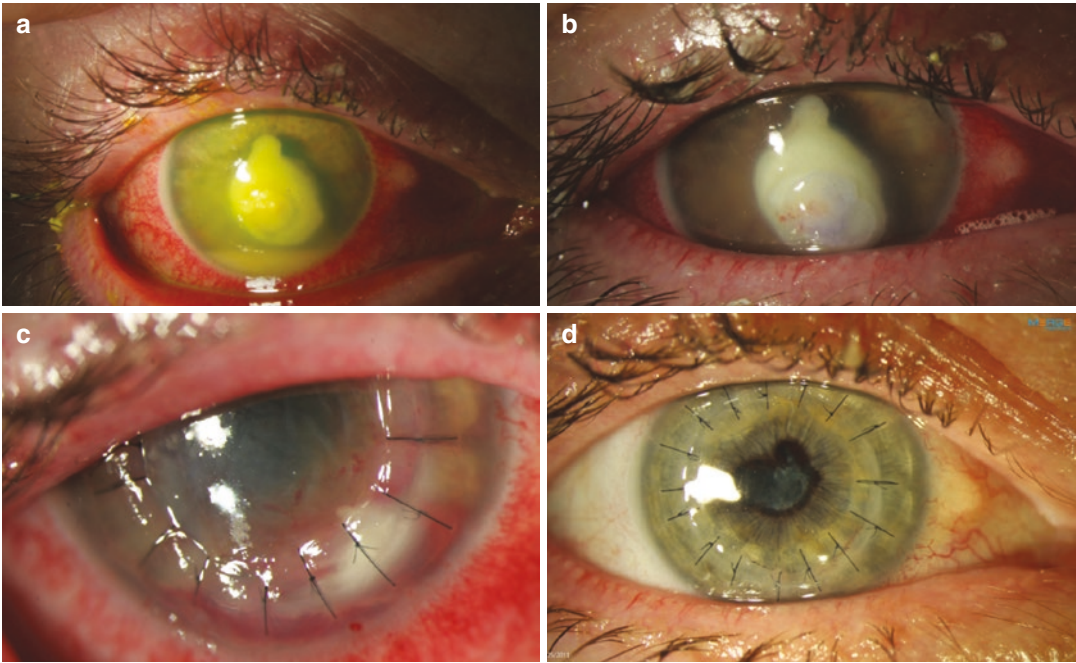


Fig. 5.2 Case 2. A 55-year-old male patient with diagnosis of fungal keratitis secondary to *Paecilomyces lilacinus*. Photo (a) shows the clinical presentation with central deep infiltrates with central melting and hypopyon. This photo demonstrates how the patient presented to our institution. Photo (b) demonstrates corneal melting with glue due to perforation. Photo (c) patient's glue did not hold, so

therapeutic corneal transplant was done, but after a couple of days, the patient developed a peripheral corneal infiltrate at 4 o'clock, so intrastromal voriconazole injections were applied every 48 hours and infiltrate resolved. Photo (d) Corneal transplant 6 weeks after corneal infection resolved

keratitis is imperative and allows better outcomes. One option to obtain a rapid diagnosis is the use of polymerase chain reaction (PCR), a highly sensitive and rapid technique to identify pathogens by extraction and amplification for small quantities of DNA. Tananuvat et al. reported a prospective nonrandomized study to compare routine microbial studies against PCR for FK. Out of 30 samples, 93.3% were positive on PCR, 40% on cultures, and 20% on potassium hydroxide. Out of the 28 PCR-positive cases, 12 were positive cultures and 16 were negative cultures. PCR did not yield any false-negative findings in the positive cultures [21].

PCR also is time efficient. The time taken for PCR is around 4–8 hours, whereas positive fungal cultures may take 1–35 days [22]. Zhao G et al. reported the use of direct PCR assays without template DNA extraction and found from 34 patients with high suspicion of FK, a positive

detection rate of 84.8% with PCR, 35% with cultures, 64.7% with smears, and 74% with confocal imaging. The sensitivity for diagnosis with direct PCR and culture were 98% and 47%, whereas specificities were 81% and 100%, respectively [23]. PCR may also be more likely to produce false positives, as the technique is at risk for contamination with incidental fungal organisms present in the examination room or the laboratory [21]. Even with the current performance of direct PCR, new molecular techniques based on amplification are under research such as nested PCR, real-time PCR, loop-mediated isothermal amplification, and dot hybridization for fungal detection.

Another diagnostic tool that can be used in cases where there are no positive cultures isolated but a high suspicion of a fungal organism is in vivo confocal microscopy. In vivo confocal microscopy (IVCM) laser scanning

Heidelberg Retina Tomography (HRT3) enables higher resolution imaging compared to white light IVCN (e.g., confoscan) allowing better visualization of cellular changes during the healing process in the cornea such as infection. Chidambaram et al. describe the cellular features of FK using HRT3 [24]. Kanavi et al. studied 133 cases of infectious keratitis and found that IVCN has a sensitivity of 94% and 78% specificity and could identify fungal keratitis in 20.3% [27] of cases in contrast to 12.0% [16] of cases by smear and culture examination [25]. However, with IVCN, patient cooperation is important as motion artifacts can affect scanning. Dense corneal infiltrates or scarring can also prevent proper tissue penetration and visualization [26].

Treatment of Fungal Keratitis: Evidence Based

Over the last 10 years, significant clinical advancement has been achieved in understanding best practices for the treatment of fungal keratitis. Table 5.1 presents a summary of the last 10 years of clinical trials. At present, no new commercially available ocular antifungal medications have been approved by the Food and Drug Administration (FDA) since natamycin was approved in the 1960s [27]. Unfortunately, natamycin has poor corneal tissue penetration, potentially limiting its effectiveness to superficial infections [28]. Newer triazoles such as voriconazole, posaconazole, or itraconazole have been suggested as better alternatives in the treatment of FK [29, 30]. Most isolates from FK have good in vitro susceptibility to triazoles. Also, increased penetration of voriconazole compared with natamycin could be advantageous, particularly for corneal ulcers with deep stromal involvement [27]. Voriconazole levels in the aqueous following topical administration have been measured to be 5 mg/ml in non-inflamed human eyes which exceed minimum inhibitory concentrations (MICs) for most pathogens [28]. Interestingly, multiple clinical studies did not show any difference between the use of

5% topical natamycin and 1% topical voriconazole against FK (Table 5.1) [27, 28, 31]. A review of the literature by Cochrane concluded that 5% topical natamycin is superior to 1% topical voriconazole in the treatment of filamentous FK [32]. In addition, repeat scraping of the epithelium during the course of treatment has been thought to be beneficial. However, Prajna et al. presented a clinical study comparing topical natamycin and topical voriconazole with and without rescraping, finding that scraping was associated with worse clinical outcomes and no difference in clinical outcomes in both topical treatments (Table 5.1) [27].

Other treatment approaches to FK include combined treatments such as topical with oral antifungal medications. Oral voriconazole exhibits excellent ocular penetration and may provide more consistent drug levels, particularly for deep stromal infections [3]. Prajna et al. studied the use of topical natamycin and topical voriconazole with and without oral voriconazole in the treatment of severe FK and found that adding oral voriconazole to topical antifungal eyedrops made no difference in the treatment of severe smear-positive, filamentous fungal ulcers. There was no decrease in the rate of perforation or need for therapeutic penetrating keratoplasty (TPK) among participants receiving oral voriconazole vs. placebo. In addition, Prajna et al. showed a significant increase in the risk of adverse events in the oral voriconazole group, including elevation of serum aspartate aminotransferase and/or alanine aminotransferase levels and visual hallucinations, well-known adverse effects of the drug (Table 5.1) [3]. Other oral antifungal therapies such as itraconazole have been studied with no significant difference in outcomes compared to voriconazole [33].

In cases where patients do not respond to standard topical medical treatment, different antifungal treatments have been described. Sharma et al. studied the use of topical and oral voriconazole against intrastromal injections of voriconazole in cases of FK which demonstrated a lack of response to 2 weeks of natamycin therapy. Intrastromal voriconazole did not display any beneficial effect over topical voriconazole (Table 5.1) [34].

Table 5.1 Summary of the last clinical trials done over the last 10-year period

Author year	Methods	Participants Setting	Groups Intervention	Outcomes	Time Fu
Mahdy 2010	Parallel Randomized controlled trial	48 eyes Egypt	Organisms: <i>Aspergillus</i> spp., <i>Candida</i> , <i>Penicillium</i> Group 1: Topical amphotericin B 0.05% and subconjunctival fluconazole 0.2% ($N = 24$) Group 2: Topical amphotericin B 0.05% alone (24 eyes)	Group 1 superior to group 2 in healing corneal ulcers, 83% vs. 67%	3 months
Prajna 2010	Parallel randomized controlled trial (multicenter)	120 eyes India	Organisms: Filamentous fungi Group 1: Topical natamycin 5% ($N = 60$) +/- corneal epithelium rescraping Group 2: Topical voriconazole 1% ($N = 60$) +/- corneal epithelium rescraping	Group 1 with no difference in final infiltrate/scar size, time to reepithelialization, or adverse events from group 2. There was a trend toward scraping being associated with worse clinical outcomes in all of the analyses	3 months
Arora 2011	Parallel group randomized controlled trial	30 India	Organisms: Most common <i>Aspergillus</i> spp., <i>Curvularia</i> spp., and <i>Fusarium</i> spp. Group 1: Topical natamycin 5% ($N = 15$) Group 2: Topical voriconazole 1% ($N = 15$)	No difference in BCVA between both groups. Average time of complete resolution of corneal infiltrate in 15 patients of group 1 was 24 days, and in 14 patients of group 2, it was 27 days. One case had corneal perforation in group 2.	Follow-up: 10 weeks or until complete resolution of the ulcer
Parchand 2012	Parallel group randomized controlled trial (multicenter)	45 Setting: Chandigarh, India	Organisms: <i>Fusarium solani</i> , <i>Aspergillus</i> spp., <i>Curvularia lunata</i> , <i>Acremonium</i> spp. Group 1: Oral and topical voriconazole 1% ($N = 15$) Group 2: Oral voriconazole and topical natamycin 5% ($N = 15$) Group 3: Oral itraconazole and topical natamycin ($N = 15$)	No difference between groups in time of disappearance of hypopyon, resolution of infiltrate, closure of epithelial defect, and final BCVA	3 months
Sharma 2013	Parallel group randomized controlled trial	40 India	Organisms: <i>Aspergillus</i> spp., <i>Fusarium</i> spp., Group 1: Topical voriconazole 1% in recalcitrant fungal keratitis ($N = 20$) Group 2: Intrastromal injections of voriconazole 50 μ g ($N = 20$) in recalcitrant fungal keratitis	Visual acuity after treatment was significantly better in the topical voriconazole group. Ulcers healed faster in the topical group by 5.5 days compared with the intrastromal group. Perforation of the corneal ulcer was seen in 1 eye in the topical group and in 4 eyes in the intrastromal group ($P 0.22$)	3 months

Table 5.1 (continued)

Author year	Methods	Participants Setting	Groups Intervention	Outcomes	Time Fu
Prajna 2013 (MUTT)	Parallel group randomized controlled trial (multicenter)	323 India	Organisms: <i>Fusarium</i> spp. and <i>Aspergillus</i> spp. Group 1: Topical natamycin 5% (<i>N</i> = 162) Group 2: Topical voriconazole 1% (<i>N</i> = 161)	BCVA outcome was better in group 1 at 3 weeks and 3 months At 6 days, group 2 had higher percentage of positive culture test. Thirty-four patients in group 2 had perforation compared to 18 patients in group 1	3 months
Prajna 2016 (MUTT II)	Parallel group randomized controlled trial	240 India and Nepal	Organisms: Most common <i>Fusarium</i> spp. and <i>Aspergillus</i> spp. Group 1: Topical natamycin 5% and topical voriconazole 1% with Oral voriconazole (<i>N</i> = 119) Group 2: Topical natamycin 5% and topical voriconazole 1% without oral voriconazole (oral placebo) (<i>N</i> = 121)	No difference between groups in rate of corneal perforation, visual acuity, rate of reepithelialization, infiltrate, and/or scar size. Group 2 had more side effects compared to group 1	3 months
Sharma 2016	Parallel group randomized controlled trial	45 India	Organisms: Most common <i>Fusarium solani</i> and <i>Aspergillus flavus</i> . Topical antifungal: Natamycin 5% and amphotericin B 0.15% Group 1: Topical antifungals+ oral antifungal (<i>N</i> = 15) Group 2: topical antifungals +intracameral amphotericin B + oral antifungal (<i>N</i> = 15) Group 3: topical antifungals + drainage hypopyon + intracameral amphotericin B + oral antifungal (<i>N</i> = 15)	No difference between groups in treatment success, visual acuity, mean time disappearance of hypopyon, and healing. Slightly higher cataract formation in groups 2 and 3 but not statistically significant	Until complete resolution of the ulcer

BCVA best-corrected visual acuity

Amphotericin B, a broad-spectrum antifungal agent, has also been used. Amphotericin B is generally effective topically for most cases of *Candida albicans*, *Aspergillus*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Coccidioides*, with limited effect on *Fusarium*, *Pseudallescheria boydii*, and *Actinomyces* [35]. Mahdy et al. compared in a clinical trial the use of topical amphotericin B and subconjunctival fluconazole against topical amphotericin B alone against treatment for

FK. The combination of topical amphotericin B and subconjunctival fluconazole was more efficient in healing FK (Table 5.1) [35]. In these recalcitrant cases, FK can be difficult to treat since the fungi can penetrate the intact Descemet's membrane and enter the anterior chamber, resulting in hypopyon. Sharma et al. compared the use of topical antifungals (natamycin 5% and amphotericin B 0.15%) and oral voriconazole with or without intracameral amphotericin B (ICAMB) and

determined no significant difference in the treatment success rate between the topical antifungal group and ICAMB groups. Microbiology analysis of the hypopyon did not reveal any positive cultures (Table 5.1) [36]. The finding suggests that ICAMB, as an additional modality of targeted drug delivery, may not be of benefit in management of mycotic keratitis. However, other studies have shown better outcomes in the intracameral amphotericin injection group compared to topicals [37, 38].

In spite of proper medical treatment, the infection can continue to progress causing significant ocular comorbidity such as corneal perforation with the secondary need for therapeutic corneal transplant. Corneal perforation is a common complication seen in up to 50% of cases of FK. In the mycotic ulcer clinical trial II (MUTT), they found from a total of 240 patients 122 (50.8%) developed full-thickness corneal perforation or needed to undergo therapeutic corneal transplant [3]. According to a recent sub-analysis by Prajna et al. from the MUTT II trial, participants with hypopyon at baseline and corneal infiltrate involving the posterior third of the cornea had an increased risk of corneal perforation and/or need for TPK for every 1 mm increase in mean penetration of the infiltrate [39]. Resistance to antifungal medication may also develop. A recent sub-analysis from MUTT 1 by Prajna et al. reported a 2.14-fold increase per year in voriconazole MICs. The same correlation was not found when testing at baseline natamycin MICs after controlling for the organism species [40].

Surgical intervention is an option for patients with disease that is refractory to medical treatment or corneal perforations secondary to fungal keratitis. The most common surgical intervention is penetrating keratoplasty (PK). PK should be delayed in cases of active fungal keratitis because infection may be introduced into the anterior or posterior chamber and the use of corticosteroids after PK may increase the chances of recurrence of fungal infection. Another option is deep anterior lamellar keratectomy (DALK) which reduces risk for immunological rejection, reduces use of topical steroids, and prevents infection from being introduced into the anterior chamber in contrast to PK [41].

In a retrospective review, big bubble DALK technique was performed on 23 eyes with deep fungal keratitis secondary to FK. Twenty-three grafts (100%) remained clear at the final follow-up. Fungal recurrence was only found in two patients, and corneal graft rejection was noticed in one patient [42].

Novel Treatments

Fungal corneal infections in general are responsible for more ocular comorbidity than bacterial corneal ulcers. Perforation is a common complication and limited treatment options exist. Adjuvant therapies are needed to prevent FK-related complications. Corneal cross-linking (CXL) is a promising adjuvant treatment due to its presumed antimicrobials effects (PMID: 29499665) and cross-linking effect which increases corneal tensile strength and resistance to collagen corneal fibers for enzymatic degradation [43, 44]. Corneal CXL is a combination treatment with ultraviolet (UV) light and riboflavin (vitamin B2) proposed by Wollensak in 2003 [45]. Corneal CXL has become an established treatment option for improving biomechanical stability and resisting the progression of keratoconus. The first therapeutic application of CXL for infectious keratitis was reported in 2008 by Isely et al., where two of the five cases were fungal keratitis [46]. The exact mechanism for the elimination of fungal microorganisms is unclear. It has been thought that Riboflavin and UV-A may both damage nucleic acids by direct electron transfer, production of singlet oxygen, and generation of hydrogen peroxide with the formation of hydroxyl radicals, so pathogens with DNA and RNA may be affected in the absence of oxygen [47].

CXL may also have a potential therapeutic effect on corneal grafts, where CXL may prevent the recurrence of infection in cases of FK after TPK, increasing the survival rate of corneal grafts [48–50]. Titiyal et al. published a prospective interventional study which evaluated the outcomes of TPK using riboflavin CXL-treated and non-treated donor corneas in fungal keratitis.

Eighty percent of patients in the CXL-treated donor group showed clear corneal grafts by 6 months compared to 22% of patients in the non-treated group. None of the patients in the CXL-treated donor group had recurrence of infections compared to 22% in the non-treated CXL group [48]. A possible mechanism for the benefits experienced by corneal graft donors treated with CXL may be that CXL regresses pre-existing blood and lymphatic vessels by the induction of apoptosis in vascular endothelial cells and upregulates T regulatory cells [49].

However, the evidence supporting CXL for FK is less robust than for bacterial keratitis. Martins et al. demonstrated in an in vitro study that riboflavin CXL was ineffective against *Candida albicans* [51]. Similarly, in a prospective randomized clinical trial, Uddaraju et al. found that CXL was not helpful in deep stromal fungal keratitis. Five eyes in the CXL group and three eyes in the non-CXL group experienced treatment failure by 6 weeks, and the CXL group experienced more perforations than the non-CXL group (4 compared to 0) [52]. In a retrospective analysis, Vajpayee et al. found no difference in average healing time and final visual acuity between groups with medical management alone and medical management with CXL. The authors concluded that CXL as an additional treatment has no advantage over medical treatment alone for moderate fungal keratitis cases [53]. Also, Sauer et al. showed that *C. albicans*, *Fusarium* spp., and *Aspergillus fumigatus* did not show any response to cornea CXL. It only showed significant inhibition of fungal growth if it was pre-treated with amphotericin B. The authors concluded that CXL enhances the effect of amphotericin B on fungal cell membranes [54]. In contrast, beneficial results were found by Zhu et al. in a study of the effectiveness of corneal riboflavin CXL on an in vitro and in vivo mouse keratitis model. Riboflavin CXL significantly decreased colony-forming units of *Fusarium solani* and reduced corneal lesion size, corneal opacity, neovascularization, and depth of ulceration [44]. The use of riboflavin CXL has also been reported in several case reports and case series with good outcomes [46, 55, 56]. In a

patient with corneal ulcer secondary to *Aureobasidium pullulans* fungal infection, accelerated riboflavin CXL was successful as a first line of treatment. Similarly, Li et al. reported successful use of CXL on eight cases of microbiologically proven FK. Healing of the corneal epithelium and ulcer was achieved in all cases 3–8 days after CXL treatment. None of the cases required corneal transplantation [55].

As an alternative to riboflavin CXL treatment for FK, research by the Ocular Biophysics Laboratory at Bascom Palmer Eye Institute has been focused on the in vitro antimicrobial efficacy of photodynamic antimicrobial therapy (PDAT) using different ophthalmic photosensitizers. In contrast to riboflavin PDAT, rose bengal PDAT was more effective at killing against organisms, such as *Fusarium solani*, *Aspergillus fumigatus*, and *Candida albicans* [57], and demonstrated complete antimicrobial efficacy against *Fusarium solani*, *Aspergillus fumigatus*, *Candida albicans*, *Pseudallescheria boydii*, and *Paecilomyces variotii* (Figs. 5.3 and 5.4). The first reported clinical case of rose bengal PDAT efficacy was demonstrated in a patient with severe *Fusarium keratoplasticum* keratitis. The patient was treated successfully with rose bengal PDAT and had no complications during 10 months of follow-up [58, 59]. The safety of rose bengal and green light has been established in vivo [60]. Resistance to treatment has also not been observed with PDAT. Previous studies have attempted to artificially engineer PDAT resistance by repeatedly performing PDAT and regrowing bacterial samples, but resistance was never developed. The structure of fungi makes them inherently more permeable to external substances than Gram-negative bacteria, and thus PDAT has been actively pursued as a novel antifungal agent [47].

Another adjunctive innovative strategy for the treatment of resistant cases of FK is the use of argon laser photocoagulation of the corneal ulcer. It was first described by Pellegrino et al. in 2013. Their publication describes the treatment of two cases of recalcitrant FK with argon laser plus standard medical treatment [61]. Khater et al. reported a prospective randomized trial comparing the use

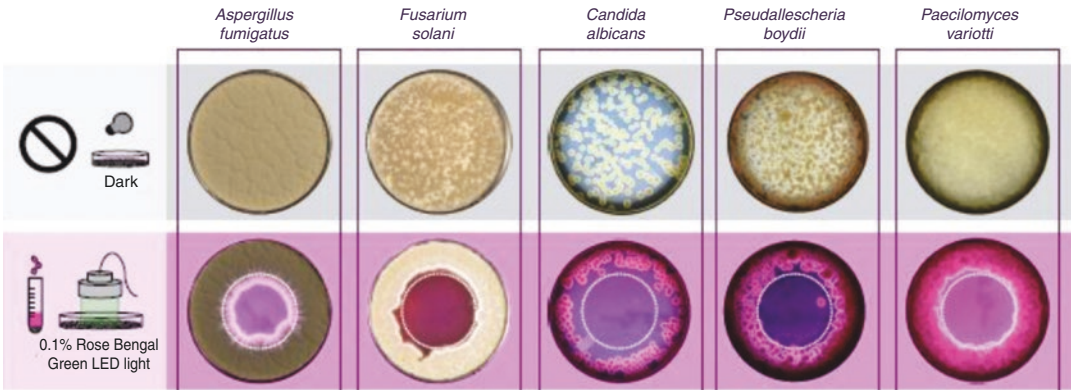


Fig. 5.3 Results from in vitro experiments testing antimicrobial efficacy of photodynamic antimicrobial therapy against fungal microorganisms isolates; white circle indi-

cates the area of the agar plate irradiated by the custom-made LED sources



Fig. 5.4 Rose bengal photodynamic antimicrobial therapy computer controlled 525 nm light-emitting diodes console; photo shows the actual procedure during the green light phase

of argon laser and amniotic membrane and debridement as well as amniotic membrane placement in cases of treatment resistance. Interestingly, the authors found better results in healing of the argon laser group compared to the debridement

group with no adverse events [62]. Khater et al. also reported comparing the use of argon laser to intrastromal voriconazole in recalcitrant cases of FK, where argon laser group again demonstrated superior healing [63].

In summary, the diagnosis and treatment of FK continues to be a challenge. Molecular techniques may help in the early detection and guidance for treatment. Novel potential treatments such as riboflavin cross-linking (CXL) and rose bengal photodynamic antimicrobial therapy (PDAT) have shown some success but still need to be evaluated in prospective and appropriately controlled clinical trials.

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Ocular Herpes Simplex

6

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Disease Entity

Ocular viral infections range from benign to malignant and are caused by various DNA and RNA viruses. Of these, the DNA virus, herpes simplex virus (HSV), causes significant ocular morbidity as it affects nearly every ocular tissue by both infectious and immune pathogenic mechanisms [1].

Epidemiology

HSV is the most common infectious cause of corneal blindness in the developed world. The global incidence of ocular HSV is estimated to be 1,000,000 new cases and 9,000,000 recurrent episodes each year [2]. The annual incidence of ocular HSV infections has recently been estimated at 11.8 per 100,000 people in the United States [3] and 13.2 per 100,000 in France [4]. Extrapolation of epidemiological data to the total US population in 2011 yielded an estimate of 64,499 new and recurrent cases annually. Comparison of the recent data to the reported incidence of 8.4 per

100,000 and prevalence of 149 cases per 100,000 in the early 1980s suggests an upward trend in both incidence and prevalence of ocular HSV [5].

The mean age for the first occurrence of ocular HSV -1 in the United States is 37.4 years, with autopsy studies on the trigeminal ganglia showing that >90% of the population is infected with latent HSV-1 by 60 years of age. Reported seroprevalence rates of HSV-1 in the United States, Germany, and Tanzania are >50%, >75%, and >90%, respectively, and are related to age and socioeconomic status. While 30% of children and 70–80% of adolescents in lower socioeconomic class are seropositive, the overall seroprevalence of HSV-1 in adolescents in the United States is about 30% [6]. While HSV-1 is shown to be responsible for most ocular herpes simplex infections, HSV-2 is the usual cause of neonatal HSV infection [7]. Recent studies indicate a changing epidemiology with 30% increase in HSV-2 seroprevalence in the United States over the past 3–4 decades. The incidence of neonatal HSV ranges from 5.8 to 11.5 per 100,000 live births in the United States, with 13–20% of neonates with HSV having ocular manifestations [8]. Of these, dendritic epithelial keratitis is the most common, with the incidence in the United States being 63%. The reported incidence of blepharoconjunctivitis, stromal keratitis, and uveitis is 54%, 16%, and 4%, respectively [5], while in the French study, the incidence rates were 56%

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dendritic keratitis, 10% geographic keratitis, and 30% stromal keratitis [4].

Economic Burden

In the United States, it is estimated that 500,000 people have ocular HSV, requiring between 4 and 6 ophthalmologist visits, leading to the total treatment cost of \$ 17.7million and a loss of 444,000 work days annually [9].

Etiology

Herpes simplex virus belongs to the alpha subgroup of the *Herpesviridae* family which is characterized by a double-stranded linear DNA genome encased within an icosahedral viral protein capsid surrounded by a lipid bilayer envelope. The herpes viral genome can also survive without the viral capsid which allows it to lie quiescent and establish latency – a feature unique to herpes viruses. HSV-1 and HSV-2 have different virus-specific antigens. HSV-1 preferentially affects the oropharynx, while HSV-2 colonizes the genital tract. Both HSV-1 and HSV-2 can cause ocular herpetic disease although HSV-1 is more common.

Pathophysiology

The transmission of HSV is by direct contact with the saliva and/or genital secretions of infected individuals and is more common in periods of asymptomatic shedding.

At the time of initial acquisition, the virus replicates in the skin, cornea, or mucosal surfaces of the orofacial region. It then travels via the trigeminal nerve axons in a retrograde fashion and becomes latent in the trigeminal nerve ganglion. In order to establish latency, the viral genome is circularized to form an episomal DNA element packed in histones. In this state of latency, the virus employs various immune-evasive mechanisms and can exist for a variable amount of time before reactivation. One such mechanism is

induction of intracellular accumulation of CD1d molecules in antigen-presenting cells. This helps the infected cells to evade recognition by natural killer T cells. HSV-1 has several other mechanisms by which it downregulates various immunologic cells and cytokines. Viral reactivation is believed to occur in response to various stimuli that induce HSV lytic gene expression, virion replication in the ganglia, and linearization of the DNA. The various environmental and systemic stressors that trigger reactivation are emotional stress, fever, UV light, menstruation, and hormonal changes. In vitro studies have shown that during periods of cellular stress, there is transient interruption of protein synthesis. This in turn affects the activity of mTOR kinase activity and causes mRNA translation in the neuron harboring the viral episome leading to viral replication. It can then travel back down any branch of the trigeminal ganglion. Recurrences tend to occur at sites with high distribution of sensory receptors such as cornea, lips, and oral mucosa. Reactivation in the ophthalmic division causes recurrent corneal disease [10].

Risk Factors

A majority of the adult population above 60 years of age is serologically positive for HSV-1. A study by Kaufman et al. showed HSV-1 DNA shedding in the tears and saliva of 98% of subjects, at least once in a 30-day study [11]. The detection of HSV-1 DNA on the corneal surface is, therefore, very common. Despite this frequent shedding of HSV-1 DNA in humans, the incidence of HSV-1 ocular disease is very low. Various factors that determine viral reactivation and disease recurrence include genetic makeup of the host, genetic makeup of the virus, and immune status of the host. Genetic factors in the host include the presence of the apoE ε4 genotype. Viral factors include virulence, antigenic differences, and viral load.

Host factors include underlying immune insufficiencies, inherited and acquired, and local tissue health. Immunocompromised individuals, for example, organ transplant recipients or

patients with diabetes mellitus or HIV, experience more severe disease and more frequent recurrences. CD8+ T cells produce interferons and related factors and inhibit viral activation in the infected neurons. In periods of immune deficiency, the activity of CD8+ T cells is reduced leading to viral replication. Several case reports have shown that patients infected with measles have altered T cell function and are more likely to develop HSV. HIV patients have been shown to have a higher recurrence rate compared to immune-competent individuals. Atopic conditions also lead to altered cell mediated immunity, and such patients are susceptible to HSV infections. It has been shown that severe atopy has 2.6 greater odds of developing ocular HSV [12]. These patients tend to have bilateral, severe disease and are less responsive to treatment. Although the HEDS trial concluded that age does not affect the recurrences, other retrospective studies have shown that children have more severe disease with more recurrences, with the recurrence rate within first year of an episode being 45–50% as compared to 18% in adults. Likelihood of bilateral HSV keratitis is also more in children.

Further, corneal health is an important factor that determines the susceptibility to HSV factors. Trauma, postsurgical inflammation, and medications have been implicated in increased risks of HSV keratitis. Topical anti-glaucoma drops, particularly prostaglandin analogs, have been shown to cause recurrences of HSV keratitis and blepharitis [13]. Corticosteroids, topical, intravitreal, and/or systemic, all alter the immune response and predispose to HSV epithelial keratitis. A few case reports have also shown HSV epithelial keratitis after intravitreal anti-VEGF injections. A recent case report shows HSV keratitis following botulinum toxin injection for epiphora. Laser procedures including laser photokeratectomy, laser-assisted in situ keratomileusis (LASIK), laser iridotomy, and laser trabeculoplasty have also been strongly implicated as a risk factor for HSV. This may be due to trauma to the corneal nerves from the laser or inflammation. Several reports have shown viral reactivation after cataract surgery, penetrating keratoplasty, and deep

anterior lamellar keratoplasty. Following PKP, recurrence can occur due to reactivation from the trigeminal ganglion.

Clinical Disease

Ocular herpetic disease may be primary neonatal, primary, or recurrent. Primary disease is the infectious disease of the nonimmune host. Recurrent disease occurs in the previously immune host, with or without a known history of herpes, and may be either infectious, immune, or both [6, 14] (Fig. 6.1).

Primary Ocular Herpes

Acute Neonatal Ocular HSV

Neonatal HSV is rare and affects about 1 in 10,000 infants, with 80% of the infection caused by HSV-2. Most exposure occurs during passage through the infected birth canal, while about 5% occurs in utero. Clinical manifestations include local disease involving the skin, eye, or oral mucosa, CNS infection, or disseminated disease with visceral organ involvement. The CNS and disseminated infections have very high mortality rates, 6% and 31%, respectively [15]. Ocular involvement mostly presents as conjunctivitis and ulcerative epithelial keratitis with punctate, dendritic, or serpiginous epitheliopathy. Stromal involvement is very rare and suggestive of intra-uterine infection. Long-term ocular complications include cataracts, necrotizing chorioretinitis, and optic neuropathy. Strabismus and opsoclonus can develop due to CNS infection and subsequent damage [16].

Primary Ocular HSV (POHSV)

Primary ocular HSV (POHSV) is the first HSV infection of the nonimmune host with less than 4% presenting as overt disease. Clinically, overt disease begins about 1 week after exposure to an infected carrier and typically manifests itself as a vesicular periocular dermatitis or blepharitis, follicular conjunctivitis, keratitis, iritis, and a nonsuppurative preauricular adenopathy. The skin eruption remains fairly localized to the

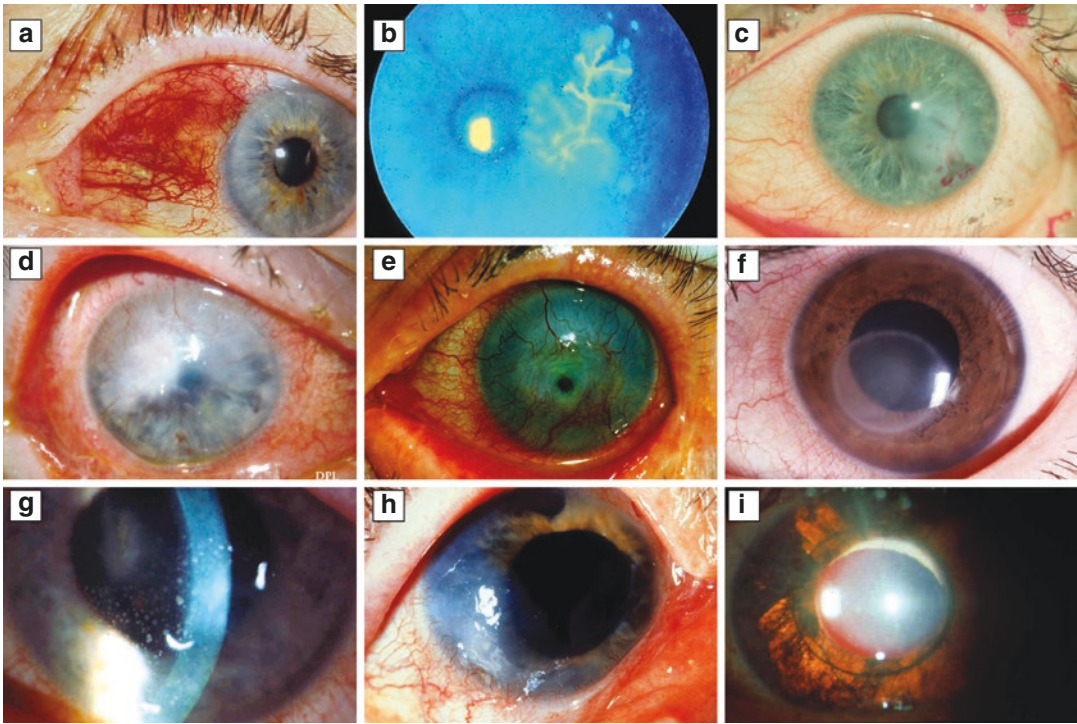


Fig. 6.1 Clinical involvement in ocular herpes simplex keratitis. Slit lamp photography. (a) Scleritis and episcleritis showing superficial and deep focal injection. (b) Epithelial keratitis showing dendritic lesion with fluorescein staining of ulcer bed. (c) Epithelial and stromal keratitis showing rose bengal staining of ulcer edges with underlying stromal haze. (d) Acute stromal interstitial

keratitis with focal corneal edema and haze. (e) Stromal keratitis showing peripheral corneal neovascularization with stromal edema. (f) Immune stromal keratitis showing circular haze. (g) Endotheliitis showing keratic precipitates and stromal edema. (h) Iritis showing iris inflammation. (i) Iris atrophy with transillumination defects

periocular area in the immunocompetent host and is a self-limited disease that resolves without scarring. A study conducted at the Moorfields Hospital on patients with primary ocular HSV reported that 84% of patients had conjunctivitis, 38% blepharitis, 15% epithelial dendritic keratitis, and 2% disciform keratitis. While POHSV is generally unilateral, 19% of these patients had bilateral disease which tends to be seen in children or patients with underlying atopic diseases.

Recurrent Ocular HSV

Blepharoconjunctivitis

HSV blepharitis presents with vesicular rash on the eyelids and along the lid margins. These lesions shed viruses for a few days and heal in

about a week. Conjunctivitis is generally follicular and may be pseudomembranous.

Episcleritis and Scleritis

While HSV episcleritis is generally an infectious process, HSV scleritis is either an infectious or an immune process and presents as a focal or diffuse deeply injected red eye that is hypersensitive to palpation. In review studies of deep sectoral scleritis, the majority of patients have tested positive for HSV and responded to treatment with acyclovir and steroids.

Keratitis

Corneal HSV can involve the epithelium, stroma, or endothelium; and the disease process, underlying pathophysiology and clinical picture, differs in each [17, 18].

- *Epithelial keratitis*

Infectious epithelial keratitis presents with redness, decreased vision, and photophobia. In dendritic keratitis, the cornea shows ulceration in a dendritic pattern with linear branches ending in terminal bulbs. The edges have active virus and stain with rose bengal (RB) stain; the center is devoid of active virus and does not stain with RB, instead picking up fluorescein stain. As the dendritic ulcer progresses when left untreated or in patients with compromised immunity and poor healing, it can lose its dendritic shape and spread out. It is then called geographic ulcer. The edges retain active virus and stain with RB. Neurotrophic or metaherpetic ulcers develop due to damage of the corneal subbasal nerve plexus by HSV. Decreased corneal sensation is seen in about 80% cases of epithelial keratitis. This damage to nerves leads to chronic ocular surface disease and epitheliopathy as corneal nerves produce trophic factors for corneal epithelium. Secondary inflammation produces further damage to nerves and epithelium and sets up a vicious cycle. This presents as irregular epithelium with punctate keratopathy that progresses to interpalpebral epithelial defect (PED) with thick rolled edges. However, unlike dendritic or geographic ulcers, the edges do not contain virus and do not stain with RB stain. This PED does not heal easily due to underlying damage to corneal nerves and can progress and cause stromal thinning and possible corneal melt and perforation [19, 20].

- *Stromal keratitis*

Stromal keratitis presents often with eye pain and blurred vision. The stromal involvement in HSV can either be due to direct viral invasion or due to immune reaction to the antigens in the stroma.

Immune stromal keratitis may present many years following initial HSV infection. The risk of developing stromal keratitis has been reported to be 21% within 2 years of the initial episode of infectious epithelial keratitis. Stromal inflammation in HSV keratitis is due to the activation of CD4 cells from HSV-specific cells, cytokine acti-

vation, and autoimmune reaction due to HSV antigen binding to host autoantigens. These CD4 cells initiate an immune destructive response and the stroma is infiltrated by inflammatory cells including neutrophils. Depending on the extent of inflammation, these antigen-antibody complex-mediated herpetic stromal keratitis can present in various forms. *Limbal vasculitis* is sectoral and presents as areas of focal hyperemia and edema and resolves over time without scarring. *Wessely immune rings* precipitate in the anterior to mid-stroma and may have a hazy edema within the ring and exhibit neovascularization. *Interstitial keratitis* (IK) is characterized by necrotic, blotchy, cheesy-white stromal infiltrates. Neovascularization, stromal scarring, and stromal edema develop subsequently. Hence without proper treatment, stromal keratitis is vision threatening and recurrence is common. In the Herpetic Eye Disease Study, 18% of patients diagnosed with HSV-1 ocular disease experienced a recurrence involving the stroma with stromal keratitis representing 44% of all recurrences. Furthermore, a history of stromal keratitis is a significant risk factor for future recurrences [18, 21].

- *Endotheliitis*

HSV endotheliitis can be both an infectious or immune-mediated process and may present in linear, disciform, or diffuse patterns [6]. *Linear endotheliitis* manifests as a line of keratic precipitates (KP) that extends from the limbus toward the center with edema of the peripheral stroma and epithelium. *Disciform keratitis* is a focal area of stromal edema without necrosis. The endothelium develops an inflammatory response to viral antigens, characterized by deposition of focal KP. The underlying endothelium decompensates and manifests as focal disc-shaped edema, hence the name disciform. *Diffuse endotheliitis* represents a more diffuse severe form of endothelial inflammation with KP over the entire cornea, diffuse edema, anterior chamber inflammation, and trabecular meshwork inflammation leading to elevated intraocular pressure.

Iridocyclitis

HSV iridocyclitis is the most common presentation of herpetic uveitis and is responsible for about 9% of all cases of nontraumatic iritis. It generally presents as unilateral anterior uveitis with non-granulomatous fine KP. Focal areas of iris inflammation result in sectoral iris atrophy.

Acute Retinal Necrosis

ARN is a syndrome characterized by retinal vasculitis, necrosis, vitritis, papillitis, and uveitis. It has been thought to be caused by herpes viruses, most commonly VZV followed by HSV-1. In immunocompromised patients, ARN may present in association with HSV-1 keratitis. In the acute viral phase, viral particles in the retina invoke an intense inflammatory response with retinal vasculitis and necrosis. In the late cicatricial phase, contractile membranes form in the vitreous and on surface of the necrotic retina causing retinal detachments in the majority of patients within 3 months [22].

Diagnosis

Diagnosis of ocular HSV is mostly made based on clinical impression only. However, objective tests are available to confirm the diagnosis in cases of neonatal infections and difficult cases.

Laboratory Approaches

1. Microscopic examination of skin, conjunctival, or corneal scrapings (Tzanck smear) or tissue obtained at keratoplasty after staining with Geimsa or Papanicolaou. Herpetic infections are characterized by multinucleated epithelial cells with ballooning degeneration and a mixed mononuclear and polymorphonuclear leukocyte (PMN) reaction. Intranuclear eosinophilic viral inclusion bodies of Lipschutz also called Cowdry A inclusions are seen in the epithelial cells.
2. Detection of HSV antigens by direct fluorescent antibody (DFA) or enzyme-linked immunosorbent assay (ELISA) test. DFA is rapid

and has high specificity; however, it requires an ultraviolet microscope. Also, topical fluorescein prior to specimen collection can lower the yield. Immunologic ELISA diagnostic kits are available commercially; they however have been shown to have low sensitivity [23].

3. Detection of HSV DNA by polymerase chain reaction (PCR) [24]. PCR has been used to detect HSV in the tear film and corneas of patients and is more sensitive and specific compared to cell cultures. PCR has further been used to identify HSV DNA in iridocorneal endothelial and in Posner-Schlossman syndromes. Commercially available kits are based on the amplification and simultaneous detection of a specific region of the HSV-1 and HSV-2 genome using real-time PCR.
4. Viral culture. The recovery rate from acutely infected ulcers is about 70% if the specimen is taken within 2–3 days of the appearance of the lesion. Although isolation of HSV-1 by cell culture has excellent specificity, the use is limited by low sensitivity and length of time need for positive results. Cultures obtained after corneal rose bengal staining may be falsely negative due to virucidal effects of the stain [25].
5. Serology. This has a limited role as HSV infections are generally due to reactivation of a latent infection. However, it is useful in certain circumstances. As IgM does not cross the placental barrier, finding IgM in a newborn is diagnostic of intrauterine infection. Serology may also be useful in patients with atypical disease. Serial titers tested 1 month apart can be informative. Quantitative documentation of a fourfold rise in either IgM or IgG strongly supports a diagnosis.

Imaging

In addition, newer imaging modalities such as in vivo confocal microscopy and anterior segment OCT can help to assess inflammation, corneal nerve damage, and corneal scarring. In vivo confocal microscopy studies in HSV keratitis have shown decreased superficial epithelial cells

with increase in cell size and squamous metaplasia with a decrease in subbasal corneal nerve density correlating with decreased corneal sensation seen clinically. Immune dendritic cells (DC) are increased in both epithelial and endothelial layers. Interestingly, endothelial cell density is decreased in cases of endotheliitis as well as in cases of epithelial and stromal keratitis with decreased corneal nerve density. Moreover, these changes of decreased corneal nerve and endothelial cell density have been shown in contralateral unaffected eyes as well suggesting a potential bilateral involvement in clinically unilateral disease. Studies have also studied changes in DC corneal nerve and endothelial cell density during the therapeutic phase to help guide treatment [20, 26, 27].

Antiviral Susceptibility Testing

This may have a role in the diagnosis of ocular HSV, specifically in recurrent and treatment non-responsive cases. Acyclovir resistance mediated by mutations in thymidine kinase may be as high as 6.4% in immunocompetent patients. These patients are also immune to other thymidine kinase-dependent drugs such as valacyclovir, ganciclovir, and famciclovir. In such cases, foscarnet, cidofovir, and trifluridine can be good alternatives [28].

Differential Diagnosis

Differential diagnosis of HSV keratitis includes other infectious etiologies such as viral and acanthamoeba and noninfectious etiologies such as contact lens overwear, neurotrophic keratitis, and Thygeson's superficial punctate keratitis. HSV interstitial keratitis includes other viral, bacterial, or fungal etiologies as well as immune-mediated conditions such as sarcoidosis, Cogan's syndrome. HSV endotheliitis includes other viral etiologies, corneal graft failure, inflammatory conditions such as Fuchs heterochromic iridocyclitis, and Posner-Schlossman syndrome.

Treatment

Medical Treatment

The mainstay of treatment is antiviral therapy in topical or oral form or both [18, 29].

The topical antiviral drugs approved by the US Food and Drug Administration (FDA) are trifluridine (TFT) solution and ganciclovir gel. Although topical acyclovir is not approved in the United States, it is widely used outside. Aqueous humor concentrations are adequate with both topical TFT and acyclovir. While TFT penetration doubles in presence of corneal epithelial defect, topical acyclovir achieves adequate levels even in intact corneal epithelium. While there are no clinical trials directly comparing topical ganciclovir gel to trifluridine solution, several clinical trials have compared each to topical acyclovir ointment and have concluded that efficacy of the three agents are similar [30]. While both TFT and ganciclovir are considered safe, prolonged topical TFT causes toxic epitheliopathy, allergic conjunctivitis, and punctal stenosis.

There are three oral antiviral agents that demonstrate activity against HSV infections and have favorable safety profile – acyclovir (ACV), valacyclovir (VCV), or famciclovir. Clinically, all three have been shown to have equal efficacy. The therapeutic dose of ACV is 400 mg orally 5×/day, for 7–10 days [31]. VCV and famciclovir require less frequent dosing (VCV 500 mg 2×/day or famciclovir 250 mg 2×/day for the same time period as acyclovir) but are more expensive. VCV is hydrolyzed back into ACV but has five times the bioavailability, and famciclovir has a longer intracellular half-life than ACV. Oral antiviral agents should be used with caution in elderly patients (>65 years old) and those with renal impairment, as all three oral antiviral agents have the potential to cause nephrotoxicity. All three oral antivirals are designated Pregnancy Category B as the use of oral valganciclovir, foscarnet, and cidofovir is limited by a poor safety profile [32].

Primary Ocular HSV

Although POHSV can resolve spontaneously, specific antiviral therapy results in earlier

resolution. ACV is the most commonly used drug with dosage altered according to age of patients. For children less than 8 years of age, oral acyclovir, available as a suspension of 200 mg/5 ml, is administered at a dosage of 20 mg/kg every 8 hours. Post-pubertal children are treated at the same dose as adults – ACV 400 mg 5×/day. Alternatively, VCV 500 mg twice daily or famciclovir 125–250 mg three times daily can be used [33]. Herpes simplex keratoconjunctivitis is treated with topical TFT 5×/day until resolution. For ocular and mucocutaneous HSV infections in the immunocompromised host, therapy consists of IV acyclovir at 5 mg/kg every 8 hours for 7–14 days [30].

Acute Neonatal Ocular HSV

Pregnant females with frequent recurrences of genital herpes should receive suppressive acyclovir therapy beginning at 36 weeks gestation. In women with active lesions at the onset of labor, caesarean section should be done to prevent infection [34]. Neonates delivered through an infected birth canal should be screened between 24 and 48 hours of age with viral cultures of eyes, nasopharynx, mouth, and rectum and if positive, they should be treated with acyclovir even if asymptomatic. An emergency pediatric or infectious disease consultation should be obtained. Treatment is with high-dose intravenous acyclovir (60 mg/kg per day in three divided doses) for 3 weeks, with adjustments made for infants with renal or hepatic insufficiency. Infants with disease localized to the skin, eyes, and mucous membranes can be treated for 2 weeks if the CSF PCR reaction assay is negative for HSV DNA. Decreasing the acyclovir dosage or administering granulocyte colony-stimulating factor should be considered if the absolute neutrophil count falls and remains below 500/mm³ for a prolonged period [35].

Recurrent Ocular HSV

Blepharitis, Dacryoadenitis, and Conjunctivitis

ACV 400 mg orally 5×/day or TFT 5×/day for 10 days or until the lesions have scabbed.

Keratitis

- *Epithelial keratitis*: Mainstay of treatment is antiviral therapy, either topical with TFT or ganciclovir gel for 7–10 days or oral antivirals in therapeutic doses; there is no reported benefit of combining oral and topical therapy. Debridement alone is not as effective. Topical antibiotic is added when active ulceration is present and corticosteroids are contraindicated in these cases [17, 32].
- *Neurotrophic keratitis*: Antiviral therapy has a limited role. Treatment of meibomian gland dysfunction and copious lubrication is needed in early stages of NK to prevent epithelial breakdown. In case of epithelial defect, autologous serum tears, cord blood serum, and platelet-rich plasma have all shown efficacy in healing of ulcers. In addition, bandage contact lenses, scleral lenses, and amniotic membrane Prokera™ amniolens may be used to promote epithelial healing. More recently, recombinant human nerve growth factor (cenegermin) has shown promise in moderate to severe NK. Surgical interventions are reserved for nonhealing ulcers or progressive corneal melts and perforations [19].
- *Stromal keratitis*: As stromal keratitis is an immune-mediated process. The mainstay of treatment is topical corticosteroids with antivirals for at least 10 weeks in cases of stromal keratitis without ulceration. In case of epithelial ulceration, corticosteroids are held or limited in strength or frequency until the epithelium heals, and topical antibiotic drops are added.
- *Uncontrolled inflammation itself may interfere with healing*. The above guidelines were established based on the HEDS study, a double-blind, randomized clinical trial that compared prednisolone with TFT versus placebo with TFT [21]. Only 26% of the patients in the prednisolone group failed treatment as compared to 73% in the placebo group. Further, the study showed that the minimum recommended treatment period is 10 weeks. There was no added benefit of oral antivirals to the treatment regimen with topical antivirals and prednisolone. However, given poor

corneal concentration and unfavorable safety profile of the topical antivirals currently available in the United States, oral antivirals in the therapeutic dose were used [17].

- *Endotheliitis*: The recommended treatment for HSV endothelial keratitis includes a topical corticosteroid in conjunction with an oral antiviral agent similar to stromal keratitis; however, the mean healing time for endotheliitis is less compared to stromal keratitis; hence the treatment is recommended for 3 weeks [17, 21].

Iridocyclitis

Mild iritis is treated with cycloplegics, while more severe disease requires topical steroids with slow taper. Oral antivirals in the prophylactic dose are often recommended as well.

Acute Retinal Necrosis (ARN)

ARN is a serious disease with very poor prognosis as retinal detachments often develop in the necrotic thinning retina. Prompt hospitalization with intravenous antiviral therapy and oral steroids is needed. Intravitreal injection of antiviral medication may also be performed.

Surgical Treatment

Surgical treatment is required acutely for progressive ulceration with corneal melting and perforation, severe neurotrophic keratitis, and visual rehabilitation in case of corneal scarring. In case of progressive thinning, tissue adhesive glue or ocular surface reconstruction with amniotic membrane or conjunctival and pedicle grafts may be needed. These procedures are indicated to provide tectonic support to the cornea. In case of advanced NK, adjunctive lateral tarsorrhaphy is often very helpful. In further progression, therapeutic keratoplasty may be indicated [36]. A novel surgical technique has been proposed recently for restoring corneal sensation in case of neurotrophic damage. This corneal neurotization involves grafting sural nerve to the ophthalmic division of the trigeminal nerve and has shown improvement in corneal sensation in 3–6 months.

Surgical options for optical reasons depend on the extent of scarring and degree of visual impairment. For partial-thickness scars, deep anterior lamellar keratoplasty (DALK) can be performed. In case of full-thickness scars, penetrating keratoplasty may be performed when the disease is quiescent. A comparison of DALK and PKP in herpetic infections has shown lower rejection, recurrence, and failure rates in DALK, although most studies have been retrospective with small sample sizes [37]. The Boston keratoprosthesis may be indicated in cases that cannot sustain a PKP [38, 39].

A major challenge in corneal transplantation in herpetic keratitis is poor graft survival. The reasons are manifold. Grafts done in case of active inflammation have less than 25% chance of survival at 1 year. Corneal neovascularization in the graft with subsequent graft rejection and failure is a common complication [40]. This occurs due to deep corneal neovascularization in stromal keratitis and also release of VEGF by viral antigens in the residual scar. Corneal hypesthesia further reduces chances of graft survival. Recurrence rate of HSV keratitis in patients without oral antiviral prophylaxis was reported at 32% at 4 months, 39–46% at 1 year, and 27–50% at 2 years after transplantation [41]. A recent review of randomized controlled trials to assess the efficacy of oral antivirals after corneal transplantation in ocular HSV showed that oral acyclovir reduces HSK recurrence by about 23 cases less per 100 grafts and graft failure by 13 fewer cases per 100 cases [42].

Thus, four major factors are key to long-term survival of keratoplasty in herpetic eyes: (1) waiting till the eye is quiescent; (2) intensive postoperative topical steroids to suppress inflammation and neovascularization; (3) removing the scar in its entirety and adjunctive lateral tarsorrhaphy; and (4) oral antivirals, e.g., ACV 400 mg po bid for 12–18 months (or longer as needed) as prophylaxis against recurrence of infection in the graft. The recurrence rate of HSV keratitis following penetrating keratoplasty decreases with increased length of oral acyclovir treatment resulting in reported recurrence rates of 0% at 16 months and 5% at 2 years. Further, it appears reasonable

following keratoplasty to continue prophylaxis with oral antivirals for as long as the patient remains on topical corticosteroids.

Prognosis

A major factor contributing to the prognosis of ocular HSV is the high recurrence rate. The HEDS study estimated the cumulative probability of recurrence in any type of ocular HSV to be 32% at 1 year [43]. While a previous history of epithelial keratitis does not significantly increase the risk of epithelial keratitis, a history of stromal keratitis increases the rate of recurrence by ten-fold [43]. Leisegang et al. reported recurrence rates of 9.6% at 1 year, 36% at 5 years, and 63.2% at 20 years after an initial episode [44]. The rate of recurrence increases significantly with the number of previous episodes; patients with one recurrence following an initial episode had a recurrence rate up to 38% at 1 year and 67% by 5 years. The relative risk of recurrence is 1.41 in 2–3 previous episodes and 2.09 with more than 4 previous episodes. A more recent study of recurrence rates reports 27% at 1 year, 50% at 5 years, 57% at 10 years, and 63% at 20 years [3].

Prophylaxis

Inhibition of reactivation and preventing recurrent infection is one of the most important aspects in the management of ocular HSV to prevent vision-threatening complications. The HEDS study was the first landmark clinical trial to establish the efficacy of systemic antiviral therapy to decrease recurrences in ocular HSV. It showed that oral prophylaxis with 400 mg of acyclovir 2×/day over a period of 12–18 months significantly decreased the recurrence rate ($p < 0.05$). Further, in patients with a history of HSK, the rate of recurrence decreased from 28% in the placebo group to 14% in the treatment group [21]. Further, there was no role for adding prophylactic dose of systemic antivirals in patients with epithelial keratitis being treated with topical antivirals to reduce rates of iritis and stromal keratitis.

However, as the number of recurrences has been shown to be an important predictor of the recurrence rates, two or more episodes of epithelial keratitis per year warrants oral prophylactic therapy. In terms of the oral antiviral agent used, studies have showed that efficacy and safety profiles of acyclovir 400 mg 2×/day is similar to valacyclovir 500 mg/day and either may be used.

Further, as HSV patients undergoing laser and surgical procedures are at an increased risk of reactivation, they should be protected with prophylactic oral antiviral therapy starting at least 24 hours pre-op and continuing for about 2 weeks. Similarly, patients undergoing laser refractive surgeries, collagen cross-linking, and cataract are at risk of reactivation and must be protected with oral prophylactic dose in the peri- and postoperative period. Oral prophylactic therapy has shown to be critical in the graft survival after corneal transplantation. Acyclovir 800 mg 3–5×/day initially for about 3 months and then tapered to 400 mg 2×/day or valacyclovir 500 mg 2–3×/day for 3 months and then tapered to 250 mg 2×/day for up to 30 months has shown dramatically reduced rates of HSV recurrence after PKP [42]. It is recommended to continue oral prophylactic therapy till the patient remains on a topical corticosteroid.

Prevention

There is much ongoing research for development of an HSV vaccine to prevent primary infection and recurrence. Epitope-based vaccines, recombinant viral vaccine vectors, and DNA vaccines show promise. HSV envelope glycoproteins are ideal immunogens as these are the targets for antibody production in HSV infection. One such subunit vaccine based on glycoprotein gD has been developed and studied for primary prevention of genital HSV [45]. The clinical trial in phase III has shown that this subunit vaccine was effective in preventing HSV-1 infection with an efficacy of 58% [46]. So far, there has been no vaccine for prevention of primary ocular infection as most systemic vaccines fail to induce local ocular immune responses. There has been

some research to develop strategies to reduce local shedding in tears of asymptomatic carriers. A study demonstrated that topical ophthalmic administration of human CD 8 T cells epitope-based vaccine to latently infected rabbits reduced the spontaneous viral shedding in tears [47].

There is also emphasis on developing vaccines for the prevention of HSV reactivation. CD 8 T cells mount an immunological response to combat viral reactivation in the trigeminal ganglion. A recent animal study demonstrated that a HSV envelope glycoprotein epitope-based vaccine was associated with mobilization of antiviral CD 8 T cells into the cornea and trigeminal ganglia, thus protecting against ocular HSV [48]. Heat-inactivated HSV-1 vaccine can boost CD 8 T cell response in the latently infected ganglia in hope to inhibit reactivation. A small clinical trial in humans showed a significant reduction in ocular HSV recurrence rate after administration of heat-inactivated HSV-1 vaccine subcutaneously [49]. However, one major risk of this approach is conversion of a non-virulent strain to an active virulent strain through mutation. Thus, the development of an effective and safe vaccine to prevent HSV warrants prospective, randomized, controlled, and long-term clinical trials.

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Herpes Zoster and the Zoster Eye Disease Study (ZEDS)

7

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Herpes zoster (HZ)/shingles is caused by the reactivation of latent varicella zoster virus (VZV) in persons who have had varicella/chicken pox, the primary infection caused by VZV. After primary VZV infection, latent VZV is present diffusely in sensory ganglia. The cause of localized reactivation is incompletely understood, although it is known that decline in VZV-specific cellular immunity due to age, immunosuppression, or other reasons are important factors. HZ is usually associated with a typical unilateral, painful, often vesicular rash in a dermatomal/radicular distribution. Unilateral pain precedes the rash in a majority of cases, and the pain is unlike any pain the person has experienced before [1]. The diagnosis is usually not made until the typical rash develops. However, Herpes Zoster Sine Herpete, the term used to describe radicular pain due to VZV in the absence of a rash can occur, and without a rash the diagnosis is often not considered and missed [2]. Herpes zoster ophthalmicus (HZO) refers to HZ when the fifth cranial/trigeminal nerve is affected and accompanied by ocular involvement.

Relatively new information regarding the epidemiology of HZ, vaccines to prevent HZ and its complications, and treatment of HZ, including current and possible new treatment under investigation, will be reviewed. Evidence regarding the increasing incidence and decreasing age at the onset of disease, as well as the possibility that VZV is the trigger for temporal/giant cell arteritis will be presented. Current information on the efficacy, safety, Food and Drug Administration (FDA) approvals [3, 4], and Centers for Disease Control and Prevention (CDC) [5] recommendations for two vaccines against HZ, the Zoster Vaccine Live (ZVL), and the Recombinant Zoster Vaccine (RZV) will be discussed. Current recommended treatment for HZ and HZO will be reviewed, and possible new treatment for HZO using prolonged suppressive antiviral medication currently under investigation in the Zoster Eye Disease Study (ZEDS), a multicenter randomized controlled clinical trial (RCT) funded by the National Eye Institute (NEI) of the National Institutes of Health (NIH), will also be discussed. Participation in this clinical trial is a unique opportunity to determine whether or not suppressive antiviral treatment of HZO will reduce complications and improve outcomes in eye disease caused by VZV, as it has been proven to do for herpes simplex virus eye disease.

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Epidemiology

HZ is a common disease with over 1,000,000 new cases per year in the USA [6] and with up to 20% involving the trigeminal nerve, resulting in possible HZO [7]. According to the CDC, over 99% of people aged 40 years and older born in the USA have had varicella/chicken pox, whether they know it or not, and they are at risk for HZ [5]. Approximately 30% of people will have HZ during their lifetime. Although HZ is more common and severe in immunocompromised persons, over 90% of people with zoster are not immunocompromised [8]. It is a common misconception that healthy people are not at risk for HZ and its potentially disabling sequelae, when in fact they are. Although the incidence of HZ increases with age, the greatest number of cases of HZ, including HZO, occur in people in their fifties [9–11]. The incidence of HZ rises significantly in people in their forties, and sharply in people in their fifties [12]. A common misconception is that HZ is a disease of the elderly, when in fact it affects a large number of people under the age of 60 years. In fact, the mean age of the onset of HZO has decreased from the sixties to the fifties [13, 14].

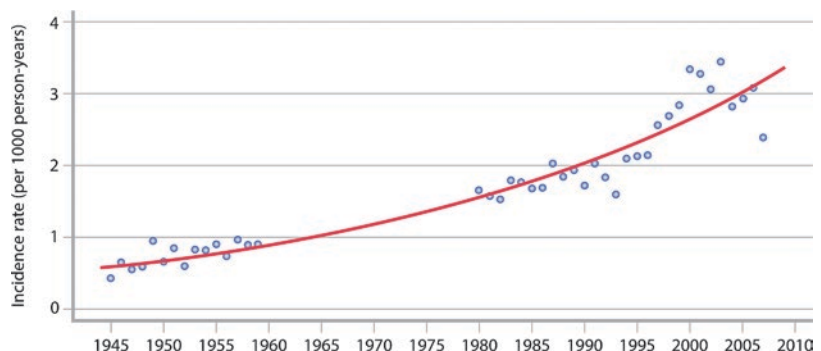
The incidence of HZ has increased over four-fold during the past 60 years across all age groups for unknown reasons [15] (Fig. 7.1). Although many people think that the vaccination of children against varicella contributes to the increase in HZ incidence by limiting boosting of immunity to VZV by exposure to chicken pox according to the Hope Simpson hypothesis, the evidence does not support this [8, 16]: the increase began before the introduction of childhood varicella

vaccination in the USA in 1995 and has continued afterward. In addition, routine vaccination against varicella is not widespread in Europe, and the incidence of HZ is similar there to that in the USA [10, 16]. The increase in HZ cases also cannot be accounted for by an aging population or by the rate of immunocompromise, and thus the reasons for it remain unknown.

There is an ever-growing list of risk factors for the development of HZ in addition to the well-known ones, including increasing age, immunocompromise, and female sex [17]. This list includes family history, depression, stress, past history of zoster, heart failure, traumatic brain injury, diabetes, asthma, acute kidney disease, and autoimmune disease. An interesting recent addition to the list is the use of statins, which in a large population-based study was associated with a modest 13% increased risk that was dose dependent and doubled with use of high dose statins, and it decreased with duration of time since statin use, consistent with a causal effect [18]. Rather than considering the implications regarding the importance of vaccination against zoster in patients with a wide variety of medical conditions, it is more efficient to recommend vaccination for all people aged 50 years and older, as discussed in the next section.

There is also an expanding list of complications after HZ, including neurologic conditions, cardiac issues, and possibly giant cell arteritis/temporal arteritis, in addition to the most common ones of postherpetic neuralgia (PHN), eye disease after HZO, and hearing loss when the eighth cranial nerve is affected (Ramsey Hunt). PHN, defined as pain beyond 3 months after the onset of zoster, is the most common complica-

Fig. 7.1 Incidence of herpes zoster in Olmsted County, Minnesota, 1945–2007, showing the temporal trend beginning prior to the introduction of antiviral medications (mid-1980s) and extending beyond the introduction of childhood varicella vaccinations (1996)



tion, and it occurs in approximately 30% of HZO patients, primarily with the onset of HZO at age 65 years or more [19]. Risk factors for PHN, in addition to increasing age, include HZO, amount of acute pain, and severity of the rash [20]. HZ has been found to be a risk factor for the development of major depression and reported to be the most common cause of suicide due to pain in people age 70 years and older [21, 22]. Postherpetic neuralgia often has a devastating effect on the quality of life in older patients with zoster, that frequently lasts for the rest of their lives. It is not prevented by recommended acute antiviral treatment of HZ. As of right now, the only way to prevent PHN is to prevent HZ through vaccination.

HZO is frequently associated with dendritic keratitis, stromal keratitis, and iritis/uveitis, often accompanied by elevation intraocular pressure, and neurotrophic keratopathy, but it can involve all parts of the eye, orbit, and optic nerve and result in permanent loss of vision [23]. Acute antiviral treatment within 72 hours of the onset of the rash is recommended with high dose oral valacyclovir (1000 mg 3 times daily), famciclovir (500 mg 3 times daily), or acyclovir (800 mg 5 times daily) for 7 days, and this has been shown to reduce eye disease at 6 months after onset from 50% to 30% [24]. It is very important to begin treatment as soon as possible, as complications occur after 72 hours, and one cannot predict who will have complications. Non-PHN complications, including eye disease, are as common in younger as older HZ patients [8]. HZO can be complicated by chronic and/or recurrent eye disease, with the latter increasing over time to 25% by 5 years [25]. Many HZO patients require chronic topical steroids, with their potential complications, to control anterior segment inflammation. Cataract surgery in well-controlled HZO can result in initial improvement in visual acuity (VA), followed by decreased VA due to retinal problems, which may be underdiagnosed in HZO [26].

HZ has long been known to be a risk factor for potentially fatal strokes, and in recent years in population-based studies, it has been reported to be a risk factor for heart disease [27]. These vascular complications occur in relatively young

patients. The risk of stroke is greater after HZO than after HZ in other locations, most likely because the virus spreads directly from the nerves to arteries, causing inflammation [28, 29].

Of great importance to ophthalmologists is evidence that VZV may be the trigger for giant cell/temporal arteritis (GCA/TA) [30]. In studies with 50 sections per temporal artery biopsy, VZV antigen was found in three quarters (61/82, 74%) of the biopsies that were positive for giant cells, and in sections adjacent to VZV antigen, giant cells were located in almost 90%. This led to the conclusions that GCA may be VZV vasculopathy of the temporal artery, and antiviral treatment of steroid-treated GCA may be beneficial, and it merits further study. These findings have been questioned in another study in which a minority (3/25) of giant cell positive TA biopsies were positive for VZV, including one case where TA developed soon after HZO [31]. The discrepancy in the results was attributed to false positives for VZV antigen in calcifications and muscle in the biopsies. The answer is not yet known on this subject, but it merits further study as it has important implications regarding the optimal treatment of GCA.

Vaccination Against Herpes Zoster

There are currently two vaccines against HZ: the Zoster Vaccine Live (ZVL, Zostavax, Merck) and the Recombinant Zoster Vaccine (RZV, Shingrix, GlaxoSmithKline). ZVL has been CDC recommended since 2008 for immunocompetent adults aged 60 years and older, and FDA approved since 2011 for immunocompetent adults aged 50 years and older [3]. RZV was FDA approved for all adults aged 50 years and older in October 2017 [4], and CDC recommended for immunocompetent adults aged 50 years and older in January 2018. CDC recommends RZV for people who have been previously vaccinated with ZVL, and as preferred over the ZV [32].

ZVL was approved after it was shown to decrease the burden of disease by 61%, PHN by 66%, and the incidence of HZ by 51% [33]. However, it was more effective in reducing the

incidence of HZ in persons aged 60–69 years (64%) than 70 years and older (38%). FDA approval of ZVL for immunocompetent adults aged 50 years and older followed evidence that it decreased the incidence of HZ by 70% in people aged 50–59 years, but the CDC recommendation remains unchanged [34]. Contraindications for ZVL include diseases and treatments impairing cell-mediated immunity, because the vaccine contains a live attenuated virus that is a real safety risk for these populations. In addition, anaphylactic reaction to neomycin or gelatin as well as pregnancy are contraindications. Since the live attenuated virus is sensitive to antivirals, vaccine recipients must be off of valacyclovir, famciclovir, and acyclovir 1 day before and 2 weeks after vaccination. The most common side effects to ZVL are local injection site reactions, which are more common in vaccine recipients in their fifties than in older vaccine recipients [33, 35]. In addition to reduced efficacy against zoster in older persons, another limitation of ZVL is that its efficacy in persons aged 60 years and above wanes after 3–10 years [36–38].

RZV, also referred to as the herpes zoster subunit (HZ/su) vaccine, contains a recombinant VZV protein in a novel immunogenic adjuvant. It is a two-injection series given intramuscularly 2–6 months apart, and it is refrigerated, in contrast to the ZVL, which is a single injection given subcutaneously and requires frozen storage. In an RCT conducted in 2010–2011 of immunocompetent adults aged 50 years and older, it was 97% effective in preventing HZ compared to placebo [39]. When results were pooled with a concomitant RCT of immunocompetent adults aged 70 years and older, it was approximately 90% effective in reducing the incidence of HZ in adults aged 70 years and older, which is much more effective than ZVL [40]. In addition, efficacy against HZ remained at 85% after 4 years, also superior to ZVL. However, grade 3 acute local and systemic reactions interfering with normal activities were reported in up to 17% of vaccine recipients, and this raised concern about adherence to the 2-dose schedule required for efficacy outside of a clinical trial in a real-world setting.

The FDA approved RZV for all adults aged 50 years and older, and the CDC recommended it

for immunocompetent adults aged 50 years and older [32]. The FDA label notes that immunosuppressive treatment may reduce the effectiveness. The CDC limited its recommendation to immunocompetent adults, because immunocompromised adults were excluded from the RCTs. The FDA label allows for the administration of the two injections 2–6 months apart, which facilitates giving them at the time of routine office visits, based on evidence that the humoral antibody response was not inferior when given 6 months compared to 2 months apart [41]. In addition, according to the label, there is no evidence of interference with the immune response when given at the same time of a quadrivalent influenza vaccine. This is supported again by evidence of lack of interference with the humoral antibody response, but it is unclear if even this can be extrapolated to influenza vaccines in general [42, 43]. According to the FDA label, the only contraindications to the vaccine are a history of anaphylaxis to a vaccine component or to the first dose. Information about relatively common acute local and systemic reactions to the RZV are provided in the label, including that they are more common in persons aged 50–69 years than people aged 70 years and older. In addition, local reactions occur at a similar frequency after the first and second injection, whereas grade 3 systemic reactions are more common after the second injection. The label emphasizes the importance of completing the two injections on schedule, and informing people in advance about the possibility of adverse reactions. The vaccine requires two shots to stimulate cell-mediated immunity against VZV in order to be effective in preventing zoster. Of note, gout occurred in 0.18% of vaccine recipients, compared to 0.05% of placebo within 30 days, but a causal relationship was not determined.

The CDC recommends RZV for immunocompetent adults aged 50 years and older, including persons who received ZVL at least 2 months ago. There is evidence of a strong immune response and comparable adverse reactions to RZV in people aged 65 years and older who received ZVL at least 5 years previously [44]. The CDC does not specify when to give RZV after ZVL, but given the limited efficacy of ZVL against HZ in persons aged 70 and older, it is reasonable to have

then get the RZV as soon as possible. Vaccination against zoster is recommended for persons with a past history of zoster, as second episodes of disease can occur in 6% within 8 years [45]. Local and general reactions to RZV are more common in persons with a past history of zoster within 4 years compared to 5 or more years prior to vaccination [46]. The CDC recommends RZV as preferred over ZVL due to its greater and more long-lasting efficacy. The CDC recognizes that continued monitoring for adverse events using the Vaccine Adverse Events Reporting System (VAERS) is particularly important for RZV due to its novel adjuvant with high reactogenicity and immunogenicity.

Under usage of ZVL for a variety of reasons has been a problem, limiting its impact on HZ, and hopefully vaccination against zoster will increase with the current approval and recommendations for RZV. According to CDC, as of 2015, the most recent year with available data, 31% of immunocompetent persons aged 60 years and older have received ZVL, while only 6% of those aged 50–59 years received it, probably due to the lack of CDC recommendation for this age group [47, 48]. In the USA, emergency room visits for HZ between 2006 and 2013 increased overall due to an increase among people aged 50–59 years, but decreased among age groups where the CDC recommended vaccination [49].

There are a number of barriers to vaccination against HZ, including high cost, complex and partial insurance coverage, and lack of strong recommendation by physicians [50, 51]. Despite interventions targeted to increase vaccination against zoster including education, increased availability, and electronic medical record reminders and alerts, primary care doctors do not consider vaccination against zoster as important as vaccination against influenza and pneumonia [52, 53]. The American Academy of Ophthalmology (AAO) issued a clinical statement in 2016 (cosponsored by the American Dermatological Association in 2017), updated in 2018, and accepted for publication as a policy statement in *Ophthalmology*), recommending vaccination against zoster for adults aged 50 years and older without contraindications [54].

A number of factors influence attitudes of physicians regarding vaccines, including evidence on the benefits of the vaccine, insurance coverage, cost to supply in the office, and whether or not it is tracked as a performance measure [51]. Hopefully, the increased efficacy of RZV will result in stronger recommendations for vaccination against HZ. Although RZV is more cost-effective than ZVL [55], cost remains high, and insurance coverage is complex with coverage under medical insurance for non-Medicare patients versus Medicare Part D, making it difficult to administer in office for the Medicare patients. Tracking vaccination according to current CDC recommendations as a performance measure is a relatively easy change to implement to try to improve attitudes and practices. Vaccination rates against HZ go up when family members are affected in proportion to the severity of their disease, but physicians should not need to experience this to improve [56]. Increasing vaccination among doctors and other health-care workers is a strong evidence of the importance of vaccination and is a good preventive medicine [57]. The bioethicist Arthur Caplan teaches that “A moral obligation to do the right thing is necessary to improve vaccination rates”.

The Zoster Eye Disease Study (ZEDS)

ZEDS is a very important study to determine whether or not prolonged suppressive antiviral treatment with valacyclovir reduces complications of HZO, including eye disease and/or postherpetic neuralgia. It is a multicentered randomized controlled clinical trial funded by the National Eye Institute that has over 200 study investigators and will enroll 1050 study participants at 60 participating clinical centers across the USA. Successful completion of this study is necessary to determine if suppressive antiviral treatment is effective and should become the standard of care for HZO. While acute high-dose oral antiviral treatment is approved and recommended for HZO, there is no standard approach to the antiviral treatment of the complications of HZO.

The rationale for ZEDS is twofold: firstly, the relatively recent evidence of the infectious pathogenesis of complications of HZ, including HZO; and secondly, the significant benefit of suppressive antiviral treatment for herpes simplex virus (HSV) keratitis, which has become the standard of care and has reduced recurrences. Evidence of recurrent varicella zoster virus (VZV) infection has been found by polymerase chain reaction (PCR) to cause dendriform epithelial keratitis (DEK) and iritis due to HZO [58–60]. The Herpetic Eye Disease Study (HEDS) Acyclovir Prevention Trial (APT) showed that suppressive antiviral treatment reduced recurrent HSV by 45% after 1 year of treatment [61]. ZEDS is analogous to HEDS APT for ocular disease caused by VZV, a different herpes virus, in that immunocompetent study participants aged 18 years and older will be randomized to suppressive antiviral treatment versus placebo for 1 year and followed every 3 months for 18 months.

To be eligible for ZEDS, one must have a history of a typical unilateral rash and a documented episode of dendriform epithelial keratitis, stromal keratitis, endothelial/disciform keratitis, or iritis within 1 year prior to enrollment. Study participants will be randomized 1:1 to double-masked valacyclovir 1000 mg daily versus placebo in four strata based on age at the onset of HZO and disease duration at the time of enrollment. Exclusion criteria, in addition to immunocompromise, are primarily the contraindications to valacyclovir, including renal insufficiency and pregnancy, or need for antiviral treatment of HSV disease.

The primary objective of ZEDS is to determine whether or not suppressive valacyclovir compared to placebo delays the time to occurrence of dendriform epithelial keratitis (DEK), stromal keratitis without ulceration (SK), endothelial keratitis (EK), iritis (IR) or stromal keratitis with ulceration (SKU) (Fig.7.2). The

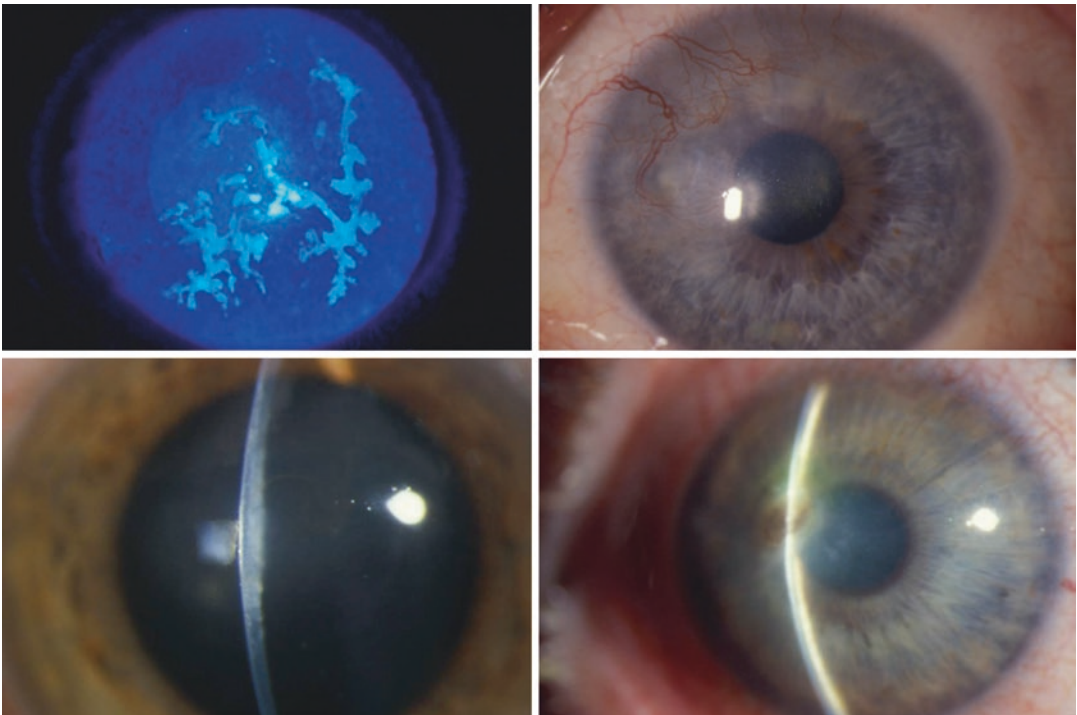


Fig. 7.2 Top left: Dendriform epithelial keratitis. Top right: Stromal keratitis without ulceration. Bottom left: Endothelial keratitis. Bottom right: Stromal keratitis with ulceration

second aim of the study is to determine whether or not suppressive valacyclovir treatment compared to placebo reduces the incidence, severity, and duration of postherpetic neuralgia after 12 months of treatment, and if it persists for 6 months afterward.

Support of ZEDS by the adequate enrollment of study participants is critical to achieve our purpose of determining optimal treatment for HZO and improving outcomes. Approximately half of our study investigators currently use suppressive antiviral treatment for HZO, and even more think it is effective [62]. However, rather than starting this treatment, which is currently not evidence based, we urge ophthalmologists to refer their HZO patients for possible enrollment into ZEDS so that it can be determined if this treatment is effective, and if so, it will become the standard of care and improve outcomes in HZO. For further information regarding ZEDS, please refer to our website at <https://med.nyu.edu/research/zoster-eye-disease-study/zoster-eye-disease-study>.

Conclusion

Herpes zoster is a common preventable disease with serious life-threatening and altering complications, including eye disease, chronic pain, and neurologic diseases. It is very important for health-care professionals, including ophthalmologists, to strongly recommend vaccination against zoster for adults aged 50 years and older. Participation in the Zoster Eye Disease Study is necessary in order to determine the efficacy of suppressive antiviral treatment in reducing chronic and/or recurrent eye disease due to HZO, as well as postherpetic neuralgia, and to determine optimal evidence-based standard of care to improve outcomes.

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Fuchs Endothelial Corneal Dystrophy: Rethinking an Old Disease with Insights from the Laboratory and Clinical Practice

Viridiana Kocaba and Kathryn Colby

Introduction

Fuchs endothelial corneal dystrophy (FECD), estimated to affect 4% of the US population [1], is the leading cause of endogenous corneal endothelial degeneration and the primary indication for keratoplasty worldwide [2]. FECD is a slowly progressive bilateral corneal disease that is characterized by abnormal corneal endothelial morphology, such as pleomorphism and polymegethism, decreased cell density, thickening of the Descemet membrane (DM), and formation of extracellular matrix (ECM) excrescences called guttae [3], which lead to derangements in endothelial cell function. Quality of vision is slowly compromised by corneal edema secondary to gradual failure of corneal endothelial pump action and guttae-induced visual disturbances such as decreased contrast sensitivity [4] and higher order aberrations [5]. Its pathogenesis is proposed to be an interaction between endogenous and exogenous factors [6]. The disease manifests in the early third to late fifth decade of life and has a female preponderance [7–9]. Since the first description of the disease by Ernest Fuchs more than 100 years ago

[10], major advances in understanding the nature and pathogenesis of FECD have been made and are described in this chapter.

Prevalence

The prevalence of the disease varies depending on the age, origin, and ethnicity of the population. FECD affects people over 50 more frequently, from 3.3–4.1% in the Japanese population [8, 11, 12], 3.9–6.6% in the US population [13, 14], and 9.2% in residents in Reykjavik, Iceland [9]. Despite a slightly higher prevalence in Chinese Singaporean population (6.7%) [8], the prevalence of FECD appears higher in Caucasians compared to Asians. In addition, regardless of geographical distribution, females are at greater risk of developing the disease at a ratio of 2.5:1 to 3.7:1 [7–9, 12, 13].

Corneal Endothelial Composition and Function

Corneal Endothelial Cells

The corneal endothelium (CE), the innermost layer of the cornea, is located between the stroma and the anterior chamber of the eye and forms a monolayer of hexagonal cells derived from the neuroectoderm [15]. The corneal endothelial cells (CECs) are highly metabolically active,

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contain numerous intracellular mitochondria, and have cytoplasmic extensions allowing intercellular exchange [16].

The endothelial cells are arrested in the G1 phase of the cell cycle at birth [17], and thus do not proliferate, resulting in a gradual decrease in endothelial cell density during life [18]. CEC are contact-inhibited, meaning that cells stop migrating once a neighboring cell is encountered. At birth, the human CE contains approximately 5000 cells/mm [2, 19]. There is a gradual decrease in endothelial cell density [20] during life at a rate of 0.36 to 0.6% of the endothelial cells per year [8, 20–22]. In late adulthood, the normal count ranges from 2000–3000 cells/mm [2] or about 700,000 cells in total. In a normal eye, this gradual reduction of CECs has no effect on corneal transparency. In a healthy CE, the size of CECs is remarkably homogeneous (coefficient of variation of approximately 0.27–0.28) with a hexagonality percentage of 60%. The decrease in endothelial cell density leads to cellular size and shape changes; polymegethism occurs when cell size increases and pleomorphism when cell shape changes. Both result as an adaptation of the endo-

thelial cells to their decreasing density to maintain the continuous cellular monolayer.

Barrier and Pump Function (Fig. 8.1)

The purpose of the endothelium is to maintain the constant hydration state of the cornea and, therefore, its transparency (Fig. 8.1). The CE provides stromal deturgescence both by a passive mechanism (barrier function), provided by the intercellular tight junctions [23], and an active mechanism, via Na^+/K^+ ATPase pumps [24], that returns solutes and water from the cornea to the anterior chamber.

Its intercellular junctions prevent water from the aqueous humor from entering the stroma. As an interface between the cornea and the aqueous humor of the anterior chamber, the CE allows both trans- and paracellular transport of nutrients while providing resistance to the entry of the aqueous humor into the stroma [25].

The endothelial pump mechanism is still not completely understood. It is not strictly a mechanism of active transport of water molecules at

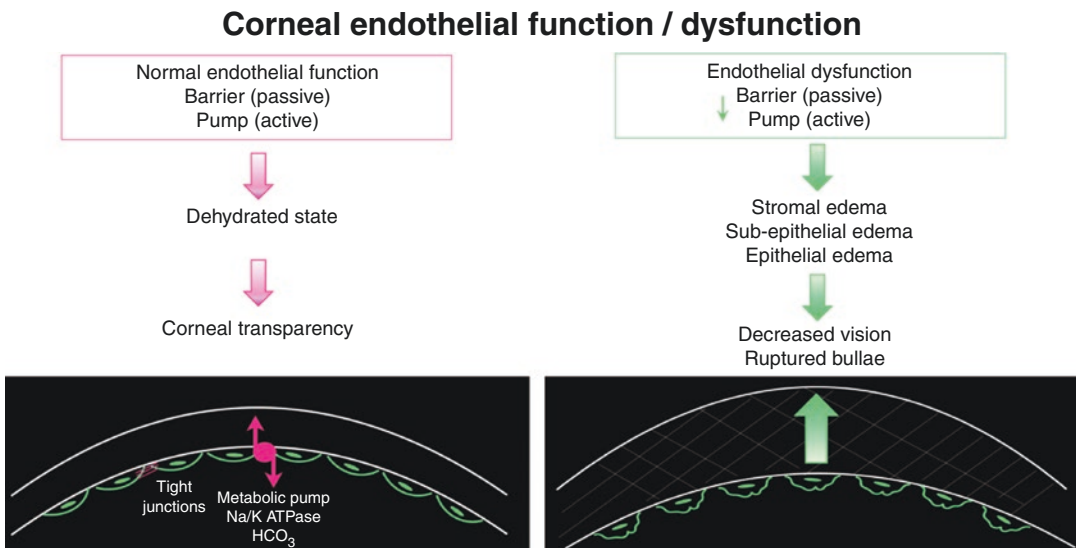


Fig. 8.1 Illustration of the endothelial pump function/dysfunction mechanism. Left, corneal endothelium in physiological state. The maintenance of corneal transparency is ensured by the passive endothelial barrier function (tight junctions) and by active Na^+/K^+ ATPase and HCO_3^-

pumps. Right, when the number of endothelial cells is insufficient to maintain the endothelial pump function, intrastromal edema appears, and vision decreases. Fluid infiltrates the subepithelial layer, elevating the corneal epithelium (bullae) which can break, causing pain

the level of the endothelium; in fact, the movement of water through the endothelium is essentially based on the osmotic gradient created by the different pumps. The path of the water molecules is mainly paracellular, and a small amount of water moves via type 1 aquaporins (AQP1). The water is transported from the stroma to the aqueous humor at a rate of 6–8 mL/h/cm [2]. The endothelial pump mechanism is most likely based on Na^+/K^+ ATPase pumps located on the basolateral sides of the endothelial cells, on intracellular carbonic anhydrase, on Na^+/H^+ and $\text{HCO}_3^-/\text{Cl}^-$ exchangers, on $\text{Na}^+/\text{HCO}_3^-$ and $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporters, and on an apical elimination route of bicarbonate [24].

Descemet Membrane

The Descemet membrane (DM), initially described by the French physician Jean Descemet in 1770 [26], is secreted by CECs [27] and is located between the posterior stroma and the monolayer of CECs. Like all basement membranes, the DM is a compact layer composed of extracellular matrix (ECM) biomolecules, including glycoproteins and glycosaminoglycans [28]. Its composition and ultrastructure vary with the stage of development and the presence of pathological conditions [29] with distinct layers forming over time [30]. During fetal development, the DM acquires a complex lamellar structure; it is 3 μm thick at birth and reaches 6–10 μm in adulthood [30, 31]. The anterior part of the DM is formed during the prenatal period, approximately from 12 weeks after conception and until birth [30]. From 16 weeks of gestation, striations appear to constitute the anterior banded layer (ABL), termed the fetal layer, which is composed mainly of collagen IV and III [32, 33].

At birth, the secretion of collagen VIII by CECs decreases when the cells pass from the proliferative to a nonproliferative state while that of collagen IV continues [27, 34]. The secreted ECM then loses its striated appearance and gradually becomes a nonbanded layer (PNBL), termed the neonatal layer [29]. In adulthood, the DM is composed mainly of collagen IV, VIII, and

I [35]. Its porous structure allows the exchange of stroma towards the endothelium and the anterior chamber; in fact, it has pores with an average size of 38 nm that specifically serve this purpose [36]. Over time, membrane material can accumulate in certain parts of the DM; in particular, in the periphery of the adult cornea, endothelial deposits, termed Hassall-Henle bodies, can be observed. These represent posterior growths of the DM and are only visible histologically [31].

The DM is elastic [37], permeable to water, solid, and resistant to the proteolytic action of metalloproteases. As the basement membrane of endothelial cells, it participates in the maintenance of dehydration of the corneal stroma.

Pathogenesis of Fuchs Dystrophy

Overview

FECD is characterized by an accelerated, progressive decline in the number of CECs, which leads to polymegethism and pleomorphism [38]. At first, only central corneal endothelial cells are affected, but later in the disease course peripheral endothelial cells are also involved. The DM ECM homeostasis is compromised, as evidenced by the formation of guttae and the disorganization of ECM collagen fibrils [35]. Recently, evidence has established that guttae size might play a role in FECD by creating a noxious environment for the CECs [39, 40]. After a significant decrease in endothelial cell density (below 350–500 cells/mm [2]) [41], the endothelial pump is not able to ensure the deturgescence of the cornea (Fig. 8.1). This results in elevated corneal hydration levels, disruption of the collagen fiber arrangement, and loss of corneal transparency. The water present in the aqueous humor infiltrates the cornea, causing stromal edema, and eventually resulting in the formation of painful epithelial bullae [42]. The progressive corneal edema prevents light from being properly transmitted in the eye and thus compromises vision. While FECD is the most common cause of endothelial dysfunction, other causes include postsurgical, inflammatory, infectious, or traumatic.

Exogenous Factors

Smoking [43] and ultraviolet radiation [44] have been reported as exogenous factors associated with Fuchs dystrophy and are suggested to increase oxidative stress, resulting in accelerated apoptosis of corneal endothelial cells.

Endogenous Factors

Genetic Factors

The genetic basis of FECD is complex and heterogeneous, demonstrating variable expressivity and incomplete penetrance. It was not until the early 1950s that the initial reports of its heritability emerged [45–47]. Over the past decade, significant progress has been made in understanding the genetics of Fuchs dystrophy. Studies in families with multiple generations of members with Fuchs dystrophy have identified several chromosomal loci associated with the pathology, classified according to the affected chromosome (IC3D classification [48]): FECD1, chromosome 1; FECD2, chromosome 13; FECD3, chromosome 18; FECD4, chromosome 20; FECD5, chromosome 5; FECD6, chromosome 10; FECD7, chromosome 9; and FECD8, chromosome 15 (Fig. 8.2).

With the identification of four single-nucleotide polymorphisms (SNPs; namely, rs17595731, rs613872, rs9954153, and rs2286812), the *TCF4* gene has been associated with Fuchs dystrophy, with the SNP rs613872 being the marker for the most robust of the

pathologies [49] due to the production of a CTG trinucleotide expansion sequence in the 3rd intron of *TCF4* within chromosome 18q21.1 (henceforth referred to as the ‘CTG18.1’ locus) [50]. The prevalence of CTG18.1 in FECD varies depending on ethnicity [51–56] with up to 79% of FECD Caucasian patients harboring these expanded sequences [50, 51, 57]. *TCF4* encodes the E2-2 transcription factor, which serves a regulatory function in the expression of multiple other genes [58]. Similar to myotonic dystrophy type 1 (DM1), which is characterized by an expanded CTG trinucleotide repeat sequence in the Dystrophia Myotonica type 1 protein kinase (DMPK) gene [59], these repeat expansion sequences negatively affect cellular function via the formation of toxic RNA foci [50], which sequesters critical transcription factors and, in turn, may cause dysfunction of the cytoskeleton and cell adhesion. Additionally, evidence has also emerged to suggest a direct correlation between repeat length at the CTG18.1 locus and FECD disease severity [53]. Furthermore, the predictive value of CTG18.1 trinucleotide repeat expansion (TNR) length on clinical progression of FECD has been characterized showing that patients harboring an expanded CTG 18.1 allele are more likely to experience clinical progression of FECD [60]. Recently, evidence has also suggest that TNR may contribute to disease pathogenesis through production of cytosolic repeat-associated non-ATG (RAN) translation products, a mechanism suggested in other repeat expansion diseases [61].

	Early onset	Late onset						
Type	FECD1	FECD2	FECD3	FECD4	FECD5	FECD6	FECD7	FECD8
Age	< 40	> 50						
Loci	1p34.4-p32	13pter12.11-q12.13	18q21.2-q21.32	20p13-p12	5q33.1-q35.2	10p11.22	9p24.1-p22.1	15q25
Genes	<i>COL8A2</i>		<i>LOXHD1</i> <i>TCF4</i>	<i>SLC4A11</i> <i>TCF8</i>		<i>TCF8</i>		

Fig. 8.2 Genetics of Fuchs dystrophy

Additional genes, such as *KANK4*, *LAMC1*, and *ATP1B1*, have recently been linked to Fuchs dystrophy [62]. Although additional studies are still required for understanding the exact involvement of these genes in the pathophysiology of Fuchs dystrophy, it is known that *KANK4* and *LAMC1* play an important role in modulating intra- and extracellular interactions. Similarly, *ATP1B1* is the gene that encodes the Na⁺/K⁺ ATPase pump, the central mechanism of normal endothelial function [63].

Nonsense mutations encoding the A2 chain of collagen VIII (COL8A2, p.R155Q, p.R304Q, p.R434, and p.Q455K) have been demonstrated in early Fuchs dystrophy (FECD1) [64], while mutations in the *TCF8* gene (or *ZEB1*) have been detected in posterior polymorphic dystrophy [65] and Fuchs dystrophy [66]. At the same time, mutations in the *SLC4A11* gene have been reported as causes of congenital hereditary endothelial dystrophy (CHED) [67] and were associated with Fuchs dystrophy in 2007 [68].

Although the vast majority of FECD cases appear to be associated with the trinucleotide repeat expansion on chromosome 18, further research on the genetics of Fuchs dystrophy is still required to determine how each of the associated genetic defects participate in disease pathogenesis.

Cellular Dysfunction

Oxidative Stress and Mitochondrial Dysfunction

In the eye, CECs have the second highest level of aerobic metabolism (after retinal photoreceptors) [69], which makes them highly sensitive to oxidative stress. In 2010, Jurkunas et al. demonstrated oxidative stress as an integral part of the pathophysiology of Fuchs dystrophy [70]. In this study, the authors showed that some antioxidative regulators, such as NRF2, are downregulated and that oxidative DNA damage and apoptosis are significantly higher in patients with FECD. Subsequent studies have confirmed this finding and have demonstrated that oxidative damage to mitochondrial DNA leads to mito-

chondrial dysfunction, cellular stress, senescence, and apoptosis [16, 44, 71–73].

Toxic Environment

Aberrant Secretion of ECM and Toxicity of Guttae

In addition to endothelial cell abnormalities, Fuchs dystrophy is characterized by a thickening of the DM associated with the presence of guttae in the posterior part of the cornea (Fig. 8.3). The accumulation of glycation end products [74] and certain proteins has also been described as a contributory factor in FECD. An increase in collagen IV, laminin, fibronectin, and several other components of ECM has been demonstrated in the DM of FECD patients [75].

The ECM, which is present in all tissues, provides mechanical support for cells and influences differentiation, migration, activation, behavior, and cell death [76]. Thus, changes in ECM composition due to the aberrant production of ECM proteins affect the physiological activity of cells and can lead to their dysfunction. Although the genesis of guttae is still poorly understood, several studies support the hypothesis that, in FECD, the homeostasis of the ECM of the DM is compromised with disease severity related to the number and size of the guttae, which promote disorganization of ECM collagen fibrils [35]. The exact composition of the guttae is still unclear. However, the expression of transforming growth factor-beta-induced protein (TGFBIP) and clusterin was found to be increased in guttae-forming deposits [77]. A recent study from Kocaba and colleagues demonstrated that guttae cause cellular stress in CECs, promoting senescence and apoptosis [39].

Barrier and Pump Dysfunction

As endothelial cell damage progresses in FECD, the number of CECs decreases and the cells lose their tight junctions, causing loss of the barrier function and reduction in the ability to actively pump solutes from the corneal stroma due to endothelial pump dysfunction. Additionally, thickening of the DM might also negatively impact fluid exchange from the stroma to the

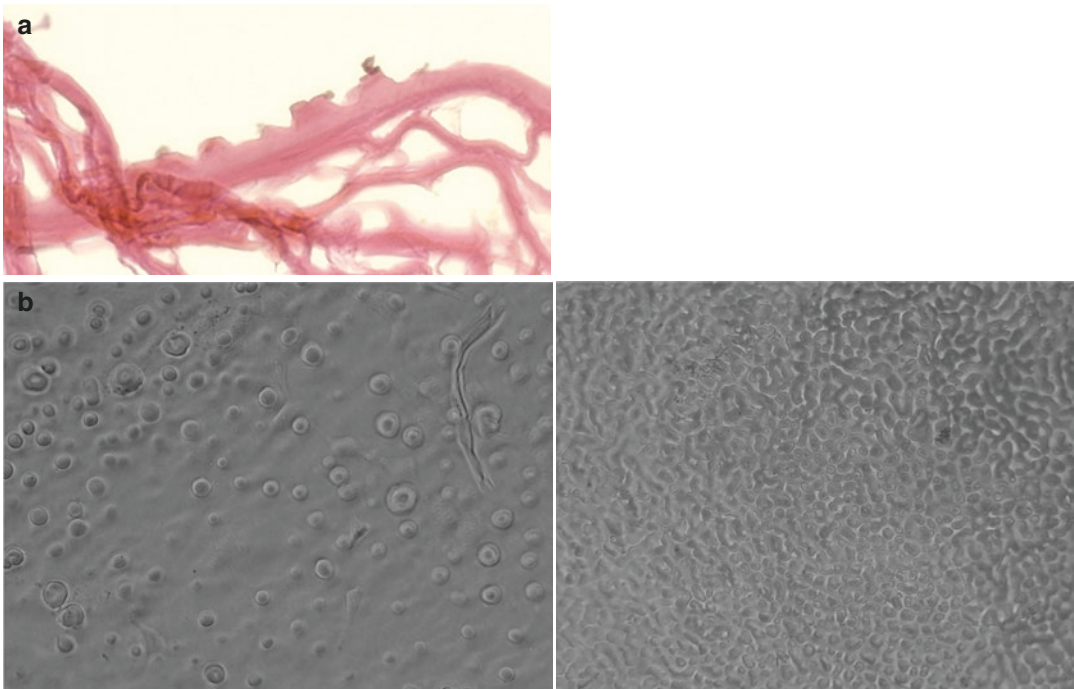


Fig. 8.3 Guttatae viewed in a histological section (a) and by bright field optical microscopy (b). (a) Histological section of the DM. Guttatae are the dome-shaped excrescences of the DM, varying in size and diameter. (b) Bright field optical microscopy of cornea. Left, extreme corneal

periphery; right, corneal center. The guttatae are present in the center and extend towards the periphery. Their size and number increase from the periphery to the center. In advanced stages of the disease, guttatae become confluent

anterior chamber. In advanced stages of the disease, the severe damage to the endothelium leads to endothelial decompensation: the capacity of the barrier and the aqueous pumps is exceeded, and intracorneal edema occurs, adversely impacting corneal transparency and visual acuity (Fig. 8.1).

Towards a Unified Theory of FECD Pathogenesis

Vicious Cycle of Fuchs Dystrophy

We have already discussed the role of oxidative stress, mitochondrial dysfunction, and perturbations of the ECM in FECD (Fig. 8.4). These are not the only cellular processes purported to be involved in this disease, however. Others include the unfolded protein response and epithelial-mesenchymal transition (EMT) [78]. Genetic factors and environmental stressors, such as UV

exposure and hormonal influences, also contribute in yet unclear ways, to the end result of increased apoptosis and endothelial dysfunction. How these pathways interact to cause FECD is not yet known. Many questions remain. Despite genetic heterogeneity, FECD has remarkable phenotypic homogeneity, which must be accounted for by any theory of disease pathogenesis. A recent review has put forth the first unified theory of FECD pathogenesis (Fig. 8.5), seeking to tie together the disparate cellular processes and genetic mutations that have been implicated thus far and placing mitochondrial dysfunction at the core of disease pathogenesis [78].

Insights from Neurodegenerative Diseases

CEC are derived from the neuroectoderm via the neural crest and it has been suggested that Fuchs can be considered as a “neurodegenerative” disease [79]. Indeed, many of the same cellular

Fig. 8.4 Vicious cycle of Fuchs dystrophy. DM Descemet membrane, ECM extracellular matrix, UV ultraviolet

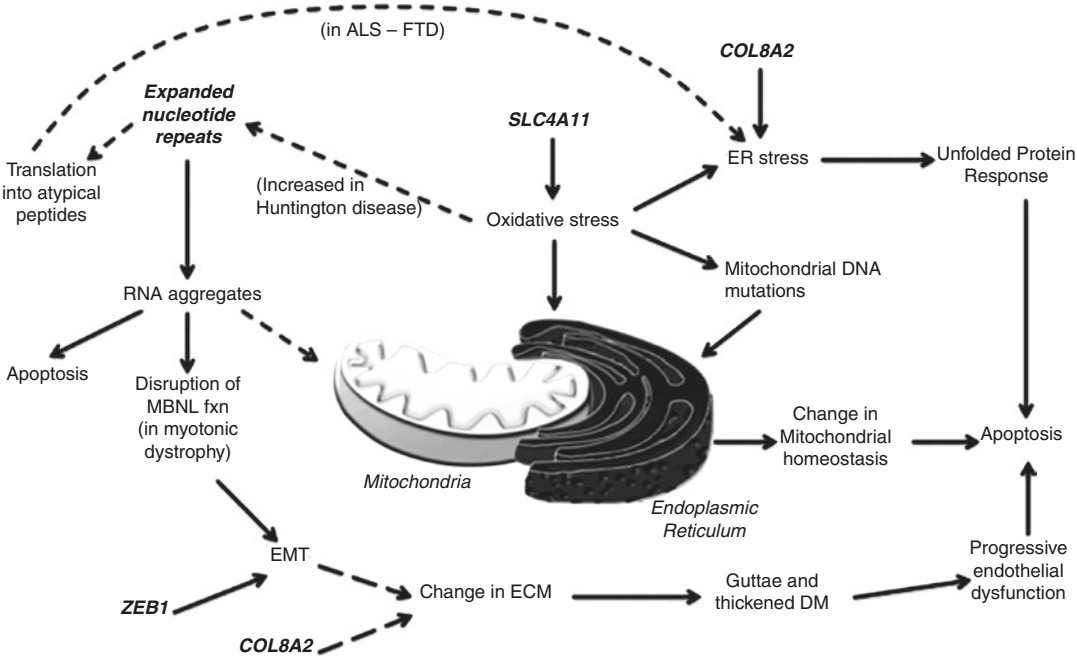
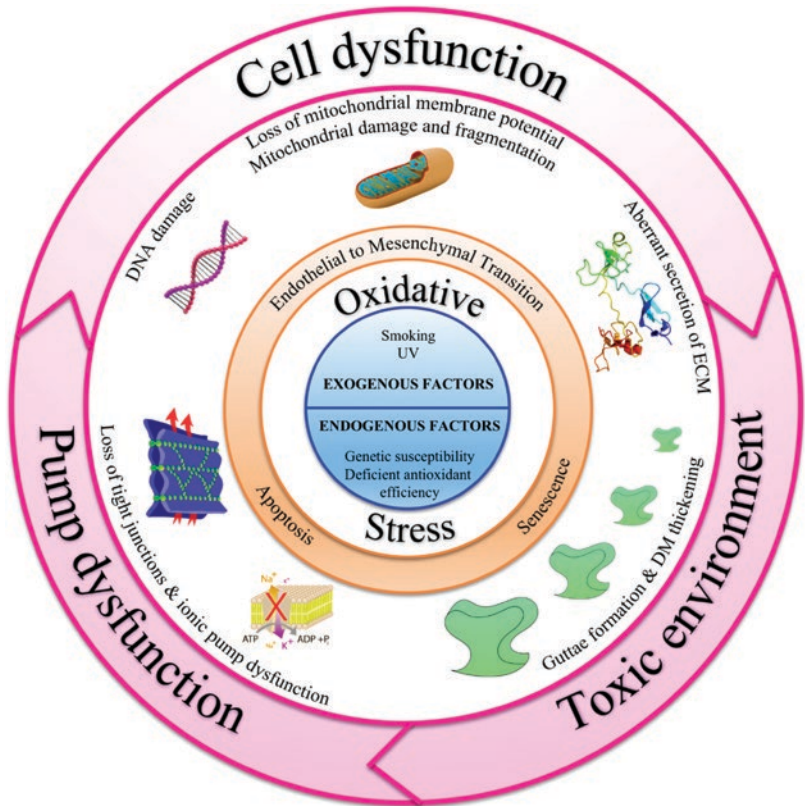


Fig. 8.5 Working theory of FECD pathogenesis. (From Sarnicola et al. [78]. Open access). ALS-FTD amyotrophic lateral sclerosis with front-temporal dementia

mechanisms (mitochondrial dysfunction, oxidative stress, repeat expansion) implicated in FECD have been shown in classic neurodegenerative diseases including Parkinson, Alzheimer, and Huntington diseases and amyotrophic lateral sclerosis [80–83]. It is likely that FECD studies will be aided by lessons learned from these disorders. Conversely, it is likely that the study of classic neurodegenerative diseases will be enhanced by insights garnered in the study of diseased endothelium from FECD, which is routinely removed as part of surgical management.

Diagnosis of FECD

Diagnosis of FECD is mainly clinical, based on slit lamp findings, corneal pachymetry, and endothelial cell imaging. Multiple staging scales for FECD have been described. One of the most commonly used one (Krachmer classification) classifies the disease in four clinical stages [84]:

Stage 1 (Fig. 8.6) represents the initial manifestation of the disease. Nonconfluent guttae are present in the posterior part of the central cornea and are visible as dark spots by direct illumination, highlighted in retroillumination. Patients are usually asymptomatic at this stage; however,

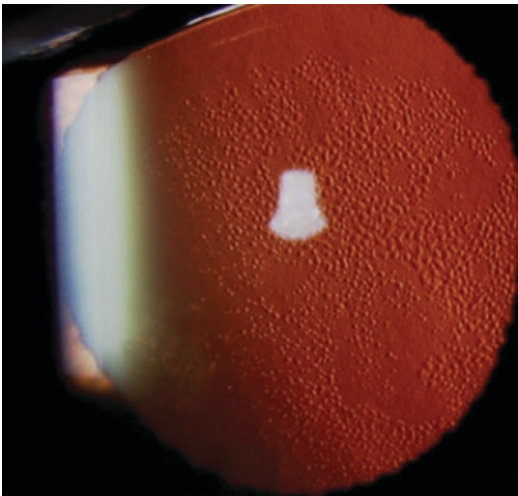


Fig. 8.6 Retroillumination photo of nonconfluent central corneal guttae in FECD

visual quality may deteriorate due to light scattering by the central guttae. *Stage 2* is defined by a reduction in the number, increase in the size (polymegethism), and loss of the hexagonal shape (pleomorphism) of corneal endothelial cells, as shown by specular or confocal microscopy. Guttae become confluent centrally and extend towards the peripheral cornea. Corneal edema may be evident on exam or by measurement of corneal thickness. Early morning vision is often affected due to increased corneal edema after eye closure during the night. In *stage 3*, vision deterioration begins to last longer and may be present throughout the day. Corneal stromal edema increases, eventually leading to epithelial and subepithelial bullae. Pain, epiphora, and light sensitivity occur if the bullae rupture. In *stage 4*, prolonged and chronic corneal edema induces subepithelial fibrosis, stromal opacification, and neovascularization of the cornea. This stage is rarely seen in the developed world due to the success of modern corneal transplantation techniques.

Additional examinations are useful for the diagnosis of early disease and follow-up. Corneal pachymetry can be used to monitor endothelial function during the evolution of the disease. Attention should be paid to the time of the day the measurement is made since this can impact corneal deturgescence. Specular microscopy allows the observation and measurement of guttae, which appear as dark spots. Moreover, observation of decreased endothelial cell density, accompanied by polymegethism and pleomorphism, is beneficial for early diagnosis of the disease (Fig. 8.7).

With an axial resolution of 11–18 μm , the anterior segment optical coherence tomography (AS-OCT) is potentially useful for visualizing changes in the endothelium and the DM during the progression of FECD (Fig. 8.8) [85]. The DM appears hyperreflective, thickened, irregular, and multilayered (Fig. 8.8a). Epithelial and subepithelial changes can also be observed: subepithelial bullae, thickening of the epithelium, and subepithelial fibrosis are seen in advanced cases (Fig. 8.8b).

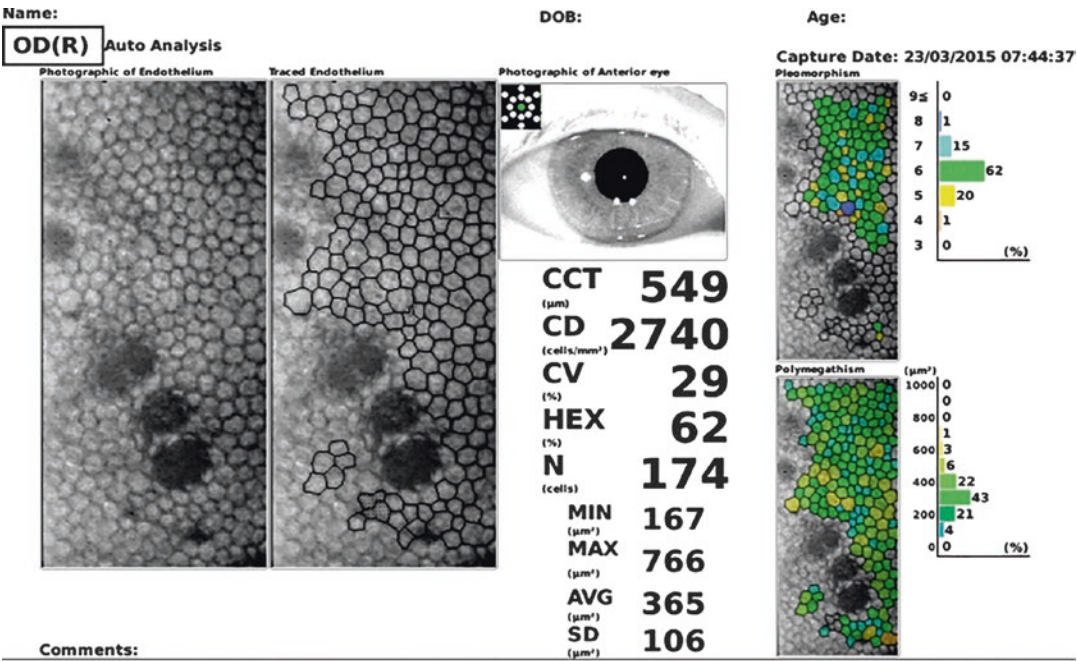


Fig. 8.7 Specular microscopy of a patient with early FECD

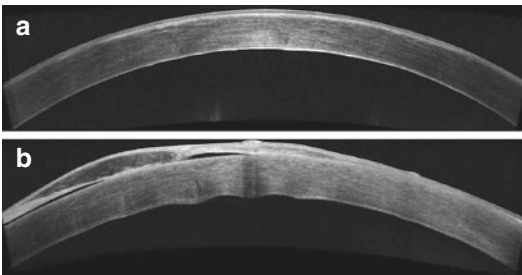


Fig. 8.8 AS-OCT of cornea from FECD patients. (a) Hyperreflectivity and thickening of the DM, subepithelial fibrosis and thickening of the epithelium. (b) Undulation of the DM and subepithelial bullae and major thickening of the epithelium in advance FECD

In vivo confocal microscopy (IVCM) allows a superior image compared to specular microscopy and is helpful for the diagnosis of the disease especially in case of corneal edema or fibrosis [86]. Guttata appear as hyporeflective zones, with a hyperreflective center that corresponds to the apices of the guttae (Fig. 8.9). IVCM can demonstrate of epithelial bullae, subepithelial fibroblast and collagen infiltration, reduction of subbasal corneal nerve den-

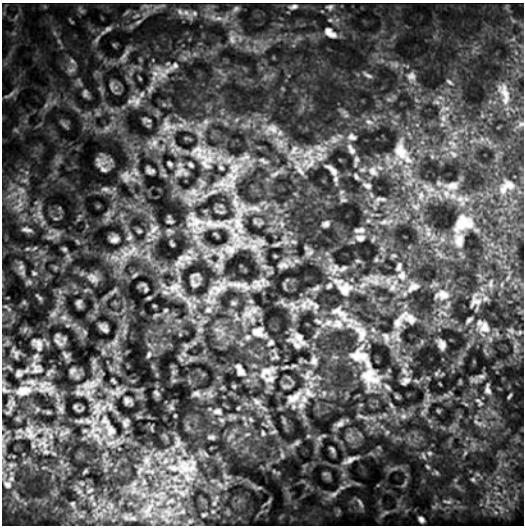


Fig. 8.9 In vivo confocal microscopy on a FECD patient with advanced disease. Guttatae are defined by hyporeflective zones with hyperreflective center; no endothelial cells are visible

sity, reduced anterior keratocyte density, and fibroblastic transformation of stressed keratocytes in the stroma.

Management of Fuchs Dystrophy

Medical management of early Fuchs dystrophy consists of topical hypertonic saline drops or ointment to shorten morning edema. Patients may use the ointment at bedtime and/or the drops upon awakening, typically one drop every 5 minutes for a total of three to four drops. This treatment does not change the course of the disease. Patients do note stinging with these agents, which reduces acceptance. A hairdryer held 5–10 inches from the cornea may also be used to facilitate corneal deturgescence. As the disease progresses, these interventions will no longer be effective. There is no convincing evidence to support the use of topical steroids in the management of Fuchs dystrophy.

Once patients are symptomatic from their FECD, surgery is the preferred treatment. Surgical management of endothelial dysfunction has undergone a revolution in the past 20 years. Interested readers who wish a historical perspective on this field are referred to two recent reviews [78, 87]. In 2019, selective endothelial keratoplasty (EK) is the most common transplantation technique, having surpassed penetrating keratoplasty in 2011. Selective endothelial transplantation techniques continue to evolve. At present, Descemet stripping endothelial keratoplasty (DSEK) is the most common technique, although Descemet membrane endothelial keratoplasty (DMEK) is increasing in popularity. These two surgeries vary in the amount of donor stroma that is transplanted, with DMEK having none and DSEK having 70–150 microns. DMEK provides more rapid visual recovery but has more complications in the immediate postoperative period. However, both DSEK and DMEK represent a real advance in the management of endothelial dysfunction, compared to penetrating keratoplasty.

Advances continue to be made. Kinoshita and colleagues have pioneered the use of cultured cells in the management of endothelial dysfunction and have recently published their initial case series [88]. Cultured cells offer the ability to treat multiple (potentially up to 100) patients from a single donor cornea. However, substantive regu-

latory hurdles (and insurance issues in the United States) must be surmounted before this technique will enjoy widespread acceptance.

Another exciting advance in the treatment of FECD is Descemet stripping only (DSO), independently pioneered by Colby in the United States and Moloney in Australia since 2014 [89, 90]. Multiple lines of evidence in 2012–2013 suggested that the endothelium in FECD might be capable of “self-rejuvenation.” There had been anecdotal case reports of corneal clearance after inadvertent removal of Descemet membrane or of attempted EK in which the donor tissue was unable to be placed [91, 92]. Melles presented a series of DMEK cases in which the donor tissue did not adhere to the host cornea, yet corneal clearance occurred in Fuchs dystrophy patients, but not in cases of pseudophakic bullous keratopathy [93]. Koizumi published a single case of a patient with FECD who attained corneal clearance after endothelial destruction with cryotherapy and treatment with a topical Rho kinase (ROCK) inhibitor [94]. Finally, an initial small series of deliberate Descemet removal was performed, although results were inconsistent [95].

The series by Borkar, Veldman, and Colby was the first to demonstrate success of DSO, with 10 of their 13 patients achieving corneal clearance, at an average of 12 weeks after a 4 mm deliberate Descemet stripping [89]. The first surgery in this series was done in January 2014. The patient, now 5 years out, retains a clear, compact cornea with excellent vision and an endothelial cell count of approximately 900 cells/mm [2]. Moloney published his initial series in 2017 and was the first to report the use of a topical ROCK inhibitor after DSO [90].

After a several year period where DSO was treated with skepticism, the corneal community has begun to embrace DSO as a treatment for focal Fuchs dystrophy. Subsequent studies have suggested that a “peeling” rather than a “stripping” technique may improve corneal clearance rate [96]. In an investigator-initiated randomized clinical trial of ripasudil, a ROCK inhibitor approved for treatment of glaucoma in Japan, corneal clearance occurred more rapidly and final

endothelial cell count was increased in DSO patients treated with ripasudil compared with those who did not use this medication [97]. A multinational industry-sponsored study of ripasudil as an adjunct for DSO is currently in the planning stages. Finally, a recent head-to-head comparison of DMEK vs. DSO showed equivalent visual outcomes. Although DSO patients took longer to clear than DMEK patients, DSO was associated with fewer postsurgical complications [98].

Descemet stripping only is a cost-effective and time-efficient surgery. Further studies are needed to determine the optimal patient population for this nongraft treatment, but its potential is enormous.

Conclusions

Our understanding of the biological basis of FECD has improved dramatically in the last 20 years, as have our options for surgical management. Future strategies for management such as gene-editing remain as exciting possibilities. Even more appealing is the possibility of preventive strategies to address this very common corneal condition.

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Pathophysiology of Corneal Graft Rejection

9

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General Issues

The most common form of solid organ transplantation in the United States is corneal allotransplantation [1]. Each year, approximately 40,000 patients in the United States receive a corneal transplant for vision rehabilitation [1]. The success rate in patients that are deemed “low risk” is extremely high, greater than 90% with the use of topical steroidal therapy alone. Much of the rationale for this high success rate is attributed to the immune privilege of both the cornea and anterior segment [2]. On the other hand, “high risk” patients (10–30% of hosts) with vascularization of their cornea preoperatively have a corneal graft failure rate between 60–90% [3–6]. This is particularly relevant in other countries where the percentage of “high risk” patients who receive a

corneal transplant is closer to 90% [7, 8]. While systemic immunosuppression to minimize immunologic rejection in these high-risk patients has shown some efficacy in the literature, their usage has been limited by the complexity of the regimens as well by associated systemic complications associated with these therapies [5, 7, 8].

The most common cause of corneal graft rejection is immunological rejection [2, 3, 7, 9]. In order for immunological rejection of a corneal allograft to occur, the coordinated recruitment and infiltration of effector T cells that are alloantigen-primed are essential steps [10, 11]. When allo-specific T cells infiltrate into the corneal allograft, apoptosis and graft rejection result [12]. Therefore, the prevention of T cell-mediated immune rejection is the primary goal of postoperative medical therapy [2, 4].

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Penetrating Keratoplasty vs. Endothelial Keratoplasty

Over the past decade, rates of endothelial keratoplasty have increased dramatically [13]. This surge is due, in part, to reports that endothelial keratoplasty procedures enjoy lower rates of rejection relative to penetrating keratoplasty [14–16]. However, in the high-risk setting (previous penetrating keratoplasty or corneal neovascularization), corneas are often scarred and thus require more extensive surgery than endothelial keratoplasty alone [17]. Furthermore, endothelial keratoplasties

performed in high-risk hosts have substantially higher rejection rates than those performed in low-risk hosts compared to, and in some cases exceeding, those for penetrating keratoplasty [18, 19].

Risk Factors

Normally, the cornea and anterior segment enjoy immune privilege for numerous reasons [8]. Indeed, in a physiologically normal eye, the cornea is devoid of both lymphatics and blood vessels. Furthermore, there is a relative paucity of antigen presenting cells within the cornea. The continuous expression of Fas ligand (FasL) helps to promote apoptosis of immunocytes bearing Fas. Immunosuppressive cytokines circulating in the aqueous humor (such as TGF β , α -MSH and VIP) promote immune quiescence [8]. However, when inflammation of the cornea or anterior segment occurs, this results in the breakdown of the vital blood-eye barrier which allows for violation the immune privilege found in normal corneas and anterior segments.

Blood Vessels

A severe inflammatory response can result in corneal vascularization, which permits immune rejection of the corneal allograft. This inflammatory response can result from a variety of etiologies including viral or bacterial infections, trauma, or rejection of a previous corneal graft [8]. Patients who have suffered from these inflammatory conditions are deemed to be at a higher risk of rejection and failure than patients who receive a transplant for conditions such as keratoconus, where the patients often have a non-vascularized recipient corneal bed.

Vascularization of the corneal bed preoperatively is known to worsen the prognosis of a corneal transplant [6]. Studies have shown that the degree of corneal vascularization in the recipient cornea is directly proportional to the incidence of graft rejection and failure [3]. Additionally, in more highly vascularized corneas, the average time between the surgical transplantation and

allograft rejection has been shown to be significantly shorter than in those recipients with less preoperative corneal vascularization [3].

Anterior Synechia

Preexisting anterior synechia is thought to be a poor prognostic sign in patients undergoing a keratoplasty. Studies suggest that fewer corneal allografts remained clear in patients with anterior synechia, and additionally that the risk of allograft rejection was higher [4, 6, 20].

Anterior Chamber Inflammation

Preoperative nonspecific anterior chamber inflammation, or persistent postoperative inflammation, is considered to predispose patients to allograft rejection [4].

Chemical Burns

In the Collaborative Corneal Transplantation Studies, eyes that had suffered prior chemical burn injuries had the highest rate of corneal allograft failure from all causes [6].

Loss of Immune Privilege

Other risk factors for graft failure are related to the loss of immune privilege of either the cornea or anterior chamber. The risk of allograft rejection or failure increases in patients who have a lack of aqueous suppressive factors, loss of expression of FasL, or loss of anterior chamber-associated immune deviation which normally would serve to prevent the suppression of delayed type hypersensitivity reactions to alloantigens.

ABO Blood Group Incompatibility

The rate of corneal rejection from any cause has been shown to be approximately 10% lower in

ABO-matched donors as compared to ABO-incompatible donors. Whereas HLA-antigen matching has not been shown to reduce the rates of graft failure, transplanting ABO-compatible corneas may be effective at decreasing the risk of allograft failure [5].

Prior Ocular Surgery

While prior anterior segment surgery is considered to increase the risk of rejection, the strongest correlation between a prior ocular surgery and allograft rejection is having undergone a prior corneal transplant. Furthermore, the incidence of allograft reaction increases and the time to onset of reaction decreases with each additional prior graft [3, 6].

Pathophysiology

Studies of transplant immunobiology have elucidated the complex sequence of molecular and cellular events that drive the immune rejection of corneal grafts. Initially, the tissue damage that occurs with surgery triggers an innate immune response, with infiltration of antigen-presenting cells into the cornea and the local release of pro-inflammatory factors [21]. Having acquired MHC class II and co-stimulatory molecules in this inflammatory milieu, APCs egress from the cornea and are trafficked via lymphatic vessels to draining lymph nodes [22]. Here, mature APCs present donor antigens to naïve host T cells, prompting the differentiation and clonal expansion of graft attacking type 1 T-helper (Th1) cells. The events leading to the generation of IFN γ -secreting CD4⁺ Th1 cells in the lymph nodes can be understood as the “afferent arm” of the alloimmune response. Following a chemokine gradient, Th1 cells migrate via blood vessels toward the graft site, where they employ a variety of mechanisms to mount a delayed-type hypersensitivity immune response against the allogeneic corneal tissue (i.e., the “efferent” arm of the immune response) [23]. However, these pro-inflammatory pathways are modulated by an array of intrinsic

immunoregulatory responses that promote graft tolerance, including regulatory T cells, tolerogenic dendritic cells, immunosuppressive epithelium-derived molecules, and neuropeptides [24]. It is the balance between these effector and regulatory immune mechanisms that determines whether allogeneic corneal grafts undergo immune rejection.

APC Activation

There are two pathways by which alloantigens are presented by APCs from the site of transplantation to T cells: the direct and indirect pathways. In the indirect pathway, recipient-derived APCs present antigens to T cells and thereby induce allosensitization [2]. This indirect pathway is considered to play a significant role in corneal allograft rejection although it is less efficient at doing so than the direct pathway [25, 26]. Donor-derived dendritic cells (DC) serve as the APCs in the direct pathway. This pathway is considered to play more of a role in high-risk patients and is approximately 100 times more effective than the indirect pathway. The increased effectiveness is a result of allogeneic MHC antigens bypassing the involvement of host APCs by directly activating naïve T cells [2, 27]. A single donor-derived DC is able to stimulate 3000 allospecific primed T cells [28, 29]. This priming is a pivotal step in rejection, and conditions that increase the number of donor-derived APCs, such as corneal neovascularization, will further increase the risk of corneal allograft rejection [2].

Effector Mechanisms

Chemokines are a superfamily of heparin-binding chemoattractant cytokines that direct leukocyte trafficking. One family of chemokines, CXC chemokines, recruits neutrophils and macrophages, along with other potent chemokines such as IP-10, Mig and I-TAC which all attract activated T cells. This family of CXC chemokines includes three chemokines, CXCL1/KC, CXCL9/Mig, and CXCL10/IP10 that are known to be produced

in high-risk allografts with corneal vascularization [30]. Additionally, these neutrophil attractant chemokines (i.e., CXCL1/KC) are upregulated in high-risk corneal allografts by pro-inflammatory cytokines like TNF- α and IL-1 α , produced at the site of transplantation.

CXCL1/KC acts by recruiting neutrophils early and thereby causes acute damage to the allograft. The infiltrating neutrophils mark the corneal graft which then directs the recruitment of allo-specific effector T cells and other leukocytes. It also results in late production of CXCL9/Mig and CXCL10/IP10 and promotes the infiltration of T cells, macrophages, and natural killer (NK) cells into the allograft [30]. In fact, if CXCL1/KC is neutralized, graft survival increases, demonstrating that both the early infiltration of neutrophils and late recruitment of alloantigen-reactive T cells play an important role in allograft rejection [30].

Two of the IFN- γ -induced CXCR3 binding chemokines, CXCL9/Mig and CXCL10/IP10, are potent chemoattractants for allo-specific activated T cells bearing the CXCR3 chemokine receptor [30]. When CXCL9/Mig is neutralized, allograft survival has been shown to increase. CXCL10/IP10 is slightly more complicated and there is thought to be a regulatory component to rejection controlled by CXCL10/IP10, as complete neutralization of the chemokine has been shown to result in a faster and more robust rejection of the allograft.

Immune Regulation

Regulatory T Cells

Regulatory T cells (Tregs) are critical mediators of immune tolerance, and function by a variety of mechanisms to maintain immunological unresponsiveness to alloantigens [31]. These mechanisms include (i) the release of immunoregulatory cytokines, (ii) modulation of dendritic cell (DC) function, (iii) induction of cytotoxicity (via granzyme-B and perforin-dependent pathways), and (iv) competition for metabolic substrates such as IL-2.

The promotion of graft survival by CD4⁺CD25⁺Foxp3⁺Tregs has been demonstrated in adoptive transfer experiments using a mouse model of corneal transplantation [32–34]. Notably, expression of the transcription factor Foxp3 has been shown to be far more relevant to the immunoregulatory capacity of Tregs than frequency, with high levels of Foxp3 expression associated with increased immunosuppressive cytokine production and greater suppression of effector T cell activation [32]. Although Tregs are known to limit the immune response both at the site of inflammation and draining lymphoid tissue [35], evidence suggests that Tregs primarily modulate the immune response to corneal alloantigens by limiting T cell priming in the draining lymph nodes [32]. Following corneal transplantation, the Tregs of allograft acceptors, but not rejectors, have been shown to colocalize with APCs in the paracortical areas of draining lymph nodes [36]. Furthermore, Tregs from allograft acceptors express higher levels of the chemokine receptor CCR7 than allograft rejectors [36]. CCR7 is known to mediate the migration of Tregs to the paracortical areas of draining lymph nodes via high endothelial venules, where they suppress the priming of T cells by DCs [37]. Of note, the amplification of CCR7 expression by ex vivo culture of naïve Tregs with CCL21, followed by intravenous infusion of manipulated Tregs, has been shown to be an effective strategy to enhance Treg homing to the lymph nodes and promote corneal transplant survival [37].

The majority of Foxp3⁺Tregs are generated in the thymus, and are referred to as thymus-derived Tregs (tTregs) [38]. This subset contrasts with Tregs generated from Foxp3⁺ conventional T cells in peripheral tissues, termed peripherally derived Tregs (pTregs) [38]. Using a high-risk model of murine corneal transplantation in which intrastromal sutures were placed prior to transplantation to induce host bed inflammation and angiogenesis, it has been shown that pTregs (but not tTregs) are susceptible to dysfunction in the inflammatory microenvironment [34]. Indeed, pTregs sourced from high-risk hosts exhibited decreased suppression of conventional Tregs relative to low-risk hosts, and were also associated

with decreased expression of the immunoregulatory cytokines IL-10 and TGF- β but increased expression of the pro-inflammatory cytokine IFN- γ [34]. These data suggest that although pTregs promote tolerance to allografts in uninflamed host beds, they are in fact liable to *promote rejection* in the high-risk setting through the secretion of pro-inflammatory factors. This observation is consistent with evidence that Tregs exhibit considerable phenotypic plasticity, and may repolarize toward different fates according to cues from their microenvironment [39, 40].

Treg plasticity has been investigated in corneal transplantation using a double transgenic mouse model that permits Foxp3 lineage tracing [41]. This study demonstrated that a fraction of Tregs lost expression of Foxp3 in the inflamed setting, becoming IFN γ ⁺ or IL-17⁺ “exFoxp3” cells that were phenotypically identical to effector Th1 or Th17 cells, respectively [41]. Moreover, the investigators showed that pTregs are particularly liable to conversion to exFoxp3 cells [41]. Tregs have developmental similarities with Th17 cells and reflected in their mutual requirement for TGF- β , and both cell types exhibit considerable plasticity [42]. These observations are highly pertinent to transplantation immunology, not least given reports describing a Th17-mediated alternative pathway of allograft rejection [43, 44]. Interestingly, studies of the effect of Th17 cells in murine corneal transplantation have inconsistent results, with some groups describing a disease-promoting role for Th17 cells [45], but others reporting exacerbated graft rejection following depletion of the Th17-associated cytokine IL-17A [46]. The plasticity and functional adaptability of Tregs have important implications for cell-based therapeutic strategies to increase allograft survival, in consideration of the risk that therapeutic Tregs may convert to pro-inflammatory phenotypes.

Tolerogenic Dendritic Cells

In addition to Treg-based therapies, there has been research interest in the use of ex vivo-manipulated DCs to promote transplant tolerance

[47, 48]. In a murine model of corneal transplantation, CD200R3⁺ regulatory DCs (DCregs, generated ex vivo by culturing bone marrow cells with GM-CSF, IL-10, and TGF- β) have been shown to promote corneal allograft survival [49]. Indeed, DCreg administration was observed to reduce CD4⁺IFN- γ ⁺ T cell frequencies and increase Foxp3 expression by Tregs [49]. In a separate murine corneal transplantation study that defined tolerogenic APCs (tolAPCs) as CD11c⁺MHC class II^{lo}CD40^{lo}CD86^{lo} cells, subconjunctival injection of recombinant IL-10 and TGF- β into donor eyes was observed to augment the population of corneal tolAPCs [50]. Furthermore, transplantation of these tolAPC-enriched corneas decreased CD4⁺IFN- γ ⁺ T cell frequencies, diminished graft infiltration of CD45⁺ and CD4⁺ cells, and significantly promoted corneal graft survival [50]. Whether by the administration of ex vivo-manipulated cells or by cytokine conditioning of donor corneal tissue, these reports offer strategies to augment tolerogenic APC populations and thus promote graft survival.

Corneal Avascularity

The healthy human cornea does not contain blood and lymphatic vessels. The absence of lymphatics limits the trafficking of APCs to the draining lymph nodes (i.e., the afferent arm of the immune response), and the absence of blood vessels curbs the trafficking of effector T cells to the graft site (i.e., efferent arm) [24]. Still, the cornea can become vascularized due to a range of corneal pathologies and surgical trauma. There is an array of antiangiogenic and anti-lymphangiogenic factors present at the ocular surface that maintain corneal avascularity, thereby impeding allosensitization and promoting graft tolerance. The corneal epithelium secretes an endogenous spliced variant of soluble VEGFR-2 (sVEGFR-2) that suppresses lymphangiogenesis by blocking VEGF-C, thereby limiting the afferent arc of the immune response [51]. As illustration of its tolerogenic properties, intracorneal administration of sVEGFR-2 has

been shown to increase graft survival by more than double in a murine model of corneal transplantation [51]. Inflammatory angiogenesis is suppressed by sVEGFR-1 and VEGFR-3 that are constitutively expressed by the corneal epithelium, thus curbing the efferent arc of the immune response [52, 53]. Endostatin is a 20-kDa collagen fragment expressed by multiple ocular tissues, including corneal and conjunctival epithelial cells [54]. By inhibiting neovascularization and delaying the recruitment of allospecific T cells, endostatin has been shown to prolong corneal allograft survival in murine studies [54]. The corneal epithelium expresses the immunoregulatory molecules thrombospondin (TSP)-1, programmed death ligand-1 (PD-L1), and pigment epithelium-derived factor [PEDF], which also suppress corneal angiogenesis [55–57] and lymphangiogenesis [58]. By inhibiting the development of lymphatic and blood vessel networks, this amalgam of factors work in concert at the cornea to restrict the alloimmune response.

Corneal Epithelial Immunoregulatory Factors

In addition to limiting hemangiogenesis and lymphangiogenesis, immunoregulatory factors expressed by the corneal epithelium have been shown to modulate the alloimmune response in a variety of ways. Corneal epithelial cells express Fas ligand (FasL), which engages the cell death surface receptor Fas, thus mediating apoptotic cell death via caspase activation [59]. In murine corneal transplantation studies using FasL⁻ mice, FasL⁻ corneas have been observed to have numerous inflammatory cells without apoptosis, associated with graft rejection rates of almost 100% [60, 61]. In contrast, FasL⁺ grafts were observed to contain apoptotic mononuclear cells with an acceptance rate of approximately 50% [60, 61]. These studies provide evidence for the importance of FasL expression in regulating the alloimmune response. PDL-1 is constitutively expressed at high levels by the corneal epithelium, and through its interaction with PD-1 on T cells has

been shown to suppress T cell proliferation, limit IFN- γ production and promote corneal allograft survival [62, 63]. A role has been reported for interleukin-1R antagonist (IL-1Ra), also expressed constitutively by corneal epithelium, in maintaining ocular immune quiescence by suppressing APC migration [64]. Topical application of exogenous IL-1Ra has been shown to promote graft survival in both the normal-risk and high-risk settings [65]. TSP-1 has been demonstrated to activate the immunoregulatory cytokine TGF- β [66]. TSP-1 is expressed by corneal epithelium as well as APCs, and has been shown to suppress allosensitization and promote graft survival in a murine model of corneal transplantation [67]. In this study, almost all TSP-1 null allografts were rejected, compared to 50% of wild-type allografts. TSP-1 null APCs expressed increased levels of MHC class II and CD80 maturation markers, indicating increased APC maturation in the absence of TSP-1 [67]. By the expression of these immunoregulatory molecules, the corneal epithelium helps to foster a tolerant immune microenvironment and promote allograft survival.

Neuropeptides

Recent research has shed light on the cross talk between the immune and nervous systems in the setting of corneal transplantation. The neuropeptides alpha-melanocyte stimulating hormone (α -MSH) and vasoactive intestinal peptide (VIP), constitutively expressed in the cornea, have been shown to promote the survival of allogeneic grafts in murine studies [68, 69]. Subconjunctival injection of α -MSH following corneal transplantation has been reported to decrease graft infiltration of mononuclear and polymorphonuclear cells, and to significantly reduce graft rejection [68]. VIP has been shown to suppress IFN- γ - and TNF- α -induced corneal endothelial cell loss, preserve corneal endothelial cell density and promote allograft survival in high-risk murine model of corneal transplantation [69]. The immunoregulatory roles of other neuropeptides (including calcitonin gene-related

peptide and neuropeptide Y) at the cornea have been described and are the subject of ongoing investigations [70].

Future Prospects in Regulating Immunity and Cytoprotection

Despite substantial advances in our understanding of the effector and regulatory mechanisms that underlie the alloimmune response to corneal grafts, there is a deficit of novel therapeutics available to the corneal transplant surgeon. Indeed, corticosteroids remain the first-line therapy in most instances, despite their considerable toxicity and limited efficacy in high-risk hosts (with previous rejection episodes or vascularized graft beds) [71]. However, there are a number of promising avenues for the translation of benchside discoveries to the clinic.

Cell-based therapies include the local or systemic administration of immunomodulatory cells such as Tregs [32, 36, 72, 73] and mesenchymal stem cells (MSCs) [74, 75]. However, the translational potential of cell-based therapies is constrained by multiple issues, including (i) the challenge of consistent production of clinical-grade cells, (ii) strict regulatory standards, (iii) substantial cost (both resources and time) of complying with regulatory standards, and (as discussed previously) (iv) phenotypic and functional plasticity of immune cells. Various approaches to circumvent these issues have been proposed, including the *in vivo* expansion of immunoregulatory cells such as Tregs [33, 76], and the identification of critical anti-inflammatory molecules secreted by immunoregulatory cells that are of potential therapeutic value (e.g., MSC secretion of tumor necrosis factor- α stimulated gene/protein 6 [75]). Alternative strategies to enhance the survival of corneal allografts include targeting heme/lymphangiogenesis with anti-VEGF agents [77–79]. Neuropeptides are also candidate therapies; for example, local VIP administration following high-risk corneal transplantation has been shown to decrease opacity and promote graft survival with increased corneal endothelial cell density observed [69]. These approaches may permit

the development of novel treatments that decrease rates of corneal transplant rejection and/or graft endothelial cell loss, particularly in the high-risk host.

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Ocular Disease in Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis

10

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Abbreviations

AMT	Amniotic membrane transplantation
BKP2	Type 2 Boston keratoprosthesis
IVIG	Intravenous immunoglobulin
LSCD	Limbal stem cell deficiency
MMG	Mucous membrane graft
MOOKP	Modified osteo-odonto-keratoprosthesis
OSDI	Ocular surface disease index
PED	Persistent epithelial defects
PROSE	Prosthetic replacement of the ocular surface ecosystem
SCORTEN	SCORE of toxic epidermal necrolysis
SJS	Stevens-Johnson syndrome
TEN	Toxic epidermal necrolysis

Introduction

Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) are forms of a rare hypersensitivity reaction affecting both the skin and various mucosal surfaces, including the eyelid and ocular surface. Initially thought to be erythema multiforme-like until 1922, SJS/TEN has since been recognized as a distinct diagnosis with its own unique clinical course [1]. SJS/TEN occurs on a spectrum of severity depending on total body surface area (TBSA) involvement; SJS encompasses less than 10% TBSA, toxic epidermal necrolysis (TEN) includes cases with a TBSA greater than 30%, and the diagnosis of SJS/TEN overlap includes any cases with a TBSA in between. Despite the combined low incidence of these diseases at roughly 2–7 cases per million per year, the mortality for these conditions is as high as 35% [2–4].

SJS/TEN is characterized by widespread keratinocyte apoptosis which manifests as sloughing of the skin and mucosal surfaces in the clinical setting. Clinically, the initial stage of the disease includes a non-specific prodromal syndrome followed by the onset of mucosal ulcerations (typically two or more mucosal surfaces are involved) and the development of cutaneous vesiculobullous lesions over the body. These lesions can progress aggressively requiring intensive care unit or burn center level of care for management of severe airway complications and symptom

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control. SJS/TEN is typically diagnosed by patient history, clinical presentation, and confirmed by skin biopsy which may show necrotic keratinocytes and full-thickness epidermal necrosis. The majority of cases are reactions to medications, though infections may also be a significant cause [8–10]. Antibiotics, especially sulfa-based ones, and anti-epileptics are among the most common etiologic drugs. Analysis of growing SJS/TEN databases from around the world continues to provide novel information regarding drug classes and individual drugs implicated in SJS/TEN [11, 12].

The three most commonly affected mucosal surfaces are oral, ocular, and genital surfaces [13, 14]. Specifically, the eye is reported to be one of the most commonly affected mucosal surfaces with acute involvement in up to 99% of patients and chronic involvement in up to 80% [15–19]. As access to and quality of acute supportive care improves, particularly in the developed world, mortality rates are decreasing resulting in more survivors, and presumably, an increase in patients with the potential for devastating chronic ocular disease including corneal blindness.

Ocular Manifestations

The relationship between the acute systemic manifestations of SJS/TEN and disease of the ocular surface is poorly understood. Some studies have shown that the SCORTEN, a disease severity score oftentimes calculated in the intensive care unit and burn centers, does not necessarily correlate with ocular disease severity [20, 21]. Furthermore, the relationship between disease type (SJS vs. TEN) and ocular complication severity is poorly understood [22]. A recent study of 15 patients comparing ocular outcomes of SJS vs. TEN showed that TEN correlated with worse visual acuity in the chronic phase [23].

The ocular surface is often involved in the acute phase of the disease as both rapid-onset keratinocyte apoptosis and associated inflammation can affect the integrity of the epithelial surfaces. Manifestations range from conjunctival hyperemia to complete sloughing of the corneal

epithelium to ulceration of the tarsal conjunctiva and the eyelid margin [24, 25]. Severe inflammation can lead to pseudomembrane formation, early symblepharon formation, corneal ulceration, and even corneal perforation [26, 27]. Inflammation of the puncta and meibomian glands may also be seen [28]. For this reason, it is crucial an ophthalmologist is involved in the acute care of SJS/TEN patients at the time of admission.

Natural history of the disease shows that these acute ocular manifestations develop into chronic ocular disease with significant visual and quality-of-life impairment [29–32]. Conjunctival ulcerations and membrane formation can cause permanent symblepharon, restricting eyelid and ocular motility. Tarsal conjunctival involvement in the acute phase can lead to eyelid malpositions as well as other adnexal pathology such as trichiasis/distichiasis, meibomitis and meibomian gland dropout, punctal occlusion, and keratinization of the eyelid margin. If not managed appropriately, mechanical degradation of the corneal epithelium by eyelid margin complications and repeated epithelial disruption can lead to corneal and conjunctival scarring and keratinization, corneal neovascularization, limbal stem cell deficiency (LSCD), and consequently significant visual impairment [33–35]. The most dreaded complication of all is blindness from irreversible corneal damage [36, 37]. SJS/TEN can drastically alter vision and consequently quality of life. While there are no SJS/TEN-specific quality-of-life assessment tools, the ocular surface disease index (OSDI) and National Eye Institute Visual Function Questionnaire 25-item (NEI VFQ-25) have been utilized in various studies to quantitatively assess the quality-of-life impairment these patients can face secondary to visual disturbances [38–40].

Ocular Disease Classification

Given how rare yet potentially severe SJS/TEN is, quantifying ocular disease severity can be helpful in informing acute and chronic management. Several grading schemes for both the acute and chronic phases have been proposed to help in guiding management [41–43]. See Tables 10.1 and 10.2. The

Table 10.1 Ocular manifestations of SJS/TEN

	Acute manifestations	Chronic manifestations
Eyelid margin	Epithelial defects, ulceration, meibomitis	Trichiasis/distichiasis, ectropion/entropion, keratinization, meibomian gland dysfunction and dropout, punctal scarring
Conjunctiva	Hyperemia, pseudomembrane formation, early symblepharon formation	Chronic hyperemia, symblepharon, keratinization
Cornea	Epithelial defects, ulceration, perforation (subacute)	Superficial punctate keratitis, neovascularization, opacification, keratinization, scarring, limbal stem cell deficiency, loss of palisades of Vogt, perforation

Table 10.2 Acute stage ocular disease classification/grading in SJS/TEN

	Categories	Components
Acute stage classification schemes		
Power et al. (1995) ^a	Mild	Conjunctivitis, conjunctival pseudomembrane
	Moderate	Conjunctival membrane, corneal epithelial defects
	Severe	Fornix foreshortening, symblepharon formation, corneal ulceration
Sotozono et al. (2015) ^a	None	No ocular involvement
	Mild	Conjunctival hyperemia
	Moderate	Either ocular surface epithelial defect or pseudomembrane formation
	Severe	Both ocular surface epithelial defect and pseudomembrane formation
Gregory (2016) ^a	Mild	No lid margin or corneal staining, conjunctival hyperemia without stain
	Moderate	Stain <1/3 of lid margin, no corneal stain, conjunctival staining <1 cm
	Severe	Stain >1/3 of lid margin, any epithelial defect more than punctate staining, conjunctival staining <1 cm
	Extremely severe	Stain >1/3 of lid margin on more than one lid, any epithelial defect more than punctate staining, multiple areas of conjunctival staining >1 cm

^aRefs. [41, 38, 43] (order as shown in table)

advent of these acute grading tools has provided better insight into identifying disease severity, allowing for early intervention in severely affected eyes and providing a framework by which disease can be followed and comparative studies can be performed.

Table 10.3 Chronic stage ocular disease classification/grading in SJS/TEN

Sotozono et al. (2007) ^a	Cornea	Superficial punctate keratitis, epithelial defect, loss of the palisades of Vogt, conjunctivalization, neovascularization, opacification, and keratinization
	Conjunctiva	Conjunctival hyperemia, symblepharon formation
	Eyelid	Trichiasis, mucocutaneous junction disease, meibomian gland dysfunction, punctal involvement
De Rojas et al. (2007) ^a	Mild/moderate	Conjunctival scarring without corneal involvement
	Severe	Conjunctival scarring with corneal involvement
	Ocular surface failure	Stem cell deficiency leading to corneal neovascularization and keratinization
	Recurrent inflammation	Episodic conjunctival inflammation
Yip et al. (2007) ^a	Scleritis	One or more episodes of scleritis
	None	Severe dry eyes, trichiasis, symblepharon, distichiasis, visual loss, entropion, ankyloblepharon, lagophthalmos, corneal ulceration

^aRefs. [42, 30, 41] (order as shown in table)

In addition to the development of acute grading tools, several chronic ocular complication grading schemes have been proposed to further assist in management of patients in the chronic phase [30, 44, 45]. See Table 10.3. These classification schemes have been used in

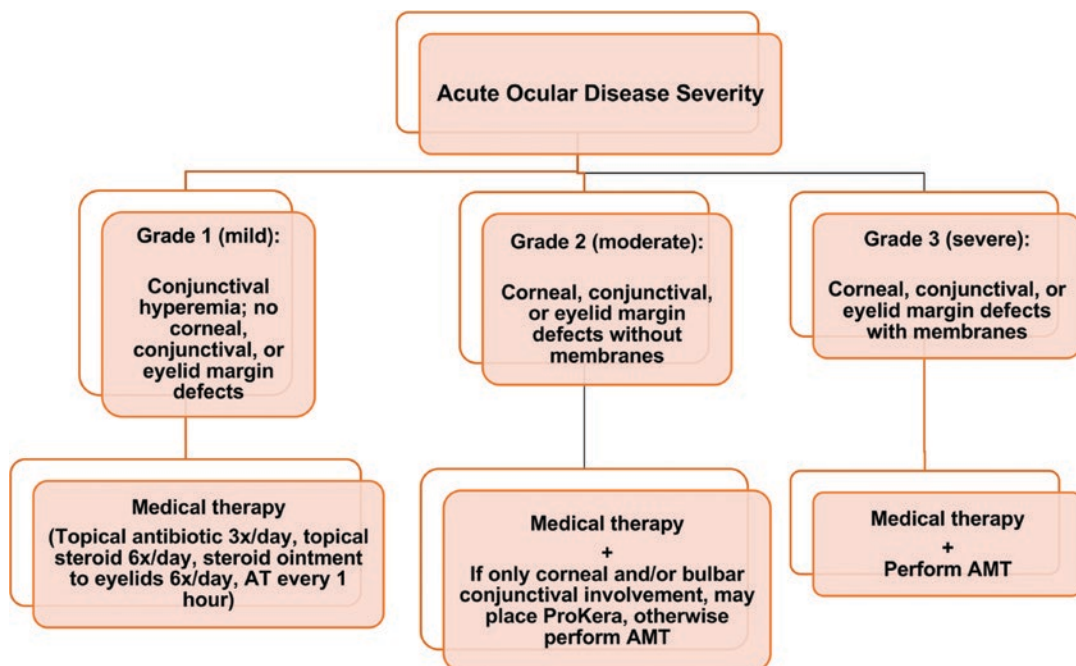


Fig. 10.1 Suggested management for acute ocular disease in SJS/TEN. (Adapted from Refs. [24, 42, 43]). AT artificial tears, AMT amniotic membrane transplantation

a few prospective studies but remain somewhat difficult to employ in retrospective studies given their detailed and specific nature [46] (Fig. 10.1).

Acute Systemic Therapy

The role of systemic therapy in the acute phase of SJS/TEN was first documented in 1988 [47]. It has been theorized that systemic steroids and intravenous immunoglobulin (IVIG) may have acute preventative and therapeutic functions. Other immunomodulators have also been advocated for in the acute phase, including calcineurin inhibitors, cyclophosphamide and TNF-alpha inhibitors [48]. However, to date, only corticosteroids and IVIG have been studied for their potential benefit in mitigating chronic ocular disease, and even so, the current data is conflicting [18].

This conflicting data includes a case series by Kim and colleagues in 2013 in which 51 patients

with SJS/TEN were studied. The authors discovered that early treatment with both steroids and IVIG can improve ocular outcomes, but that the individual therapies used alone had no impact on visual acuity or ocular outcomes [49]. Another study by Araki and colleagues examined five patients undergoing intravenous pulsed steroid with methylprednisolone in the acute phase and found therapeutic benefits with regard to visual acuity restoration and corneal health [50]. However, another multi-center comparative study with 43 patients found no therapeutic benefits to systemic immunomodulatory treatments in the visual outcomes and complication scores in patients with SJS/TEN [51]. Provided the equivocal evidence for various systemic immunotherapies in the acute phase, definitive recommendations for their use for ocular disease cannot be made. An article published by Le et al. in 2016 found that clinical use of systemic steroids in burn centers is around 65% and that only 4% of the surveyed centers utilize IVIG in their treatment protocol for SJS/TEN [52].

Acute Ocular Management

Several algorithms, mostly based on the aforementioned disease classifications schemes, have been proposed for initial ocular therapy in SJS/TEN [18, 24, 42, 43, 53]. In addition to early symblephara and pseudomembrane removal, these algorithms propose medical regimens and, in more severe cases, amniotic membrane transplantation (AMT). The most common medical ocular therapies employed in the acute phase include lubrication, topical steroids, and topical antibiotics [24, 45, 53, 54]. Prospective studies evaluating the benefits of these treatments are lacking.

Amniotic Membrane Transplantation (AMT)

In the presence of more severe acute ocular disease, additional therapy is required to stabilize the ocular surface. Amniotic membrane transplantation was first described in 1995 in the setting of severe ocular diseases by Kim and Tseng [55, 56]. Amniotic membrane is the membrane found on the innermost surface of the human placenta. Its efficacy on the ocular surface lies in its antimicrobial and immunomodulatory properties and in its promotion of epithelialization [57].

The first report of amniotic membrane use in SJS/TEN was described by Zhou et al. in 1999 followed by a similar study by Honavar et al. in 2000 [58, 59]. It was not until 2002 that amniotic membrane transplantation was described in a series of patients in the acute phase of SJS/TEN [60]. It has since been used and studied in the context of acute SJS/TEN with remarkable benefits being shown [61–64]. However, to date, only one prospective randomized control study validating the effectiveness of adjuvant AMT in acute SJS/TEN has been performed [65].

Indications for AMT therapy in the acute phase include moderate to severe ocular surface disease, eyelid epithelial defects, and/or pseudomembrane formation. Ideally, it is recommended to perform AMT within 5 days of onset

of SJS/TEN symptoms, although this is often-times difficult to accomplish given the step-wise progression of the disease and the resultant delayed presentation. AMT involves laying down and stretching a cryopreserved amnion over the entire ocular surface, including the fornices and eyelid margins. The membrane is typically secured to the ocular surface using 8-0 nylon or prolene suture and bolsters. In more recent years, securement of amniotic membrane to the ocular surface in a variety of ocular diseases has been trialed with cyanoacrylate or fibrin glue [66–69]. Our unpublished data on sutureless, glued AMT application in SJS/TEN shows it to be as effective as sutured technique and significantly less time consuming. We have previously described this technique and it is shown in Fig. 10.2 [70].

Dissolution of the amniotic membrane typically happens between 3 and 10 days post transplantation [61]. AMT can be performed by the bedside in sedated or cooperative patients but can also be performed in the operating room under general anesthesia. AMT should not be confused with application of a ProKera® (Biotissue, Inc.™) device, a commercially available amniotic membrane that is fused to a symblepharon ring. A ProKera device alone will not suffice given its limited ability to cover all fornices and eyelid margins and has been shown to be less effective than complete coverage of the ocular surface by AMT [70]. If a patient is unable to receive AMT (uncooperative or medically unstable patient), a ProKera device may be used as a last resort.

Chronic Ocular Management

Up to 80% of patients with SJS/TEN develop chronic ocular disease and regular follow-up with a cornea specialist is crucial. Chronic disease is typically a direct result of the changes that occurred in the acute phase. The following are current techniques for managing the various chronic ocular manifestations of the disease as well as the future direction of therapy.

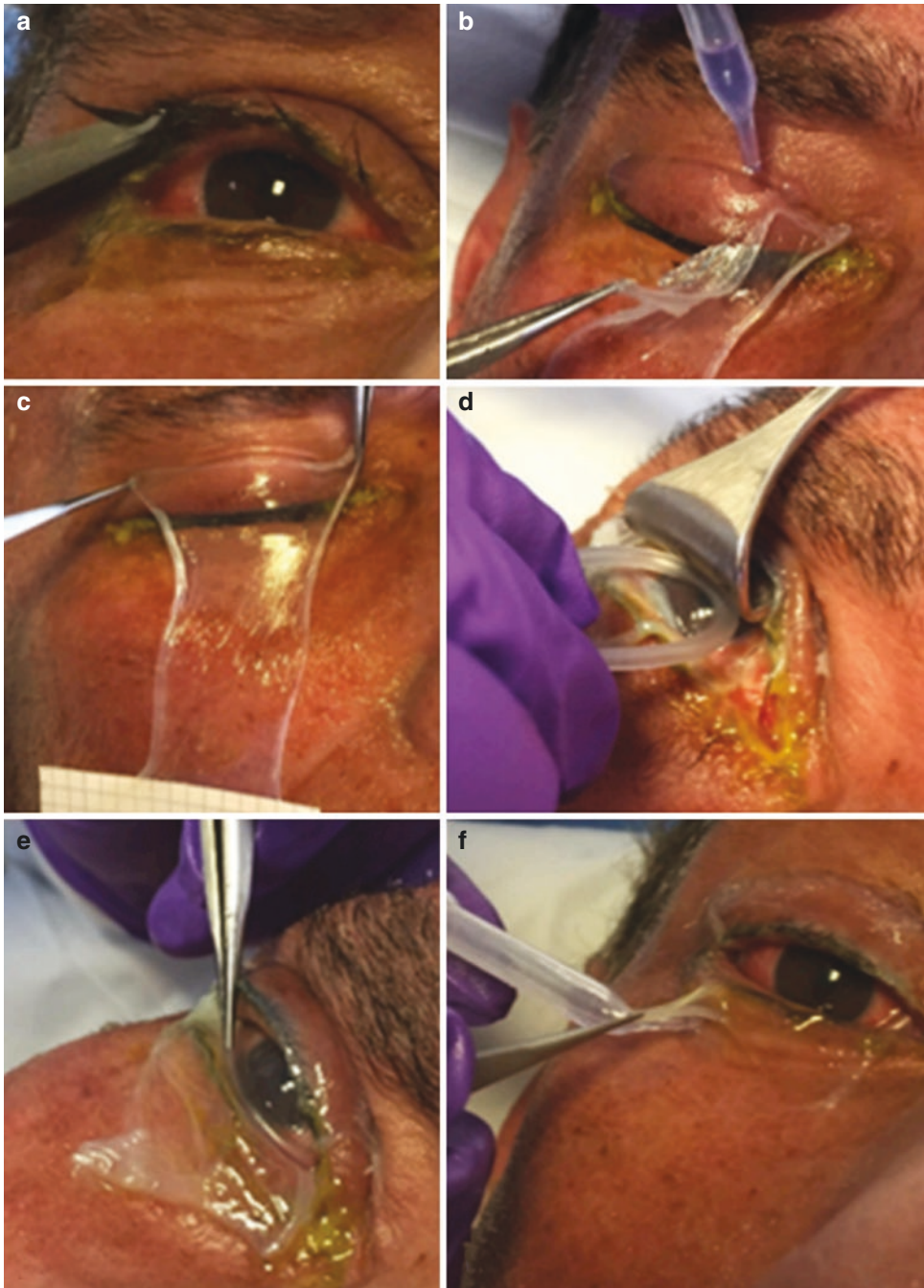


Fig.10.2 Amniotic membrane transplantation – sutureless technique. Procedure for sutureless amniotic membrane transplantation (AMT) in the right eye of a patient with Stevens-Johnson syndrome with ocular surface and eyelid margin epithelial defects. (a) Upper and lower eyelashes are trimmed. (b) Cyanoacrylate glue is used to secure the amniotic membrane (AM) sheet (5 × 10 cm) to the upper eyelid. Care is taken to ensure glue is placed at least 4 mm above the eyelid margin. (c) AM is held across the upper eyelid for 30 s

to ensure adhesion. (d) Desmarres lid retractor is used to retract the upper eyelid to allow the symblepharon ring to be placed in the superior fornix. (e) The symblepharon ring is then tucked into the inferior fornix. (f) The inferior margin of the AM is secured to the lower eyelid with cyanoacrylate glue. The patient should be able to close the eye without any lagophthalmos caused by the symblepharon ring. (Reprinted from Shanbhag et al. [70]. Copyright (2019) with permission from Elsevier)

Eyelid Malpositions and Misdirected Lashes

Lagophthalmos, eyelid malpositions, and misdirected lashes can result in a disruption of the normal tear film, and exposure and mechanical keratopathy, and directly damage the corneal surface. Lagophthalmos and eyelid malpositions can be treated by minor procedures such as cicatrix release or by tarsorrhaphy. Eyelid malpositions can also be addressed via reconstructive procedures including lamellar repositioning, canthoplasty, amongst others. Lastly, eyelash misdirection can be addressed by hyfrecation or bulb extirpation. Manual epilation is not a definitive solution.

Dry Eye

It is reported that up to 59% of SJS/TEN patients suffer from dry eye in the chronic stage [16, 44]. The inflammatory processes in the acute phase can lead to scarred lacrimal ducts, a reduction in goblet cell density, and severe meibomian gland inflammation causing eventual gland dysfunction and dropout [24, 28, 35]. In such cases, topical medications such as cyclosporine and frequent application of preservative free tears are used to improve goblet cell density and provide lubrication, respectively. Some cases may require punctal cauterization, though puncta are often already occluded by scarring from the disease process. Salivary gland transplantation may also be employed, although data on this is limited [71, 72]. In cases of severe dry eyes, some have advocated for scleral contact lenses that bathe the ocular surface in a liquid reservoir [73]. Anecdotally, serum tears may also improve symptoms. Future studies are warranted to investigate the impact of other interventions in the acute phase, such as manual meibomian gland expression, in restoring the functionality of glands and to reduce potential gland dropout.

Posterior Eyelid Margin Keratinization

Repeated microtrauma from eyelid margin keratinization can result in severe ocular surface disease

including neovascularization and conjunctivalization of the cornea. Nonsurgical intervention includes the use of scleral contact lenses that vault over the cornea and protect it and have been shown to be beneficial in restoring vision in SJS/TEN [79, 80]. A more customized scleral lens system, called the Prosthetic Replacement of the Ocular Surface Ecosystem (PROSE, BostonSight, Needham, MA), is beneficial in patients who may not be candidates for standard scleral lenses [30, 38, 39, 81, 82].

In cases of extensive symblephara preventing the use of scleral lenses or in cases with continuous corneal insult secondary to heavily keratinized eyelids, reconstructive procedures are warranted. A mucous membrane graft (MMG) is an autologous surgical option that is employed in keratinized eyelids, which replaces the diseased conjunctiva with viable and healthy labial or buccal mucosa. Such procedures help restore the integrity of the mucocutaneous junction and allow for improvement in visual acuity and ocular surface health [74–77]. In addition to the structural benefits of a MMG, the procedure has recently been shown to play an important immunological role in down regulating the presence of anti-fibrotic cytokines to reduce intractable, chronic inflammation [78].

Persistent Epithelial Defects

Persistent epithelial defects (PED) can lead to severe corneal complications and it is critical to address them as early as possible. Standard therapies for management of recurrent defects in non-SJS/TEN patients include persistent lubrication with ointments and artificial or serum tears. ProKera devices can also be used to promote re-epithelialization. However, PED in the setting of eyelid margin disease require an intervention that protects the ocular surface from the eyelid. These interventions include bandage contact lenses, scleral lenses, and mucous membrane grafting. The vaulting of a scleral lens off of the corneal surface allows the surface to heal but also protects it against any mechanical insult. PROSE devices have been shown to significantly improve vision and heal PED in SJS [30, 38, 39, 81, 82].

In cases with persistently injured cornea, limbal stem cell deficiency may occur, but management via limbal stem cell transplantations in the SJS population has shown to be largely ineffective [83]. Keratoprosthesis may be needed in the most extreme cases as discussed below.

Cases of corneal PED should be cultured. Our unpublished data show a possible increased risk of corneal infection in SJS/TEN, even in the absence of a frank infiltrate. Unrecognized infection and ulceration can lead to perforation of the cornea.

End Stage Disease

For patients with severe corneal disease not amenable to more conservative approaches, artificial corneal transplantation may be considered. Postoperative complications and device retention are thought to be worse in patients with SJS/TEN than in any other population, but keratoprosthesis may be the only option for visual rehabilitation in end stage corneal blindness [84–87]. Transplanting ophthalmologists must be aware of the potentially increased risk of infectious keratitis and endophthalmitis, sterile melt, and glaucoma. The type 1 Boston keratoprosthesis may be used in eyes with a wet ocular surface and good eyelid function. Many cases of end stage disease in SJS/TEN do not fulfill these criteria as eyes have developed a xerotic, keratinized surface, often with eyelid abnormalities. These cases require a type 2 Boston keratoprosthesis (BKP2) or a modified osteo-odonto-keratoprosthesis (MOOKP). Both of these surgeries are complex, cosmetically displeasing, and time consuming. The BKP2 is performed as a single surgery while the MOOKP is staged over two or more surgeries and requires at least one viable autologous cuspid tooth. Endophthalmitis and glaucoma continue to be significant concerns in these eyes [88, 89]. Both of these unique transplant options require additional surgical training and are usually only performed at tertiary care referral centers with experienced clinicians who can appropriately manage complex disease [18].

The Future of Ocular Disease in SJS/TEN

SJS/TEN is a rare and debilitating disease which can profoundly affect one's life and vision. The disease in the acute stage can lead to devastating chronic outcomes for these patients. While advances in acute care have significantly improved outcomes in the chronic phase, SJS/TEN continues to carry a large burden of visual morbidity. Furthermore, developing countries have not seen the decrease in chronic complications that developed nations have as access to appropriate acute care is limited and access to culprit drugs is often unrestricted. In the near term, educational programs that promote early involvement of ophthalmologists in the care of SJS/TEN patients must be pursued. Even in the United States, only 66% of burn centers routinely consult ophthalmology for SJS/TEN admissions, resulting in the loss of a large window of opportunity for optimal acute care [52]. In the long term, given the potential ocular and systemic severity of the disease, the future of care in SJS/TEN lies in prevention of the disease through identification of at-risk individuals. In 2004, HLA-B*1502 was found to be strongly associated with carbamazepine-induced SJS/TEN in the Han Chinese [90] and now genetic testing for this HLA is recommended by the FDA prior to starting this medication in specific populations. Since then, associations to this degree have not been found, but discovery of drug and population-specific genetic risk factors is being actively pursued [91–97].

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Treatment of Chemical Burn to the Eye: A Changing Picture

11

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In spite of continuing preventive efforts to reduce the incidence of sight-reducing chemical burns to the eye by industrial safety legislation; increased agrarian, laboratory, and household precautions; and reduced criminality, severe burns are still causing substantial blindness worldwide, especially in the developing world. The victims are often in their early working years, and treatment is woefully inadequate (Fig. 11.1).

The literature on how to deal with chemical burns is huge and frequently repetitive, but excellent clinical reviews of standard treatment are available (e.g. Hughes [1], Wagoner [2], Pfister and Pfister [3, 4]). In this brief review, there will be no attempt at repeating the established literature, unless controversial issues require. Nor will unilateral burns with their better prognosis and

different treatment be addressed. Rather, recent findings on the biology of severe chemical burns, which have changed our concept of how the chemicals can lead to blindness via inflammation, corneal ulceration, and, particularly, via glaucoma, will be discussed⁵. Based on these developments, a modified clinical treatment paradigm will be proposed.

Corneal Ulceration After Chemical Burn

Following chemical burn injury to the cornea, severe inflammation and limbal stem cell damage can contribute to further corneal ulceration, neovascularization, opacification, and, ultimately, blindness. While the inciting chemical leads to direct tissue damage (such as protein hydrolysis, and saponification by alkali and the formation of neutrophil chemoattractant molecules) [9], it is the continued and disordered expression of inflammatory cytokines and matrix metalloproteinases (MMPs) that drive the often destructive response associated with chemical injury [10–13]. The corneal epithelium, inflammatory cells, and keratocytes each contribute such mediators and enzymes. However, most of the response seems to originate anatomically from the *corneal surface*. Simple experiments with glued-on contact lenses after chemical burn have provided some additional clues about this attack from the surface (Fig. 11.2). After a large

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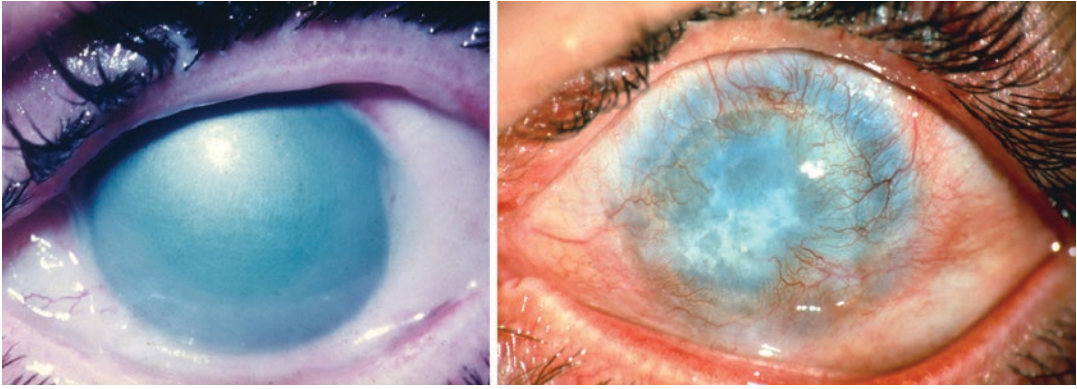


Fig. 11.1 On the left, a discouraging picture of an eye of a 12-year-old boy who 2 days earlier suffered a severe, bilateral alkali burn. The conjunctival-scleral ischemia and the corneal marbling are obvious signs that are very ominous for the future. The boy is now a man in his 60s. In spite of intensive medical attention and numerous

operations, he has been blind for most of the time since the accident. Several standard keratoplasties have been failures (above). Subsequent keratoprosthesis, although anatomically successful, could not restore vision due to advanced glaucoma. Glaucoma is now the main obstacle to visual rehabilitation after a chemical burn

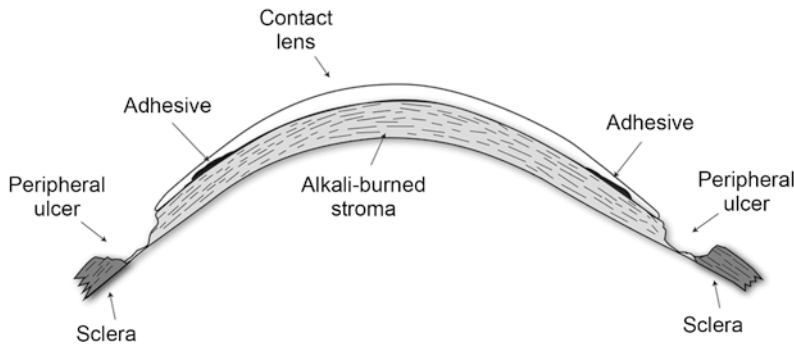


Fig. 11.2 Diagram of a large contact lens glued with cyanoacrylate adhesive to the de-epithelialized corneal stroma – a technique developed by Gasset and Kaufman, 1968 [6]. After an alkali burn, in rabbits, the stroma beneath such a glued-on lens remains totally intact

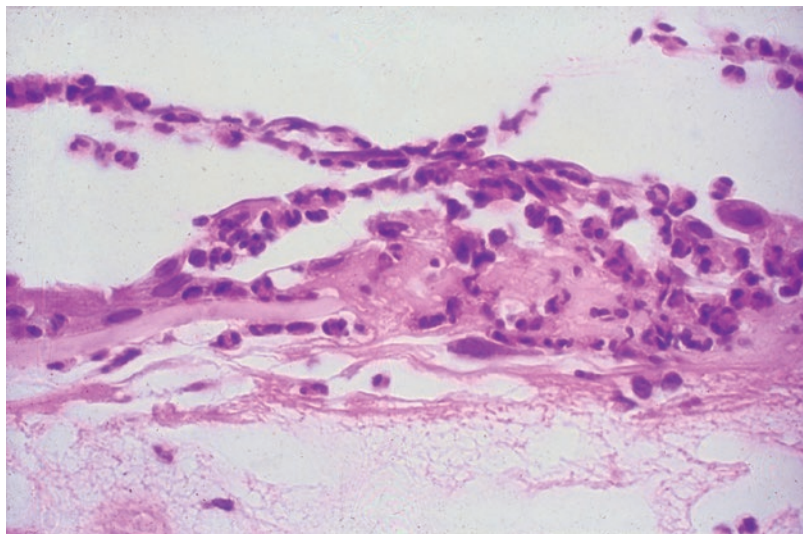
whereas the exposed periphery can severely ulcerate and perforate within 2–3 weeks. Thus, corneal ulceration of the cornea originates on the surface from contact with elements in the tear film of the inflamed eye. (Drawing based on paper by Dohlman et al. [7], with permission)

contact lens is attached to the denuded corneal stroma by a peripheral ring of cyanoacrylate adhesive, the underlying stroma remains completely intact without a trace of neutrophilic infiltration or ulceration [7]. Outside the contact lens, the stroma ulcerates rapidly. Also ulceration quickly ensues when the contact lens is removed. The main source of the ulceration seems to be tear film components of the inflamed eye. Histopathological evidence points to tear neutrophils adhering to the surface of the denuded stroma (Fig. 11.3) where they presumably release their tissue-degrading MMPs [8].

Several additional studies have confirmed this concept [14, 15]. This neutrophil adhesion to denuded stromal surface seems to be a key factor in the development of corneal ulceration.

It is well known that MMPs play a central role in corneal ulceration – the collagenolytic concept was introduced by Gross and Lapiere [16], and for cornea by Slansky [17] and by Brown et al. [18]. Collagenase inhibitors have been extensively studied in an effort to prevent or retard corneal ulceration. Medroxyprogesterone inhibits collagenase synthesis [19], and EDTA-Ca, cysteine, acetylcysteine, and other chelators have shown

Fig. 11.3 After a chemical burn, polymorphonuclear leukocytes (PMNs) accumulate in the tear film of the inflamed eye. Where corneal epithelium is absent, the PMNs tend to stick to the denuded stroma, releasing proteolytic enzymes, causing ulceration. (Kenyon et al. [8], with permission)



some efficacy [20–22], albeit rather weak. Doxycycline or derivatives seem to be more effective, systemically or topically [23, 24]. Corticosteroids are still by far the most used traditional anti-inflammatory drug after a chemical burn although their efficacy in protecting the cornea from subsequent ulceration and scarring is limited. Severe burns can be so destructive that no standard pharmacological means are apt to markedly modify the clinical outcome.

It is now known that the chemically burned cornea is also the source of dramatic increases of several proinflammatory cytokines (see review) [13]. The roles of these cytokines are sometimes hard to interpret at the present time, and a few studies have been in part contradictory. Of particular clinical interest, however, are reports where inhibitors to these cytokines have been identified as strongly anti-inflammatory or otherwise protective. Thus, suppression of IL-1 by receptor antagonist [25], blockade of IL-6 receptor [26], and the potential role of tocilizumab [27] have been reported. Particularly, the role of TNF- α after chemical burns and the therapeutic possibilities of TNF- α inhibitors (in particular infliximab) have been the subject of considerable interest [28–32] (also see below). Corticosteroids and cyclosporine can reduce inflammatory cytokines in the cornea [33], and amniotic fluid, if applied topically, reportedly accelerates healing

[34]. More recently, stem cells (limbal epithelial or mesenchymal) have been shown to attenuate inflammation in the alkali burned cornea [35, 36]. Stem cells also have been shown to clear stromal opacities – an exciting new therapeutic possibility [37]. In short, a number of known mediators are responsible for the corneal surface ulceration and disordered wound healing seen after chemical burns (Figs. 11.4 and 11.5).

Glaucoma After Chemical Burn

Glaucoma is now recognized as the most severe long-term complication after a chemical burn [38–40]. Patients with burn injuries are known to often progress rapidly to end-stage glaucoma despite pressure-lowering treatment [40]. Although glaucoma originally was believed to occur by pH diffusion to the retina and its ganglion cells, recent animal experiments ruled out this possibility and showed that alkali is effectively buffered at the iris-lens level [29, 31]. In more detailed mechanistic investigations, oxygen and redox changes were also found to be restricted to the anterior segment. However, these changes cause the release of inflammatory cytokines that rapidly diffuse posteriorly and damage the retina [29, 31, 40]. Importantly, these events can occur in the absence of elevated

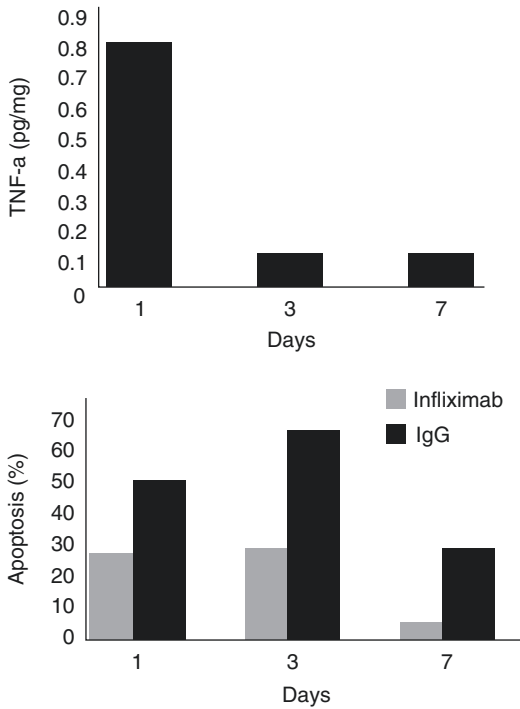


Fig. 11.4 Upper panel: Markedly elevated TNF- α level in the posterior segment of mice eyes after alkali burns (first experiment). Lower panel: Apoptosis rate in retinal ganglion cells after the burn. Intraperitoneal infliximab causes (here moderate) reduction of the apoptosis rate vs. IgG controls. (Cade et al. [29], with permission)

IOP [31]. TNF- α elevation was a major feature of the observed inflammatory responses, leading to blood–retinal barrier disruption, invasion of blood-derived monocytes along with added activation of retinal glial cells, again within 24 hours of injury in a mice model [41]. The role of retinal microglia in this progressive retinal degeneration is likely not trivial. Peripheral monocytes/macrophages do not only infiltrate into the retina after injury but also engraft permanently, causing neuroglia remodeling. This process is highly neurodegenerative as these cells continue to express major histocompatibility complex II (MHC-II), interleukin-1 beta (IL-1 β), and tumor necrosis factor alpha (TNF- α) for the long term [41]. These events aggravate the inflammatory process that injures the ganglion cells in the retina, which in turn is suspected to be a major cause of glaucoma [31, 42].

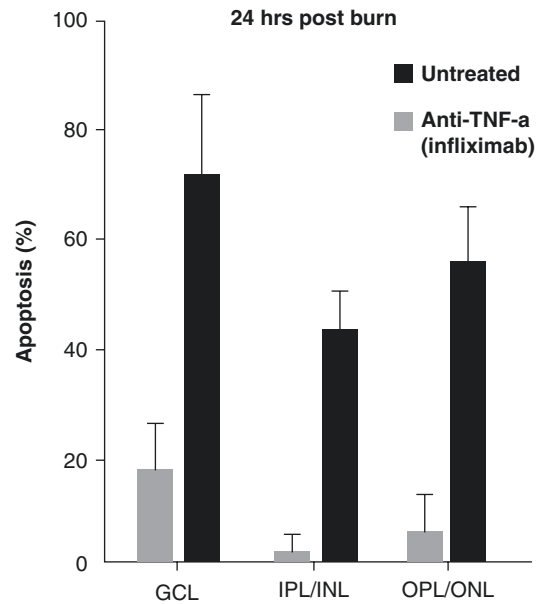


Fig. 11.5 Subsequent experiments in mice showed damage to other retinal layers as well, and a marked neuroprotective effect from infliximab (GCL ganglion cell layer, IPL inner plexiform layer, INL inner nuclear layer, OPL outer plexiform layer, ONL outer nuclear layer). (Paschalis et al. [31], with permission)

The most important outcome of these animal studies has been the protective effect of TNF- α inhibition (infliximab) on the retina, repeatedly demonstrated by our investigators [29–31]. TNF- α inhibition markedly reduces inflammation in the anterior segment of the burned eye if given promptly, and it can be assumed that less TNF- α can therefore become available to diffuse posteriorly. The TNF- α reaching the retina should be further blocked by the systemically administrated infliximab. The net result is a neuroprotective effect manifested by significantly lowering the degree of apoptosis in the retinal ganglion cell layer and thereby reducing axonal degeneration in the optic nerve [29, 31]. This interpretation is also supported by similar antiinflammatory and neuroprotective effects seen in TNF- α receptor 1 and 2 gene knockout animals [31]. Experiments were also performed using a new drug-eluting device loaded with a low dose of infliximab, placed subconjunctivally in rabbits. After an initial burst and with subsequently less than 1 μ g of the drug

released per day over a month, significant neuro-protective effect was still observed [32].

These results introduce highly promising possibilities for long-term protective treatment of patients by using TNF- α inhibitors after an accident [5]. Other TNF- α blockers, as well as antibodies against different inflammatory cytokines (IL-1 β , IL-6), may also be candidates in the future. Etanercept, another TNF- α inhibitor, has already been shown to prevent retinal ganglion cell loss in a rat model with elevated intra-ocular pressure [43]. Corticosteroids such as Kenalog (triamcinolone acetonide), injected sub Tenon, also protect against ganglion cell apoptosis (unpublished), but their complications restrict their long-term use.

Neuroprotection by prophylactically lowering the intraocular pressure to the lowest safe level after chemical burn would also be expected to be beneficial.

Parenthetically, the described retinal inflammatory mechanism may also result from other types of injury to the anterior segment as well, such as mechanical trauma, infections, and penetrating corneal surgery (keratoplasty, keratoprosthesis), etc. There may be an unmet need for long-term anti-inflammatory treatment beyond corticosteroids also in these situations. These possibilities have been the subject of considerable interest using animal experiments [30, 44–46].

Emergency Treatment

It remains obvious that lavage with any quickly obtainable fluid – usually tap water – should be attempted as rapidly as possible after a chemical exposure. Speed of action – and availability of irrigation fluid – are of the essence, whereas the exact composition of the fluid at that moment is probably of less importance. Wearing protective goggles and availability of showers in the work place or laboratory should be mandated by law.

It is also reasonable to continue lavage at the hospital emergency facility until neutral pH, by litmus test, although efficacy of this exercise is

questionable [47]. Reim and his collaborators have conducted a series of studies on fluid for irrigation, ex vivo and in patients, and they prefer tap water over physiologic saline for the lavage, if nothing else is available. However, emergency rooms are advised to be prepared to administer a borate buffer (Cederroth®) or diphoterine (Prevor®), both of which have more specific action [48, 49]. Their availability varies between countries.

The recent findings that alkali burn to the cornea can result in retina damage by diffusion posteriorly of inflammatory cytokines such as TNF- α within hours or days of the exposure (see above) have changed therapeutic priorities after chemical burns [29]. Very early apoptosis of retinal ganglion cells – the hallmark of glaucoma – is likely to explain the glaucoma manifesting even a long time later. Effective prevention against such a “time bomb” would be expected to require protective action very promptly after the accident [5]. Standard topical treatment with corticosteroids may not protect the retina sufficiently. Also, TNF- α inhibitors cannot be given before a TB test has been proven negative – which will presently require 24 hours. Therefore, it seems reasonable to augment the topical steroids with a sub Tenon injection of 40 mg Kenalog (triamcinolone) on arrival at the emergency room. After clearance of TB, Humira (adalimumab) – another TNF- α inhibitor – can be given as a 40 mg subcutaneous injection every 2 weeks until the intra-ocular inflammation has subsided. Supporting this suggestion are reports of highly favorable outcomes of treatment with TNF- α inhibitors in non-infectious uveitis patients [52–54]. If such biologics are to be used in chemical burn settings, supervision by a rheumatologist with pertinent expertise is recommended. The duration of such anti-inflammatory treatment might be guided by periodic measurement of the titer of TNF- α in blood [55].

For unclear reasons, topical corticosteroids are only moderately effective in the anterior segment after chemical burns [31] and should be tapered, beginning after a week, to avoid facilitating corneal ulceration [50].

Since glaucoma after chemical burns is so frequent and often severe, it seems reasonable to guard not only against an effect of cytokines on the ganglion cells but also against a possible IOP-related etiology as well, regardless of what pressure measurements are recorded, especially when angle closure is observed [5, 51]. We therefore recommend prophylactic pressure lowering to the lowest safe level, starting in the emergency facility. We prefer carbonic anhydrase inhibitors to glaucoma drops which can be painful and difficult to administer. One can start with acetazolamide 250 mg twice daily and gradually increase the dose to four times daily if needed. It seems reasonable to aim for an IOP level of 5–10 mm Hg. This pressure-lowering prophylaxis should probably be continued for months or years, if need be, but with observance of possible side effects.

Prophylactic anti-ulcerative treatments have been suggested (see above) but are not often employed. Doxycycline 100 mg three times daily p.o. can be given [24]. Topical medroxyprogesterone 1% 2–4 times has at least some anti-inflammatory effect [19]. Ascorbic acid and citric acid treatment have been proposed but are still controversial.

Emergency surgery is rarely necessary. In very severe, ischemic cases, Reim is proposing advancement of Tenon's capsule over the cornea – an effective treatment [56]. Amniotic membrane has received considerable popularity (Tseng and others) [57]. After a chemical burn, it can briefly protect the de-epithelialized corneal stroma from tear PMNs, but after a few days, it usually becomes digested away. Amnion in this setting is probably no more effective than a more simply applied and better retained soft contact lens (e.g. Kontur® 16 mm lens).

A glued-on contact lens effectively protects the corneal stroma from ulceration (see above) but is impractical. Prompt paracentesis and replacing aqueous with buffer [47, 58] have been suggested but are not very effective and may also come too late.

If a later corneal ulceration advances towards perforation, a surgical intervention with a total Gundersen-style conjunctival flap invariably stops further ulceration. All corneal epithelium

must have been removed and limbal sutures been employed to ensure healing. This restores an intact epithelial lining (a barrier for PMNs) and brings in blood vessels (α_2 -macroglobulin in blood has anti-collagenase properties). A flap limits vision to 20/200 or less and may require a subsequent penetrating graft (PK). A more extensive approach is a lamellar graft – riskier but may result in slightly better vision. A frank perforation needs sealing with cyanoacrylate adhesive (pioneered by Slansky [59]) or, if larger, with a therapeutic PK.

Visual Rehabilitation

Standard efforts to restore vision in cases of profound corneal opacification usually involve corneal transplantation in one of its permutations – normally PK. In the case of blindness after a chemical injury, however, PK is notoriously unsuccessful – why? In a classic study by Abel, Binder, Polack, and Kaufman in 1975 [60], a series of cases did initially very well (sometimes for months) but then gradually developed surface irregularities, vascularization, and stromal scarring, resulting in failure. In our clinical experience this sequence of events is related to the limited survival of the graft epithelium. In many cases the island of donor epithelium can be seen gradually shrinking and finally be shed off, being replaced by the (genetically?) damaged host epithelium which cannot sustain a normal, clear stroma beneath it.

A Boston keratoprosthesis (B-KPro) does much better than standard PK after a burn [61]. The “Boston Keratoprosthesis” is much less a design and materials concept and more a surgical technique and management paradigm, based on surrounding tissue biology. Thus, transparent non-toxic polymethyl methacrylate (PMMA) was introduced by industry already in the late 1940s, followed by proposals for virtually every conceivable KPro design in the 1950s, by Stone [62], Strampelli [63], Cardona and DeVoe [64], Barraquer [65], Falcinelli [66], and many others (see historical review [67]). The insight into the biological aspects (inflammation, nutrition, evaporation, infection, antimicrobial prophylaxis,

Fig. 11.6 In the chemical burns to the eye, the destructive chemicals are usually acid or alkaline. It has been long held that acid burns, in general, result in a more benign clinical outcome than after alkali burns. This is confirmed after keratoprosthesis surgery following severe burns, shown in the above diagram. (From Cade et al. [39], with permission)

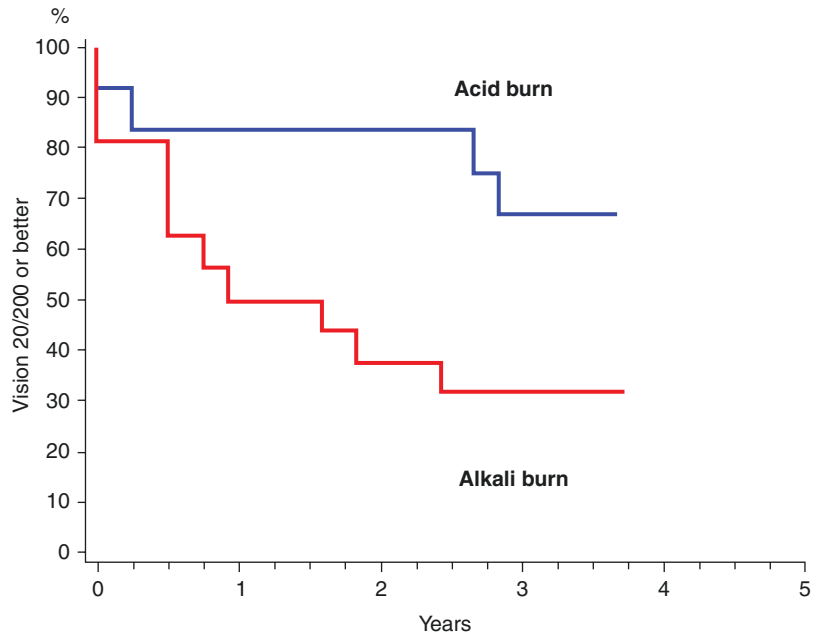
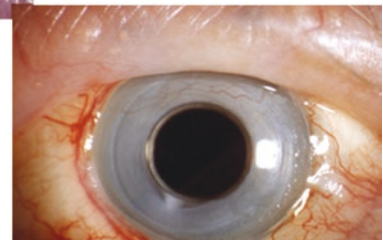
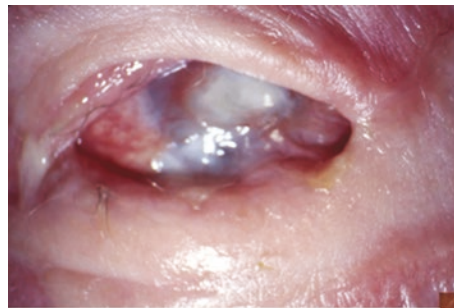


Fig. 11.7 Acid burn pre- and postoperatively. Boston keratoprosthesis Type I (early model) since 15 years. Vision 20/40 – but with advanced glaucoma. (Dohlman et al. [61], with permission)



ulceration, glaucoma, etc.) came later [61]. The B-KPro is now a standard surgical procedure with a total of over 15,000 implantations performed worldwide. The surgical technique is relatively simple, but postoperative follow-up requires regular visits and compliance with prophylactic antimicrobials, etc. [68] – which can be burdensome in countries with limited resources. There is now no question, however, that a KPro of some model is indicated in most cases of bilat-

eral corneal blindness following a chemical burn (Fig. 11.7). Thus, in a series of 28 cases with blindness from a severe chemical burn (light perception to finger counting), a B-KPro restored vision to a remarkable level of 20/300 to 20/20 in 86% of these eyes. Retention was acceptable and media clarity surprisingly good. However, pre- and postoperative glaucoma were substantial problems and a threat to long-term safety [39] (Figs. 11.6 and 11.7). This brings urgency to our

efforts to develop anti-inflammatory and neuro-protective treatments for immediate use after a chemical burn and after B-KPro surgery in the hope of forestalling later development of devastating glaucoma.

Summary

Recent clinical observations and experimental research are likely to result in improved treatment of chemical burns⁵:

1. Background: In patients with autoimmune disease (Sjögren's syndrome, Stevens-Johnson syndrome, etc.) who have had a keratoprosthesis implanted which was complicated by later corneal ulceration, intravenous administration of infliximab (antibody against TNF- α) could be remarkably effective in reducing inflammation and prevent ulceration [61, 69, 70]. These clinical observations led to a number of subsequent clinical and experimental investigations, summarized below.
2. In patients who have suffered a chemical burn to the eyes, glaucoma is very common, even with normal IOP. Following a subsequent Boston keratoprosthesis, glaucoma is even more frequent and is the major obstacle to long-term visual success [39, 40].
3. In experimental animals, a chemical burn to the cornea can lead to severe *retinal* damage as well, including ganglion cell apoptosis (the hallmark of glaucoma) and optic nerve damage [29, 31].
4. The posterior segment damage is not directly caused by pH changes. Alkali is effectively buffered at the iris-lens level and posteriorly the pH remains neutral [29, 31].
5. Rather, inflammatory cytokines such as TNF- α are generated in the anterior segment by the burn and rapidly diffuse posteriorly to reach the retina within hours or days after the accident [29, 31].
6. In addition to the ganglion cell damage, other retinal layers are affected as well. Disruption of the blood-brain barrier and invasion of blood-derived monocytes follow.

Retinal glial cells are activated, again within 24 hours of injury [31, 41].

7. Infliximab (TNF- α antibody), if given promptly after the eye burn in rabbits or mice, is markedly neuroprotective and reduces ganglion cell apoptosis [29]. (This effect supports the assumption that TNF- α is instrumental in mediating glaucoma after corneal burn). The corticosteroid triamcinolone acetonide has similar protective effect.
8. It has become very likely from these animal experiments that in order to be maximally neuroprotective against later glaucoma, the anti-inflammatory treatment should be initiated as promptly as possible after the accident [29]. Both corticosteroids (short-term) and biologics would be expected to be beneficial. However, before a TNF- α inhibitor can be administered, screening for latent TB must be done – requiring a test that at present takes 24 hours. Therefore, in the interest of speedy protection, we presently recommend a sub Tenon injection of 40 mg Kenalog (triamcinolone) on the patient's arrival to the emergency area. Standard topical steroids are indicated as well.
9. Corticosteroids are presently the clinical anti-inflammatory mainstay after chemical burn but their well-known side effects severely limit their usefulness in the chronic setting [50]. Biologics, due to their safety profile, promise to increasingly assume a steroid-sparing role. Adalimumab, another TNF- α inhibitor, is already being successfully used in chronic, non-infectious uveitis [52]. We therefore recommend that, following the initial steroid injection and TB test, subsequent TNF- α inhibition makes use of Humira (adalimumab), 40 mg injected subcutaneously every 2 weeks, as long as the TNF- α level in blood is substantially elevated. These recommendations are likely to be continually revised.
10. Chemical burns can also result in aqueous outflow obstacles (closed angle, etc.), occasionally causing IOP rise [51]. Regardless of the IOP level, however, prophylactic, long-term carbonic anhydrase inhibitors would be expected to be beneficial [5].
11. The Boston Keratoprosthesis has considerably better outcome (vision, retention) than a

standard corneal transplant, after chemical burn [39]. Surgery should be delayed until a more quiet stage has been achieved, usually 3–6 months post-accident [68].

12. In patients, it has been found that the TNF- α and TNF-R2 levels in the blood are often above normal many years after B-KPro surgery [55]. These levels of cytokines should serve as markers for sub-clinical inflammation which can result in not only glaucoma but also retinal detachment, retropsthesis membrane, vitritis, macular edema, etc. Cytokine inhibitors may be needed on a chronic base in order to assure a satisfactory postoperative outcome.
13. For long-term delivery of biologics, a drug-eluting device, to be placed subconjunctivally, has been developed [71]. In rabbits with less than 1 μ g infliximab released per day over a month, significant retinal neuro-protective effect is still observed [32].

The priorities for treatment of chemical burns are clearly changing. Corneal opacification is now less of a problem due to improving safety of a keratoprosthesis as rescue procedure. Rather, it has become clear that irreversible glaucoma is the main obstacle to long-term visual rehabilitation. Armed with these new insights and with better and more timely means for tissue protection and more effective corneal surgery, it is expected that not only chemical burns but also other severe corneal conditions and surgeries will benefit in a way that has not been possible in the past.

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Cicatrizing Disorders of the Ocular Surface

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Maintenance of corneal clarity and integrity depends upon the sustained health and homeostasis of elements that are of intimate relation to the ocular surface, including the eyelids, conjunctiva, lacrimal apparatus, and other accessory structures. Pathology involving cicatricial changes to conjunctiva has been well known to lead to blindness by way of progressive scarring and neovascular changes to cornea and, in severe cases, loss of the eye altogether. Cicatrizing conjunctivitis is characterized by chronically active inflammatory disease starting with subepithelial inflammatory cell infiltrate, later progressing to subepithelial fibrosis, foreshortening of the fornices, symblephara formation, and, finally, ankyloblephara via keratinization of lid and mucosal surfaces. This, in turn, leads to secondary complications like corneal neovascularization and scar formation, goblet cell depletion, severe ocular surface dryness, high risk of secondary infectious infiltrate, limbal stem cell deficiency, contractile entropion, trichiasis and distichiasis, canalicular stenosis, and obliteration of eyelid anatomy [1]. These conditions may arise from various local and systemic causes, making it absolutely essential to identify the etiology early to avoid not only severe vision loss but also the potential morbidity associated with these condi-

tions. Unfortunately, the wide spectrum of causes and typical delayed awareness of the severity of the process involved often lead to more advanced disease prior to the initiation of proper therapy, leading to a large percentage of these patients presenting with various blinding complications when first seen by the ophthalmologist. Also, there is an unfortunate tendency by some investigators to assume that one versus another process is at the root of inflammation without proceeding with necessary testing.

Cicatrizing conjunctivitis is caused by an extremely wide range of pathology including non-infectious or autoinflammatory, infectious, and malignant disorders (Table 12.1). Noninfectious causes include many of the blistering skin disorders, notably ocular cicatricial pemphigoid (OCP), more formally recognized as conjunctival mucous membrane pemphigoid (MMP), and linear IgA disease, which is classified as a subset of MMP [2]; severe atopic disease; severe reactive epithelial diseases such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and erythema multiforme (EM); graft versus host disease (GVHD); blepharoconjunctivitis and ocular rosacea; as well as other disorders such as sarcoid, lichen planus, discoid lupus, and secondary (i.e., medication, trauma, radiation) induced inflammation. Infectious etiologies can include ocular trachoma, *Corynebacterium diphtheriae*, adenovirus, and herpetic disease. Masquerade disorders, such as squamous metaplasia or carcinoma, must also be kept in mind. Table 12.1 illustrates a list of

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Table 12.1 Differential diagnosis of cicatrizing ocular surface disorders

<i>Noninfectious</i>
Blistering skin disorders
Cicatricial pemphigoid (MMP/OCP)
Linear IgA disease
Bullous pemphigoid
Epidermolysis bullosa acquisita
Atopic disease
Vernal keratoconjunctivitis
Atopic keratoconjunctivitis
Stevens-Johnson syndrome
Toxic epidermal necrolysis
Erythema multiforme
Graft versus host disease
Ocular rosacea/blepharoconjunctivitis
Sarcoidosis
Lichen planus
Discoid lupus
Systemic sclerosis
Medication-induced
Trauma or radiation
<i>Masquerade syndromes</i>
Squamous cell carcinoma/carcinoma in situ
Mucosa-associated lymphoid tissue lymphoma
Sebaceous cell carcinoma
<i>Infectious</i>
Trachoma
<i>Corynebacterium diphtheriae</i>
Adenovirus
Herpetic disease

these and other disorders that can be associated with cicatricial changes of the ocular surface. With appropriately aggressive investigation including fully comprehensive history taking and review of systems, thorough medical examination, and targeted laboratory testing, one may be able to narrow the broad spectrum of possible causes, even in early stages of disease.

Of note, some of the important topics included in this discussion are covered more thoroughly in other sections of this manuscript; thus focus will not be put on these here.

Epidemiology

Occurrences of diseases of this type are likely underappreciated, as some go unrecognized in their early forms, only to be categorized once the

more significant findings of scarring or other secondary complications are seen. OCP is often regarded or reported as the most common cause of presenting cicatricial changes [3]. Early attempts to estimate prevalence place it anywhere from 1 in 8000 to 60,000 [4–7] although it is also noted that this likely represents only progressed cases, with some stating it impossible to accurately assess [7]. Reports generally show the disease has a mean age onset in the sixth to seventh decade [1, 8, 9] but may occur much earlier and has a female predilection of 2:1 or more with no geographic or racial bias noted [1, 8, 10]. Linear IgA is rare, occurring in only a few per million individuals per year [11].

Atopic disease is common, with various forms affecting upwards of an estimated one-fourth of the US population [12], but severe forms such as atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC) are sparse. AKC can occur at almost any age, generally reported more in males age 30–50 [13, 14]. VKC occurs more commonly in males in warm climates, a severe but typically self-limiting disease manifesting and resolving in the first two decades in 89% in a large series with long-term follow-up [15]. Thankfully, SJS and TEN are rare, with incidence estimates at only a few cases per million occurring yearly [16].

GVHD is by convention uncommon, manifesting as a complication of allogeneic hematopoietic cell transplantation (HCT). Incidence rates vary widely in both acute (19–90%) and chronic forms (33–80%) depending on age, HLA-matching, and product given [17, 18], with lower rates seen in recipients with better donor parity and those receiving cord blood versus peripheral blood stem cells (PBSC) or bone marrow [19].

Rosacea is seen more commonly in lighter-pigmented populations with prevalence reported from 2–22% [20–23], and speculation of bias toward more easily distinguished facial flushing in this group. Ocular involvement is reported in ranges from 10 to over 50% [24–26], most commonly reported around the fifth decade with a strong preponderance of females [26, 27]. Keratopathy may occur due to several associated

mechanisms; however, the disease severe enough to progress to the level of cicatrizing changes is infrequent [28].

The exact prevalence of sarcoidosis is unknown despite many attempts to classify this, largely due to its occult nature in asymptomatic patients. Screening methods using lung imaging studies have concluded it in the range of 1–50 in 100,000 individuals; however, autopsy studies have estimated it to be much larger in the range of 320–640 per 100,000 [29]. Eye findings are more common in females.

Infectious causes of ocular inflammation tend to be more prevalent in developing regions, primarily in areas of lower socioeconomic status. Ocular trachoma is thought to cause vision impairment in 2.2 million individuals worldwide with over half of these being permanently affected, many being women and children, with cicatricial disease occurring more often in middle age [30].

Diphtheria is much less common in the USA now than in the early twentieth century prior to immunization, and there are recent years where no cases were reported. However, some endemic areas remain with reduced burden compared to prior decades, particularly in the newly independent states formed after the dissolution of the Soviet Union in the 1990s [31].

Adenovirus is an extremely common form of infectious conjunctivitis, typically self-limiting; however, the severe epidemic form is seen much less often, the degree of which is unknown as most cases are managed by local providers and not reported or recognized. Other forms of viral conjunctivitis include those due to the herpes simplex group, which more often causes corneal scarring via a primary keratitis rather than cicatrizing inflammation followed by secondary corneal changes.

Masquerade conditions may mimic, and, in some cases, be very difficult to distinguish from noninfectious inflammatory disease such as OCP, especially when less commonly found in the forniceal rather than bulbar conjunctiva – the former would be more likely to appear like pemphigoid. Squamous cell carcinoma or intraepithelial neoplasia may present in various stages and affect

conjunctiva in this manner and is the most common malignancy of conjunctiva occurring in less than 0.02–3.5 per 100,000 yearly in the USA, most often seen around the fifth to sixth decades of life [32]. Incidence is thought to be higher in areas of greater sun exposure as in equatorial regions.

Common Ocular features

In earlier stages, the features of diseases leading to cicatrix are typically indistinguishable from more common forms of conjunctivitis, and may even remain occult for years, disguised as chronic or stubborn “dry eye” in many clinics. Early diagnosis is rare and difficult to come by, often occurring because of clinical suspicion due to other systemic signs or symptoms or by chance in the case of conjunctival resection done for other reasons, i.e., superior resection to treat superior limbic keratoconjunctivitis. There may also be mixed presentations of chronic diseases, and it may be impossible to determine if the disease processes occurred independently or if one was brought on by another.

Patients usually complain of redness, irritation, foreign body sensation, dryness, burning, and light sensitivity. It is usually of indolent onset, many patients regarding the condition as a minor nuisance at first, dealing with symptoms and treating on an as needed basis until symptoms persist to the point of frustration or are significant enough to warrant concern and desire to be evaluated. Early findings include injection and conjunctival thickening, possibly involving a diffuse papillary reaction seen as worse in the superior or inferior palpebral conjunctiva. Large follicles may form and corneal neovascularization may eventually appear. Other differing early features may also be present depending on the etiology, such as bullae, hemorrhage, ulceration, or pseudomembrane formation [33]. Irritation and symptoms may be present in the absence of clinically active appearing disease and may be due to subclinical inflammation but is often concomitant dryness occurring secondarily.

Scarring first appears in the form of lacy white subepithelial fibrotic strands that coalesce and contract, causing a buckling of adjacent conjunctiva into ridges. These changes are often found in a perivascular distribution and in the inferior fornices [1, 34]; however, it is not uncommon for the bulbar and inferior forniceal surfaces to appear fairly uninvolved in a patient who is exquisitely symptomatic but then to evert the lids to find tarsal conjunctiva with significant injection and cicatrix formation, often asymmetric. Progression of disease leads to loss of peripheral architecture, including foreshortening of fornices and shallowing of canthal recesses, at times with obliteration of plica and caruncle. Various stages and depths of forniceal foreshortening may be present. Symblephara or fibrous bands occurring between bulbar and palpebral surfaces may form at any time, including prior to foreshortening, often occult but becoming more prominent when one retracts the lid in the opposite direction of a patient's gaze, superiorly or inferiorly. Ankyloblepharon formation is the hallmark of advanced disease, "end game" in most cases, where fusion of lid to bulbar surface has occurred, typically with marked keratinization of the ocular surface that remains visible, many times involving adhesions from lid to cornea (Fig. 12.1).

Secondary complications of scar formation can be just as damaging, all presenting a threat to sustained corneal clarity. As mentioned above, associated dryness is the most common presenting complication, occurring with loss of goblet cells or irreversible damage to the lacrimal apparatus, including main or accessory salivary glands and ducts. Contraction of fibrosis may cause infolding or irregularity of adnexal borders or lamellae leading to entropion as well as lash follicle misdirection or trichiasis, both leading to constant abrasive scraping of lashes against an already inflamed and irritated cornea. This along with lagophthalmos and exposure may be present, together contributing to worsening corneal neovascularization, limbal stem cell deficiency, and then possible central ulceration, secondary infectious processes, or perforation. There are even suggestions that cicatrix formation in some disease may cause susceptibility to ocular hypertension or glaucoma via failure of normal limbal structures involved in aqueous outflow [35]. Lastly, therapeutic doses of corticosteroid vis-à-vis drops, pills, injectables, or intravenous administration can also predispose to conjunctival thinning or masking of poorly controlled disease, which may be seen to advance despite a lack of injection on examination.

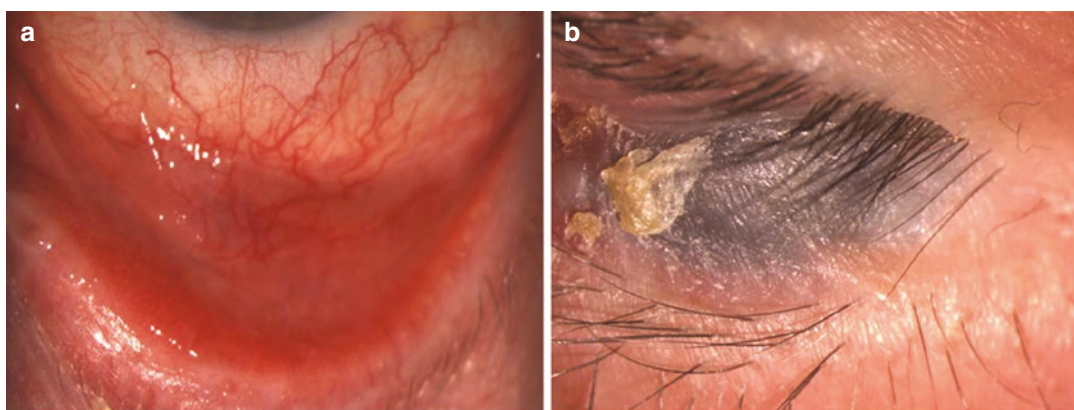


Fig. 12.1 Common ocular features of cicatrizing conjunctivitis including (a) subepithelial fibrosis, forniceal foreshortening, symblephara, and (b) ankyloblepharon (with complete corneal keratinization)

Distinguishing Features and Pathophysiology

While the abovementioned entities may lead to a similar phenotypic presentation, they are, in fact, quite distinct, stemming from differences in pathogenesis and systemic involvement. In some cases the diagnosis may be obvious; however, findings are often only first discovered after symptoms may have been present for several years. Management varies widely, and a thorough evaluation and attempt to definitively identify the causative disorder is crucial given the significant morbidity of delayed diagnosis or poor or misguided therapeutic approach. It must also be considered that more than one offending entity may be present, co-conspiring in scar formation (Fig. 12.2).

Ocular Cicatricial Pemphigoid/ Mucous Membrane Pemphigoid

Patients with MMP present with blistering inflammation and ulceration of mucosal tissues, most commonly oral cavity [36], but also naso-

pharynx, esophagus, trachea, and vagina. Cutaneous lesions are less commonly found [1, 9], and it is debated whether this may be an isolated presenting feature (Brunsting-Perry variant) versus a localized variant of another blistering disease, bullous pemphigoid [37, 38], which infrequently affects conjunctiva. Questioning for oral and nasal blisters or hemorrhage, vaginal irritation, hoarseness, heartburn, dysphagia, and respiratory distress is critical; diagnostic screening via endoscopy for nasopharyngeal, esophageal, and tracheal involvement may detect significant active disease even in asymptomatic patients. A consensus of international experts published accepted definitions and outcome measures in an attempt to more reliably compare data from differing studies and meta-analyses in this field; however, it was noted there is not a consensus on how to stage ocular involvement of MMP [39]. Currently, two staging systems are widely referred to for this, including the Foster system [1, 40], which stages disease by progression of clinical signs of cicatrix formation, and the Mondino and Brown system [41], which stages disease by loss of inferior forniceal depth or foreshortening (Table 12.2).

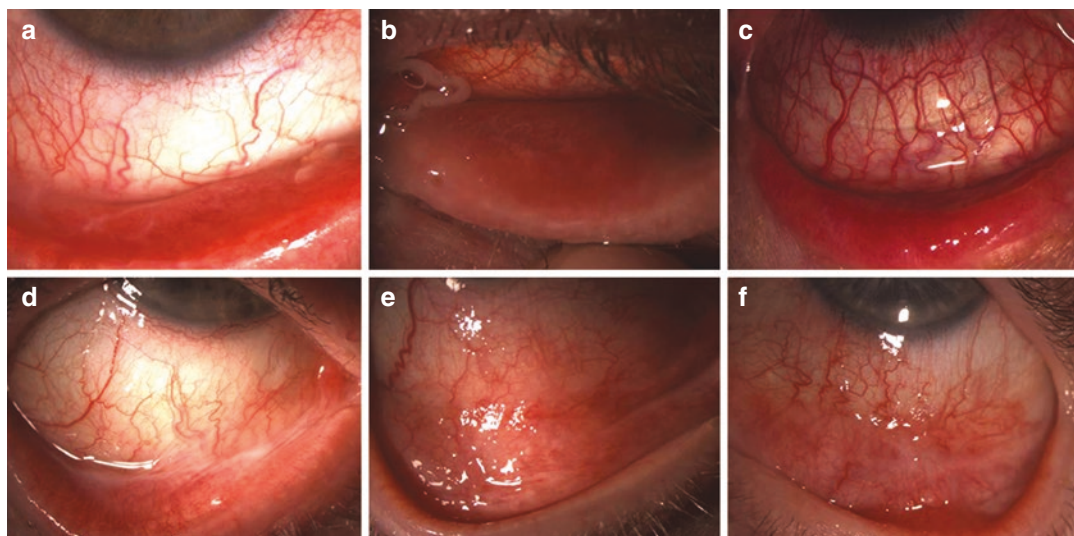


Fig. 12.2 Chronic cicatrizing conjunctivitis manifesting from (a) ocular cicatricial pemphigoid, (b) atopic keratoconjunctivitis, (c) Stevens-Johnson syndrome, (d) graft

versus host disease, (e) sarcoidosis, and (f) squamous cell carcinoma in situ

Table 12.2 Staging systems for ocular cicatricial pemphigoid

	Foster	Mondino and Brown
Stage 1	Subepithelial fibrosis	0–25% forniceal foreshortening
Stage 2	Forniceal foreshortening	25–50% forniceal foreshortening
Stage 3	Symblepharon formation	50–75% forniceal foreshortening
Stage 4	Ankyloblepharon formation	75–100% forniceal foreshortening

Inflammation originates within the lamina lucida of the basement membrane zone of mucosal epithelium, with antibodies mostly directed against the cytoplasmic domain of the beta-4 subunit of hemidesmosome integrin [42–44], inciting complement activation and recruitment of varying types of inflammatory infiltrate (including plasma cells, lymphocytes, macrophages, neutrophils, eosinophils) in both acute and chronic diseases. Other targets have also been suggested, including laminin 332 (previously described as laminin 5, epiligrin, kalinin, nicein/BM600), found in disease which presents with pathology indistinguishable from classic OCP but is highly associated with an increased relative risk of malignancies [45]. Several inflammatory markers are elevated in OCP patients such as tumor necrosis factor- α (TNF- α), interleukin-1 α (IL-1 α) and - β (IL-1 β) [46], and interleukin-5 (IL-5) [47], while tumor growth factor- β (TGF- β) 1 and 3 levels are elevated in conjunctival samples [48]. Patients with the HLA-DQB1*0301 allele have a demonstrated increased risk of disease, thought possibly to bind to the beta-4 subunit [49]. It is not completely understood how fibrosis is induced specifically here, but it has been shown that conjunctival fibroblasts from OCP patients possess a “profibrotic phenotype” compared to controls [50].

Other blistering diseases, including bullous pemphigoid and epidermolysis bullosa acquisita, also involve inflammation resulting from immune signals interacting with various targets in the hemidesmosome, such as BP180 and collagen VII. Conjunctival involvement is much less frequent in these than MMP. They can usually be

clinically distinguished via systemic findings, mostly large or diffuse skin lesions not usually seen in MMP. The final diagnosis may depend on biopsy of affected tissues.

One must also keep in mind the possibility of multiple processes contributing to stubborn or uncontrolled chronic cicatricial disease. A closed-minded provider may miss these, only to struggle in the “unsuccessful treatment” of one problem while actually plagued with the persistence of another. One such example is coexistence of atopic disease with MMP, as in the recent report from our group where 230 consecutive biopsy-proven pemphigoid patients were studied with 33 patients having clinical symptoms of atopic disease (eczema, asthma, hay fever) and 23 patients with evidence of atopy suggested in biopsy specimens, who later successfully responded to antiallergy medications or infusion therapy directed against both diseases [51]. We have also found, in our clinical experience, that evidence of OCP may be suggested via biopsy in individuals with other known disease, i.e., SJS or GVHD, perhaps via epitope spreading, and that these patients may respond to appropriate anti-inflammatory therapy for pemphigoid while their other disease remains inactive. It would seem this may be the mechanism by which OCP, responding to immunosuppressive therapy, is also sometimes found in developmental disorders such as ectodermal dysplasia [52]. Infectious co-conspirators must also be remembered.

Atopic Disease

Systemic manifestations of atopic disease are more obvious, typically making determination of the cause of ocular surface scarring less difficult. Seasonal and perennial allergic conjunctivitis do not present with scarring; however the more severe processes vernal keratoconjunctivitis (VKC) and particularly atopic keratoconjunctivitis (AKC) can result in fibrosis and neovascular changes in cornea, with potential permanent irreversible vision loss.

Itching is the most common and suggestive symptom of allergy, though patients also

complain of burning, intermittent blurring, dryness, light sensitivity, lid fullness, and tearing. Early on, congested papillary, or “boggy,” conjunctiva commonly found in either superior (more VKC) or inferior (more AKC) tarsal surfaces and fornices or including bulbar surfaces is seen. Thick ropy mucous, which patients often describe pulling out in strings in the “mucous fishing syndrome,” is found often in vernal catarrh, and this activity can exacerbate disease. Darkened or scaly periorbital epithelium is often present in AKC as is the characteristic nasal crease from repetitive upward nasal rubbing or wiping, termed the “allergic salute.” Corneal findings include shield ulcers (VKC), Horner-Trantas dots (VKC more than AKC) and neovascular pannus, as well as secondary complications of ulceration and infection. Chronic disease may progress to cicatrizing changes in the fornices and eventually the bulbar surface. Allergic rhinitis and hay fever are often seen, and there is a high association of eczema or allergic dermatitis and asthma with both VKC [53] and AKC [54]. Presentation and demographic commonly help differentiate VKC and AKC, with VKC occurring more in young males in warmer tropic climates.

Mast cells are activated and seen in higher numbers in all allergies. They are strikingly present in conjunctival epithelium on light microscopy of biopsy specimens of VKC and AKC, often degranulating alongside eosinophils, neither seen in normal conjunctiva [55, 56]. Fibroblasts and collagen are found in greater abundance, which may stem from a large percentage of mast cells releasing basic fibroblast growth factor (bFGF) [57] and which may lend to the incidence of cicatrix formation by a mechanism as yet still poorly understood. Epithelial hyperplasia with pseudotubule formation is commonly seen. Mechanisms of both Gell and Coombs type I and IV hypersensitivity drive inflammation, with a varied leukocytotic infiltrate and severe ocular surface and corneal damage eventuated by the eosinophil-derived enzymes major basic protein (MBP) and eosinophil cationic protein (ECP). Tears and serum of both patient types contain elevated levels of IgE, and in AKC, ECP, among other important factors

in this process. Eosinophils, as well as MBP and ECP, have been isolated in corneal ulceration in AKC but also under intact corneal epithelium [58]. More recent studies are looking into the role of the epithelial nuclear protein interleukin-33 (IL-33), which is upregulated in conjunctiva in AKC and may mediate disease [59].

Stevens-Johnson Syndrome, Toxic Epidermal Necrolysis, Erythema Multiforme

These disorders present acutely with distinct findings that are almost never confused with other diseases in this section. Studies including histopathology and serologic evaluation are limited due to the nature of disease; however, those done typically disclose very severe diffuse blistering disease involving skin and mucous membranes, involving microangiopathy, thought to be induced by various medications or infectious processes. One should be mindful, however, of the possibility of a concomitant disease process occurring later on after disease has clearly passed through the acute stages. More on this can be found in the dedicated section in this manuscript.

Graft Versus Host Disease

GVHD occurs in a select population of individuals who have typically received allogeneic HCT for hematologic malignancies and benign disorders and thus is relatively rare. Both acute and chronic stages have been described, which are proposed to have distinct mechanisms of actions. Acute disease models are based mainly on animal studies, while chronic disease is less well understood with hypotheses that are more speculative. In contrast to the proposed 3-phase “cytokine storm” [60] of acute disease consisting of antigen-presenting cell (APC) conditioning, T-cell activation, and target cell apoptosis, chronic GVHD is thought to result from complex development of improper thymic function whereby autoreactive T-cells are no longer

removed. Chronic disease often resembles features of autoimmune disorders; it occurs less often in T-cell depleted marrow recipients [61] and is associated with decreased levels and function of T_{reg} lymphocytes [62].

Target antigens of donor T-cell-mediated inflammation in the acute stages mainly involve recipient skin, gastrointestinal organs, and liver but may also involve lung, oral mucosa, and the eye. Classic symptoms of acute disease include maculopapular rash, nausea, anorexia, diarrhea, cholestasis but may only be conjunctivitis [63]. Various stages of acute and chronic disease have been previously proposed, with more advanced disease involving pseudomembranous and epithelial sloughing in both, but cicatricial changes occur more frequently in chronic disease, along with the common complication of significant keratoconjunctivitis sicca via various secondary mechanisms. Of course, the most agreed upon risk for chronic GVHD is having had acute GVHD. Co-conspiring mechanisms may also include concurrent autoimmune disease, such as OCP, evidence of which we personally have seen in our own conjunctival biopsies taken from chronic GVHD patients.

Ocular Rosacea, Blepharoconjunctivitis

Rosacea and blepharoconjunctivitis are common presenting findings in patients with cicatrizing ocular surface changes, however, are not usually attributed as the direct cause. It may even be that such patients attributed to have this as the cause of their cicatrizing disease may actually have an unrecognized secondary inflammatory etiology, such as atopy, MMP (perhaps with “negative” biopsy), or adverse reaction to topical medications. On top of this, secondary processes often stemming from or associated with lid margin disease, such as phlyctenular keratoconjunctivitis, staphylococcal marginal disease or keratitis, trichiasis and distichiasis, or Demodex infestation, may contribute to corneal changes.

Rosacea is generally classified into four disorders based on morphological characteristics:

erythematotelangiectatic, papulopustular, phymatous, and ocular [64]. Cicatrizing changes are uncommon but were found in one study to be associated with rosacea in MMP-negative biopsies in 12 of 131 patients referred for evaluation of cicatrizing disease [28]. Findings include erythema, telangiectases, papules, and pustules in areas around the face and neck, including the lid margins. Fibrosis is commonly associated with the phymatous form of rosacea, but there may be overlap in these categories in nature. It stands to reason that cicatrizing disease should only be thought attributable to rosacea when other etiologies have been ruled out via thorough history and conjunctival biopsy.

The exact mechanism of this disease is unknown; however, it is felt to be a product of innate immune system or neurovascular dysregulation triggered by one or more of various factors in a genetically susceptible individual [65, 66]. Multiple cell types are thought to be involved, and there is evidence that intercellular adhesion molecule-1 (ICAM-1), transient receptor potential (TRP) ion channels, toll-like receptors (TLRs), and even the angiogenic CD105 (endoglin) may be involved [67]. Innate immune response cascades to epithelial microbial challenge may produce proinflammatory cytokines and antimicrobial peptides such as cathelicidin; the latter is seen to be elevated in rosacea patients, and a cleavage product of this (LL-37) is felt to contribute to all subtypes of rosacea [68]. Type IV hypersensitivity is also thought to be involved [69].

Other Noninfectious Disorders

Several other noninfectious disorders may present with cicatrizing changes of the ocular surface, including sarcoid, lichen planus, discoid lupus, malignancies, as well as those changes induced by medication, trauma, or radiation. Some of these processes are described more in other sections.

Sarcoidosis is a diagnosis typically made by clinical suspicion supported by granulomatous changes seen on biopsied tissues of involved

organs. Systemic features include fever, arthralgia, cough, dyspnea, weakness, and fatigue; however, many patients are asymptomatic. Ocular findings are present in 20–80% of cases [70], and of several possible presenting findings, the most likely is anterior uveitis [71]. Chest imaging with highly suspicious findings in the proper clinical setting will often be enough to make the diagnosis, though biopsy is always more valuable to confirming diagnosis if it is possible to attain.

Lichen planus may present identical to OCP; however, biopsy will reveal absence of immunoreactant deposition and a shaggy appearance of fibrinogen at the BMZ [72]. Etiology is unclear, and it more commonly affects other tissues such as genital or oral mucosa.

Discoid lupus is also a rare cause of conjunctival cicatrix formation, more often affecting oral or anogenital mucosa. An accompanying diagnosis of lupus may already be known, and serologic investigation may reveal typical findings such as antinuclear antibodies (ANA), anti-Ro (SS-A), anti-La (SS-B), and anti-double-stranded DNA (dsDNA) antibodies. Biopsy may show thickening, disruption, or duplication of the BMZ on immunofluorescence studies [73, 74].

Drug-related conjunctivitis is also addressed thoroughly elsewhere in this manuscript but is among the most common reasons for cicatrizing disease of the ocular surface.

As discussed earlier, malignancies have been associated with a less common variant of MMP [45]. Primary malignancies involving conjunctiva may also mimic and sometimes be indistinguishable clinically from, or present concomitantly with, MMP [75]. However, in squamous cell carcinoma and conjunctival intraepithelial neoplasia, histologic evaluation will reveal dysplasia in various degrees of severity, with or without invasion of stromal structures beyond the BMZ. Human papillomavirus (HPV) types 16 and 18 [76] is strongly associated with lesions, and other entities including human immunodeficiency virus (HIV), cyclosporine, and xeroderma pigmentosum have also been implicated in the pathogenesis of CIN. Lesions do not necessarily have to originate at the commonly appreciated site of the limbus. Corkscrew

vessels, leukoplakia, and invasion to underlying tissue may make the diagnosis more suspicious. Exfoliative or impression cytology may be performed, as may excisional biopsy in some cases, helping to yield the diagnosis.

Trachoma

Infectious etiologies, as a cause for cicatrizing disease, are much less common in the developed world, but elsewhere still account for a significant amount of vision loss worldwide due to conjunctival scarring and eventual secondary corneal pathology. Transmission of *Chlamydia trachomatis*, an obligate intracellular gram-negative organism, is typically spread to the eye via hand or genital contact from infected individuals, and when suspected, both chlamydial and gonococcal (commonly coinfectious) urine cultures should be obtained. Differing serotypes are associated with types of infection – serotypes A to C are associated with ocular trachoma, serotypes D to K with genitourinary infections, and L1 to L3 with lymphogranuloma venereum. Two components, comprised of the inactive yet infectious elementary body and active yet noninfectious reticulate body, make up the organism. Chronic inflammation results, reinfection is common due to the inability of immune surveillance to eradicate the pathogen completely, then leading to scarring of conjunctiva and cornea.

Active chlamydial conjunctivitis presents in stages, which have been classified in various schemes. The World Health Organization (WHO) scheme is the most recent [77] and documents the development of follicular conjunctivitis in the upper tarsus, which worsens with thickening and eventual fibrosis, trichiasis, and corneal opacification. Both innate and cell-mediated immune responses are thought to contribute to cicatrix formation. Th2 lymphocytes may be more important in scar formation, and increased cytokine formation (IL-1 β , TNF- α , TGF- β) has been associated with scarring as well [78]. Histopathology in acute childhood disease reveals epithelial hyperplasia with mixed inflammatory infiltrate involving macrophages, T lymphocytes, and

neutrophils, with plasma cells also being found beneath epithelium and around accessory lacrimal glands. Lymphoid follicles are found in stromal tissue. Cicatricial disease, more frequently seen in adults, is formed by chronic inflammatory infiltrate with squamous metaplasia or atrophy, T lymphocyte predominance, follicles, and subepithelial fibrous membranes [79]. Various available lab tests as well as culture using cycloheximide-treated McCoy cells can also determine the diagnosis.

Corynebacterium diphtheriae

Acute membranous conjunctivitis due to *C. diphtheriae*, a gram-positive bacillus primarily affecting respiratory mucosa, is extremely rare in the USA due to wide and effective immunization. Localized generation of a true membrane from organism, necrotic mucosa, and fibrin is seen which is adherent to underlying stroma and leads to hemorrhage when removed. Epithelial denudation leads to worsening infiltrate and eventual corneal opacification [80]. Systemically, hematogenous spread of exotoxin may cause fever, myocarditis, renal tubular necrosis, and neurodemylination.

Adenovirus

Adenovirus is the most common cause of infectious red eye and conjunctivitis worldwide [81]. Highly contagious via transmission by fomites, water, and fecal oral contamination, human adenovirus types 8, 19, 37, and 54 are thought to be responsible for most outbreaks of epidemic keratoconjunctivitis (EKC) [82]. Adenovirus is a nonenveloped double-stranded DNA virus which penetrates ocular surface epithelial cells via binding to cell surface integrins, incorporating into vesicles, and eventually releasing viral DNA into cell nucleus. Both innate and cell-mediated immune mechanisms respond to viral infection involving natural killer cells, monocytes and macrophages, as well as Th1 and cyto-

toxic T lymphocytes. The virus may then lyse the cell, releasing virions that may go on to infect other cells.

Clinical presentation of EKC includes extreme versions of typical follicular conjunctivitis findings, including superficial punctate keratitis and, classically, pseudomembrane formation. However, it has been demonstrated that early angiogenesis and associated chemokine formation are present in these membranes, which may in turn lead to true membrane formation, subepithelial fibrosis, and eventual symblephara [83]. An accompanying lymphadenopathy and pharyngitis may be present. Infiltration of virus into corneal epithelial and, eventually, stromal cells leads to accumulation of lymphocytes, macrophages, and activated fibroblasts [84]. There is no gold standard testing performed in clinical settings; however, some rapid detection kits exist and are used with a significant degree of efficacy [85]. Polymerase chain reaction analysis of infected tears may also confirm viral DNA is present.

Herpetic Disease

A wide variety of pathology may present due to *Herpesviridae* viruses, including conjunctival, corneal, uveal, retinal, optic nerve, and orbital manifestations. The contributions of this family of infectious entities to ocular surface disease are addressed in another section in this manuscript.

Approach to Diagnosis: Conjunctival Biopsy

Perhaps the most important thing to take from this section is the concept of proper approach to diagnostic biopsy, particularly, in MMP, where too often we hear that conjunctival biopsy yield in the diagnosis is poor or inconclusive. Patients may also not respond as expected to therapy when biopsy is performed with limited analyses, yielding the so-called DIF-negative MMP that is then treated as MMP. This likely stems from various deficiencies in the diagnostic approach

taken. Previous thoughts on why this is so have ranged from feelings that antibody levels are low or undetectable, or that DIF in conjunctival specimens is lower than cutaneous samples, or even that there may be a subset of patient who develop T-cell-mediated disease without detectable auto-antibody at the BMZ [86–88]. It has even been stated that some have abandoned the use of biopsy in routine management of these patients, rationalized by the notion that all other possible confounding etiologies are always somehow able to be ruled out by other means and that DIF-negative cases would be treated the same way regardless of this using immunosuppressive therapy [89]. It is fair to say, and we would agree, that cases of DIF-negative MMP exist that should be, due to strong clinical suspicion, labeled and treated as MMP despite recommendations by the First International Consensus on Mucous Membrane Pemphigoid [2]. Strangely absent from these discussions, however, despite previously published recommendations [1], is mention of other more likely reasons for why diagnostic yield may be poor.

Easily correctable is the surgical approach. Attention should be given toward a “one-touch” technique with careful handling thereafter of specimen, in an effort to prevent disturbance of the very fragile conjunctival epithelium – epithelium is easily disrupted or displaced from underlying stromal tissue, thus rendering the sample uninterpretable with respect to BMZ. Biopsy should also ideally be taken in an area of or directly next to inflamed or affected tissue, as one might expect a lesser yield during quiescence via disease inactivity or suppression of inflammation by steroid or other anti-inflammatory therapy.

Ideal processing of the specimen has previously been described [1], yet despite common practice we do not wish to discount the existence of an unusually fair amount of biopsied tissue, as seen in our experience done by other providers prior to presentation in our lab, for which DIF was not performed at all, with only histologic evaluation performed under microscopy. DIF, of course, is the standard, during

which a characteristic brilliant pattern of IgG in stromal cells is commonly seen, which can also make the sample uninterpretable by way of the inability to recognize and distinguish a distinct linear deposition of IgG at the BMZ. Lastly, there is almost a frank unwillingness to even acknowledge the exceedingly more sensitive approach to detection of autoantibodies or complement at the BMZ by way of avidin-biotin complex immunoperoxidase technique. Admittedly, this is more labor intensive and costly than DIF, but is approximately 1000 times more sensitive, and may detect the suspected low amount of immunoreactant deposition more readily, in addition to other possible confounding features (i.e., observation of high levels of IgE possibly implicating co-conspiracy of atopic disease) (Fig. 12.3).

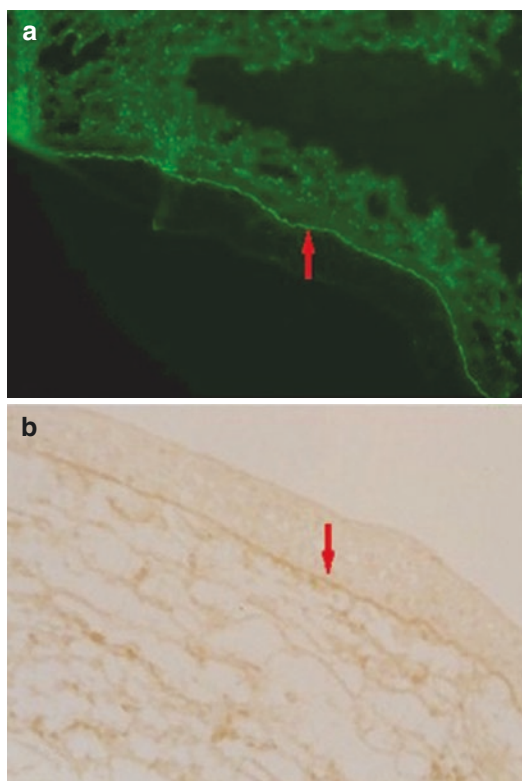


Fig. 12.3 Conjunctival biopsy showing distinct immuno-reactant positivity at the BMZ (red arrows) via use of (a) direct immunofluorescence and (b) avidin-biotin complex immunoperoxidase technique

Medical Management

The goals of treatment in the cicatrizing disorders of the conjunctiva are first, to abort inflammation and prevent further tissue damage; second, to offer symptomatic relief; and third, to improve the ocular surface microenvironment and to restore corneal clarity in order to improve vision. While the treatment of infectious causes is more straightforward, that for the autoimmune disorders is not often clear-cut and may call upon clinical experience. Therapeutic decisions are often made on a case-by-case basis. In OCP, acute inflammation is addressed with topical and systemic corticosteroids, but its inevitable relapse-remission cycle will ultimately result in disease progression if only such therapy is implemented. Thus, the authors advocate for a step-ladder approach of steroid-sparing agents. It has been clearly demonstrated by many investigators that systemic immunomodulatory therapy can effectively diminish the tissue-destructive processes caused by inflammation in patients with OCP, in turn saving vision and ocular function [90–93]. In milder cases of OCP, dapsone 50–100 mg daily may offer inflammatory control. However, the majority of cases encountered at our practice are recalcitrant to dapsone alone. If there is no contraindication, methotrexate (MTX) is frequently our first-line therapy for OCP, starting at a dose of 10–15 mg weekly. The dose can be escalated up to 25–30 mg weekly, and it may be switched to subcutaneous administration to increase bioavailability. The two other antimetabolites commonly employed in the treatment of OCP are azathioprine (AZA, 100–250 mg daily) and mycophenolate mofetil (MMF, 1–1.5 g twice daily, which must be taken on an empty stomach). Calcineurin inhibitors such as cyclosporine and tacrolimus are not typically used in OCP as the disease is predominantly B lymphocyte driven. TNF- α inhibitors such as adalimumab and infliximab, while very effective in other ocular inflammatory diseases including uveitis, scleritis, and retinal vasculitis, are not typically used in OCP. In fact, TNF- α inhibitors have been associated with drug-induced bullous pemphigoid in patients with rheumatoid arthritis,

psoriasis, and ulcerative colitis [94–97]. However, there have been reports of these medications being successful in treating refractory MMP or OCP in the literature [98–100], and we have had some personal success with this as well. On the other hand, rituximab, a B-cell-specific biologic, has proven very effective in inducing remission in OCP [101]. When conventional immunosuppressive therapy fails to bring forth disease remission, the authors recommend a combination of rituximab and intravenous immunoglobulin (IVIg), which is described elsewhere [102]. Finally, oral or intravenous pulse cyclophosphamide is employed in OCP cases not responsive to aforementioned medications, though it carries a higher systemic risk profile.

In severe atopic diseases, topical antihistamine/mast cell stabilizers and corticosteroids are frequently insufficient, and chronic systemic corticosteroids carry inappropriate long-term risks. Tacrolimus dermatologic ointment is available in 0.03% and 0.1% concentrations and is used for periocular dermatitis. Ophthalmic topical preparations of cyclosporine A (up to 1%) and tacrolimus (0.1%) can be specially formulated and may offer relief in less recalcitrant cases. Our experience in severe atopic cases, however, is that systemic immunomodulatory therapy is necessary for long-term inflammatory control without propagation of tissue damage. Although antimetabolites (MTX, AZA, MMF) have demonstrated variable efficacy in atopic diseases, we prefer T-cell inhibition via cyclosporine A (3–5 mg/kg/day divided dose) or tacrolimus (0.1–0.2 mg/kg/day divided dose). Of note, atopic patients have reduced innate immunity and are more susceptible to infections. Hence, concurrent bacterial or herpetic keratitis must be kept in mind and treated appropriately when ocular surface inflammation presents in these patients. Omalizumab, a monoclonal anti-IgE antibody approved by the FDA for allergic asthma, has demonstrated efficacy in AKC and VKC in several reports [103–105]. Monthly infusion of AK002 (Allakos Inc., San Carlos, CA), a fully humanized monoclonal antibody that targets Siglet-8, which is an inhibitory receptor on the surface of mast cells, eosinophils, and some basophils, is currently under investigation in the treatment of a variety of

diseases driven by mast cells and eosinophils, including eosinophilic gastroenteritis, indolent systemic mastocytosis, chronic urticaria, and severe allergic conjunctivitis.

Although the ocular surface inflammation is generally regarded as acute in SJS, we have through the years encountered patients whose inflammation became chronic, with progressive conjunctival cicatrix and corneal blindness the result. Conjunctival biopsy reveals an OCP-like picture, with IgG, IgA, and complement deposition at the basement membrane zone. In these cases, we recommend a therapeutic stepladder as outlined above. The only significant difference is that TNF- α inhibition appears more effective in these “chronic” SJS cases than in OCP.

An effective treatment strategy for severe rosacea blepharoconjunctivitis must implement both topical and systemic measures. We recommend lid hygiene employing effective (lasting, wet compresses typically cool down too quickly) warm compresses and manual expression of meibum, artificial tears containing lipids, and omega-3 supplementation as the foundation of the therapy. Topical tetracycline and cyclosporine can then be used. Short-term corticosteroids are appropriate in acute keratitis to prevent scarring and neovascularization, but long-term topical corticosteroids must be avoided. A specially formulated povidone-dimethylsulfoxide ointment was recently shown to be an effective topical therapeutic for rosacea blepharoconjunctivitis [106]. In the USA, the only FDA-approved oral therapy for rosacea is a modified-release doxycycline (40 mg daily), which has only anti-inflammatory and no antimicrobial properties, and in vivo studies demonstrated no long-term effects on the normal body flora at this dose [107]. However, in severe cases of ocular rosacea resulting in conjunctival cicatrix and corneal neovascularization, we find it necessary to use doxycycline at a higher dose of 50–100 mg twice daily, tapered gradually over a course of at least 6 months. In the pediatric population under the age of 8, oral erythromycin is a first-line therapy and is recommended at a dose of 40 mg/kg/day given over 6 months for moderate rosacea and 12 months for severe cases [108].

Demodex is a parasite that normally lives on the human skin and does not usually cause dermatological issues. However, when it penetrates the dermis, various skin manifestations, including rosacea, can occur. Its prevalence is higher in patients who are elderly or who have poor hygiene and may represent an important cause of chronic blepharoconjunctivitis in patients who fail standard rosacea treatment. A careful examination of eyelashes must be conducted to look for the mites. The treatment for Demodex blepharitis involves improvement in hygiene (washing bed sheets and pillow cases in hot water and drying in high heat), lid scrub with baby shampoo or commercially available cleaning pads, and hypochlorous acid/tea tree oil/shampoo.

Surgical Management

As mentioned above, secondary complications of conjunctival cicatrix can be just as threatening to the corneal integrity and clarity, and trichiasis due to cicatricial entropion must be addressed promptly. This can be done with mechanical epilation with forceps on a regular basis. Epilation can also be accomplished with electrolysis, radiofrequency wave, and argon laser, although these procedures may result in permanent lid scarring and do not necessarily prevent hair regrowth. While cryotherapy has been used for large, confluent areas of trichiasis, it is inappropriate in inflammatory conjunctival cicatrizing disorders as it can worsen symblepharon and entropion. We normally recommend at least 6 months to 1 year of disease quiescence, employing both topical and systemic agents, before entropion surgery, or any significant anterior segment surgery, in fact, is attempted. In patients with aggressive OCP that requires alkylator or biologic therapies, a longer period of inflammatory disease inactivity is desirable, and the clinician must be prepared for postsurgical flare even in the setting of a quiescent eye preoperatively. We recommend perioperative high-dose oral prednisone, starting 1 week before surgery, and tapered after surgery based on degree of postoperative inflammation. What is often striking, and

must be kept in mind by the treating physician in any OCP patient undergoing surgery, is the possible lack of conjunctival inflammation after surgery yet with eventual complete breakdown of corneal epithelium. In these cases, bandage contact lens or amniotic membrane with or without temporary tarsorrhaphy, along with intravenous corticosteroids, may be employed to abort the inflammation.

Once corneal blindness occurs, keratoplasty is required to restore vision. Anterior segment inflammatory disease is a risk factor for corneal graft rejection, owing to increased cytokine production, upregulated expression of HLA-DR in the graft, and facilitation of lymphocytic migration via increased expression of adhesion molecules [109]. Also, penetrating keratoplasty (PKP) alone is associated with poor visual outcomes in the setting of limbal stem cell deficiency. However, limbal stem cell allograft has a better survival rate, with proper systemic and topical immunosuppression, in other causes of conjunctival cicatrix such as chemical injury and AKC [110]. There are various approaches to limbal stem cell transplants, and the details are described elsewhere in this text and the literature.

Therefore, in the majority of cicatrizing disorders that lead to corneal blindness, especially OCP, atopic, SJS, and herpetic diseases, PKP has a lower long-term success rate and worse visual outcomes, in comparison to other indications such as pseudophakic bullous keratopathy and keratoconus. If one were to attempt PKP in the cicatrizing disorders, we recommend achieving disease quiescence with systemic therapy for at least several months (and possibly 1 year or longer in OCP), along with appropriate perioperative systemic and topical corticosteroids. These patients often require a keratoprosthesis (KPro) (Fig. 12.4) rather than a primary PKP in our experience, but this may represent a bias in our tertiary referral center. In patients who still have reasonable tear production and lid function, as well as fornix enough to retain the absolutely required bandage contact lens, we use the Boston KPro type 1 exclusively, because of the relative ease of its insertion and good retention rate. Of course, the viability of the keratoprosthesis is

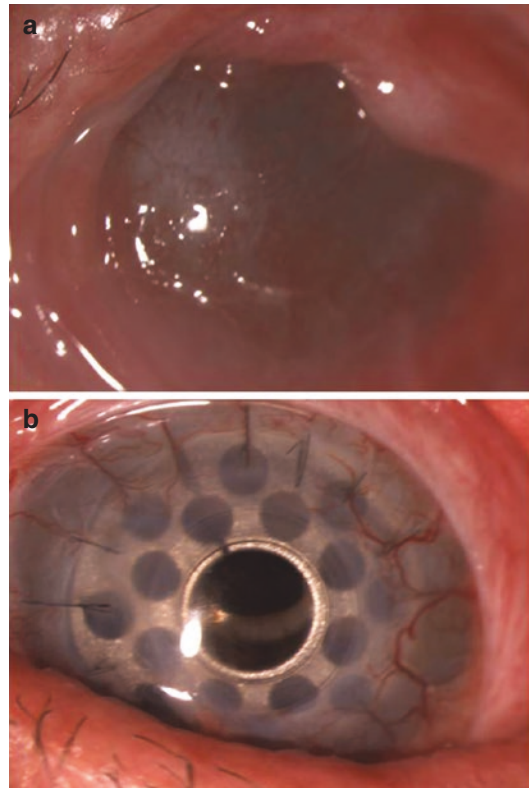


Fig. 12.4 Severe keratopathy, with thick keratosymblephara and neovascularization, in stage 3 OCP (a) requiring keratoprosthesis (b) for visual rehabilitation

highly contingent upon the eye's inflammatory status and ocular surface health. Unfortunately, even when the surgery is successful and keratoprosthesis retained, the long-term visual outcome is suboptimal, with only 11% of the eyes achieving 20/60 vision [111]. A study at the Massachusetts Eye and Ear Infirmary examining KPro type 1 in OCP patients offers an even grimmer picture: only 1 of the 8 eyes had vision of 20/200 or better after a mean follow-up of 3.2 years [112]. Therefore, keratoprosthesis remains a last resort in our practice, being offered only to patients who are on the verge of bilateral blindness. The osteo-odonto-keratoprosthesis (OOKP) and the Boston KPro type 2 may restore vision in those with advanced ocular surface disease, characterized by little to no tear production and poor lid closure, but these procedures are performed by at a limited number of centers in the USA and worldwide.

Final Words

Cicatrizing disorders of the ocular surface can present a tremendous challenge to the ophthalmologist both in diagnosis and in management thereafter. It must be emphasized again that not all cicatrizing diseases of the ocular surface, when one fails to recognize the disease process via more definitive diagnostic means, are necessarily going to be cicatricial pemphigoid. A good understanding of the possible causes of this presentation in patients, and the ability to discern when more than one process may be at work, is key in the successful approach to management, especially when disease is particularly severe. The approach to successful management is contingent upon a thoughtful and open-minded yet appropriately aggressive approach, with action taken to preferably avoid secondary complications if possible by optimizing the ocular surface vis-à-vis successful treatment of systemic inflammatory disease as well as accompanying dryness, lid disease, and limbal stem cell deficiency. It is by these means that corneal opacification and neovascular changes may be avoided, which are the lasting and blinding results of uncontrolled cicatricial disease of the ocular surface. Lastly, as these diseases are often systemic in nature, the provider must recognize and address all suspected extraocular manifestations, which in time may present greater morbidity than ocular disease.

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Part III

Surgery & Alternatives to Surgery



Kenneth R. Kenyon, Kathryn M. Hatch,
and Roberto Pineda

Introduction

With deference to *The Band*: “In the winter of ’65, we were hungry, just barely alive.” (*The Night they drove Old Dixie down*, 1969). A scant century later in 1965, still hungry, having weathered college in Boston, and now commencing med school at Johns Hopkins in Baltimore, I (KRK) was serendipitously invited to join the Wilmer-based lab team of the post-doc who had patiently shepherded my undergraduate thesis. Soon thereafter, my lifetime first surgical exposure occurred when summoned to the Wilmer OR by the renowned Professor A E Maumenee as he performed a PK (penetrating keratoplasty) on a teenage boy with densely opaque corneas. After completing trephination, the surgeon literally dropped the corneal disc into my waiting fixative and bid me to “go study this with your electron microscope.” Proceeding as directed, the immediate result was the initial ultrastructural description of congenital

hereditary corneal dystrophy (CHED) [1], while the long-term consequence became my fascination with corneal surgery. The Maumenee keratoplasty, by the way, was altogether up to date, securing with 10-0 Ethilon nylon, a cornea manually trephined from a whole donor eye, aided by a Zeiss operating microscope.

Fast-forward another decade, and I was again instructed by Ed Maumenee to take the Boston cornea fellowship with Dr. Claes H. Dohlman (CHD) and company. Thus the summer of 1976 was especially highlighted by keratoplasty on a patient of Claes whose multiple graft rejections necessitated a tissue matched cornea from, as it happened, Baltimore, where corneal preservation in tissue culture media had become standard. However, when confronted intraoperatively with the corneo-scleral donor cap but without a compatible trephination block, I rapidly retrieved the required Teflon punch block from my Cornea Service stash. As the patient was already under general anesthesia, the punch block was rapidly sterilized and rinsed, and with the certainty of recent Wilmer experience, I deftly punched the precious donor tissue, only to recoil in horror as the pristine cornea disc immediately assumed the consistency and transparency of a potato chip! The Teflon block, although not perceptibly warm to surgical-glove-insulated hands, was nonetheless sufficiently hot to literally cook the cornea! This was my first encounter with CHD’s somewhat guttural throat clearing, then as now, a

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uniform indicator of his dismay if not displeasure. Undaunted, with co-fellow Steve Foster, also witness to the catastrophe, we took this crispy cornea to the Eye Research Institute, performed ultrastructure on the tissue and thermal absorption studies on the block. Proudly offering our manuscript to Claes in anticipation of his coauthorship, his throaty response coupled with “I don’t think so” again conveyed tacit dissent. Of course, “Damn the torpedoes...,” Steve and I published nonetheless [2].

The Ghost of Keratoplasty Past : 1975–2000

The era of the late 1970s and 1980s, when I was privileged to direct the MEEI Cornea Service, offered a Periclean Age. Apart from the overarching inspiration of then Harvard Department Chair and Player-Coach CHD, the enormity of contributing colleagues included Deborah Langston, the Herpes Queen; Dick Thoft, self-styled Ocular Surface King; and Steve Foster, Creator of Corneal Immunology, plus scores of high energy clinical and research fellows who ultimately flew higher and faster than us all. Keratoplasty, although remaining almost exclusively penetrating (Fig. 13.1), was advancing on many fronts: Eye banking uniformly involved donor tissue uniformly preserved in tissue culture medium

(Fig. 13.2), extending viability from two days to two weeks, and increasingly networked tissue sharing to eliminate interminable locally limited waiting lists. As refractive surgery progressed out of incisional infancy, the simplistic notion that a clear corneal graft was a success, irrespective of



Fig. 13.2 Eye Banking, circa 1975. With transition from enucleated whole eye donor preparation to storage of the excised corneo-sclera in tissue culture medium (TC-199 as shown), viability of donor cornea tissue (at 4 degrees Centigrade) was greatly extended from 2 days to 2 weeks, thereby facilitating tissue sharing and elective surgical scheduling as well as eye bank networking, both regionally and internationally

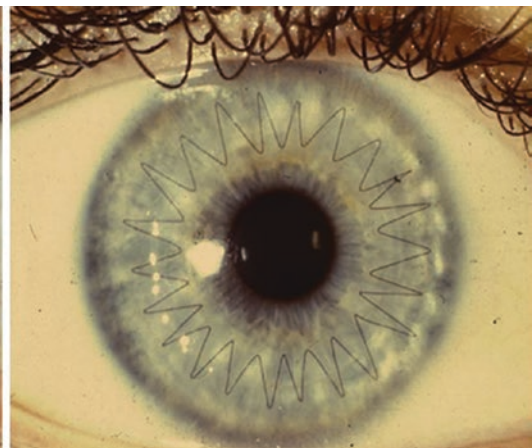
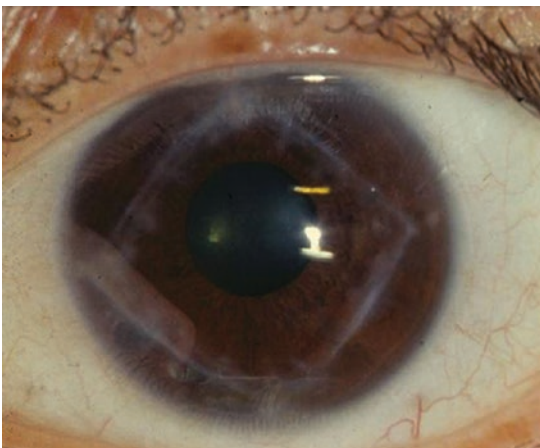


Fig. 13.1 Evolution of the PK. Left: Square Castroviejo-style graft, vintage 1960. Still clear 40 years later! Right: PK sutured with 24-bite 10-0 nylon running suture for accelerated visual recovery and astigmatic adjustment capability

refractive and astigmatic issues, became more visually sensitized by development of topographic instrumentation. Transplantation immunology advanced at the basic level but remained slow in clinical translation as even large-scale clinical trials of HLA matching seemed of too limited cost/benefit to warrant broad adaptation, although advances in topical and systemic immunosuppression extended the survival of high-risk keratoplasty (Fig. 13.3). Importantly, as phacoemulsification improved throughout the 1980s, IOL materials and designs processed from anterior to posterior chamber location although the unanticipated tsunami of pseudophakic corneal edema consequent to closed loop anterior chamber IOLs busied keratoplasters for more than a decade with PK plus IOL exchanges (preferably with PC IOL replacement) [3] (Fig. 13.4).

During this period, contemporaneous developments in three distinct but related areas particularly served to enhance keratoplasty outcomes:

First, anterior segment reconstruction techniques evolved from the strategies of ocular trauma and cataract surgical complications. The penetrating keratoplasty itself greatly facilitated open sky access to performing iris- or scleral-fixated PC IOL. Mechanical and/or visco dissection of irido-corneal synechiae with iridoplasty closure recreated a central pupil and reduced adhesion recurrence with secondary glaucoma [4]. Such anatomically and visually restored eyes enjoyed long-term graft survival [5] (Figs. 13.5, 13.6, 13.7 and 13.8).

Second, extending Thoft's conjunctival autograft insights and influenced by identification of limbal basal epithelium as stem cells of the corneal epithelium in the early 1980s, we initiated limbal autograft transplantation, predominantly for unilaterally chemically burned eyes [6] (Fig. 13.9). As grafted limbal stem cells (LSCs) became "the gifts that keep on giving," definitive and permanent restoration of the ocular surface,

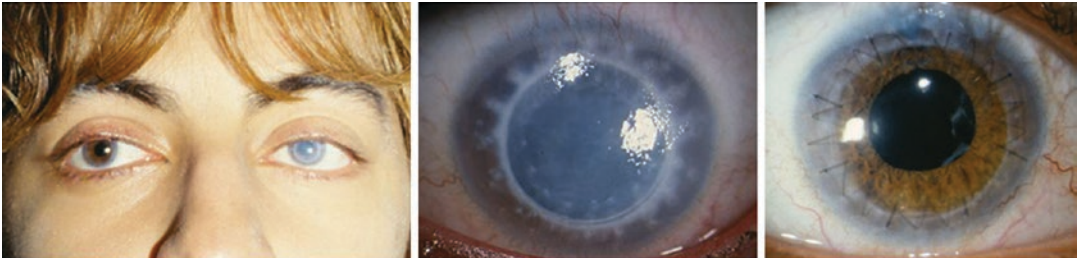


Fig. 13.3 Immunosuppression for high-risk keratoplasty. This 29-year-old woman with CHED (left) had undergone 3 penetrating keratoplasties in each eye, all rejected (middle). In 2005, PK no. 4 was performed for each eye while

utilizing oral prednisone, cyclosporine, and mycophenolate (right). At 10-year follow-up, both grafts remained clear while utilizing only topical corticosteroid

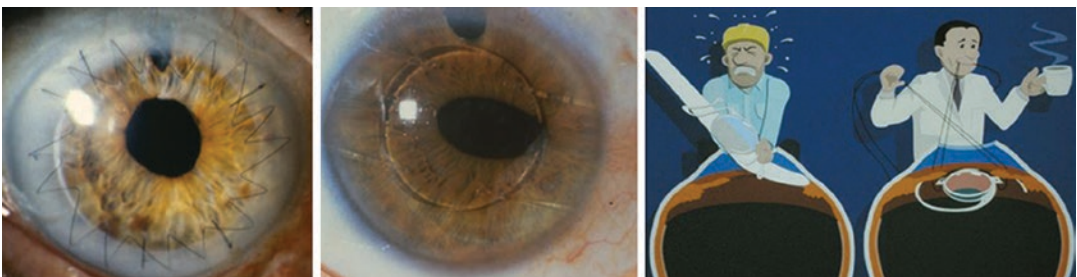


Fig. 13.4 PK and IOL exchange. During the 1980s and 1990s, the use of closed loop anterior chamber IOLs became a leading cause of corneal and macular edema (left). Penetrating keratoplasty and IOL exchange, here

with iris-sutured posterior chamber IOL, was frequently required (middle). Although use of a flexible haptic anterior chamber IOL remained an option, a comparative clinical trial [3] favored the posterior chamber IOL (right)

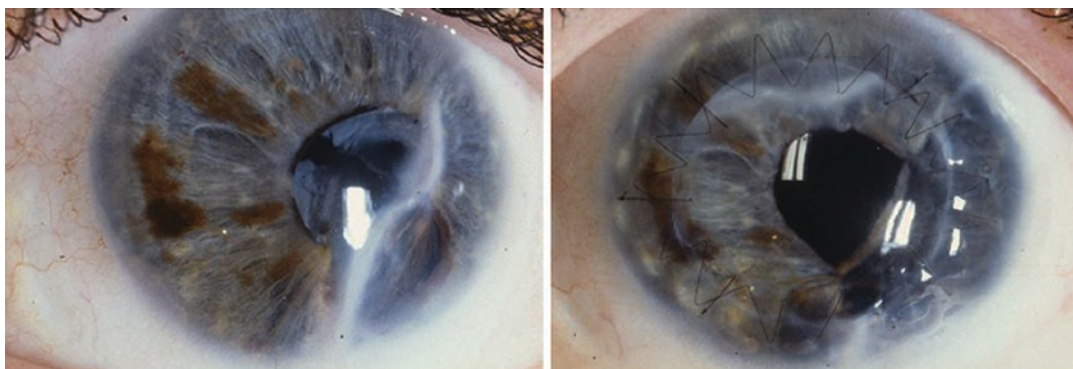


Fig. 13.5 Anterior segment reconstruction: Rotating autograft. Left: Following corneal laceration although the central cornea remains clear, the linear stromal scar, irido-corneal adhesion and iris tears, lens remnants, and vitreous prolapse must be managed. Right: A rotating corneal

autograft repositioned the scar superiorly beneath the upper lid. Removal of cataract remnants, iris-sutured posterior chamber IOL, synechiolysis and iridoplasty completed anatomical restoration and visual rehabilitation while retaining the patient's own cornea



Fig. 13.6 Anterior segment reconstruction: Synechiolysis. Top left and middle: In the presence of irido-corneal synechiae and iris ruptures, excision of the corneal disc is carefully performed with Vannas scissors to avoid further iris damage and to retain iris tissue segments for recon-

struction. Bottom left and right: A dry cellulose surgical sponge is useful to reduce iris synechiae from the cornea and angle, supporting the keratoplasty wound margin anteriorly while depressing the adherent iris posteriorly

thereby reducing corneal conjunctivalization, inflammation, neovascularization and ulceration greatly improved keratoplasty prognosis, especially if anterior lamellar keratoplasty could be performed to obviate endothelial rejection risk (Fig. 13.10). Subsequent extensions of this fundamental concept have included limbal allograft transplantation, mini-limbal biopsies expanded in tissue culture on various substrate membranes, simplified limbal epithelial transplantation

(SLET) (Fig. 13.11), and nasal or oral mucous membrane transplantation [7]. Although the requirement for concomitant immunosuppressive therapy complicates management of limbal allograft variants for bilateral LSC-deficient cases (Fig. 13.12), advances in immunotherapy have improved outcomes in such “desperate times call for desperate measures” situations. The certain to be forthcoming adaptation of pluripotent mesenchymal or other stem cells via either

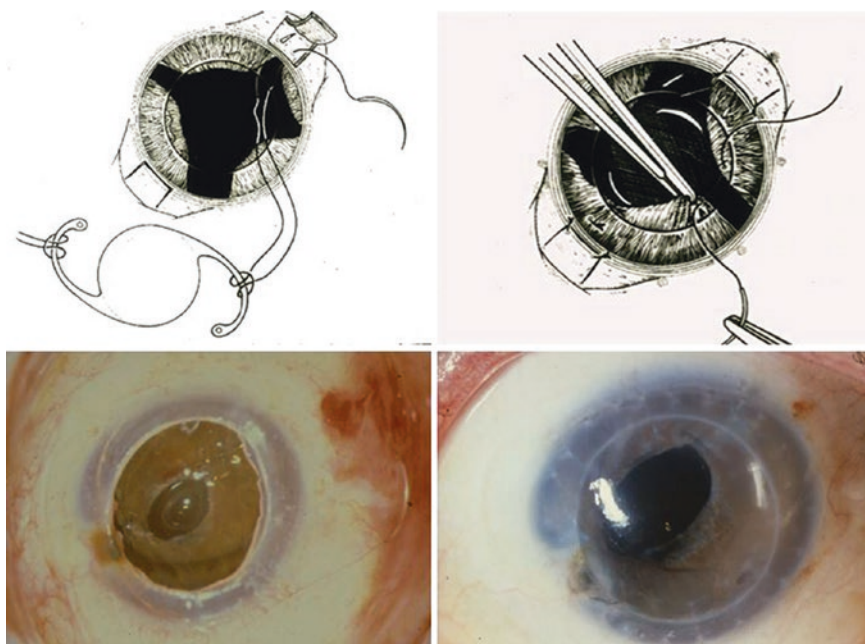


Fig. 13.7 Anterior segment reconstruction: secondary IOL and iridoplasty. Top left: The posterior chamber IOL is secured by sutures (9-0 or 10-0 polypropylene or Goretex) attached to IOL haptics and buried beneath partial thickness scleral flaps. Top right and bottom left:

Iridoplasty utilizes multiple interrupted sutures of 10-0 nylon or polypropylene. Bottom right: 3 years postoperatively, the PK remains clear, anterior chamber deep, pupil round and central, IOL stable, and intraocular pressure normal

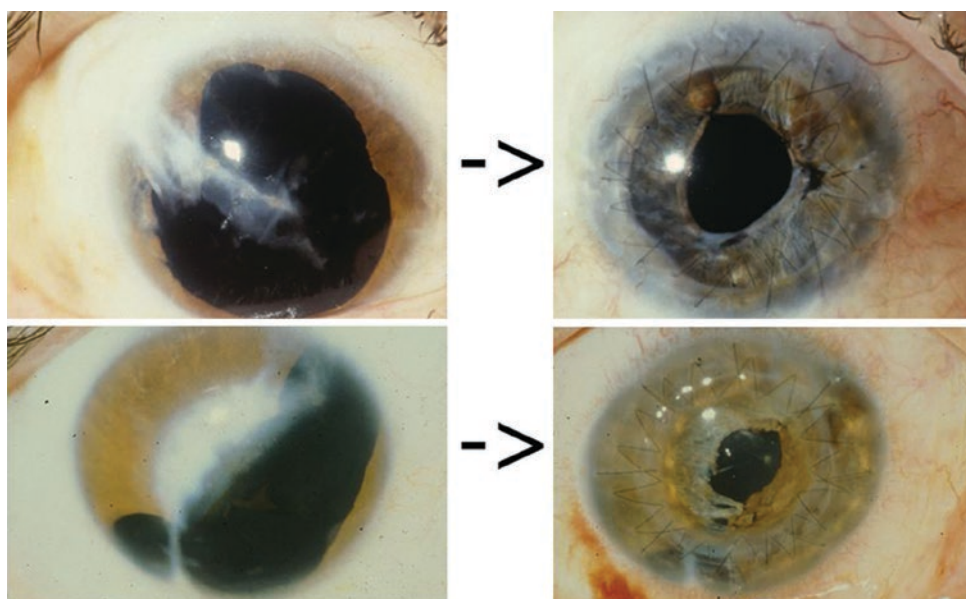


Fig. 13.8 Anterior segment reconstruction: selected cases. Top left: Monocular auto accident survivor with corneal scar, extensive iris rupture, and aphakia. Top right: Elaborated iris remnants recreate a central pupil in conjunction with PK and posterior chamber IOL. Bottom left:

Seaman sustained extensive corneal and iris laceration but remarkably no lens injury. Bottom right: PK included vitreous decompression to deepen anterior chamber, whereupon 3.00–6.00 iris remnant could be released and sutured to restore central pupil while still retaining clear lens!

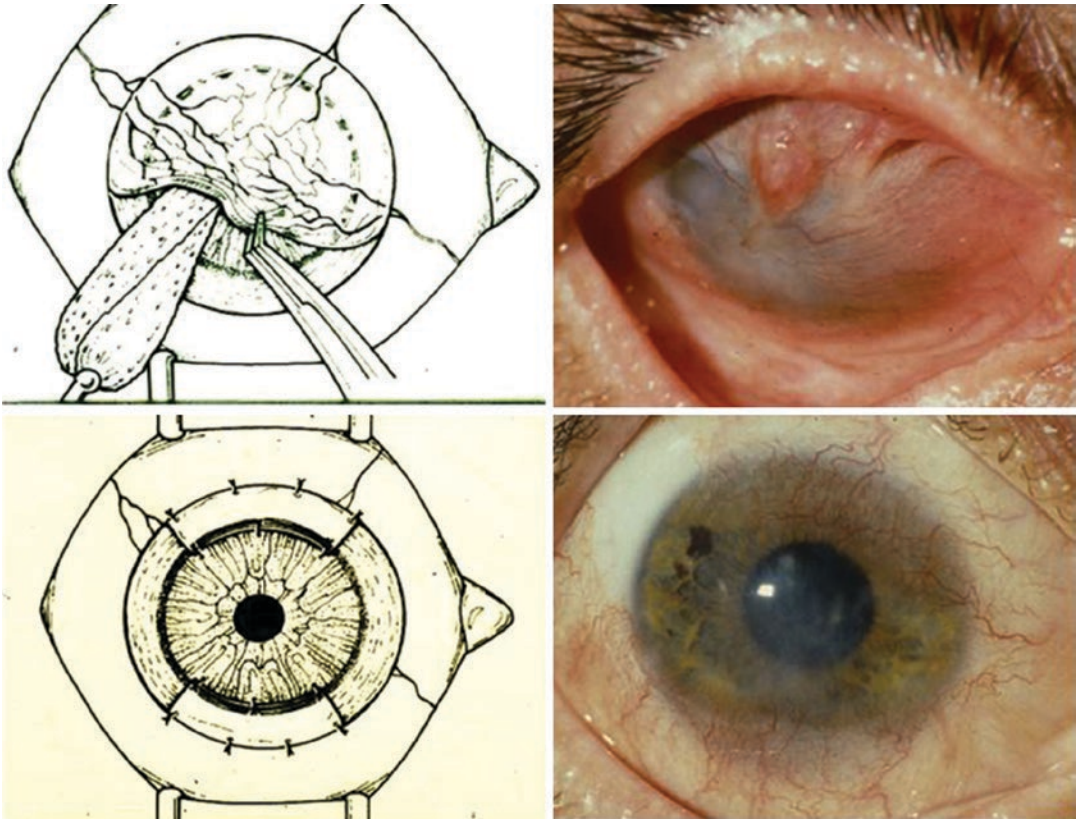


Fig. 13.9 Ocular surface reconstruction: limbal autograft for chemical injury. Top left: Following peritomy, the fibrovascular pannus is peeled by superficial keratectomy. Bottom left: Two limbal strips from donor eye are transferred superiorly and inferiorly. Top right: Following

chemical assault, vision is reduced to hand motions, as dense pannus with superior symblepharon have developed. Bottom right: Following limbal autograft alone, vision recovers to 20/60 and cosmesis is improved such that keratoplasty is not required



Fig. 13.10 Ocular surface reconstruction: limbal autograft and lamellar keratoplasty. Left: In 1986, this 6-year-old boy sustained severe chemical injury OS by alkaline bathroom cleaner. Middle: Following limbal autograft, cornea is stable without conjunctivalization. Right: 1 year

following deep anterior lamellar keratoplasty (DALK), vision is 20/40 as cornea is clear and avascular. This patient returned for more than 20-year follow-up with continuing stable visual and anatomical outcome, attesting to longevity of transferred limbal stem cells

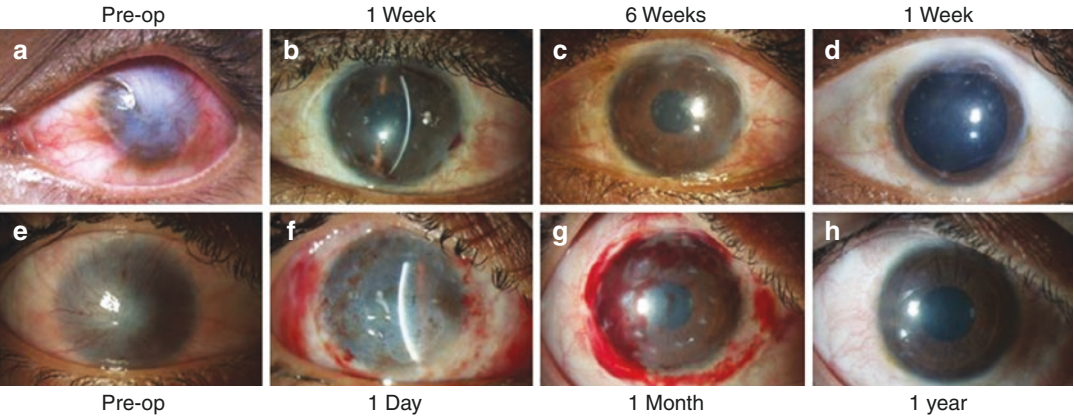


Fig. 13.11 Ocular surface reconstruction: simplified epithelial limbal transplantation (SLET). Two ocular chemical injury cases with 1-year postoperative follow-up

demonstrate remarkable ocular surface and visual recovery. (Reproduced courtesy of V Sangwan and S Basu)



Fig. 13.12 Ocular surface reconstruction: limbal allograft. Top left: A 32-year-old woman with Stevens-Johnson Syndrome and active corneal neovascularization. Bottom left: Following limbal transplant from identical twin sister, corneal surface is restored. Top right: A

64-year-old man following bilateral chemical injury. Bottom right: After limbal graft from brother, followed by PK, cataract + IOL and iridoplasty, corneal clarity improved. Patient lived 30 years thereafter with adequate functional vision

direct or cultured application should soon render ocular surface restoration routine.

Third, by the mid-1990s, the role of amnion membrane both as a collagen matrix membrane but especially for its intrinsic epi- and neurotrophic growth factors, anti-inflammatory substances and neovascular inhibitors afforded a paradigm shift in the management of persistent epithelial defects, neurotrophic keratopathy and conjunctival replacement, among multiple other ocular surface applications. Various preservation and application methods have been devised by Tseng and others [8], and with respect to keratoplasty, adjunctive amnion applications for limbal transplantation and for neurotrophic corneas have greatly improved these otherwise poor surgical prognosis situations [9] (Figs. 13.13 and 13.14).

Back to the Future: The Keratoplasty Revolution of Selective Lamellar Keratoplasty: 2000–Present

For the half century since initiation of modern corneal transplantation, keratoplasty almost invariably meant penetrating keratoplasty. Although increasingly successful, the common risks and comorbidities of PK, such as graft failure or rejection, glaucoma, cataract, high or irregular astigmatism, begged for less invasive and vulnerable techniques to improve vision, speed recovery and reduce complications. Despite even earlier interest in lamellar keratoplasty (LK), dating to von Hippel in 1888, the technical difficulties and imperfect visual outcomes limited its adaptation even for kerato-

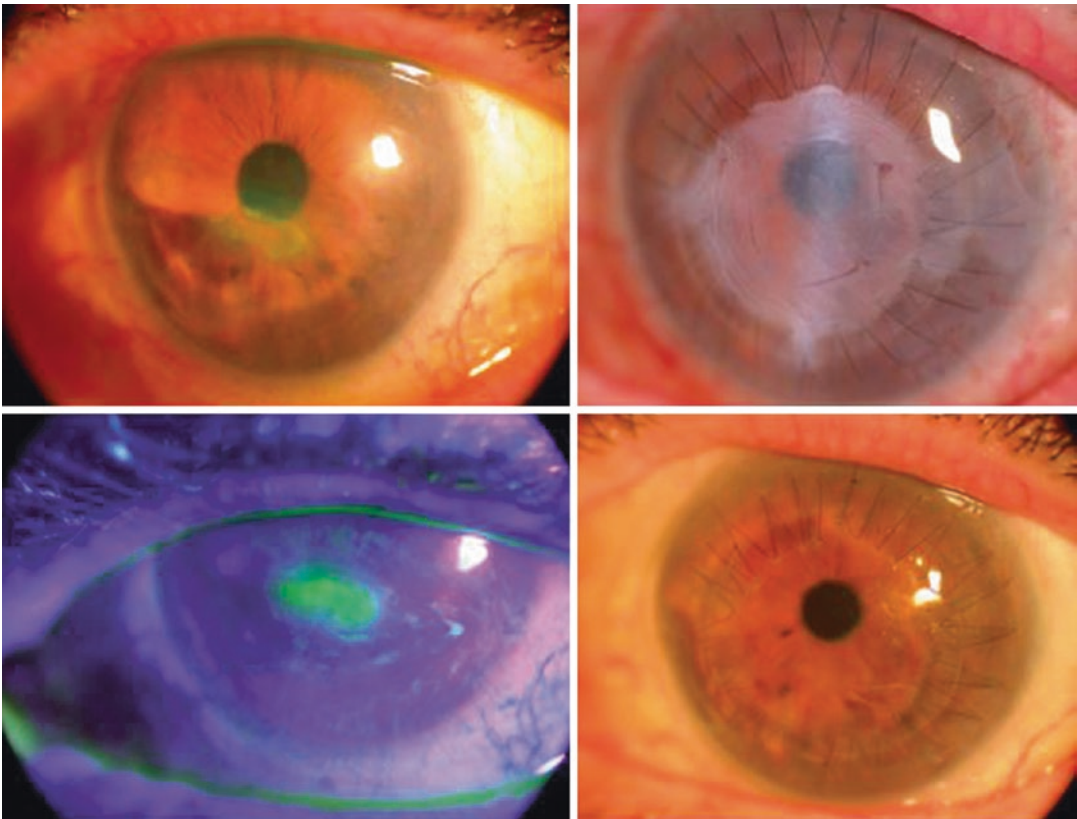


Fig. 13.13 Amnion membrane and DALK for high-risk keratoplasty. Left: Following herpes zoster, neurotrophic keratopathy with PED, stromal thinning and scarring reduce visual acuity to 20/400. Top right: Following

DALK with amnion membrane overlay (remnants present centrally) and lateral mini-tarsorrhaphy. Bottom right: Eventual corneal surface stability improves visual acuity to 20/30 and allows tarsorrhaphy release

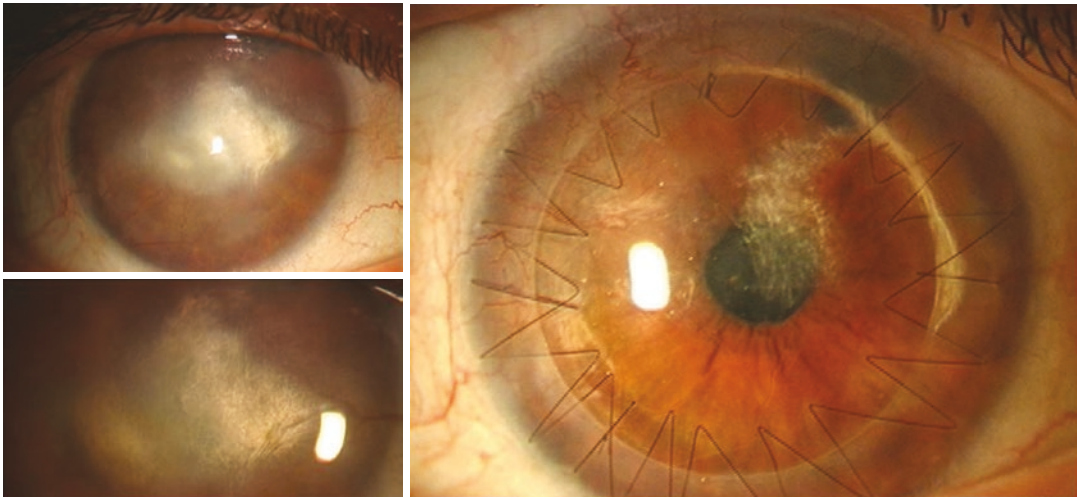


Fig. 13.14 Amnion membrane and DALK for high-risk keratoplasty. Top left: A 36-year-old male with herpes zoster reducing vision to hand motion due to dense corneal scar. Bottom left: Higher magnification resolves active stromal neovascularization releasing lipid and cholesterol into stroma. Right: At 6 months after DALK,

amnion membrane overlay and mini-tarsorrhaphy, followed by cataract extraction, vision improves to 20/40. Note residual deep cholesterol deposits which after 12 years postoperatively have absorbed completely. Neovascularization has never recurred

conus and stromal scars. However, with the new millennium came the Keratoplasty Revolution, shifting from “open sky” full thickness corneal surgery to selective tissue layer replacement targeted to disease-specific indications. Thus anterior lamellar keratoplasty (ALK) for stromal replacement and endothelial keratoplasty (EK) for endothelial failure have consequently and continually reduced PK surgery, such that by 2013, in the United States, EK had exceeded PK procedures.

Revival of Anterior Lamellar Keratoplasty: Historically among the first successful keratoplasties, ALK offered solely stromal tissue replacement for scarring and keratoconus without risk of immune-driven endothelial rejection. Although the visual results of early ALK were hampered by interface irregularity, the breakthrough of deep ALK (DALK), predominantly thanks to Anwar’s “Big Bubble” technique, allowed dissection to the level of Descemet membrane and offered major visual advantages to surgeons able to master the technical challenges (Figs. 13.13, 13.14 and 13.15). ALK is especially advantageous in the developing world where donor cornea supply is limited and pos-

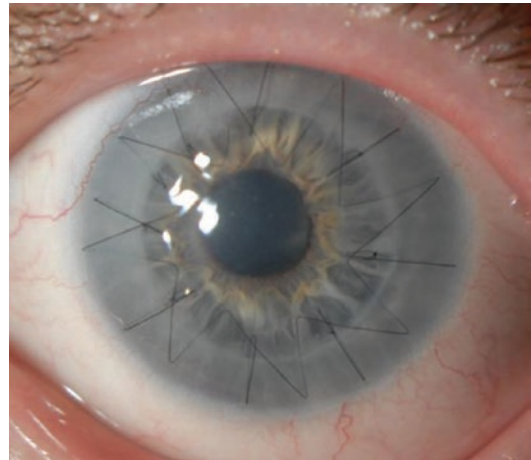


Fig. 13.15 Deep anterior lamellar keratoplasty: In a young patient with mucopolysaccharidosis Type I-H (Hurler syndrome), DALK greatly improves clarity of stroma densely infiltrated by storage accumulations while avoiding risks of PK

sibly of lower endothelial quality. Even in developed areas, such as the U.S., for example, 40% of potential donor corneas are discarded predominantly for unhealthy endothelium [10]. As ALK relies solely on clear stromal matrix but not viability of donor epithelial or endothelial

cells, long-term preservation methods further extend the feasibility and economy of this approach. Gamma-irradiated corneal tissue, for example, the Vision Graft Sterile Cornea (VSC, Tissue Banks International), affords such an alternative to fresh tissue. Although limited to ALK and corneal patch grafts, this tissue can also be utilized to reinforce leaking conjunctival filtering blebs, exposed glaucoma shunt tubes or eroded IOL haptic or sutures materials [11, 12]. Such grafts also have room temperature shelf life of at least one year, thereby enhancing availability and distribution, and their sterility reduces risk of donor-to-host microbial infection. Additionally with the advent of femtosecond lasers, the keratoplasty incisions for both PK and ALK can be customized in zigzag or mushroom configurations to facilitate earlier suture removal and reduced astigmatism [13]. The femtolaser can also facilitate determining the optimal lamellar dissection plane for Big Bubble DALK technique, but its use is hampered by limited access, substantial cost and application only within clear stroma.

Trends in Endothelial Keratoplasty: Since the early 2000s, EK has progressively become standard therapy for endothelial dysfunction, notably Fuchs dystrophy, pseudophakic corneal edema, and keratoplasty rejection. The evolution of EK is in itself epic, commencing with posterior lamellar keratoplasty (PLK) by Melles, followed by deep lamellar endothelial keratoplasty (DLEK) of Terry, then culminating with Melles's Descemet stripping endothelial keratoplasty (DSEK), which he yet further advanced in 2006 with Descemet membrane endothelial keratoplasty (DMEK) [14, 15] (Fig. 13.16). Although a highly challenging surgical technique, DMEK is equally highly advantageous, having optimal visual acuity, rapid visual recovery and reduced allograft rejection risk [16] (Fig. 13.17).

As technically daunting as EK itself is the preparation of the donor tissues, requiring thin (100 μ or less) stromal or solely Descemet membrane disc carriers of delicate endothelium. With the adaptation by eye banks to utilize the microkeratome for preparation of uniformly thin posterior stromal/endothelial discs for so-called

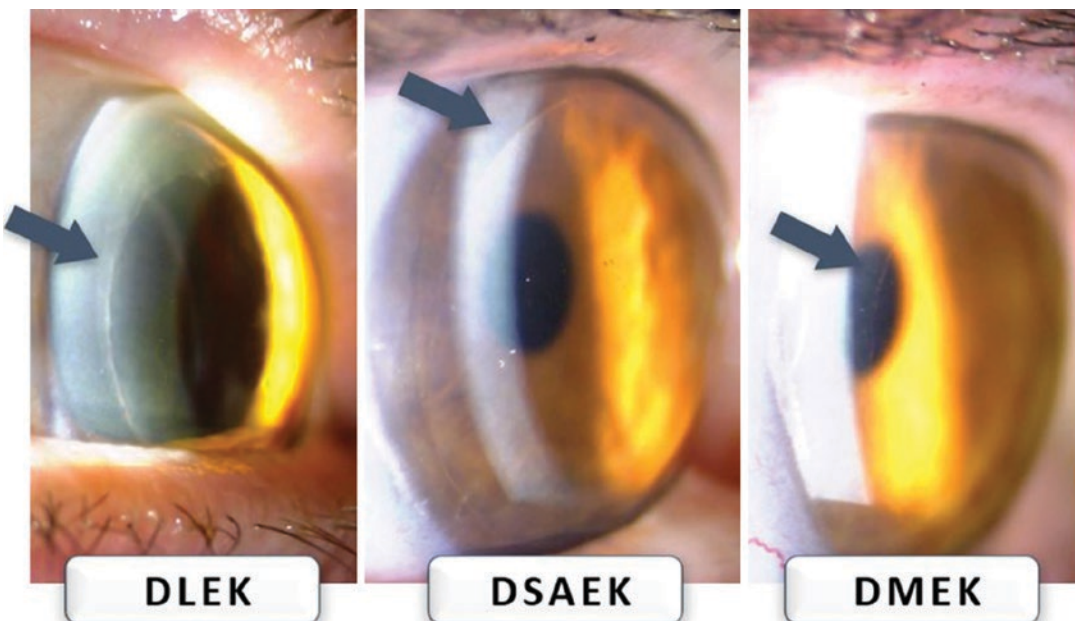


Fig. 13.16 Evolution of endothelial keratoplasty. In less than two decades, endothelial keratoplasty has advanced from deep lamellar endothelial keratoplasty (DLEK, left) to

Descemet stripping automated endothelial keratoplasty (DSAEK, middle) to Descemet membrane endothelial keratoplasty (DMEK, right). (Reproduced courtesy of T John)

Fig. 13.17 Descemet membrane endothelial keratoplasty. Top left: Preparation of Descemet membrane graft facilitated by trypan blue staining. Center: Descemet graft inserted as scroll into anterior chamber. Top right: Manipulation and air bubble position and secure graft. (Reproduced courtesy of T John)

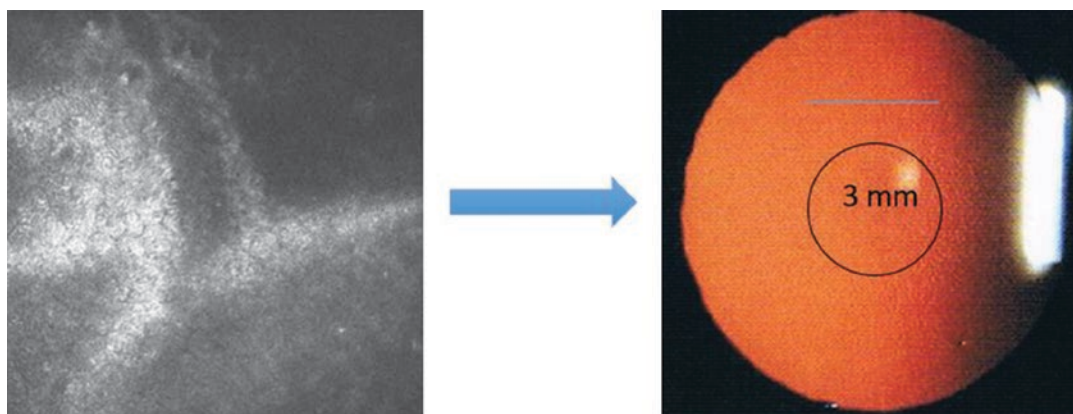
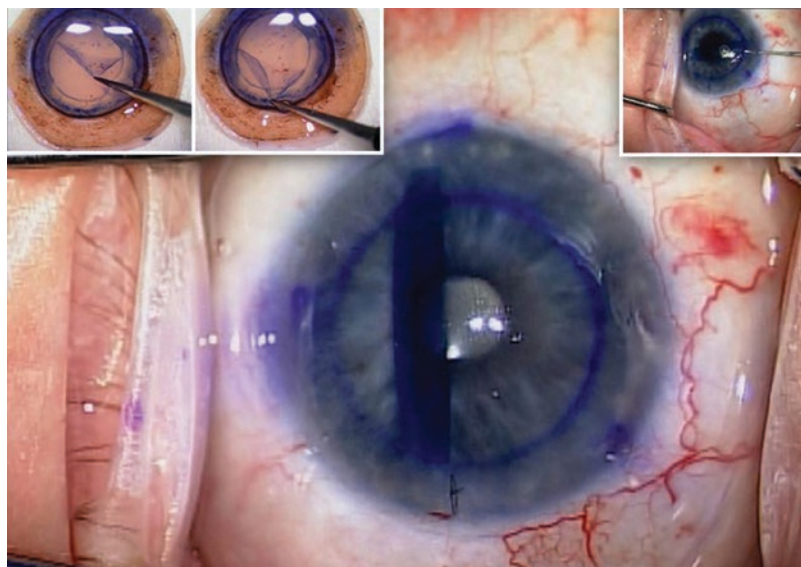


Fig. 13.18 Descemet stripping only (DSO). Central stripping of Descemet's membrane without endothelial keratoplasty allows more viable peripheral corneal endothelial cells (left, specular microscopy) to migrate cen-

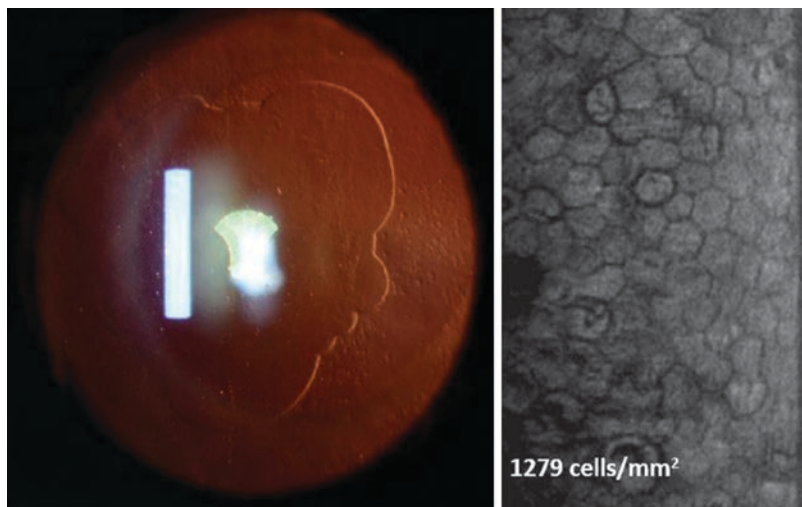
trally and repopulate the central cornea (right, slit lamp retroillumination), ultimately resulting in deturgescence and visual clarity. (Reproduced courtesy of P. Veldman)

Descemet stripping automated endothelial keratoplasty (DSAEK) and the extension to DMEK tissue preparation, the increasingly routine provision of “tissue ready” DSEK or DMEK tissues, often provided within single-use insertion instruments, is indicative of the increasingly adept contribution of eye banking to the Keratoplasty Revolution.

Yet other variations on the endothelial regenerative theme are of merit. For patients with moderate Fuchs dystrophy having largely central guttae and a preserved peripheral endothelium, primary descemetorhexis alone without replace-

ment by a donor graft has attracted major interest (see Kocaba & Colby Chap. 8, this volume). This technique, termed descemetorexhis without endothelial keratoplasty (DWEK) or Descemet stripping only (DSO), involves removal of the central 4–5 mm disc of endothelium and Descemet membrane by manual descemetorhexis alone and thereby stimulates peripheral endothelial cells to migrate and cover the central defect (Figs. 13.18 and 13.19). Recognizing that post-operative corneal edema requires weeks to months for resolution as the central endothelial

Fig. 13.19 Descemet stripping only (DSO). Left: Slit lamp retroillumination 3 years postoperatively discloses restored corneal clarity and curved margins of Descemetorhexis. Right: Specular microscopy of central cornea resolves restoration of endothelial cell mosaic with normalized endothelial cell count and minimal pleomorphism. (Reproduced courtesy of K. Colby)



cell layer is regeneratively restored, the visual outcomes of successful DSO can be comparable to DMEK-treated eyes [17], and if unsuccessful, subsequent EK can still be favorably performed (see Kocaba & Colby Chap. 8, this volume).

Yet another exciting variation on corneal endothelial regenerative theme are extraordinary advances of the Koizumi and Kinoshita teams in Japan where the intracameral introduction of cultured human corneal endothelial cells in the presence of Rho-kinase (ROCK) inhibitor agents has clinically achieved remarkable visual recovery (see Kinoshita Chap. 18, this volume), and most recently even without endothelial cell augmentation, DSO plus topical application of a ROCK inhibitor (ripasudil, Galanatec) has proven successful for Fuchs dystrophy [18].

Keratoconus and Ectasia: Keratoconus (KCN) and post-refractive ectasia are corneal degenerations characterized by progressive stromal thinning and steepening plus irregular astigmatism. Typically commencing in puberty, variably progressing and often stabilizing during the third decade, the incidence of KCN is 0.001–0.03% [19]. Post-refractive ectasia is rarely consequent to surgery-induced mechanical destabilization that occurs in 0.04–0.6% of predominantly LASIK cases [20]. Management of both conditions range from spectacle or contact lens use for milder cases to keratoplasty for more advanced cases. A multicenter observational study of KCN

determined the 8-year incidence of penetrating keratoplasty to be 15% for patients younger than 40 and 8% for those 40 and older [21].

Commencing approximately 20 years ago, intrastromal rings, arcuate segments of rigid PMMA (Intacs and others) were devised for surgical implantation in the peripheral stroma to support and reduce irregular astigmatism (Fig. 13.20), and although not capable of stopping ectatic progression, variable improvement of corneal shape and acuity has reduced the need for keratoplasty. More recently, intrastromal rings have been found to be increasingly synergistic in conjunction with corneal cross-linking (CXL) [22].

Indeed, CXL, the real game changer for ectasias since the late 1990s [23], is a relatively minimalist procedure capable to halt, but not reverse, ectatic progression. Involving application of riboflavin (vitamin B2) solution followed by ultraviolet radiation (UV-A) to the corneal stroma to produce free oxygen radicals which promote formation of new covalent bonds between stromal collagen fibrils, clinical results of CXL (see Hersh Chap. 16, this volume) include arresting progression of the condition, vision preservation and possible improvement of topographic shape, thereby greatly reducing need for future keratoplasty [24].

With the 2016 US FDA approval of CXL, a paradigm shift in the treatment of ectatic corneal

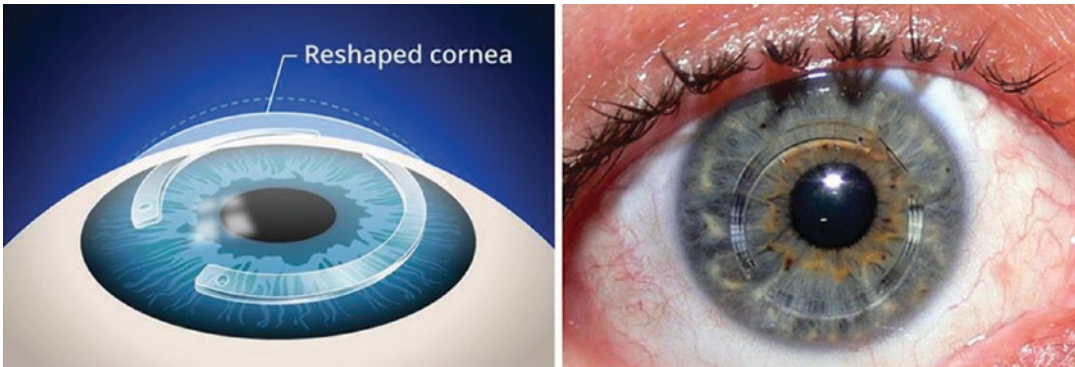


Fig. 13.20 Intrastromal ring segments. To reduce irregular astigmatism of keratoconus and other ectasias, arcuate PMMA (polymethyl methacrylate) segments (usually 2) are surgically implanted within the mid-peripheral stroma.

Although progression of the primary disorder is not arrested, corneal flattening (left) improves refraction and contact lens tolerance

conditions (and certain infectious keratitis cases) has occurred. Combined with advances in contact lens materials as well as scleral, hybrid and PROSE lens designs (see Jacobs Chap. 24, this volume), keratoconus management has become far less surgically invasive. Moreover, the integration of CXL, intrastromal rings and even topography-guided excimer refractive surgery in selected eyes lacking extreme corneal deformity and/or refractive regulatory can further improve long-term vision [25].

Perspective on Penetrating Keratoplasty in a Lamellar and Keratoprosthesis World

As selective keratoplasty increasingly replaces the “one size fits all” of PK, the menu and algorithm of choices have become increasingly well defined. Thus, for predominantly ocular surface issues, including LSC deficiency, the performance of LSC transplantation variations can in itself be restorative and require augmentation with ALK only when stromal scarring or thinning coexist. The application of ALK, especially in developing areas where infectious or traumatic corneal scarring is prevalent and perhaps utilizing preserved donor stroma, is promising for efficiency as well as economy and especially avoidance of immune rejection. Purely endothelial dysfunction or dystrophy will be entirely

within the purview of EK, and increasingly DMEK, at least until the further refinement of DSO + rho kinase inhibitor strategies. Thus, only somewhat more specialized circumstances will merit DSAEK or PK. Specific scenarios in which DSAEK is potentially preferable over DMEK include aphakia, pseudophakia with anterior chamber IOL, as well as eyes post-filtering procedures (especially, tube shunts) or post-vitreectomy. PK remains more than justified in settings of both combined stromal (e.g., scarring or ectasia) plus endothelial (e.g., edema) abnormality and especially where more complex anterior segment reconstruction (e.g., IOL exchange, synechiolysis, iridoplasty, vitreolysis) is mandated. Keratoprostheses remain required for extreme ocular surface conditions (e.g., severe dry eye, lid and sensation abnormality) and multiple immune failures with potentially broader application pending improved biocompatibility. Hence, the “Compleat Keratoplaster” is obligated to maintain an increasingly broad skill set and to devise from her/his Tool Box the most situation-specific strategy.

The Bright Bioengineered Future

As previously observed, the limited supplies and increasing costs of conventional eye banking and tissue preservation doom the ability of human donor corneas to ever meet demand, especially in

the developing world. Fortunately, many previously theoretical approaches are now under active development, including xenotransplantation, tissue-engineered corneas, and cell-based therapies as well as other approaches to biologic corneal regeneration.

Xenotransplantation: Offering potential to close the gap between need versus availability of transplantable corneal tissue, the initial clinical corneal xenotransplantation was performed by Kissam in 1838 [26], over 70 years before Zirm's historic first corneal allotransplantation [27]. Given the many anatomical and optical similarities between pig and human corneas, use of porcine corneas has been extensively researched [28]. Few recent studies of clinically relevant pig-to-nonhuman primate models report that although porcine corneas transplanted into rhesus monkeys were rejected, immune suppression with steroids prolonged graft survival [29], and acellular porcine corneal stromas (APCS) grafts both in vitro and in rabbit models showed good histocompatibility and low immunogenicity [30]. Approved for clinical trials in China, a human clinical study utilizing APCS for ALK in fungal keratitis cases demonstrated no recurrent infections, nearly complete epithelialization and substantial visual improvement [31]. Recent progress in the genetic manipulation enables pig tissues to resist the primate immune response, suggesting that as the remaining immunological barriers are overcome, pig to human corneal transplantation may become a wider reality [32].

Tissue Engineering and Regenerative Biology: The priorities for considering prosthetic corneal alternatives are transparency, tissue strength, and biocompatibility to improve integration. The cul-

mination of CHD's 50-year focus in developing the Boston Keratoprosthesis, from FDA approval since 1992 to the most successful artificial cornea to date, is nonetheless limited by lack of biointegration of its polymethyl methacrylate core (also see Dagher Chap. 15, this volume).

Fortunately, interest in tissue engineering and regenerative biology rapidly advances the fabricated cornea cause [33]. In principle, the two primary approaches to tissue engineering are cell-based and scaffold-based. In the former, specific cell types, such as the corneal epithelium and endothelium, play the main role in tissue engineering the cornea; in the latter, a stromal scaffold affords a substrate mimicking the microenvironment supporting the constituent cell populations. Hence, many research groups now pursue developing biomimetic, cytocompatible and transplantable stromal replacements, utilizing materials ranging from classical collagen gels, films and sponges to less traditional components such as silk, fish scales, gelatin and polymers [34, 35].

An alternative strategy is to create a corneal implant that is refractively suitable and biointegratable but does not attempt to perfectly mimic the cytoarchitecture of native cornea. Materials that have been investigated for this concept include natural polymers such as gelatin, semisynthetic polymers such as gelatin methacrylate, and synthetic polymers. The most direct ways in which 3-D printing could be employed in corneal implants are to print a scaffold upon which cells are seeded (either in vitro or in vivo), the resultant synthetic or semisynthetic construct employing a "core and skirt"-like model (Fig. 13.21). The concept of 3-D bioprinting stem cells or col-



Fig. 13.21 Diagrammatic concept for 3-D printing of artificial cornea. (a) Image acquisition via ultrasound (US) and optical coherence tomography (OCT) for modeling artificial cornea; or gathering measurements relating to the surrounding structures. (b) Image segmentation constructs

a 3-D model. (c) Model is converted to standard tessellation language (STL) and sliced. (d) 3-D model printing by inkjet or extrusion-based system. (e) 3-D model implanted by a cornea surgeon similar to a standard allograft. (Modified from Fig. 2 Ludwig et al. [44])

lagen into a corneal scaffold is additionally promising. The ability to regenerate the cornea with autologous cells could dramatically improve as the clinical use of cell-based corneal replacements within tissue-engineered matrices offers major advantages including relative resistance to infection, inflammation, and immune rejection, as well as organic integration into the host tissue and formation of well-differentiated epithelia [36]. A UK group recently reported proof of concept for a 3-D-printed corneal construct of alginate and collagen containing human corneal stromal cells which were able to survive and grow [37], suggesting that tissue engineering, with the aid of 3-D printing and host cultured components, may be possible. Due to its consistent microstructure, limited cellular components and avascularity, the cornea is a near ideal candidate to become one of the first ink-printed tissues. The ability to integrate corneal imaging data and create a specific shape and curvature based on the patient's own optical parameters is additionally exciting.

Stem Cells: Stem cell-based therapies may eventually usurp conventional corneal transplantation even accepting the many challenges regarding each unique corneal cellular layer. As such, intensive research has focused on corneal stem cells as a source of regenerative cell-based therapy, based on increased awareness of the specific stem cell types located in each corneal layer [38].

Our knowledge of stem cells and their role in regenerative medicine has expanded greatly over the past four decades, as use of limbal stem cells (LSC) for ocular surface rehabilitation, first as *in vivo* autografts [6] and subsequently as *ex vivo* expansion grafts on amnion or collagen membranes [7], has become clinically routine for the restoration of intrinsic and acquired LSC deficiencies ([7]; see Rosenblatt and Djalilian Chap. 20, this volume). Complementary applications for stromal and endothelial stem cells, however, have remained more theoretical. Nevertheless, with identification of corneal stromal stem cells, their application as a cell-based therapy for corneal stromal scars is promising, as, for example, injection of human corneal stem cells in lumican-null mice can restore corneal transparency [39].

Similarly, identification of a higher density of corneal endothelial precursor cells in the corneal periphery holds promise for promoting *in vivo* endothelial cell proliferation. Apart from these intrinsic ocular sources of stem cells, several alternative sources outside the ocular milieu have also been identified, including derivatives from oral mucosal epithelium, dental pulp and hair follicle. Oral mucosal stem cells, which notably express limbal stem cell markers, have already been clinically applied for ocular surface regeneration with some success [40].

In addition to such defined stem cell sources, mesenchymal stem cells, notable for their pluripotency, express embryonic and mesenchymal stem cell markers, and can differentiate into cells of the three embryonic layers [41]. Such cells have been identified from adipose tissue, amniotic membrane, bone marrow and umbilical cord, and in particular, bone marrow-derived cells applied to animal corneas seem capable of inhibiting inflammation and angiogenesis as well as promoting wound healing [42]. Likewise, adipose-derived stem cells have been demonstrated in rabbit corneal stroma to aid in repair and regeneration through differentiation into functional keratocytes with production of cell-specific products [43]. Further work involves the use of adipose-derived stem cells to transform as human corneal endothelial cells.

To summarize, advances in corneal stem cell research hold particular promise of their use in regenerative medicine and tissue engineering. Although transplantation of LSC has become increasingly routinized, attentions now turn to use of adult and/or pluripotent embryonic stem cells for corneal epithelial restoration. Additionally, recent developments for human stromal stem cells, their immune privilege and their potential to secrete organized collagen lamella may be applicable in corneal tissue engineering. This concept should also be extendable to stem cells of the corneal endothelium and the conditions required to stimulate their proliferative activity. Unlike the selective keratoplasty principle of today, tomorrow's cell-based therapies will target specific cellular strategies to solve specified corneal diseases.

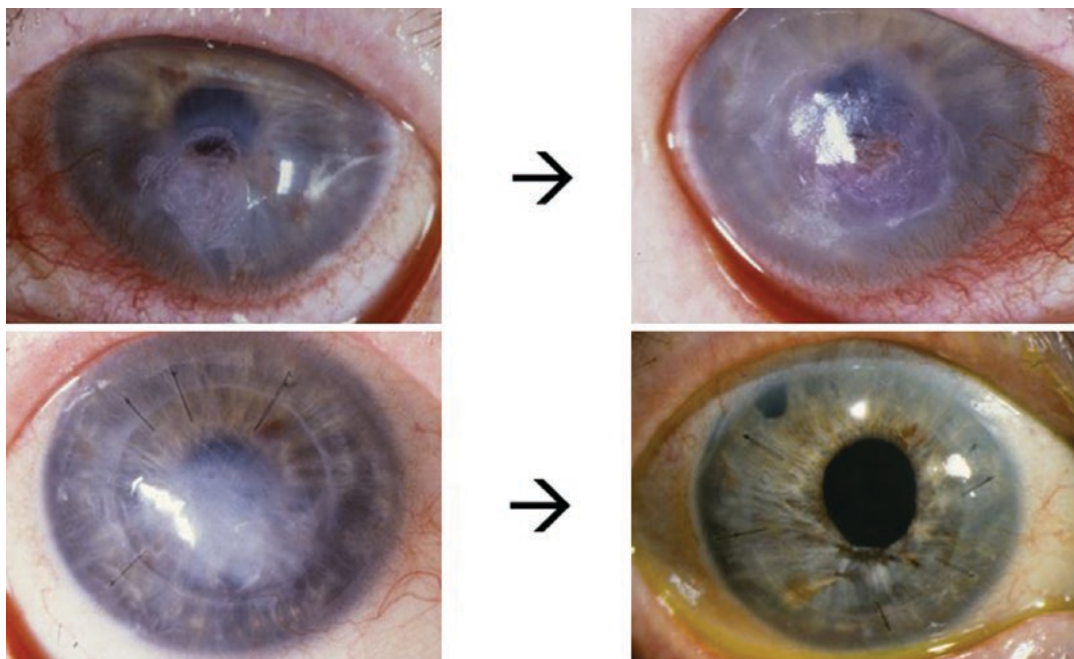


Fig. 13.22 The creative choreography of keratoplasty. Staged application of various pharmacologic and surgical therapies requires strategy appropriate to the clinical situation. Top left: This 72-year old woman with rheumatoid arthritis and keratitis sicca presents sterile stromal perforation initially stabilized with cyanoacrylate tissue adhesive.

Top right: Progressive stromalysis requires second tissue adhesive application. Bottom left: 6 months later, lamellar keratoplasty affords tectonic stability but stromal scar limits vision. Bottom right: After additional 6 months, penetrating keratoplasty, synechiolysis + iridioplasty and ECCE with posterior chamber IOL achieve 20/50 visual rehabilitation

Conclusion

In its near 120-year history, corneal transplantation may be divisible into 3 epochs.

The first half century from Zirm in 1906 through the mid-1950s established the all-important proof of concept but was hampered by relatively primitive instrumentation and optics for microsurgery, limited capabilities in eye banking and few anti-infective and anti-inflammatory medications. The ensuing five decades through the close of the twentieth century were hallmarked by linear advances in keratoplasty microsurgical technique and technology, consistent eye banking preservation and networking, plus adjunctive surgical strategies for the ocular surface, anterior segment reconstruction and cataract, as well as broad pharmacologic developments, with which multifaceted strategies could be crafted to suit complex clinical challenges (Fig. 13.22). With the new millennium,

the Keratoplasty Revolution has launched two decades of exponential growth, as selective keratoplasty and keratoprotheses have opened the door through which tissue bioengineering and stem cell therapies will advance. Cell-based therapies for each corneal layer will target specific disorders and a single donor cornea might potentially treat many patients, thereby reducing the global burden of corneal blindness.

In viewing this ever-accelerating growth curve of keratoplasty, we can see clearly now that the Best is yet to Come! What a privilege to observe and operate within this ever-exhilarating and advancing scene.

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Striving for Perfect Vision: Insights from Refractive Surgery

14

Asim Farooq, Pushpanjali Giri, and Dimitri Azar

Overview

Refractive surgery involves the surgical correction of the refractive errors of the eye such as myopia, hyperopia, astigmatism, presbyopia, and cataracts. As one of the rapidly evolving fields in ophthalmology, it has come along a long way since 1948 when Father Waclaw Szuniewicz pioneered the concept of refractive surgery by experimenting to change the shape of the corneal curvature. Today, femtosecond lasers have revolutionized the surgical procedure by providing precision, safety, and reproducibility like never before. The major milestones of refractive surgery include the introduction of keratomileusis in 1964, the invention of radial keratotomy in 1970, the invention of the excimer laser between 1973 and 1983, the first excimer laser use for corneal ablation in 1987, the first LASIK use in the 1990s, the approval of PRK and LASIK by the FDA in 1995 and 1996, respectively, the approval of wavefront technology in

2003, and the evolution of the femtosecond laser for refractive surgery in 2001 [1].

Optics

The human eye as an optical system is very similar to a camera, where the cornea (diameter: 12 mm, central thickness: 0.55 mm, refractive index: 1.3771) and lens (actively accommodating) refract light. However, the eye is a much simpler optical instrument compared to many artificial optical instruments in terms of its design and complexity. Only two positive lenses, the cornea and the crystalline lens, comprise the optical instrument of the eye, and these initiate the visual process by projecting the images into the retina. Still, the usual field of the view of the eye is relatively large as compared to most optical instruments, at least $160 \times 130^\circ$ [2]. The wave-like nature of light governs the quality of the retinal images under most normal viewing conditions. Wavefront aberration and point-spread function (PSF, the image of a point source) are used to describe the optical quality under such conditions. To form a perfect retinal image, the wavefront aberrations would have to be null or constant; an eye without any aberrations would do just that, where the retinal image would depend only on the pupil diameter [2]. In conditions of very low luminance after dark adaptations, the particle nature of light plays a role in the

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vision. In these conditions, photoreceptors absorb photons in discrete quanta, the probability of a photon being absorbed imposes a fundamental limit to the vision related to the photon statistics.

Wound Healing

The cornea accounts for approximately 70% of its refractive power; hence, the way a cornea heals after keratorefractive surgery is of paramount interest and importance. The healing process of the corneal wound affects the efficacy and the safety of the refractive procedure. Corneal wound healing involves complex and coordinated cascades of molecular and cellular pathways, involving cytokines and growth factors that result in a highly variable biologic response. Epithelial, stromal, and endothelial cells are the three main types of cells involved in corneal wound healing. Epithelial and stromal cells interact with each other during wound healing, while endothelial cells are relatively less affected. Roughly, the wound healing process follows inflammation (latent phase), myofibroblast differentiation (migration), extracellular matrix (ECM) deposition (proliferation), and fibrosis development (epithelial reattachment) (Fig. 14.1) [3].

Epithelial Wound Healing

Epithelial wound healing involves limbal stem cells and their progeny under the action of growth factors and cytokines from tears and cells, as well as the basement membrane proteolytic remodeling, with no actual cell transformation [4]. The therapies to facilitate epithelial wound healing include limbal stem cell transplantation, gene therapy, miRNA, and nanocarriers. Limbal stem cell transplantation significantly improves corneal reepithelialization. Gene therapy, miRNA, and nanocarriers are emerging therapies. The miRNAs are considered to play important roles in many phases of corneal epithelial wound healing, with some miRNAs promoting and others inhibiting healing [5–9]. The inhibitors of miRNA (antagomir), specifically miR-146a, have been reported to enhance wound healing in human diabetic organ-cultured corneas [8].

Further investigations of the miRNA targets and affected pathways are still needed to understand normal epithelial healing, and its dysregulation in chronic wounds and diseased corneas. Gene therapy has a good potential for normalizing epithelial wound healing. Viral- and nanocarrier-based gene therapies are the two kinds of available gene therapies. Viral-based therapies (recombinant adenoviruses, rAV; adeno-associated viruses, rAAV; and lentiviruses) have been successfully used to deliver specific genes into the cornea to produce high-level expression of the target gene rapidly. The viral vectors are chosen depending upon the desired level of expression and desired target cell; rAV transduction has been reported to produce considerably higher expression of the green fluorescent protein (GFP) reporter than with rAAV transduction [10]; however, the transduction appears to reach only the epithelial and endothelial cells. rAAV transduction is seen on all layers of corneal cells: the epithelial, stromal, and endothelial cells [11]. Nanocarrier-based therapies include metal-, lipid-, and polymer-based systems, and they allow several different plasmids to combine in one particle to increase the desired gene expression [12] and effective drug delivery [13]. Nanoparticles coated with all-trans retinoic acid, lactide-co-glycolide and loaded with antifibrotic drug pirfenidone, and elastin-like polypeptides loaded with mitogenic protein lacritin, have been reported to promote corneal epithelial wound healing [14–15].

Stromal Wound Healing

Stromal wound healing involves keratocyte transformation from inactive keratocytes into activated fibroblasts and subsequently into myofibroblasts. The myofibroblasts, expressing α -SMA, contribute to wound contraction. The keratocyte transformation is influenced by cytokines and growth factors derived from the epithelial cells, immigrating immune cells, and stromal cells themselves [4]. The process of wound healing after refractive surgery involves epithelial-mesenchymal interactions, stepwise transformation of corneal cells during healing, and the involvement of TGF- β signaling. Antifibrotic agents appear to be promising

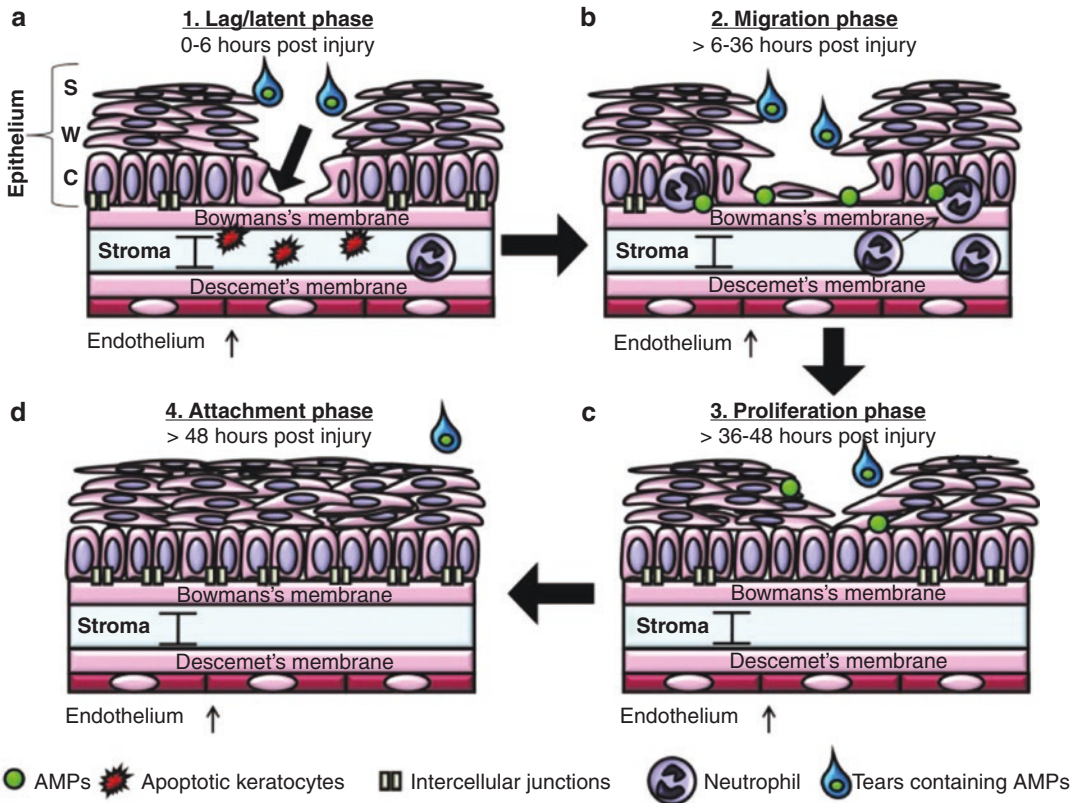


Fig. 14.1 Four main stages of corneal epithelial wound healing. Corneal epithelial wound healing can be described to occur in four main phases (a–d). The initial lag or latent phase (a) of the wound-healing process takes place during the first 6 h after injury. The lag or latent phase can be marked with a reduction in the number of intercellular junctions, the apoptosis of anterior keratocytes and the beginning of some neutrophil infiltration into the cornea. During this phase, the basal epithelial cells (arrow) forming the columnar layer also prepare to migrate. During the migration phase (b) occurring 6–36 h postinjury, the epithelial cells continue to migrate to close the gap and begin to adhere to the basement membrane. A primary wave (at 18 h) and a secondary wave (at 30 h) of

neutrophils containing AMPs infiltrate into the stroma. The corneal epithelial cells produce AMPs such as CAP37 during this time. During the proliferation phase (c) occurring between 36 and 48 h postinjury, the basal epithelial cells from the columnar layer begin to proliferate before differentiating into wing and stratified corneal epithelial cells. The last phase in the process, the attachment phase (d), occurs 48 h postinjury as the cells firmly adhere back to the basement membrane and the number of intercellular junctions increase. The tear film is present throughout this process and is a known source of AMPs that may modulate the wound healing process. AMP antimicrobial peptide, CAP37 cationic antimicrobial protein of molecular weight 37 kDa [3]

treatments for stromal haze and scarring. Emerging TGF- β inhibition-based new interventions using gene therapy, introduction of specific ECM components, implantation of stromal equivalents, and nanotechnology for drug delivery are being investigated for stromal wound healing. Stem cell therapy is a promising approach to deal with fibrosis and haze. Treatment with femtosecond lasers for LASIK and keratectomy is thought to lead to better

refractive outcomes, potentially due to less damage to the stromal cells [16–18].

Endothelial Wound Healing

Endothelial wounds are relatively rare, however, they do occur, usually as a consequence of burns or surgeries meant to replace dysfunctional endothelial cells, such as DSEK or DMEK [19–21]. The process of endothelial wound healing is substantially different from that of epithelial and

stromal cells; they involve cell migration and spreading. The emerging progress in endothelial wound healing treatment include ROCK inhibitors (in the form of eye drops) to facilitate cell migration, and SMAD 7 gene therapy to suppress fibrotic changes [4].

Instrumentation

The major instrumentation in refractive surgery includes excimer lasers, laser (femtosecond) and mechanical microkeratomes, eye trackers, and instruments for collagen crosslinking. Excimer lasers and microkeratomes are crucial to LASIK, LASEK, epi-LASIK, and PRK procedures. With the development of wavefront and topography-guided excimer laser platforms, LASIK procedures have become more precise, safer, customizable to patient needs, and more widely applicable to refractive correction of all kinds of ametropia. Intralase (60 kHz, 150 kHz, 500 kHz) and VisuMax are the two kinds of femtosecond laser platforms that are in routine use as laser microkeratomes. Nd:glass (amplification glass matrix mixed with neodymium) is used to create laser energy, and the delivery system comprises of two perpendicular galvanometers to allow the three-dimensional scanning of the laser. Comparative studies of both femtosecond lasers have shown similar safety, efficacy, and predictability, and both produce excellent visual results [22], although different levels of tear proteins are reported to arise with these different laser platforms due to induction of distinct biological responses in the cornea and ocular surface [23]. The FDA has approved the use of VisuMax femtosecond laser for correction of myopia through the new, increasingly popular SMILE procedure. The collagen crosslinking instrumentation is possible through UV LEDs and optical beam delivery system. Proper wavelength selection with consideration of temperature influence on the wavelength and the power output is important when choosing the appropriate UV-light source. Appropriate output shaping and control of the optical beam are key factors of crosslinking equipment, and the factors to consider include

optical beam spot size, optical output power density distribution, optical beam aiming and positioning, as well as auxiliary beam aiming. The LED UV emitters in the crosslinking equipment system must comply with the IE 60825 safety regulation [24]. IEC 60601-1-11:2015 contains the most common regulations adopted by several countries. Opto X-Link, Avedro, Avedro KXL system, Kestrel-Intacs-XL, CSO Vega, and CCL Vario SwissMed are some representative cross-linking models that are available in the market.

Incisional Surgery (AK, RK, LRI, FEMTO-AK; FEMTO Wedge Resection)

Incisional corneal surgery has been employed in a variety of refractive procedures. Radial keratotomy (RK) was first attempted by Sato in the 1930s and was popularized by Fyodorov in the 1970s [25]. This technique was used to treat myopia by using radial incisions to flatten the central cornea (Fig. 14.2). The method developed by Fyodorov involved a series of paired radial incisions made through the epithelium and into the deep stroma. While relatively effective in reducing myopia overall, there was significant refractive fluctuation in the first 3 months, partly caused by varying degrees of stromal hydration [25].

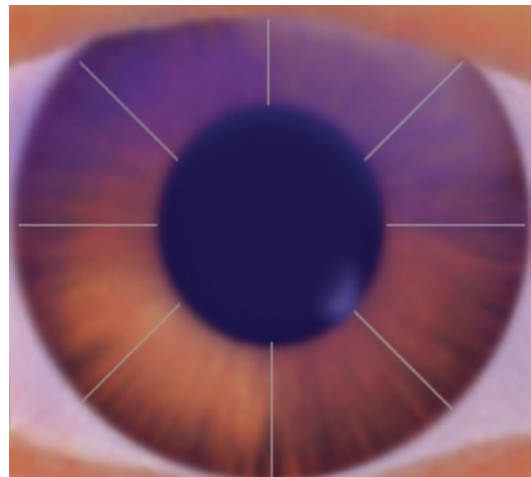


Fig. 14.2 Radial keratotomy

This procedure was ultimately replaced with newer refractive surgery techniques, such as photorefractive keratectomy (PRK), which was also studied as a method of treating residual refractive error after RK, and laser-assisted in situ keratomileusis (LASIK) [26].

Astigmatic keratotomy (AK), also known as arcuate keratotomy (when made in an arcuate configuration), was described by Binder in 1984 [27]. Using a blade, an incision, or a pair of opposing incisions, is made centered on the steep meridian of the cornea, thereby reducing corneal astigmatism; initially, this was at times paired with RK, although now it is often performed at the time of cataract surgery, or as an independent procedure (Fig. 14.3). The length and depth of the incisions, as well as the distance from the limbus, may be varied based on the amount of astigmatism and degree of effect desired. A number of nomograms were developed to guide the surgeon in AK placement [28]. A subset of AK, limbal relaxing incisions (LRIs) are made in the peripheral cornea near the limbus in an arcuate configuration. While AK causes flattening in the meridian of the incision, it tends to cause steepening in the opposite meridian as would be predicted by Gauss' law of inelastic domes, leading to a negligible effect on the spherical equivalent [29].

Femtosecond laser arcuate wedge-shaped resection was described by Ghanem and Azar in

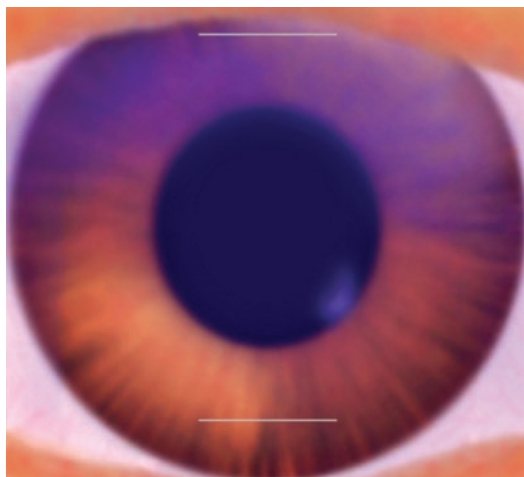


Fig. 14.3 Astigmatic keratotomy.

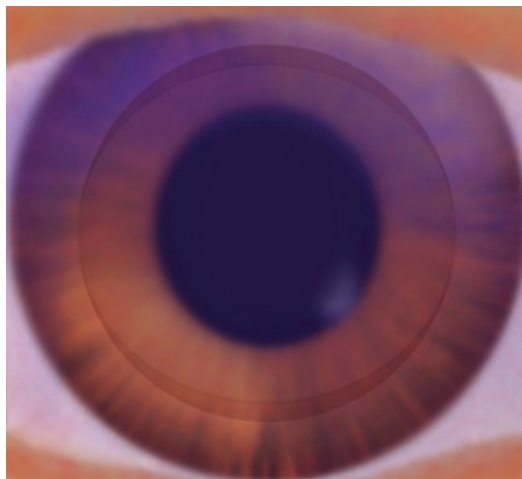


Fig. 14.4 Femtosecond laser arcuate wedge-shaped resection.

2006 (Fig. 14.4) [30]. This technique utilized intersecting arcuate incisions created by a femtosecond laser to perform a wedge resection for the correction of high corneal astigmatism. After the development of a formula to calculate the relative decentration of arcuate cuts, the technique was tested in porcine corneas, and then carried out in a postpenetrating keratoplasty patient with 20 diopters of astigmatism. The procedure resulted in a nearly 15 diopter reduction of astigmatism, which was significantly greater than what had been demonstrated with AK alone.

Soon thereafter, femtosecond laser-assisted AK (FLAK) was described by Harissi-Dagher and Azar [31]. Two patients with high corneal astigmatism after penetrating keratoplasty underwent FLAK comprised of paired arcuate incisions within the donor cornea [31]. The corneal astigmatism improved from 8.5 to 4.9 diopters in the first case, and from 7.0 to 4.3 diopters in the second case, with improvements in best corrected visual acuity of 20/100 to 20/30, and 20/200 to 20/60, respectively. FLAK has subsequently been found to be a reliable and effective procedure to address corneal astigmatism [32].

The relatively recent advent of femtosecond laser-assisted cataract surgery (FLACS) has popularized FLAK, which may be performed in the same session as wound creation, capsulotomy, and lens fragmentation. Similar to manual AK,

the location, length, and depth of FLAK incisions may be varied, and a nomogram may be used to guide placement. FLAK incisions may include the anterior corneal surface or start within the anterior stroma, and typically end within the deep stroma (e.g., at 90% depth). The biomechanical properties of the cornea (i.e., corneal hysteresis and corneal resistance factor), as well as astigmatism type, may influence the efficacy of FLAK [33].

PRK, LASEK, and EPI-LASIK

Photorefractive keratectomy (PRK) was first performed in the USA by Dr. Stephen Trokel in the 1980s after collaborating with research scientists at IBM to modify an excimer laser for ophthalmic use [34]. The procedure involved the removal of corneal epithelium, followed by the use of an excimer laser to ablate and reshape the anterior corneal stroma (Fig. 14.5). The following year, Munnerlyn et al. published a study describing the use of the excimer laser to reshape rabbit corneas [35]. The authors also described a theoretical formula (subsequently known as the Munnerlyn formula) that estimated the depth of corneal ablation required in excimer refractive surgery: the depth per diopter of intended treatment equaled the square of the treatment zone diameter in millimeters divided by three.

The surface-based excimer refractive procedures also include laser epithelial keratomileusis (LASEK) and epi-LASIK. LASEK was first performed at the Massachusetts Eye and Ear

Infirmery by Dr. Dimitri Azar in 1996. This procedure involved the use of a semisharp circular instrument that was placed on the cornea and filled with alcohol for approximately 30 seconds, after which modified Vannas scissors were used to create a hinged epithelial flap. An excimer laser was then applied to the stroma, followed by replacement of the epithelium [36]. Epi-LASIK was described by Dr. Ioannis Pallikaris and colleagues at the University of Crete [37]. This procedure was similar to LASEK, except that instead using alcohol, a microkeratome was used to create an epithelial flap. Subsequent work revealed that LASEK and epi-LASIK had similar visual outcomes, epithelial closure time, pain, and haze formation, regardless of whether the epithelial flap was retained or discarded [38]. Similar visual outcomes between PRK, LASEK, and epi-LASIK, as well as other factors (including the challenge of successfully replacing the epithelial flap), led many surgeons to favor PRK among the surface-based refractive procedures.

PRK was ultimately approved by the Food and Drug Administration (FDA) in 1995. Early studies revealed the development of anterior stromal haze after PRK, and its incidence and duration appeared to correlate with stromal ablation depth [39–40]. Majmudar et al. [41] described the use of mitomycin C (MMC) in the treatment of corneal haze postrefractive surgery (including cases of RK and PRK). In 2005, Gambato et al. [42] reported the results of a prospective, randomized clinical trial that showed intraoperative MMC was effective in the prevention of anterior stromal haze after PRK.

Despite the wide adoption of LASIK, in part due to faster visual recovery and less postoperative pain, there remains an important role for the surface-based excimer refractive procedures. For example, due to the decreased ablation depth of surface-based procedures in comparison to LASIK, there is a decreased risk of postrefractive surgery ectasia. The surface-based procedures also do not carry a long-term risk of flap-related complications, such as epithelial ingrowth, traumatic flap dehiscence, and flap-related infections.

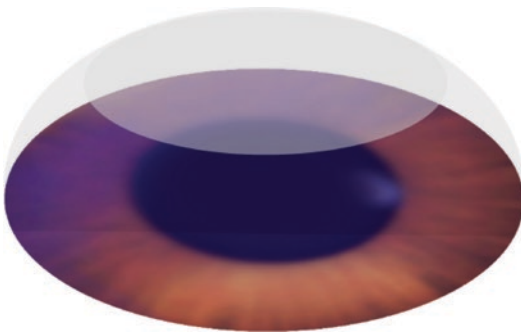


Fig. 14.5 Photorefractive keratectomy.

Indications and Contraindications

The primary indication for surface-based refractive surgery is the correction of refractive error, including myopia, hyperopia, and corneal astigmatism. Importantly, surface-based refractive surgery may be considered as an alternative in eyes that, with LASIK, would have a thin residual stromal bed (e.g., less than 250 microns), leading to an unacceptable risk of postrefractive surgery ectasia. There are other indications (and subindications) that have also been described. For example, phototherapeutic keratectomy (PTK) followed by PRK has been reported in the treatment of conditions such as epithelial basement membrane dystrophy with concurrent refractive error. Recent technological advancements, including the advent of topography-guided custom PRK, have facilitated the reduction of irregular astigmatism in postpenetrating keratoplasty eyes [43].

There are several contraindications to surface-based refractive surgery, some of which are considered absolute. These include pregnancy, corneal inflammation or infection (including herpes simplex virus or herpes zoster virus keratitis), neurotrophic keratopathy, and autoimmune disorders such as rheumatoid arthritis. Patients with a visually significant cataract should also be excluded, as well as those who would have a thin residual stromal bed after surface-based refractive surgery. Other contraindications include an unstable refraction (e.g., progressive myopia), moderate to severe dry eye syndrome, and the use of certain medications including isotretinoin. Patients with topographic signs of corneal ectasia (including forme fruste keratoconus) should also be excluded from surface-based refractive surgery alone; it should be noted that good outcomes have been reported with corneal collagen crosslinking followed by topography-guided PRK, and this technique may become more widely adopted [44].

Surgical Techniques

PRK involves the instillation of a topical anesthetic, antisepsis (e.g., using povidone-iodine),

and the placement of an eyelid speculum. This is followed by the removal of the corneal epithelium overlying the treatment zone. This step may be performed with the assistance of alcohol, mechanically, or with a transepithelial laser; however, alcohol-assisted epithelium removal is most common and appears to provide a smooth underlying surface for subsequent ablation. Alcohol-assisted epithelium removal involves the use of a circular instrument that is placed over the cornea and filled with 18–20% ethanol for approximately 30 seconds at which time the alcohol is absorbed using a small sponge. The instrument is removed, and the epithelium within the alcohol exposure zone is gently debrided using another small sponge. The excimer laser is then applied. MMC may then be applied to prevent haze formation (e.g., a 6 mm round sponge soaked in MMC 0.02%, placed over the ablation zone for 30 seconds), after which the eye is copiously irrigated. Finally, a steroid drop, antibiotic drop, and bandage contact lens are placed.

The initial steps in LASEK are similar to PRK. Once the eyelid speculum is placed, the cornea is marked with overlapping 3 mm circles in the periphery. A semisharp circular instrument is then placed centrally into which 18–20% ethanol is placed. After approximately 30 seconds, the alcohol is absorbed using dry sponges, and if needed alcohol is placed (and absorbed) again. Next, the semisharp circular instrument is removed, and a modified Vannas scissor is used to create a hinged epithelial flap. Once the excimer laser and MMC have been applied to the stroma, the epithelium is gently floated back over the treatment zone using a balanced salt solution on an anterior chamber cannula (e.g., 27 gauge). The flap is carefully realigned using intermittent irrigation, after which it is allowed to dry for at least 2 minutes. A steroid, antibiotic, and bandage contact lens are then placed.

Epi-LASIK also involves the creation of an epithelial flap, but instead of alcohol (as in LASEK), it is created using a microkeratome; Pallikaris et al. first described the use of a modified microkeratome with an oscillating blade for this purpose. After placement of the eyelid speculum and irrigation of the corneal surface using an

anterior segment cannula, the surface is dried with a sponge, and the cornea is marked using a LASIK marker. Next, the microkeratome is applied to eye, and once suction is achieved, the oscillating blade is advanced, causing epithelial separation and creating a 2–3 mm nasal hinge. The suction is then released, and the microkeratome is removed from the eye. The epithelial sheet is then reflected nasally. The remaining steps are similar to LASEK.

Conclusion

Continued developments are likely to improve visual outcomes and patient satisfaction from this category of refractive surgery. For example, topography-guided custom PRK is now available in the USA, and the results thus far are promising [44]. Corneal collagen crosslinking (CXL) followed by topography-guided custom PRK in eyes with keratoconus appears to improve both uncorrected and best corrected visual acuity compared to CXL alone and appears to have a low risk of worsening ectasia [45]. Transepithelial PRK has also been the subject of recent investigations [46]. Further research is required to determine the long-term safety and efficacy of these techniques.

LASIK and SMILE

LASIK is a well-established and commonly used refractive procedure worldwide. SMILE, on the other hand is a newer technique, but rapidly gaining popularity since its availability in 2011. SMILE is gaining increasing popularity primarily because of the noninvasive nature of the procedure while producing comparable refractive and visual outcomes to that of LASIK. The noninvasive nature comes from the absence of flap in SMILE, instead involving the direct extraction of a stromal lenticule through a 2 mm keyhole incision. The flap absence contributes to better corneal biomechanical integrity during wound healing. The corneal biomechanical integrity is maintained better in a flapless procedure theoretically because both the stromal cohesive and tangential tensile strength

are stronger in the anterior portion of the cornea than in the posterior portion. In fact, the cohesive tensile strength at the anterior 40% of the corneal stroma is thought to be at least 50% stronger than at the posterior 60%. Flap-based procedures such as LASIK sever the stronger anterior corneal lamellae, leaving the cornea with the weaker cohesive tensile strength [47–52]. Severing the anterior corneal lamellae also contributes to stromal thickening during the postwound healing phase as the peripheral anterior lamellae are no longer under tension, and they relax and spread out.

The corneal biomechanical integrity has been studied using conceptual and computational modeling studies. A study comparing the contralateral eye after SMILE and flap-based corneal refractive surgery resulted in a 49% (of 10 eyes, range: 2% to 87%) greater mean reduction of effective stromal collagen fiber stiffness within the flap region compared to the SMILE eyes [53]. Mechanical strain is linked to higher chances of corneal ectasia. Another study comparing posterior corneal elevation showed backward shift of central posterior surface in both LASIK and SMILE at postoperative 3 months, while at postoperative 3 years, SMILE showed stable posterior mean elevation (PME), and LASIK showed more posterior shift of PME [54].

Patient Evaluation for LASIK and SMILE

LASIK

When evaluating patients for LASIK, patient history, physical examination, and LASIK testing are important. Patient history includes past ocular/medical history – systemic diseases (diabetes, autoimmune diseases), ocular conditions (ocular herpes, peripheral keratitis), previous history of strabismus, and patients with thin corneas. The ocular conditions are likely to resurface after LASIK surgery, and the systemic diseases may alter the normal wound healing process. Medications such as ImitrexTM (for migraine), AccutaneTM (for severe acne), and other over the counter antihistamines can alter the wound healing process and can cause dryness of the ocular surface. Other patient history to consider are

patient lifestyle, family history of corneal transplant, and patient expectation of the visual outcomes after surgery. LASIK testing includes dry eye, contrast sensitivity, and pupil testing, as well as pachymetry, keratometry, corneal topography, and wavefront analysis. Other general physical examination such as visual acuity, refraction, and complete eye examination, including posterior dilated exam and tonometry, are also done.

SMILE

The important parameters to consider when performing the procedure are the desired refractive correction, the optical zone, lenticule side cut angle, minimum lenticule thickness, cap diameter and thickness, cap side cut angle, and fluence levels. Other parameters to consider are the patient age, refractive error, residual stromal bed thickness, and scotopic pupil size. In this respect, the SMILE patient evaluation parameters are similar to the LASIK procedure.

For SMILE patient evaluation, it is ensured that the patient has myopia of 1.00 D to -8.00 D and astigmatism of ≤ -0.50 D; this is the treatment range that has been approved by the FDA in the USA [55]. Outside the USA, myopia of up to -10.00 D combined with astigmatism of up to -5.00 D has been treated [56]. The problem associated with managing thin lenticules in low myopia correction has been attempted to be resolved experimenting with a wider optical zone. The FDA reported good visual outcomes with a minimum peripheral lenticule thickness of $15\text{ }\mu\text{m}$ [57–58], and with increasing surgeon experience, a $10\text{ }\mu\text{m}$ thick lenticule is recommended to avoid excess removal of stromal tissue. The SMILE procedure is still under investigation regarding hyperopic correction. However, encouraging preliminary results have been reported from using a lenticule profile of 7-mm optical zone with a 2-mm transition zone [59].

Surgical Techniques

LASIK

LASIK involves refractive correction by combining lamellar corneal surgery with the accuracy of

the femtosecond laser. The surgical procedure involves two steps: (1) flap creation in the cornea and (2) ablation of the stromal tissue depending upon the desired refractive correction (Fig. 14.6). The flap creation process takes only a few seconds, with the use of either a mechanical microkeratome or a femtosecond laser. Femtosecond laser offers more precision and safety than the microkeratome. The flap is gently lifted to expose the stromal tissue, which is to be ablated. The ablation step involves ordinary, wavefront- or topography-guided excimer laser to permanently remove the desired amount of stromal tissue. In myopic ablation, the central cornea is flattened relative to the periphery, while in hyperopic ablation, the central cornea is made steeper relative to the periphery. Larger ablation diameters are needed for effective treatment of hyperopia compared to myopia. For hyperopic astigmatism, the flat meridian is steepened by ablating tissue along the paracentral area. Mixed astigmatism is corrected employing the crosscylinder and bitoric techniques [60]. In the crosscylinder technique, the cylinder power is divided into two symmetrical parts, and one-half is treated on the positive meridian while the other-half is treated on the negative meridian [60]. In the bitoric technique, a combination of paracentral and cylindrical ablation is employed to flatten the flat meridian, so that the axis is steepened [60–64].

The patient's refractive needs are considered when choosing wavefront- or topography-guided excimer laser profile. Wavefront technology can measure both the higher and lower order aberrations, as well as provide higher precision in increments of 0.10 D or smaller for the standard 0.25 D adjustment ceiling. Topography-guided LASIK takes into consideration the spherocylindrical correction as well as the corneal shape to calculate the ablation profile.

SMILE

The curved contact glass surface of the femtosecond laser is docked onto the patient's cornea. As the contact is made between the cornea and the contact glass, a tear film meniscus appears, and the patient is able to see the fixation target (a flashing green beam of light) clearly (Fig. 14.7a).

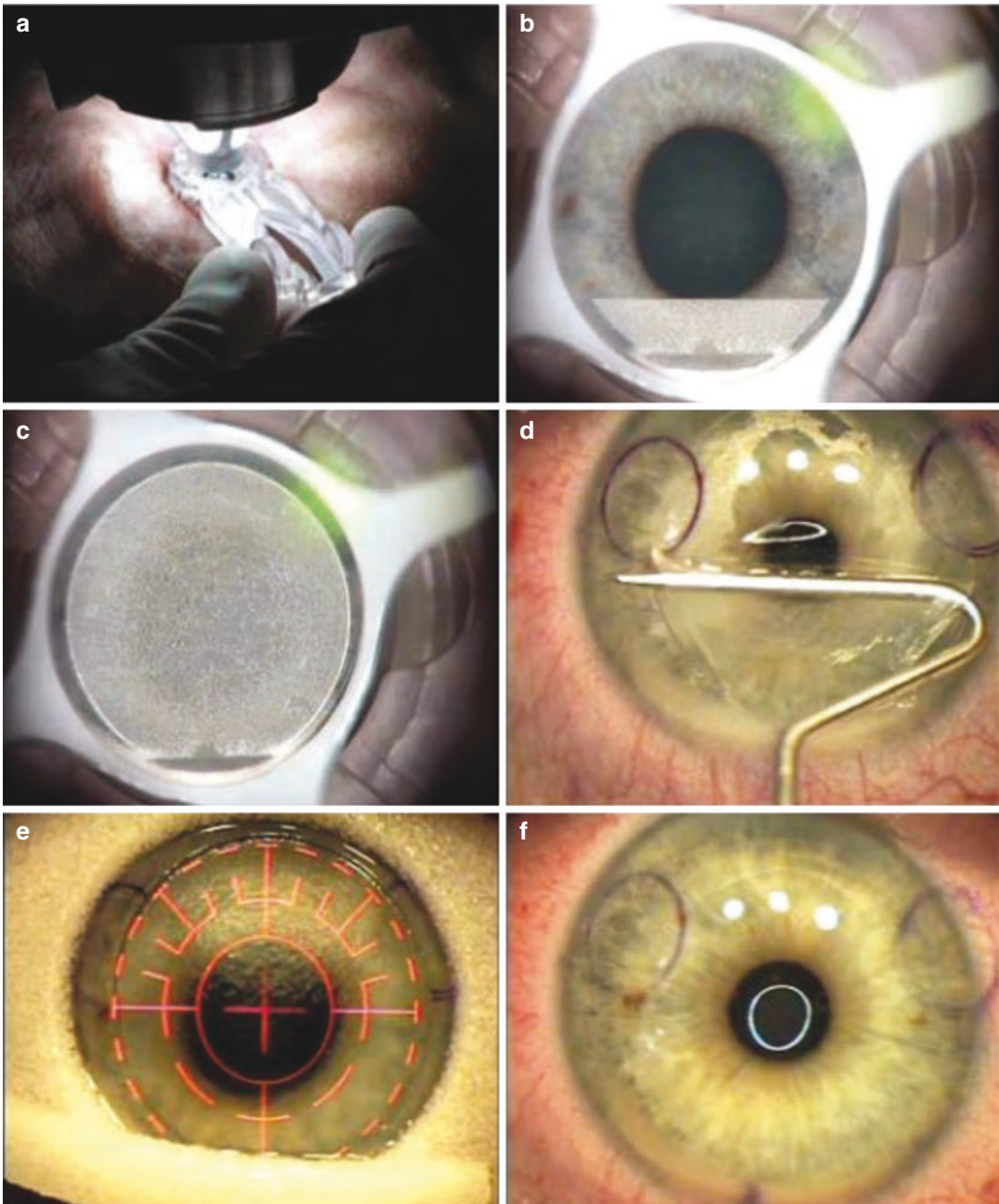


Fig. 14.6 Intralase™ femtosecond laser flap construction. (a) Placement of low pressure suction ring to align and stabilize the globe. A flat contact lens attached to the laser system is used to appanate the cornea. (b) Pocket is first constructed to collect the gases, then laser pulses are

delivered in previously programmed raster pattern. (c) Construction of side cuts. (d) Lifting of flap from stromal bed after disappearance of cavitation bubbles. (e) Laser ablation of stromal bed. (f) Reposition of flap. (Reprinted with permission from: Hallak et al. [176])

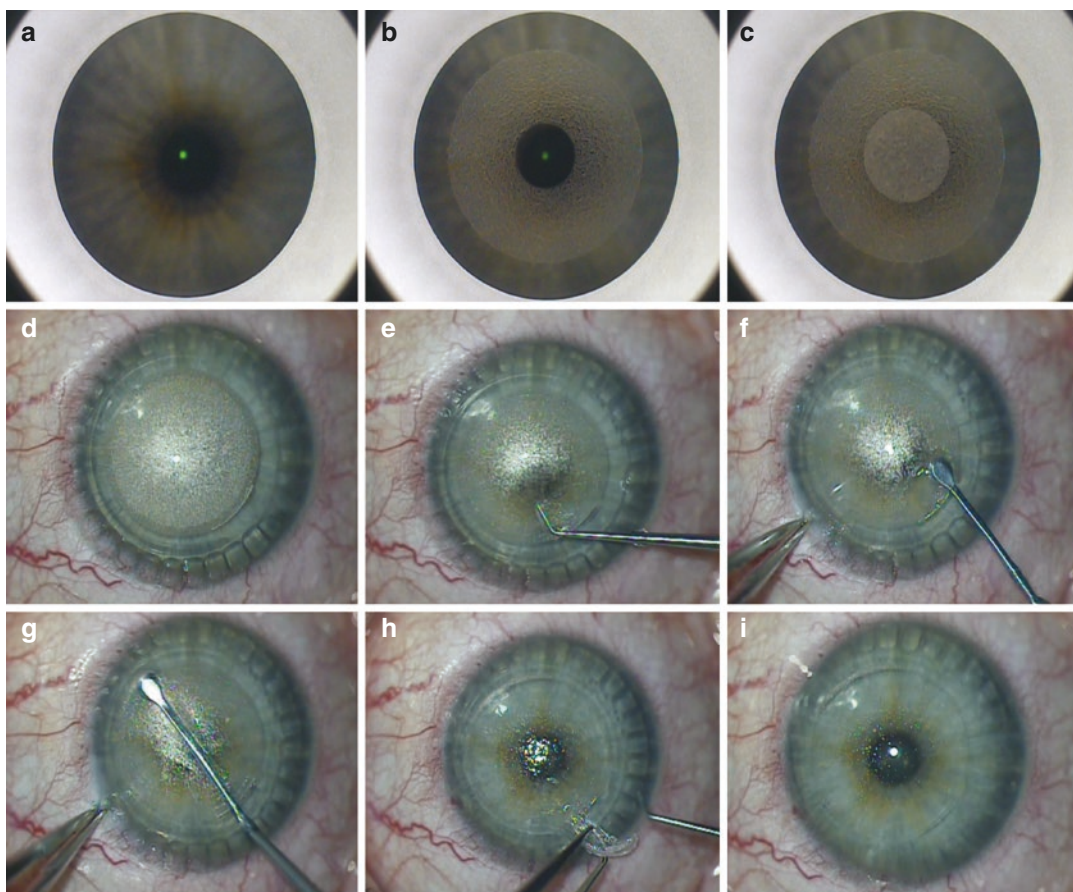


Fig. 14.7 SMILE surgery on a right eye. (a) Patient is asked to fixate green light. Note, this may not coincide with the center of the entrance pupil. Patient interface is aligned and suction started. (b) Refractive posterior surface of lenticule is created by spiraling-in application of femtolaser spots. (c) Anterior surface of lenticule is created parallel to anterior surface of the cornea (spiraling-out). (d) Complete laser application with superotemporal

incision. (e) Sidecut is opened with a semisharp tip. First, the upper lenticular surface is entered. (f) Upper interface is separated using a blunt spoon-shaped SMILE spatula (custom-made). (g) Lower interface is separated. (h) Lenticule is extracted with microforceps. (i) Finished SMILE procedure. (Reprinted with permission from: Giri et al. [177])

The vergence of the fixation beam is focused according to the patient's refraction to allow for this clear sight of the fixation target. When the patient focuses directly on the fixation light, the corneal suction port is activated to fix the eye in position and to align the visual axis. Besides this patient-controlled centration approach, the surgeon can also use infrared light to confirm the centration and then activate the laser. After the initial stage of adequate suction, the patient is usually able to maintain fixation as the intraocular pressure (IOP) rise is relatively small.

To make the lenticule cut, the lower interface of the intrastromal lenticule is created in a spiral in pattern first (Fig. 14.7b), followed by a 360° sidecut, which is followed by a spiral out pattern creation of upper lenticule interface (Fig. 14.7c), and then finally by a 2–4 mm superior or superotemporal incision for access (Fig. 14.7d). The access incision connects the upper lenticule interface (also known as cap) to the corneal surface. Total suction time is independent of the refractive error treated and ranges from 25 to 35 s depending upon the mode used.

When it is time to remove the lenticule, the tissue planes are defined by opening up the small incision and by identifying the anterior and posterior interfaces of the lenticule (Fig. 14.7e). A blunt, circular tip dissector is used in a windshield wiper–like fashion with the fulcrum centered at the incision to separate the upper interface (Fig. 14.7f). The lower layer is dissected very similarly (Fig. 14.7g). A pair of microforceps is used to grasp the lenticule and extract the lenticule after the interface separation (Fig. 14.7h). The lenticule can also be directly scooped out from within the pocket using the latest lenticule separation dissector. Minimal washing of the interface with a balanced salt solution at the end of the procedure helps clear the Bowman's membrane folds and better visual outcomes on postoperative day 1 [65] in terms of uncorrected distance visual acuity (UDVA) and contrast sensitivity.

A variation of the lenticule extraction technique, called “lenticuloschisis,” involving the gentle peeling of the lenticule off the stroma in a rhexis-like pattern instead of the actual dissection of the planes has also been reported [66]. The authors reported less surface roughness and irregularity on postoperative day 1 compared to the typical dissection technique. Prerequisites such as ideal bubble pattern, optimized energy levels, myopia of >3 D and good experience in conventional lenticule dissection technique were emphasized, however, before attempting the “lenticuloschisis technique.”

Long-Term Visual Outcomes After SMILE

From 5-year comparison studies of SMILE and FS-LASIK for myopia, no statistically different refractive stability was found between the two groups, although myopic regression was observed in terms of total corneal refractive power (TCRP) [67]. Another 5-year study of the visual outcomes in 616 astigmatic myopic eyes showed that uncorrected visual acuity (UVAC) was better in the fifth year compared to the immediate aftermath of the procedure, with 88% eyes having

UVAC of logMAR 0.1. The safety index was reported to be better on the fifth year than right after the invention with 92% eyes achieving refraction of 0.5 D within the SE target and 77% eyes losing no visual line. During the 5-year period, the SMILE-treated eyes showed regression of 0.24 D [68]. A third 5-year study of 56 myopic and myopic astigmatic eyes reported stable refractive outcomes with no significant changes in comparison to the 6-month follow-up results [69]. In this study, the spherical equivalent was reported to be -0.375 D with 32 of the 56 eyes gaining 1–2 Snellen lines, and no eyes losing 2 or more lines, and a long-term regression of 0.48 D. A 3-year follow-up study of the post-SMILE irregular astigmatism and curvature changes in 50 myopic astigmatic eyes showed a reduction of posterior astigmatism in high refractive corrections [70]. However, despite the compensatory effect of the posterior corneal surface, increase in irregularities was seen [70]. In contrast to the excimer laser–assisted techniques, the almost intact anterior lamellae and Bowman layer after SMILE could cause different kind of remodeling of anterior and posterior corneal surfaces after surgery. Han et al. reported 4-year refractive, wavefront aberrations and quality of life outcomes after SMILE for 47 moderate-to-high myopic eyes, concluding that the SMILE-corrected eyes showed predictable and stable refractive correction [71]. The reported efficacy index was 1.07 ± 0.16 with 89% eyes achieving the correction of ± 0.5 D of the intended refractive correction.

Complications of SMILE and LASIK

Intraoperative complications during SMILE include suction loss, opaque bubble layer, incision bleeding, incision abrasion, incision tear, epithelial defect, subconjunctival hemorrhage, difficult lenticule extraction, tear, unintended posterior plane dissection, inaccurate laser placement, and cap perforation [72–81]. The postoperative SMILE complications include dry eye, ectasia, diffuse lamellar keratitis (DLK), and interface lamellar fluid [82–88].

Intraoperative complications of LASIK include inadequate exposure, suction loss, corneal epithelial defect, irregular or incomplete cut, decentered flap, free cap, buttonhole, pizza slicing, and limbal hemorrhage [89–101]. Femtosecond-specific intraoperative complications include vertical gas breakthrough, anterior chamber bubble, and opaque bubble layer [89, 102–106]. The photoablation-related intraoperative complications include decentration, central islands, uneven ablation, overcorrection, and undercorrection [89]. Other intraoperative complications include flap destruction, interface debris, and wrinkle. Early postoperative complications of LASIK include interface debris, flap displacement and flap folds, flap striae and flap folds, sliding or dislodged flap, flap/cap loss, DLK/shifting sands of Sahara, pressure induced stromal keratopathy (PISK), central toxic keratopathy, epithelial ingrowth, flap melt, and infectious keratitis [89, 107–110]. The late postoperative complications include regression, induced or iatrogenic keratectasia, night vision problems and glare, transient light sensitivity syndrome, rainbow glare, dry eye, and neurotrophic epitheliopathy [89, 111–118].

The most-noted advantage of the SMILE procedure over LASIK is less occurrence of dry eye, owing largely to the preservation of corneal nerves in SMILE from the absence of the flap cut. Overall, analyzing the various indicators for the dry eye such as TBUT, Schirmer's test, OSDI score, and tear film osmolarity indicates that the SMILE procedure does not appear to exacerbate dry eye symptoms, whereas FS-LASIK appears to do so to some extent, at least up to 6 months. The major meta-analyses of SMILE vs. FS-LASIK outcomes have reported significantly higher corneal sensitivity in SMILE than FS-LASIK, especially until postoperative 3 months [119–122]. Although no significant differences in the Schirmer's test have been reported between the two procedures by the major meta-analyses, TBUT scores have been reported to be significantly better for SMILE than for FS-LASIK [119–123]. The comparison of the subjective OSDI score have also been reported to be significantly worse in FS-LASIK by the five major meta-analyses studies [119–123].

In case a retreatment is desired after the SMILE procedure, surface ablation has been reported to be a safe method of secondary enhancement by Siedlecki et al. who enhanced 43 of 1963 SMILE-treated eyes (2.2%) with intraoperative mitomycin C and surface ablation [124]. A second SMILE procedure below the existing interface also has been reported to be feasible by Donate and Thaeon [125]. When the lenticule cap is thin between 100 and 110 μm , FS-LASIK can be performed by converting the cap into the flap although the usable optical zone is limited with this method. A special software called "Circle software" is offered by VisuMax for cap-to-flap conversion where the flap is larger in diameter than the original cap [126].

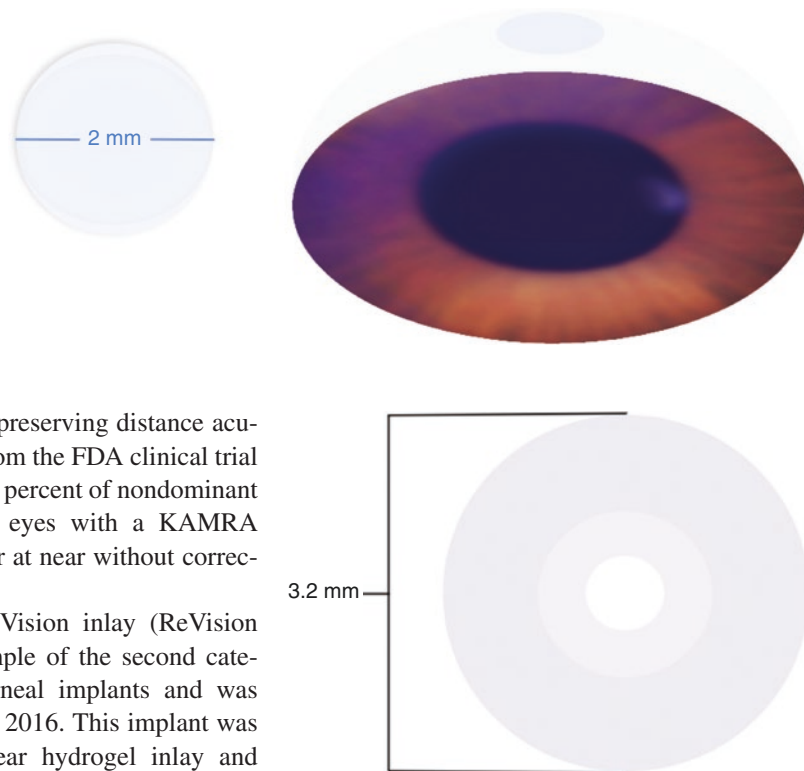
Presbyopic Corneal Implants and ICRS for KC

The concept of refractive corneal implants was first introduced by Dr. Jose Barraquer in 1949 [127–128]. Refractive corneal implants in use today can be divided into two broad categories: those that seek to address presbyopia and those that seek to primarily address irregular corneal astigmatism, such as in the setting of keratoconus. Presbyopic corneal implants are generally placed within the anterior stroma of the nondominant eye. These can be further divided into three categories: (1) those that have a small aperture and work by creating a pinhole effect (2) those that work by reshaping the central anterior corneal curvature and (3) those that have concentric rings with add power within the outer ring, similar to a multifocal contact lens or intraocular lens.

Presbyopic Implants

An example of the first category of presbyopic corneal implants is the KAMRA inlay (AccuFocus, Inc.). This implant was approved by the FDA in 2015. KAMRA is approximately 6 microns in thickness and 3.8 mm in diameter, with a 1.6 mm central opening. The small central opening creates a pinhole effect, allowing for

Fig. 14.8 Raindrop
Near Vision Inlay



sharp near vision while preserving distance acuity. Three-year results from the FDA clinical trial cohort revealed that 87.1 percent of nondominant emmetropic presbyopic eyes with a KAMRA inlay saw 20/40 or better at near without correction [129].

The Raindrop Near Vision inlay (ReVision Optics, Inc.) is an example of the second category of presbyopic corneal implants and was approved by the FDA in 2016. This implant was a 2.0 mm diameter clear hydrogel inlay and worked by increasing the central corneal curvature, thereby creating a hyperprolate anterior corneal surface; near objects could be viewed using central rays while distance objects could be viewed using paracentral rays (Fig. 14.8). Whitman et al. reported the results of a 1-year safety and efficacy study in which 373 nondominant emmetropic presbyopic eyes received the Raindrop inlay [130]. This study showed that 93 percent achieved an uncorrected near visual acuity of 20/25 or better. Eighteen inlays required replacement, usually due to decentration soon after placement; however, these eyes tended to have excellent visual outcomes. Revision Optics removed the Raindrop from the market in early 2018, presumably due to poor adoption among refractive surgeons.

Finally, an example of the third category of presbyopic corneal implants is the Presbia Flexivue Microlens (Presbia PLC). This implant is a 3.0 mm diameter clear hydrogel inlay, with a plano central zone, and a surrounding ring with an add power ranging between +1.50 diopters and +3.50 diopters (Fig. 14.9). It is currently

Fig. 14.9 Presbia Flexivue Microlens

undergoing FDA trials in the USA. In a study by Malandrini et al. from Italy, 26 presbyopic emmetropic eyes received the implant, and the mean uncorrected near visual acuity was 20/25 at 36 months postoperatively [131].

Intracorneal Ring Segments

The second broad category of refractive corneal implants, which primarily seek to address irregular corneal astigmatism, is comprised of intrastromal corneal ring segments (ICRS) (Fig. 14.10). These were first proposed by Fleming et al. in 1989 [132]. The authors constructed a mathematical model to determine the change in corneal curvature that may occur with the placement of an intrastromal corneal ring. Subsequently, Intacs corneal implants (Addition Technology, Inc.) were approved by the FDA in 1999 for use in the correction of mild to moderate

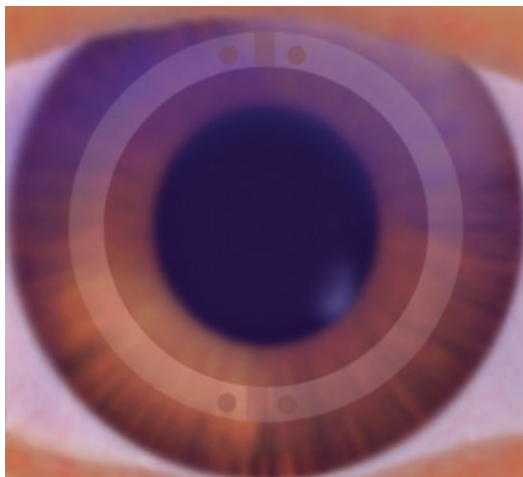


Fig. 14.10 Intracorneal ring segments

myopia, and in 2004 received a Humanitarian Device Exemption for use in the treatment of keratoconus. Unlike presbyopic corneal implants, which are typically placed in the anterior corneal stroma, ICRS are typically placed in the posterior corneal stroma, which facilitates their flattening effect.

In addition to Intacs, there are other ICRS types with varying specifications, including Ferrara (Mediphacos, Inc.), Myoring (DiopTex), and Bisantis (Opticon 2000 SpA and SolekoSpA). The position of ICRS can be determined with high precision using anterior segment optical coherence tomography [133]. Complications may include anterior or posterior extrusion of the ring segment as well as continued intolerance or inability to wear contact lenses for refractive correction.

It should be noted that the number of patients who require either ICRS or corneal transplantation in the setting of keratectasia may be decreasing due to the increased utilization of corneal collagen crosslinking (CXL), which was approved by the U.S. Food and Drug Administration in 2016. In addition to decreasing or halting the progression of keratectasia, CXL can lead to improvements in corneal astigmatism and visual acuity in some patients. As noted previously, its use has also been described in conjunction with PRK.

Indications and Contraindications

Presbyopic corneal implants may be considered for the nondominant eye in presbyopic patients, typically between 40 and 60 years of age, who have not yet developed a visually significant cataract. The primary indication is the correction of presbyopia. The central and paracentral cornea must be clear, and the corneal stroma must have adequate thickness. Although they may be placed in presbyopic emmetropic patients, they may also be considered in patients with refractive error as well as at the time of LASIK surgery. Similar to other refractive procedures, contraindications include corneal inflammation or infection (including herpes simplex virus or herpes zoster virus keratitis), neurotrophic keratopathy, and autoimmune disorders such as rheumatoid arthritis. Patients with a large angle kappa may be poor candidates for this procedure.

Patients with steep corneas that are not amenable to correction with a rigid contact lens (e.g., due to contact lens intolerance) but have adequate corneal thickness in the mid-periphery (at least 400 to 450 microns) may be candidates for ICRS. The central and paracentral cornea must be clear. The contraindications for ICRS are similar to other refractive procedures.

Surgical Techniques

A presbyopic corneal implant is placed into the patient's nondominant eye. It may be placed either within a stromal pocket or beneath a flap. After instillation of topical anesthetic, antisepsis (e.g., povidone-iodine), and placement of an eyelid speculum, the stromal pocket or flap may be created using a microkeratome or a femtosecond laser. A stromal pocket may be preferable due to less corneal nerve damage as well as a lower risk of decentration of the implant, and a femtosecond laser provides a more consistent depth and configuration (for either a stromal pocket or flap). The depth of insertion within the stroma depends on which implant is being placed.

The implant is typically inserted using instruments that are specifically designed for the device. Appropriate positioning of the implant is critical. The Purkinje light reflex (with the patient

fixating on the light) should be used as a primary guide with positioning over the pupillary center as a secondary guide. As noted above, patients with a large angle kappa may be poor candidates for this procedure. A topical steroid and antibiotic are given postoperatively.

The initial steps for ICRS placement are similar to those for presbyopic corneal implants. After placement of an eyelid speculum, the area of the cornea overlying the pupillary center is marked with a Sinsky hook. Using a mechanical technique, a 1–2 mm radial incision is made at approximately 70–80% depth using a preset diamond knife (the depth is determined using preoperative pachymetry). A semiautomated suction ring is then placed around the limbus after which semicircular dissectors are inserted in each direction through the radial incision (clockwise and counterclockwise) to create stromal pockets. The suction ring is then removed, and the ICRS are inserted into the stromal pockets. A circular stromal pocket may also be created using a femtosecond laser at 70–80% stromal depth. A topical steroid and antibiotic are given postoperatively.

Conclusion

Presbyopic corneal implants may improve near visual function in the appropriately selected patient population. Further research and development may lead to broader acceptance and utilization of this technology. ICRS may be used to reduce corneal astigmatism in patients with keratectasia, including from keratoconus and postrefractive surgery ectasia. Similar to presbyopic corneal implants, appropriate patient selection is critical for success with this procedure.

Lenticular Refractive Surgery

Lenticular refractive surgery includes surgery involving the crystalline lens of the eye. The lens can be absent (aphakia) or present (phakia) and the surgeries can involve procedures to correct cataracts, astigmatism, and presbyopia. Cataracts most often cause aphakia, but aphakia can also occur

due to certain injuries that damage the lens, or partially (subluxation)/completely detach the lens of the eye, or they can be congenital due to genetics. Surgeries for treating aphakia can include removing the damaged lens if necessary (pseudophakic), and then implanting artificial lens. The surgical outcomes are typically good; however, some complications such as aphakic glaucoma, and vitreous and retinal detachment are known to occur. Phakic lenticular surgery involves implanting a special type of intraocular lens to correct myopia or myopic astigmatism, leaving the natural lens of the eye untouched. The presbyopia-correcting lenticular surgery involves implanting multifocal, accommodating and extended depth of focus IOLs.

Phakic IOLs

Phakic IOLs are becoming increasingly popular due to the comparable visual and refractive outcomes as LASIK. In contrast to LASIK, the phakic IOLs do not require tissue ablation, instead they work by combining the power of the implanted lens with that of the natural lens to achieve 20/20 or better vision. A major advantage of the phakic IOL over LASIK is the capability of refractive correction of very high myopia levels of up to 23D, high hyperopia levels of up to 21D, and astigmatism of up to 7D [134]. Numerous studies have reported better visual outcomes after pIOL implantation in highly myopic patients than after LASIK [135–140]. Other advantages common for all pIOL models include excellent refractive stability, improved visual acuity, retention of accommodation, rapid visual recovery, and reversibility [136, 138, 141–142]. Complications include increased intraocular pressure from blockage of aqueous outflow, and intraocular tissue injuries although these are rare overall [143–144]. The IOLs are made of biomaterials carefully chosen to ensure great long-term uveal and capsular biocompatibility [145], material adhesiveness (expectation is that the IOL gets fused with the anterior and posterior capsule to prevent decentration and rotation) [146], and nutritional health of the cornea. Overall, phakic IOLs are the best option for the surgical correction

of high refractive errors. The two broad varieties of phakic IOLs available for clinical use include anterior chamber phakic IOLs (AC PIOLs) and posterior chamber phakic IOLs (PIOLs).

Anterior Chamber Phakic IOLs (AC PIOLs)

The anterior chamber models are of two subtypes: [1] angle supported and [2] iris claw. The anterior chamber angle-supported phakic IOLs include AcrySof Cachet (Alcon, Fig. 14.11a), Visian ICL (Staar Surgical, Fig. 14.11b), and Veriflex IOL (Fig. 14.11c). AcrySof IOL is only available for myopia correction (-6.00 to -16.50 D) and comes in four sizes (12.5 mm, 13.0 mm, 13.5 mm, and 14.0 mm). The Visian ICL is available for myopia correction (-0.25 to -18.00 D, hyperopia correction (0.50 to 10.00 D)

and astigmatism correction (-6.00 to 6.00 D, the brand name is Toric ICL). It is designed to fit in the ciliary sulcus and comes in four sizes for myopia/myopic astigmatism (12.1, 12.6, 13.2, and 13.7 mm) and in four sizes for hyperopia (11.6, 12.1, 12.6, and 13.2 mm) [147]. The Veriflex IOL is available for myopia correction (-2.00 to -14.50 D) and astigmatism correction (up to -5.00 , provided that the sphere plus cylinder does not exceed -14.50 D) [148]. It was developed based on the Verisyse platform and can achieve precise centration over the pupil and high rotational stability but requires some surgical skills for enclavation [149].

Posterior Chamber Phakic IOLs (PIOLs)

Three phakic posterior chamber IOLs are currently available: the Implantable Contact Lens

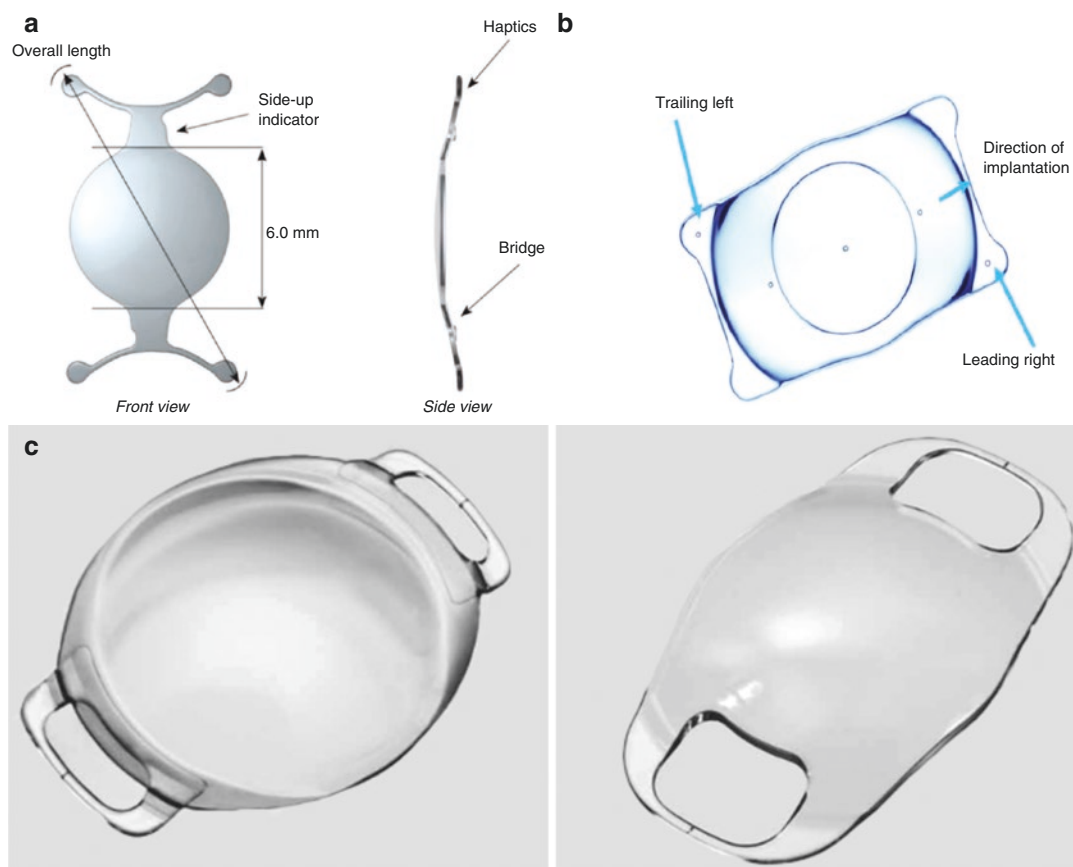


Fig. 14.11 Anterior chamber phakic IOLs (AC IOLs). (a) AcrySof Cachet (Alcon) IOL. (b) Visian ICL. (c) Veriflex IOL

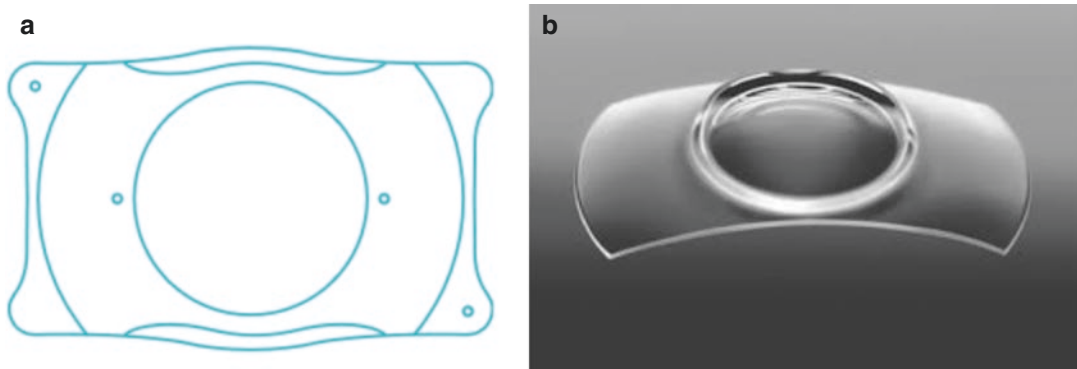


Fig. 14.12 (a) STAAR surgical ICL – made of collagen copolymer (acrylic and less than 0.1% porcine collagen) with a refractive index of 1.45 at 35 °C; optical zone diameter between 4.5 and 5.5 mm for myopia and 5.5 mm for hyperopia; available powers of -3 to -23 D for myopia and $+3$ to $+21.5$ D for hyperopia. The new (2011) model, V4c Visian ICL with KS Aquaport, VICMO

incorporates a central 0.36 mm diameter port that precludes the need for preoperative iriditomies (b) CIBA PRL – made of ultrathin silicon polymer, with refractive index of 1.46; length of 10.8 or 11.3 mm (myopia) and 10.6 mm (hyperopia); and width of 6.0 mm; available powers of -3 to -20 for myopia and $+3$ to $+15$ for hyperopia

(ICL; STAAR Surgical), the Phakic Refractive Lens (PRL; CIBA Vision, Embrach, Switzerland), and the Sticklens, (IOLTech, France), which is currently under evaluation (Figs. 14.12a, b). Reported complications include endothelial cell loss and lens opacification. A 8-year follow-up study reported the opacification rate of 5% at 8 years, with phacoemulsification rate of 5% (41 eyes total) [150] while a 10-year follow-up study reported 28% at 10 years, with phacoemulsification rate of 17% at 10 years (111 eyes total), respectively [151]. Another 10-year follow-up study reported 55% lens opacification (CI 45–63%) with 18% phacoemulsification rate (CI 10–26%) out of 133 eyes [152]. With the zonular-supported PRLs, although quite infrequent, cataract formation and a rare, specific complication of PIOL, posterior luxation, are reported. Preservation of corneal anatomy and asphericity, image magnification, potential gain in vision lines, and less reduction of contrast sensitivity are some of the advantages of PIOLs over refractive surgery. Compared to AC PIOLs, the advantages of PIOLs are fewer incidences of halos and glare and less endothelial cell destruction. However, some complications that may occur include cataract formation, endothelial cell damage, pupillary block glaucoma, pigment dispersion,

inflammation, and infection. Cataract formation incidence is also higher for PC PIOLs compared to AC PIOLs because of the normal nutrition impairment of the natural lens due to proximity between it and the IOL [153].

Toric IOLs

Toric IOLs are suitable for treating both cataract and astigmatism simultaneously. The first toric IOL was designed by Shimizu et al. in 1992 to correct corneal astigmatism during cataract surgery [154]. This lens was a three-piece polymethyl methacrylate (PMMA) nonfoldable IOL that required a large 5.7 mm corneal incision. Two kinds of toric phakic IOLs are available that are suitable for postkeratoplasty surgery: [1] the iris-fixated toric Artisan/Verisyze and [2] the posterior chamber Visian T-ICL (toric implantable Collamer lens). The IOLs can be placed opposite a clear corneal incision, on the corneal incision on the steep meridian, on the peripheral corneal relaxing incision (up to 9D) to treat astigmatism [155]. It is important to measure preoperative corneal astigmatism accurately, and these can be achieved by methods of corneal topography, manual and automated keratometry, and Scheimpflug imaging. The IOL power calculations can be done by calculation programs available; standard astigmatism vector analysis based individually calculated

personalized IOL calculation is more accurate than standard calculation method.

Typically, good visual outcomes are reported toric IOL implantation with a very small amount of residual astigmatism [156–158]. Multifocal toric IOL implantation can offer spectacle independence for near, distance, and intermediate vision independent of corneal astigmatism [159–160]. However, there are chances for more complications after multifocal toric IOL implantations such as accurate estimation of corneal astigmatism and rotational stability [159]. Overall, toric IOLs are reported to provide satisfactory astigmatism correction, often providing better results than monofocal IOLs or limbal relaxing incisions (LRIs) [156]. Bilateral toric IOLs are even reported to improve subjective vision quality [157].

APHAKIC IOLs

Charles Kelman started the modern era of cataract surgery with the introduction of phacoemulsification surgery in 1967 [161]. Cataract surgeries can be performed with multifocal, accommodating or toric IOLs.

Cataract Surgery with Multifocal IOLs (MFIOLs)

Multifocal IOLs are usually the preferred option for achieving spectacle independence across a wide range of distances postcataract and postrefractive lens exchange surgery. Most important factors that influence the choice of MFIOLs include patient's age, needs, lifestyle, and psychological profile; patient's pupil reactivity and size in various light conditions; patient's ophthalmic condition and associated eye comorbidities (especially relating to contrast sensitivity function); evidence from peer-reviewed literature, especially the defocus curve of the lens; and surgeon's attitude, education, and experience [162]. Three designs of available MFIOLs are refractive, diffractive, and a combination of the former two designs. Refractive MFIOLs can be rotationally symmetric or asymmetric, and work by providing appropriate focus for both near and distant objects through the annular zones of various

refractive powers. Pupil size dynamics and decentration, intolerance to kappa angle, rough areas between zones (contributing to potential halos and glare), and loss of contrast sensitivity affect the visual outcomes of refractive MFIOLs [162]. Diffractive MFIOLs contain diffractive microstructures in concentric zones and decreasing distance between the annular zones, called the Fresnel-zone plate to produce optic foci. Near multifocality is achieved by the combination of anterior and posterior surface powers along with 1st order diffraction, while distance multifocality is achieved by the combination of anterior and posterior surface powers along with 0th order diffraction [162]. Compared to the refractive MFIOLs, the diffractive MFIOLs are more tolerant to the decentration and kappa angle, and less pupil size dependent, but they have a higher potential for glare and halos due to the nontransition areas. Some commonly used MFIOLs include Restor bifocal IOL, AcrySof refractive-diffractive IOL, PanOptix (Alcon) trifocal/refractive IOL, At Lisa (Carl Zeiss Meditec) bifocal/trifocal diffractive IOL, and Mplus Lentis (Oculentis) bifocal refractive IOL [155].

A systematic review and meta-analysis [163] of multifocal vs monofocal IOL outcomes based on 21 randomized controlled trials (RCTs) with 22,951 subjects found that MFIOLs performed better on uncorrected intermediate VA (at 60 cm) and uncorrected near VA, as well as distance corrected intermediate VA (at 60 cm) and distance corrected near VA compared to the monofocal IOLs. No statistically significant differences were found between the two groups for uncorrected and corrected distance VA. In terms of contrast sensitivity and spectacle independence, the MFIOL group performed better than the monofocal group; however, the patients experienced greater amounts of glare and halos in the MFIOL group [163]. A few other studies also have reported more dysphotopsia, glare, and halo (3.5 times more) in MFIOLs in comparison to monofocal IOL implantations [164–165], although dysphotopsia tends to reduce over time due to neuroadaptation. Other issues resulting from multifocal IOLs are night driving and low-contrast issues [166]. Another interventional case

series study [167] of 26 emmetropic presbyopic patients who underwent trifocal diffractive IOL implantation following femtolaser-assisted cataract surgery (FLACS) and refractive lens exchange (RLE) found satisfactory near, intermediate, and distance visual outcomes at 6 months. No intraoperative or postoperative complications were observed, and 96% [24] patients said that they would recommend the procedure to their family and friends [167].

Cataract Surgery with Accommodating IOLs

Accommodating IOLs work by providing dynamic increase of dioptric power as per the focus needs at near, intermediate or distance vergence [168–169]. The IOL mechanisms can be truly accommodating or pseudoaccommodating. Pseudoaccommodative mechanisms include miosis, higher-order aberration induction and lens tilt [168]. Shape changing, single or dual optic position changing, lens filling, and refractive index modulating mechanisms are some of the design strategies that are employed in designing accommodating IOLs [168]. The Crystalens® (Crystalens Bausch and Lomb, Inc., Rochester, NY) was the first accommodating IOL to be approved by the FDA – the original version was approved in 2003, the “high-definition” version in 2008, and the toric version in 2010. Trulign® (also by Bausch and Lomb) is another accommodating IOL to be approved by the FDA, but it is currently only allowed to be described as offering “a broad range of vision” instead of accommodating [170]. The Crystalens is a biosil-based hinged plate-haptic IOL, which is thought to provide accommodation through changing the position and shape of the axis [168]. A 2010 meta-analysis [171] comparison of accommodating and monofocal IOLs restoring accommodation after cataract surgery looked at 12 RCTs with 727 eyes and found greater anterior displacement of accommodating IOLs (an average of 0.84 mm displacement has been reported from cyclopentolate cycloplegia to pilocarpine-stimulated accommodation [172]), although heterogeneity was reported among different studies using different testing methodologies.

Good visual outcomes are reported in the literature with the implantation of accommodating IOLs. In a study of patient satisfaction levels at a mean of 5.4 years after cataract surgery implantation with bilateral accommodating IOL, 90% of 68 patients reported being “very satisfied” [173]. The study compared the patient satisfaction rates between accommodating IOL and multifocal IOL implantations; patients in MFIOL group experienced more glares and halos compared to the accommodating IOL group [173]. A complication that can occur during the accommodative effort with Crystalens is capsular contraction syndrome, occurring from the changes in the tilt/shape of the IOL (called “accommodative arching”). The “accommodative arching” can temporarily induce myopic astigmatism and/or higher-order aberrations. These effects are varied depending on the variability of capsular bag size, fibrosis, and medications with anticholinergic side effects. In some cases, the “accommodative arching” can alter the intended position of the IOL optic, resulting in a z-syndrome with asymmetric capsular contraction leading to astigmatism along the IOL axis [168]. Some methods to mitigate the complication of z-syndromes include early treatment of capsular fibrosis and striate with Nd:YAG laser, insertion of capsular tension ring and IOL exchange depending on the severity of the condition [168, 174]. Measures to avoid the formation of z-syndrome includes creating a round, central capsulorhexis with anterior capsule covering plate haptics, polishing the underside of anterior capsular leaflets, meticulous cortical cleanup, rotation of IOL vaulted posteriorly along the posterior capsule, and construction of a water-tight wound [175].

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The Role of Keratoprosthesis in the Treatment of Corneal Blindness

15

Mona Harissi-Dagher

Introduction

The recovery of vision in patients with corneal blindness has become increasingly successful with advances in keratoplasty techniques progressing since the beginning of the twentieth century. While it is possible for grafts to remain clear for decades, graft failure for all diagnostic categories is not insignificant [1–4]. The prognosis for subsequent graft failures is worse for all subgroups, especially in the multiple regrant failures category [5–7]. Furthermore, generally accepted risk factors for graft failure such as glaucoma, multiple surgeries, presence of inflammation, and severity of neovascularization are usually more prevalent in subsequent grafts and tend to develop with time in initial grafts [2]. At present, keratoprosthesis surgery appears to be a reasonable option for this regrant failures group of patients.

A considerable amount of research on the topic of keratoprosthesis development has been undertaken by several groups of investigators. Recent advances aimed at preventing and treating early complications after keratoprosthesis surgery have improved the outlook and prognosis of patients undergoing the surgery.

History

The concept of an artificial cornea in the treatment of corneal blindness was first suggested in writing by the noted French surgeon, Pellier de Quengsy, in 1789, at the time of the French Revolution [8]. This was followed by further efforts in design and insertion techniques [9–13]. However, extremely high incidences of early complications were associated with those keratoprosthesis, which typically failed owing to tissue necrosis with subsequent leak, infection, and extrusion of the device. After 1906, when the first successful human-to-human corneal graft was performed, attention was diverted away from keratoprosthesis development until many years later when it was realized that penetrating keratoplasties would not be successful in all cases [14, 15].

After 1950, keratoprosthesis research gained momentum. During World War II, it was noticed that polymethylmethacrylate (PMMA) splinters imbedded in the corneas of pilots were well tolerated. This led to experiments showing that PMMA discs could be retained in the cornea of rabbits [16–18]. Soon thereafter, human applications followed, and many ophthalmologists attempted to refine their procedure using these new inert plastics. Once again, however, many of these cases were associated with severe complications, and the procedure lost favor with many surgeons. Some ophthalmologists, nevertheless,

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persevered in developing their techniques and polished them over the years.

The current keratoprosthesis effort is maintained primarily in approximately a dozen centers worldwide and diverse approaches exist [19–32].

This chapter is dedicated to the Boston Keratoprosthesis created by Dr. Claes Dohlman in the 1960s. The promising outcomes of implantation of this device at the Cornea Service of the Massachusetts Eye and Ear infirmary and, later on, in several centers internationally over the last 50 years will be outlined. This chapter describes the Boston keratoprosthesis devices, design and material, surgical techniques, and follow-up routines.

Design and Material

For a number of years, a PMMA keratoprosthesis of double-plated collar button design was used [33–35]. The device consists of two plates joined by a stem, which constitutes the optical portion

and locked in position. It has undergone a number of important design changes since the mid-1960s. The diameter of the front and back plates, the stem diameter, the absence or presence of holes, and their diameter in the back plate have varied, the presence of a locking ring or a snap on back plate have all been developed to improve its design and retention.

The double-plated devices we currently use come in two main designs (Fig. 15.1) [36]. Type I, the simple collar button, is the most frequently used. It is favored in eyes with reasonable blink and tear secretion mechanisms. The advantages of this design include a short optical stem, which provides a good view with the slit lamp, a generous visual field, and good stability because the wide plates prevent tilting of the device off the visual axis. Another noteworthy change in the design is the addition of holes within the backplate, which allows for better access for nutrition from the aqueous as well as rehydration of the corneal stroma adjacent to the stem. This helps to prevent necrosis of the surrounding tissue [37]. The profile of the keratoprosthesis

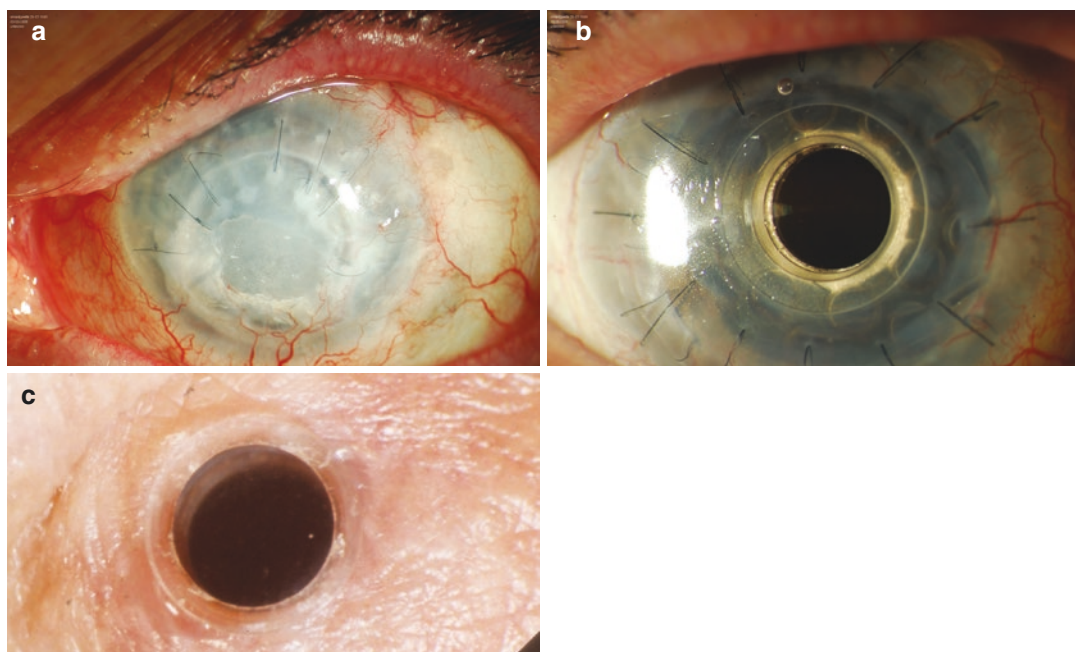


Fig. 15.1 Successful implantation of type I and type II Boston Keratoprosthesis. (a) Prekeratoprosthesis type I. (b) Postkeratoprosthesis type I – collar button-shaped device

used in eyes with adequate tear secretion and blink mechanism. (c) Keratoprosthesis type II – device with an added nub to be implanted through the eyelid in end-stage dry eyes

was also redesigned both to minimize a dellen effect and allow a contact lens to fit comfortably over it [38].

Type II is reserved for end-stage dry eye conditions. It is similar to keratoprosthesis type I except that it has a 2 mm long anterior nub designed to penetrate the skin. It is believed that if the prosthesis is allowed to protrude through the skin, protection of the ocular surface is enhanced and extrusion rate is consequently reduced.

Optically, both types of devices are manufactured with a range of dioptric powers, allowing selection to approximately fit the axial length of the patient’s eye. They are prepared for pseudophakia and aphakia in case the IOL or the natural lens is removed.

Indications and Prognostic Categories

Keratoprosthesis is a procedure in evolution, and because the outcome of keratoprosthesis surgery differs markedly among various corneal diseases, the indication for such surgery should be categorized accordingly. In general, some criteria must be fulfilled before qualifying for the procedure. First, end-stage retinal pathology, optic nerve disease, or end-stage phthisis constitute a contraindication. Second, monocular status, young age, or poor general health should be taken into consideration since they raise more concern. In patients with bilateral involvement who have little or no chance of success with a standard penetrating keratoplasty, the only hope lies in a keratoprosthesis. Whether a procedure is advisable is dependent not only on the afflicting condition but also on the experience and time commitment of the surgeon. This said, guidelines can be suggested.

One of the principles that we have recognized is that among diagnostic categories, there exists a prognostic hierarchy [39]. This hierarchy begins with repeat graft failure patients, including those with a diagnosis of HSV; these patients tend to fare best. Patients having sustained chemical burns, in whom glaucoma is perhaps the most important long-term complication, are more difficult. Finally, the autoimmune and inflamma-

Table 15.1 Keratoprosthesis prognostic categories

From best to worst
1. Noninflammatory conditions: graft failure, dystrophies
2. Infectious: HSV, HZV, bacterial and fungal ulcers
3. Chemical burns
4. Autoimmune: ocular cicatricial pemphigoid, Stevens-Johnson syndrome

tory diseases such as Stevens-Johnson syndrome and ocular cicatricial pemphigoid have the worst prognosis with any surgical intervention, including keratoprosthesis surgery (Table 15.1) [40]. Because the long-term retention of visual acuity and the integrity of the eye are critical end-points in assessing traditional grafts, the importance of the concept of Kaplan Meier survival/life table analysis in reviewing the success and failure of keratoprosthesis and its alternative procedures cannot be overemphasized. Although keratoprosthesis retention has traditionally been a significant barrier to the long-term success of such devices, today’s reality is much more encouraging.

Patient Evaluation

History

A detailed history of the ocular condition as well as any important systemic disease is mandatory. This usually reveals the underlying cause of the corneal condition whether traumatic, surgical, or inflammatory in nature. Duration of symptoms, laterality of the condition, and its episodic or progressive course can be elicited. Details and dates of previous surgery (keratoplasty, cataract extraction, glaucoma shunt, retina repair, etc.) should be solicited. History of glaucoma is particularly important in predicting outcome, especially following chemical burns.

Visual Acuity

Visual acuity should be recorded in the standard fashion using a Snellen chart. Relative contributions of the cornea, cataract, retina, or optic nerve

are difficult to ascribe in eyes so severely damaged that keratoprosthesis is a necessity. If the corneal surface is highly irregular in the presence of only moderate stromal opacities, hard contact lens refraction can be revealing. A standard visual field test is rarely applicable in these cases; alternatively, gross projection of a strong light source is useful to assess. Testing central fixation, and, particularly, light projection nasally is often helpful. If nasal projection is lost, end-stage glaucoma must be suspected.

Intraocular Pressure

Severe corneal damage often makes exact intraocular pressure measurement impossible and precludes view of the optic nerve. Recording intraocular pressure can be fraught with error. Simple digital palpation, even if imprecise, is frequently a dependable approach.

Blink Rate and Tear Secretion

On examination, blink mechanism and tear secretion are important factors in assessing keratoprosthesis prognosis. Evaporative damage to the corneal tissue around a keratoprosthesis can be detrimental especially if a soft contact lens cannot be retained [38]. Blink rate and completeness can be verified when the patient does not feel observed. Lagophthalmos and frank chronic exposure are extremely important to recognize. Finally, tear break-up time may be valuable in assessing the health of the ocular surface.

Slit-Lamp Examination

This step of the evaluation is the cornerstone of the patient evaluation. Eyelids should be inspected for marginal incongruities. Conjunctival inflammation, surface keratinization, and fornix foreshortening or symblephara should be noted. The corneal surface should be examined for irregularity, keratinization, epithelial defects, and subepithelial vascularization. Stromal opacity from scarring or edema as well as deep vascularization

should be evaluated. Anterior chamber depth and reaction, and the status of the iris, pupil, and lens (or intraocular lens) all merit detailed notes. The fundus is often not observable in keratoprosthesis candidates, but when possible, an effort should be made to examine disc cupping and macular changes. Disc cupping has high prognostic importance and may dictate aqueous shunt implantation. Gross changes in the posterior pole, such as massive age-related macular degeneration, are vital to observe. Special attention should be given to signs of inflammation throughout the examination as its presence influences the prognosis of keratoprosthesis surgery.

Special Examination

Ultrasound examination is necessary in most cases. B-scan can reveal a retinal detachment or massive debris behind an opaque cornea or lens but it cannot measure glaucomatous optic nerve cupping with precision. A-scan measurement of the axial length of the eye is also required for proper selection of a keratoprosthesis with the correct dioptric power in aphakic eyes.

Documentation

Pre- and postoperative external photography of the eyes help document baseline and allow assessment of progress and outcome of the surgery.

Patient Selection

If a standard corneal transplant has a good chance of giving longstanding vision, this would be the preferred technique. However, if one or more graft failures occur within months after surgery, decreasing vision to finger counting or less, a keratoprosthesis may be considered.

Keratoprosthesis Type I

Keratoprosthesis type I is indicated in the noninflammatory graft failure group where blink and

tear mechanisms are reasonably normal and visual acuity is less than 20/400. As well, the status of the fellow eye must be factored in. The opposite eye should have suboptimal vision, such as 20/100 or less. Furthermore, the age of the patient is a consideration. If the long-term survival of the keratoprosthesis is questionable, it follows that elderly patients have a greater chance of trouble-free course than younger patients.

Patients with heavy exposure to evaporative forces may still be candidates for the procedure but would need extensive tarsorrhaphy and other lid reconstruction in order to avoid exposure of the surrounding tissues, limiting it to the PMMA surface.

Keratoprosthesis Type II

Keratoprosthesis type II is preferable in end-stage dry eyes as observed in ocular cicatricial pemphigoid, Stevens-Johnson syndrome, and chemical burns [39, 40]. This involves not only more complicated surgery but also a closer life-long follow-up regimen and more frequent revisions.

Postoperative Care

Postoperative care is primordial for the success of the KPro. Follow-up visits should be individualized but very frequent.

Type I

Prophylactic antibiotic after surgery must be maintained indefinitely. Excluding patients with autoimmune diseases or severe chemical burns, it seems adequate to treat patients with a fourth-generation fluoroquinolone for life. To enhance endophthalmitis prevention [41], vancomycin 14 mg/ml with benzalkonium once daily for life may be given. Compliance should be stressed.

Corticosteroid drops usually as prednisolone acetate 1% are given four times a day for some time, occasionally extended with lower doses for a year or two depending on the presence of post-

operative inflammation, always monitoring for steroid response.

The intraocular pressure is difficult to measure since essentially only finger palpation is possible. Disc appearance and visual field must be followed frequently. Topical glaucoma medications can penetrate into the eye despite the plastic barrier albeit slower than normal. Oral carbonic anhydrase inhibitors have the usual effect [42].

In cases of herpes simplex, addition of systemic antivirals is recommended on a permanent basis.

Type II

Sustained postoperative prophylactic antibiotics are even more important after type II than type I. Topical vancomycin 1.4% in addition to the standard fluoroquinolone is used no less than twice daily [43]. The drops are administered on an indefinite basis to the crevice around the nub.

Corticosteroids in high doses are essential during the first postoperative month to abate prolonged intraocular inflammation common in patients requiring a type II keratoprosthesis. Topically administered steroids do not reach the inside of the eye, and systemic administration has a less favorable risk-benefit than subtenon delivery.

In keratoprosthesis type II, an oral carbonic anhydrase inhibitor is the most reliable medical treatment for elevated intraocular pressure.

Complications

In past times, primarily tissue necrosis around the device, extrusion, and/or endophthalmitis ended the KPro effort, often with loss of the eye. During the last few decades, however, thanks to the work of several groups of surgeons and investigators, the picture has been much brighter. In most cases, the severe complications are seen within the first year after surgery; however, the patient is never safe from potential complications and requires frequent and close monitoring (Fig. 15.2).

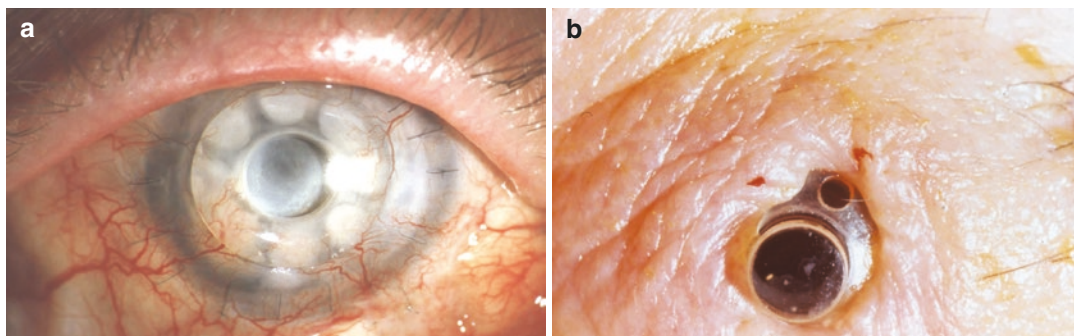


Fig. 15.2 Keratoprosthesis complications. (a) Retroprosthetic membrane in type I. (b) Beginning skin retraction around the nub in type II

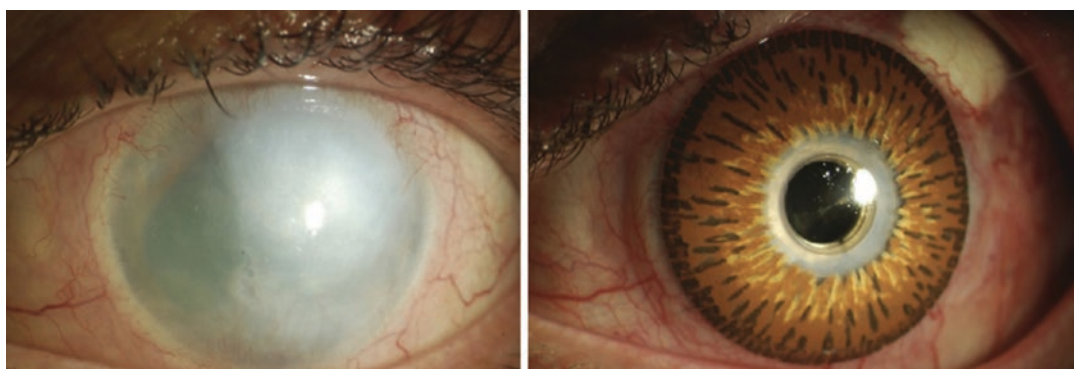


Fig. 15.3 Cosmetic in addition to therapeutic advantage of soft contact lenses with Boston Keratoprosthesis

Tissue Necrosis and Melt

Tissue necrosis and subsequent melt are now rare with type I keratoprosthesis. Adding fenestrations to the back plate has improved nutrition and hydration to the overlying corneal tissue. Prompt intervention is wise should the melt occur with a consequent leak and hypotony. A new keratoprosthesis in a new fresh graft should be implanted and protected by a soft contact lens.

In type II keratoprosthesis, skin can retract away from the nub secondary to evaporative damage of the skin edge and is hard to avert. Skin revision is advisable when skin retracts to the edge of the front plate. A new keratoprosthesis in a new fresh graft is recommended if a leak occurs [44, 45].

Soft Contact Lens Loss

Addition of a soft contact lens after keratoprosthesis type I surgery and its retention or replacement for an indefinite time has added a remarkable benefit to the health of the tissue around the device. Without a soft contact lens, evaporation and irregular drying of corneal tissue around the double-plated keratoprosthesis can be a disturbing problem. Drying, dellen formation, epithelial defects, and stromal thinning can occur with long-term undesirable consequences. However, the hydrophilic soft contact lens worn around the clock has been found to be highly protective. The lens seems to diffuse evaporative forces well and to allow better hydration [46]. At times, inadvertent loss of the lens requires replacement adding to the overall costs (Fig. 15.3).

Inflammation

In autoimmune diseases such as ocular cicatricial pemphigoid, Stevens-Johnson syndrome, graft-versus-host disease, a chronic low-grade intraocular inflammation complicates the course. Consequently, a retroprosthetic membrane, epiretinal membrane, and angle closure glaucoma may supervene. Corticosteroids are the standard treatment to suppress such developments. In type I keratoprosthesis, topical prednisolone drops are routine, sometimes augmented by peribulbar/subtenon injections of triamcinolone. Systemic steroids are used less commonly because of less favorable risk-benefit ratio. After type II surgery, drops cannot reach the anterior chamber, and, therefore, peribulbar/subtenon injections or systemic steroids are the only means to influence intraocular events.

Retroprosthetic Membrane

Intraocular inflammation post keratoprosthesis surgery can be prolonged and severe in autoimmune eyes. This frequently leads to a retroprosthetic membrane with decline in vision. Steroids are indicated at the first sign of such a membrane formation. Once formed, it is worthwhile to open the membrane with an Nd:YAG laser before it becomes too thick or vascularized [47]. Laser pulses with energy above 3.0 mJ are inadvisable because they can crack or pockmark the plastic. If the membrane becomes thick, leathery and, particularly, if vascularized, a closed vitrectomy under high infusion pressure and membranectomy are required to restore vision [48].

Infectious Endophthalmitis

This is the most dreaded complication after keratoprosthesis surgery. Vision can be lost permanently within hours. In our experience, the infectious agents have all been gram-positive organisms. However, bacterial endophthalmitis

has been nearly eliminated with adherence to the regimen of antibiotic prophylaxis of vancomycin and a fluoroquinolone [42, 43]. It is extremely important to impress upon the patient that meticulous compliance for life is mandatory. Should an endophthalmitis still occur, immediate tap and inject are crucial. Topical fortified antibiotics may be required. A vitrectomy may be deemed necessary in severe cases.

Sterile Uveitis: Vitritis

A sudden massive vitritis has been observed in a few patients with reduction of vision to hand motion. This vitritis masquerades as an infectious endophthalmitis with no accompanying pain, tenderness, or redness. Bacteria are usually not isolated in these cases. Still, these patients might be treated for suspected bacterial endophthalmitis. Within a few weeks or months, the vitreous clears and the vision returns back to the baseline level prior to the event [49].

Glaucoma

With the drastic reduction in endophthalmitis, glaucoma is now the most serious complication after keratoprosthesis surgery. Its pathogenesis is probably multifactorial, but inflammation and gradual closure of the anterior chamber angle are the most likely causes of marked aggravation of intraocular pressure. It is, therefore, vital to monitor the intraocular pressure and nerve damage postoperatively. Tonometers are useless in this setting, and digital palpation of the globe is the main method available to obtain a rough estimate of intraocular pressure. Glaucoma drops are effective only in keratoprosthesis type I but not type II. Oral carbonic anhydrase inhibitors have side effects and should be used with caution in patients with Stevens-Johnson syndrome and completely avoided in patients with sulfa allergy. Since medical control of glaucoma is often insufficient, an Ahmed valve shunt is indicated at the

time of the keratoprosthesis in autoimmune diseases, chemical burns, and in patients with preexisting glaucoma [42].

Retinal Detachment

Retinal detachment is not a common complication. It can be rhegmatogenous or tractional in nature. It is diagnosed by direct visualization or by B-scan ultrasonography. Three-port vitrectomy is performed, and a long-acting gas or sometimes silicone tamponade is used. The prognosis is ominous.

Conclusion

In conclusion, although standard penetrating keratoplasty has an excellent prognosis in the noninflamed virgin eye, the prognosis for repeat keratoplasties for graft failures, especially multiple graft failures, is relatively poor. On the other hand, the device retention and vision rehabilitation achievable with a keratoprosthesis type I compares favorably with repeat keratoplasty for multiple graft failures in nonimmune diseases [50] providing rapid visual rehabilitation.

The Boston Keratoprosthesis has excellent optics, stability, wide visual field, and good retention. The surgery is carried out reasonably well but postoperative follow-up is demanding. Complications may arise but have become less common through prevention, early recognition, and appropriate management. Successful outcome requires considerable patient compliance with antibiotics, glaucoma treatment, and frequent follow-up. Temporary postoperative tissue coverage, antiinflammatory medicine, and glaucoma shunts are among the factors that have improved keratoprosthesis survival. Although the management of keratoprosthesis patients requires a solid long-term commitment from the patient and the surgeon, the potential reward in terms of the visual rehabilitation in an otherwise hopeless clinical situation can truly be gratifying. The success of the Boston Keratoprosthesis will undoubtedly continue to spread owing to

rapid rehabilitation and improving long-term outcomes. In a career that now spans six decades, Dr. Dohlman has revolutionized the treatment of corneal blindness by developing and pursuing research on the Boston KPro, offering patients with little hope a viable option to recover sight.

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Corneal Crosslinking for Keratoconus and Corneal Ectasia

Peter S. Hersh and Steven A. Greenstein

Introduction

Corneal collagen crosslinking (CXL) is a treatment designed to decrease the progression of keratoconus [1], in particular, and other corneal thinning processes such as post-LASIK/PRK ectasia [2–5]. Additionally, studies have suggested that crosslinking also can have beneficial visual and optical effects, such as an improvement in corneal steepness, visual acuity, topography irregularity indices, higher-order aberrations, and subjective visual function in some patients [6–13].

Mechanism of Corneal Crosslinking

In the corneal collagen crosslinking procedure, riboflavin (Vitamin B2) is administered in conjunction with ultraviolet A (UVA – 365 nm) irradiation. Riboflavin acts as a photosensitizer. The reactive oxygen species (singlet oxygen) produced by this interaction as well as UVA-excited molecules of riboflavin result in the crosslinking effect and causes mechanical stiffening of the

cornea [14]. Most of this “crosslinking” occurs within the collagen molecules themselves and the corneal proteoglycan matrix [15]. Whether there are actual “crosslinks” between collagen fibers remain unclear. However, these are unlikely given the distances between the actual fibers [16–20]. Independent of UVA light penetration, the interwoven collagen fibrils in the anterior stroma, compared to the posterior stroma, appear to enhance the stiffening effect of “crosslinking” as well [21, 22].

The Crosslinking Procedure

The procedure for the US multicenter collagen crosslinking trial was based on the original corneal crosslinking procedure described by Seiler and colleagues [1]. In brief, a topical anesthetic is administered and the central 9 mm epithelium is removed by mechanical debridement. Riboflavin is then administered topically every 2 minutes for a total of 30 minutes. Following riboflavin administration, riboflavin absorption is confirmed on slit-lamp examination (Fig. 16.1a, b). At this time, pachymetry measurements are performed, and if the cornea is <400 μm , hypotonic riboflavin is administered, one drop every 10 seconds for 2 minute sessions, after which pachymetry measurements are performed again to confirm that the stroma had swelled to $\geq 400 \mu\text{m}$. The goal of this is to provide adequate corneal thickness to absorb the

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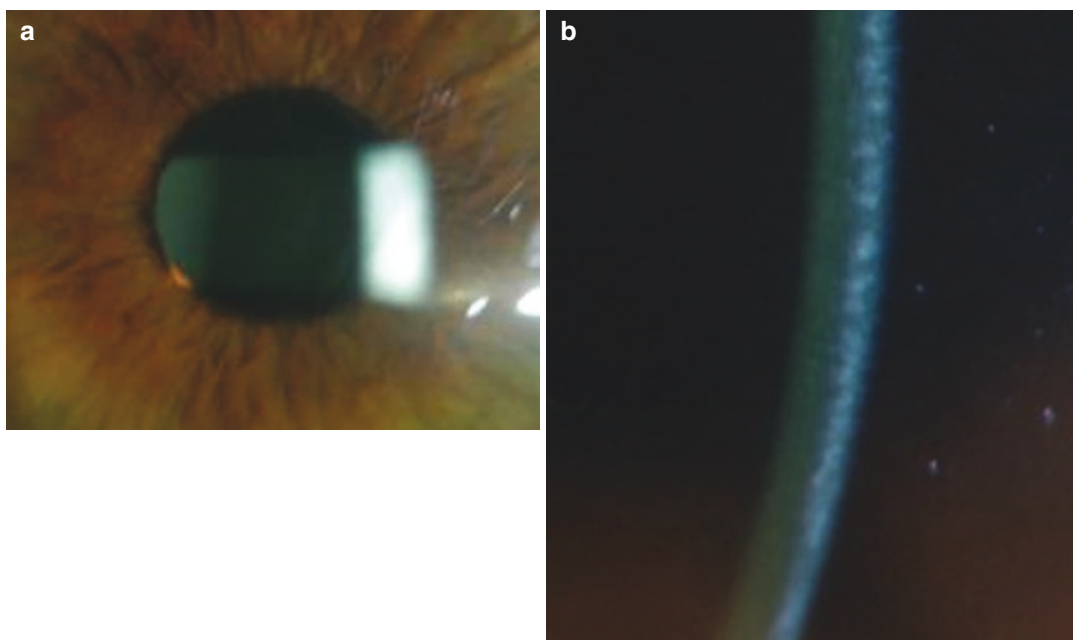


Fig. 16.1 (a) Slit-lamp image confirming corneal riboflavin uptake and anterior chamber flare. (b) Slit-lamp image confirming corneal riboflavin uptake

incoming UV light in order to protect the endothelium from damage by the UV-riboflavin interaction. The cornea is aligned and then exposed to UVA 365 nm light for 30 minutes at an irradiance of 3.0 mW/cm². While the cornea is exposed to UVA light, riboflavin administration is continued every 2 minutes. Postoperatively, antibiotic and corticosteroid drops are administered, a soft contact lens bandage is placed, and the eye is reexamined by slit-lamp examination. The contact lens is then removed after the epithelial defect is closed.

Clinical Outcomes

Visual Acuity

Generally, CXL appears to stabilize visual acuity, and in many cases offers a modest improvement in uncorrected and best-corrected vision. Studies have shown that, on average, at 1 year postoperatively,

uncorrected and best-corrected vision changed by 0–2.7 Snellen lines [13, 23, 24]. In the US multi-center trial of keratoconus treatment, uncorrected and best-corrected visual acuity improved by an average of 4.4 logMAR letters and 5.7 letters, respectively [25, 26]. Individually, best-corrected visual acuity improved by 10 letters or more in 28% of eyes treated, 5% of eyes worsened by 10 letters or more, and all other eyes remained within 10 letters of pre-operative visual acuity, 1 year after CXL treatment.

Topographic Outcomes

In more recent studies, additional topographic indicators, such as the point of maximum flattening and the regularization index, have been studied to better interpret the effect of CXL over the entire corneal contour [27, 28]. However, maximum keratometry remains the standard topographic indicator of CXL success, since it measures, to some extent, the severity of the

keratoconic cone. In the literature, average flattening of maximum keratometry ranges from about 1D to 3D, 1 year after CXL [1, 13, 23, 24, 29, 30]. In the US multicenter trial, maximum keratometry flattened by 1.6D at 1 year postoperatively. Individually, 31% of eyes flattened by ≥ 2 D, 6% of eyes steepened by ≥ 2 D, and all other eyes remained within 2D of preoperative maximum keratometry measurements. In other studies, long-term (≥ 3 years), maximum keratometry remained stable in over 90% of adult patients (>18 years old) and 75% of pediatric patients (<18 years old) after CXL [31–33].

Postoperative Timecourse

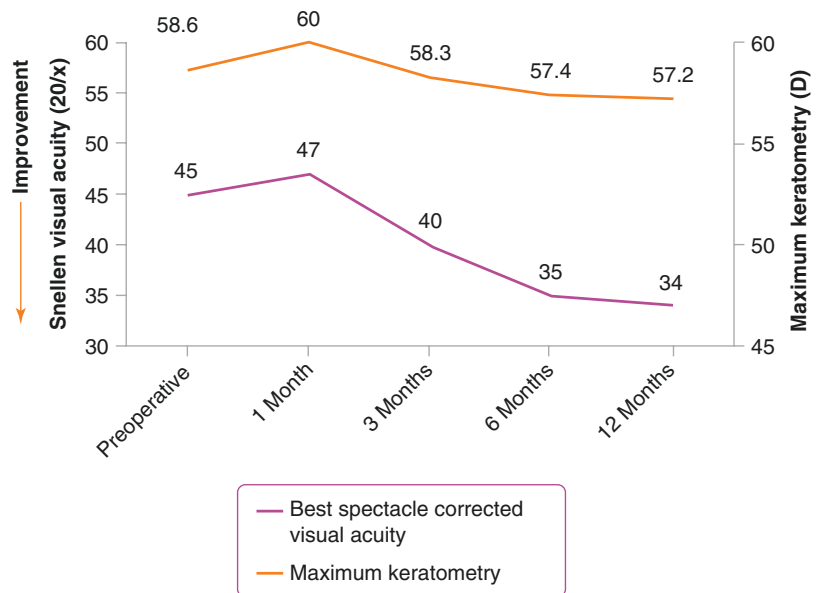
In our study of the clinical timecourse after crosslinking, there was a significant worsening of vision and steepening of the keratoconic cone at 1 month postoperatively [13]. These changes appear to plateau at 3 months and improve thereafter (Fig. 16.2). Interestingly, these postoperative outcomes appear to be congruous with the postoperative thinning and crosslinking-associated corneal haze changes over time. These findings suggest stromal and epithelial remodeling over time after crosslinking.

Biomechanical Effects of CXL

Wollensak et al. [14] reported that immediate stress measurements increased by 71.9% and 328.9% in porcine and human corneas, respectively, after CXL. In rabbit corneas, these increases in stress measurements were maintained between 69.7% and 106% at 8 months postoperatively [19]. These increases corresponded to an increase in Young's modulus. A postoperative increase in Young's modulus was further demonstrated with collagen hydrogels exposed to UVA/riboflavin therapy [20]. Since this work, additional studies have demonstrated increased corneal stiffness, ex vivo, after CXL as well [22, 34].

To date, it has been difficult to quantify these biomechanical changes in vivo [35, 36]. The Ocular Response Analyzer (ORA, Reichert Inc., Buffalo, NY USA) and the Corvis® (Oculus, Wetzlar, Germany) are commercially available devices designed to obtain in vivo measurements of corneal biomechanical properties [37–39]. Previous studies were able to demonstrate changes in Corvis and ORA measurements consistent with corneal biomechanical changes after CXL; however, the clinical reliability of these measurements are still to be determined [40, 41].

Fig. 16.2 Change in best spectacle-corrected visual acuity (BSCVA) and maximum keratometry over time [1]. (Modified from: Hersh et al. [13])



Our previous work, analyzing ORA measurements after CXL, did not show a significant change in CH or CRF measurements 1 year after CXL [10]. In the future, techniques such as Brillouin microscopy may prove to be useful in quantifying in vivo biomechanical changes after CXL therapy [42–44].

Complications

Early in the postoperative course after CXL, patients are subject to the typical complications of the epithelial wound healing process. In the US multicenter trial for CXL in keratoconus patients, 22.5% of patients had a remaining epithelial defect 1 week after their procedure. One patient developed an ulcerative keratitis on postoperative day number three, which resolved with

topical antimicrobial therapy. At 1 year, of the 102 eyes treated in the study, a corneal scar was noted in one eye, endothelial folds in another eye, and an irregular corneal epithelium was noted in one eye as well [26]. The complications after CXL for patients with post-LASIK/PRK ectasia were similar; however, one post-LASIK patient developed epithelial ingrowth after CXL, which required a flap lift and epithelial removal at that time [25].

Postoperative Haze

On clinical examination, corneal haze has been noted after the crosslinking procedure (Fig. 16.3a). Crosslinking-associated corneal haze is different in clinical character from haze after other procedures such as excimer laser photorefractive kera-

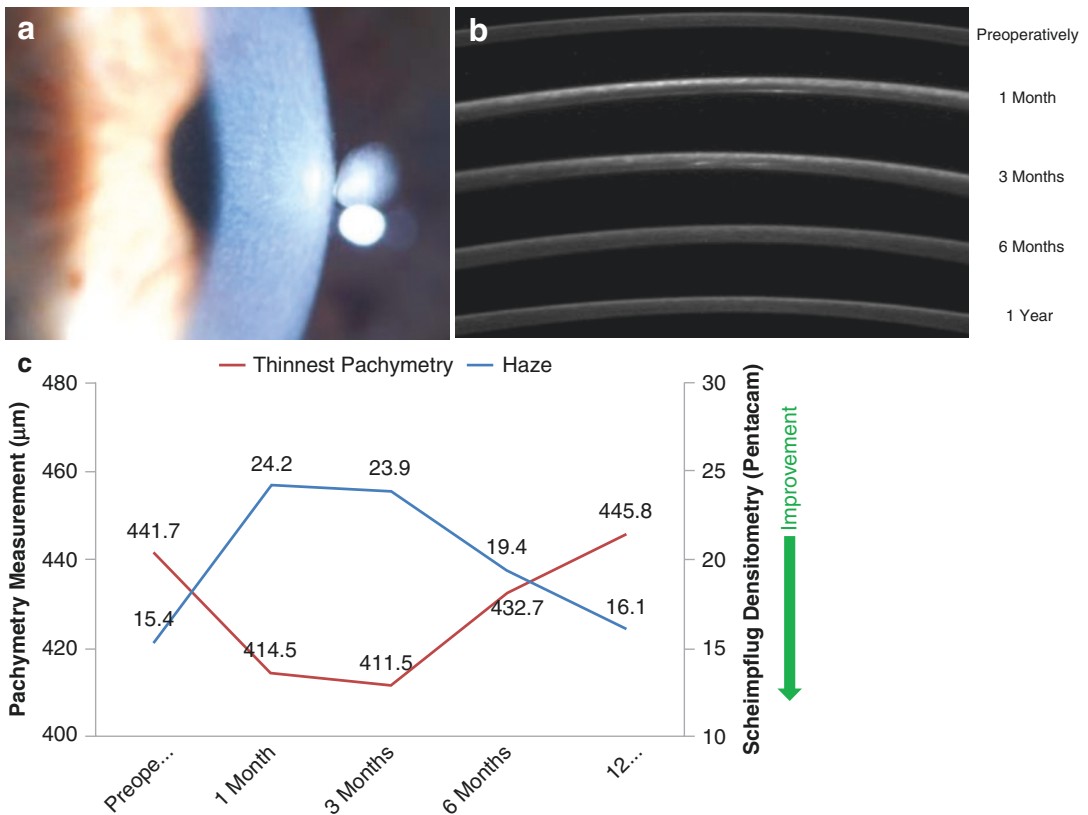


Fig. 16.3 (a) Representative slit-lamp image of clinical haze after collagen crosslinking. (b) Scheimpflug images of crosslinking-associated corneal haze over time [2]. (c) Change in thinnest pachymetry (Pentacam) and crosslinking-

associated corneal haze over time [2, 3]. (Reprinted with permission from: Greenstein et al. [7]. Reprinted with permission from: Greenstein et al. [12])

tectomy. The former is a dust-like change in the corneal stroma or a midstromal demarcation line [45], whereas the latter has a more reticulated subepithelial appearance. Crosslinking-associated corneal haze is most likely a measure of backscattered and reflected light, causing decreased corneal transparency [22]. Corneal haze has been confirmed using confocal microscopy as well and can be objectively quantified using Scheimpflug densitometry (Fig. 16.3b) [46].

Similar to the timecourse of clinical outcomes after crosslinking, there appears to be an increase in haze, which peaks at 1 month, and plateaus between 1 and 3 months. Between 3 and 6 months the cornea begins to clear and continues to return towards baseline at 1 year (Fig. 16.3c).

To date, it remains unclear whether this postoperative haze is a true complication or rather a desired wound healing effect demonstrating the efficacy of the crosslinking procedure. Activated keratocytes, important for the remodeling the corneal stroma, may contribute to the development of the haze seen with Scheimpflug imagery [47].

Corneal Pachymetry

Corneal thinning is a general concomitant of the early CXL postoperative course (Fig. 16.3c) [3–6, 14–17]. Postoperatively, similar to the timecourse of crosslinking-associated corneal haze, and crosslinking clinical outcomes, the cornea appears to thin at 1 and 3 months and to rethicken between 3 and 12 months. The physiology of this initial thinning and subsequent rethickening is, as yet, unclear. Epithelial remodeling is a possible early factor in corneal thickness changes (Fig. 16.4a, b). Moreover, anatomic and structural changes of corneal collagen fibrils such as compression of collagen fibrils (especially the more transverse-oriented anterior fibrils) [10, 23], changes in corneal hydration [24] and edema [25, 26], keratocyte apoptosis [13, 27, 28], changes in glycosaminoglycans [29], and other processes might be implicated in the distinct clinical timecourse after CXL.

Predictors of Outcomes

The essential goal of collagen crosslinking is to stabilize the progression of the ectatic cornea. We performed an analysis to determine which patients would be good candidates or contraindicated for crosslinking [11]. Our multivariate analyses revealed the only independent predictor of a change in postoperative best-corrected vision after CXL was preoperative best-corrected visual acuity. Those eyes with worse preoperative best-corrected visual acuity were more likely to experience an improvement of ≥ 2 Snellen lines. Specifically, eyes with a preoperative Snellen visual acuity of 20/40 or worse were 5.9 times more likely to improve by two lines or more (Fig. 16.5a). With regard to postoperative topography, eyes with a maximum K ≥ 55 D were 5.4x more likely to have topographic flattening ≥ 2 D after CXL compared with eyes with flatter corneas (Fig. 16.5b).

From the viewpoint of clinical decision-making, since no independent predictors of failure of crosslinking to stabilize topographic disease progression were identified, it is reasonable that all eyes with progressive keratoconus should be considered for crosslinking treatment with the goal of diminishing disease progression. With regard to postoperative best-corrected vision, from our current knowledge, eyes with worse vision initially would expect the greatest chance of actual visual improvement. Furthermore, all eyes, irrespective of preoperative best-corrected visual acuity, are equally likely to remain stable (within 2 lines of best-corrected visual acuity); however, eyes with initially good vision (better than 20/40) may be somewhat more susceptible to a loss of 1 line (Fig. 16.6) of best-corrected vision postoperatively. Therefore, eyes with good visual acuity and progressive disease, or eyes with a high risk for progressive disease (e.g., pediatric patients), may still benefit from crosslinking treatment, but the ophthalmologist should be aware of the possible complications, and the patient should be properly counseled.

Finally, preoperative cone location may play an important role in the efficacy of the

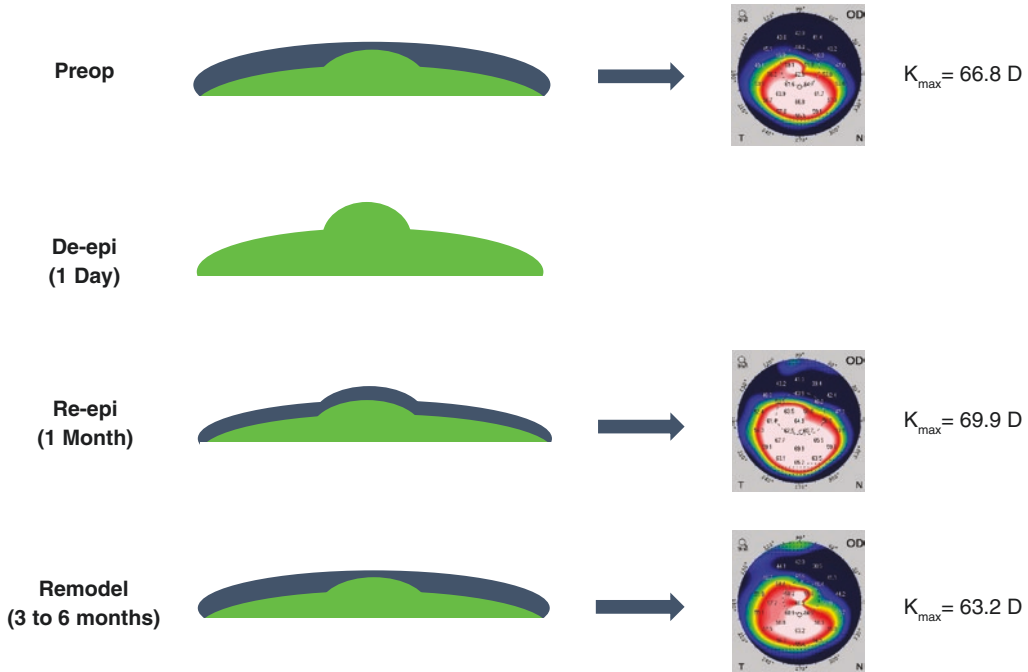


Fig. 16.4 Diagram of postoperative epithelial remodeling after epithelial removal and CXL treatment

crosslinking procedure [48]. There appears to be more topographic flattening in those eyes with centrally located cones. In our previous work, maximum keratometry flattened by 2.6D in eyes with centrally located cones, and by only 1.0D and 0.05D, in those eyes with para-central and peripheral cones, respectively. Patients with post-LASIK/PRK ectasia, who are more likely to have peripheral cones, and those keratoconic patients with more peripheral cones, may benefit from other procedures to “centralize” the cone, for instance, by intra-corneal ring segments or by conductive keratoplasty [48]. These procedures may subsequently lead to a more robust crosslinking response.

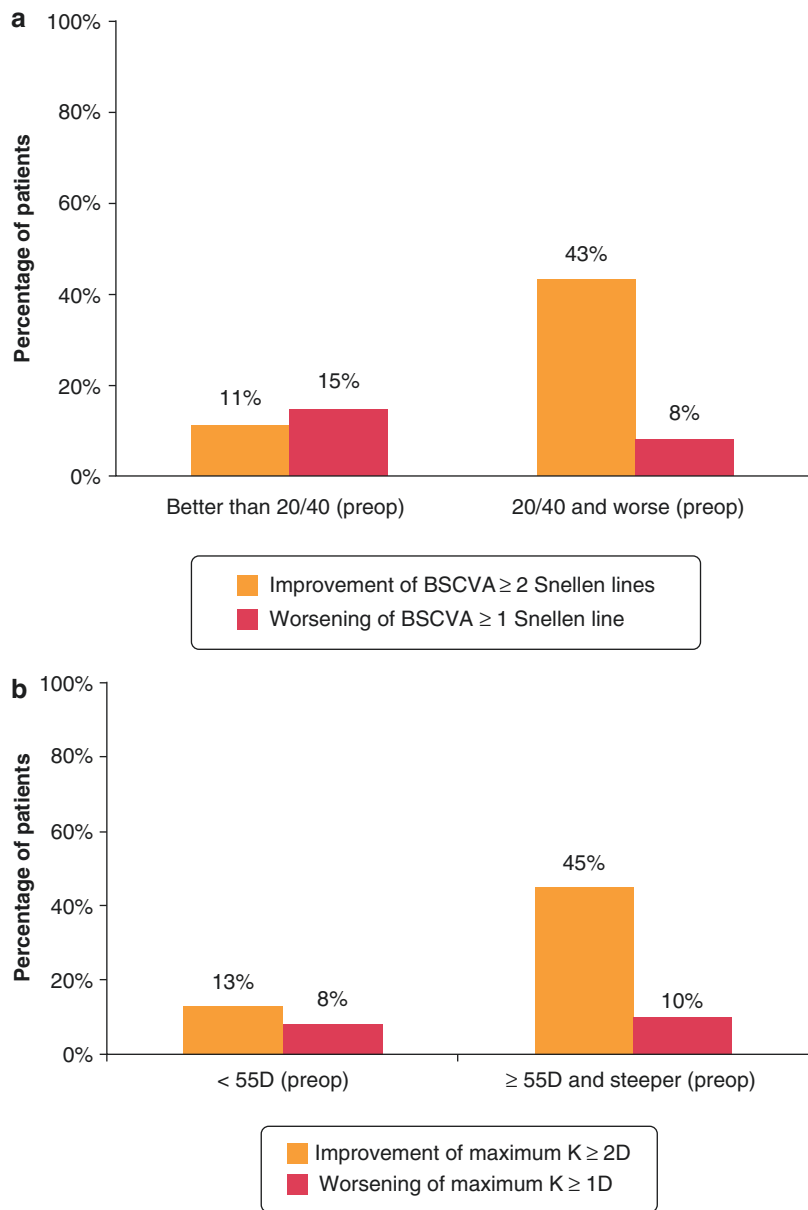
Future of Corneal Collagen Crosslinking

There are several modifications to the FDA-approved CXL procedure that are currently under investigation. Transepithelial crosslinking, a vari-

ation of the standard crosslinking procedure in which the corneal epithelium is not removed, offers several possible advantages, including faster recovery time, and less wound healing complications. Early results of transepithelial crosslinking have been mixed [49–54]. Multiple substances have been used to promote riboflavin penetration through the epithelial layer of the cornea. Additionally, iontophoresis has been used to improve the penetration of riboflavin through the corneal epithelium and to improve transepithelial CXL outcomes [55].

In addition to the riboflavin used for transepithelial CXL, modifications have been made to the standard dextran riboflavin used for traditional CXL therapy. Standard dextran riboflavin has a dehydrating effect on the corneal stroma, and frequently hypotonic riboflavin is required to swell the cornea before UV light therapy is initiated. Hypotonic forms of riboflavin, methylcellulose riboflavin, and more concentrated versions of riboflavin have all been studied for CXL therapy [56–58]. Early studies appear to indicate that CXL

Fig. 16.5 The effect of preoperative BSCVA on CXL outcomes [4]. **(a)** Percentage of eyes in which BSCVA improved by ≥ 2 Snellen lines 1 year after CXL (blue), and percentage of eyes in which BSCVA worsened by ≥ 1 Snellen line 1 year after CXL (red). **(b)** Percentage of eyes in which maximum K flattened by ≥ 2 D 1 year after CXL (blue), and percentage of eyes in which maximum K steepened by ≥ 1 D 1 year after CXL (red). (Modified from: Greenstein et al. [11])

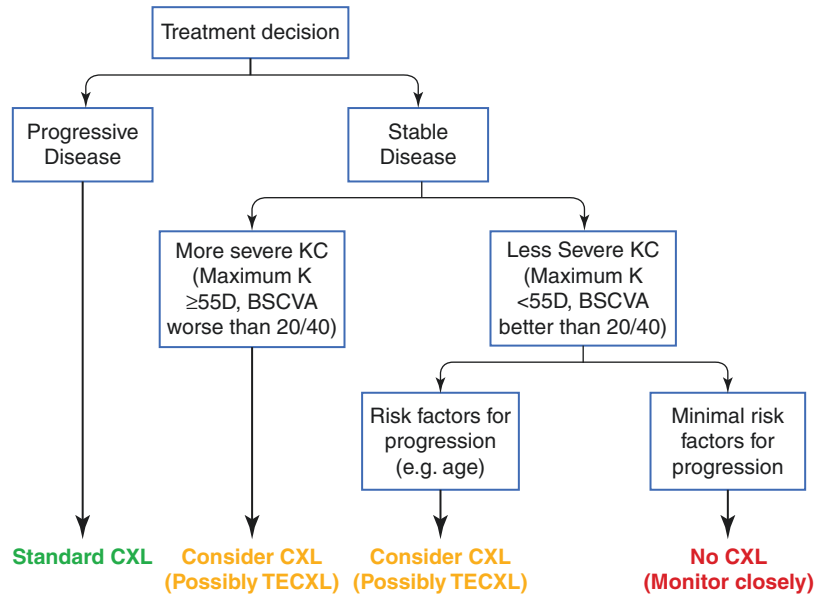


therapy is successful in conjunction with most of these riboflavin forms; however, long-term and prospective studies are still required to determine the optimal riboflavin composition for CXL.

Another variation to the standard crosslinking procedure is accelerated corneal collagen crosslinking. In this procedure, the cornea is exposed to a higher power of UV light over a significantly shorter period of time [59, 60]. Ex vivo studies have shown that despite a constant total dose of

5.4 J, increased irradiance levels decrease post-operative mechanical stiffening of the cornea compared to standard treatment [61]. Higher irradiance levels likely cause the free radical conversion of oxygen to outpace oxygen replenishment of the cornea, and, therefore, less of a crosslinking effect. In vivo, however, studies have shown that accelerated CXL (mostly 9 mW/cm² for 10 minutes) may have similar results to the standard CXL procedure, and pulsing the UVA light

Fig. 16.6 Treatment algorithm for CXL patient selection [4]. (Modified from: Greenstein et al. [11])



may increase oxygen replenishment in the cornea during the accelerated procedure as well [62–64]. Moreover, despite delivering a higher power of UV light, the safety and integrity of the corneal endothelium remains intact secondary to the shorter exposure time and less penetration depth [65]. Further modifications to the CXL procedure, including topographically “customized” CXL, which focuses UV light directly on the cone itself, rather than diffusely over the entire cornea, are under investigation [28].

Finally, there has been increasing interest on the use of corneal collagen crosslinking as part of a larger treatment algorithm for patients with keratoconus and post-LASIK/PRK ectasia. While there are many patients who may benefit from crosslinking alone, there are other patients, who may benefit from crosslinking as an adjunct procedure to stabilize the cornea. Other procedures such as intracorneal ring segments [27, 66–69] and PRK [70–72] may induce more initial flattening of steeper cones and improve the contour of the ectatic cornea; however, corneal collagen crosslinking may be required to better stabilize these changes over time.

Corneal collagen crosslinking is a promising new treatment to stabilize and even improve the visual acuity and topography of patients with keratoconus and post-LASIK/PRK ectasia. In the

future, faster and more precisely guided UV light delivery systems, as well as new forms of riboflavin, may continue to improve the safety and efficacy of this new procedure. Further studies being conducted will likely continue to reveal the patients who will most benefit from corneal collagen crosslinking whether it is as a standalone procedure or in conjunction with other procedures designed to improve the contour and optical quality of the ectatic cornea.

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Part IV

Frontiers in Corneal Research

Claes H. Dohlman's Legacy in Corneal Research

17

Ilene K. Gipson

Claes Dohlman recognized early on in his career that understanding the basic aspects of the physiology and function of corneal tissues was key to treating corneal blindness and corneal disease. His early research that commenced during his training years was guided by research mentors, including Jonas Friedenwald, MD, and Endre Balazs, PhD. After receiving his MD and PhD at the University of Lund in 1957, he moved to Boston where within a very short period, he established the Department of Cornea Research at the then Retina Foundation (1962) and shortly thereafter the Cornea Service at the Massachusetts Eye and Ear Infirmary (1964). He assembled a group of basic scientists and cornea fellows and a very productive period ensued, one in which multiple avenues of cornea research were pursued that yielded much new information and established the cornea research specialty within ophthalmology.

Since Dr. Dohlman attracted both basic scientists and clinical fellows, many aspects of his research in corneal tissue biology, physiology and function, and corneal disease were researched. Each of the areas he and his team investigated became prominent areas of specialty within the cornea field. These topics include the following:

(A) corneal epithelial function and corneal wound healing, (B) the extracellular matrix of the corneal stroma especially as it relates to corneal edema, (C) corneal physiology and nutrient transport, (D) corneal collagenases and their role in corneal stromal dissolution in corneal ulceration, (E) tear film biology and composition, and as the result of experience from research in these areas led to (F) keratoprosthesis development.

The following account of Professor Dohlman's research in these topics is not an exhaustive account of all his contributions, rather it highlights the topics of his early research which led to his extensive work in development and optimization of the Boston Keratoprosthesis (KPro) as well as his efforts to understand the etiology of KPro failure in specific corneal diseases. His contributions to corneal disease by way of specific treatments are not covered herein. This short review also highlights his leadership contributions to expansion and development of the corneal research field.

Corneal Epithelial Function and Corneal Wound Healing

The earliest of Dr. Dohlman's corneal-related research papers, published during his training years with Jonas Friedenwald, reported on mitotic activity of the corneal epithelium and the healing of corneal epithelial wounds in

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hypophysectomized rats and in rats treated with several endocrine hormones [30]. Reduced mitotic activity in the corneal epithelium occurred after hypophysectomy, but healing of a pinprick wound was unimpaired. Upon completion of training, and development of the Corneal Research Department at the Retina foundation, there followed a very productive 10–15 years of research, including a series of experiments that yielded fundamental new information on the function of the corneal epithelium.

Much of his epithelial/ocular surface/corneal work is beautifully summarized in a publication of Dr. Dohlman's his [6] Friedenwald lecture entitled "*The function of the corneal epithelium in health and disease*" [6]. For those of us working in this field, reading this 1971 paper with its classic experiments summarized, demonstrates the fundamental work that the Dohlman group contributed to our current understanding of the ocular surface epithelium and its function.

Dr. Dohlman's interest in the corneal epithelium and its role in corneal health and disease led to efforts to experimentally replace the epithelium as a potential way to treat corneal defects and corneal ulceration. With Miguel Refojo, PhD, in particular, experiments with synthetic polymers were done. Contact lenses were placed on experimental animals to see if they protected the corneal stroma post chemical burns (see [7]), and human studies followed [11]. One suspects that experiments such as these were early ones in a progression of leading to the development of the Boston Keratoprosthesis.

Corneal stromal wound healing was also investigated during these early years. One of Dr. Dohlman's most cited papers is on the "*Tensile strength of corneal wounds*" [14]. Other highly cited papers report early use of adhesives to facilitate wound strength [27, 32] (Table 17.1).

The Extracellular Matrix of the Corneal Stroma

As noted above, during his training years Dr. Dohlman was a research fellow with Endre Balazs at the Retina Foundation in Boston (renamed Eye

Research Institute of the Retina Foundation, then the Schepens Eye Research Institute) – one of his earliest papers described their work "*Chemical Studies on Descemet's Membrane of the Bovine Cornea*" [8]. Thus an early interest in the extracellular matrix of the cornea with Balazs sparked a series of studies on stromal composition, physiology, and stromal wound response. Investigations on mucopolysaccharides (glycosaminoglycans) of the stroma [9, 10] led to investigations on factors effecting corneal swelling pressure. With BO Hedbys, Dohlman developed methods for measuring swelling pressure in vitro, the resulting publication being another of his most-cited papers (Table 17.1) [18]. He was particularly interested in how swelling pressure related to corneal edema, a serious clinical problem [12] also [34].

Corneal Physiology and Nutrient Transport

The investigations of the Dohlman group on oxygen and nutrient sources for the corneal epithelium are classics in the field. By a series of experiments with oxygen-permeable versus non-permeable contact lenses, they demonstrated that with nonpermeable lenses, cellular edema occurred, glycogen content decreased markedly, and lactic acid content increased. Thus they ascertained that oxygen was obtained through the anterior surface of the cornea. The source of glucose for the corneal epithelium was also investigated. Using clever methods of inserting impermeable membranes in the corneal stroma (as only a surgeon would do!), they found that glycogen disappeared in the region of the epithelium and stroma anterior to the membrane. Epithelium over a central hole placed in the membrane retained its glycogen levels, demonstrating that the source of the glucose is directly posterior to the membrane, and upon further investigation that the source is the aqueous humor. These experiments are summarized in the publication of Dr. Dohlman's Friedenwald lecture [6]. The knowledge gained from these experiments on the sources of nutrients and the complex physiology showing interdependency of the different cell layers of the

Table 17.1 Twenty most-cited papers, CH Dohlman, M.D., Ph.D. from Web of Science, 2018

1. <i>Use of 5-Iodo-2'-Deoxyuridine (IDU) in treatment of herpes simplex keratitis.</i> Kaufman, HE; Dohlman, C; Martola, EL. <i>Archives of Ophthalmology.</i> (Kaufman et al. [21])
2. <i>Keratoprosthesis: Preoperative prognostic categories.</i> Yaghouti, F; Nouri, M; Abad, JC; Power, W; Doane, M; Dohlman, C. <i>Cornea.</i> (Yaghouti et al. [33])
3. <i>Precorneal tear film. 1. Factors in spreading and maintaining a continuous tear film over corneal surface.</i> Lemp, MA; Holly, FJ; Iwata, S; Dohlman, CH. <i>Archives of Ophthalmology.</i> (Lemp et al. [22])
4. <i>Tensile strength of corneal wounds.</i> Gasset, AR; Dohlman, CH. <i>Archives Of Ophthalmology.</i> (Gasset and Dohlman [14])
5. <i>Endophthalmitis after keratoprosthesis – Incidence, bacterial causes, and risk factors.</i> Nouri, M; Terada, H; Alfonso, EC; et al. <i>Archives of Ophthalmology.</i> (Nouri et al. [25])
6. <i>A new method for the determination of the swelling pressure of the corneal stroma in vitro.</i> Hedbys, BO; Dohlman, CH. <i>Experimental Eye Research.</i> (Hedbys and Dohlman [18])
7. <i>Corneal edema and intraocular pressure. 2. Clinical results.</i> Ytteborg, J; Dohlman, CH. <i>Archives of Ophthalmology.</i> (Ytteborg and Dohlman [34])
8. <i>Use of adhesive for closure of corneal perforations.</i> Webster, RG; Slansky, HH; Refojo, MF; Boruchoff, S; Dohlman, C. <i>Archives of Ophthalmology.</i> (Webster et al. [32])
9. <i>Penetration routes of topically applied eye medications.</i> Doane, MG; Jensen, AD; Dohlman, CH. <i>American Journal of Ophthalmology.</i> (Doane et al. [5])
10. <i>Evaporation rate of water from precorneal tear film and cornea in rabbit.</i> Iwata, S; Lemp, MA; Holly, FJ; Dohlman, C. <i>Investigative Ophthalmology.</i> (Iwata et al. [20])
11. <i>Double-blind clinical study of adenine arabinoside therapy of viral keratoconjunctivitis.</i> Pavanlangston, D; Dohlman, CH. <i>American Journal of Ophthalmology.</i> (Pavan-Langston and Dohlman [26])
12. <i>A drug-eluting contact lens.</i> Ciolino, Joseph B.; Hoare, Todd R.; Iwata, Naomi G.; Behlau, I; Dohlman, C. <i>Investigative Ophthalmology & Visual Science.</i> (Ciolino et al. [4])
13. <i>Glaucoma associated with keratoprosthesis.</i> By: Netland, PA; Terada, H; Dohlman, CH. Conference: 100th Annual Meeting of the American-Academy-of-Ophthalmology. Location: Chicago, Illinois. Date: Oct 27-Nov 01, 1996. <i>Ophthalmology.</i> (Netland et al. [24])
14. <i>Function of corneal epithelium in health and disease – Friedenwald, JS memorial lecture.</i> Dohlman, CH. <i>Investigative Ophthalmology.</i> (Dohlman [6])
15. <i>Evaluation of adhesives for corneal surgery.</i> Refojo, MF; Dohlman, CH; Ahmad, B; Carroll, J; Allen, J. <i>Archives of Ophthalmology.</i> (Refojo et al. [27])
16. <i>Collagenase in corneal ulcerations.</i> Slansky, HH; Gnadinge, MC; Itoi, M; Dohlman, C. <i>Archives of Ophthalmology.</i> (Slansky et al. [31])
17. <i>The Boston keratoprosthesis in Stevens-Johnson syndrome.</i> Sayegh, Rony R.; Ang, Leonard P. K.; Foster, C. Stephen; Dohlman, C. <i>American Journal of Ophthalmology.</i> (Sayegh et al. [29])
18. <i>Amniotic membrane inlay and overlay grafting for corneal epithelial defects and stromal ulcers.</i> Letko, E; Stechschulte, SU; Kenyon, KR;.....Dohlman, C,.....et al. <i>Archives of Ophthalmology.</i> (Letko et al. [23])
19. <i>Successful prevention of bacterial endophthalmitis in eyes with the Boston keratoprosthesis.</i> Durand, Marlene L.; Dohlman, Claes H. <i>Cornea.</i> (Durand and Dohlman [13])
20. <i>Structural alterations affecting transparency in swollen human corneas.</i> Goldman, JN; Benedek, GB; Dohlman, CH; Kravitt, B. <i>Investigative Ophthalmology.</i> (Goldman et al. [16])

cornea on one another can be seen as guiding Dr. Dohlman's design of the KPro; in that he recognized the need of a full thickness prosthesis to replace the entire tissue.

Corneal Collagenases and Their Role in Corneal Ulceration

In the early 1960s, Jerry Gross at the nearby Massachusetts General Hospital discovered tissue collagenase (previously only known in bacte-

ria) and showed its degradative effect on collagen molecules [17]. Soon thereafter Dohlman and colleagues described the appearance of collagenases in ulcerating corneas [15, 19], and major efforts of the Dohlman group were to identify collagenase in various corneal diseases and search for inhibitors of the enzyme (then thought to be one enzyme, now known to be a whole series of matrix metalloproteinases). Mike Berman, PhD, a basic scientist expert on collagenase was hired and a spate of papers related to collagenase activity ensued [2, 3].

While a “magic bullet inhibitor” to prevent collagenase and stop ulceration did not appear, much has been learned about the “collagenases” (now known as matrix metalloproteinases, or MMPs) by the Cornea Research Group at Schepens, and other Dohlman Group offspring (see Dimitri Azar, MD, > Elizabeth Fini, PhD). To this day, Dr. Dohlman retains an interest in these enzymes, particularly as it relates to KPro retention and to diseases in which KPro has not been as successful [1, 28].

Tear Film Biology and Composition

In the mid-1960s, Dohlman’s group developed an interest in the tear film, particularly, as it relates to dry eye. With early colleagues/fellows such as S. Mishima, Michael Lemp, David Maurice, Frank Holly, and Jules Baum, the rate of turnover of tears was measured, the thickness of tears and their layers were described, and they attempted to measure the contribution of lipids and “mucus” on tear film spread and tear film stability. Alterations in tear film stability were observed in various ocular surface pathologies, and in some cases were ascribed to mucin deficiency. For review, see the Dohlman Friedenwald lecture [6]. These early discoveries/experiments have led to the development of the specialty of ocular surface and tear biology that has gained in importance and participation and is a fruitful avenue of investigation to this day. Many of the investigators in this field are direct, first-, second- or third-generation descendants of the Dohlman group.

Early Research Culminated in Keratoprosthesis Development

Having worked in most aspects of corneal biology and physiology, particularly relating each to disease processes of the cornea, Dr. Dohlman settled into KPro development and optimization. From the areas that he had researched in his early years in the Cornea Research Department at the Retina Foundation, he brought information and expertise through Marshal Doane, especially, that

impacted and guided this development. Early experiments with synthetic polymers, placing contact lenses on the cornea, to replacing both the corneal epithelium and endothelium, plus insertion of nonpermeable membranes into the stroma provided the information of the requirement of all movement of vital nutrients to the cornea. Thus he designed a full thickness prosthesis. His experiments testing wound strength after use of glues to anneal wound margins may have influenced his use of carrier donor corneas for the KPro so that it could be better integrated into the host. His attempts to prevent infections and to prevent carrier melt around the KPro undoubtedly related to his early experience with collagenase and remains a topic of interest and concern especially in those patients with diseases in which KPro has not been successful [33].

Research Impact by Leadership, Group/Faculty Development, and Training

While Dr. Dohlman’s research accomplishments developed and influenced the field greatly, it is his accomplishment in hiring and bringing both basic and clinical research together in the same group that may be one of his most important contributions. Upon his establishment of the Cornea Research Department at the Retina Foundation (Now Schepens Eye Research Institute), he brought a number of scientists onto the faculty.

These included Miguel Refojo, PhD, Marshal Doane, PhD, David Maurice, PhD (consultant), Frank Holly, Mike Berman, PhD, Charles Cintron, PhD, Art Neufeld, PhD and Ilene Gipson, PhD. These scientists worked beside and in collaboration with the clinical faculty including, Richard Thoft, MD, Ken Kenyon, MD, Debra Langston, MD, Mathea Allansmith, MD, Stephen Foster, MD, as well as many corneal fellows over the years. The contributions of this group not only in research, but also in training and populating the cornea research field, have been an important component to advancing the field. During that period of integration of the

basic scientists with the clinical scientists, weekly seminars were held in which the clinics were shut and the faculty met to review their research efforts. Claes Dohlman was a continual presence at these meetings and to this day attends the less frequent seminars of the group.

Not only did Claes bring basic researchers into the field, he has continually kept apprised of basic science developments as he has been an avid reader of the basic research literature. Over the years he has sent faculty papers demonstrating new developments in the basic sciences that could be used in understanding corneal biology. As a personal example, when cyclins and the control of the cell cycle was first discovered and published, Claes suggested to me (author of this chapter) that these findings be applied to the non-replicative corneal endothelium. I suggested to Nancy Joyce, PhD, then looking for an independent field of study, that she pursue this field. She did, and important findings were made that influence work in the field today.

Summary

Dr. Dohlman has been the ultimate translational scientist; he constantly has applied his research in basic corneal physiology to studies of corneal disease mechanisms and treatment. It is clear that his early basic research as well as his continual and constant reading and awareness of the research literature led him to keratoprosthesis development. This effort demonstrates Dr. Dohlman as one of the first translational researchers in the field, and one who based his translational work in fundamental biologic knowledge of the cornea, much of which he gained by his own laboratory efforts.

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Cultivated Cells in the Treatment of Corneal Diseases

18

Shigeru Kinoshita and Morio Ueno

Introduction

At present, there are two major corneal regenerative medicine pathways that involve the use of cultured cells. The first involves the transplantation of a cultured epithelial cell sheet for reconstruction of the ocular surface. Following this direction, both autologous and allogeneic corneal epithelial stem cell sheet transplantations and cultivated oral mucosal epithelial sheet transplantation (COMET) have been devised [1–9]. These cultured epithelial cell sheets are used for ocular surface reconstruction in the disease state, such as corneal epithelial stem cell deficiency or conjunctival epithelial stem cell deficiency, or both, in order to preserve mucosal epithelial integrity on the ocular surface. Ideally, preservation of the properties of the corneal epithelium is required, or at least, the preservation of the properties of the mucosal epithelium.

Although the results of autologous corneal epithelial stem cell sheet transplantation appear to be promising [3], at least to some extent, it is difficult to establish as a standard treatment that

can be available to many patients unless the time and financial costs required for preparation can be lowered. Against this backdrop, simple limbal epithelial transplantation (SLET) [10], a novel surgical limbal stem cell transplantation technique that requires no cultured cells, avoids the need for sophisticated laboratory facilities, and does not require a cell culturing process, has begun to gain acceptance for the treatment of patients with unilateral limbal stem cell deficiency (LSCD). Moreover, attempts have been made to perform COMET for the treatment of devastating ocular surface diseases such as Stevens-Johnson syndrome [6–9, 11]. Furthermore, the preparation of a corneal epithelial sheet from induced pluripotent stem cells (iPS cells) is on the near horizon [12]. Cultured epithelial cells used for reconstruction of the ocular surface or skin regeneration need to continuously proliferate after in vivo transfer, and how much of the so-called stem cell population is contained within the cultured cell sheet is key to the success of the clinical outcome [3].

On the other hand, and as will mainly be discussed in this chapter, are methods focused on corneal endothelial regeneration. These methods are primarily based on using cultured corneal endothelial cells (CECs), and the focus of this research has primarily been on establishing the degree in which in vivo properties of the cells are maintained in in vitro culture conditions rather than the transplantation of stem / progenitor

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cells. To that end, the key aspect is whether in vitro CECs in culture are well differentiated and retain fully adequate cellular function. What is required for the culturing of CECs and neurons, which probably do not proliferate in vivo [13], must be fundamentally different from what is required for the culturing of ocular-surface epithelial cells, including corneal epithelial cells. When cultured cells are used as a means of regenerative medicine, it is vital to clearly determine which one of the above procedures is the primary aim and then proceed.

The third direction of corneal regenerative medicine is the treatment of inflammatory corneal stromal scarring with mesenchymal stem cells. In this approach, researchers are aiming at therapeutic effects by releasing beneficial biological factors from transplanting mesenchymal stem cells to modulate the inflammation and fibrosis pathway [14]. Thus, this type of corneal regenerative medicine is clearly distinct from a true cell-based therapy. In any case, groundbreaking corneal regenerative medicine using cultured cells is sure to develop from those lines of cornea-related scientific research. This chapter will mainly describe a cell-based treatment focused on cultured CEC injection therapy, one of the current novel therapeutic approaches.

Background

Early Stage: Successful Culture of CECs in Primates

In the 1980s, when the culture of corneal epithelium and corneal stromal cells was first actively carried out, most researchers considered that CECs do not proliferate in vivo and that is extremely difficult for CECs to proliferate even in vitro. Thus, few research groups were focused on the cultivation of CECs, and there were no success stories. Under such difficult circumstances, the partial success of the cultivation of human CECs was reported by researchers such as Engelman et al. [15], Joyce et al. [16], Miyata et al. [17], etc.; cornea researchers immediately became aware that human CECs could be cultured.

Meanwhile, unique treatment concepts were arising and being investigated. For instance, corneal endothelial cells were cultured, then being transplanted with bare corneal stroma on a bullous keratopathy (BK) animal model as an alternative to corneal transplantation with partial success being reported [18, 19].

Second Stage: Animal Experiments Using a Cultured CEC Sheet

When the first-stage CEC culture became successful, several research groups worldwide began to cultivate human CECs and reported successful cases of cultured CEC sheet transplantation in animal experiments [20, 21]. In addition, a unique method of magnetizing cultured cells and injecting these cells into the anterior chamber was also explored [22, 23]. On the other hand, considerable progress has been made in the culturing method, and several groups have investigated better culture protocols for the cultivation of human CECs [24–26]. However, the cultured CECs at that time contained a substantial amount of epithelial-to-mesenchymal transition (EMT) cells, which is now considered to have been at the dawn for cultured CECs for clinical use.

Third Stage: Modification of the Cultivation Protocol and Its Use for CEC Injection Therapy in an Animal Experiment

Following these early stages, culturing methods for primate CECs have been extensively explored and partially established, and cultured CEC sheet transplantation has now become possible in animal experiments [27]. However, since an appropriate carrier material for cultured cells had yet to be found, and since, simultaneously, corneal endothelial transplantation procedures such as Descemet stripping automated endothelial keratoplasty (DSAEK) [28] and Descemet membrane endothelial keratoplasty (DMEK) [29] became popular for the

treatment of BK, the interest in a tedious and labor-intensive cultured CEC sheet transplantation gradually faded.

On the other hand, a culture method of primate CECs holding a reasonable pump function and barrier function without contamination of EMT cells was established, and it has become possible to prepare a large amount of cultured CECs from one single donor cornea. The discovery of the use of a few factors in the culture method has greatly advanced in the procedure [30–33]. Furthermore, via animal experiments, it was previously reported that the CEC layer could be reconstructed by injecting cultured CECs together with a ROCK inhibitor into the anterior chamber, thus initiating a huge paradigm shift in corneal endothelial therapy [34, 35]. Those series of experiments were carried out using monkey and rabbit CECs, thus confirming that reconstitution of the CEC layer and restoration of corneal transparency is possible in a BK animal model. Those experiments also revealed that there was no increase of intraocular pressure (IOP), no intraocular inflammation, and no ectopic proliferation of cells [35], and that the cells that leaked out intravenously quickly disappeared.

Current State-of-the-Art Cultured HCECs Injection Therapy

Cultured HCECs for Clinical Use

Although numerous previous studies have investigated the culture of HCECs, very few studies, other than those from our group, have focused on the investigation of the cellular quality of cultured CECs. Initially, we focused more on the ability of the cells to proliferate than on the degree of maturation of cultured CECs in vitro. However, we now place our focus on the creation of cells for injection that contain a high purity of mature cells that is close to in vivo HCECs in order to obtain an optimal outcome and a higher CEC density post surgery (Fig. 18.1). For creation of such cells, merely the expression of Na⁺-K⁺ ATPase and ZO-1 is not good enough as it is necessary to define cell maturation by cell density, several cell surface markers, etc. [36–38]. However, we cannot deny the possibility that the basic concept may change in the future based upon the further accumulation of knowledge leading to a deeper understanding of cellular differentiation and the proliferation of cultured HCECs.

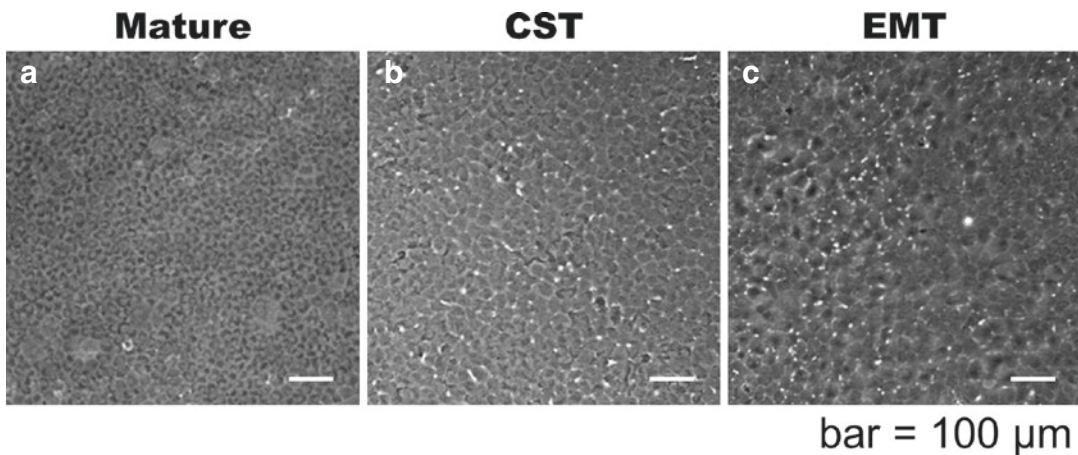


Fig. 18.1 Phase-contrast microscopy images of in vitro HCECs. (a) Mature cells (i.e., the ideal cells for clinical use), (b) cell state transition (CST) cells, (c) epithelial-to-mesenchymal transition (EMT) cells. Most of the

mature cells are small and uniform in shape, but the shape of the CST cells and the EMT cells are different from that of the mature cells

In addition, no contamination by mycoplasma in the cultured cells, as well as the sterility of the final culture media, via bacterial testing, viral testing, and an extremely low endotoxin concentration, must be confirmed in order to verify that the cells are suitable for clinical application [38]. Since many cultured cells are prepared from a single donor cornea, and are provided to a large number of patients as “one lot,” that single lot not only brings effective clinical results but may also bring adverse events. Thus, a great deal of care is required to guarantee safety. Incidentally, mature cultured CECs are not tumorigenic and do not show chromosomal abnormalities [39].

Clinical Trials of Cultured HCEC Injection Therapy

Since December 2013, we have been performing clinical trials on the injection of cultured HCECs for the treatment of corneal endothelial dysfunction including Fuchs endothelial corneal dystrophy, and to date, the trials have involved over 60 cases. All of the several clinical trials were prospective, either nonrandomized, “single-group” studies or randomized, double-blind multicenter studies, and were performed in compliance with either the Japanese guidelines on clinical research with human stem cells or the recently established Japanese laws in regard to regenerative medicine. Since the total number of *in vivo* HCECs located at the posterior corneal surface in normal subjects is approximately 2.4×10^5 cells, the amount of cells that was injected into the anterior chamber in our initial clinical study was approximately 1×10^6 cells, and the cells in the liquid suspension used for injection were comprised of cultured cells obtained from the culture cells in the flask a few hours prior to injection. Under local anesthesia, after removal of the abnormal extracellular matrix and/or degenerated CECs on the host Descemet’s membrane, the cultured HCECs in combination with the ROCK inhibitor were injected into the anterior chamber using a small-gauge needle [38]. The patients were then immediately placed in the prone position to enhance the adhesion of injected cells (Fig. 18.2).

The foundation of these series of novel surgical procedures is based on our previously reported experiments involving animal models [34, 35].

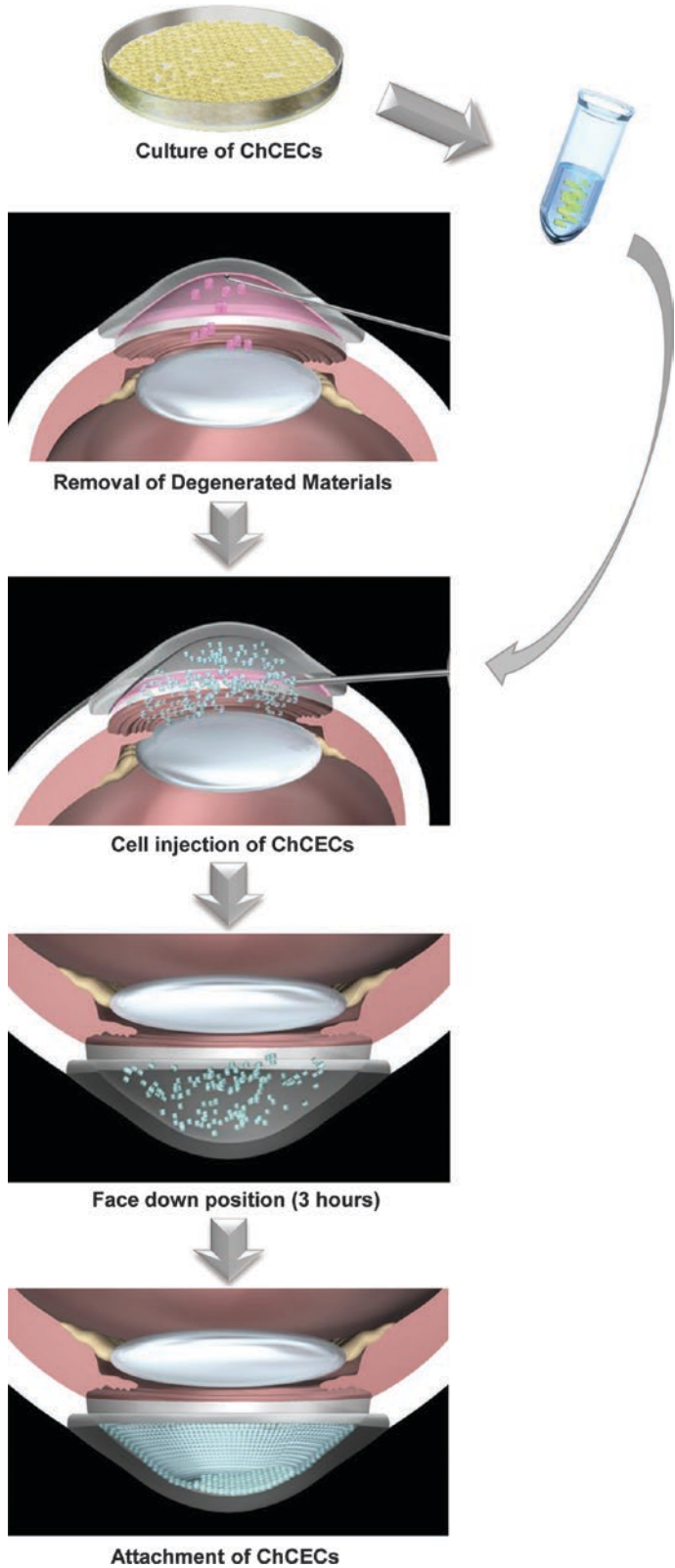
In addition, it should be noted that there were a few cases in which the patient’s Descemet’s membrane was partially peeled off, and even in such cases, good cell adhesion was obtained [38]. Those results mirrored the phenomenon that we observed in our previously reported animal experiments [40]. In the initial 11 cases of cell injection therapy, complete restoration of corneal transparency with a normal range of thickness was attained in all eyes within 12 weeks postoperatively. At 2 years postoperatively, the mean CEC density was 1534 cells/mm² and best-corrected visual acuity was significantly improved in all treated eyes (Fig. 18.3). No adverse reactions directly related to the cultured ‘HCEC injection therapy’, such as uveitis, elevation of IOP, or endothelial rejection, were observed [38]. In this way, the finding that the cells injected into the anterior chamber adhere to the posterior surface of the cornea and can self-organize is a revolutionary biological phenomenon, (Fig. 18.4) and it may be possible to repeat a similar event in other organs if it involves a close and confined space.

Although excessive cultured HCECs can escape from the corneas and migrate into the circulatory system, properly performed “cell trace” experiments revealed no obvious ectopic proliferation at sites other than the cornea, probably due to the occurrence of spontaneous cell digestion or cell death within 2-weeks post injection [35].

Evaluation of Antigenicity of Allogeneic Cells and Fate of Escaped Cells from the Eye

Since cultured CECs are basically created from allogenic donor corneas, strict and thorough consideration must be made toward immunological reactions. In fact, there is more frequency of corneal endothelial reaction in cases undergoing penetrating keratoplasty (PKP) than in those undergoing DSAEK as well as in cases

Fig. 18.2 Schema diagram showing the procedure of cultured HCEC injection therapy



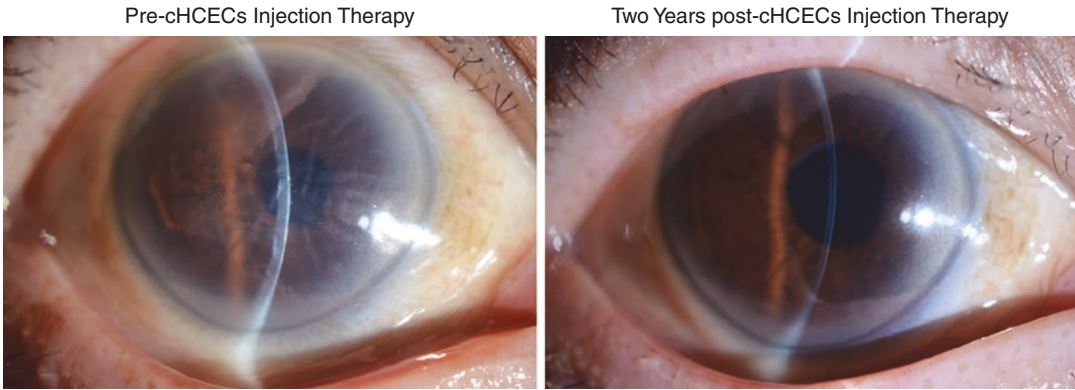
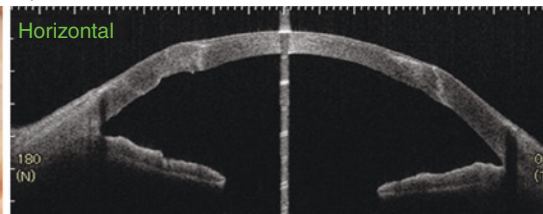
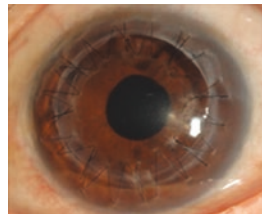


Fig. 18.3 Cultured HCECs injection therapy for bullous keratopathy. (a) Preoperative image of the eye of a 74-year-old female with bullous keratopathy (BK). (b)

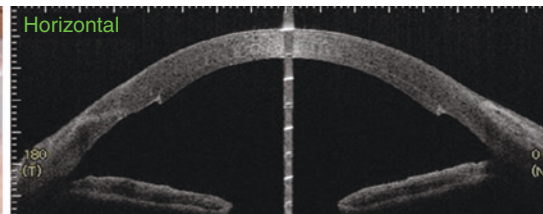
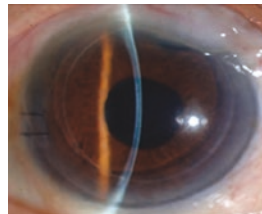
Image of the eye of 2 years post cultured HCECs injection therapy. The cornea post chCECs injection therapy is transparent

Fig. 18.4 A comparison via optical coherence tomography (OCT) images of the surgical outcomes in eyes post penetrating keratoplasty (PKP), Descemet stripping automated endothelial keratoplasty (DSAEK), and cultured HCEC injection therapy. The cornea of the eye that underwent cultured HCEC injection therapy resembles that of a normal healthy eye

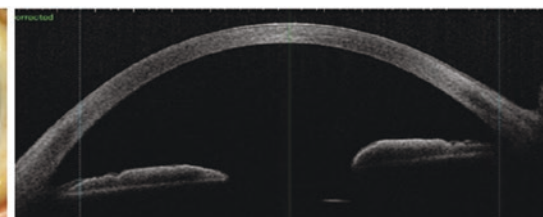
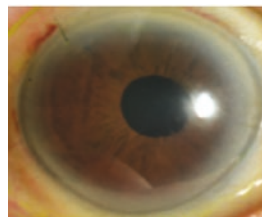
Penetrating Keratoplasty (PKP)



Descemet stripping automated endothelial keratoplasty (DSAEK)



Cultured hCEC-injection



undergoing DSAEK than in those undergoing DMEK [41], and an immunological aspect has yet to be well elucidated in regard to cell injection therapy in the clinical setting. However, in mouse experiments, an immune tolerance was reportedly obtained by injecting allogeneic CECs into the anterior chamber, and similar results occurred when administered intravenously [42]. In addition, Streilein et al. reported

that an immunological privilege could be maintained via anterior chamber-associated immune deviation (ACAID) to protect allogeneic cells in the anterior chamber [43]. Therefore, one can assume that there is minimal immunological reaction after cell injection therapy. Furthermore, the expression of human leukocyte antigen (HLA) class I in the cultured CECs decreased in the cells with a higher degree of cell maturation [36], thus

indicating that from the view of immunogenicity, matured cells are ideal cells in clinical use. Indeed, no immunological event occurred in the eyes of the patients in the clinical trials, although it should be noted that those patients did undergo topical steroid administration post surgery. Thus, the amount of ophthalmic steroids may be reduced in the future to prevent the events of steroid-induced increase of IOP and unexpected infection. Moreover, cultured CECs that escaped from the anterior chamber into the circulatory system produced no adverse events both in the clinical trials and in the animal experiments [34, 35, 38].

Future Directions

Seeds and Soil Theory in the Clinical Setting

The causes of BK include a wide diversity of diseases, such as Fuchs endothelial corneal dystrophy, pseudoexfoliation syndrome, cytomegalovirus corneal endothelitis, and corneal endothelial trauma in intraoperative ophthalmic surgeries, including cataract, glaucoma, and vitreoretinal surgery, etc. Among these diseases, it is fundamental to understand what kinds of pathological disease states are the most appropriate candidates for CEC injection therapy. For instance, it would be ideal if CEC injection therapy is effective in failed graft and/or BK induced by glaucoma filtration surgery. In the future, a clinical feedback is necessary to see the relationship between seeds in terms of the quality of cultured CECs, and soil in terms of corneal endothelial diseases, and the anterior chamber environment as a reverse translational research. Through this process, one would obtain the deep understanding of HCEC biology.

Combination Therapy with ROCK Inhibitor Eye Drops

As a treatment for corneal endothelial dysfunction, at least two treatment pathways can now be

considered: one pathway involves the supplementation of CECs via surgical treatment, such as corneal endothelial transplantation and cultured CEC injection therapy, while the other pathway is a medical treatment involving the topical application of drugs that maintains, at least to some extent, the health of aging cells and EMT cells. One of the representative candidates is a ROCK inhibitor eye drop. It has been reported that ROCK inhibitor is effective for promoting cell migration and cell proliferation and inhibiting cell death in cases of corneal endothelial injury [30, 44]. In addition, it should be noted that certain drugs may reduce oxidative stress and endoplasmic reticulum stress in CECs [45–49]. By using these eye drops for a certain time period from before and/or after cultured CEC injection therapy, there is a possibility that the injected CECs can be kept in a better state. In addition, theoretically, only topical eye-drop instillation after Descemet's membrane stripping in the central portion of the cornea may be beneficial. However, if it is intended to construct a high CEC density, some type of corneal endothelial transplantation or CEC injection cannot be avoided. In order to study this combined therapy, it is first necessary to establish "proof of concept" in each treatment and obtain official regulatory approval from the data in each clinical trial.

Advancement of Cell Delivery and Cell Storage

If CEC injection therapy eventually becomes accepted as a treatment for BK worldwide, the impressive wave in the methodological efficiency of cell culture and simplification of the surgical procedure is sure to quickly follow. From this aspect, and through advanced research and product development, the understanding of CEC biology will broadly deepen and interest in the methods of cell delivery and preservation will simply widen. In order to attain this high level, the cells must retain the high quality of cultured CECs as the ideal cells for clinical use. If this final, yet formidable, barrier can be overcome, the clinical use of cultured CECs is set to become

a global standard. As an extension, it appears that a topical drug application therapy based upon the essence of CEC biology may also develop. In any event, since the fact that cultured CECs injected as a cell suspension in the anterior chamber can self-organize and reconstitute the endothelial cell layer with corneal restoration has now been confirmed, an amazing advancement in medical science is sure to arise in the near future.

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New Developments in Dry Eye Research

19

Kazuo Tsubota and Norihiko Yokoi

Introduction

In the last few decades, research on dry eye disease has focused on the etiology of dry eye disease [1–3], the involvement of inflammation [4], the association with visual display terminal (VDT) use [5–9], and the use of contact lenses (CL) [8–12]. Research into the involvement of inflammation in dry eye led to the development of anti-inflammatory eye drops, such as cyclosporine A, and these drops are now the main treatments for dry eye in the USA and other countries. As dry eye is a lifestyle disease, with the people most at risk being those whose work involves prolonged daily use of VDTs and who have a sedentary lifestyle, dry eye research teams at Keio University, and others, have started investigating the relationship between an individual's systemic condition and dry eye disease [9, 13–18]. We review these relatively new areas of research in the first part of the chapter. In the second part of this chapter, we discuss the emerging field of tear-film breakup science [19, 20].

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Systemic Condition, Reactive Oxygen Species (ROS), and Dry Eye

A sedentary lifestyle and prolonged daily use of VDTs are risk factors for dry eye disease. A sedentary lifestyle is also one of the risk factors in diseases such as atherosclerosis, cancer, and diabetes whose incidence rates increase with age [21]. Atherosclerosis is associated with silent inflammation caused by modern lifestyles, involving lack of exercise and overeating. Chronic inflammation is also known to be involved in dry eye etiology, and so we hypothesized that dry eye may also be partly caused by poor lifestyle choices. If dry eye is a lifestyle disease, the prevention or treatment of dry eye should start with lifestyle modification rather than medication. In 1993, we reported that tear evaporation increases in VDT workers, suggesting a direct link between VDT work and dry eye [22].

Caloric Restriction and Reactive Oxygen Species

How should we initiate research to demonstrate that lifestyle modifications can alleviate dry eye? Calorie restriction (CR) is a major lifestyle modification that is known to benefit health and increase longevity, so we wondered if CR would help alleviate dry eye. We had previously

developed a dry eye animal model, which mimics the dry eye seen in VDT workers, by placing the animals (mouse) on jogging boards (swings) to disrupt blink frequency and disrupt tear dynamics [23]. We used this animal model to ascertain if the dietary intervention of CR could have an effect on dry eye. It is known that tear secretion decreases with aging; however, we demonstrated that tear production was maintained in our dry eye animal model in aging animals that had been reared on a calorie-restricted diet (Fig. 19.1) [24]. This was a surprising observation because we did not expect to be able to maintain tear production with one sole intervention, in this case CR. The mechanism by which CR has its effect has not been fully elucidated, but we do know that reactive oxygen species (ROS) are suppressed in the lacrimal gland of the CR animal model. Whereas, in the untreated animals, increased ROS levels in the lacrimal glands suppress tear secretion, resulting in decreased tear production [7, 25].

Superoxide dismutase (SOD) is an important enzyme in the respiration pathway, removing

ROS from cells. Increased levels of ROS are thought to be a major mechanism of aging [26], and so we speculated that ROS could cause, or at least worsen, dry eye. We previously found that the SOD mutant mouse (SOD mouse), in which ROS accumulates from the early stages of life, showed aging phenomena in the retina [27] or nerve fibers [28]. Also, we have observed that dry eye and meibomian gland dysfunction (MGD) develop with early onset of dry eye development, with excessive ROS in both the lacrimal gland [29] and meibomian gland [30].

Dry Eye and Obesity

Obesity is another risk factor for accelerated aging, so we investigated tear production in an obese mouse. Tear production was greatly reduced in the db/db mouse, the obese mouse which lacks leptin receptors [31]. In contrast, exercise is beneficial for systemic health, so we hypothesized that exercise may alleviate dry eye. We found that exercise in the mouse increased tear production, and ROS suppression in the lacrimal gland was observed [31]. This research supports the hypothesis that an individual's systemic conditions and factors that affect ROS levels are involved in dry eye pathogenesis. Tears have abundant antioxidant molecules [32], and these are dramatically reduced in dry eye [33]. Thus, dietary supplementation with antioxidants may alleviate the condition of dry eye [34].

Epidemiological Study of Dry Eye Patients' Systemic Condition

The Osaka epidemiological study, designed to discover the risk factors of dry eye, was based on our previous research into systemic involvement in dry eye [35–41]. The Osaka study provided an estimate of the prevalence of dry eye in the Japanese population and demonstrated the relationship between dry eye incidence and lifestyle choices. First, we looked at the association between obesity and dry eye. Historically, the main type of dry eye seen was the tear-deficient

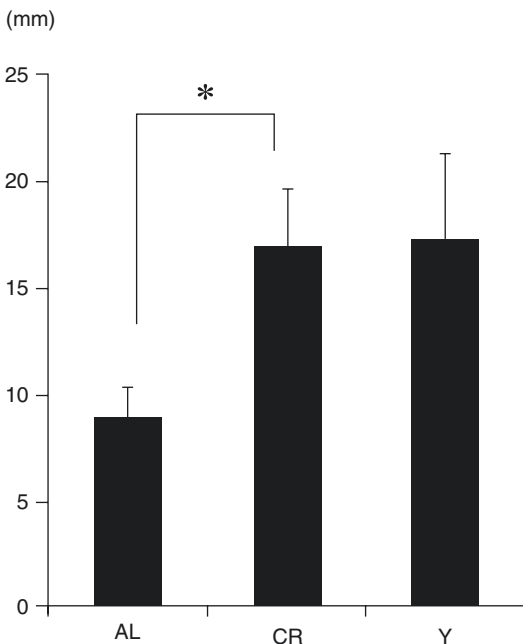


Fig. 19.1 Tear function change by CR effect. Cotton thread test. AL, ad libitum group; CR, caloric restriction group. $*p = 0.02$. (Reprinted from Kawashima et al. [24], Copyright (2010), with permission from Elsevier [24].)

dry eye characteristic of Sjögren's syndrome. This leads to the assumption that typical dry eye patients are thin elderly women. However, the Osaka study showed that BMI is negatively related to tear production [37]. In other words, we found a higher incidence of dry eye in people who are obese. Sports participation is considered to be good for health, and we investigated dry eye in individuals who exercise frequently. As we expected, we found a lower incidence of dry eye in individuals with healthy lifestyles that included more exercise compared with those individuals who had unhealthy lifestyles, with less exercise [42]. This result had been suggested by results from the animal study that showed exercise increased tear production [31].

Getting sufficient sleep is another important lifestyle factor; and optimal sleep patterns are thought to be associated with optimal health [43]. We found that poor sleep quality is associated with dry eye incidence [41]. The mechanism of this relationship is unknown, but there are two possibilities: dry eye may cause poor quality of sleep, meaning that dry eye was the primary event; or poor sleep quality may worsen dry eye. In the latter case, sleep quality would be one of the etiologies of dry eye, which would be interesting because many dry eye patients complain of symptoms from the point of awakening. We performed a series of clinical studies related to sleep, which confirmed the association between dry eye and poor sleep quality [44–46]. We carried out a simple clinical study to demonstrate causality, asking the question “Does dry eye treatment improve quality of sleep for dry eye patients?” Our study demonstrated that initial dry eye treatment improved the patients' quality of life [47], leaving the possibility that dry eye may interfere with sleep quality through a vicious circle. This has the effect that when the patient's dry eye is well controlled, the patient's quality of life improves dramatically. Chronic pain patients are known to suffer from sleep disorders [48, 49], and as dry eye is now categorized as one of the pain syndromes, an association between dry eye and poor quality sleep seems highly likely.

In our study, we were interested to note a negative association between subjective happiness

levels and dry eye symptoms (Fig. 19.2) [40]. The study showed that the signs of dry eye, such as the Schirmer test result or ocular surface staining, are not related to the level of happiness but the symptoms are related to the level of happiness; patients with no signs of dry eye but who had dry eye symptoms had the lowest happiness scores, suggesting that happiness might be related to the neuropathic component of the ocular surface. This research shows that the prevalence of dry eye is related to lifestyle and the quality of life in modern society.

New Systemic Approach for the Treatment of Dry Eye

If an individual's systemic condition is involved in the pathogenesis of dry eye, systemic treatments should be looked at in addition to topical therapies, such as eye drops. We began investigating the effects of lifestyle in the treatment of dry eye, focusing on diet, exercise, and stress control as the lifestyle factors likely to be involved.

Dry Eye and Dietary Supplements

We initially surveyed several fruit ingredients and supplements and found that some of them were effective against dry eye [50]. Lactoferrin is an important protein found in milk and tears and is known to suppress systemic inflammation [51, 52]. We hypothesized that lactoferrin intake would alleviate dry eye. An animal study showed increased tear production [53] with the reduction of ROS in the lacrimal gland. A clinical study in Sjögren's patients also demonstrated the beneficial effect of lactoferrin [16]. As lactoferrin intake is related to the modification of the intestinal microbiome, we further hypothesized that microbiome-oriented supplements would be beneficial for dry eye. The combination of lactoferrin and *Enterococcus faecium* (WB2000 by Wakamoto Pharmaceutical Co., Ltd.) has proved to be effective in clinical trials [18]. The combination supplement was introduced to the Japanese

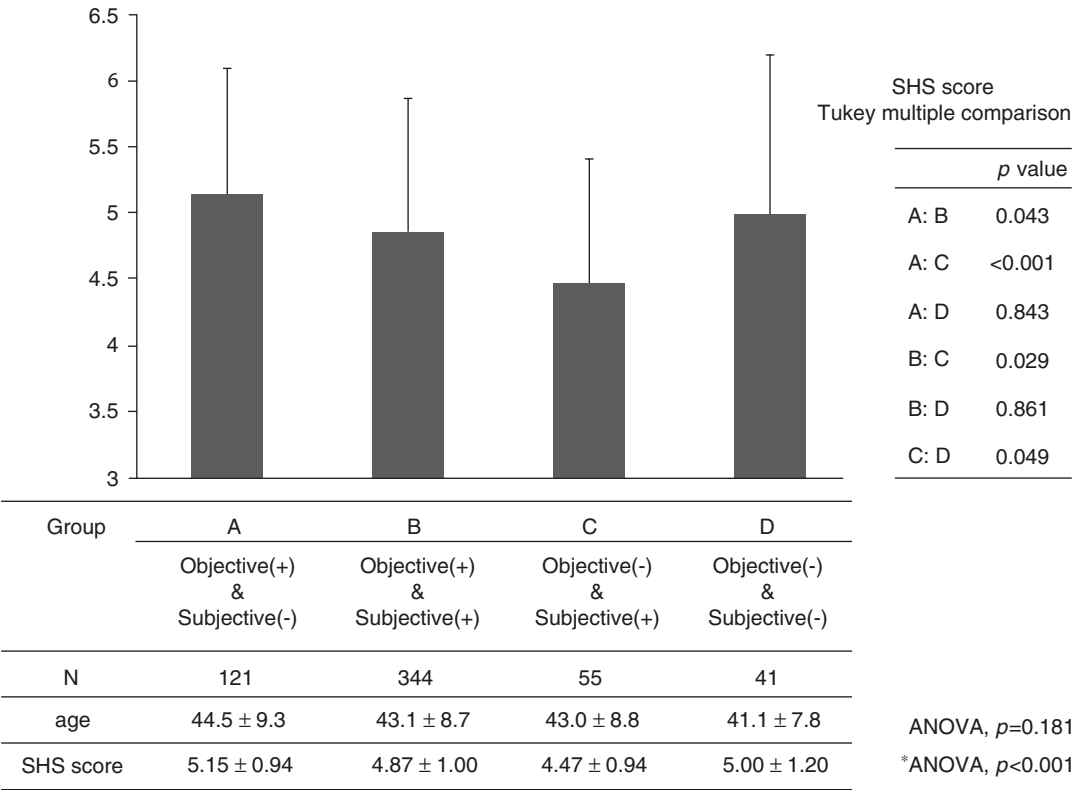


Fig. 19.2 Comparison of SHS score between objective and subjective classifications. SD standard deviation. (Reprinted with permission from Kawashima et al. [40].)

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market and has been well received as a therapeutic option for treating dry eye, in addition to the use of eye drops or moisture glasses.

Berries are healthy foods, which people are now encouraged to consume [54, 55]. We have surveyed several types of berries and found that consumption of the maqui berry is associated with stimulation of the lacrimal gland [56]. It was not previously known that different types of berries had different absorption properties and consequently had different effects on dry eye. Thus, the general concept of all berries being good for health might not be applicable to dry eye as some berries will work better toward alleviating this condition than others.

Honey or royal jelly is also considered to be good for health. For dry eye, we found that only royal jelly is effective and did not see an effect with honey [57]. The ingredients of royal jelly appear to increase the calcium concentration of

the lacrimal gland acinar cells and increase tear secretion. Both the animal study and the clinical pilot study showed the effectiveness of royal jelly [17], making royal jelly a promising food supplement for controlling dry eye.

Dry Eye and Depression

In addition to the muscarinic acetylcholine receptor, a new receptor for serotonin in the lacrimal gland was found (Fig. 19.3) [58]. Tear production was dramatically reduced when mice were fed on 5-hydroxytryptophan (5-HTP) deficient feed; 5-hydroxytryptophan is the amino acid precursor of serotonin. When serotonin levels in the body decreased after 5-HTP depletion, the lacrimal gland did not work. This is a recently described receptor along with the previously reported muscarinic and adrenergic

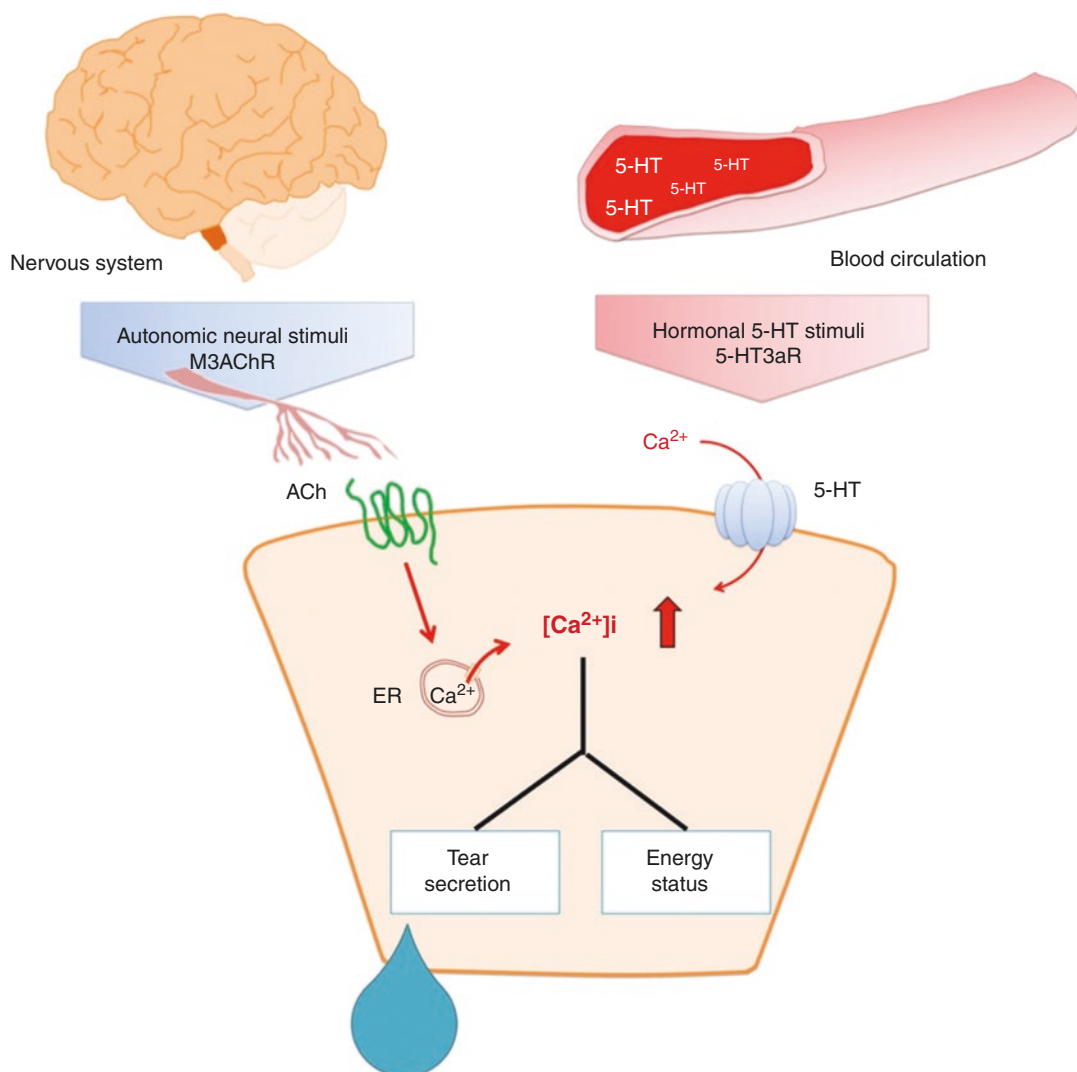


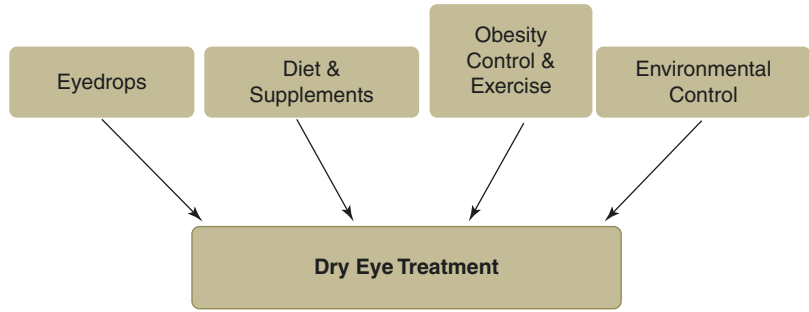
Fig. 19.3 Outline of hormonal regulation of tear secretion by serotonin. 5-HT_{3aR}, a ligand-gated ion channel that induces intracellular calcium ($[Ca^{2+}]_i$) mobilization through entry of extracellular Ca^{2+} into the cell, is located in the basolateral lacrimal gland (LG). $[Ca^{2+}]_i$ in the LG acinar cells, which are responsible for maintaining aqueous tear secretion and cellular energy, is regulated by the interaction between 5-HT_{3aR} and m3AChR activity via blood 5-HT and neuronal parasympathetic stimulation,

respectively. Tear secretion is maintained at an optimum level by the simultaneous action of blood 5-HT and the neural parasympathetic pathway. ACh, acetylcholine; ER, endoplasmic reticulum; m3AChR, M3 muscarinic acetylcholine receptor; 5-HT, serotonin; 5-HT_{3aR}, serotonin type 3a receptor. (Reprinted with permission from Imada et al. [58]. © Imada T, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License [58].)

receptors. Serotonin is believed to be related to depression and studies show that 5-HTP supplementation can alleviate depression [59–61]. As recent research has shown an association between depression and dry eye [44–46, 62], it is possible that the serotonin receptors in the

lacrimal gland may be involved in this relationship between mental health and dry eye. Taken together, it is apparent that an individual's systemic condition affects lacrimal gland function and could be used as a future therapeutic target (Fig. 19.4).

Fig. 19.4 Systemic approach for the treatment of dry eye



Tear-Film Breakup Science

The other emerging field of research in dry eye is the importance of tear-film breakup [19, 63, 64]. Although strong emphasis has been placed on inflammation in the pathogenesis of dry eye, it has become apparent that abnormal tear-film breakup is the fundamental phenomenon of dry eye disorders [63, 64]. The importance of abnormal/short tear-film breakup is a major factor for dry eye and has been included as part of the definition of dry eye in DEWS I, DEWS II, which is the international consensus about the definition of dry eye, as well as the Korean Corneal Disease Study Group, Chinese Medical Association Ophthalmic Branch Cornea Group, Asia Dry Eye Society, and AAO Dry Eye Preferred Practice Patterns [2, 65–67, 111, 112]. The Asia Dry Eye Society has proposed that abnormal tear-film breakup is an essential component of dry eye pathogenesis [68]. Dry eye disease consists of two parts: the symptoms and the signs. Symptoms include a variety of unpleasant sensations, such as heaviness, burning, grittiness, etc., which are expressions of pain [1, 69–74]. In addition to the subjective symptoms, impaired visual function is also considered to be a part of the symptoms of dry eye (see section on Visual Impairment Caused by an Unstable Tear Film).

What are the most important dry eye signs? Several signs have been proposed in the past, including fluorescein dye staining, rose-bengal dye staining, lissamine-green dye staining, inflammatory markers in tears and tear osmolarity, but no one parameter is directly related to the

symptoms. The neuropathic pain components are now considered to be very important in the pathogenesis of dry eye, so researchers are investigating the sensation on the corneal epithelium [73, 74]. One theory on dry eye pain is that subnormal stimulation through early tear breakup may accelerate the pain sensation through receptors such as TRPM8 [75], thus the cornea should be covered by a stable tear film all the time to avoid subnormal stimulation. It is thought that scleral lenses alleviate severe pain in dry eye patients by avoiding the subnormal stimulation on the cornea as the sclera lens' water reservoir provides a substitute for the unbroken tear layer.

In 1995, we described the novel concept of dry eye with short tear-film breakup time (BUT) [19], in which there is a short tear-film breakup time, no ocular staining but severe symptoms. After our publication, other researchers also demonstrated the importance of this type of dry eye [76–82]. This type of dry eye is easily misdiagnosed because there is no staining, and the appearance of the eyes in the slit-lamp examination is almost normal. Diagnosis rests on the measurement of tear-film breakup. Recently, we reported that the sensation pattern is abnormal in this short BUT-type of dry eye and proposed the presence of neuralgia in the pathogenesis of short BUT-type dry eye (Fig. 19.5) [83]. The definition and diagnostic criteria proposed by the Asia Dry Eye Society, which showed that an unstable tear film with symptoms is sufficient for a dry eye diagnosis, is based on this concept in cases of abnormal sensitivity in dry eye patients [68].

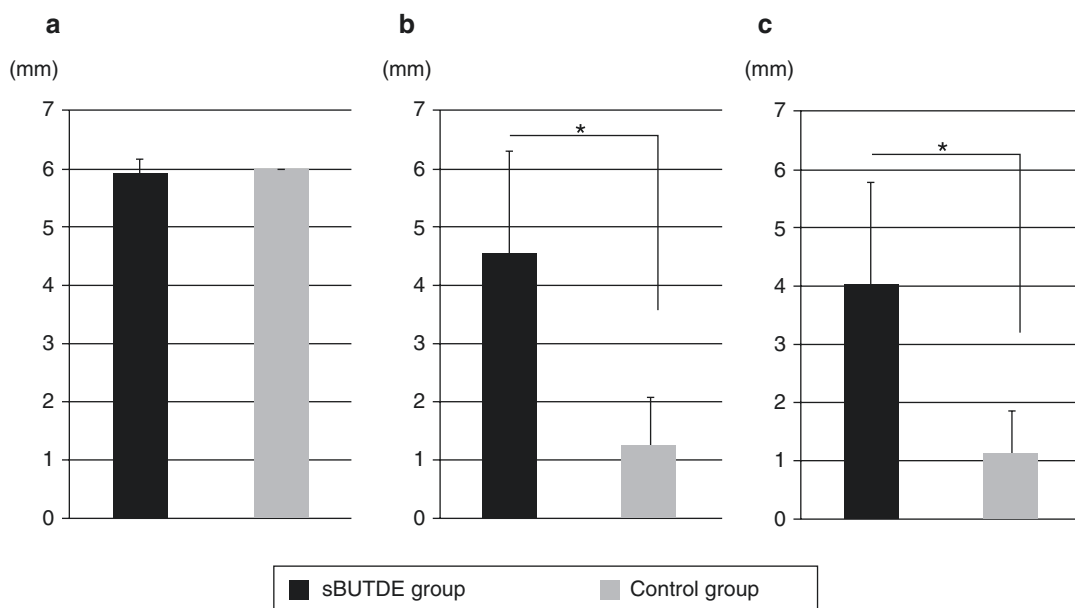


Fig. 19.5 Corneal sensitivity scores in the symptomatic and asymptomatic groups. (a) S-touch, the sensitivity at which the subject perceives touch. (b) S-blink, the sensitivity at which blinking is induced. (c) S-pain, the sensi-

tivity at which the subject feels pain. (Reprinted with permission from Kaido et al. [83]. © Kaido M, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License [83].)

Visual Impairment Caused by an Unstable Tear Film

The importance of tear-film breakup is supported by the relationship between visual impairment in dry eye and tear breakup time. As the first part of the eye the light enters is the tear-film layer, this smooth stable film is essential for good visual function. When the tear film becomes unstable, light entering the eye is scattered on the surface of the tear-film layer interfering with visual function [84–88]. Dry eye patients suffer from impaired visual activities, such as night driving or reading [89, 90]. This is caused by the unstable tear film. The visual function related to the unstable tear film can be detected; this is called functional visual acuity (Fig. 19.6) [91]. Koh et al. described higher order aberration changes in dry eye patients [85, 92]. The impaired functional visual acuity can be recovered by treatment of the eyes by punctal occlusion [76, 77] or using diquafosol sodium that stabilizes the tear film [93–96]. Furthermore, visual impairment resulting from unstable tear film explains why dry eye patients

suffer more when doing VDT work. Modern VDTs use blue light LEDs as a light source, which contains an abundance of blue light components. As blue light is a short wave length of light, it is easily scattered by uneven surfaces, which means that dry eye patients, as a result of their unstable tear films, have more difficulty seeing in blue light. Thus, reducing blue light components provides a better light environment for dry eye patients [97]. The recent introduction of blue light reducing glasses for computer users should help dry eye patients.

Fluorescein Breakup Patterns for the Diagnosis of Dry Eye Subtypes

Yokoi et al. recently reported the classification of fluorescein breakup patterns (BUPs), which appears to be based on different pathophysiologies, as a simple method of diagnosing DE subtypes [20]. Several studies attempted to show the importance of tear-film BUPs in DE, yet no

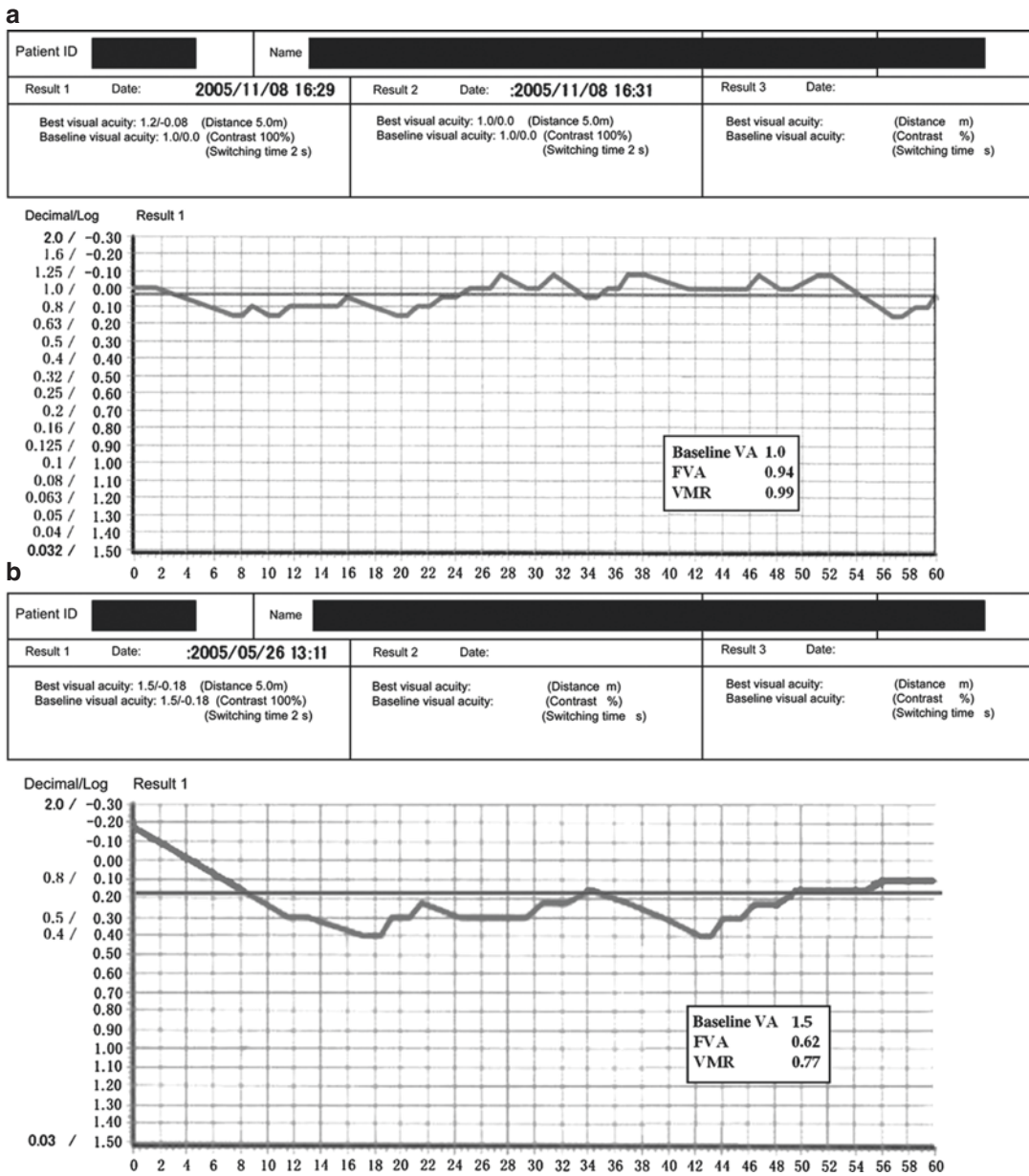


Fig. 19.6 Results of functional visual acuity (FVA) measurement: graphs from subjects with normal and dry eyes. (a) Results from the left eye of a 22-year-old woman without dry eye: Schirmer score, 28 mm; no vital staining; and tear-film breakup time, 7 seconds. Her baseline decimal visual acuity, FVA, and visual maintenance ratio (VMR) scores were 1.0, 0.94, and 0.99, respectively. (b) Results

from the right eye of a 52-year-old man with dry eye: Schirmer score, 5 mm; rose-bengal score, 8 points; fluorescein-staining score, 4 points; and tear-film breakup time, 1 second. (Reprinted with permission from Kaido et al. [91] by Wolters Kluwer Health, Inc. https://journals.lww.com/corneajrnl/fulltext/2007/10001/Concept_of_Functional_Visual_Acuity_and_its.7.aspx [91].)

conclusions were obtained [98]. The representative abnormal values for tear-film breakup time (BUT) are reportedly less than 5 or 10 seconds [68]. However, this value provides no

information about DE subtypes. According to the classification by Yokoi et al., fluorescein BUPs were categorized into five categories, and each BUP is caused depending on the insuffi-

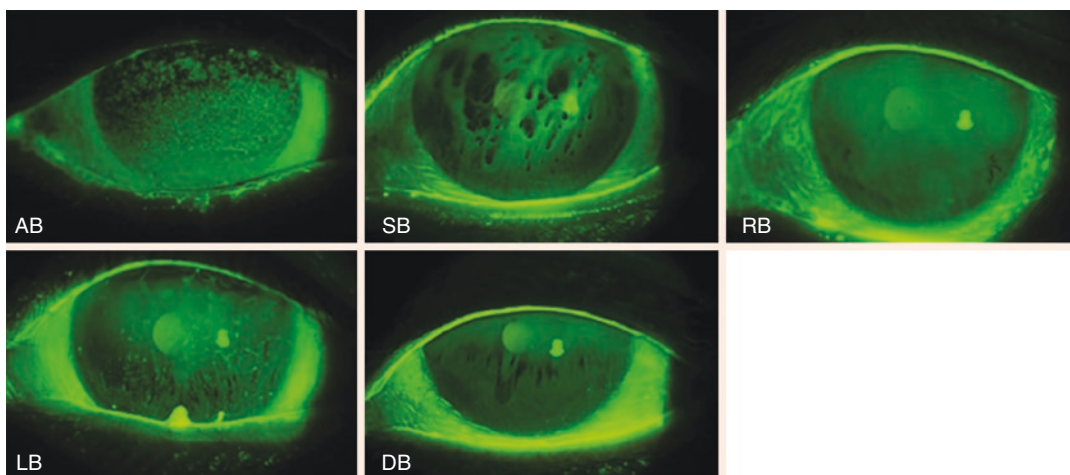


Fig. 19.7 Classification of fluorescein breakup patterns. Fluorescein breakup patterns after the eye is opened can be classified into five essential categories. Immediately after eye opening, an “Area break (AB)” or a “Spot break (SB)”, respectively, can be seen in relation to severe aqueous tear deficient dry eye (ATDDE) or decreased wettability DE

(DWDE). During the upward movement of fluorescein, a “Line break (LB)” or a “Dimple break (DB)”, respectively, can be seen in relation to mild to moderate ATDDE or DWDE. After cessation of the upward movement of fluorescein, a “Random break (RB)” can be seen in relation to increased evaporation dry eye (IEDE)

cient components of the ocular surface, including aqueous tears, secretory mucins, and membrane-associated mucins of the ocular-surface epithelium in relation to DE subtypes (Fig. 19.7).

Tear-film-oriented therapy (TFOT) is a newly developed concept in which DE is treated by improving tear-film breakup via the supplementation of insufficient ocular-surface components, and fluorescein BUPs are useful for determining which components are insufficient. This method of diagnosis is termed “Tear-film-oriented diagnosis” (TFOD).

Treatment for the Improvement of Abnormal Tear-Film Breakup

Previously, it was difficult to effectively treat the symptoms in short-BUT DE with treatments other than punctal occlusion [76, 77], in which increased aqueous tear volume results in counteracting the tear-film breakup and leads to the reduction of symptoms. However, side effects such as epiphora with resultant blurred vision, or epiphora alone, or discharge can occur, so punctal occlusion is recommended only in patients

with severe aqueous tear deficiency. In DE, controlling inflammation is important, yet it has no direct effect on tear-film stabilization.

Diquafosol sodium eye drops and rebamipide eye drops increase aqueous tears/mucins and mucins alone, respectively, both of which can increase fluorescein BUT [93], thus leading to improved comfort and visual function. These treatments are now available as eye drops and are the first topical treatments, which stabilize the tear-film layer [93, 95, 96, 99–107]. Stabilizing the tear-film layer also improves functional visual acuity.

Another non-invasive method for the treatment of short BUT is environmental modification. Adverse environments, such as windy or very dry conditions, exacerbate dry eye [108]. In contrast, moisture-rich environments stabilize tear evaporation (Fig. 19.8) [109]. In theory, if there is no evaporation, the tear film should be stable, unless corneal wettability is otherwise disrupted (Fig. 19.9) [110]. Thus, environmental humidity is important for the prevention and treatment of dry eye. Swimming goggles, moisture glasses, and room humidifiers are the most popular interventions. For environmental moisture control, the passive increase in humidity

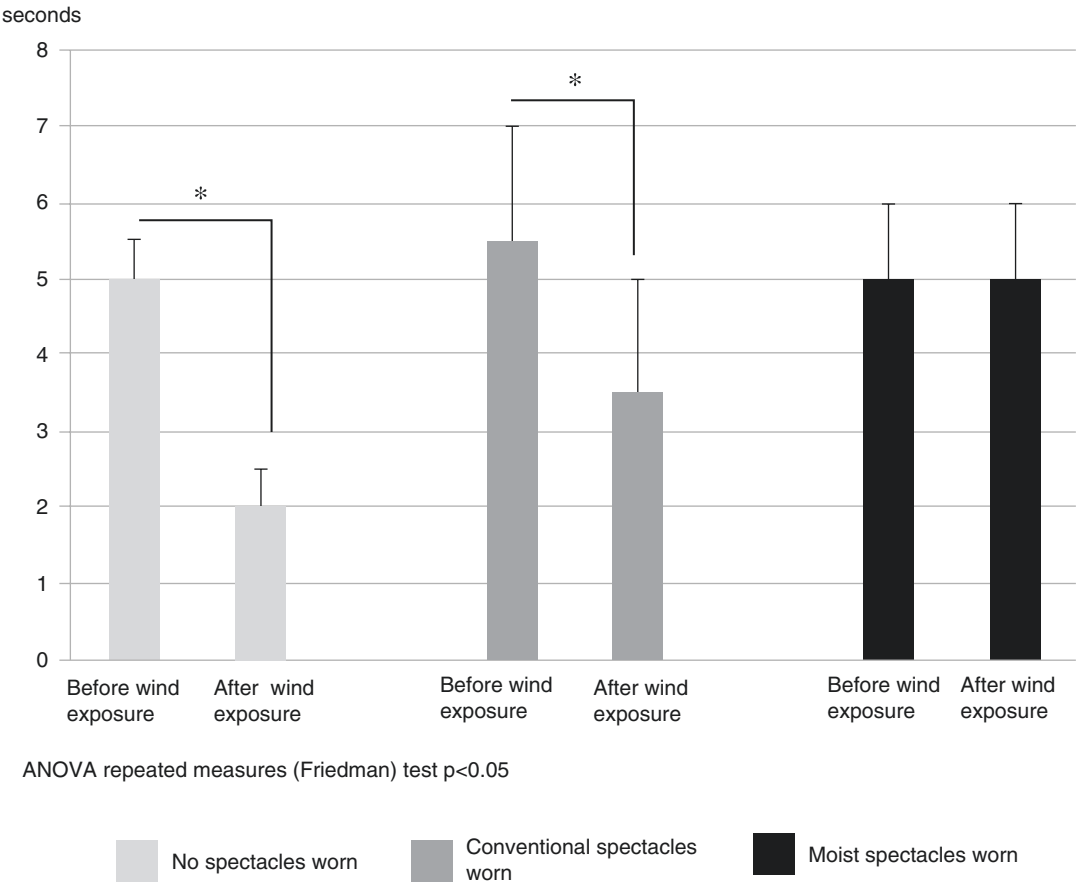
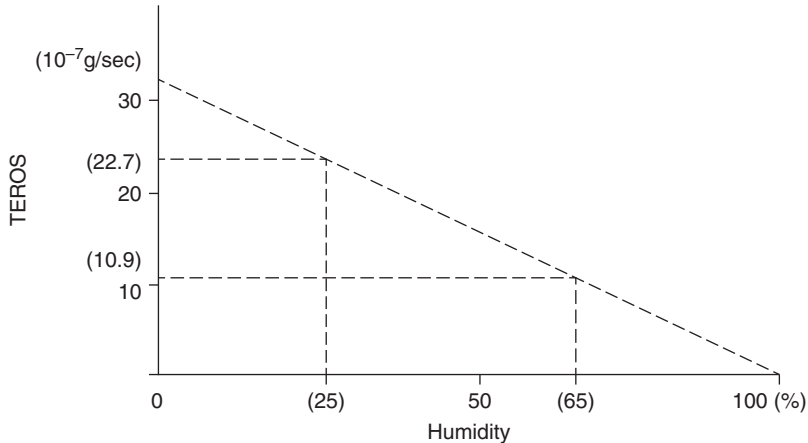


Fig. 19.8 Comparison of tear breakup time scores between moist spectacle wearers and non-wearers after adverse environmental exposure. Note the significant decrease in tear-film breakup time scores under wind exposure with or without moist chamber spectacle wear.

$*P = 0.05$. ANOVA, analysis of variance. (Reprinted with permission from Ogawa et al. [109] by Wolters Kluwer Health, Inc. https://journals.lww.com/claajournal/full-text/2018/11000/Evaluation_of_the_Effect_of_Moist_Chamber.5.aspx [109].)

Fig. 19.9 The relationship between the environmental humidity and tear evaporation rate from ocular surface (TEROS). When the humidity is 100%, the tear evaporation is zero. (Reprinted with permission from Tsubota and Yamada [110]. © Association for Research in Vision and Ophthalmology [110].)



afforded by commercially available moisture glasses is an easy way to begin treatment. Future developments in these glasses are expected.

Conclusion

In this chapter, we have discussed two aspects of dry eye research. One is the relationship between dry eye and an individual's systemic condition, as dry eye is a lifestyle disease most often seen in people who work with computers and have a sedentary lifestyle. Dry eye is an additional risk factor for lifestyles that are also associated with diseases such as diabetes, obesity, and insomnia. Although the current treatment modality uses eye drops as the first line of treatment, we believe that lifestyle modification should be the first intervention. We have reviewed several aspects of the relationship between dry eye and systemic condition and have suggested possible approaches including foods and dietary supplements.

In the second half of this chapter, we focused on tear-film breakup. It has become apparent that neuropathic components are important in dry eye development. The pain point of the cornea is the ocular surface that is supposed to be completely covered by the tear film. When the ocular surface is not covered by the tear-film layer where there is an unstable tear film, the pain point is stimulated and this causes pain. Consequently, tear-film stability is an area of research that is currently attracting attention, and we described the recent developments in this field, including tear-film breakup pattern categorization and short breakup time. Future research should focus on the relationship between the systemic condition of the individual, including their lifestyle, tear-film stability, and dry eye symptoms. Once all the factors in dry eye have been established, new treatments and preventative measures against dry eye could be developed.

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Conflicts of Interest Kazuo Tsubota reports consultancies and research funding received from Santen Pharmaceutical Co.,

Ltd., and Otsuka Pharmaceutical Co., Ltd., as well as research funding from Wakamoto Pharmaceutical Co., Ltd., Toshiba Materials, R-Tech Ueno, and Kowa Company. He holds the patent rights for the method and the apparatus used for the measurement of functional visual acuity (US patent no: 255 7470026), and is the inventor of JINS Moisture eye glasses by JINS, Inc., patent holder. In addition, he is an investor for Tissue Tech, Inc., Tear Solutions and Tsubota Laboratory, Inc. where he holds the position of CEO. Outside the submitted work, Kazuo Tsubota reports consultancies for Novaliq GmbH, Laboratoires Thea, AMO Japan K.K., Ocular Surface Research & Education Foundation, and NIDEK Co., Ltd.

Norihiko Yokoi reports personal fees from Santen Pharmaceutical Co., Ltd., and Otsuka Pharmaceutical Co., Ltd.

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Novel Approaches for Restoring the Function of the Limbal Stem Cell Niche

20

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Introduction

Various congenital, traumatic, toxic, and autoimmune processes may lead to limbal stem cell dysfunction (LSCD). In the past, patients with LSCD suffered from permanent loss of visual function as the condition was difficult to manage. In recent decades, research in this area has led to novel treatment paradigms. It was discovered that significant corneal injury in patients with LSCD can lead to not only a decreased number of corneal epithelial progenitor cells but also a disturbance in the limbal stem cell niche. Under these pathological conditions, a pro-inflammatory environment persists in patients with LSCD (Fig. 20.1), leading to continued activation of inflammatory cytokines, impaired macrophage phagocytosis, and pathological hemangiogenesis. Thus, the current strategy in corneal regeneration in this area is a two-pronged approach, including both direct repopulation of limbal epithelial stem cells (LESCs) and also restoration of the limbal stem

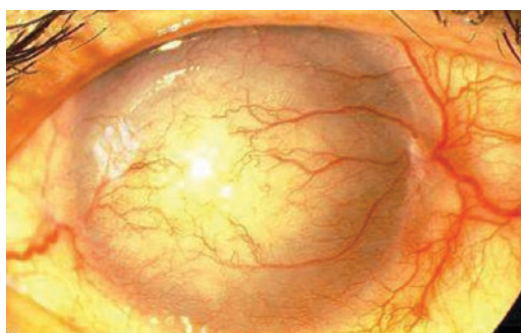


Fig. 20.1 Extensive scar formation and neovascularization in a patient with LSCD

cell niche. In this chapter, a summary of current approaches to corneal epithelial regeneration will be provided. Novel research into possible future approaches to corneal regeneration including the use of mesenchymal stem cells will also be discussed.

Physiology of the Corneal Limbus

Previously, it was found that corneal epithelial stem cells reside in the undulating ridges of palisades of Vogt at the corneal limbus. In this region, corneal epithelial progenitor cells are able to survive to proliferate and differentiate and interact with various other cell types, including immune cells, nerve cells, vascular cells, stromal cells, and melanocytes in a specialized microenviron-

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ment, or the limbal stem cell niche. The limbal niche is critical to the maintenance of healthy limbal epithelial stem cells (LESCs). Recent advances in imaging technology have led to an improved understanding of the limbal niche anatomy. Specifically, it appears that the limbal epithelium extends more deeply than central epithelium and is marked by intervening stromal areas. Although distinct distribution patterns of LESC have been identified in different individuals, LESC are commonly found in the limbal epithelial crypts at the basal layer of the limbal epithelium [1–3]. Confocal microscopy also revealed unique clusters of hyperreflective CD90 and CD105 positive mesenchymal stem cells (MSCs) in the anterior limbal stroma adjacent to the basal epithelium (Fig. 20.2) [4]. In addition to their anatomical proximity to corneal epithelial progenitor cells, these corneal mesenchymal stem cells were shown to interact with limbal epithelial stem cells through various molecules, including aquaporin-1, vimentin, and chondroitin sulfate [5, 6], and promote epithelial clonal growth and differentiation via various signaling pathways, including SDF-1/CXCR4, BMP/Wnt, and IL-6/STAT signaling [7, 8]. As mesenchymal

stem cells were found in most other types of adult tissues, over the past decade, these cells were increasingly investigated for their therapeutic potentials in a range of human diseases. Due to their anti-inflammatory and potential regenerative properties through their secreted factors, it is increasingly more plausible that mesenchymal cells are critical to LESC function.

Repopulation of Limbal Epithelial Stem Cells

Limbal Tissue Transplantation

Over the last few decades, developments in novel limbal tissue transplantation techniques, in conjunction with advances in immunosuppressive agents, have offered new hopes to patients with disabling ocular surface diseases and stem cell deficiency. For the surgeon, it is important to follow an algorithm for treating patients with stem cell deficiency. Prior to attempting any limbal tissue transplantation techniques, it is important to manage any comorbid ocular conditions. For example, prior

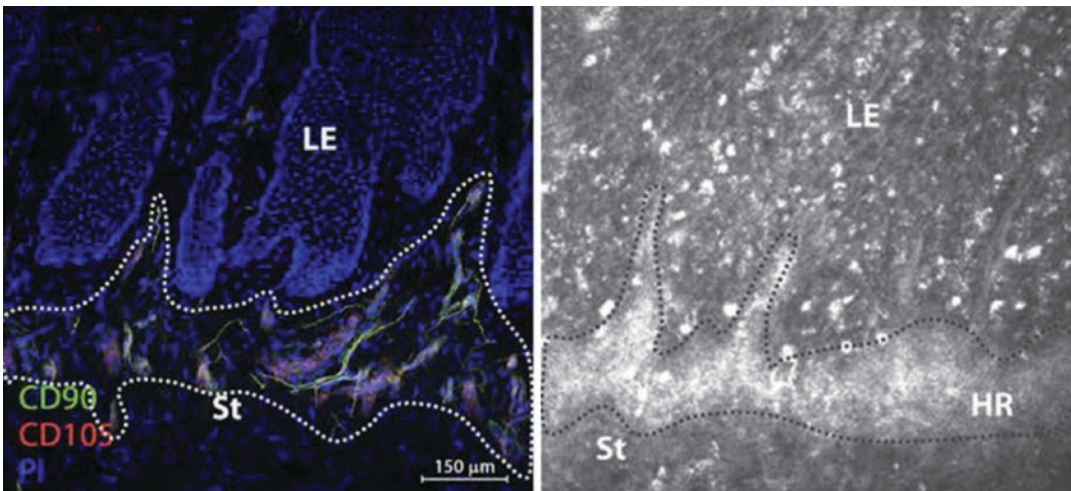


Fig. 20.2 Comparison of sequential confocal microscopic images of immunostained [CD90-FITC (green); CD105-Alexa 633(red); propidium iodide (blue)] tangential sections of cadaver limbus with in vivo confocal microscopic images (IVCM) of superior limbus in a healthy subject. Based on the location and morphological

features, the unique clusters of CD90- and CD105-positive mesenchymal stem cells observed in donor limbal sections appear as hyperreflective (HR) structures in the anterior limbal stroma by IVCN (LE, limbal epithelium; St, stroma). All IVCN images are 400 × 400 μm (reprinted with permission from: Mathews et al. [4])

tube shunt surgeries may be considered for patients with glaucoma on multiple toxic ocular medications; patients with any eyelid or eyelash abnormalities should have these corrected and optimized first. Ocular surface inflammation should also be minimized as much as possible. Several transplantation techniques are currently available clinically depending on whether stem cell deficiency is unilateral or bilateral. Furthermore, the source of stem cells used can be either an autograft or allograft, which can be obtained from either a living relative or cadaver.

For patients with unilateral LSCD, either a conjunctival limbal autograft (CLAU) or a simple limbal epithelial transplant (SLET) may be performed [9]. With either procedures, it is ideal that the patient's donor eye be free from any condition that may later predispose it to the development of stem cell deficiency. In CLAU, two trapezoid-shaped limbal grafts measuring 6 mm from the superior and inferior limbus of the healthy eye are excised and transplanted to the diseased eye. The procedure for SLET is similar; however, a smaller 2 by 2 mm area centered on the superior limbus with minimal conjunctival tissue is isolated from the healthy eye. This tissue is then cut into 8–10 small pieces and placed on amniotic membrane and distributed in a circular fashion around the center of the disease cornea (Fig. 20.3). As patient's own tissue is used for CLAU and SLET, the advantage of both procedures is that no sys-

temic immunosuppression is required. In patients with bilateral LSCD, a couple of tissue transplantation options are currently available. One option is living-related conjunctival limbal allograft (lr-CLAL), in which the procedure is similar to CLAU, and conjunctival and limbal allografts are harvested from a relative from the superior and inferior limbus and then secured into place on the recipient's eye with glue. Another option is keratolimbal allograft (KLAL), in which limbal tissue attached to a corneal carrier and a 1-mm scleral rim harvested from one and a half cadaveric donor eyes is then secured onto the recipient's eye with nylon sutures and/or fibrin glue. An advantage of KLAL over lr-CLAL is that a large number of stem cells can be harvested from a donor and thus can be used to treat patients with severe LSCD. Additionally, in patients with significant conjunctival loss, a combined conjunctival limbal and keratolimbal allograft (C-KLAL) transplantation can be considered. It is important to keep in mind that with all allograft transplantation techniques, systemic immunosuppression is required.

Ex Vivo Stem Cell Expansion

With recent improvements in tissue engineering techniques, we now have the ability to expand limbal stem cells in culture *ex vivo*. In this technique, limbal tissue is obtained from the

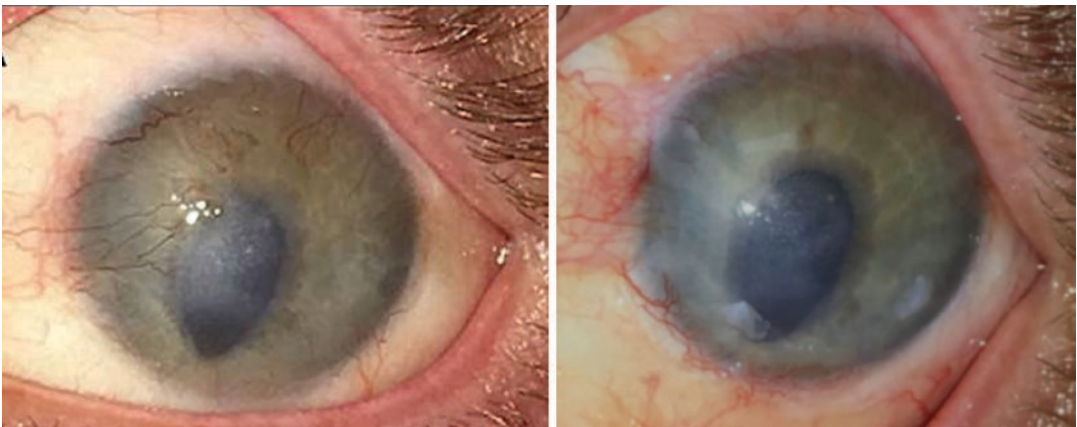


Fig. 20.3 A patient with sectoral LSCD in one eye with corneal neovascularization underwent successful SLET with regression of neovascularization and relatively improved central corneal clarity

donor eye corneoscleral ring and divided into segments of 1–2 mm in length. Donor stem cells are then expanded *ex vivo* in complete growth medium with the addition of lethally gamma-irradiated 3T3 feeder cells [10–12]. Subsequently, confluent cell sheets of two to three layers in thickness are then released and transferred to the recipient eye using a variety of substrates, including amniotic membrane, and other matrices such as collagen and fibrin. Currently, research is active in this area to improve carriers for cultivated epithelial stem cells with various natural or synthetic biomaterials and also to substitute a xenogeneic cell culture system (containing animal serum and growth factors) with human or recombinant materials [13–18].

Non-Limbal-Based Epithelial Cell Transplantation

Given that patients with bilateral disease do not have a source of autologous limbal stem cells, various other sources of epithelial cells have been explored with varying degrees of success. One such example is cultivated oral mucosal epithelial transplantation (COMET). In a clinical study using autologous cultivated oral mucosal epithelial sheets on human amniotic membrane transplantation for patients with complete LSCD, ocular surface stability was achieved in 72% of patients. However, since oral mucosal epithelium is fundamentally different from corneal epithelium, clarity of the recipient corneas were lacking; modest visual improvement was only seen in 48% of the patients [19, 20]. Another approach is to use autologous conjunctival epithelial cells cultivated *ex vivo*, which can form cell layers and basement membrane-like structures on human amniotic membrane [21, 22]. This method also appeared to be able to stabilize the ocular surface with moderate success [21]. However, no long-term clinical results have been reported.

An exciting area in current research is the generation of corneal epithelial cells from induced pluripotent stem (iPS) cells. Recently, it was found that iPS cells were able to generate a self-formed ectodermal autonomous multi-

zone of ocular cells, spanning the ocular surface ectoderm, lens, neuro-retina, and retinal pigment epithelium [23, 24]. Cells isolated from the ocular surface ectodermal zone can subsequently be sorted and expanded *ex vivo* to form corneal epithelium-like structures [24]. Although various ethical concerns will need to be addressed, this area of research, albeit still in early experimental stages, is potentially transformative in the future.

Rebuilding the Limbal Stem Cell Niche, Looking into the Future

As mentioned previously, chronic inflammation and dysfunction of the extracellular matrix (ECM) and resident cells in the limbal cell niche were observed in LSCD. Thus, the simple addition of epithelial stem cells onto a hostile ocular surface environment will lead to the eventual loss of transplanted epithelial cells or tissue [25–27]. To address this issue, recent strategies in the management of LSCD include decreasing ocular surface inflammation and rebuilding the limbal niche.

Various hemoderivatives produced from the patient's own blood are valuable options currently used in the clinic to help optimize the ocular surface health and decrease inflammation. These include autologous serum, platelet-rich plasma, and plasma rich in growth factors. Hemoderivatives are made preservative-free and provide natural substitutes for many proteins, vitamins, and lipids active in the human tear. Autologous serum drops have been shown to suppress apoptosis in the conjunctival and corneal epithelium [28]. Autologous serum tears are especially important in patients with severe aqueous tear deficiency and deficiency in reflex tearing as seen in Stevens-Johnson syndrome or following alkali or thermal injuries. Recent studies have also shown that platelet-rich plasma and plasma rich in growth factors may be superior than autologous serum in certain situations as these preparations are rich in growth factors, including epidermal growth factor, transforming growth factor, fibroblast growth factor, and

insulin-like growth factor-1, which are potentially important in the modulation of the immune system [29–31].

Bioactive Extracellular Matrix

Various other strategies in regenerative medicine aim to rebuild the ECM of epithelial stem cells. Currently, the most important and widely used scaffold for ocular surface reconstruction is the human amniotic membrane. Similar to basement membrane structures of the conjunctiva, the basement membrane of amniotic membrane has collagen and laminin structures [32], which may facilitate epithelial cell migration, adhesion, proliferation, and differentiation [33–35]. The basement membrane of amniotic membrane also has anti-inflammatory, antiangiogenic, and antifibrotic properties. These properties make amniotic membrane a great substrate in tissue engineering and cell delivery. For example, amniotic membrane is currently used as one of the candidates for the cultivation and delivery of limbal epithelial stem cells [33, 36, 37]. Although amniotic membrane currently serves as good ECM substitute, it has several downsides – it is an opaque tissue with low tensile strength; the effect of amniotic membrane is also temporary as it is typically digested shortly after transplantation; it also carries a small risk of transmitting infectious diseases [33]. These shortcomings of the human amniotic membrane make improved options for ECM substitutes more desirable.

Several novel ECM substitutes are currently being explored in animal models to assess their feasibility in maintaining human corneal limbal epithelial cells. For example, ECM hydrogels have been shown to have good biocompatibility and ability to fill an irregularly shaped space [38]. To reduce their antigenicity, hydrogels have been produced from decellularized porcine corneal ECM by using detergents and ribonucleases to remove all cellular components [38, 39]. Another method utilizes 3-D printing technology by mixing collagen, elastin, and laminin as bio-inks to fabricate corneal limbal ECM constructs [40]. A variety of other biological materials have also

been proposed as carriers for cultivated limbal epithelial cells, including collagen, fibrin, siloxane hydrogel contact lenses, and silk fibroin [14, 17, 18, 41–43]. Recently, it was shown that biomaterials such as silk fibroin can be transformed into highly transparent films and modified to create nanoscale patterns using soft lithography to mimic the ECM structures [42, 43]. These biomaterials will allow for the engineering of transplantable corneal epithelial sheets and may offer promising options for patients with limbal stem cell deficiency.

Mesenchymal Stem Cells

Mesenchymal stem/stromal cells that can differentiate into fat, bone, or cartilage are known to play an important role in tissue repair and maintenance and have been extensively studied for regenerative therapies in other areas of medicine. As mentioned earlier, limbal mesenchymal stem cells appear to have a major role in the maintenance of the limbal stem cell niche. In addition to their physical proximity to limbal epithelial stem cells, limbal mesenchymal stem cells exert several paracrine effects to improve the survival of epithelial stem cells. For instance, mesenchymal stem cells have been found to secrete anti-inflammatory cytokines including hepatocyte growth factor and interleukin-1 receptor antagonist, which have been shown to be able to restore corneal clarity [44–47]. They also attenuate the expression of immunomodulatory molecules including TNF- α and INF- γ , which are secreted by the injured corneal epithelium [45]. In various experimental studies, these cells were found to decrease tissue oxidative stress leading to decreased tissue neovascularization [48–51]. Due to these properties, the treatment that uses mesenchymal stem cells have been shown to speed healing and reduce scarring in experimental models of eye injury [51]. Recently, a clinical trial is underway to look at the effect of bone marrow-derived mesenchymal stem cells from a healthy donor on patients with ocular surface injuries and nonhealing defects. Currently, this study is in a phase 1 trial that aims

to determine its safety and the optimal dosage of cells to promote corneal healing. This is a promising future line of treatment because the extraordinary healing factors produced by mesenchymal stem cells will help to prevent damages caused by an overblown immune response to injury, which can ultimately cause more tissue damage than the initial injury.

In summary, LSCD is caused by both a deficiency in the number limbal epithelial stem cells and a dysfunction of the limbal niche, which is composed of a specialized ECM, signaling molecules, and various niche cells including mesenchymal cells. In addition to epithelial tissue-based therapies that are currently in clinical practice, mesenchymal stem cells, with their potent immunomodulatory properties and the ability to potentially support epithelial cells, offer a promising candidate for the restoration of the limbal stem cell niche. These cells have the potential to help patients with severe ocular surface disease due to a variety of etiologies.

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Corneal Angiogenesis and Lymphangiogenesis

21

Felix Bock and Claus Cursiefen

Corneal Lymphangiogenic Privilege and Causes for Corneal Neovascularisation

Due to several corneal diseases [1] (Table 21.1) and after surgery [2], blood and lymphatic vessels can grow into the normally avascular cornea. This neovascularization starts for both blood and lymphatic vessels at the limbal vascular plexus. Blood vessels impair significantly the visual function of the cornea due to opacification by blood vessels themselves but also by secondary effects such as oedema and lipid keratopathy in the corneal stroma. Lymphangiogenesis in contrast is visually not disturbing, but a key risk factor for immune reactions after corneal transplantation [3]. Hem- and lymphangiogenesis do not only occur as a consequence of diseases but can also be the reason for infectious or inflammatory corneal diseases. Both hem- and lymphangiogenesis are an essential part of the worldwide most frequent reasons for corneal blindness (trachoma) [4] and the most frequent reason for infectious blindness – herpetic keratitis – in the western civilization [5].

The causes for corneal avascularity (“corneal lymphangiogenic privilege” [6]) are only partly

Table 21.1 Causes of corneal angiogenesis

1. <i>Primary corneal neovascularization</i>
(a) Infectious
(i) Viral keratitis (e.g. herpes keratitis)
(ii) Bacterial keratitis
(iii) Mycotic keratitis
(b) Inflammatory
(i) Corneal foreign body (sutures), injury, chemical burn
(ii) Sclerokeratitis, autoimmune
(iii) Autoimmune components of the herpetic stromal keratitis
(iv) Transplant rejection
(v) Persistent epithelial defects
(vi) Cogan syndrome
(c) Hypoxic
(i) Contact lenses
(d) Barrier defects
(i) Innate or acquired limbus stem cell insufficiency
(ii) Aniridia
(iii) Chemical burn, burn, contamination
2. <i>Secondary corneal neovascularization</i>
(i) After penetrating keratoplasty (suture orientated)
(ii) After corneal suture, block excision, or amniotic membrane placement (suture orientated)

Modified from Cursiefen et al. [2]

understood yet. To maintain this status, the effect of anti(lymph)angiogenic factors has to outweigh the effect of the (lymph)angiogenic factors in the cornea. Under inflammatory or hypoxic circumstances, this balance can be shifted to an increased release of proangiogenic factors and this leads in consequence to an ingrowths of vessels into the

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cornea. In the human cornea, antiangiogenic factors such as pigment epithelial-derived factor (PEDF), angiostatin, endostatin, thrombospondin 1 and tissue inhibitors of metalloproteinase (TIMPs) have been found [7]. These factors are mainly localized in the corneal epithelium, corneal endothelium and the basal membrane of the cornea. Corneal avascularity is redundantly organized, so that the loss of only one of the antiangiogenic factors does not cause spontaneous corneal neovascularization. This was demonstrated by many laboratories, including ours [8], e.g. for thrombospondin 1 and 2.

In addition to anti(lymph)angiogenic factors, the cornea has developed several receptor decoy mechanisms trapping angiogenic growth factors, such as ectopically expressed VEGF receptors 1, 2 and 3 [9–11]. These contribute also to corneal lymphangiogenic privilege.

Finally, the aqueous humour also contains numerous inhibitors of angiogenesis and lymphangiogenesis, such as VIP and alpha-MSH [12].

Angiogenic factors responsible for pathologic vessel ingrowths in the vascularized human cornea are, e.g. vascular endothelial growth factor (VEGF) A, VEGF C and tumour necrosis factor- α (TNF α) [2, 7, 13]. Sources of these factors can be both endogenous and exogenous: the corneal epithelium can generate endogenously VEGF both in consequence of hypoxia (e.g. induced by contact lenses) [14] and induced by inflammatory mediators (e.g. herpes virus infection) [15, 16]. Exogenous sources of VEGF are invading neutrophils, granulocytes and macrophages [15, 16]. New vessels are mostly orientated to the point of the causing factor (*focus of angiogenic factors*).

The signal cascades of each disease that causes angiogenesis have not been fully clarified in detail. During inflammation-induced corneal neovascularization, the damage of the epithelium and stroma leads to an increase of interleukin 1 (IL 1), which in turn upregulates the expression of intracellular adhesion molecule 1 (ICAM 1) on the limbal vessel endothelial cells. The increased expression of ICAM 1 leads to the invasion of inflammatory cells, mainly neutrophils [17]. The infiltrating neutrophils secrete VEGF which attracts further neutrophils and the

sprouting of capillaries via IL 8. Whereas the clinical and histological identification of corneal blood vessels was well defined far over 100 years, the identification of clinically invisible lymphatic vessels was very difficult. The discovery of specific markers of the lymphatic endothelium like the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), the VEGF C/D receptor VEGFR3, podoplanin and Prox-1 [18] made the histological identification of lymphatic vessels possible. With these markers and ultrastructural examinations, the existence of clinically invisible lymphatic vessels in the human cornea was demonstrated [2, 19]. The lymphatic vessels made up 10% of all detected vessels in vascularized corneas and they were significantly more frequent directly after initiation of corneal neovascularization and the amount of lymphatic vessels was correlated with the amount of blood vessels [19]. Corneal lymphangiogenesis is triggered by the lymphangiogenic growth factors such as VEGF C and D as well as by its receptor VEGFR3 [2]. Macrophages have been shown to play a key role in the immune amplification cascade leading to inflammatory hem- and lymphangiogenesis, mainly via secretion of VEGF A and C [20].

New sprouting capillaries in the cornea pass through a phase of vessel maturation, which is characterized by degeneration of capillaries of the initial dense vessel network called pruning [21]. During this phase which is morphologically characterized by the absence of pericytes, immature capillary endothelia get apoptotic if the local concentration of angiogenic factors like VEGF falls below a certain threshold. In contrast, mature, already pericyte-covered vessels seem to be independent from an exogenous supply with VEGF. Also during human corneal neovascularization new vessels become covered by pericytes, whereas 2 weeks after initial corneal neovascularization, 80% of the vessels are already covered by pericytes [22]. So molecular angioregressive therapies might be only successful in the initial phase of corneal neovascularization. Therefore, physical methods to regress blood and lymphatic vessels are developed. Using fine-needle diathermy [23], photodynamic therapy [3], or UV crosslinking [24], mature blood and lymphatic

vessels could be regressed in a mouse model corneal neovascularization. This approach of “temporary preoperative selective regression of corneal lymphatics prior to transplantation” was shown to significantly promote corneal graft survival thereafter [16–18].

Corneal angiogenesis can be the cause of corneal diseases like herpetic keratitis, dry eye diseases, or limbal stem cell deficiency [6]. In an animal model of herpetic keratitis, the inhibition of the corneal neovascularization led secondarily to an inhibition of the herpetic neovascularization, which was formerly seen as causal. The phenomenon might be explained by the assumption that CD4+ T cells need blood vessels to reach the cornea [15, 16].

In models of evaporative, acute dry eye disease, the growth of lymphatic vessels unaccompanied by blood vessels towards the central cornea was described [25]. A similar phenomenon was observed in a model of acute corneal oedema [26]. In this setting, a physiological role for lymphangiogenesis into the primarily avascular cornea could be described. By using a mouse model of perforating corneal injury which induced an acute oedema with significant deposition of extracellular fluid in the corneal stroma, it was demonstrated that lymphatic vessel grew transiently and selectively into the cornea and contributed to the resolution of corneal oedema. This opens the avenue for curative lymphangiogenic therapies for oedematous corneal diseases.

In limbal stem cell deficiency (LSCD), the barrier function of the limbal transition zone from the highly vascularized conjunctiva is disrupted. Here, limbal epithelial stem cells (LESC) are located, which replenish the corneal epithelium. The LESCs are located in the so-called palisades of Vogt, which are embedded in a vascular plexus providing nutrition in addition to that obtained from the aqueous humour and the tear film [27]. Reasons for LSCD can be of congenital origin like aniridia or are acquired such as after chemical and thermal burns, during inflammatory eye disease like ocular pemphigoid, in allergic diseases like Stevens-Johnson syndrome, or hypoxia induced by extensive contact lens wearing [28] as well as pterygium. It was demonstrated that also

UV exposure reduced the antilymphangiogenic properties of limbal stem cells and induced a loss of stem cell markers in limbal epithelial cells and fibroblasts. Furthermore, pro-inflammatory and macrophage-recruiting cytokines such as TNF α , IFN γ and MCP1 were significantly upregulated following cell irradiation [29].

Role of Blood and Lymphatic Vessels in Corneal Graft Rejection

Efferent blood vessels have the role of an entrance for immune effector cells, the so-called efferent arm of the immune reflex arc, whereas lymphatic vessels represent the afferent arm. The role of blood versus lymphatic vessels in corneal allograft survival was determined by implanting donor corneas into either uninflamed, inflamed avascular, prehemvascularized only, or prehemvascularized and prelymphvascularized murine recipient eyes [30]. To selectively inhibit corneal lymphangiogenesis, anti-VEGF receptor 3 antibodies and anti-integrin α 5 small molecules were used. Grafts implanted into only prehemvascularized recipient beds showed a comparable good graft survival as grafts placed into completely avascular, normal recipients. In contrast, pre-existing lymphatic vessels significantly decreased corneal graft survival [3]. This important role of lymphatic vessels in corneal graft rejection was further supported by the finding that the antiangiogenic factor semaphorin 3F had an isolated antilymphangiogenic effect in the cornea when applied topically as eye drops which subsequently improved corneal graft survival [31]. In summary, pathologic corneal lymphatic vessels seem to be the key mediator of immune reactions after transplantation and not the blood vessels [3].

Clinical Consequences of Loss of Corneal (Lymph)Angiogenic Privilege

The bio-microscopic identification of corneal blood vessels is mostly unambiguous. In contrast corneal lymphatic vessels cannot be detected at

the slit lamp. The corneal neovascularization does not only impair the transparency and visual acuity per se, but also by associated complications. These complications are corneal oedema due to the leakage from newly formed vessels, yellow lipid aggregation (lipid keratopathy), corneal band degeneration and bleedings into the cornea. Subjective discomfort of a patient due to corneal neovascularization and secondary transformations are a reduced visual acuity up to blindness, blurry vision, photophobia, foreign body sensation, pain, epiphora and cosmetic deformation.

In an ophthalmopathologic evaluation of explanted corneas, 20% of all corneas which underwent keratoplasty showed evidence of hemangiogenesis [1]. These new vessels are either subepithelial (pannus) or stromally located [1]. Sometimes the location of the vessels allows to draw conclusions on the pathogenesis, for example, in deep stromal corneal neovascularization after congenital lues (parenchymatic keratitis).

Pre-existing corneal blood and lymphatic vessels are a major risk factor for immune reactions after penetrating keratoplasty (KPL) [32]. Since the rate of immune reactions in avascular graft beds is at approximately 10% [33], the rate of immune reactions increases in vascularized, so-called high-risk eyes up to 50%. As far as it was recently demonstrated that in vascularized corneas also lymphatic vessels occur [2, 19, 34], a transplant loses its immunologic privilege if inserted into such a prevascularized bed [18]. Within the corneal lymphatic vessels, antigen-presenting cells (APCs) and donor antigens [35] are able to leave the cornea first into the limbal and conjunctival lymphatic vessels and afterwards into the regional lymph nodes [2]. The outstanding role of the draining lymph nodes for immune rejections was recently demonstrated in an animal model by the removal of the regional lymph nodes either after normal- or high-risk keratoplasty, which lead to nearly complete survival of the grafts [36].

Corneal neovascularization does not only occur prior to but also after KPL [2]. The ves-

sels reach the graft in every tenth patient and are mostly located at the 12 o'clock and 6 o'clock position, are less in the palpebral fissure and are orientated to the outer suture endings, which suggest a release of angiogenic factors in these areas [2]. In comparison to the motor trephination, the atraumatic excimer laser trephination in penetrating keratoplasty induces significantly less corneal neovascularization after KPL [2]. So besides the epithelium-suture interaction, also the wound healing seems to play a role in its pathogenesis. Further risk factors are blepharitis, suture knots in the host cornea and large graft diameters [37]. In addition to the suture removal, also antiangiogenic therapies can be used. The therapy with conventional steroids does not have a significant impact on the incidence and degree of corneal neovascularization 1 year after keratoplasty.

Until now there are no reports about corneal neovascularization after refractive surgery. In contrast, contact lenses can lead to relative hypoxia in the corneal epithelium and stroma which in turn leads to upregulation of angiogenic factors like VEGF and in last consequence to corneal neovascularization [14]. This vascularization is often located between the epithelium and the Bowman lamellae (vascular pannus) or, more rarely, stromally or in deep stromal [38, 39]. The vascular pannus can impair visual acuity and the peripheral visual field, disturbs cosmetic patterns and probably increases the risk of immune reaction after KPL.

Therapeutic setups for anti(lymph)angiogenic therapy at the cornea can be separated into three different categories [34]: (a) angiostatic setting with inhibition of newly sprouting blood and/or lymphatic vessels, (b) angioregressive setting with regression of persisting vessels and (c) angioocclusive setups to induce functional vessel obliteration.

The following will give an overview of current therapies used in the above-mentioned setups. As an angiostatic therapy, anti-inflammatory steroids and cyclosporine A are used but with only small effect [33, 40].

Pharmacological Inhibitors of (Lymph)Angiogenesis

In ophthalmology, topical corticosteroids are still the main treatments after keratoplasty because they are believed to be antiangiogenic and anti-inflammatory [7]. On the other hand, it is known that these nonspecific immunosuppressive agents can cause severe side effects (cataract, keratitis, glaucoma, etc.) in the eye [41]. Therefore developing more specific drugs to prevent corneal graft rejection is an important aim in ophthalmology [34]. The observation that tumour growth might be dependent on angiogenesis in the 1970s [42, 43] boosted the investigation on antiangiogenic drugs. Today several drugs at least including antiangiogenic activity like celecoxib, thalidomide and iressa were approved by the FDA. Recently drugs with exclusively antiangiogenic properties like VEGF_{R1R2} trap and VEGFR3 antibody which are in part already in clinical trials or in the clinic were developed. Four VEGF-specific drugs were already approved by the FDA: bevacizumab, ranibizumab and pegaptanib and aflibercept. Ranibizumab, pegaptanib and aflibercept are developed for intravitreal injection into the vitreous of the eye for the treatment of age-related maculopathy (AMD). Avastin was shown to inhibit effectively angiogenesis in the cornea when used off-label as eye drops in patients [44]. In the following the three drugs analysed in this work for their antihem- and especially for their antilymphangiogenic properties are introduced.

Anti-VEGF-A Therapeutics

Bevacizumab

Many endogenous [45] and exogenous [46] antiangiogenic factors are known already. In contrast, to inhibit *lymph*angiogenesis so far there exist only a few experimental approaches, e.g. the use of a VEGF_{R1R2} trap [47] or of a blocking anti-VEGFR3 antibody [48], but there is no FDA-approved substance available yet.

Bevacizumab (rhuMAB VEGF) is a recombinant, humanized, monoclonal antibody that binds to VEGF A and prevents VEGF A from ligating to its receptor. The antibody was engineered by assembling VEGF-A-binding residues from the murine-neutralizing antibody into a framework of a human immunoglobulin [49]. Bevacizumab was FDA-approved in 2004 as a first-line treatment for patients with metastatic colorectal cancer. In addition, it is now widely used off-label for the treatment of neovascular forms of age-related maculopathy. In a prospective, open-label, noncomparative study, it could be shown that topical bevacizumab reduces the severity of corneal neovascularization [50]. Furthermore, it was demonstrated that bevacizumab not only inhibits corneal hem- but also lymphangiogenesis [51].

VEGF_{R1R2} trap

On the basis of a soluble decoy receptor in which VEGFR1 is fused to the Fc portion of human IgG1 [52, 53], several variants of a parental VEGF trap with reduced positive charges were engineered. Further steps reducing the protein charging of several Ig domains resulted in the VEGF_{R1R2} trap. This fusion protein combined the high specific binding affinity to VEGF with better pharmacokinetic properties [54]. It was approved for the treatment of wet AMD by the FDA in 2011.

Cursiefen et al. [47] demonstrated that this antibody effectively inhibits hem- and lymphangiogenesis in the cornea. In a model of normal-risk keratoplasty, no blood or lymph vessels were detectable immediately after normal-risk transplantation in either donor or host cornea in both treatment group and control group. Hem- and lymphangiogenesis occurred 3 days after transplantation. Both vessel types reached donor tissue 1 week after allografting and similarly after syngeneic grafting in the control group. Early postoperative trapping of VEGF A significantly reduced both hem- and lymphangiogenesis and

significantly improved long-term graft survival. They concluded that there is VEGF-A-dependent hem- and lymphangiogenesis after normal-risk keratoplasty within the preoperatively avascular recipient bed. Inhibition of hem- and lymphangiogenesis after normal-risk corneal transplantation improves long-term graft survival, establishing early postoperative hem- and lymphangiogenesis as novel risk factors for graft rejection even in low-risk eyes.

The same is also true for mouse models of vascularized high-risk eyes. Here also inhibition of additional postoperative hem- and lymphangiogenesis by blocking VEGF A signalling using VEGF trap can significantly promote corneal graft survival [55, 56].

Furthermore, it was shown that local VEGF trap application not only inhibits the outgrowth of blood and lymphatic vessels but also modulates the corneal immune microenvironment. Local VEGF depletion prior to transplantation could increase the expression of pro-inflammatory and also immune-regulatory cytokines exclusively in the corneal microenvironment. Topical VEGFR1/R2 trap treatment significantly inhibited the invasion of CD11c+ dendritic cells towards the central cornea. Within successfully transplanted corneal graft, an upregulation of the tolerogenic factor Foxp3 was observed. So, locally restricted VEGF depletion seems to increase graft survival by modulating the microenvironment of the recipient and by inducing tolerogenic mechanisms [57].

VEGFR3 Antibody mF4-31C1

In a recent study it was shown that by modulation of angiogenesis and especially lymphangiogenesis *after* normal-risk keratoplasty, one can improve graft survival in the murine model of keratoplasty [47]. The murine antibody mF4-31C1 is a blocking, monoclonal antibody binding the VEGF receptor VEGFR3. It antagonizes the prolymphangiogenic factor VEGF C and therefore has an antilymphangiogenic effect. Recently, it was demonstrated in a mouse model of spontaneous, low to non-inflammatory corneal hem-

and lymphangiogenesis (*corn1*-mouse model) that blocking of VEGFR3 by the specific antibody mF4-31C1 decreases the spontaneous outgrowth of blood and lymphatic vessels in the cornea by competitive inhibition [48].

Antisense Oligonucleotides Against IRS-1

The most advanced clinical experience regarding local inhibition of pathological corneal hem- and lymphangiogenesis exists with eye drops containing antisense oligonucleotides against insulin receptor substrate 1 (IRS1). Here phase II and III clinical trials could demonstrate a significant inhibitory and regressive effect in patients. Interestingly, in patients with herpetic keratitis there even was a disease course modification observed [40, 58].

Lymph Vessel Regression and Graft Survival

In preclinical most and clinical studies, strategies to stop *progressive* corneal hem- and lymphangiogenesis have been established. But most patients come with already pre-existing mature blood and lymphatic vessels, so regression of both vessel types is even more important to improve graft survival after corneal grafting. We could demonstrate that corneal lymphatic vessels regress over time and that graft survival is enhanced in recipient beds with partly regressed inflammatory neovascularization [56]. The current angioregressive therapies are limited to the treatment of the clinically visible *blood* vessels by fine-needle cauterization or photodynamic therapy [59, 60] – whether both affect lymphatic vessels is unknown.

Molecular Regression Strategies

Interrupting, e.g. the angiopoietin/Tie 2 signal pathway or blocking the lymphatic endothelial marker LYVE-1 or VEGF C might be strategies to

force the regression of blood and lymphatic vessels. It is well known that the Ang/Tie system plays a crucial role in blood and lymphatic vessel development and adult blood vessel maintenance. A Tie2-Fc fusion protein can inhibit the Ang1-mediated outgrowth of lymphatic vessels in a corneal pellet implantation assay [61], and in a cell culture model the stabilizing function of this system on isolated human lymphatic endothelial cells is abolished by a blocking anti-Tie2 antibody [62]. Thereby it is very well possible that an anti Ang/Tie therapy on already existing corneal lymphatic vessels could lead to forced regression of this vessel type and thereby allow the restoration of normal-risk situation prior to keratoplasty.

Physical Methods to Regress Corneal Blood and Lymphatic Vessels

Recently, three novel approaches have been developed and tested preclinically for lymphangioregression in the cornea.

Photodynamic Therapy (PDT)

An even more direct method to induce lymph vessel regression would be the specific destruction of this vessel type. As already mentioned above, corneal blood vessels are destroyed by the use of systemically applied intravenous verteporfin (Visudyne®), a benzoporphyrin derivative in photodynamic therapy (PDT). Verteporfin is a photosensitizer which is approved to eliminate the abnormal blood vessels in the eye associated with conditions such as the wet form of macular degeneration. Verteporfin produces, when stimulated by non-thermal red light with a wavelength of 693 nm in the presence of oxygen, highly reactive short-lived singlet oxygen and other reactive oxygen radicals, resulting in local damage to the endothelium and blockade of the vessels. Very recently this method was transferred to the treatment of tumour-associated lymphatic ves-

sels in the skin. The photoreactive agent was injected intradermally into the tip of mouse ears and subsequently treated by PDT. This resulted in the specific destruction of the lymphatic vessels [63]. From there a study was conducted to assess the possibility to regress both blood and lymphatic vessels in the cornea after systemic intravenous injection of the verteporfin, the influence of timing of PDT after injection of the photosensitizer and the effect on graft survival in subsequent experimental murine high-risk keratoplasty. PDT after intravenous injection of verteporfin could selectively regress pre-existing corneal blood vessels or both blood and lymphatic vessels depending on the timing of PDT after injection of the photosensitizer (Fig. 21.1). Performing PDT at least 1 hour after a systemic photosensitizer injection destroyed both vessel types. Pretreatment of recipients with PDT and verteporfin intravenously promoted graft survival in prevascularized high-risk recipients [3].

Fine-Needle Diathermy

Fine-needle diathermy (FND) is one of the clinical options for managing pathologic corneal neovascularization. Using the murine model of high-risk corneal transplantation, it could be demonstrated that FND destroys not only blood but also lymphatic vessels and thereby promotes graft survival. FND caused significant regression of both blood and lymphatic vessels and graft survival was significantly prolonged when transplants were placed into recipient beds of FND-pretreated high-risk eyes. This again supports the novel concept of temporary selective preoperative regression of host lymphatic vessels to promote graft survival after subsequent corneal transplantation (Fig. 21.2).

UV-Light Crosslinking

CXL is currently used for the treatment of progressive keratoconus [64]. Furthermore, it is used

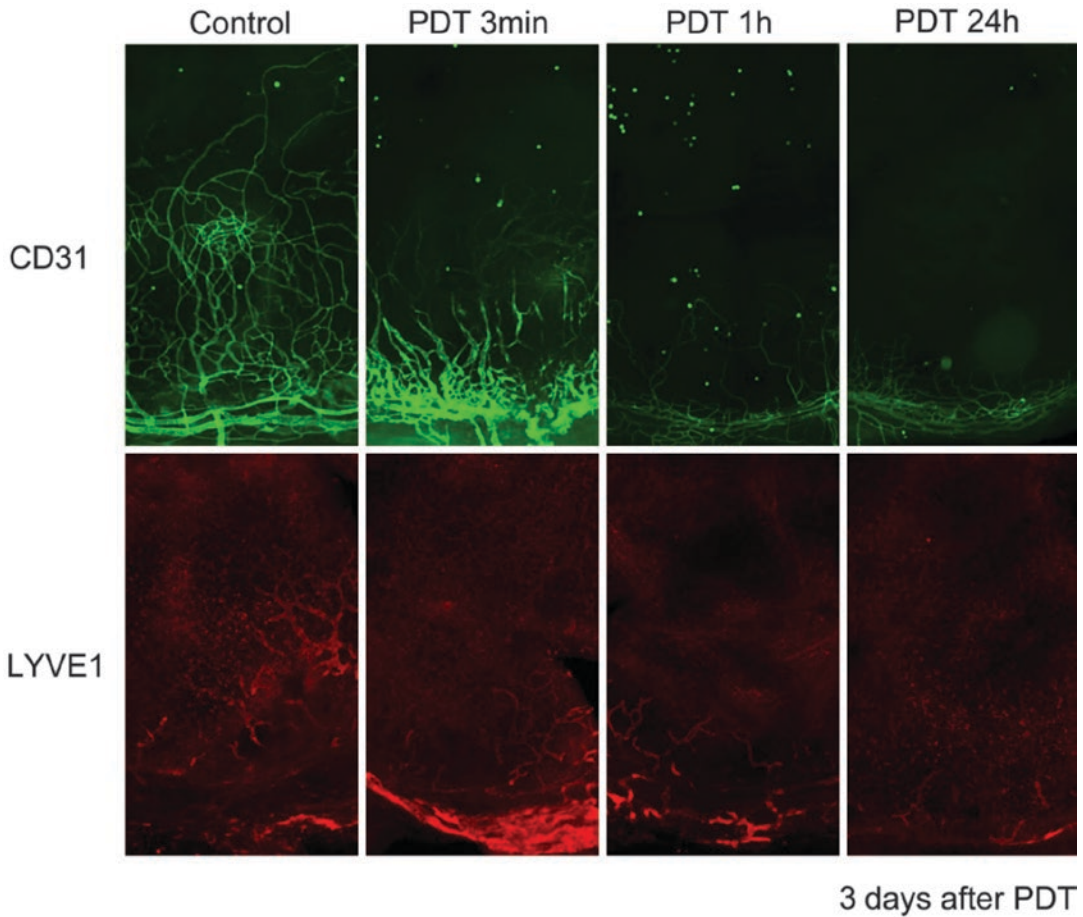


Fig. 21.1 Fluorescent images of whole-mount mouse corneas stained for CD31 (blood vessels) and LYVE1 (lymphatic vessels) following PDT after verteporfin iv injection. Regression of both blood and lymphatic vessel

can be observed (modified from Hou et al. [3]). Reprinted via Creative Commons License: <http://creativecommons.org/licenses/by/4.0/>

in corneal ulcers, pellucid marginal degeneration, corneal melting and iatrogenic keratectasia after laser eye surgery [65]. Riboflavin is a photosensitizer and can be photoactivated by UV light to produce reactive oxygen radicals [66]. UV radiation damages DNA as well as RNA of pathogens and is therefore used as an antimicrobial procedure [67]. For corneal blood and lymphatic vessel, it was shown that UV-light CXL using local UV-A irradiation and topical riboflavin application regressed both pre-existing lymphatic vessels and blood vessels (Fig. 21.3) [18]. In addition, macrophages as well as CD45+ cell were decreased in CXL-treated corneas. Accompanied

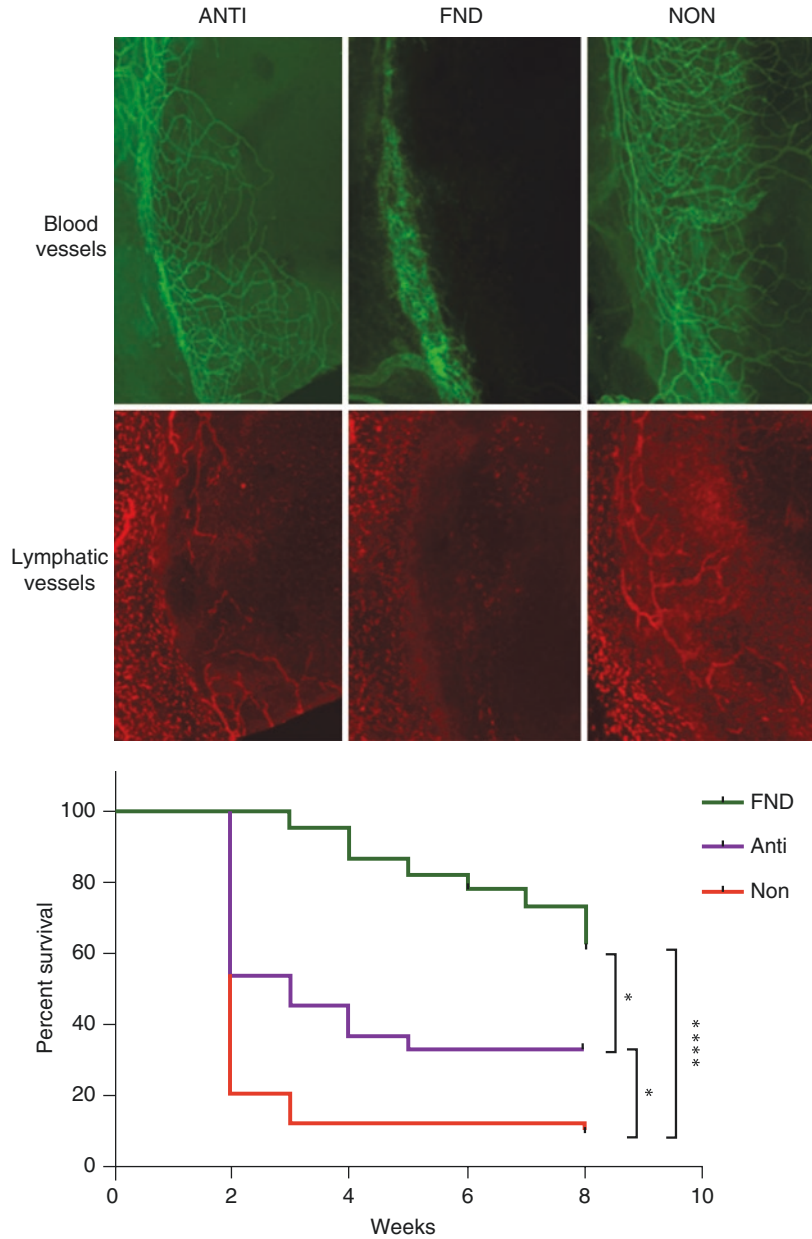
by an increased frequency of FoxP3+ regulatory T cells, the graft survival was increased.

These new lymphangioregressive strategies now need to be translated into the clinic to promote graft survival after high-risk corneal transplantation.

Imaging of Corneal Lymphatic Vessels

Pathologic corneal lymphatics are clinically invisible at the slit lamp. As a prerequisite for clinical trials, very recently two novel methods

Fig. 21.2 CD31/LYVE-1 immunohistochemistry pictures at day seven after FND treatment showed that FND significantly reduced areas of blood and lymphatic vessels as compared to controls (ANTI and NON). B. The graft survival was significantly prolonged when transplants were placed into recipient beds of pretreated groups (with either FND or anti-inflammatory drops). FND treatment significantly improved the graft survival in comparison to isolated anti-inflammatory therapy (FND versus ANTI: 60.87% vs. 33.33%, $p < 0.05$) (modified from Le et al. [23]). Reprinted via Creative Commons License: <http://creativecommons.org/licenses/by/4.0/>



have been developed to detect these vessels also in the clinical setting: a. intrastromal fluorescein injection and subsequent detection of lymphatics using HRA [68] as well as noninva-

sive imaging of lymphatics using microscopic OCT pictures [69]. This will allow to define endpoints for clinical antilymphangiogenic trials (Fig. 21.4).

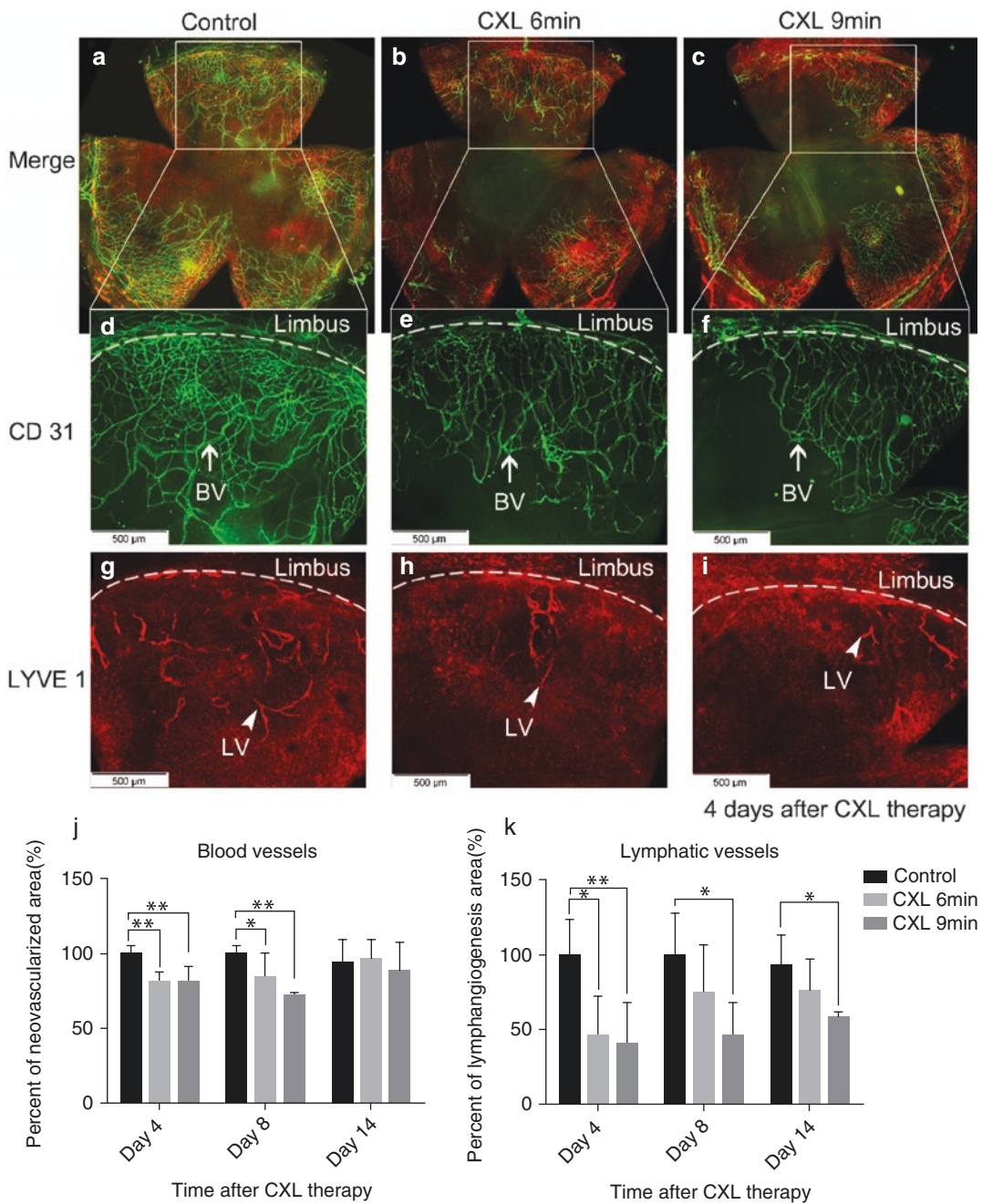


Fig. 21.3 Regression of mature corneal blood and even more so lymphatic vessels after corneal crosslinking (Hou et al. [24]). Reprinted via Creative Commons License: <http://creativecommons.org/licenses/by/4.0/>

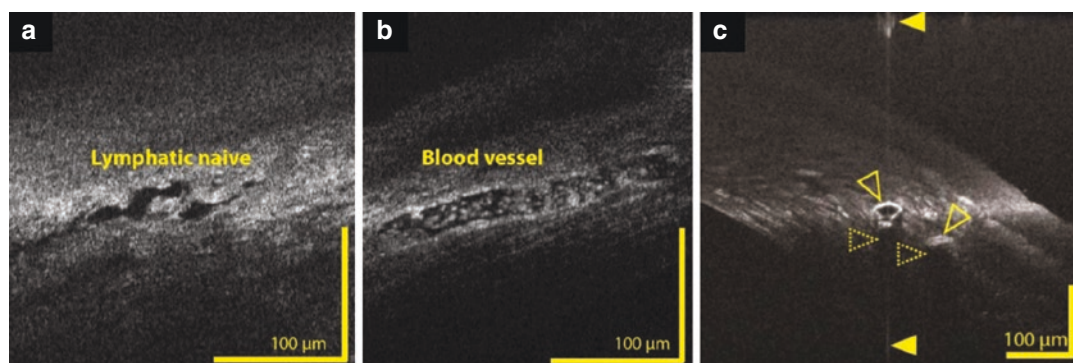


Fig. 21.4 Longitudinal microscopic OCT cross-sections of corneal lymphatic vessel (a) and corneal blood vessel (b). Lymphatic vessels appeared as dark mainly cell-free lumen, whereas blood vessels were densely populated with fast-flowing cells. In contrast to lymphatic vessels, blood vessels exhibit a hyperreflective endothelium. (c): OCT cross-section of a vascularized cornea with intrastromal injection of India ink 24 h prior to imaging. Ink-specific artefacts such as an increased signal at the tissue/

ink interfaces due to high reflection (marked by solidly outlined arrow), a shadowed area underneath the vessels (marked by dashed outlined arrow) and vertical line artefacts due to the high reflection of the ink (solid arrow) were observed. This gives further proof of the presence of ink inside the lymphatic vessel (modified from Horstmann et al. [69]). Reprinted via Creative Commons License: <http://creativecommons.org/licenses/by/4.0/>

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Natalie A. Afshari and Ashlie Bernhisel

Introduction

Genetic mutation can result in insignificant corneal changes that can simply be incidental findings on exam or can result in defects of enormous visual consequence. The more substantial phenotypes tend to be secondary to mutations that cause changes in gene expression early in embryogenesis. Less devastating mutations are those that cause a slow degeneration over time. In this chapter we will explore both ends of the spectrum. We will also highlight diseases that express variability in penetrance as well as those that exhibit allelic and locus heterogeneity.

Anterior Segment Dysgenesis (ASD)

Critical anterior segment formation occurs during the seventh week of gestation, and although neural crest migration is the main contributor to the anterior segment, mesoderm, surface ectoderm, and neuroectoderm also make up portions of the anterior segment. During the 8th week of embryogenesis the cornea further differentiates

with the endothelium becoming distinct through the direction of PIT2X, while PAX6 directs the differentiation of the iris and angle structures [1]. FOXC1 has also been implicated. Disruptions in the migration of neural crest and various genetic mutations can lead to abnormalities in the cornea, iris, ciliary body, angle structures, and lens formation. Regarding the cornea in particular, these mutations can lead to isolated anomalous corneal shape and structure or a combination of corneal and anterior segment changes. Up to 50% of individuals with anterior segment dysgenesis will develop glaucoma [2].

Posterior Embryotoxon

Posterior embryotoxon is defined as an anterior displacement of Schwalbe's line, which is the location in which the trabecular meshwork meets the termination of Descemet's membrane. Found in isolation, the posterior embryotoxon is not pathologic and occurs in about 10% of the population [3]. It does however have associations with other pathologic eye diseases and certain systemic genetic disorders including Alagille syndrome.

Axenfeld–Rieger Syndrome

This is an autosomal dominant disease with 100% penetrance, but with highly variable phenotypic

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Fig. 22.1 Microdontia from National Human Genome Research Institute (open domain) [6]

manifestations. Axenfeld anomaly, Axenfeld syndrome, and Reiger syndrome were all terms that were previously defined as separate pathologic entities. However, due to an increased understanding that they are all a part of the same genetic process, they are now classified together. Ocular findings include posterior embryotoxon, iris hypoplasia, angle dysgenesis, and ectropion uvea. Rarely there can be iridocorneal adhesions. Managing glaucoma is the most important strategy for this disease, as glaucoma tends to be the most vision-threatening aspect of the syndrome. Systemic manifestations include redundant peri-umbilical tissue, dental abnormalities, and craniofacial abnormalities. Various combinations of ocular and systemic findings are associated with this syndrome [4].

Forty percent of cases have been localized to the *FOXC1* and *PITX2* genes. In the other 60% of cases the genetic etiology is unknown. Over half of the mutations occur *de novo*. This disease exhibits allelic and locus heterogeneity with at least 87 *PITX2* mutations and 54 *FOXC1* mutations having been identified. In general, *FOXC1* mutations are more likely to cause phenotypes in which the anterior segment alone is affected, whereas *PITX2* is more likely to cause the classic systemic manifestations, in addition to other more rare systemic abnormalities, such as cardiac malformations [5] (Fig. 22.1).

Peters' Anomaly

This malformation is defined by the absence of endothelium, Descemet's membrane, and in some instances the posterior stroma. This

abnormality typically presents with central or paracentral opacification at the time of birth.

Peters' anomaly is further divided into two groups. Type I Peters' is defined by a central opacity and iris strands between the opacity and the pupillary border. Type II is defined by lens adherence to the cornea and possible cataract formation. In general, type I Peters' anomaly has higher reported success rates after penetrating keratoplasty compared to type II. There appears to be a stronger association between bilateral cases of Peters' anomaly with systemic disease compared to unilateral cases, though the genetics of such has yet to be fully illuminated [7].

Differentiating Peters' anomaly from sclerocornea may seem obvious to the clinician but often the two are confused and ultrasound biomicroscopy can be helpful in confirming the disease process. Ultrasound can also be helpful in differentiating type I Peters' anomaly from type II, which may help guide surgical planning [8].

The majority of cases occur sporadically; however, autosomal dominant and autosomal recessive transmission have been described. Eighty percent of the cases are bilateral and over half of these cases are associated with angle closure [9].

Mutations in homeobox genes *PAX6*, *PITX2*, and *FOXC1* have all been implicated in causing this anomaly. However, *CYP1B1* mutations may be responsible for to up to 20% of cases, despite the fact that it is not a homeobox gene [10]. The *FOXE3* gene has been implicated in type II Peters'. This mutation results in failure of the normal separation of the lens vesicle from the surface ectoderm.

Congenital Causes of Irregular Corneal Dimensions

Megalocornea

Megalocornea is typically a nonprogressive X-linked recessive disease. Autosomal dominant and autosomal recessive forms do exist but are uncommon. This disease is a bilateral disease that is present at birth with a corneal diameter of

13–16.5 mm. There is typically global, rather than focal, corneal thinning. Intraocular pressure is typically normal. Later in life these patients may develop mosaic corneal degeneration (central cloudy dystrophy), pigment dispersion, arcus juvenilis, mild iris atrophy, and early cataracts. Of note, zonules may be weak and therefore care needs to be taken during cataract surgery. This propensity for weak zonules can also lead to ectopia lentis [11].

In 2012 the causative gene was found to be chordin-like 1 gene (CHRD1) on Xq23, which codes for ventroptin. Ventroptin has been shown to be expressed in the human cornea starting at week 7 gestation. Associated systemic abnormalities and syndromes include Marfan syndrome, Down syndrome, craniosynostosis, Alport syndrome, osteogenesis imperfecta, and mucopolidosis II [12].

Microcornea

This is an autosomal dominant and rarely autosomal recessive mutation that can occur unilaterally or bilaterally and is defined by a corneal diameter of less than 9 mm in a newborn and less than 10 mm in adults. The cornea can be clear or cloudy. The embryologic pathway responsible for the disease is contested. This abnormality is associated with hyperopia, congenital and juvenile cataracts, persistent fetal vasculature, optic nerve hypoplasia, colobomas, and cornea plana. Systemic associations include Ehlers–Danlos syndrome, congenital CMV, myotonic dystrophy, fetal alcohol syndrome, and achondroplasia [13].

No gene locus has been identified for microcornea when found in isolation. PAX6 is indicated in some cases. In addition, V-maf avian musculoaponeurotic fibrosarcoma (*MAF*) encodes a transcription factor that is associated with juvenile-onset cataract, microcornea, iris coloboma, and other anterior segment dysgenesis [13].

Cornea Plana

This is a bilateral autosomal dominant and recessive disease in which corneal curvature is

less than 43 D and on average is 30–35 D, which results in severe hyperopia. Slit lamp examination reveals peripheral corneal haze, an extended limbus. The abnormality is associated with cataracts, narrow angles, and associated angle closure. In addition, it often presents with microcornea, anterior and posterior colobomas, and sclerocornea. The responsible mutation has been mapped to 12q22 (KERA). This is common in those of northern Finnish ancestry and is associated with Ehlers–Danlos syndrome.

Sclerocornea

This is a rare nonprogressive congenital disorder that may occur in isolation or along with other ocular abnormalities. It is classically bilateral but asymmetric. In the periphery there is opacification and the conjunctival and episcleral vessels extend onto the cornea in a regular arcade pattern, making the limbus difficult to identify. In some cases, there can be complete opacification of the cornea. The limbus can be difficult to define. Sclerocornea is associated with corneoplana and with that comes narrow-angle glaucoma and angle closure. This abnormality is associated with a long list of syndromes and has an even longer list of associated ocular abnormalities, with cataracts being the most common. About half of sclerocornea cases are autosomal dominant, autosomal recessive, or x-linked. The remaining 50% of cases occur sporadically [14, 15] (Fig. 22.2).

Other Corneal Opacities

Congenital Corneal Keloid

This is an extremely rare entity, which typically presents bilaterally. Congenital keloids are typically associated with other ocular problems including aniridia, iridocorneal adhesions, cataracts, and anophthalmos [17]. The disease has been associated with Lowe syndrome, more correctly termed oculocerebrorenal syndrome, which is an x-linked recessive disease characterized by



Fig. 22.2 Appearance of sclerocornea in two cases of combined sclerocornea, aphakia, and microphthalmia. Open source. vis license: CC BY 3.0 [researchgate.net](https://www.researchgate.net/figure/Phenotypic-appearance-in-patients-with-sclerocornea-aphakia-and-microphthalmia_fig2_256482074) [16]. (Pantoja-Melendez et al. [16]. eCollection 2013. PubMed

PMID: 24019743. Modified: Only including 2 of 4 images. CC BY 3.0. https://www.researchgate.net/figure/Phenotypic-appearance-in-patients-with-sclerocornea-aphakia-and-microphthalmia_fig2_256482074)

cataracts, glaucoma, renal disease, intellectual disabilities, and seizures [18].

Histopathologically the corneas of affected individuals demonstrate irregular and disorganized collagen, fibrosis, and blood vessels. The gene implicated is OCRL1 which encodes inositol polyphosphate 5-phosphatase.

Congenital Corneal Anesthesia

This is a rare, usually bilateral disease that presents with recurrent eye redness, photophobia, recurrent erosions, and sterile corneal infiltrates. Vision is often threatened secondary to corneal scarring, ulcerations, and perforations. The anesthesia can be isolated to the cornea or can involve the entire trigeminal nerve and in some instances may be systemic or have other systemic associations. Corneal anesthesia has been divided into three groups, though these groups are not all-inclusive. Group I is isolated corneal anesthesia. Group II is associated with mesenchymal abnormalities such as Goldenhar syndrome, Moebius syndrome, and Riley–Day syndrome. Group III patients have corneal anesthesia associated with focal brainstem abnormalities [19].

The clinical presentation includes a history of recurrent redness and photophobia and may have abnormally low or absent tear production. On examination, patients often are found to have an irregular, dry surface and can also present with

sterile infiltrates or ulceration. Due to this presentation this is commonly misdiagnosed as HSV, vitamin A deficiency, AMBD, or toxic keratopathy [20].

Riley–Day syndrome is an autosomal recessive disease also known as familial dysautonomia (FD) and has been mapped to 9q31–q33. When pooled together in a study performed at L.V. Prasad, there seemed to be a male predilection and the average age in which symptoms were first noticed was 18 months of age. Management includes abundant preservative-free artificial tears and temporary tarsorrhaphy in cases of sterile ulceration. All cases in which PKP or DALK has been performed have resulted in rejection and/or failure [19].

Dystrophies

Dystrophies are typically bilateral and often symmetrical corneal abnormalities, which slowly progress throughout life. Many involve the accumulation of deposits and present in typical patterns, though some appearances may overlap or be atypical. For centuries these dystrophies were defined by their clinical and histopathologic appearance.

Once genotyping became more accessible, the genes responsible for these dystrophies have come to light with many of the same genes being responsible for vastly different phenotypes that

were classified in very different categories. In 2008, the International Committee for Classification of Corneal Disease (IC3D) was formed by cornea experts from various countries who changed the classification based on genetic information [21, 22]. The categories are organized by how well the genetic component is understood. Many of these dystrophies demonstrate both allelic heterogeneity and locus heterogeneity.

Category 1 – These are dystrophies in which the genetic component is well defined including gene identification and locus mapping, and the specific mutation has been identified.

Category 2 – This includes well-defined dystrophies in which gene mapping has led to a general location of the mutation; however, the specific gene has not yet been elucidated.

Category 3 – This is defined by a corneal dystrophy in which the disease is well characterized but no gene locus has been identified.

Category 4 – This is reserved for cases in which the dystrophy characteristics are unclear or may be part of another subset of dystrophy.

Epithelial and Subepithelial Dystrophies

Epithelial Basement Membrane Dystrophy (EBMD)

Most cases actually represent a degenerative process; however, molecular genetic studies have identified the *TGFBI* gene at 5q31 in some families. Eponyms include anterior basement membrane dystrophy and Cogan's microcystic epithelial dystrophy [23].

All cases are characterized by an irregularity of basement membrane and associated abnormality in epithelial health characterized by poor epithelial adhesions and resulting erosions. The degenerative type presents more commonly in women over the age of 50, whereas the inherited type appears to occur much earlier and is unclear whether there is a sex predilection. Patients often complain of pain upon awakening with associated tearing and photophobia. Same day slit lamp examination may not reveal any epithelial defects.

On examination there can be dots, map, fingerprint, or bleb patterns. Dots or "Cogan" are irregular oval-shaped gray clusters. Maps are geographical areas of gray, heaped-up epithelium in the central epithelium. Fingerprint lines are thin parallel lines. Bleb or Bron pattern is described as pebbled glass. These patterns can be seen in isolation or in combination. These are most easily viewed with a wide beam at an oblique angle or with retroillumination.

Epithelial Recurrent Erosion Dystrophy (ERED)

This hereditary disease is autosomal dominant (AD), which affects individuals beginning in the first decade of life. The bilateral erosions occur at various locations on their corneas. This is important to note because the nonhereditary type causes recurrent erosions in the same area of the cornea and is typically unilateral. Similar to nonfamilial erosions, symptoms include burning, photophobia, and pain which is often precipitated by environmental factors such as dust and lack of sleep. Frequency and severity of attacks decrease with age [24].

There is a Smolandiensis variant in which affected individuals may develop protruding subepithelial nodules that appear similar to a central keloid. About 1/4 of this subtype will eventually need a corneal transplant in at least one of their eyes.

Meesmann Epithelial Corneal Dystrophy (MECD)

This autosomal dominant (AD) dystrophy appears early in life and is characterized by intraepithelial cysts which are best viewed with retroillumination. The epithelium surrounding the cysts is clear. The cornea may have reduced sensation and may have mild thinning. There can be mild discomfort and mild reduction in visual acuity. This is caused by the mutations in either the keratin 3 genes at locus 12q13 [25].

Stocker–Holt is similar in presentation, though it may have more severe symptoms and has associated fine epithelial opacities that stain and appear in a whirl pattern. The responsible gene is keratin 12 at locus 17q12 [26].

Gelatinous Drop-Like Corneal Dystrophy (GDLD)

This autosomal recessive (AR) corneal dystrophy was first described in Japan 1914 and presents as subepithelial gelatinous material that eventually coalesce to create an elevated, irregular mass. These deposits destroy Bowman's layer and the overlying epithelium is typically thinned. In addition, superficial neovascularization is common. It is a rare disease most common to Japan, but has also been seen in the United States and Europe [27].

Patients typically become symptomatic in childhood with decrease in vision and painful erosions. There are three subtypes including the mulberry type, band keratopathy type, and the kumquat type. The lesions typically reoccur rapidly with all forms of treatment.

The gene responsible is tumor-associated calcium signal transducer 2 (*TACSTD2*) at locus 1p32 (Fig. 22.3).

Lisch Epithelial Corneal Dystrophy (LECD)

This X-linked dominant disease presents in childhood with a gray-colored feather or whirl-like pattern. Its locus is at Xp 22.3; however, the gene has not been identified. This is slowly progressive with gradual decline in vision over time. This is category 2 corneal dystrophy [28].

Bowman's Layer Dystrophies

Reis–Bucklers Corneal Dystrophy (RBCD)

This is autosomal dominant (AD) dystrophy. Affected individuals usually present with recurrent erosions in childhood and demonstrate a gradual

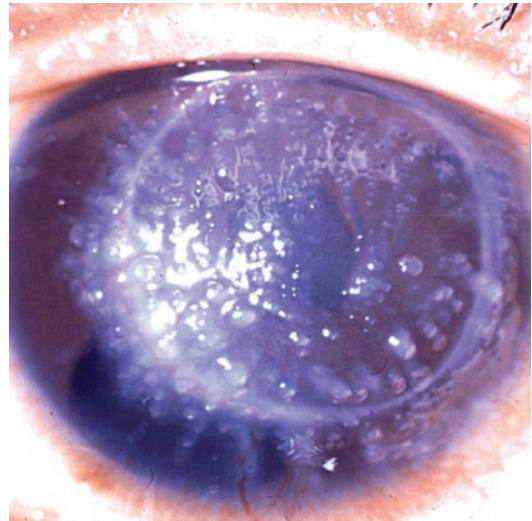


Fig. 22.3 Gelatinous drop-like corneal dystrophy. Amyloid is visible in the donor tissue of a corneal graft as well as in the surrounding host tissue (Klintworth [27]). © 2009 Klintworth; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited [27])

decline in vision. The erosions in Reis–Bucklers is typically worse than in Thiel–Behnke. Slit lamp examination reveals a central reticular appearance that eventually becomes a thick opacity that takes over Bowman's layer and the anterior stroma, and the surface becomes more irregular. As the opacity worsens, the erosions typically improve. The peripheral cornea typically remains unaffected. Vision is impaired by both the opacities and the induced corneal astigmatism and corneal sensation can become reduced. Light microscopy demonstrate Masson trichome-positive staining. Electron microscopy demonstrates rod-shaped bodies in the irregular tissue. The *TGFB1* gene is responsible and molecular genetic studies have revealed G623D, R124L, and R124C mutations [29].

Thiel–Behnke Corneal Dystrophy (TBCD)

This autosomal dominant (AD) dystrophy has its onset during childhood or adolescence with small deposits in the subepithelial layer, which then

becomes more reticular or honeycombed in appearance in a similar but less aggressive manner compared to Reis–Bucklers. The peripheral cornea is usually spared. Clinical appearance and symptoms are similar to Reis–Bucklers, which makes the two difficult to differentiate. Using anterior segment OCT one is more likely to see the sawtooth pattern in this disease. Symptomatic patients may be treated with epithelial debridement or with superficial keratectomy. The responsible gene is TGFBI which is mapped to 10q24 and 5q31. The mutation is Arg555Gln. This is a category 1 corneal dystrophy [30].

Grayson–Wilbrandt Corneal Dystrophy (GWCD)

This autosomal dominant (AD) dystrophy occurs in late childhood to early adolescence and like both Reis–Bucklers and Thiel–Behnke it results in erosions and subepithelial opacities, but often has a milder presentation. The stroma between lesions is clear. There is only a single publication looking at a family with this disease and neither the gene nor locus has been identified. This is a category 4 dystrophy [31].

Stromal Dystrophies

TGFBI Corneal Dystrophies: Lattice Corneal Dystrophy Type I (Classic LCD)

This autosomal dominant (AD) dystrophy is characterized by branching, refractile, linear amyloid deposits that spare the far periphery. The deposits start superficially in the center of the cornea and then dive deeper in the stroma but stop 1 mm from the limbus. The endothelium and Descemet's membrane are not involved. The typical depth is from anterior to mid stroma. In some cases Bowman's can be involved and epithelium can be thinned. Initially intervening stroma is clear, but later a general haze occurs. There may be asymmetry between the two eyes.

Patients usually present due to recurrent erosions. In the first 10 years of life patients may

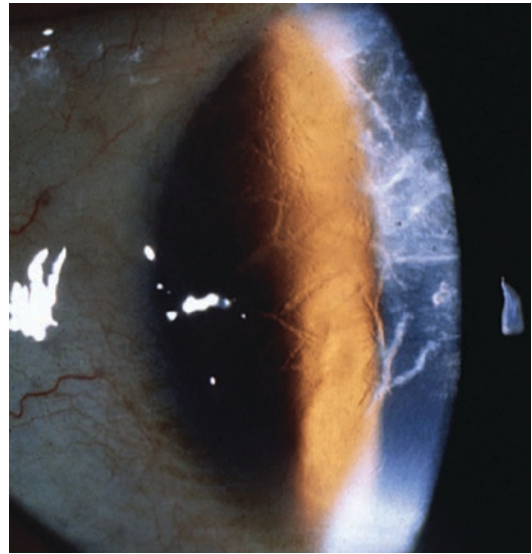


Fig. 22.4 Lattice corneal dystrophy type I (Klintworth [27]). © 2009 Klintworth; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited)

start to show the first signs of these deposits. At first the stroma in between the deposits may be clear, but will become hazy with time. Vision is usually not impaired until the fourth or fifth decade of life at which time many undergo penetrating keratoplasty.

Histopathology demonstrates amyloid deposits, which are birefringent with polarized light and stains with Congo red. The TGFBI gene encodes for keratoepithelin. About half of patients with lattice type have mutations at R124C, R124H, R555W, and R555Q. Other mutations include L568A, A546D, P551Q, and V505D [29, 32] (Fig. 22.4).

TGFBI Corneal Dystrophies: Other Subtypes of Lattice Corneal Dystrophy

The subtypes include type III, type IIIA, and type IV. These are similar to type I with the differences including the diameter of the fibrils. Type IV is less symptomatic due to the amyloid being more posterior [33].

Lattice Corneal Dystrophy, Gelsolin Type (LCD2)

This is a systemic autosomal dominant (AD) disease and therefore it is not a true corneal dystrophy. However, due to its similar appearance on slit lamp exam, it is included to help the clinician diagnose it as such. Its common name is Meretoja syndrome. The genetic cause is a mutation in the gelsolin gene, which maps to 9q34.

In lattice type II the amyloid deposits start at the limbus and spread centrally with the central cornea being largely spare, which is an important distinction to make from the other lattice subtypes. Corneal sensation is often reduced. Later in life vision may be affected and erosions may occur.

The systemic findings are largely due to the systemic amyloid deposition, which causes peripheral neuropathies including facial nerve involvement which can cause the typical facial drooping, dermatochalasis, lagophthalmos, and lip protrusion [27].

Granular Corneal Dystrophy Type 1, Classic (GCD1)

This is autosomal dominant (AD), which involves the TGFBI gene and maps to 5q31. This dystrophy results in opaque deposits described as granules, snowflakes, or bread crumbs. Children may demonstrate a vortex-like pattern. The deposits are most likely to be found in the anterior stroma and often spare the limbus. Photophobia is common and is likely related to erosions. Although opacities can be visualized as early as the first few years of age, significant changes often do not occur until much later in life. Homozygotes will have a more severe course. The substance is hyaline and stains with Masson trichrome. This disease shows 100% penetrance. The mutations identified are at R555W and R124S [34].

Successful treatment has been reported with both superficial keratectomy/PTK and PKP. PKP is only recommended for deeper involvement as reoccurrence typically occurs after just 4 years of transplantation [35] (Fig. 22.5).

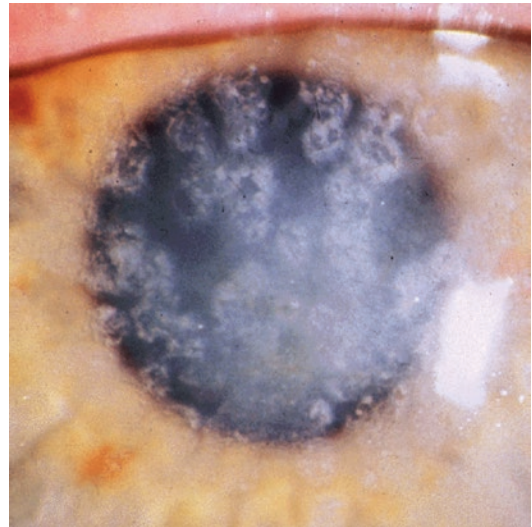


Fig. 22.5 Granular corneal dystrophy type I (© 2009 Klintworth; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited)

Granular Corneal Dystrophy Type 2 (GCD2)

This corneal dystrophy, also known as Avellino dystrophy, is an autosomal dominant (AD) dystrophy. The opacities can be in snowflake, icicle, or starbursts and may or may not have lattice lines. The cornea between the opacities is typically clear until later in life. If lattice is present, it occurs later in life and the lines are typically wider than seen in classic lattice and also are deeper in the stroma compared to the snowflake opacities. TGFBI is once again the culprit with mapping to 5q31 and the indicated mutation is R124H as well as N544S [36].

Histopathology demonstrates both hyaline and amyloid deposits.

Treatment involved PTK, lamellar keratoplasty, and penetrating keratoplasty based on the depth of the opacities [37]. Of note, granular corneal dystrophy has been reported to be exacerbated by LASIK and therefore using LASIK in these patients is contraindicated [38] (Fig. 22.6).

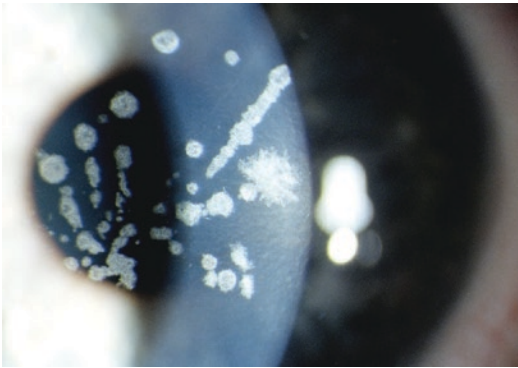


Fig. 22.6 Granular corneal dystrophy type II (© 2009 Klintworth; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited)

Macular Corneal Dystrophy (MCD)

This is one of the few autosomal recessive (AR) corneal dystrophies and is the most severe and most rare of the classic stromal dystrophies. This dystrophy, unlike many others, causes diffuse stromal haze all the way to the limbus. Only later do elevated white opacities occur which is why there is no clear stroma in between these lesions. These white opacities often coalesce and become quite visually significant in the 20s and 30s. Erosions are not classic but have been described. Photophobia can also occur even without associated erosions.

The involved gene is *CHST6*, which codes for corneal N-acetyl-glucosamine-6-O-sulfotransferase located at 16q22. The mutations are multiple including variants of insertions, deletions, nonsense mutations, and rearrangements [39].

Histopathologic examination demonstrates glycosaminoglycans or mucopolysaccharides that stain with Alcian blue. There are three subtypes that react differently to immunohistochemicals. They do not have any clinical differences.

Treatment includes PTK and PKP depending on the depth of the opacities [37]. Reoccurrence is less common compared to the other stromal opacities.

Fleck Corneal Dystrophy (FCD)

This autosomal dominant (AD) dystrophy presents at birth or within the first decade of life with diffuse clouding. It appears to be nonprogressive. It is one of the few dystrophies that can be unilateral or asymmetric though it is classically bilateral. The opacities have been described as flecks or dandruff and can be generalized or in a ring pattern. The flecks themselves are granular with clear borders and the intervening stroma is clear. It is best viewed with a broad oblique beam. The opacities extend all the way to the periphery. It involves the stroma and does not extend beyond the borders of the stroma. This dystrophy is associated with limbal dermoids, keratoconus, pseudoxanthoma elasticum, and atopy. In isolation this dystrophy is typically asymptomatic other than occasional photophobia.

Histopathology shows that keratocytes contain extra glycosaminoglycans and lipids, which stain with Alcian blue and oil red O, respectively.

This is a category I dystrophy in which phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase type III (*PIP5K3*) is indicated which is localized to 2q35 [40, 41].

Schnyder Corneal Dystrophy (SCD)

This autosomal dominant (AD) dystrophy was previously named Schnyder crystalline corneal dystrophy due to the commonly associated corneal crystals. It is important to note that only half of those affected actually have crystals. This dystrophy classically causes central haze or central subepithelial crystals as early as 2 years of life but more commonly in the second decade of life. In the following decade arcus lipoides develops peripherally. During these earlier stages patients maintain good scotopic vision; however, photopic vision is reduced [42].

Eventually the mid-periphery is also opacified and then the entire stromal bed becomes opaque. The presence or absence of crystals does not appear to affect the clinical course. About half of patients above the age of 50 have undergone PKP.

This is a category 1 corneal dystrophy. The responsible gene is UbiA prenyltransferase domain containing 1 (*UBIAD1*) mapped to 1p36. There are 21 known mutations with N102S being the most common mutation. This disease has high penetrance [43].

Congenital Stromal Corneal Dystrophy (CSCD)

This autosomal dominant (AD) corneal dystrophy presents in a congenital manner with fine, diffuse corneal opacities that extend from limbus to limbus. Corneal thickness is increased. The vision loss associated can be moderate to severe. Penetrating keratoplasty is recommended in severe cases. On histopathology the overall stromal appearance is normal; however, on electron microscopy the collagen fibril diameter is half that of average normals. The indicated gene is decorin and is located at 12q21.33. It is a category 1 corneal dystrophy [44].

Posterior Amorphous Corneal Dystrophy (PACD)

Posterior amorphous corneal dystrophy is an autosomal dominant inherited corneal dystrophy, which presents in childhood with sheet-like opacities and extends from limbus to limbus; however, there appears to be a ring of mid-peripheral cornea that is clear [45].

This disease process presents in early to late childhood with scattered gray opacities in the posterior cornea. It is nonprogressive. The cornea is flat with K <41 D and the cornea may be significantly thinned. Unlike cornea plana the Descemet's membrane and endothelium may be disturbed by these opacities. There are also associated iridocorneal adhesions, and corectopia has been noted without associated glaucoma. Other than the associated hyperopia, vision is only minimally affected by the opacities with most patients seeing 20/40 or better. Some argue to be a dysgenesis rather than a dystrophy; therefore, it is considered a category 3 dystrophy [46].

Endothelial Dystrophies

Congenital Hereditary Endothelial Dystrophy

This is a bilateral autosomal dominant disease that presents at birth with thickened Descemet's membrane and corneal edema. Histopathologic findings include thickened Descemet's membrane, diffuse edema, multinucleated cells, and scattered areas of missing Bowman's layer. Management includes endothelial keratoplasty with or without Descemet stripping. All cases should be screened for hearing loss or Harboyan syndrome. The gene responsible is SLC4A11 [47].

Posterior Polymorphous Corneal Dystrophy (PPCD)

This autosomal dominant (AD) dystrophy is characterized by endothelial lesions including broad bands with scalloped edges, discrete grey lesions, and vesicles in clusters. Early corneal edema can be managed with hypertonic salt solution. In cases without stromal opacification EK may be performed. In cases of opacity PK can be undertaken with the understanding that it may reoccur in the graft.

Histopathological findings include multilayered endothelial cells that have features that are more classically associated with epithelial cells such as positive keratin staining, microvilli, easy growth in cell culture, and proliferation. Descemet's membrane is abnormal and thickened. There can also be various degrees of corectopia, iridocorneal adhesions, and stromal edema. Both angle closure and open-angle glaucoma can occur [48, 49].

Fuchs Endothelial Corneal Dystrophy (FECD)

Fuchs endothelial corneal dystrophy has complex genetics with mixed inheritance patterns. This corneal disease most commonly presents in the fourth or fifth decade of life. It is usually

asymptomatic and noted on exam or discovered after cataract surgery. The classic finding is guttae, which are small collections of Descemet's membrane in the central cornea.

In the United States as much as 4% of the population above the age of 40 years are affected by Fuchs, though the disease usually does not start affecting vision until the fifth decade or later. Fuchs is the most common disease resulting in endothelial keratoplasty, which is now the treatment of choice over penetrating keratoplasty. On histopathology endothelial cells demonstrate polymegathism and pleomorphism. Descemet's is thickened. There is typically stromal edema.

Fuchs demonstrates significant allelic heterogeneity and locus heterogeneity. When the ICD3 was first published, only a few alleles and mutations had been observed [50]. Recently a large amount of work has been done using a genome-wide association study (GWAS) to further delineate which genes are responsible for this disease. Four major loci have been identified of which the most common and well established is TCF4 in which a trinucleotide repeat causes the disease [51]. KANK4, LAMC1, and ATP1B1 have also been identified [33]. This is a category 1 corneal dystrophy.

Where the Field Is Heading

Now that the genome has been sequenced and gene mapping is becoming more accessible and affordable, we will continue to determine various mutations that result in a wide range of corneal disease phenotypes. As diagnosis and identification become clearer, so do targets for treatment. Newer gene editing techniques involve what is called clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated 9 (Cas9) nucleases [52]. In simplified terms, artificial nucleases are directed to cleave certain areas of the genome so that defective genes can be deleted or replaced.

Within the area of corneal disease in particular targeting overexpression or expression of defective proteins could have implications for the TGFBI corneal dystrophies [53]. In addition,

trinucleotide repeats such as in the TCF4 gene in FECD may prove a promising target [54]. Furthermore, Meesmann epithelial corneal dystrophy is currently being studied as a possible disease of interest for gene therapy [55].

Other areas of upcoming promise include stem cell therapy and Rho-kinase inhibitors. Stem cells have been the hot topic for several years. Currently human pluripotent stem cells (PSCs) have been successfully generating a renewable source of endothelial cells [56]. In terms of endothelial dystrophies another area of upcoming promise is that of Rho-kinase inhibitors (ROCK inhibitors) which show promise in cell adhesion and suppression of cell death, which has large implications for the treatment of FECD [57, 58]. Lastly, epigenetics is an emerging field that may play a larger role and may give guidance in areas that we do not fully understand [59, 60].

Summary

The list above is not complete, though it is extensive. Some areas that go beyond the scope of this chapter include diseases that are not well understood in the dystrophy category such as various syndromes like Alport or Alagille syndrome. Most of the corneal dystrophies were discussed; however, very rare and poorly understood dystrophies such as central cloudy dystrophy of François (CCDF) C4 and pre-Descemet corneal dystrophy (PDCD) were not listed. In addition, the genetics of keratoconus and metabolic storage diseases will be covered in another chapter.

It is important to understand that variability in penetrance and genetic heterogeneity exist and therefore the clinician must be vigilant to determine the cause of degenerations and dysgenesis.

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The Challenge of Antibiotic Resistance in Corneal Infection

23

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The discovery and development of antibiotics for treatment of bacterial infections was one of the greatest medical advances of the last century, and it has saved millions of lives. However, as early as 1945, Sir Alexander Fleming expressed concern about the consequences of antibiotic overuse [1]. Seven decades later, antimicrobial resistance (AMR) has reached alarming levels and is now a major public health threat in the twenty-first century [2]. The emergence of multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), antibiotic-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus*, multidrug-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant Enterobacteriaceae among others [3] is raising the specter of a post-antibiotic era [4–6]. AMR organisms, which initially were largely confined to hospitals, have now found their ways into communities, posing a serious threat to otherwise healthy individuals [7]. MRSA, for instance, caused an estimated 80,461 invasive infections and 11,285 deaths in the US in 2011 [8], and are now common causes of ocular infection [9–12]. Most derive from specific MRSA lineages that have disseminated throughout the US and

other locations, and are associated with particularly serious ocular and orbital infections [13].

AMR is an inexorable consequence of the broad use of antibiotics in human and veterinary medicine and animal husbandry. Exposure of bacteria to antibiotics eliminates susceptible strains and allows the proliferation of resistant lineages, including those harboring resistance genes on mobile elements. All genes (as distinct from mutations, which will be discussed later) encoding resistance are ancient [14] and mainly derive from the producers of antibiotics to protect them from the antibiotic they produce [15]. However, the spread of these resistant elements into the human ecosystem – among pathogens that cause human or animal infection – is a recent event that followed the introduction of antibiotics into human use. In addition to acquiring transferable resistant determinants, spontaneous mutations that alter antibiotic targets can be selected in a bacterial population under antibiotic pressure.

Although most ocular infections originate in the community as opposed to the hospital, and are mainly caused by resident microbes on the patient, increasing use of topical antimicrobial agents for prevention and treatment has resulted in the emergence of antibiotic-resistant organisms isolated from ocular infections [16–20]. In the past few decades we have learned much about the epidemiology of antimicrobial resistance in ocular infections. We also have an increased

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understanding of the pharmacokinetic properties of newer topical antibiotics in ocular tissues. In this chapter we will review how the growing issue of antimicrobial resistance has become significant among causes of bacterial keratitis and its clinical relevance.

Microbial Etiologies of Keratitis

While the etiologies of infectious keratitis vary according to geographical location and predisposing risk factors, keratitis caused by bacteria is the most common worldwide [21–24]. The three most common predisposing factors in all geographies are traumatic eye injury, contact lens (CL) use, and recent surgery. Traumatic corneal injuries are most common in rural, farming communities in which there is a high risk of corneal abrasion from vegetative material or soil. In rural farming communities, particularly in warm climates, fungal causes of keratitis can be as common as bacterial causes [25]. The most common bacterial cause of keratitis following trauma include *Staphylococcus* spp., *Streptococcus* spp., and *Pseudomonas aeruginosa*. Atypical infections occur with unusually high prevalence in certain pockets of the world: *Nocardia* spp. infection accounted for 11.1% of all cases in the Steroids for Corneal Ulcers Trial (SCUT) study, in which the majority of patients were enrolled from Tamil Nadu, India [26]. Non-tuberculous *Mycobacteria* infections, while uncommon, have been reported in as much as 7.9% of all bacterial isolates in a large study from Taiwan [27].

In the developed world, microbial keratitis is most often the result of contact lens wear and constitutes 33.7–50.3% of cases [28–30]. The vast majority are bacterial, with a significant minority due to fungi and *Acanthamoeba*. A large, multicenter prospective case-control study consisting of 964 CL wearers from study sites in the UK, Australia, and New Zealand found that of 121 positive cultures, 51.2% were *Pseudomonas* spp. [31]. While *Pseudomonas aeruginosa* has been reported as the most common causative organism in contact lens wearers, Gram-positive organisms cause most of the remaining non-*Pseudomonas* cases [31, 32].

Among common corneal surgical procedures, penetrating keratoplasty (PK) and keratoprosthesis insertion carry the highest risk of complicating infection, occurring in some studies at rates as high as 5–10% [33–36]. By contrast, the prevalence of corneal infection following refractive surgeries such as laser in situ keratomileusis (LASIK) is substantially lower ranging between <0.01% and 1.5% of procedures [37–39]. Bacterial keratitis appears to be the most common etiology following PK [40, 41]. For keratorefractive surgeries, a large retrospective series conducted by members of the American Society of Cataract and Refractive Surgery, comprising an estimated 20,941 procedures, concluded that methicillin-resistant *Staphylococcus aureus* (MRSA) was the most common cause of infection post-keratorefractive surgery (28%) followed by non-MRSA staphylococci (18%) [42]. Atypical causes of post-refractive surgery infection, such as non-tuberculous *Mycobacteria*, actinomycetes, and fungal infections, typically arise as late-onset infections in 0–4.2% of cases [42].

Antimicrobial Resistance in Keratitis

Empirical use of topical broad-spectrum antibiotics remains the first-line treatment for bacterial keratitis. Combination therapy with fortified cephalosporin (e.g., cefazolin 5%) plus an aminoglycoside (e.g., tobramycin or gentamicin, 0.9–1.4%) was used for decades as the initial therapy [43, 44]. However, monotherapy using a commercial fluoroquinolone ophthalmic solution was shown, at least initially, to be as effective as combination therapy [45]. Because of the practicality of using commercially available eye drops and a reported slightly better tolerance profile compared to fortified antibiotics, many clinicians favor the empirical use of fluoroquinolones in clinical practice [46, 47]. However, exposure of bacteria capable of causing keratitis to these antibiotics during topical therapy has been associated with the selection of less susceptible bacterial populations [48]. Antibiotic use results in replacement of susceptible organisms by more tolerant subpopulations, which may then become dominant at the site of infection. This can result

in prolonged treatment and failures [49]. This problem has become especially acute with fluoroquinolone antibiotics because of the underlying genetics of resistance. Fluoroquinolones target enzymes involved in bacterial DNA replication, termed type II topoisomerases – specifically the enzymes gyrase and topoisomerase IV [50]. Resistance to fluoroquinolones can be achieved by mutations in these genes, generating enzymes that retain their function in the presence of the fluoroquinolone. Antibiotic-resistance-conferring mutations arise in about one microbe in a billion [50]. Therefore, many bacterial strains, if they reach sufficiently large populations, may arise with resistances to fluoroquinolones. In contrast, resistance to other antimicrobials, such as methicillin resistance in *S. aureus*, requires the microbe to obtain one or more genes from another donor microbe that possesses this resistance [51]. This, too, has happened as the result of the widespread use of antibiotics, but is rarer. An *S. aureus* strain causing an infection is less likely to convert to methicillin resistance by gaining the necessary genes from a donor during the course of the infection, than it is for a mutation to arise within the population that confers fluoroquinolone resistance. Therefore, in an era when fluoroquinolone therapy predominates, the generation of resistance as a consequence of therapy is an especially important consideration.

Topical ophthalmic antibiotics are formulated in high concentration to provide levels above the minimum inhibitory concentration (MIC) needed to be effective, despite washout and other anatomical and physiological barriers. These obstacles, in addition to nasolacrimal drainage, include reflex blinking, tear turnover, and absorption into the conjunctiva. As a result, approximately 95% of a topically applied solution is rapidly eliminated following instillation [52, 53]. The decreased retention time of antibiotics in the ocular surface ultimately leads to maximum drug concentration ($C_{\max}$) values in the cornea that are far smaller than the initial concentration of the eye drop. This can result in pharmacokinetics/pharmacodynamics (PK/PD) indices that are sub-optimal for organisms with increased MIC values. In corneas of pigmented Dutch-Belted rabbits, the newer fluoroquinolones besifloxacin,

moxifloxacin, and gatifloxacin achieve PK/PD indices against methicillin- and ciprofloxacin-resistant staphylococci that are approximately 15- to 120-fold lower than the minimum PK/PD target that predicts clinical efficacy against Gram positives [54]. This carries the risk of selecting for subpopulations of spontaneous mutants expressing proteins that are resistant to the effects of the antibiotics [55, 56].

Whether topical ocular antibiotic use contributes to the genesis of resistant strains, or simply selects for the outgrowth of antibiotic-resistant microbes already present among a patient's flora, is controversial. In the antibiotic era, few patients have escaped prior exposure to antibiotics. Moreover, the populations of microbes on the ocular surface is comparatively small, reducing the chance that a spontaneously resistant strain will arise locally. Nevertheless, the end result is the same – antibiotic use selects for microbes that are increasingly antibiotic resistant. Therefore, it is not surprising that selection of resistant organisms on the ocular surface following prophylactic use of fluoroquinolones has been well documented [18, 19, 57, 58]. Similar observations have been related to the topical treatment of ocular infections, including keratitis. A retrospective study associated the use of topical fluoroquinolones within 3 months before sampling with higher rates of resistant isolates *S. aureus* causing external ocular infections [17]. Further, prior topical antimicrobial therapy in patients with infectious keratitis (antibiotics used were not stated) was associated with increased isolation of organisms resistant to one or more commonly used ophthalmic antibiotic [20]. A report from the Steroids for Corneal Ulcers Trial (SCUT) demonstrated that keratitis-causing isolates from patients who were using a second-, third-, or fourth-generation topical fluoroquinolone at presentation had moxifloxacin MICs that were 1.47–3.48-fold higher than those who were not under treatment at enrollment [48].

Fluoroquinolone-resistant bacteria are expected to become increasingly common in the community in parallel with the use of these agents for treatment and prophylaxis of eye infections. In 1992, roughly 1 year following

introduction of Ciloxan (ciprofloxacin 0.3%, Alcon) in the US market, the first cases of ciprofloxacin-resistant organisms causing keratitis were reported [59, 60]. In examining isolates from a large case series from the Bascom Palmer Eye Institute, resistance to ciprofloxacin and ofloxacin was found in some *Staphylococcus aureus* keratitis isolates from as early as 1990 [61]. Resistance has increased since then: Among isolates recovered from 1990 to 1994, 7% of the *S. aureus* were resistant to the fluoroquinolones, and this grew to 21% for strains isolated between 1995 and 1998. Interestingly, a similar pattern of increase was not observed for aminoglycosides, which retained good in vitro coverage over the years evaluated [61]. Similar observations were made for keratitis isolates recovered at the Eye and Ear Institute of the University of Pittsburgh Medical Center. The observed rates of resistance to ciprofloxacin among all bacterial keratitis isolates (Gram positive and Gram negative) were near 16% in 1993. Of *S. aureus* only, 5.8% were resistant to ciprofloxacin in 1993, but by 1997 this increased to 35%. Gradual increase in ciprofloxacin resistance among coagulase-negative *Staphylococcus* was also noticed [62]. In India, rates of resistance were already much higher in the 1990s, with one report finding 32.5% of Gram-positive and 13.3% of Gram-negative keratitis isolates with reduced susceptibility to ciprofloxacin. That study observed further losses in ciprofloxacin susceptibility over time, especially for *P. aeruginosa* (0% in 1992 vs. 18.8% in 1996), alpha-hemolytic streptococci other than *S. pneumoniae* (0% in 1992 vs. 60.7% in 1996), and *Corynebacterium* species (0% in 1992 vs. 37.8% in 1996) [63].

Resistance of keratitis isolates to fluoroquinolones, including the newer agents moxifloxacin and gatifloxacin, continues to grow in many parts of the globe [64–69]. Because of decreasing susceptibility, more potent 8-methoxyfluoroquinolones, such as gatifloxacin (Zymar, Allergan) and moxifloxacin (Vigamox, Alcon), have replaced older fluoroquinolones ciprofloxacin and levofloxacin [70, 71]. The older fluoroquinolones preferentially

target either DNA gyrase or topoisomerase IV. The newer-generation compounds gatifloxacin and moxifloxacin have significant activity against both DNA gyrase and topoisomerase IV at achievable levels, making it necessary for an organism to mutate both enzymes to become resistant [71]. Nevertheless, resistance to the newer fluoroquinolones started to emerge not long after their introduction in the US market in 2003. In 2006, the first cases of ocular infection caused by 8-methoxyfluoroquinolone-resistant organisms were reported in patients following in situ keratomileusis (LASIK) and photorefractive keratectomy, both given topical moxifloxacin or gatifloxacin for prophylaxis [72]. One infection was caused by *P. aeruginosa* and the other by methicillin-resistant *S. aureus*. Both cases were resolved with topical aminoglycosides and surgical intervention. Currently, resistance to these fourth-generation fluoroquinolones among keratitis isolates has been reported to be as high as 30% for *S. aureus* and 32% for coagulase-negative staphylococci (CoNS) [23, 73]. More broadly, the ARMOR surveillance study found rates of resistance to moxifloxacin and gatifloxacin among contemporary ocular isolates in the US (including from keratitis) to now range from 26.1% to 47.8% for *S. aureus* and 27.2–40% for CoNS [74]. *Streptococcus pneumoniae* and *P. aeruginosa* remain highly susceptible to these fluoroquinolones in the US [11, 23, 73]. Conversely, the epidemiology of fourth-generation fluoroquinolone resistance among *P. aeruginosa* keratitis isolates is significantly different in other parts of the globe and that will be briefly discussed below.

Methicillin-Resistant *Staphylococcus aureus*

In North America, resistance among *S. aureus* keratitis isolates is especially notable and appears to have evolved at a greater rate than in keratitis-associated Gram-negative bacteria. Considering that in this geography, *S. aureus* is a main causative agent of bacterial keratitis,

this poses a challenge to empirical treatment of keratitis with fluoroquinolone monotherapy. National surveillance programs that monitor antibiotic resistance among ocular pathogens have found that many of the fluoroquinolone-resistant *S. aureus* are in strains that are also methicillin-resistant [9, 11]. Methicillin resistance, conferred by the acquisition of a block of genes in the form of a cassette, emerged in the early 1960s, and because of the widespread use of modified β -lactams, those lineages proliferated first in hospitals and then in the community [75]. So although methicillin resistance is unlikely to arise as the result of a particular course of therapy, methicillin-resistant *S. aureus* (MRSA) are now common [75]. Moreover, methicillin-resistant strains are more likely to also have resistances to other non- β -lactam antibiotics. In a large retrospective review spanning a 20-year period (1993 to 2012) that included keratitis cases, MRSA were most often also resistant to the fluoroquinolones used topically, including ciprofloxacin (73.8%), gatifloxacin (45.9%), and moxifloxacin (35.2%). Increases in rates of resistance to gatifloxacin and moxifloxacin were significant between the first and second decades of the study [66]. Reports of recent MRSA keratitis isolates have shown that the current rates of resistance to fourth-generation fluoroquinolones range as high as 81–100% [23, 73, 76].

The high rates of resistance to fluoroquinolones among MRSA isolates raise the prospect that multidrug-resistant strains of MRSA may have pathogenic properties that make them particularly prone to cause keratitis. In fact, a molecular epidemiology study from Japan found that MRSA keratitis isolates are predominantly members of the *Staphylococcus aureus* clonal complex 5 (CC5), as determined by multilocus sequence typing [77]. This clonal complex has been historically associated with multidrug-resistant healthcare-associated infections in the US [78], including in residents of nursing homes [79, 80]. Increasing pathogenicity of CC5 strains for corneal tissues is especially alarming since there are fewer remaining treatment options for these infections.

Pseudomonas aeruginosa

Keratitis isolates of *Pseudomonas aeruginosa* remain largely susceptible to second- to fourth-generation fluoroquinolones, third-generation cephalosporins, and aminoglycosides [11, 23, 73]. The US-based ARMOR study, which included analysis of nearly 400 *P. aeruginosa* isolates, found 6.6% resistant to ofloxacin, 5.1% resistant to ciprofloxacin, 4.5% resistant to levofloxacin, and 3.1% resistant to tobramycin [11]. It is important to note, however, that despite widespread susceptibility among many clinical isolates, multidrug-resistant (MDR, i.e., resistant to ≥ 1 drug in at least three antibiotic classes) *P. aeruginosa* are emerging in some regions of the world, which is a cause for great concern [81]. A study from L.V. Prasad Eye Institute in India found that these infections are associated with greater morbidity, including a greater likelihood of severe complications such as perforation and need for surgical intervention [82]. Antibiotics to which MDR *P. aeruginosa* retain susceptibility, such as colistin and imipenem, are not routinely available in topical formulations and, in many parts of the world where corneal ulceration is endemic, are not available at all. *P. aeruginosa* strains that show an invasive phenotype in vitro (corresponding to an *exoS*⁺/*exoU*⁻ genotype) are associated with poorer initial and final visual acuity, increased infiltration size, presence of hypopyon, and resistance to fluoroquinolones and aminoglycosides [83].

Clinical Relevance of Conventional Antimicrobial Resistance Testing

Bacteria are categorized in clinical microbiology laboratories as sensitive, intermediate, or resistant according to MIC breakpoints established by standard-setting groups, including the Clinical Laboratory and Standards Institute (CLSI) [84]. These breakpoints are defined based on consideration of (1) the in vitro susceptibility (MIC values) of strains without overt resistance genes compared to strains expressing resistance mechanisms to the antibiotic tested, (2) levels of

antibiotics achieved at the site of infection following systemic antibiotic use, and (3) clinical and microbiological outcomes from prospective trials [85]. However, these considerations rarely factor the unusual anatomy and drug distribution in the eye, or the use of topical high-dose antibiotics associated with a steep clearance curve. Thus, the relevance of CLSI-type sensitivity/resistance cut-offs for infection of the cornea has long been debated. Topical ophthalmic antibiotic solutions distribute throughout the eye achieving different concentrations in each particular tissue and compartment. Bioavailability of antibiotics decreases from external to internal ocular tissues. For the anterior segment, highest antibiotic concentrations are achieved in the tears followed by conjunctiva, cornea, and then aqueous humor [86]. Although formal susceptible and resistance definitions created for systemic use of antibiotics to treat infections at other anatomical sites may be of dubious value for predicting cure when applied topically to the cornea, it is nevertheless also true that microbes categorized as susceptible will be more easily treated at any site than microbes categorized as resistant, even in bacterial keratitis [87–91]. Rates of clinical improvement and cure of patients in a multicenter clinical trial treated with topical 0.3% ciprofloxacin were higher for infections caused by isolates with a ciprofloxacin MIC ≤ 1 $\mu\text{g/mL}$ (the systemic breakpoint for sensitivity for many bacterial species) when compared to MIC levels higher than 1 $\mu\text{g/mL}$. Further, keratitis caused by organisms with higher MICs resolved more slowly [91]. The SCUT report also noted an association between poor clinical outcome and higher MICs for moxifloxacin, the antibiotic given topically to the patients during the trial [87, 89, 90]. Increased MIC to moxifloxacin among *S. pneumoniae* and *P. aeruginosa* was associated with larger infiltrate/scar sizes and slower time to reepithelialization when corrected by causative organism [89]. At 3 weeks after enrollment, a twofold increase in MIC was followed by a 0.018 logMAR decrease in the best spectacle-corrected visual acuity and an increase of approximately 0.04 mm in the infiltrate/scar size [89]. Additionally, improvement in visual acuity was more frequent (89.3%) for infections caused by organisms with a moxifloxacin

MIC ≤ 1 $\mu\text{g/mL}$, than those with an MIC ≥ 2 $\mu\text{g/mL}$ (59.3%) [90]. Interestingly, these numbers are in agreement with the “90–60 rule” which suggests that approximately 90% of the infections caused by susceptible organisms respond to the antibiotic therapy, while 60% of those caused by resistant organisms are responsive to treatment [92]. Similarly, in a large cohort of UK patients treated for *Pseudomonas* spp., Enterobacteriaceae, and *S. aureus* keratitis who received initial monotherapy with a fluoroquinolone antibiotic, a linear association between MIC and increasing time for reepithelialization was found [88]. Therefore, in vitro antimicrobial susceptibility testing is informative for predicting clinical success in treating keratitis, although some patients with keratitis caused by formally antibiotic-resistant strains may achieve clinical resolution. Thus, despite some limitations in the accuracy of traditional in vitro testing of susceptibility, knowledge of antimicrobial sensitivities continues to be fundamental in supporting treatment decisions, especially for the subset of patients presenting with more recalcitrant infections.

The Challenge of Diagnosis

Given the growing fluoroquinolone resistance among keratitis isolates, prudent use and early antibiotic de-escalation is important to prevent selection of resistant mutants within the population. However, current diagnostic approaches to infectious keratitis are time-consuming and often insensitive, impeding efforts to rapidly tailor the treatment with the most appropriate antibiotic to a great number of patients. Culture of microbes recovered from the site of suspected infection remains the cornerstone of microbial keratitis diagnosis. While interhospital variation exists, corneal scrapings are commonly fixed using Gram and Calcofluor white stains and cultured on a combination of blood, chocolate blood, and Sabouraud's agar. In addition to taking days to weeks for an organism to grow, the sensitivity of culture from the largest retrospective studies across multiple sites remains in the order of 50–70% [61, 67, 93]. This results in heavy depen-

dence on a few broad-spectrum empiric antibiotics and selects for the development of resistance irrespective of route of administration. This has proven especially true for the empiric use of topical fluoroquinolones in the treatment of bacterial keratitis [17, 19, 48].

Novel molecular diagnostic tools, including PCR and more recently next-generation sequencing (NGS), show great promise in complementing and perhaps eventually superseding stain and culture as the mainstay of diagnosis. PCR-based studies for bacterial, fungal, and amoebic keratitis have reported sensitivities of >90% while decreasing considerably the turnaround time [94–97]. Because they are agnostic to the underlying agent, NGS of DNA from clinical samples, coupled with sophisticated bioinformatic analysis that discards contaminating patient DNA, appears especially promising [98]. NGS was recently used to determine the infectious etiology of keratitis in 16 formalin-fixed corneal samples [99], 14 of which derived from patients with failed penetrating keratoplasties. That approach was able to identify the correct etiology in 13 of 16 cases, with two of the cases having detected HSV-1 keratitis, but not at the diagnostic threshold. In most cases, DNA material was obtained from full corneal buttons but the individual case of a superficial corneal biopsy produced data of similar quality. This suggests that with further refinement, including more precise establishment of diagnostic limits and reduced cost, rapid diagnosis may be achievable by NGS or related molecular technologies using small sample volumes such as those obtained from corneal scrapings. Importantly, this technology will not only allow physicians to tailor the treatment for each patient at an early stage in disease, it may also be of value for monitoring the emergence of antimicrobial resistance.

Novel Delivery Systems for Improved Delivery

The vast majority of topical antibiotics are solutions of antibiotic in a solvent [100]. With relatively low manufacturing costs and reasonably

high patient tolerance, the most successful and widely used topical antibiotics are those which rely on the inherent characteristics of their molecular structure. Moxifloxacin, for instance, has been shown to achieve a six- to sevenfold increase in conjunctival penetration compared to other ocular fluoroquinolones because of its intrinsic lipophilic nature and biphasic solubility and solubility in the aqueous humor [101]. Nevertheless, a major limitation of topical ocular antibiotics is achieving adequate residence time along with tissue penetration of the ocular surface. It has been shown that at best the bioavailability of topical antimicrobials within the cornea hovers in the vicinity of 5% of the dosage applied [53, 102]. As previously shown, the suboptimal concentration of antibiotics achieved in the cornea during treatment may result in PK/PD indices that are far below the target for Gram-positive organisms [54]. This may result in decreased clinical effectiveness and favors the selection of antibiotic-resistant mutants in the population.

To address this, novel delivery systems that improve residence time and ocular tissue penetration and adhesion have been proposed. This includes vehicles to enhance tissue penetration, such as benzalkonium chloride [103] and cyclodextrins [104], carriers that prolong ocular residence time [105–108], and ophthalmic devices that offer sustained drug delivery [109–111]. One example of a novel drug delivery system in current commercial use is Besivance (Bausch and Lomb Inc., Rochester, NY) 0.6% suspension, which is composed of besifloxacin, bound to a polycarbophil-based mucoadhesive gel (Durasite®, Insite Vision Inc., Alameda, CA). Approved by the FDA in 2009 for the treatment of bacterial conjunctivitis, it is also commonly used as off-label treatment for superficial corneal infections and post-procedure antibiotic prophylaxis. In animal models, Besivance has been shown to achieve higher tear concentrations and durations of effect when compared to other commercially available ocular antibiotics such as Vigamox (moxifloxacin hydrochloride, Alcon) and Zymar (gatifloxacin, Allergan) at comparable concentrations [54]. This appears to be conferred by its mucoadhesive delivery matrix, which

adheres to the mucin and glycosaminoglycan-rich surface of the cornea [105].

Alleviation of lacrimal drainage of topical medications can be achieved by in situ gelling of antibiotic, thereby enhancing ocular surface residence time. Gelation may be activated by pH (cellulose acetate), temperature (e.g., polymethacrylates), or ion activation (sodium alginate) [108]. In situ gelling formulations of moxifloxacin [108] and gatifloxacin [107] have been shown to be generally stable and may provide sustained conjunctival release of up to 8 hours, which may reduce administration frequency and improve patient compliance. Impregnated or reservoir contact lenses offer the unique advantages of sustained delivery; ability to carry cyclodextrins, liposomes, and emulsions; and the potential to achieve >50% bioavailability on the corneal surface [111]. Numerous antibiotics such as norfloxacin [109] and ciprofloxacin [110] have been demonstrated to offer reservoir release and antibacterial action of up to 24–96 hours. While dosage may be potentially decreased with contact lenses, comfort for patients with acutely inflamed and painful ocular surfaces may be a limitation.

Summary

Antimicrobial resistance is a growing concern for treatment of keratitis. Fluoroquinolones that are commonly used for empirical therapy can select resistant mutants during treatment, and the rates of resistance to these antibiotics are rising among keratitis-causing organisms. Further expansion of these resistant populations in the community, which are associated with poorer clinical outcomes, will reduce the coverage of fluoroquinolone monotherapy and impact the role of these agents as first-line options for empirical treatment. Attempts to stop this growing phenomenon by moving away from prolonged empirical therapy with broad-spectrum antibiotics are significantly hampered by inadequate diagnostics. Rapid and more sensitive diagnostic technologies are expected to assist in the prompt identification of antibiotic-resistant infections and make it possible to tailor the treatment in early stages of the

disease. Novel delivery technologies that improve antimicrobial concentrations in the cornea and decrease the chances for selecting resistant mutants during treatment are expected to play an important role in the fight against antimicrobial resistance in bacterial keratitis.

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Part V

Special Topics

Therapeutic Contact Lenses in the Management of Corneal and Ocular Surface Disease

Deborah S. Jacobs and Joshua S. Agranat

Introduction

Claes Dohlman has observed, “We don’t see poly(morphonuclear leukocyte)s under a contact lens.” Clinicians appreciate that therapeutic lenses provide an improved environment for corneal epithelial function and that such lenses make patients feel better. We have yet to explain the mechanism underlying the phenomenon that Dr. Dohlman described. Advances in contact lens offer important avenues for treatment of certain conditions, including persistent epithelial defect, exposure keratopathy, neurotrophic keratitis, and ocular chronic graft versus host. Recent decades have yielded an expanded spectrum of contact lens options for corneal ectasia and irregular astigmatism, including specialty rigid gas-permeable corneal lenses, custom silicone hydrogel soft lenses, hybrid lenses, and scleral lenses. Incorporation of contact lens in therapeutic regimens can postpone or eliminate the need for keratoplasty, ocular surface reconstruction, or tarsorrhaphy. Furthermore, contact

lenses have played an important role in the success of the Boston KPro Type I.

To follow is an overview on the use of contact lens for corneal and ocular surface disease (OSD). History and principles will be reviewed for soft lenses and for scleral lenses. Specific corneal conditions in which therapeutic contact lenses have a large impact will be reviewed.

History and Principles of Therapeutic Soft Contact Lens

In the proper clinic context, a carefully chosen contact lens can aid eyes with ocular surface disease (OSD). Soft contact lenses can promote corneal healing by creating a physical barrier which provides mechanical support and protection, reduces desiccation, and alleviates pain. Vision may improve as a consequence of corneal healing or incidentally by correction of surface refractive error.

The foundation for well-tolerated soft contact lenses was laid in the 1960s by the chemist Otto Wichterle who first developed biologic hydrophilic gels, commonly known as hydrogels [1]. Over the following decades, advances in hydrogel technology has allowed for increased comfort, extended wear, and the widespread use as a temporary bandage after trauma and surgery or in disease. Reports estimate that over 70% of optometrists and ophthalmologists have

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prescribed soft contact lenses for therapeutic purposes [2].

The important parameters when evaluating the therapeutic potential of a soft lens are oxygen permeability, water content, and mechanical compatibility with the ocular surface. Oxygen permeability (Dk) is typically reported in a standardized fashion for the thickness of -3.00 diopter lens in the units Dk/L or Dk/T. In commercially available lenses, Dk ranges from <10 in the oldest hydrogel materials to up to 340 in soft lenses made entirely of silicone rubber. Advances in polymer science over the past 50 years have resulted in hydrogels with higher intrinsic oxygen permeability as well as increased water content, which in turn confers improved oxygen transmission. The earliest therapeutic lenses were the Plano T (Bausch & Lomb, Rochester NY) and Permalens Therapeutic (CooperVision, Fairport, NY) which were made of low and slightly higher Dk lenses. The water content of a lens is reported as a percentage and is another important variable to consider when choosing a therapeutic lens. Low water content lenses may be irritating, while high water content lenses may cause desiccation of the cornea and exacerbate aqueous tear deficiency. Interestingly, the hydrogels with high water content tend to fail as therapeutic lenses because they may act as sponges on dry eyes.

Currently, most lenses marketed with therapeutic indications for use are made of the higher Dk silicone hydrogel materials which confer higher oxygen transmission via the material itself. Silicone hydrogel (SiHy) soft lenses were expected to offer increased biocompatibility and reduce complications thought to be related to hypoxia including infection [3, 4]. Lower infection rates in overnight wear have not been found in population-based studies [5, 6]. These high Dk lenses are a sound choice in cases in which corneal neovascularization is already present. There are large-diameter hydrogel lenses on the market in the USA and abroad that are useful in instances in which standard 13–15 mm soft lenses are not retained on the ocular surface. Despite relatively low Dk, these lenses, such as Kontur (Kontur Kontakt Lens, Hercules CA), have played an important role used off-label as therapeutic lenses in instances of exposure or for defense against melt of the K-pro carrier tissue. Daily disposable lenses, both hydrogel and SiHy, have been introduced to the market but are not indicated for therapeutic use nor for overnight wear. Figure 24.1 illustrates the use of soft lenses in the treatment of exposure keratitis.

Pure silicone lenses are used for the optical rehabilitation of aphakia in infants. These patients require a contact lens with maximal oxygen transmission to allow for extended wear of a very thick lens because of high power requirement and

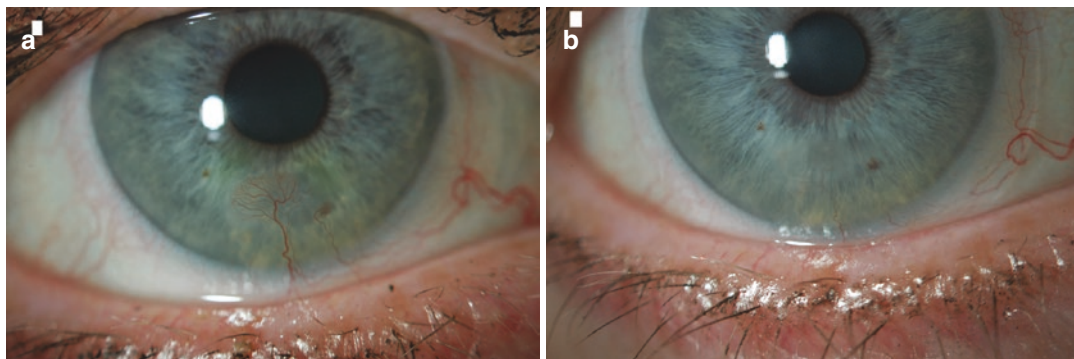


Fig. 24.1 A 65-year-old with distant history of lower lid blepharoplasty and recent inferior ulceration. **(a)** Corneal neovascularization that developed under extended wear of low Dk large-diameter hydrogel lens (Kontur) for symp-

tomatic exposure keratitis. **(b)** Regression of corneal neovascularization with switch to high Dk SiHy lens (Focus Night & Day)

somewhat rigid modulus for ease of fit and handling. The downside of pure silicone rubber lenses is their propensity to develop deposits on the lens surface which decrease their optical clarity and may reduce tolerance.

Even if the material and composition of the lens is ideal for a particular therapeutic indication, the treatment may fail due to inadequate mechanical fit of the lens to the ocular surface. The topography of the cornea and sclera are of paramount importance in guiding lens choice; the steepness of the base curvature in millimeters of the lens should be titrated to the steepness of the underlying cornea (which is routinely measured in diopters). Further, if the lens diameter is too small or if the base curve is too flat, then there may be failure to retain the lens. In situations where achieving lens retention proves difficult, such as with poor lid function or exposure, a large-diameter hydrogel lens >15 mm may be advisable and outweigh the potential to exacerbate hypoxia.

Adequate fit of a soft lens selected for extended wear can be confirmed with the following clinical findings [7]:

- Good lens centration.
- The lens should not cross the limbus with extremes of gaze or blink.
- The lens should move slightly with blink or “push-up” test (a nudge delivered through the lower lid by the examiner’s fingers, while the patient is instructed to look up).
- There should be no report of lens awareness or discomfort.
- Lens retention and absence of tight lens syndrome after 1 day of wear.

Lastly, it is important to consider the role of antibiotic prophylaxis when soft lenses are used on an extended wear basis for therapeutic or “bandage” purposes. In situations necessitating extended wear, the theoretical advantage of decreased risk of microbial keratitis must be weighed against the disadvantages related to pharmacologic toxicity of the drug and preservatives, cost, inconvenience, and selection for resistant organisms. Antibiotic prophylaxis makes

most sense when overnight wear is employed in the setting of epithelial defect or concomitant topical steroid use. In the absence of geographic epithelial defect, the theoretical advantage may not outweigh the disadvantages.

History and Principles of Scleral Lenses

In the 1980s advances in material science brought rigid gas-permeable (RGP) lenses to the market. Until that time, use of large-diameter lenses was limited by hypoxia, because the only materials available were glass or PMMA. In order to avoid epithelial hypoxia, corneal lenses had to be small and move with the blink to allow tears carrying atmospheric oxygen under the lens. Tolerance typically required a period of “adaptation” and was typically limited to 12 hours or so. Mechanical instability was a challenge for patients with the steepest or most irregular corneas; unfortunately, these were exactly the patients who could benefit most. Attempts were made to create fenestration holes for ventilation and to decrease suction in larger-diameter lenses to avoid this instability and allow access of atmospheric oxygen, but the resultant bubbles and desiccation of the ocular surface and lens limited utility. Gas-permeable polymers introduced in the 1970s allowed for enough oxygen transmission that larger diameters were better tolerated. Scleral lenses introduced in subsequent decades made of rigid gas-permeable polymers that did not require fenestration and allowed for fluid ventilation with saline or artificial tears permitted “full-time” wear and correction of very steep or highly irregular corneas [8].

Scleral lens is typically defined as an RGP lens of 17.5 mm or greater. RGP lenses with smaller diameters (13–16 mm) are sometimes classified as mini-scleral or corneoscleral lenses and tend to contact the cornea apically and/or peripherally. Mini-scleral lenses are easier to fit than their large-diameter counterparts, but the corneal contact can cause erosion, scarring, and neovascularization [9].

Achieving proper fit of an RGP scleral lens usually begins with testing various standard lenses from a trial set with attention paid to patient subjective comfort, minimal lens movement, no corneal contact, adequate reservoir depth, and alignment of the bearing haptic with the underlying sclera. Further customization may be required for satisfactory fit. These lenses are approved only for daily wear with overnight disinfection. Continuous wear with daily removal, disinfection, and reinsertion is reported for ocular surface disease that has failed other interventions [10–12].

Prosthetic Replacement of the Ocular Surface Ecosystem (PROSE) treatment was approved by the FDA in 1994 for daily wear for distortion of the cornea and for OSD and astigmatism. The treatment uses custom-manufactured and fitted highly gas-permeable prosthetic devices to replace and optimize the ocular surface. The customization of these lenses via spline functions as opposed to the traditional superposition of base curves is the uniquely defining feature of PROSE treatment and allows for infinite variation of contour in range of diameters from 17.5 to 24 mm. Best fit is achieved with a trial device that is further customized to ensure proper fluid ventilation, minimal movement, and no corneal contact. PROSE treatment supports healing, reduces pain and photophobia, neutralizes irregular astigmatism, eliminates desiccation, and protects the ocular surface from the environment and lids [10, 13, 14]. The devices used in PROSE treatment have set the bar high as far as impact in corneal ectasia [15] and impact across all diagnoses at 5 years [16]. The devices can be customized to achieve adequate fit, even in the presence of filtering blebs or tube shunts, which are relative contraindications with other lenses due to the irregular postsurgical limbal anatomy and risk of erosion or infection [17].

Areas of Impact

Reducing Need for Keratoplasty

Contact lenses have proven to be a successful intervention for severe ocular surface disease and

corneal ectasias and even delay or warrant unnecessary invasive surgeries such as penetrating keratoplasty. A common indication for penetrating keratoplasty is corneal ectasia that cannot be corrected with spectacle or contact lens. The corneal ectasias include keratoconus, pellucid marginal degeneration, post-LASIK ectasia, and keratoglobus. Typically, the vision deficits due to corneal ectasias can initially be treated with the use of spectacles. However, as ectasia progresses, irregular astigmatism may not be corrected adequately with spectacles, requiring contact lenses. At first soft lenses may be adequate, but as the ectasia progresses, the soft lens may drape over the irregular surface failing to neutralize optical irregularity, and hard lenses must be employed for satisfactory vision. If thinning and steepening (especially inferior and absolute steepening >52D) require refractive correction beyond what conventional RGP lenses can offer, specialty lenses designed specifically for ectasia should be considered. These include some RGP corneal lenses, piggyback systems, hybrid lenses, scleral lenses, and PROSE treatment.

Conventionally, contact lens failure and intolerance are accepted indications for corneal transplantation even if the cornea is clear. A longitudinal study of 1004 eyes in 518 keratoconus patients over a 30-year period found that contact lenses were a successful treatment option and postponed surgery in 99% of fittings [18]. RGP scleral lenses succeed further in a majority of patients with keratoconus who might otherwise have proceeded to surgery [19, 20].

PROSE treatment is a practical alternative to penetrating keratoplasty for eyes with advanced disease considered to be “contact lens intolerant.” A study of patients who were referred having failed contact lens treatment found mean change of -0.54 logMAR (about 5 lines improvement) in visual acuity compared to habitual correction and mean of 20 points improvement in visual function in the NEI VFQ-25 questionnaire [14]. Another study reported that 93.1% of eyes achieved a visual acuity of 20/40 or better and had a mean improvement in VFQ-25 scores of 27.6 ($P < 0.001$) on a 100-point scale with similar results for post-penetrating keratoplasty eyes. All

candidate eyes could be fit, despite including patients which had failed previous attempts at contact lens treatment using advance technologies such as specialty corneal lenses, hybrid lenses, piggyback systems, and even scleral lenses. No cone was too steep for a PROSE device [15]. Outcomes after PROSE treatment compare favorably to keratoplasty [21]. Surgeons in Belgium report similar impact on need for keratoplasty in keratoconus, with the advent of contemporary, gas-permeable scleral lens designs [22].

The advances in scleral lenses and in PROSE treatment have caused a paradigm shift in the management of corneal ectasia. Furthermore, “axial opacity” is not sufficient indication for keratoplasty unless vision has been assessed wearing a mechanically stable contact lens or PROSE device. In most situations, failure of these specialty contact lenses should be demonstrated before considering the option of penetrating keratoplasty which is not a cure and has its own set of risks and limitations.

Ocular Chronic Graft-Versus-Host Disease

Ocular chronic graft-versus-host disease (cGVHD) is a common complication of allogeneic hematopoietic stem cell transplantation. This aggressive ocular surface disease can be marked by sight-threatening processes including severe keratoconjunctivitis sicca, cicatricial conjunctivitis, corneal melt, and corneal perforation. Contact lenses have been proven to be an important therapeutic option for this chronic condition. Improvement in visual acuity and reduction in the ocular surface disease index (OSDI) score have been reported with soft and SiHy lens in studies which were also notable for lack of complications [23, 24]. Success with specialty lenses and devices such as the Jupiter scleral lens and PROSE treatment has also been reported [13, 25, 26]. Limitations of the larger-diameter specialty lenses and devices are poor cooperation in young children or others with cognitive or motor deficits and advanced cicatri-

cial symblephara which preclude proper fit and mechanical stability.

Persistent Epithelial Defect

Persistent epithelial defect is a term used when there is failure for the cornea to reepithelialize after surface trauma or breakdown. If standard measures to support ocular surface healing such as lubrication and punctal plugs are not sufficient, extended wear of SiHy lens can be considered [27]. Neovascularization is a common complication of extended soft lens wear and can especially occur if the lens is too tight or the lens Dk is too low. Extended wear of the device used in PROSE treatment with topical preservative-free antibiotics in the fluid reservoir has also been shown to be effective in treating PED [10, 28].

Exposure Keratopathy

Exposure keratopathy whether paralytic from trauma, surgery, or neurologic processes or anatomic from tumor, trauma, or surgery can be sight-threatening. Standard treatment includes aggressive lubrication regimens, eyelid weights, palpebral springs, chemodenervation causing protective ptosis, and tarsorrhaphy. Specialty contact lenses can reduce desiccation and eyelid trauma. They further have the advantage of improved visual acuity and cosmetic outcome compared to tarsorrhaphy. Recent reports demonstrate that scleral lenses can eliminate the need for tarsorrhaphy [29] and that PROSE treatment can effectively compensate for paralytic exposure due to tumor or trauma [30].

Neurotrophic Keratopathy

Neurotrophic keratopathy is typically the result of trigeminal nerve dysfunction causing impaired corneal sensation, desiccation, ulceration, and perforation. Neurotrophic corneas are at risk for infection especially if there is

epithelial breakdown. Soft contact lenses have been used as a bandage for patients with neurotrophic keratopathy and epithelial defects to support surface healing [31]. Lenses can be used in conjunction with ocular lubricants, punctal plugs, and partial tarsorrhaphy or chemodenervation with botulinum toxin injections. Scleral lens can be worn on a daily wear basis to support the epithelium and prevent breakdown, with the added benefit of maintaining or improving vision. For cases that progress to descemetocoele or small perforation, layered amniotic membrane transplantation or cyanoacrylate tissue adhesive may be used. Typically bandage soft contact lens is placed over the amnion or glue to maintain hydration and reduce any mechanical disruption by the lids. A large series reporting on the use of scleral lenses for ocular surface disease found that 9 of 10 eyes with neurotrophic cornea fit with scleral lens achieved therapeutic goals of complete reepithelialization, comfort, and protection of the ocular surface [32]. PROSE treatment has

been shown effective in the treatment of neurotrophic keratopathy [33]. Careful monitoring for infection and melt is required in patients with neurotrophic cornea. Concomitant use of topical antibiotic drops as prophylaxis against infection is warranted if a contact lens of any type is used in the presence of an epithelial defect.

Stevens-Johnson Syndrome

Therapeutic contact lenses can play a role in the management of the long-term sequelae of Stevens-Johnson syndrome (SJS) by reducing the impact of evaporation and keratinized lid margins on an ocular surface that is compromised by inflammatory insult to lacrimal glands, goblet cells, and components of the ocular adnexae. PROSE treatment results in a durable impact on quality of life in patient suffering from the chronic sequelae of SJS [34, 35]. Figure 24.2 illustrates the use of a PROSE

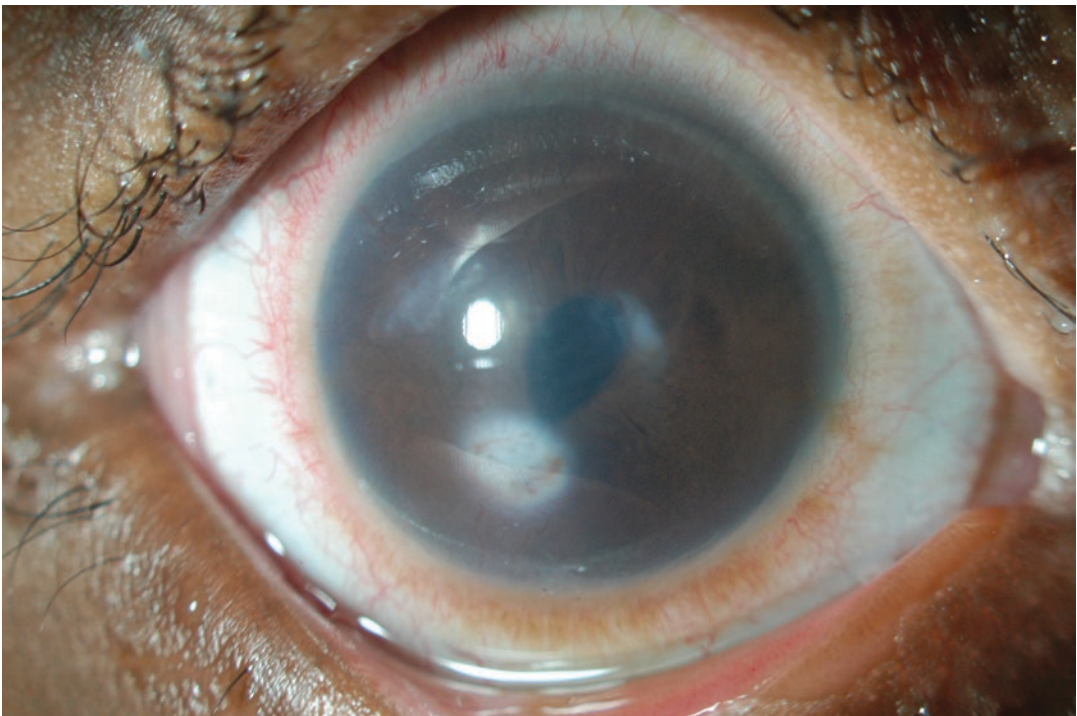


Fig. 24.2 A 12-year-old wearing PROSE device 1 year s/p SJS with history of ulceration and microperforation. Wearing of PROSE devices allowed her sufficient comfort to go outdoors and return to school

device for support of the ocular surface, and for improvement in comfort and vision, after suffering previous perforation in the setting of Stevens-Johnson syndrome.

Keratoprosthesis

The Boston Keratoprosthesis (K-pro) Type I is the most widely used artificial cornea for corneal blindness that has failed other medical and surgical interventions. The Boston KPro Type I has a front plate with optical stem, a back plate which supports donor cornea tissue, and a titanium locking ring. The K-pro and donor tissue are assembled and then the donor tissue is sutured to the host bed using standard keratoplasty technique. Carrier tissue melts, infection, and leaks characterized early experience with the Boston KPro Type I. The tendency toward these complications was substantially reduced with the addition of a bandage soft contact lens as part of the standard postoperative regimen. Large-diameter soft lenses or hybrid lenses hydrate the surface, reduce deposits at the optical axis, and reduce carrier tissue melt [36]. Contact lenses have the added benefit of refractive correction and improved comfort and cosmesis [37]. Lenses over the Boston KPro Type I do place the patient at risk for lens deposits or biofilm formation, chronic conjunctivitis, and infection [38]. The best schedule for contact lens disinfection and/or replacement in the setting of a Boston KPro Type I has not been determined. The advantage of a new or freshly disinfected lens must be weighed against the mechanical and infectious risks of manipulation of a contact lens in the presence of a stable KPro and significant underlying pathology. Continuous wear of the same contact lens for months and even years, in the absence of significant deposits, with concomitant use of antibiotic prophylaxis is a common approach.

The Boston KPro Type II device is implanted in conjunction with a complete permanent tarsorrhaphy, save for a small optical aperture which penetrates the lids; therefore, there is no role for contact lens with this device.

Conclusion

Contact lenses present an appealing and functional option for the correction of refractive error. In addition to this “cosmetic” application, contact lenses serve an important function in the management and treatment of corneal and ocular surface disease. Corneal specialists should have an appreciation of the history and role of contact lenses in the management of corneal disease. Contact lenses can improve quality of life for those with corneal disease, eliminate the need for cornea transplantation or tarsorrhaphy, and contribute to the success of the Boston KPro in the treatment of corneal blindness.

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Roberto Pineda

Dr. Claes Dohlman never veered away from a difficult corneal problem. That included those patients with severe bilateral corneal blindness not amenable to conventional keratoplasty. His research and development of the Boston keratoprosthesis as a method to treat these advanced cases of corneal blindness cannot be overstated. It was his hope that his work would help impact the burden of global corneal blindness, and it certainly has with over 1000 devices used annually, a large percentage of which are implanted internationally.

From its October 2018 key fact sheet, the World Health Organization (WHO) has estimated that 253 million people suffer from visual impairment of which 36 million are blind [1]. Eighty percent of all global visual impairment is considered avoidable with refractive error and cataracts being the leading causes. However, blindness from corneal disease is a major ophthalmic public health problem and corneal opacities are a priority eye disease for the WHO.

Corneal opacities are the fourth cause of blindness globally (5.1%) after cataract, glaucoma, and age-related macular degeneration

(AMD) according to the WHO. Trachoma affects nearly 4.9 million blind, usually due to corneal scarring and vascularization. Ocular trauma and corneal ulcerations are additional significant causes of corneal blindness. They are often underreported but are estimated at 1.5–2.0 million new cases of unilateral blindness every year [2]. Table 25.1 lists the major causes of corneal blindness [3].

A special category is corneal blindness in children. In 1997, WHO held a meeting during which the estimate of numbers of blind children was revised to 1.4 million [4]. Corneal scarring (mainly from vitamin A deficiency and measles) was one of five conditions prioritized for control [5]. Today, childhood blindness represents approximately 1.5 million cases globally with corneal blindness including xerophthalmia (350,000 cases per year), newborn conjunctivitis (affecting an est. 1.6–12% of all newborns) [6], and rarer ocular infections like herpes and keratoconjunctivitis.

Through hard work from the WHO and global community, there have been some success stories such as the control of onchocerciasis (Fig. 25.1) and leprosy, although these diseases are still significant causes of blindness, affecting approximately 250,000 individuals each. On the other hand, traditional medical eye drops have been more recently recognized and implicated as a major risk factor in the present epidemic of corneal ulcers in developing countries [2].

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Table 25.1 Major causes of corneal blindness

Infectious
Bacterial keratitis
Fungal keratitis
Viral keratitis
Trachoma
Onchocerciasis
Leprosy
Ophthalmia neonatorum
Nutritional
Vitamin A deficiency (xerophthalmia in children)
Inflammatory
Mooren's ulcer
Stevens-Johnson syndrome
Inherited
Corneal stromal dystrophies
Fuchs endothelial dystrophy
Degenerative
Keratoconus
Trauma
Corneal abrasion predisposing to microbial keratitis
Penetrating trauma
Chemical injury
Doctor-caused (iatrogenic)
Pseudophakic bullous keratopathy



Fig. 25.1 Young man from western North Sudan blind from onchocerciasis (river blindness)

It is important to recognize that the burden of corneal blindness on the individual and the wider community is enormous and often under-appreciated. Specifically, corneal blindness tends to affect younger people compared to other blinding conditions such as cataract and glaucoma. These are people whose education or work may become limited or cease completely due to vision loss. Corneal blindness also disproportionately affects poorer rural communities as well as woman and children because of the higher prevalence of diseases like trachoma and occupations such as farming with increased risk of eye injuries from contaminated objects such as plant material with limited and delayed access to treatment. This community burden of corneal blindness is not just shown by the prevalence, but because those affected are younger, there is a very high disability-adjusted life year (DALY), compared to cataract surgery [7].

Public health prevention programs are the most cost-effective means of decreasing the global burden of corneal blindness as the only effective treatment is surgery with corneal transplantation. However, conditions, such as keratoconus or hereditary corneal dystrophies, cannot be prevented and require longer-term treatment and additional resources such as corneal collagen cross-linking as well as corneal transplantation. Corneal transplantation is also considered both a high resource and high barrier solution to corneal blindness in most low- and middle-income countries due to lack of eye banks, lack of trained surgeons, and lack of access to healthcare institutions.

Even in some developed nations, the eye banking systems are often insufficient to meet patient demand. A recent survey by Gain et al. globally quantified the considerable shortage of corneal graft tissue, with only 1 cornea available for 70 needed. Their study stated that efforts to encourage cornea donation must continue in all countries, but that it was also essential to develop alternative and/or complementary solutions. A world map of the supply and demand of corneas in 148 countries highlights this point [8] (Fig. 25.2). Additionally, a recent publication in

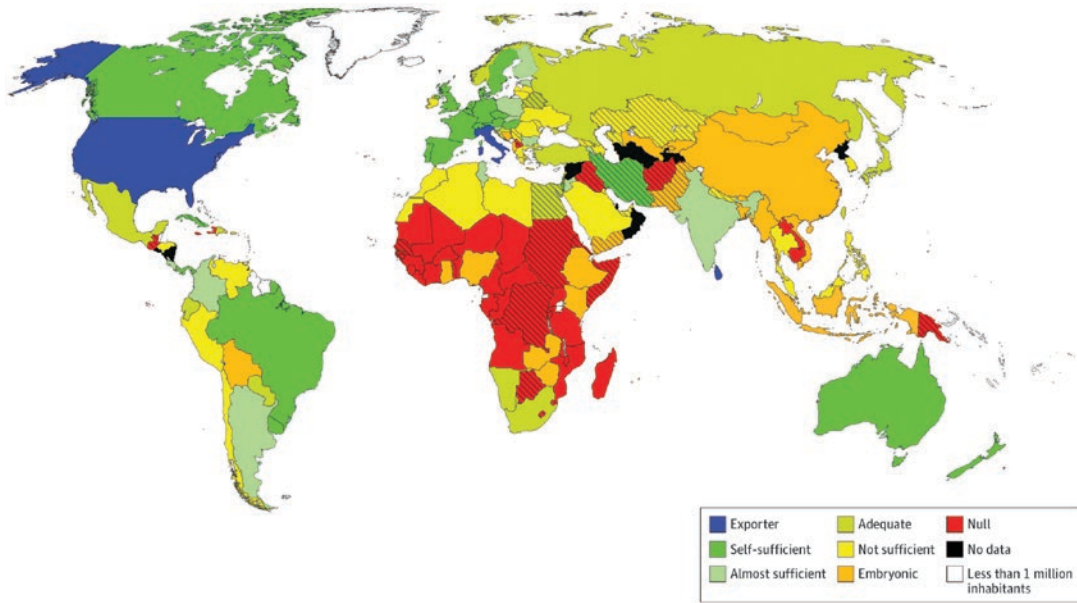


Fig. 25.2 Global map demonstrating the supply and demand of corneal transplantation across 148 countries (Reprinted with permission from Gain et al. [8])

the journal *Cornea* outlines the disparity between overall causes of corneal blindness and current practice of transplantation. Leading causes of global corneal blindness are primarily anterior in nature with healthy endothelium, but current practice indicates that endothelial keratoplasty overwhelmingly is being performed for pathology prevalent in high-income countries. This contrasts with anterior lamellar keratoplasty techniques which appears to be underutilized [9]. This makes sense as most corneal transplantation is performed in developed countries; however, a few countries such as India, Brazil, Sri Lanka, the Philippines, and Iran have developed excellent eye banking systems and are leading the way as models for low- and middle-income countries.

Numerous agencies (i.e., WHO and International Agency for the Prevention of Blindness [IAPB]) and nongovernmental organizations (NGOs), such as SightLife, SEE International, KeraLink International, and ORBIS International, are working to lessen the global burden of corneal blindness. These global organizations are working diligently to reduce the number of corneal blind affected by:

- Implementing preventative health programs
- Performing corneal grafts and transplants
- Teaching appropriate surgical techniques
- Training local eye care personnel in ophthalmology in rural and urban areas
- Strengthening local healthcare infrastructure, including encouraging the development of local eye banks

As an important cause of blindness, much effort has been targeted on controlling and eliminating trachoma, the world's leading cause of infectious blindness and a disease of poverty. Gender disparity is a defining factor of this condition with women affected 4X more frequently than men. According to the WHO data published in June 2018, 157.7 million people are at risk for trachoma with 2.8 million needing surgery to end pain and prevent further vision loss. Ethiopia is currently the most affected country (Fig. 25.3). Initiatives such as the WHO's SAFE (Surgery for trichiasis, Antibiotics for infection, Facial cleanliness, and Environmental improvements) strategy program have reduced the number of people at risk of trachoma blindness from 325 million in 2011 to 157.7 in 2018. As



Fig. 25.3 Improvised endemic trachoma area in Ethiopia

of July 2018, 38 countries are actively implementing an integrated package of interventions called the “SAFE strategy” to eliminate trachoma [10].

Forty-three countries are known to require interventions to eliminate trachoma as a public health problem. *As of September 2018, eight countries – Cambodia, Ghana, the Islamic Republic of Iran, Lao People’s Democratic Republic, Mexico, Morocco, Nepal, and Oman – had been validated by WHO as having eliminated trachoma as a public health problem, and an additional four countries have reported achievement of the elimination targets. Africa is the most severely affected, with 29 countries known to require interventions to achieve elimination, and the region where the greatest efforts to intervene are underway (Fig. 25.4) [10].*

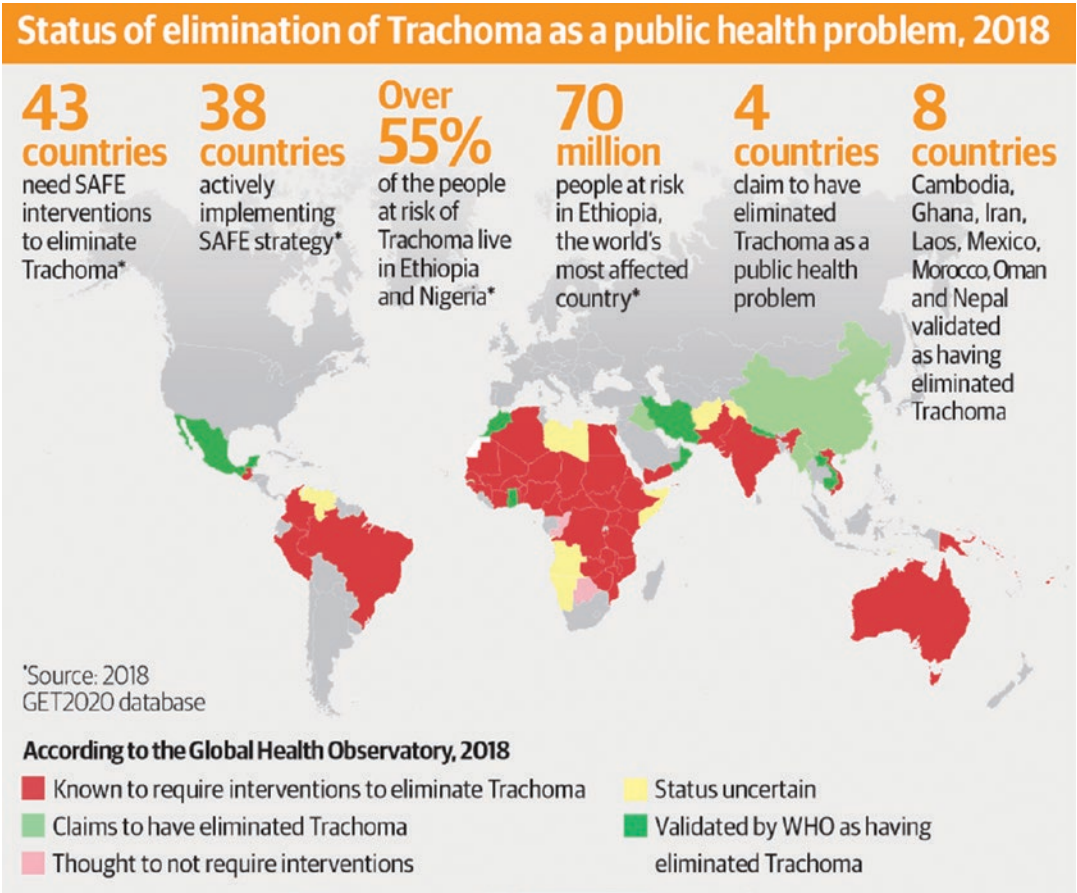


Fig. 25.4 Atlas Vision map of trachoma from the 2018 GET2020 database, accessed from the IAPB website (<http://atlas.iapb.org/vision-trends/trachoma/>)

WHO leads an international alliance of interested parties to work for the global elimination of trachoma, the *WHO Alliance for Global Elimination of Trachoma by the year 2020 (GET2020)*, established in 1996. The Alliance fosters cooperation within its worldwide partnership of governments of endemic countries, donors, nongovernmental organizations, academic institutions, and the private sector to realize the GET2020 goals.

For children, xerophthalmia, which is caused by vitamin A deficiency and sometimes precipitated by measles, accounts for more than half the new cases of childhood blindness each year (Fig. 25.5). In addition to blindness, these young children are at increased risk of death. Prevention is key: vitamin A supplementation, measles vaccination, and nutritional advice have led to a marked reduction in this condition, but it is still prevalent in areas like sub-Saharan Africa.

Microbial keratitis is responsible for many cases of corneal blindness, principally, due to delayed presentation and inadequate treatment. As mentioned previously, these cases are often not reported or included in reports particularly when they are unilateral in nature. However, new

training programs focused on educating rural healthcare providers to identify and treat these conditions may lessen the overall burden of corneal blindness as such cases are substantial.

Likewise, ocular trauma contributes considerably to global corneal blindness. Here, preventive healthcare education is the key to avoiding unnecessary blindness. Public education and government and NGO programs will be the most effective method of addressing this issue.

Advanced cases of corneal blindness are often not appropriate for standard corneal transplantation as they are high risk with nearly a 100% graft failure rate. These cases tend to occur more commonly in low-income countries with a higher percentage of corneal blindness. For these cases, a Boston keratoprosthesis may be indicated. It is currently the most common keratoprosthesis used worldwide and its effectiveness in the international ophthalmic community has been well documented [11, 12]. Although effective, like corneal tissue, the cost of the keratoprosthesis would be considered a high resource barrier for addressing corneal blindness.

As a solution to this problem, Dr. Claes Dohlman and Dr. James Chodosh have developed a low-cost keratoprosthesis called the Lucia keratoprosthesis, for approximately \$100 USD. The device is available in only one size (average axial length) and for aphakic patients only (Fig. 25.6). It was designed for low- and middle-income countries to help individuals with severe corneal blindness not eligible for standard transplantation. We have used this device with great success in patients by using their own cornea (autologous carrier), thereby making it a financially plausible option even in low- and middle-income countries (Fig. 25.7) [13].

In summary, many people still suffer from corneal blindness worldwide. Most are women and children, younger than other causes of blindness, who are of school or working age. Socially and economically, this greatly impacts their ability to lead productive lives. Dr. Dohlman recognized this years ago when he began his work on advancing technology to address the worst of the corneal blind. The



Fig. 25.5 Measles keratitis in a young girl from Ethiopia

Fig. 25.6 Current design of the Lucia keratoprosthesis

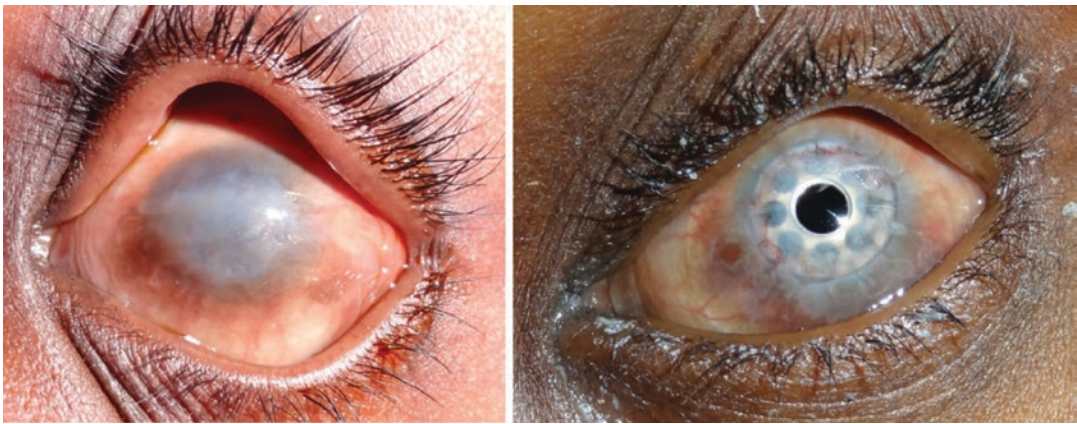
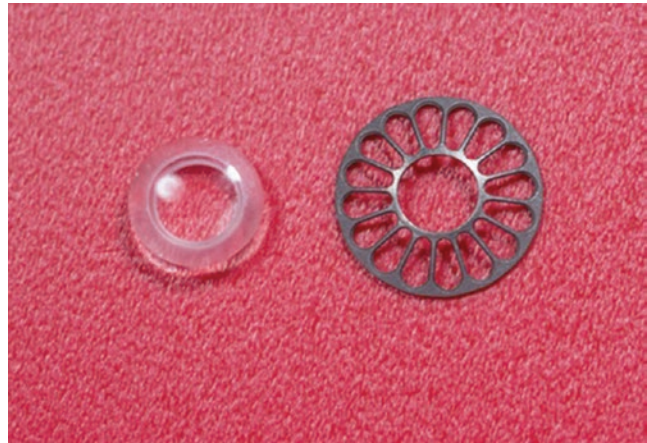


Fig. 25.7 Before and after the Lucia keratoprosthesis using the patient's own cornea as an autologous carrier

Boston keratoprosthesis has become an important stepping-stone in addressing world corneal blindness. Additionally, much successful work has been done to reduce corneal blindness thanks to the global community, coordinated by organizations like the WHO and IABP, but more work is still needed. Through organized efforts on multiple fronts including international preventative health treatment programs and eye care education, eye banking, and surgeon training, we will endeavor to eliminate global corneal blindness.

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Introduction

Epidemiology is the study of the distribution and determinants of disease and the application of this understanding to the treatment and prevention of health conditions [1]. In essence, epidemiology tells us about the “who,” “when,” “where,” and “how” of human disease. Although the spectrum of corneal disease is wide, there are many conditions that have yet to be studied in depth from an epidemiologic perspective. In this chapter we focus our efforts on diseases where epidemiology has played a significant role in furthering our understanding of the disease burden in populations, risk factors for disease occurrence or progression, natural history, and most importantly, opportunities for prevention.

First, it is important to review some basic definitions that are fundamental to epidemiologic investigations. *Prevalence* refers to the

proportion of individuals in a defined population with the condition being studied at a given time point. Prevalence may be reported as a proportion with values between 0 and 1, or as a percentage. In contrast, *incidence* refers to the occurrence of new cases in a defined population during a given time period. *Relative risk* is the incidence or rate of the condition in an exposed group to an unexposed one in a prospective or forward-looking study. *Attributable risk* (AR) is the difference in the occurrence of disease in a group that is exposed to a possible causative factor and a group that is unexposed [2]. An *odds ratio* (OR), the hallmark of retrospective or case-control studies, represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure [3]. A sample of cases (individuals with the condition of interest) is obtained and compared with a contemporaneous or matched sample of non-cases, along with exposure status in each group:

	Exposed	Not exposed
Cases	a	b
Non-cases	c	d

The odds of being exposed can be computed for cases and non-cases as a/b and c/d , respectively. The OR is then computed as $OR = (a/b)/(c/d)$.

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Dry Eye

Challenges in the Definition of Dry Eye Disease

The lack of clear and standardized disease definition and classification systems is a major challenge in the study of dry eye disease (DED). This includes the absence of a validated diagnostic test (or combination of tests) to distinguish reliably between those who do and do not have DED. Specific challenges with testing for DED include weak correlation between patient symptomatology and test outcomes, lack of test repeatability, and inherent variability in the disease process. Such classification challenges affect both epidemiologic research and clinical trials of potentially therapeutic agents.

In epidemiologic research on large populations, DED has most commonly been assessed on the basis of a variety of questionnaires that assess frequency and/or severity of symptoms and sometimes with the use of tests that are feasible to perform in the field.

Descriptive Epidemiology

Prevalence

Studies that estimate the prevalence of DED in large populations yield values that range from under 5% to over 30% – with these variations likely explained by differences in the definition of DED used and the specific characteristics of the population studied. We will outline the methodologies and findings of representative studies that look at a broad range of populations across geographic boundaries. The discussion focuses on studies that are population-based or that derive from large and likely generalizable populations.

USA

The *Salisbury Eye Evaluation (SEE)* [4] was the first large epidemiological study of DED in the USA. A questionnaire that assessed the frequency of six dry eye symptoms was administered to 2520 volunteers aged 65–84 years. These subjects represented a statistical sample from the

Medicare beneficiary rolls. Patients also underwent Schirmer and rose bengal tests with grading of corneal staining, as well as anatomic assessment of the meibomian glands. Approximately 15% of subjects reported having one or more dry eye symptoms “often” or “all the time”; 20% reported experiencing three or more symptoms “sometimes,” “often,” or “all the time.” When defined on the basis of both symptoms and signs (Schirmer test result ≤ 5 mm or high rose bengal test), there was a prevalence of 3.5%, whereas only 0.7% were both symptomatic and had both a low Schirmer score and a high rose bengal score. This study first documented the lack of correlation between signs and symptoms of dry eye in a population base.

The *Women’s Health Study (WHS)* and *Physicians’ Health Study (PHS)*, although not population-based, surveyed large populations using a validated questionnaire, the former 39,876 US women and the latter 25,444 US men. In both studies, DED was defined as the presence of clinically diagnosed dry eye syndrome (DES) or severe symptoms (both dryness and irritation either constantly or often). The studies estimated that 7.8% of women 49 years of age or older and 4.34% of men 50 years of age or older have DED in the USA. Differences by age and race will be discussed below [5].

The *Melbourne Visual Impairment Project (VIP)* and *Blue Mountains Eye Study (BMES)* were both population-based studies performed in Australia. The VIP assessed self-reported symptoms of DED by an interviewer-administered questionnaire, as well as four tests of DED. Among the 926 participants, the proportion with DED according to each measure was as follows: 16.3% by Schirmer test, 10.8% by rose bengal staining, 8.6% by tear breakup time (TBUT), 1.5% by fluorescein staining, 7.4% with two or more signs, and 5.5% with any severe symptom [6]. In the BMES, 1174 participants aged 50 or older completed a comprehensive eye examination and dry eye questionnaire. In total, 57.5% of participants reported at least one dry eye symptom, with 16.6% reporting moderate to severe symptoms and 15.3% reporting three or more symptoms [7].

The *Beaver Dam Offspring Study (BOSS)* was a cohort study of aging participants 21–84 years old. Participants were asked: “How often do you have dry eyes, a dry, gritty, or burning feeling?”, “How much does the dryness in your eyes bother you?”, “Is there a season of the year when the dryness in your eyes is the worst?”, and “Are you currently using eye drops at least once a day for dry eyes?” Participants who reported symptoms were present sometimes or more often and they were moderately bothersome or greater, or those who reported currently using eye drops at least once a day for dry eyes were considered to be cases. Questionnaire data on health history, medication use, risk factors, and quality of life were available for 3275 participants. The prevalence of dry eye in the BOSS was 14.5%. Statistically significant associations were found with female sex (OR 1.68; 95% CI 1.33–2.11), contact lens use (OR 2.01; 95% CI 1.53–2.64), allergies (OR 1.59; 95% CI 1.22–2.08), arthritis (OR 1.44; 95% CI 1.12–1.85), thyroid disease (OR 1.43; 95% CI 1.02–1.99), antihistamine use (OR 1.54; 95% CI 1.18–2.02), and steroid use (OR 1.54; 95% CI 1.16–2.06) [8].

International Studies

A population-based study undertaken in *Indonesia* [9] employed the same questionnaire developed and used in the SEE that assessed the presence of six DED symptoms: dryness, grittiness or sandiness, burning, redness, crusting, and eyes stuck shut. Demographic, lifestyle, and medical data were collected from 1058 participants aged 21 years or over spread across five rural villages and one provincial town of the Riau province, Indonesia. The prevalence of DED, defined as the presence of one or more symptoms often or all the time, was found to be 27.5%.

The same questionnaire, as well as examination of TBUT, Schirmer test, fluorescein stain of the cornea, and the meibomian glands, was used in a population-based study in *Taiwan* [10]. Of the 1361 participants, 33.7% reported having one or more symptoms of DED often or all of the time. The proportion of patients who were both symptomatic and exhibited signs of DED was higher than in the SEE: 21.1% had Schirmer

value of ≤ 5 mm (vs 2.2% in the SEE), and 20.8% exhibited meibomian gland disease (versus 0.85% in the SEE).

In a population-based study conducted in *Japan*, a 30-item self-administered questionnaire sent to 2500 randomly selected adults 20–49 years old was completed by 598 individuals. Approximately 33% of respondents endorsed symptoms of DED [11]. Participants were asked to perform a self-diagnosis based on an explanation of symptoms associated with DED, such as pain, asthenopia, stinging, and general discomfort; the study did not report whether individuals were asked to base their diagnosis on the number of symptoms experienced or their frequency. The prevalence of DED among Japanese high school students (83% male) aged 15 to 18 was also assessed in a separate study conducted at 8 private high schools in Tokyo district, Japan. The same three-item questionnaire used in the WHS and PHS was translated into Japanese and sent via mail to all 3433 students, with 99.7% response rate. Severe symptoms of DED were reported by 21% of boys and 24.4% of girls. Clinically diagnosed DED was reported by 4.3% of boys and 8.0% of girls in the study [12].

Differences by Age and Sex

Age

Meta-analysis of existing prevalence data was recently conducted by the Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) Epidemiology subcommittee to determine the prevalence of DED stratified by age [13]. Only population-based prevalence studies (24 in total) conducted since 1980 that provided rates within decade or finer age categories were included. Data were separated into subgroups based on broadly similar diagnostic criteria: symptomatic disease, WHS criteria, self-report of a prior diagnosis, and clinical signs. The percentage change in prevalence with each decade of increasing age was calculated and reported as the slope estimate. The prevalence of DED as defined by symptoms (3.43% increase per decade) and clinical diagnosis (2.01% increase per decade) showed only modest increase below

age 49, followed by a gradual increase from age 50 and more marked increase beyond age 80. Analysis of prevalence per WHS criteria showed no significant difference across age categories. Within analysis of prevalence as defined by disease signs, TBUT, Schirmer testing, and fluorescein testing showed the greatest changes in prevalence with age, with prevalence of MGD increasing at 5.23% per decade.

Sex

The WHS and PHS, two of the largest parallel studies in the USA examining the health status of over 39,000 women and 25,000 men, respectively, showed a statistically significant age-adjusted 70% greater risk of DED for women (4.34% prevalence in men versus 7.8% prevalence in women) [14–16]. The BDES similarly revealed that the age-adjusted prevalence was approximately 50% higher in women (16.7% among women versus 11.4% among men). The BOSS included younger adults (mean age 49 years versus mean age 65 in the BDES) and also showed significantly higher prevalence of DED in women (17.9%) compared to men (10.5%) [17].

While the preponderance of population-based studies has shown a higher prevalence of DED in women, several have shown no significant differences. For example, SEE ($n = 2400$ US adults age 65 or older) showed that the prevalence of one or more DED symptoms at least “often” was not significantly different at 15.6% among women versus 13.3% among men.

Similarly, most but not all of the epidemiological studies examining DED in Asia have demonstrated a higher prevalence of DED among women. Of the 17 larger epidemiological studies in Asian countries, 11 showed a higher prevalence of symptomatic DED among women than men (ranging from 16% higher to nearly three-fold higher), 2 studies showed no difference in DED prevalence, and 2 studies showed a 43%–67% higher risk among males [14].

Incidence

Data on the incidence of DED is limited. In the BDES, cumulative incidence of DED was based

on subjects’ response to the question “For the past three months or longer, have you had dry eyes?” The study demonstrated that in a Caucasian population aged approximately 50–90, 13.3% (95% CI 12.0–14.7%) developed symptomatic DED over 5 years, and 21.6% (95% CI 19.9–23.3%) over 10 years [18]. The completion of a dry eye questionnaire by participants (women aged 20–87) in the Twins UK study 2 years apart allowed for the estimation of the incidence of DED according to both WHS and BDES criteria: 4.4% (95% CI 3.5–5.3%) and 10.4% (95% CI 9.1–11.7%), respectively, during a 2-year period [19].

Risk Factors

There are a variety of risk factors for DED in specific groups that are recognized by corneal specialists. These include contact lens wear, refractive surgery, diabetes, and a host of conditions that are known to affect the ocular surface directly. However, while these patient subsets are heavily represented in the practices of corneal specialists, they are relatively rare in large, population-based studies.

Population-based studies that include young adults have found that DED is between approximately 2 [20] and 4 [21] times more prevalent among *contact lens* (CL) wearers. Among Japanese high school students, CL wear was associated with a higher prevalence of severe DE symptoms [22].

It is well known that patients can experience significant DE symptoms for several months, or even years after corneal refractive surgery such as laser in situ keratomileusis (LASIK) or photorefractive keratectomy (PRK), likely due to the severing of corneal nerves during the procedure [23]. One study showed that chronic DED after LASIK was significantly associated with female gender, greater ablation depth, and dry eye symptoms before LASIK [24]. The prevalence of DED appears to be highest immediately after refractive surgery, with rates ranging from 0.25% [25] to 48% [26]. However, Schirmer I test values tend to return to baseline by one year postoperatively [27].

Several studies have shown an association between *systemic medications* that may have a dehydrating effect and symptoms of dry eye after controlling for age, sex, and other known risk factors. For example, the PHS found a significant association (OR 1.28, 95% CI 1.12–1.46) between the use of antihypertensives and risk of DED. Conversely, the BDS reported decreased incidence of DED in patients using angiotensin-converting enzyme (ACE) inhibitors, a borderline increased incidence of DED with diuretic use, and no association of DED incidence with calcium channel blocker use [28]. Both the SEE [29] and BDS [30] reported an increased risk of DED with antihistamine use (OR 1.67 and 1.46, respectively). Antidepressant use has also been found to be positively correlated with DED in a number of population-based studies, including SEE, BDS, PHS, and Blue Mountains Eye Study. An increased risk of DED with the use of estrogens was found in the WHS, Shihpai study, and Blue Mountains Eye Study. In the WHS, the risk of DED increased by about 30% among those who had used estrogen combined with progesterone and by about 70% in those who had used estrogen alone. Prospective analysis showed that the diagnosis of DED followed the initiation of estrogen use. Moreover, each additional 3 years of hormone use increased the risk of DED by 15% [31]. In the Shihpai and Blue Mountains Eye studies, estrogen therapy increased the risk of DED by 28% and 70%, respectively.

While *diabetes* has been hypothesized to be an important risk factor for DED, most population-based studies have not found a strong association between the two conditions. For example, while the BDES showed increased risk of DED in diabetics (OR 1.38), the confidence interval includes the null hypothesis (95% CI 0.88–1.99), and the Salnes study and PHS did not find significant association. Given DED may be a consequence of neuropathy resulting in reduced corneal sensitivity, DED might only develop in the setting of inadequate glucose control. Accordingly, DED has been seen more frequently (prevalence 59.3% versus 42.4% in diabetic patients with and without diabetic retinopathy, $P = 0.02$) in patients with

diabetic retinopathy, a reflection of poor glucose control, as well as correlated with the duration of diabetes [32]. It has been suggested that studies that use self-reported symptoms as an outcome measure may underestimate the prevalence of DED in this population given neuropathy is a potential underlying mechanism [13].

Systemic autoimmune conditions, including Sjogren's syndrome (SS), rheumatoid arthritis, lupus erythematosus, and scleroderma, are known to be associated with dry eye [33]. From the perspective of a corneal specialist, the dry eye associated with such conditions is often severe and a management challenge. Severe dry eye in the setting of such diseases may well have unique risk factors. Unfortunately, and entirely for reasons relating to feasibility, insight into rates and risk factors has been largely limited to information gathered from patients followed in subspecialty clinics. However, in population-based studies of dry eye, autoimmune diseases or their serologic markers have not been associated with DED [34].

Trachoma

Clinical Description

Trachoma is caused by ocular infection with *Chlamydia trachomatis*, an obligate intracellular Gram-negative bacterium. Trachoma remains the most common cause of infectious blindness worldwide [35]. Serotypes A, B, Ba, and C are responsible for endemic trachoma. While serotypes D to K are associated with genital infections, they can also infect the eye, leading to ophthalmia neonatorum in infants or inclusion conjunctivitis in adults. In trachoma-endemic communities, where trachoma is a chronic disease, the "pool" of active inflammatory disease resides in the children [2]. Repeated episodes of reinfection and inflammation lead to serious ocular complications, including atrophy of the meibomian orifices and lacrimal ducts, entropion, trichiasis, and progressive corneal scarring and blindness.

Table 26.1 WHO simplified trachoma grading system

Grade	Clinical signs
Trachomatous inflammation follicular (TF)	Five or more follicles of >0.5 mm on upper tarsal conjunctiva
Trachomatous inflammation intense (TI)	Inflammatory thickening obscuring more than half the normal deep tarsal vessels
Trachomatous conjunctival scarring (TS)	The presence of easily visible scars in the tarsal conjunctiva
Trachomatous trichiasis (TT)	At least one eyelash rubbing on the eyeball or evidence of recent removal of in-turned eyelashes
Corneal opacity (CO)	Corneal opacity blurring part of pupil margin

In 1987, the World Health Organization published a simplified grading system to assess disease severity within a population based on the presence or absence of five clinical signs of the disease, each scored independently (Table 26.1) [36].

It is important to note, however, that the WHO grading scheme was not developed to predict evidence of infection, as the predictive probability of a positive sign or negative sign at the individual level is poor [37]. Rather, the system was developed to assess disease on a population level in a reliable manner that would be straightforward to teach to eye nurses and other health workers.

Epidemiology

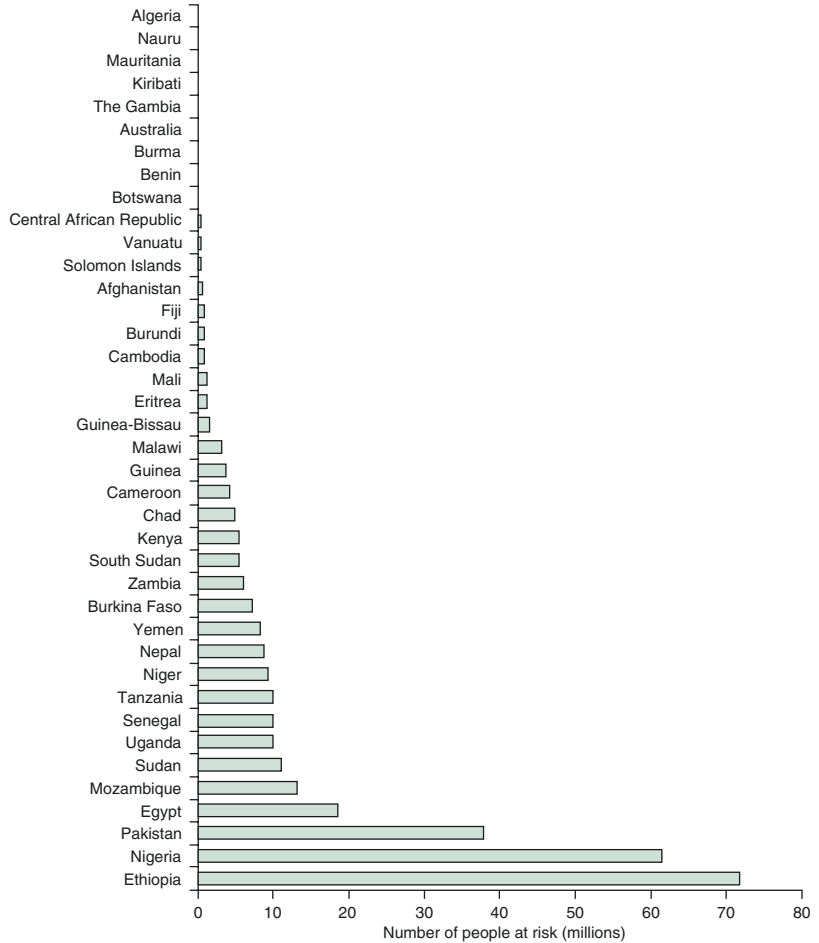
Trachoma is a powerful example of how epidemiologic studies and reasoning have the potential to effect directly the strategies for disease prevention and control. Historically, trachoma was endemic worldwide, including in the USA and UK, as well as other developed regions. In fact, prominent eye hospitals such as the Massachusetts Eye and Ear Infirmary and Moorefield's Hospital in London were established with a primary mission to treat the disease. However, trachoma rates in the developed world waned as living standards improved in the early twentieth century [38]. The disappearance of trachoma from more developed countries was

hastened by general improvements in sanitation and basic public health measures and with the introduction of sulfa drugs in the 1930s and more potent antibiotics in the 1940s [39]. However, trachoma continued to be a major scourge throughout much of the developing world with treatment limited through much of the second half of the twentieth century to topical tetracycline and with limited success of this treatment due to the challenges of sustained application and inadequate basic epidemiology. Renewed efforts at trachoma control beginning in 1999 led to significant declines in disease burden in various regions, including the Middle East, India, Latin America, and Asia. However, trachoma continues to be an endemic disease and major cause of ocular morbidity in sub-Saharan Africa, the Pacific Islands, and Aboriginal communities in Australia [2]. Worldwide, an estimated 21 million people suffer from active trachoma, with approximately 2.2 million blind or severely visually impaired as a result [40]. However, such estimates need to be approached cautiously, as many prevalence studies have been carried out in areas of countries known to be at high risk, and extrapolation to the rest of the country or region is difficult [39]. The figure below (currently labeled Fig. 26.1) shows the population at risk in trachoma-endemic countries, compiled by the International Coalition for Trachoma Control [41].

At the beginning of 2012, trachoma control activities, which we will discuss below, only covered approximately 50% of areas deemed to be at high risk. The major barrier to expansion of these activities was a lack of reliable region-specific epidemiology to prioritize interventions and their intensity [42]. Therefore, in July 2012, Sightsavers, the International Trachoma Initiative (ITI), and the London School of Hygiene & Tropical Medicine formed the Global Trachoma Mapping Project (GTMP). Following an intensive planning and piloting phase, mapping commenced in the Oromia Region of Ethiopia in December 2012, soon followed by projects in other high-risk countries [43].

Mapping for the GTMP was conducted through house-to-house cluster random sample

Fig. 26.1 Estimates of the population at risk of trachoma by country. Brazil (1.3 million), China (455 million), and India (425 million) have been excluded from the figure



surveys in evaluation units of generally 100,000–250,000 people in size. All residents aged 1 year and older in each cluster were examined for clinical signs of trachoma. In addition to individual-level demographic and clinical data, household-level water, sanitation, and hygiene data were collected via a purpose-built application on smartphones, transmitted wirelessly, analyzed, and local ministry-of-health-approved via a secure web-based portal. The main outcome measures were the unit-level prevalence of follicular trachoma (TF) in children aged 1–9 years, prevalence of trachomatous trichiasis (TT) in adults aged 15 and over years, the percentage of households using safe methods for disposal of human feces, and the percentage of households with proximate access to water for personal hygiene purposes [44].

Between December 2012 and January 2016, the GTMP examined approximately 2.6 million people in 1546 districts across 29 countries (Table 26.2 and Fig. 26.2) [45]. The GTMP identified that approximately 62 million people globally live in areas in which the TF prevalence in children is $\geq 10\%$, or approximately 28% of the global population surveyed [46]. As discussed in more detail in the section below, Management and Prevention, WHO guidelines [47] recommend that SAFE (Surgery for advanced disease, Antibiotics to clear *C. trachomatis* infection, Facial cleanliness, and Environmental improvement) interventions be undertaken for at least 3 years before review where the TF prevalence in children is $\geq 10\%$. It has been forecast that 100 million people globally will need to receive antibiotic mass drug administration according to the data gathered through the GTMP.

Table 26.2 Total countries and districts mapped under the GTMP

Region	Countries (mapped using GTMP methods)	Specific countries	Districts (mapped for baseline prevalence)
Sub-Saharan Africa	16	Benin, Chad, Cote d'Ivoire, Democratic Republic of Congo, Eritrea, Ethiopia, Guinea, Malawi, Mozambique, Nigeria, Republic of Congo, Senegal, Tanzania, Uganda, Zambia, Zimbabwe	1146
Middle East and North Africa	3	Egypt, Sudan, Yemen	213
Asia and Pacific	8	Cambodia, Fiji, Kiribati, Laos, Pakistan, Papua New Guinea, Solomon Islands, Vanuatu	183
The Americas	2	Mexico (Central America), Columbia South America	4
Total	29		1546

In certain regions, the GTMP confirmed suspicions of endemic trachoma, while in others, it revealed lower levels of trachoma prevalence than originally estimated. For example, survey results in Oromia, Ethiopia – the first region studied as part of the GTMP – confirmed that overall age-adjusted TF prevalence in 1–9-year-olds was high, at 23.4% [48], with similar findings in the Tigray (26.1%) [49] and Southern Nations, Nationalities, and Peoples' Region (25.9%) [50] of Ethiopia. On the other hand, in some areas of Mozambique, the GTMP determined the prevalence was less than 5% rather than 35% as originally thought, allowing mass drug administration to be stopped [51].

Risk Factors

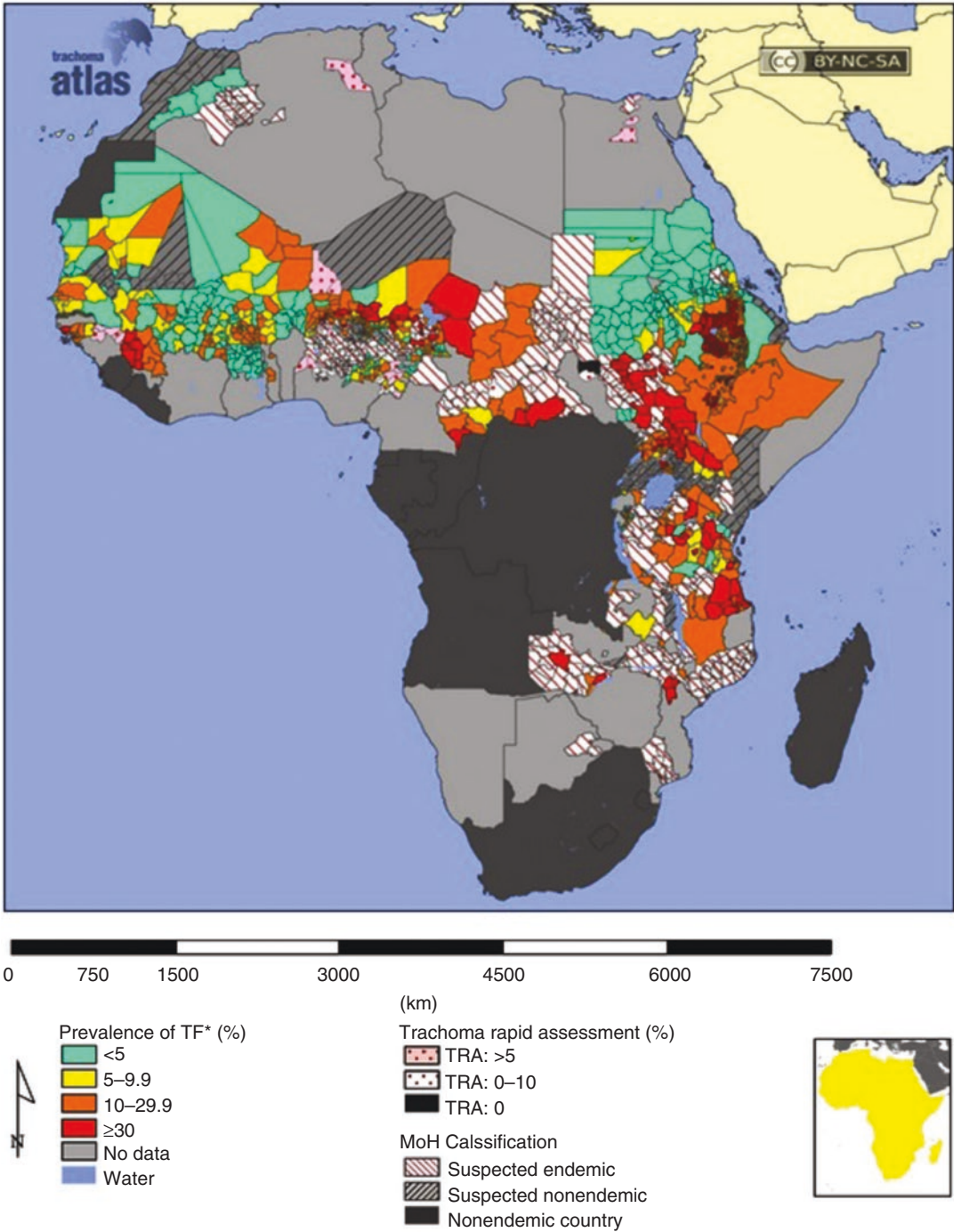
Trachoma remains endemic in regions where the *C. trachomatis* pathogen persists and where environmental conditions enable its ongoing transmission. Below we discuss the key factors that have been found to contribute to trachoma risk and that control strategies have been designed to address.

Nutrition

While acute malnutrition has not been found to be associated with trachoma risk or severity [52], an observational study in Ethiopia found that children with signs of chronic malnutrition (e.g., low height for age and low weight for age) were at greater risk. Stunted children, defined as having height for age more than 2 standard deviations below the mean, were nearly twice (OR 1.96) as likely to have active trachoma as non-stunted children (95% CI: 1.12–3.43), even after controlling for age, gender, and infection status of other household members. The study found no association between markers of acute malnutrition and infection status [53].

Personal Hygiene

Several studies have linked poor hygienic conditions with higher risk of trachoma, presumably due to a higher likelihood of contact with ocular and other secretions that are potential sources of infection. A study conducted in a hyperendemic area of Central Tanzania in which 527 preschool-aged children were examined for specific signs of unclean faces and presence of trachoma determined the specific elements of an unclean face that were related to risk of trachoma. The odds of trachoma were 70% higher in children with flies and nasal discharge on their faces, while dust and food on the face did not confer additional risk. In families, the odds of trachoma increased 4.8-fold if a sibling had trachoma and 6.8-fold if a sibling had trachoma and an unclean face [54]. A randomized community-based intervention trial conducted in Tanzania following mass topical antibiotic treatment in children showed that children who kept their faces clean were about half as



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Fig. 26.2 Prevalence of trachoma TF in districts mapped during the GTMP in Africa [164]. Creative Commons. (Figure courtesy of Trachoma Atlas)

likely to have trachoma at the end of the 1-year follow-up period. They were also a third as likely to have severe trachoma as children who did not have clean faces [55].

Water

Multiple studies have found a correlation between prevalence of active trachoma and distance to the nearest water source [56]. In a study in Tanzania, active trachoma prevalence was substantially lower in children from households where more water was used for personal hygiene, independent of the total amount of water used. Additionally, the proportion of water allocated to hygiene was predicted by lower water collection time [57]. However, the provision of water to communities does not alone ensure that infection or active trachoma rates decline [58]. As an epidemiological survey carried out in the Dodoma region of Tanzania found, community- and household-level decision-making surrounding use of water for hygiene is complex and an important factor in how much of an impact improved access to clean water has on the trachoma risk of a community [59].

Flies and Latrines

Although the presence of *C. trachomatis* has been demonstrated in flies caught in trachoma-endemic regions [60], it is unclear if they are able to transmit the infection directly. Clinical trials regarding the impact of fly control on trachoma levels have produced differing results. In the Gambia, a cluster randomized trial in which one arm received 6 months of spraying with permethrin to control flies resulted in significantly fewer fly-eye contacts in intervention villages and significant reduction in trachoma, from 7.18% to 3.69% [61]. When the same fly control methodology was employed in a clinical trial in Tanzania, there was no significant difference in the levels of trachoma between the control and intervention communities. The authors postulated that in regions hyperendemic for trachoma in Tanzania, multiple routes of transmission may have been in effect. Therefore, curbing one route of transmission would have no impact, whereas the converse

might be true in an area where flies are responsible for the majority of transmission.

The presence of a functional latrine near the house has been associated with lower trachoma prevalence [62]. The reason for this finding is unclear, but several have been hypothesized that latrines may reduce breeding sites for flies [56], or may be a marker for higher socioeconomic status and therefore the means to achieve better hygiene [63].

Management and Prevention

Given trachoma's absence in developed countries, including little trachoma by the 1930s in the USA, it was largely forgotten as a public health concern until renewed focus by the World Health Organization in the late 1990s. A resolution of the World Health Assembly in 1997 established the Global Alliance for the Elimination of Blinding Trachoma by the year 2020 (GET 2020) [64]. The following year, a World Health Assembly resolution called for trachoma elimination using a combined health and development approach to address both the risk factors and sequelae of the disease, termed *SAFE*: Surgical treatment, Antibiotic treatment for acute infection, Face washing, and Environmental changes to improve sanitation [65].

Surgery

Up until several years ago, the WHO had recommended the bilamellar tarsal rotation procedure (BLTR) for the correction of trachomatous trichiasis based on several studies [66, 67], including a randomized controlled trial ($n = 384$) conducted in Oman [68] which demonstrated that the procedure was superior to other treatment options. Tarsal rotation was successful in 80% of cases of minor trichiasis (five or fewer lashes), compared with success rates of 29% for electrolysis and 18% for cryoablation. However, the most commonly used alternative procedure, the posterior lamellar tarsal rotation (PLTR), was not included in these earlier trials. More recently, a randomized controlled trial in Ethiopia comparing BLTR

and PLTR demonstrated that the cumulative rate of recurrent trichiasis by 12 months was more frequent in the BLTR group than in the PLTR group (22% versus 13%; OR 1.96 [95% CI 1.40–2.75]; $p = 0.0001$) [69]. The WHO currently includes both the BLTR and PLTR in its recommendations regarding trichiasis surgery for trachoma [70]. The preoperative severity of trichiasis [71] and variability among surgeons [72] have been found to be the main risk factors for recurrence after surgery, although standardized training has been found to mitigate the latter [73]. Even when surgical services are available, presentation for care by afflicted subjects has found to be as low as 10% [74]. The main barriers have been found to be perceived costs and difficulty with transportation. Therefore, significantly higher utilization has been achieved with local village-based surgery compared to health-center-based [75].

Antibiotic Treatment

To break the cycle of transmission of infection in endemic communities, the WHO recommends antibiotic treatment with oral azithromycin (20 mg/kg up to 1 g) or topical tetracycline. The former is preferred given the ease of compliance with a single oral dose versus topical application for 6 weeks with the latter [76]. Mass treatment of the entire village (MDA; mass drug administration) is recommended when the prevalence of TF is greater than 10% in children under 10 years, while targeted treatment of only affected families is recommended when the prevalence is between 5% and 10% [77]. However, mass treatment has been found to be more cost-effective than targeted treatment, given the labor and material cost of ensuring that all members of a household are free of disease before deciding not to treat [78]. The WHO recommends that for TF prevalence greater than 10%, mass distribution should continue for 3 years before prevalence is reassessed [79]. This recommendation is supported by a randomized controlled trial in Tanzania in which prevalence did not drop below 5% before the third round of annual mass treatment in any of the communities studied [80]. However, a more recently conducted population-

based survey in Malwai demonstrated that in districts with TF prevalence of 5.0–9.9%, one round of azithromycin MDA led to a reduction in TF prevalence to <5% [81]. While further evidence for this approach needs to be collected, it is plausible that it could lead to a change in WHO policy to improve the cost-effectiveness and practicality of trachoma treatment. Azithromycin is expensive, and mass distribution is only cost-effective at present due to Pfizer's continuing large-scale donation program [82].

Face Washing and Environmental Change

Facial cleanliness and environmental behavior changes address the root causes of trachoma and are a critical component of the SAFE strategy [2]. Despite acknowledged heterogeneity across studies and a lack of standard definitions, a systematic review and meta-analysis demonstrated that having a clean face was significantly associated with reduced odds of TF and TI trachoma (OR 0.42, 95% CI 0.32–0.52), as was face washing at least once daily (OR 0.76, 95% CI 0.57–0.96) [83]. In a cross-sectional survey study in Sudan, the greatest decrease in trachoma was in areas with good uptake of both antibiotics and children's facial cleanliness [84]. In one of the few randomized studies assessing this prevention and treatment modality conducted in Tanzania, the odds of having severe trachoma in villages where face-washing programs were implemented were 0.62 (95% CI 0.40–0.97) compared with control villages [55].

Impact of the SAFE Program

Scaling up of the SAFE program across the globe has resulted in a significant reduction in trachoma prevalence, with the number of people at risk dropping from 325 million in 2011 to 182 million in 2016. Eyelid surgery for trachomatous trichiasis grew from 140,000 to 260,000 in 2016 [85]. In 2016, more than 190 million people lived in areas needing interventions; 85 million of them were treated by azithromycin (44.7% coverage), compared with 52 million in 2014 when an estimated 204 million people required treatment (25.4%) [86]. As of September 2018, the WHO has vali-

dated eight countries as having eliminated trachoma as a public health problem: Cambodia, Ghana, Lao People's Democratic Republic, Mexico, Morocco, Nepal, Oman, and Iran [87]. Morocco has implemented the SAFE strategy since 1997, although its efforts to curb trachoma date back to 1953, when the Ministry of Health together with the WHO and the UN Children's Fund trained medical and nursing staff at Morocco's National Ophthalmology Center to serve the poorest and most disadvantaged populations with trachoma. In the 1990s, a high prevalence of the disease in regions of the southeast led Morocco to mobilize relevant sectors such as health, education, and water sanitation to ensure implementation of the WHO-endorsed SAFE strategy [88]. By the end of 2005, Morocco had achieved the epidemiological end points defined by WHO for elimination of trachoma as a public health problem, which include [89]:

- (i) A prevalence of trachomatous trichiasis of less than 0.2% in adults aged over 15 years (approximately 1 case per 1000 people)
- (ii) A prevalence of less than 5% of trachomatous inflammation follicular in children aged 1–9 years

Nepal is a more recent example of a country that has successfully combatted trachoma and has been working toward the elimination of trachoma and other forms of preventable blindness since 1979, when it began the world's first national epidemiological survey on prevalence and causes of blindness [90]. Nepal instituted its National Trachoma Program in 2002, implementing the SAFE program in all 20 of its endemic districts. As a result, the prevalence of active (inflammatory) trachoma fell by 40% between 2002 and 2005. Intervention was multifactorial and included incentives to local communities to build and maintain latrines to improve sanitation and collaboration with the Ministry of Education to include a module on trachoma and the importance of hygiene in the school curriculum. Approximately 30,000 operations were provided to manage trichiasis, and almost 15 million doses

of azithromycin were distributed. Surveys conducted between 2005 and 2015 showed that rates of active trachoma in children had been lowered to below the elimination prevalence threshold, and these levels were maintained after mass antibiotic treatment was discontinued [91].

Ulcerative Microbial Keratitis

Clinical Description

Ulcerative microbial keratitis refers to disruption of the corneal epithelium with associated suppuration of the underlying corneal stroma and inflammatory signs such conjunctival and scleral injection, anterior chamber reaction, or hypopyon in the setting of proven or presumed microbial infection. There may be corneal necrosis and, in the most extreme cases, corneal perforation and endophthalmitis. The most common causative pathogens are bacterial, viral, and fungal, although free-living organisms such as *Acanthamoeba* can also cause corneal ulceration. Herpetic corneal infections will be discussed elsewhere in this book.

Descriptive Epidemiology

In developed country settings, the rapid rise in prevalence of contact lens wear starting in the last 25 years of the twentieth century caused a significant shift in the distribution of the most common pathogens responsible for corneal ulceration. While *Streptococcus pneumoniae* was previously the most common cause of microbial keratitis [92], the increasing popularity of soft contact lenses was associated with a shift to *Pseudomonas aeruginosa* as the most common cause of corneal ulceration in North America and elsewhere in the developed world by the 1980s [93]. We will discuss the incidence, principal pathogens by region, and the key studies demonstrating these trends. Studies that specifically examine contact lens-related keratitis will be discussed under the section titled "Risk Factors."

Incidence

In considering estimates of the incidence of microbial keratitis, it is important to understand that variation in disease definition can have a significant effect on the estimation. For example, some studies used clinical definitions alone or billing records for case ascertainment, while others required the acquisition of cultures or even the requirement of a positive culture.

The Olmsted County Study [94], the first population-based study to examine the incidence of corneal ulceration in the USA, showed that incidence rates of ulcerative keratitis had increased from 2.5 per 100,000 in the 1950s to 11.0 per 100,000 people in the 1980s. More recently, and in a more heterogeneous population, the incidence of ulcerative keratitis in Northern California was estimated to be 27.6 per 100,000 (95% confidence interval, 24.6–30.9). Although the two populations and studies are not directly comparable, it is likely that the nearly twofold greater incidence reported in the later study reflects an increased prevalence of contact lens wear over the intervening time period.

In Scotland, a prospective, multicenter surveillance study was conducted in a population of approximately three million which counted all patients presenting with suspected microbial keratitis during the 8-month study period who had positive corneal cultures. In total, 52 patients presented with microbial keratitis, yielding an incidence of approximately 2.6 per 100,000, in what was the first prospective incidence study of culture-proven microbial keratitis in Europe [95].

In Hong Kong, a prospective population-based study of microbial keratitis incidence was conducted between April 1997 and August 1998 by surveilling cases at the only two eye centers serving the Kowloon and New Territory East districts, respectively, and by sending questionnaires to the 27 private ophthalmologists in these districts during the study period. A total of 223 cases, representing an incidence of 10.9 per 100,000, were identified during the study period [96].

In contrast, incidence studies from Southeast Asia have found much higher rates. Gonzalez et al. conducted the first population-based study

in India [97]. Based on retrospective review of all ophthalmologists in Madurai who kept medical records, the annual incidence of microbial corneal ulceration was estimated at 34 per 100,000. After making assumptions about the number of cases also seen by local ophthalmologists without medical record documentation, the incidence was estimated to be 113 per 100,000, or approximately ten times the incidence reported in the Olmsted County Study in the USA. In Bhutan, a survey conducted by the World Health Organization estimated the annual incidence of corneal ulceration to be approximately 339 per 100,000 [98], although details regarding survey methodology were not provided. A similar rate, approximately 306 per 100,000, was also reported in a prospective population-based study that used volunteer village health workers to help track the rate of corneal ulcers occurring in two districts in Bhutan [99] that encompassed a total population of 32,001. In Nepal, The Bhaktapur Eye Study was a 24-month prospective population-based study that employed 81 primary eye care workers to monitor the ocular health of 34,902 individuals living in the southern half of the Bhaktapur district, a rural community east of Kathmandu. Primary eye care workers referred all cases of potential keratitis to one of the three local secondary eye study centers in Bhaktapur for examination, treatment, and follow-up by an ophthalmologist. Approximately 558 cases of corneal ulceration were reported, an annual incidence of approximately 799 per 100,000 [100]. While the rates cannot be reliably compared across studies and countries due to differences in disease definitions and methodology, it is nonetheless interesting to note the very high rates in Southeast Asia, which are at least an order of magnitude larger than estimated in the USA.

Regional Variations in Key Pathogens

In developed regions, including the USA, Europe, and Australia, *Pseudomonas aeruginosa* has been the pathogen most commonly associated with microbial keratitis followed by Gram-positive bacteria, fungi, and *Acanthamoeba* [101–103]. While *Pseudomonas* was the most

common pathogen, a longitudinal study of keratitis in a major teaching hospital in Australia demonstrated seasonal patterns in causative organisms over 5 years. *Pseudomonas aeruginosa* and fungi were more common during summer and autumn months, whereas *Streptococcus pneumoniae* was more common during winter months [104]. Similarly, a retrospective study at a major tertiary referral center in southern Florida that analyzed corneal culture results over 9 years demonstrated that while *Pseudomonas* was the predominant bacterial organism, fungi were isolated in approximately 36% of positive cultures, highlighting the role that regional variations in temperature and humidity may play in the pathogens implicated in microbial keratitis [105]. A definite history of ocular trauma, usually with vegetable matter in the setting of agricultural work, was elicited in 60% of patients with fungal keratitis, compared with 22% of patients with *Pseudomonas* ulcers. Studies with similar case series methodologies reporting cases with positive cultures from the northeast, Midwest, and west coast of the USA consistently report bacteria as the predominant organisms (89–99%), with fungi and *Acanthamoeba* comprising only 3.5–11% [106, 107].

In Hong Kong, despite its semitropical climate, *Pseudomonas aeruginosa* was the most common pathogen in culture-positive cases in both contact lens wearers and non-wearers [13]. The explanation offered by the authors was the urbanization of Kowloon and the adjacent New Territory East in which the study was conducted.

The high prevalence of fungal pathogens in tropical climates has been demonstrated in multiple studies, including those in Bangladesh [108], Ghana [109], Tanzania [110], southern India [111], and eastern India [112]. In fact, fungi predominated as the causal pathogen in the latter three, causing 51%, 50%, 53%, and 63% of ulcers in those regions, respectively. A more recent study in southern India confirmed the relative stability of these figures, with fungi causing 49% of culture-positive ulcers, while bacteria accounted for 46% [113]. In Bangladesh, even though 53.5% of cases were bacterial, with *Pseudomonas* being the most common (24%), fungal pathogens were respon-

sible for nearly 36% of cases. Fungal ulcers are seen rarely in Bhutan, accounting for fewer than 2% of corneal ulcers, perhaps owing to its location in the Eastern Himalayas and more temperate climate [100].

Risk Factors

Contact Lenses

In developed country settings contact lens wear is the most common risk factor associated with ulcerative keratitis. Poggio et al. [114] conducted the first population-based incidence study of ulcerative keratitis among wearers of contact lenses. The investigators estimated the annualized incidence of ulcerative keratitis to be 20.9 per 10,000 persons using extended-wear soft contact lenses for cosmetic (refractive correction) purposes and 4.1 per 10,000 persons using daily-wear soft contact lenses for cosmetic purposes. In a separate study, using a case-control methodology, Schein et al. showed that the relative risk of ulcerative keratitis for extended-wear versus daily-wear contact lenses was approximately 4 [115]. Further, users of extended-wear contact lenses who wore them overnight had a risk 10–15 times greater than those using daily-wear contact lenses and not sleeping with them. Users of daily-wear contact lenses who sometimes wore them overnight had 9 times the risk of the users with such CLs who did not. Finally, among users of extended-wear lenses, the risk of ulcerative keratitis was incrementally related to the extent of overnight wear.

The FDA reduced its approval for extended-wear contact lenses from 30 to 7 consecutive days based on the results of these studies published in 1989. More than a decade later, a new generation of soft contact lenses made from silicone hydrogel materials gained FDA approval based on safety demonstrated in small preapproval studies and the logic and hope that the increase in oxygen transmissibility of these new material would yield lower rates of keratitis. However, when Schein et al. [116] conducted a prospective surveillance study of microbial keratitis associated with CIBAVision's fluorosilicone

hydrogel CLs, the overall annual rate of presumed microbial keratitis was estimated at 18 per 10,000, very much in line with prior estimates, suggesting that the enhanced oxygen permeability had not conferred the desired added safety. Subsequently, a case-control study at Moorfields Eye Hospital [117] and an Australian national surveillance study [118] also found that extended wear of silicone hydrogel lenses increased the risk of microbial keratitis compared to daily use of soft lenses and that the risk was comparable to that associated with overnight wear of hydrogel lenses. The Australian population-based incidence study found that CL-related corneal infection affects approximately 4.2 per 10,000 wearers in Australia per year, with the highest incidence associated with overnight wear of silicone hydrogels at 25.4 per 10,000. The results of these studies in Australia were remarkably consistent with those reported in the USA some 10 years earlier, again suggesting a lack of significant protective effect of the newer contact lens materials.

When incidence studies of contact lens-related keratitis are performed, the rates estimated essentially reflect the rates of bacterial keratitis among users of soft contact lenses, since the most common organisms are bacterial and the population wearing rigid contact lenses has been steadily diminishing. Although the most important risk factor for bacterial keratitis associated with soft contact lens wear has consistently been demonstrated to be overnight or extended wear, there are several other risk factors that have been identified with some consistency across several large studies. These include smoking [119], low socioeconomic status [120], male gender [121], and youth [119]. A variety of factors associated with contact lens and lens case hygiene have also been implicated, including length of time the case has been used [122], cleaning the lens case [101], chlorine disinfection (versus peroxide), and infrequent disinfection [120]. The use of rigid gas permeable contact lenses has been associated with a reduced risk of ulcerative keratitis, perhaps due to rare overnight use and improved tear exchange [123]. The preponderance of severe bacterial infections related to contact lens wear are due to *Pseudomonas* species. Such infections

are rarely reported in the setting of daily-wear soft lenses that are disposed of daily.

Contact Lens Solutions

Although rare in comparison to disease caused by bacteria, contact lens-related corneal infection due to fungi and *acanthamoeba* are important due to the severity of associated disease. Contact lens-related corneal infections due to these organisms appear to occur in both sporadic and epidemic fashion. Regarding fungal keratitis in the setting of contact lens wear, a baseline incidence is not known. However, there was a dramatic increase in the number of fungal keratitis cases caused specifically by *Fusarium* that was observed first in Singapore [124] and then subsequently in France [125], Hong Kong [126], the USA [127], and elsewhere between 2004 and 2006. A case-control study of 164 US cases performed by the Centers for Disease Control and Prevention (CDC) found that use of the contact lens solution ReNu with MoistureLoc was strongly associated (OR 13.3; 95% CI 3.1–119.5) with contact lens-related *Fusarium* keratitis, as was reusing contact lens solution already in the lens case (OR, 3.2; 95% CI 1.2–9.4) [127]. These findings resulted in a regional recall of the product involving Singapore and Hong Kong in February 2006 followed by voluntary recalls in the USA (April 2006) and globally (May 2006). A subsequent surveillance study of contact lens-related fungal keratitis at 10 major eye centers in the USA showed that the count of cases returned to its low baseline after removal of that product from the market [128]. The precise pathophysiology of this epidemic of *Fusarium* keratitis was never fully determined, but was felt to be related to the buildup of a protective biofilm in contact lens cases that was in some way potentiated by that specific solution [129, 130].

More than 85% of cases of *Acanthamoeba* keratitis (AK) are associated with contact lens wear [131]. There have been several attempts to estimate the rate of keratitis associated with *acanthamoeba*. In the USA, extrapolation from CDC data yields an annual incidence of 1.65–2.01 cases per million contact lens wearers [132]. In the UK, the estimated incidence was

found to be between 17.53 and 21.14 cases per million contact lens wearers [133]. In Scotland, a prospective population-based cohort study revealed the incidence for AK was 1.49 per 10,000 soft contact lens wearers; the infection was not detected in the absence of contact lens wear nor with use of rigid gas permeable contact lenses [134]. Less than a year after the multi-country *Fusarium* outbreak described above, an increasing number of AK cases were reported from major centers in the USA [130, 135]. Case-control studies conducted in Chicago and by the CDC [136, 137] confirmed the presence of an AK outbreak and identified the use of a specific lens care solution, AMO Complete Moisture Plus, as a significant risk factor for the development of contact lens-related *Acanthamoeba* keratitis (OR 16.67; 95% CI 2.11–162.63) [138], leading to an immediate worldwide recall (May 2007). Although less associated, additional hygiene-related variables (solution “reuse,” lack of “rubbing,” and showering with lenses) seemed to confer additional risk [138].

In comparing the *Fusarium* and *Acanthamoeba* outbreaks, although odds ratios for excess risk with specific contact lens solutions were the highest relative to other risk factors in both cases and similar in magnitude, follow-up CDC surveillance [139] has demonstrated that AK cases in Chicago and nationally did not return to baseline after withdrawal of AMO Complete Moisture Plus from the market. This development, totally different from the effect on fusarium infections after withdrawal of ReNu with MoistureLoc, demonstrates that (1) it can be unreliable to extrapolate attributable risk directly from odds ratios derived from case-control studies, and (2) the underlying pathogenesis of the AK outbreak was likely more complex, pointing to other factors such as, perhaps, an increasing bioburden of amoebae in our water supply and hence contact lens cases [140]. Unlike bacterial keratitis among CL users, overnight wear has not been shown to be an important risk factor for AK. Instead, factors related to CL and case hygiene, such as use of homemade saline, topping off (adding new solution to an existing volume of used solution in the lens case), lack of adequate disinfection, and

improper case cleaning or replacement, appear to be primary [141–143]. While the use of soft contact lenses is a well-documented risk factor for *Acanthamoeba* eye infection, AK reports in patients who wear rigid contact lenses are limited. A case-control study conducted by the CDC to identify modifiable risk factors of AK in rigid gas-permeable (RGP) contact lens wearers demonstrated the following significant risk factors: wearing lenses for orthokeratology (OR undefined, $P = 0.02$), wearing lenses while asleep (OR 8.00, $P = 0.04$), storing lenses in tap water (OR 16.00, $P = 0.001$), and topping off contact lens solution in the case (OR 4.80, $P = 0.01$). After stratifying by use of RGP lenses for orthokeratology, storing lenses in tap water and topping off remained significant exposures [144].

In summary, ulcerative keratitis among contact lens wearers is most often caused by bacteria, and the most important risk factor is overnight wear of soft contact lenses. By contrast, the principal risk factors for contact lens-associated keratitis due to fungi and *acanthamoeba* are hygiene and contact lens case related. Therefore, epidemiologic research can directly lead to recommendations to reduce both the rate and severity of disease. Adherence to strict daily disposal of soft contact lenses (no solutions or contact lens cases) should accomplish both.

Trauma and Other Risk Factors

While multiple risk factors may lead to corneal ulceration in the developing world, trauma is the most significant predisposing factor. In Nepal [100], southern India [111], and northern China [145], more than 50% of patients with infectious corneal ulceration reported a history of ocular trauma. Agents responsible for trauma are mainly agricultural and animal products, such as tree branches, insects, stones, or corn stalks. In Bangladesh, the most frequent injury was due to rice grains or leaves, although antecedent trauma was reported at a lower rate in this population, with approximately 35% of patients having experienced eye trauma prior to the development of microbial keratitis [108]. In addition, the majority of ulcer patients in India were agricultural workers or laborers (78.8%), an occupation profile

similar to Nepal (72%). Presumably a higher level of physical activity and thereby higher risk of ocular injury – especially in an environment replete with vegetative matter – confer a higher risk of microbial keratitis.

Despite access to excellent care at the Aravind Eye Hospital in southern India, 37.3% of patients also used some kind of traditional or herbal topical treatment in the eye (e.g., breast milk, plant juice, honey, chicken blood) which may potentially have increased the risk or severity of the corneal infection.

Trauma is also an important risk factor in the developed world, accounting for between 12% [107] and 27% [146] of all cases of ulcerative keratitis – with more recent studies demonstrating a lower percentage of ulcers caused by trauma likely due to an increase in the prevalence of contact lens wear and associated microbial keratitis. Luchs and associates [147] reported that approximately 4.7% of patients with bullous keratopathy experienced a corneal ulcer during a 10-year period, though a more recent study showed that a low percentage (1.9%) of patients developed ulcerative keratitis as a result of bullous keratopathy – potentially due to improved surgical techniques and greater availability of corneal donor tissue [107].

Other risk factors, although less rigorously studied, include chronic dacryocystitis, leprosy, non-leprous lagophthalmos, Bell's palsy with exposure keratitis, trachoma, keratoconjunctivitis sicca, and neurotrophic keratitis from herpetic and non-herpetic causes [100, 121, 148].

While it might be presumed that there is lack of access to medical care in the developing world, there is a great deal of variation. In the study in southern India, over 60% of the patients in the study presented for examination during the first week of their illness, while in Nepal, only 43.7% of patients were seen in the first week, and 19.3% took longer than 1 month to make the journey to the hospital. Multiple studies have demonstrated that late presentation leads to worse outcomes, which holds true for bacterial [149], fungal [150], and acanthamoebic [151] keratitis. Therefore, improving awareness and increasing access to care should be at the forefront of any strategy to

reduce the burden of corneal blindness caused by AK.

The early postoperative period after refractive surgery is known to pose risk for the development of microbial corneal ulceration, with rates ranging between 0.2% and 1.5% [152, 153].

Other known risk factors, but for which rates are unknown, include previous ocular surface disease, prior ocular surgery, and systemic diseases such as diabetes, HIV, and end-stage renal disease [103, 107, 154].

Prevention

Just as risk factors for microbial keratitis vary geographically, so too should strategies for prevention.

As previously discussed, correct use of daily-wear disposable contact lenses, if widely adopted, would be expected to lower rates of contact lens keratitis significantly and also reduce the rate of the more severe infections caused by *Pseudomonas*, fungi, and *Acanthamoeba*. Additionally, the lack of an effective and sustained surveillance program by the FDA and/or industry and profession aimed at assessing the rates of severe cases of lens-related microbial keratitis has delayed detection of outbreaks.

In the developing world, where trauma is the most common potentially addressable risk factor for corneal ulceration, multiple studies have demonstrated that severity of disease can be lessened through large-scale, community-based programs. The Bhaktapur Eye Study, a large 2-year study in Nepal, demonstrated a benefit to early treatment of corneal abrasions with topical 1% chloramphenicol ophthalmic ointment three times a day for 3 days. Of the 442 patients with corneal abrasion enrolled in the study, 424 (96%) healed without infection, and none of the 284 patients who were started on treatment within 18 hours after the injury developed ulcers. Four of the 109 patients (3.7%) who presented 18–24 hours after injury developed infections, and 14 (28.6%) of the 49 patients who presented 24–48 hours subsequently developed corneal ulceration [100].

Success with the Bhaktapur Eye Study led to corneal ulcer prevention programs in three different countries in South East Asia: Bhutan [99], Burma [155], and India [156]. In Bhutan, all study patients presenting with a corneal abrasion were treated with 1% chloramphenicol ointment three times a day for 3 days. None developed corneal ulceration, despite the fact that 17.3% of patients presented more than 18 hours after injury, including 7.8% of patients presenting between 25 and 48 hours after their injury. Given approximately one-third of corneal ulcers in Burma are caused by fungal pathogens, 1% clotrimazole antifungal ointment and 1% chloramphenicol ointment were applied three times a day for 3 days to all eyes with confirmed corneal abrasion. No cases of microbial keratitis occurred in the study population, of which 9% reported within 0–6 hours of the injury and 97% within 18 hours.

In a study from south India, patients were randomized into two groups and treated with either 1% chloramphenicol and 1% clotrimazole ointment or 1% chloramphenicol and a placebo ointment three times a day for 3 days. Over the 18-month study period, no patients in either study arm developed corneal ulceration. This outcome might reflect some antifungal activity of chloramphenicol [157, 158] or simply the fact that the ointment enhances epithelialization and therefore healing and infection prevention.

Other Conditions

Keratoconus

There have been few studies examining the epidemiology of keratoconus. The most commonly cited study on keratoconus prevalence is a registration study conducted between 1935 and 1982 in Olmsted County, Minnesota. All patients in whom keratoconus had been newly diagnosed during the study period were identified through the electronic record system at the Mayo Clinic, with a reported annual incidence of 2 per 100,000 and prevalence of 54.5 per 100,000 [159]. More recently, a study that relied on extracting data

from the nationwide health registry in the Netherlands found the incidence of keratoconus to be 13.3 per 100,000. The prevalence of keratoconus, estimated based on the annual incidence, mean age at diagnosis, and average life expectancy, was 265 per 100,000 [160]. A cross-sectional study using corneal topography in a college student population sample in Jerusalem yielded a much higher prevalence, at 2340 per 100,000 [161]. These wide variations may be attributed to differences in definition of disease, sensitivity of diagnostic modalities, regional differences with respect to accessibility of health care, differences in study design, and/or ethnic differences with respect to the incidence of keratoconus [160]. More comprehensive, population-based studies that employ standardized disease definitions and methods of screening are needed to gain a more complete understanding of the worldwide burden of keratoconus.

Corneal Dystrophies

There is even greater paucity of data regarding the epidemiology of corneal dystrophies. Many estimates of the relative frequency of corneal dystrophies have relied primarily on information from corneal transplant registries and large case series from corneal surgeons. For example, according to the Australian Corneal Graft Registry, which provides detail on the percentages of reported corneal transplants that were performed for specific indications, Fuchs was the corneal dystrophy that predominated (16%), followed by the following dystrophies: granular (0.19%), macular (0.08%), lattice (0.07%), posterior polymorphous (0.04%), Schnyder (0.03%), Reis-Buckler (0.02%), and Meesman (0.003%) [162]. However, there is bias toward more severe cases in transplant registries and referral and selection biases in reports arising from corneal surgeons' practices. To minimize the effect of some of these biases, a study completed by Musch et al. relied on extracting data from the records of eight million enrollees in a national managed-care network in the USA. To provide a conservative estimate, at least two consistent

codings for the specific corneal dystrophy during the defined time period were required. This approach yielded prevalence data (per million covered lives) for various corneal dystrophies as follows: endothelial (535.9), macular (9.7), Meesman (7.8), lattice (6.9), granular (5.3), and unspecified (240.5) [163]. However, this study also had significant limitations, including, most importantly, suboptimal diagnostic specificity. For example, the nonspecific diagnosis anterior corneal dystrophy was the second most common, accounting for 15.6% of those diagnosed with a corneal dystrophy, while 26.0% of patients were classified as having an unspecified dystrophy. A population-based survey using internally consistent diagnostic criteria is the only method by which to circumvent the limitations described above. However, the size and cost of such a survey to describe accurately the epidemiology of diseases with a very low estimated prevalence would likely be prohibitive.

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Eye Banking: History and Future Direction

27

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History of Eye Banks

Origins of Corneal Transplants

The first successful human corneal transplant was performed in 1905 by the Austrian ophthalmologist Dr. Eduard Zirm. This achievement was the first of its kind, predating all other successful solid organ transplants by 49 years. An 11-year-old boy who had suffered a penetrating injury to the sclera had his eye enucleated and donated the cornea for two corneal grafts to a 45-year-old farm laborer who sustained severe bilateral alkali burns from lime used to clean a chicken coop. Despite the eventual failure of the right graft, the left graft improved the recipient's left eye acuity from counting figures to 6/36 by 6 months after surgery. Since Dr. Zirm's first procedure over a century ago, corneal transplantation has become increasingly prevalent. Improvements in technology, surgical methods, and under-

standing of ophthalmologic physiology have led to better outcomes and decreased rejection rates. Dr. Vladimir Filatov, a Russian ophthalmologist known for the development of tissue therapy, recognized the relative paucity of donor tissue and postulated that cadaver corneas could be used to overcome this supply issue [1]. This has led to the development of eye banking and the vital role eye banks play in restoring vision in patients with corneal blindness globally.

Origins of Eye Banks

In 1944, Dr. Townley Paton was returning home after performing corneal recovery surgery at a New York City prison when he conceived of the idea of an eye bank. In the years prior, Dr. Paton had been performing corneal transplants using eye tissue taken from executed prisoners. His vision was to approach both living people and the next of kin of recently deceased individuals, who could agree to donate their eyes or the eyes of their relatives. These donated eyes would be stored at an eye bank until they could be used to restore vision to a patient in need. In the 10 years following the creation of the Manhattan Eye Bank for Sight Restoration in 1944, 3800 corneas were supplied. Furthermore, the number of surgeons who could perform keratoplasty rose significantly from 15 to several hundred [2].

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By the 1950s, there was a strong push toward an eye bank accreditation process along with increased coordination and standardization of eye banking practices. This was driven by the existence of eye banks that had been founded without medical direction and with questionable fund-raising practices. In 1961, under the guidance of the American Academy of Ophthalmology and Otolaryngology, the American Medical Association, the American Ophthalmological Society, and the Association of Research in Ophthalmology, the Eye Bank Association of America (EBAA) was founded with a focus on improvement of patient safety via standardization of tissue recovery, processing, and preparation. In 1964, 46 member banks joined the new association, representing all American eye banks that met the standards. During the 1980s, the EBAA established the first set of national standards regarding the collection, preservation, and equitable distribution of donor tissue. These standards have allowed for the fair and ethical recovery and distribution of corneal tissue. Since their establishment, the EBAA medical standards have continued to evolve to reflect updated best practice guidelines. In addition to establishing national medical standards for corneal tissue, the EBAA also established an accreditation system based on inspection of member banks [2].

In 1944, when Dr. Paton partnered with Dr. John McLean to establish the Eye-Bank for Sight Restoration, the world's first eye bank, the Central Staten Island Lions Clubs were eager to assist. They founded the Sight Restoration Society to help people in their community receive necessary transplants. In 1945, the Buffalo Host Lions Club raised over \$10,000 via fund-raisers and raffles to start the second eye bank in the United States, the Buffalo Eye Bank and Research Society. As other Lions Clubs followed suit and began to establish eye banks of their own, it was clear that a standardized set of policies would be needed to ensure that new eye banks were staying abreast of current best practice guidelines. The Lions Clubs International Eye Bank Program was adopted in March of 1984 to standardize policies and help the establishment of new eye banks. As of 2015, the Lions

had established 55 Lions Eye Banks worldwide, of which 43 are located in the United States and Canada. Thirty-nine of these eye banks are still tied to the Lions Clubs to this day. In 2016, 68,192 corneas were recovered from 34,374 donors by Lions Eye Banks, of which 40,324 were used for transplantation while 16,165 of those corneas were used for research and education. In addition to running these eye banks, Lions Clubs also provide philanthropic support and promote donor registration [3].

Present

Growth

In the years since the initial founding of eye banks, eye donations have increased from 6000 in 1967 to 135,203 in 2017 [2, 4]. With this exponential growth in availability of eye donations, there has been an accompanying increase in demand both domestically and internationally (Fig. 27.1). Several factors help to explain this greater than 20-fold increase over the last six decades. Laws aimed at making organ donation more feasible, standardization of practices leading to increased confidence in the eye banking system, an aging population, and surgical advances have all contributed to this surge in both supply and demand [2].

The increase in corneal supply for the eye banking system is largely credited to the passage of four laws (Table 27.1). In 1968, the Uniform Anatomical Gift Act was passed to allow next of kin to donate tissues and organs via signature in front of a witness. Prior to the passage of this law, there was an extensive amount of paperwork that was required to donate body parts, making organ donation far less frequent. The Uniform Anatomical Gift Act was further revised in 2006 to permit the use of life support systems to maximize the time period for which organ procurement remained viable [5]. In 1975, the Medical Examiner/Justice of the Peace Law was passed and currently exists in 12 states. This law gives the medical examiner (ME) or justice of the peace (in counties without an ME) the right to

Fig. 27.1 Corneal grafts of US origin used over the last 10 years, domestic (blue) and international (orange)

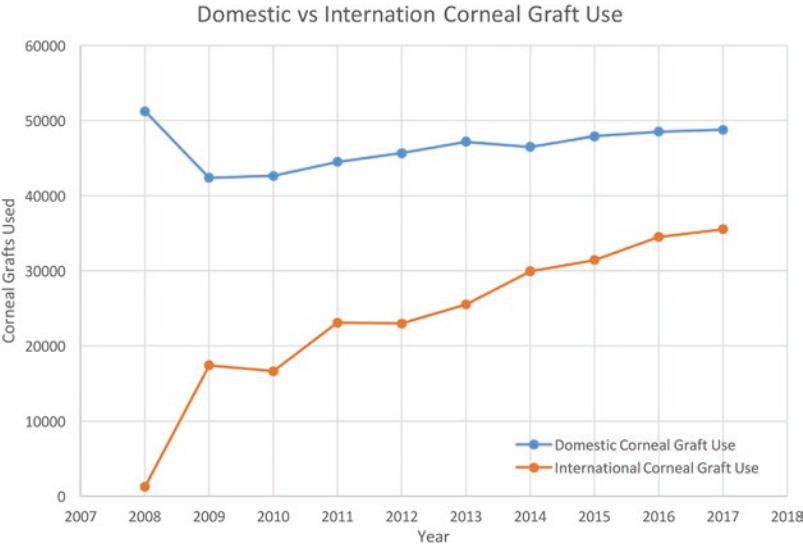


Table 27.1 Laws that contributed to the growth of eye banking and cornea donation

Year passed	Law	Description
1968 (revised in 2006)	Uniform Anatomical Gift Act	Allows next of kin to donate tissues and organs via signature and witness; revision permitted the use of life support to maximize time period for organ procurement
1975	Medical Examiner/ Justice of the Peace Law	Grants medical examiner right to approve corneal donation when there is no known familial objection
1970s	Driver' License Laws	Allows driver's license to indicate approval for anatomical donation
1982	Routine Inquiry/ Required request Laws	Hospitals that participate in Medicare reimbursement are required to offer patient's next of kin the chance to donate body parts

approve corneal donation, when there is no known family objection. Also, in the 1970s, a majority of states passed Driver's License Laws which allowed a driver's license to indicate approval for anatomical donation. Finally, in 1982, the Routine Inquiry or Required Request Laws were passed in 43 states. These laws stated that, in order for a hospital to participate in Medicare reimbursements, they must offer the

patient's next of kin a chance to donate body parts. Despite this policy applying to organs only and not corneas, its precedent paved the way for increased corneal donation as well [2].

In addition to the increased corneal supply that has been seen with the advent of eye banking, there is also an increased demand. This is due, in part, to the increased confidence that physicians have in eye banks. In the 1980s, the Eye Bank Association of America created its medical standards program to ensure safe and efficient storage, retrieval, and utilization of corneal tissue supplied by eye banks [2, 6]. In addition to the implementation of stringent medical standards, there has been an increase in demand for donated corneas due to an increasing number of indications for corneal transplantation. This includes the growing number of cataract surgeries performed. Although not commonly reported now, bullous keratopathy is a complication that can occur with cataract surgery [2, 6]. Public perception has also led to an increase in demand for corneal tissue, as individuals have become more comfortable with this intervention as a method to improve sight [2]. Moreover, there has been an increase in the number of surgeons capable of performing these transplants. In 1970, there were a total of 10 US fellowships available in the cornea [2], compared to the nearly 100 programs that are currently available in 2018. This growth in the number of

corneal specialists is in large part due to the contributions of Dr. Claes Dohlman, affectionately dubbed the “Father of Modern Corneal Science.” [7] Dr. Dohlman created the first organized corneal subspecialty with a structured corneal fellowship program at Massachusetts Eye and Ear. Over the course of his career, he has directly trained more than 200 corneal specialists. His reach is far greater when considering the number of corneal specialists who have trained under his trainees. Dr. Dohlman’s contributions to modern-day corneal training have allowed the number of corneal surgeons to keep pace with eye bank growth and thus corneal tissue supply growth. This is in contrast to eye banking systems in other countries where there are not enough cornea specialists to meet demand. In India, where the eye banking system has grown immensely over the past decade, it has been predicted that the corneal tissue supply will outpace corneal surgeon capacity in the near future [8]. In fact, this may prevent India from reaching its goal of 100,000 corneal transplants by 2020.

Along with an increase in the number of people capable of performing corneal transplant surgeries, there has also been an increase in the number of surgical procedures that require corneal tissue [9]. Since the late 1990s, a series of procedures have been created, including deep lamellar endothelial keratoplasty (DLEK), Descemet’s stripping endothelial keratoplasty (DSEK), Descemet’s stripping automated endothelial keratoplasty (DSAEK), and Descemet’s membrane endothelial keratoplasty (DMEK) [9].

Eye Bank Structure

Each eye bank is unique in terms of the size and volume of tissue processing that occurs. However, there are fundamental roles that must be performed at each eye bank. These roles include determination of donor eligibility for providing corneal tissue, along with tissue recovery, processing, and distribution (Tables 27.2 and 27.3).

The *director of the eye bank* is appointed by the Board of Directors, Board of Regents, or another governing body [10]. As outlined in the

latest Medical Standards published by the EBAA, the director of the eye bank is responsible for all administrative functions, day-to-day operation of the eye bank, and compliance with national standards [10]. The director is also responsible for appointment of a well-qualified technical and support staff, including eye bank technicians, who are able to perform the necessary laboratory tests and procedures [10].

The *medical director* at an eye bank is typically an ophthalmologist with corneal fellowship training or who has demonstrated some expertise in the field either through clinical practice or research [10]. This individual is responsible for overseeing all medical aspects

Table 27.2 Resources required for eye bank function

Facility resources	Eye bank laboratory. Equipment, maintenance and cleaning, instrument maintenance, procedures manual, infection control and personnel safety, waste disposal

Table 27.3 Services provided by eye banks

Donor eligibility	Documentation on donor information, medical examiner/coroner/pathologist documentation, method of consents, eye maintenance prior to recovery
Recovery, tissue evaluation	Preocular tissue recovery and donor preparatory procedure, enucleation, in situ corneal excision, and blood drawing, gross examination, slit lamp examination, and endothelial density check
Processing, preservation, storage	Whole eye storage for surgical use, excision of the corneoscleral rim from enucleated whole eyes, laboratory microkeratome anterior or endothelial lamellar processing, transfer of corneal tissue, femtosecond laser processing of donor cornea, laboratory microkeratome anterior or endothelial lamellar processing of donor tissue for DMAEK/DMEK, scleral presentation
Quality assurance	Quality control, testing, microbiologic culturing, infectious disease testing, HIV screening, hepatitis B screening, hepatitis C screening, non-required laboratory results, conflicting serology reports, and tissue recall, adverse reaction reporting, eye bank record entry and entry correction
Distribution of tissue	Procedures established by the individual eye bank

of the eye bank's laboratory operations, including formulation, approval, and implementation of medical procedures, participation in training and oversight of technical staff, operation of a quality assurance program, and verification of competency in tissue recovery and preservation by personnel including those applying for CEBT certification [10]. The medical director must also observe the designated staff trainer performing specific procedures on an annual basis [10]. These procedures include an in situ corneoscleral disc excision or laboratory corneoscleral disc removal from a whole eye, a posterior lamellar processing procedure that utilizes a microkeratome, a laser-shaped processing procedure, or a manual dissection processing procedure for endothelial keratoplasty [10].

In the standards, there are also written directives establishing a formal quality assurance program. It is the responsibility of the *quality assurance director* to ensure that the program meets the requirements outlined in the Medical Standards. Quality assurance requirements include periodic audits of eye bank operations, establishment of a process for donor eligibility determination, identification of problems and complaints related to eye bank activities, and development of corrective action plans [11, 12]. The quality assurance program is also responsible for establishing a system for reporting, documenting, and investigating deviations in practice. Moreover, the bank's quality assurance programs must provide a protocol for the receiving surgeon to report adverse reactions from transplantation of different tissue types.

Infectious Disease Testing

All donors of ocular tissue must be tested for relevant communicable pathogens, including human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2), hepatitis B virus (HBV), hepatitis C virus (HCV), and *Treponema pallidum* or syphilis [10]. Any donor whose specimen is reactive for any of these infections, apart from syphilis, must be ruled ineligible for donation.

Eye Bank's Role in Education

Eye banks participate in education at all levels of ophthalmology training. ACGME requirements state that all ophthalmology programs are required to have surgical skills development resources, i.e., wet lab and/or virtual reality simulator [11]. To that end, eye banks provide wet lab locations and tissue for residents to learn and practice their surgical skills.

Eye banks also assist in the education of surgeons who may have completed their fellowship training prior to the advent of some of the newer, partial-thickness corneal surgeries. For example, with many DMEK training programs, training surgeons observe surgery followed by travel to a local eye bank where they observe DMEK graft preparation by an eye bank technician, before completing a wet lab session where they have the opportunity to perform the DMEK procedure using human cadaver eyes.

Additionally, eye bank training includes education of its own staff. In fact, newly appointed medical directors are required to attend a Medical Director's Symposium and a Medical Advisory Board Meeting within 1 year of appointment [10]. Physicians who provide verification of competency for tissue recovery and preservations are also required to attend the Medical Director's Symposium once every 3 years and a Medical Advisory Board Meeting once every 3 years [10]. At these meetings, evidence-based procedures and protocols are introduced, already existing methods are reviewed, and other topics are discussed including ethical dilemmas and what the appropriate actions are when these situations arise.

Eye Bank Participation in Innovation/Scientific Inquiry

As the field of corneal transplantation has evolved, eye banks have participated in seminal studies that have contributed to advances in knowledge and procedural technique.

Participation in Clinical Trials

Cornea Donor Study

In 2008, the Cornea Donor Study Investigator Group published the results of the Cornea Donor Study [13]. At the time that the study was being conducted, many US eye banks had arbitrarily set an upper limit for age of donation at 65 or younger. Many surgeons were reluctant to use corneas from older donors; however, there was no scientific evidence to support this practice.

The study followed 1090 subjects, age 40–80 years old, with corneal disease (primarily Fuchs' dystrophy and pseudophakic corneal edema) over 5 years and included 43 participating eye banks. Patients whose eyes were either at low risk or high risk for graft failure were excluded from the study. Follow-up included as needed for the first 6 months, once between 6 months and 12 months, and then annually for 5 years after the surgery.

Overall, 5-year success rate was 86% for grafts performed with donors age 12–65 as well as with donors age 66–75. The data was also analyzed using donor age as a continuous variable, and again there was no significant difference based on age for the range of 12–75. There was a slightly higher success rate for donor corneas at the younger end of the donor age range; however, the donor pool at that age is so limited (<15% of donors are under the age of 40) that this conclusion did not have practical implications. Results of the study showed no significant difference in five-year graft survival rates for corneal transplants at moderate risk for failure between donors 66 years of age and older and those younger than 66. These results significantly increased the potential donor pool for corneal transplantation.

Cornea Preservation Time Study

In 2017, the results of a three-year study on the effect of corneal preservation time on DSAEK outcomes was published. Prior to this study, it was common for surgeons in the United States to refuse to accept donor corneas that had been preserved for longer than 7–8 days. In fact, this practice was widespread despite the US FDA approval of 4 °C solutions for up to 14 days. The Cornea

Preservation Time Study was initiated to determine whether the three-year graft failure rate after EK with donor corneas that had been preserved for 8–14 days was truly inferior to failure rate of donor corneas that had been preserved for fewer than 7 days [14].

The study enrolled 1090 participants with corneal disease-associated endothelial dysfunction, ages 42–90. Most participating patients had Fuchs' endothelial corneal dystrophy (94%) with the rest having pseudophakic or aphakic edema. Patients were randomly assigned to receive a donor cornea from one of two groups: (1) preservation time 0–7 days or (2) preservation time 8–14 days.

The results of this study indicated that preservation time up to 11 days had little clinical influence on the 3-year graft success after DSAEK [15]. The study did, however, find that there was a significantly greater endothelial cell loss associated with longer preservation time. Despite this finding, endothelial cell loss over the 4–13 day preservation time range was clinically similar. In addition, there was a higher frequency of graft failure for those corneas that had been preserved for 12–14 days; however, even these grafts may be suitable if necessary as the DSAEK success rate was still high.

Tissue Processing Techniques

Eye banks prepare the tissue that is used in more recently developed procedures, including DSAEK and DMEK. There are also ongoing studies related to preloaded DMEK and associated outcomes.

DSAEK (Descemet's stripping automated endothelial keratoplasty) is a procedure that is used to treat corneal endothelial disorders that is performed by peeling a patient's native Descemet's membrane and replacing it with a graft that includes posterior stroma, Descemet's membrane, and endothelium [16]. The corneoscleral tissue is excised from the donor eye and kept in cold storage. An artificial anterior chamber is then created by mounting the corneoscleral tissue on a holder and filling the holder with balanced salt solution or preservation medium. Once the tissue is positioned on the artificial anterior

chamber, microkeratome cutting depth is adjusted for control over the thickness of the resulting graft. Once the automated microkeratome cutting is complete, the tissue is then stored in cold storage until transplantation [17].

DMEK (Descemet's membrane endothelial keratoplasty) is another procedure that is used to treat corneal endothelial disorders. DMEK and DSAEK are similar; however, in DMEK, the transplanted tissue includes only Descemet's membrane and corneal endothelium. Retrospective studies have demonstrated that DMEK has associated faster visual recovery and improved visual acuity relative to DSAEK [18]. However, DMEK is technically more challenging in both graft preparation and surgical implantation. Additionally, the graft preparation techniques of DMEK are not as standardized as those used for DSAEK. There are many techniques that have been described for harvesting DMEK tissue, of which three will be described. The first method is the manual peeling method, originally described by Lie et al. [19] and Melles et al. [20]. This technique involves immersing the donor corneoscleral rim in BSS and then peeling the Descemet's membrane using forceps. The second technique is the submerged corneas using backgrounds away (SCUBA) method [21]. In this procedure, the cornea is submerged in Optisol or BSS which reduces surface tension and allows the Descemet's to settle onto the stroma. The third and final technique we will describe is pneumatic dissection. This technique was originally described by Anwar and Teichmann [22] in anterior lamellar keratoplasty. In this method, air is injected into the donor cornea to separate stroma and Descemet's membrane.

Though DMEK surgeons receive prestripped tissue, they are still responsible for punching the tissue, separating it from the unpeeled hinge point, staining it with trypan blue, rinsing it with balance salt solution, and loading the tissue into the injector [23]. Recent studies have explored the feasibility of preloading DMEK grafts into an injector as a means of decreasing OR time and minimizing the risk of tissue damage during the final preparation. Additionally, the preloaded tissue can be evaluated prior to being shipped for

quality assurance purposes [24]. One method of preloading that was explored by Parekh et al. involved removing excess periphery from the prestripped membrane, folding the membrane manually from two ends with a forceps, and moving the membrane from the tissue base to an intraocular lens cartridge (IOL) which was then sealed with a rubber plug on both the funnel and rear exit [25]. In this method, the tissue was made into a trifold while taking care to touch the Descemet side only so as to avoid endothelial damage. This method of tissue preparation may become more widespread in the near future as preliminary studies on this method have shown that these preloaded DMEK tissues minimize surgical time and costs without sacrificing outcomes [26].

Future of Eye Banks

Corneal Tissue Engineering

Despite the current national success of US eye banks in terms of meeting overall demand, there remains a cornea tissue shortage worldwide [27]. With the aforementioned trend toward more endothelial keratoplasty (EK), a more labor-intensive procedure compared with traditional penetrating keratoplasty (PK), this shortage will continue and potentially worsen [28].

One proposed method to meet this new demand would be to create bioengineered corneas. Construction of corneal, epithelial, stromal, and endothelial equivalents to be used for drug testing and biomedical research was first accomplished by Griffith and colleagues in 1999. These bioengineered corneas were used in drug testing and biomedical research because of their ability to mimic human corneal morphology, biochemical marker expression, transparency, ion and fluid transport, and gene expression [29].

Though this technique has been successful in vitro, in vivo success has yet to be achieved. Endothelial cell function, in particular, is affected by many disease processes and is essential for cornea function. Endothelial cells are responsible for the pumping function of the

cornea and allow for normal vision. One option that is being investigated for replacing corneal endothelial function is to culture corneal stem cells and use them to create a bioengineered graft. Such a process would involve harvesting, culturing, and creating a scaffold for the cells to be transported on and subsequently transplanted from.

Though corneal endothelium is presumed to lack the regenerative abilities of the corneal epithelium, in recent years there have been both *in vitro* and *in vivo* studies carried out that have demonstrated the potential presence of corneal endothelial progenitor cells. Telomerase-based studies used to detect the presence of dividing cells have also been conducted and have shown telomerase activity in the middle and peripheral portions of the cornea without activity in the center [30–33]. Such studies have led to the hypothesis that there may be slow regeneration of human corneal endothelial cells from the peripheral regions of the cornea [34].

As in the past, eye banks will have a key role to play in the advancement of tissue culture growth. In order to maximize efforts in this domain, eye bank personnel will need further training in the culturing of endothelial and epithelial cells, along with the creation of a cell sheet for transportation and transplantation.

Cell-Based Therapy

Other studies have investigated cell-based therapies in the hopes of allowing the host to regenerate endothelium. This therapy is based on injection of cultured corneal endothelial cells (CEC) into the anterior chamber of the eye. However, when these CECs are injected alone, they are washed off by aqueous humor flow which results in poor adhesion of the injected cells onto the corneal tissue.

One method that has been used to enhance adhesion of injected CECs to the corneal tissue is via magnetic attachment of iron powder or superparamagnetic microspheres that is incorporated into cultivated CECs. This method has been demonstrated to work in rabbit models [35, 36].

In 2016, another method of ensuring CEC adhesion was explored by Okumura and colleagues [37, 38]. They demonstrated an improved ability to regenerate endothelium by including a Rho-associated protein kinase (ROCK) inhibitor in the suspension with CECs, in monkeys. In a subsequent 2018 study [39], human CECs with supplementary ROCK inhibitor were used to successfully treat 11 patients with bullous keratopathy. At 24 weeks post-injection, all 11 of the treated eyes had a CEC density greater than 500 cells per square mm, and 10 of the 11 had a CEC density greater than 1000 cells per mm². In addition, 10 of the 11 treated eyes had a corneal thickness of less than 630 micrometers at 24 weeks after injection with an improvement in best corrected visual acuity of two lines in nine of the 11 treated eyes. The authors of the study hypothesized that the injected CECs repopulated the Descemet's membrane and the bare posterior surface of the corneal stroma.

If this cell-based therapy becomes a widely used treatment modality, one can foresee a future in which eye banks will assume responsibility for harvesting cells and preparing suspensions for injection.

Conclusion

The evolution of eye banking has made surgical correction of refractory diseases more prevalent, and its contribution to innovation in the field has made treatment more successful. From the early beginnings of eye banking when the only goal was to increase available supply of donor corneas through the surgical innovations that have changed treatment modalities, eye banks have had a significant impact on the treatment of corneal disease. The rapid growth of eye banking was due in no small part to changes in organ donation laws, the contributions of Lions Clubs, and early recognition of the need for organization and standardization of eye banking. The founding of the EBAA and establishment of the Medical Standards were landmark events that allowed for the early success of eye banks.

Eye banks have played a key role in advancements within the field of corneal transplantation both in terms of increasing tissue availability and improving surgical techniques. Landmark trials in corneal transplantation have demonstrated that more tissue is viable than was previously being used. This finding has significantly increased the availability of corneas for transplantation. Though the original procedures for corneal transplantation involved full-thickness transplantation, developments in the field have led to partial-thickness transplantation becoming the procedure of choice relative to penetrating keratoplasty. Partial corneal transplantation procedures are associated with improved outcomes and faster healing time. The development of these procedures would not have been possible without eye bank participation. Eye banks are now responsible for the preparation of this delicate tissue prior to implantation. In the future, eye banks will continue to play a significant role both in scientific inquiry and the preparation of new treatment modalities prior to implantation.

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Insights from Clinical Trials in Corneal Surgery

28

Jonathan H. Lass and Rony R. Sayegh

Introduction

Advances in corneal surgery have been marked by the successive introduction of innovative techniques by pioneers in the field. Since Dr. Eduard Zirm performed the first successful penetrating keratoplasty (PKP) in 1905 [1], a number of novel corneal transplantation techniques have been introduced including Descemet stripping automated endothelial keratoplasty (DSAEK),

Descemet membrane endothelial keratoplasty (DMEK), and deep anterior lamellar keratoplasty (DALK), as well as advances in keratoprosthesis surgery (Fig. 28.1). Each technique once introduced by innovators has been subsequently refined with additional surgical experience, typically at the same single site, and with longer follow-up, resulting in greater insights as to factors impacting graft success, visual results, astigmatism, and endothelial cell loss. Larger case-control, cohort, and less commonly randomized clinical trials (RCT) frequently follow, performed in one or multiple centers, further validating the procedure and reporting outcomes.

RCTs are the gold standard of study designs, producing the highest level of evidence [2]. The Collaborative Corneal Transplantation Studies (CCTS) [3], the Cornea Donor Study (CDS) [4] and its Specular Microscopy Ancillary Study (SMAS) [5], and the Cornea Preservation Time Study (CPTS) [6–8] are seminal RCTs that have provided greater insights as to factors impacting graft success and endothelial cell loss. These studies represent the few number of National Eye Institute-supported RCTs, since its founding over 50 years ago that have significantly impacted our understanding and practice of corneal surgery. In addition, the Keratoprosthesis Cross-Linking Study is an ongoing RCT stemming from the pioneering work of Dr. Claes Dohlman in the development of the Boston keratoprosthesis, originally

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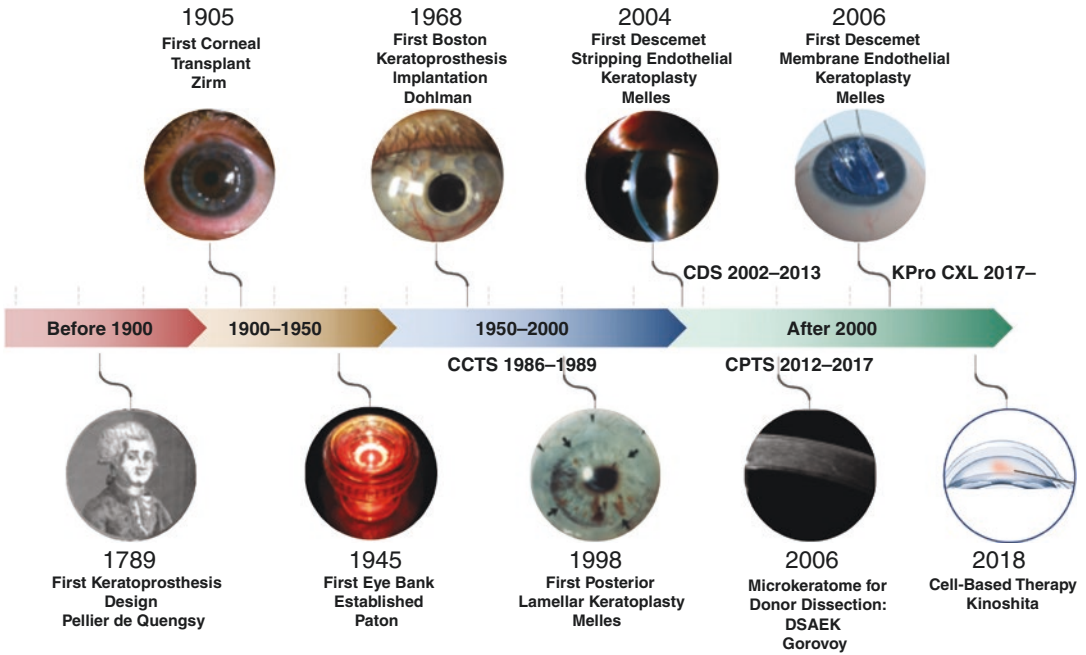


Fig. 28.1 Timeline of the major advances in corneal surgery and the seminal clinical trials discussed in the text (not drawn to scale). (Reprinted with permission from: Bibliothèque de l'Académie nationale de médecine. *Expert Review of Ophthalmology* 2016;11(5):377–82,

Duraent Biologicals Ltd., duraent.com, *Ophthalmology* 107:1850–1857. Fried S, Sayegh RR. Standardizing DMEK. Biomedical Illustration and design thesis. *N Engl J Med* 2018;378 (11):995–1003)

conceived of in the 1970s [9]. This procedure has offered hope for those patients where keratoplasty is no longer a viable alternative.

Collaborative Corneal Transplantation Studies

In 1991, 40,000 PKPs were being performed in the United States yearly, 10% of which were considered high risk, comprising failed prior PKPs and vascularized corneas from various etiologies. The 2-year survival probability was 90% for low-risk PKPs and 60% for high-risk ones [3]. CCTS was designed as a prospective, randomized, multicenter trial looking at the value of histocompatibility-matching and cross-matching in PKP [10]. Four hundred nineteen high-risk recipients from eight clinical centers were included with corneal vascularization involving two or more quadrants or prior grafts failure from rejection. Eyes with ocular surface problems

such as severe chemical burns, uncontrolled glaucoma, and aniridia were excluded. A standardized postoperative topical corticosteroid regimen was instituted. Donor corneas were randomly allocated in a double-masked manner to be either positively or negatively matched on the basis of the following serologically defined antigens: HLA-A, HLA-B, and HLA-DR. The CCTS found that matching for HLA-A, HLA-B, and HLA-DR antigens had no effect on overall graft survival, the incidence of irreversible rejection, or the incidence of rejection episodes. Indeed, at 3 years after surgery, 24% of the grafts in the low match group and 25% of the grafts in the high match group had failed due to rejection [3].

Donor-recipient ABO blood group compatibility was determined but not used for recipient selection. At 3 years after surgery, the estimated proportion of eyes with graft failure was 41% for the ABO-incompatible group and 31% for the ABO-compatible group. The estimated proportion of eyes with failure from rejection by 3 years

was 30% for the ABO-incompatible group and 16% for the ABO-compatible group. As such, the ABO-matched group had fewer graft failures, rejection failures, and rejection episodes than the negative group; however, these differences were not statistically significant [11].

Subsequent analysis of this high-risk corneal transplantation cohort identified young recipient age, the number of previous grafts, history of previous anterior segment surgery, preoperative glaucoma, quadrants of anterior synechiae, quadrants of stromal vessels, a primary diagnosis of chemical burn, and blood group ABO incompatibility as among the strongest risk factors for graft failure. Donor and corneal preservation characteristics had little influence on graft outcome [12]. Although this study did not show the value of histocompatibility-matching for high-risk keratoplasty, the study did pave the way for the conduct of masked prospective clinical trials to study important questions surrounding eye banking and keratoplasty and stressed the value of frequent follow-up and good compliance. There also was no need for the added time and expense awaiting an HLA-matched cornea [13]. Finally, CCTS negative findings spurred continued efforts to understand the immunology of keratoplasty rejection in order to find novel methods to prevent rejection [14] as well as the development of the Boston keratoprosthesis program under Dr. Dohlman [15].

Cornea Donor Study (CDS)

The CDS was designed as a prospective, multicenter, double-masked, controlled trial to determine the role of the age of the corneal donor tissue (younger than 66 years compared to 66–75 years) for long-term graft survival following PKP for moderate risk for failure conditions including Fuchs' dystrophy and pseudophakic/aphakic corneal edema (PACE) [16]. The study was developed by Drs. Edward Holland and Mark Mannis to address the concerns regarding the use of older donors over 60 years for these indications and if successful, showing non-inferiority, expanding the donor pool. Enrollment

for the study was completed in 2002 and included one eye from each of 1090 recipients 40–80 years of age. Eligible corneas were from donors 10–75 years old with a central endothelial cell density (ECD) of 2300–3300 cells/mm² and good to excellent rating by the eye bank. PKPs were performed by 105 surgeons at 80 sites in the United States. Graft failure was defined as a cloudy cornea that was sufficiently opaque to compromise vision for at least three consecutive months or the performance of a repeat graft.

The results showed the cumulative probability of graft success was 86% at 5 years [17] and 75% at 10 years in this intermediate-risk population [18]. The two main donor age groups (12–65 years and 66–75 years) had no significant difference in graft survival rate at 10 years, but there was a suggestion of a donor age effect at the extremes of the age range [19]; a slightly higher success rate was noted in PKPs performed with corneas from donors aged 33 years and below and a slightly lower rate of success for donors aged 72 years and above [17]. The graft failure rate was significantly higher in eyes with PACE compared to those with Fuchs' dystrophy (30% vs. 20%), as well as in eyes with a previous history of glaucoma, especially if prior glaucoma surgery had been performed. While lens status in Fuchs' did not seem to influence the probability of success, eyes with PACE and having an anterior chamber IOL were associated with a higher risk of graft failure (59%) which was reduced to 23% if exchanged for a posterior chamber IOL. The ECD and corneal thickness at 6 months, 1 year, and 5 years were strongly associated with an increased probability of graft failure [20].

Donor characteristics including gender, history of diabetes, and cause of death, as well as retrieval and timing factors including type of tissue retrieval (enucleation vs. in situ), time from death to preservation, whether the body was refrigerated, time from death to refrigeration, and time from death to surgery, had no significant impact on graft survival outcomes. Similarly, donor cornea characteristics including endothelial characteristics such as baseline ECD, polymorphism/polymegathism, and endothelial cell damage, as well as epithelial and stromal changes,

did not impact graft survival [21]. Conversely, in a subset of 500 grafts, 17 of which failed, which had preoperative and postoperative ECDs obtained, a strong correlation was found between the ECD at 6 months with graft failure from endothelial decompensation. In fact, the 5-year endothelial failure rate of grafts with fewer than 1700 cells/mm² at 6 months was six times higher (13%) that of those with 2500 cells/mm² or more (2%) [20]. The probability of graft failure at 10 years was 29%, 10%, and 2% for graft with an ECD at 5 years of less than 500 cells/mm², 500 to 1499 cells/mm², and more than 1500 cells/mm², respectively. In a multivariate analysis, both 1-year corneal thickness and ECD were associated with subsequent graft failure ($P = 0.002$ and 0.009) [22]. No other donor factors, including ABO matching, donor age [19], and diabetes history [23], and no operative factors correlated with graft failure at 10 years.

The cumulative probability of definite rejection was 10% during the first 5-year period, and this increased to 12% by the end of the 10-year follow-up period [24]. According to recipient diagnosis, the cumulative probability of a rejection event among patients with Fuchs' dystrophy was 13% and increased to 21% among those with PACE. Among eyes with Fuchs' dystrophy, a higher probability of a rejection event was observed in postoperatively phakic eyes compared with those that underwent cataract extraction with or without intraocular lens implantation during PKP. There was a trend for increased risk of rejection in older patients (70 years or older), patients with a history of smoking, and those of African American race. Female gender was also associated with a higher risk of rejection. No significant association was found with donor age, ABO compatibility, or any other donor factors [24–26]. At the 10-year follow-up, the only baseline factor that was found to be significantly associated with a higher risk of definite graft rejection was a preoperative history of glaucoma, especially if previous glaucoma surgery was performed and anti-glaucoma medications were used at the time of PKP. Of those patients who experienced a definite rejection event, 69% went on to graft failure by the 10-year follow-up.

The CDS paved the way for the use of older donor tissue for keratoplasty and provided more flexibility in tissue placement not tied to the highest preoperative ECD for graft success. This has proven extremely helpful as endothelial keratoplasty has favored the use of older donor tissue to assist in lenticule positioning.

Cornea Preservation Time Study

Hypothermic (2–8°C) corneal donor storage solutions are approved by FDA for storage of donor corneas for up to 14 days; however, we reported in a survey [6] that 20,852 (96%) of donor corneas used for keratoplasty in the United States in 2011 and 2012 from 19 eye banks were less than 8 days preserved despite the lack of scientific evidence showing superiority of tissue with shorter preservation time (PT).

In response to this practice, the CPTS was a randomized clinical trial that was conducted at 40 clinical sites and 70 surgeons with the objective of determining the effect of PT on graft success and endothelial cell loss 3 years postoperatively in eyes undergoing DSAEK for cornea conditions associated with endothelial dysfunction and moderate risk for graft failure, Fuchs' dystrophy, or PACE [6]. A total of 1090 recipients (1330 eyes) were included. The median age was 70 years, 60% were female, 90% were white, 18% were diabetic, 52% were phakic, and 94% had Fuchs' dystrophy [6]. Eyes were randomized to receive donor corneas stored ≤ 7 days or 8 to 14 days. Eyes with failed PKP, failed DSAEK, tube shunts, uncontrolled glaucoma, anterior chamber intraocular lens, or anterior synechiae for more than 3 clock-hours were excluded.

Of the 1330 donors in the study, 49% were older than 60 years of age, and 27% were diabetic. The median eye bank-determined screening ECD was 2688 cells/mm², and 74% had their lamellar dissection for DSAEK eye bank prepared [6]. Donor corneas met EBAA standards for DSAEK. Surgeons were masked to all donor and tissue information.

The 3-year cumulative probability of graft success was significantly higher in the 0–7-day PT

group at 95.3% compared to 92.1% in the 8–14-day PT group; however, the difference in rates was small when PT was less than 12 days [8]. Graft failure was observed in 30 out of the 675 grafts (4%) in the 0–7-day group and 49 of 655 grafts (7%) in the 8–14-day group. Primary and early failure rates in the first month were about twice as high in the longer PT group. This difference between the two groups was small afterward, suggesting that loss of endothelial cell function during longer-term storage may lead to early failure in a small number of donor corneas. Donor positioning issues occurred in 16% of the 0–7-day group and 22% of the 8–14-day group. Recipients of grafts with longer PT, from diabetic donors, and with operative complications had increased risk for postoperative graft dislocation [27].

A total of two eyes (0.15%) developed fungal and one eye (0.08%) developed bacterial infections after DSAEK in the CPTS. Surgeon-prepared tissue remained a significant risk for positive fungal cultures, whereas younger donors and accidental death were at a greater risk for positive bacterial cultures. This reflects a low rate of postoperative infections and a lack of significant effect of PT on the rate of positive rim culture [28].

At the initial eye bank tissue screening, the mean central ECD was 2746 ± 297 cells/mm² in the 0–7-day preservation-time group ($n = 485$) and 2723 ± 284 cells/mm² in the 8–14-day group ($n = 460$). At 3 years, the mean ECD decreased from baseline by $37 \pm 21\%$ to 1722 ± 626 cells/mm² in the 0–7-day group and by $40 \pm 22\%$ to 1642 ± 631 cells/mm² in the 8–14-day group. When analyzed as a continuous variable (days), longer PT was associated with lower ECD. Endothelial cell loss was comparable for donors preserved for 4–13 days [7]. Donor PT 12–14 days, donor diabetes, and intraoperative surgical difficulty were associated with higher endothelial cell loss at 3 years. A recipient diagnosis of PACE compared to Fuchs' dystrophy was also associated with increased cell loss at 3 years [29].

The CPTS subsequently examined through a multivariable analytic approach the donor (besides PT), recipient, and operative factors impacting graft success and cell loss following

DSAEK [29, 30]. Confirming previous DSAEK studies, PACE and the occurrence of operative complications led to a higher risk for graft failure. But surprisingly, diabetes in the donor also led to a higher risk for graft failure, particularly primary and early failures, unlike previous PKP, DSAEK, and DMEK studies [30]. Similarly, greater endothelial cell loss occurred in the clear grafts at 3 years in the donors with diabetes and as expected in those eyes with preoperative PACE and operative complications [29]. These findings we believe will stimulate greater interest through prospective trials as to the role of the severity of diabetes in the donor and recipient in order to delineate which diabetic donors are suitable for keratoplasty and which are not.

Finally, the CPTS examined the donor, recipient, and operative factors for graft rejection and the consequences of the rejection episode [31]. The cumulative probability of definite DSAEK rejection was low (3.6%) at 3 years in the CPTS. Surprisingly the only factor that was associated with a higher rejection rate was younger recipient age in a study cohort mostly >50 years old. This finding from the CPTS is the first trial to show that age is a factor in higher risk for rejection in the adult population for an intermediate-risk indication, Fuchs' dystrophy, and PACE, for a primary keratoplasty procedure supporting more prolonged topical corticosteroid usage in this population and closer observation postoperatively.

Keratoprosthesis Cross-Linking Study

The Boston keratoprosthesis (KPro) was developed as an alternative to PKP in eyes who will fare poorly with traditional corneal transplantation [32]. These include eyes with multiple failed PKPs and those with cicatrizing, immune conditions such as Stevens-Johnson syndrome and chemical burns [33]. Despite a high retention rate of around 95%, corneal melts remain an important complication in eyes implanted with the Boston type 1 keratoprosthesis occurring in around 16% of cases [34]. Significant risk factors

for corneal melt include previous infectious keratitis and a diagnosis of Stevens-Johnson syndrome, mucous membrane pemphigoid, or chemical injury [35]. Anecdotal evidence shows that collagen cross-linking (CXL) of donor corneas confers resistance to keratolysis and prevents corneal melting in high-risk patients [35, 36].

The KPro CXL Study was therefore designed as a phase I/II prospective, randomized, multicenter, double-masked, vehicle-controlled clinical trial evaluating the safety and efficacy of CXL the KPro carrier tissue in subjects who are candidates for high-risk KPro implantation. The study is currently enrolling 84 patients who are being randomized to receive either a de-epithelialized cornea cross-linked with riboflavin 0.1% with dextran 20% solution and ultraviolet A light or a de-epithelialized cornea that is exposed to only riboflavin 0.1% with dextran 20% solution but no ultraviolet A light (control arm). Patients will be followed for 2 years, and the primary outcome measured is time to device loss or replacement. Outcomes and complications will be assessed over the duration of the trial.

Insights and Impact of Clinical Trials

RCTs remain the gold standard for evaluating novel corneal surgery techniques. They provide the robust, high-quality data, needed for comparative effectiveness of the various corneal interventions. The CCTS and CDS had a tremendous impact on the way surgeons have managed corneal conditions and CPTS is well on its way to do the same [37]. With DMEK gaining momentum as the next standard in endothelial keratoplasty, this procedure may benefit from the insights obtained into DSAEK outcomes from the CPTS, particularly as donor and donor cornea risk factors may be common to both procedures [38]. Furthermore, novel corneal procedures are continually being introduced including Descemet stripping without endothelial keratoplasty (DSO) [39] or descemetorhexis without endothelial keratoplasty (DWEK) [40] for Fuchs' dystrophy, cell-based therapy with the injection of cultured corneal endothelial cells

into the anterior chamber supplemented with a rho-associated protein kinase inhibitor [41], and 3D bioprinting of corneal constructs [42], all of which could benefit from RCTs in the future as they find their place into the cornea surgeon's armamentarium.

This rapid rate of innovation in the field of corneal surgery poses a challenge to RCTs which typically require extensive preparation that can significantly delay the initiation of the trial. There may also be selection bias for the surgeon with a certain skill level and patients with access to participating medical centers, which also need to have sufficient resources to participate in trials. The increasing popularity of big data and registries such as the American Academy of Ophthalmology's IRIS Registry [43] provides the opportunity to effectively address many of these issues by providing observational data, improving the accuracy of sample size calculation and patient inclusion criteria, and standardizing data designation, nomenclature, and exchange.

Besides the challenge of developing these technologies, and the methodology for the RCTs, funding these studies remains an important challenge that partnership between the government, academia, private practitioners, and industry is crucial in addressing. We must continue to be able to conduct these studies to maintain corneal health and ultimately the quality of lives of our patients.

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Clinical Trials in Dry Eye Disease: What We Have Learned and What We Still Need to Understand

29

Gary N. Foulks

Introduction

Clinical trials in dry eye disease (DED) have taught us much about the disease but have often failed to provide evidence sufficient for regulatory approval of new pharmaceutical therapies [1]. Some of the difficulty is due to the nature of dry eye disease, while much of the problem is due to clinical trial design issues. The two major types of clinical trial in dry eye disease are environmental trials and controlled adverse environment (CAE) trials which differ in population selection and operational detail [2]. The environmental trial includes a broad selection of subjects who qualify based upon a confirmed diagnosis of DED and who are evaluated in their usual environment and activity level. The CAE trial selects subjects based upon their response to the controlled adverse environment that includes controlled humidity, airflow, and level of visual activity [3, 4]. CAE trials are particularly helpful in demonstrating a pharmacological effect in an enhanced population, but those results often have less generalizability to the broader population. Currently the CAE method is accepted by the US Food and Drug Administration (FDA) as a basis for approval, but no pharmaceutical agents for

treating DED yet have been approved solely upon such trials (Gary Novack, PhD, April 2018, personal communication). The benefits and accomplishments of CAE trials have been recently reviewed [3, 4].

Although a number of novel device approaches to treating aspects of DED, including LipiFlow™ and TrueTear™, have been approved by the FDA, the discussion of this chapter will focus rather on clinical trials for pharmaceutical treatments of DED.

Results of Previous Clinical Trials

An extensive review of clinical trials for the various treatments of dry eye in Sjögren's disease has been conducted and reveals that in the past 15 years there have been published results from 22 randomized, controlled clinical trials involving 9 topical, 10 oral, 2 intravenous, and 1 subcutaneous modalities of treatment that reported ocular outcomes [5]. All of the topical trials enrolled small numbers of subjects, only two were double-masked, and the reports did not comply with Consolidated Standards of Reporting Trials (CONSORT). Of the topical treatments, fluorometholone was more effective than nonsteroidal drops [6]. Of the oral treatments, pilocarpine was superior to topical lubricants or punctal plugs but with a high incidence of adverse gastrointestinal effects, and cevimeline was superior to placebo.

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Neither is approved in the USA for treatment of dry eye but rather for treatment of dry mouth [7–9]. The parenteral treatment trials of etanercept and rituximab failed to show benefit in ocular signs and symptoms [10–12].

Numerous clinical trials have been conducted in non-Sjögren (DED), but only two prescription pharmaceutical agents have been approved by the FDA: topical cyclosporine (Restasis™) and lifitegrast (Xiidra™) [13, 14]. Clinical trial work on improved lubricants continues [15, 16], but the most extensive clinical trials address anti-inflammatory options [17, 18].

Vagaries of Clinical Trials in DED

There are many unique features of dry eye disease that complicate design of clinical trials. The multifactorial etiologies of tear film instability and ocular discomfort include reduced tear secretion, increased evaporation, elevated tear osmolarity, inflammation of the ocular surface, and neuropathic changes in the eye. These features are recognized in the recent definition of dry eye disease published by the TFOS International Conference on Dry Eye (DEWS II) in 2017 [19]. One of the major problems is the often poor correlation of signs and symptoms in dry eye disease, which has been well documented in numerous clinical trials [20–22]. Some of this lack of correlation could be due to the paradox of increased severity of surface disease leading to decreased sensation of the ocular surface with lower symptoms despite increased objective severity of disease [23–26]. Likewise, in early disease, there can be a heightened sensitivity of the cornea that results in greater symptoms despite little objective evidence of DED [25].

The features of tear film instability and inherent variability of some parameters of the tear film have implications regarding clinical trial design. The methods to measure mechanical instability of the tear film have been shown to be quite variable, although newer techniques have been reported to reduce that variability [27]. Osmolarity of the tears also has variability in the dry eye patient but not in the normal patient and

therefore can be a reliable diagnostic parameter but requires a more comprehensive measurement in a clinical trial of efficacy, i.e., describing the change in degree of variability as well as a mean or individual measurement [28, 29].

Very importantly for clinical trial design is the problem of placebo effect [30]. All published clinical trials but one have demonstrated pronounced placebo response which may be a combination of true placebo response but also lubricant response to vehicle [31–34]. A true placebo is considered a maneuver, instruction, or substance that in itself provides no benefit to the condition being treated and hence could be useful to exclude any apparent beneficial effect that would be attributable to the patient's desire to respond to therapy [31]. In dry eye disease, however, there is the possibility that increased lubrication of the ocular surface due to a placebo or the vehicle will improve both symptoms and signs of the disease, complicating comparison with the treatment under study [32]. In the following pages, an attempt is made to provide an overview of some specific challenges and pitfalls of clinical trials in dry eye disease and the modifications in clinical trial design that may remove such obstacles in future trials.

Challenges and Pitfalls Identified in Early Clinical Trials in DED and Progress to Date

Recognition of some inherent difficulties in conducting clinical trials in dry eye disease prompted in the mid-1990s a conference composed of academicians, eye care practitioners, members of the pharmaceutical industry, staff of the National Eye Institute, and representatives of the US Food and Drug Administration to develop a categorization of dry eye patients and recommendations for standardized testing for the classification, diagnosis, and evaluation of dry eye disease and its resulting damage to the ocular surface [35]. The designation of two broad categories of dry eye, aqueous deficient and evaporative, was further refined to identify two subcategories of aqueous deficient due to

Sjögren's syndrome and non-Sjögren's dry eye in recognition of the greater severity and difficulty in treating the Sjögren's dry eye. Subsequent studies have shown that the concurrence of aqueous deficient dry eye and meibomian gland dysfunction MGD, the most common form of evaporative dry eye, can be from 40% to 85% [36, 37].

Some specific challenges and pitfalls in clinical trials in dry eye disease have been reviewed in a publication from 2002 (Table 29.1) [30]. Avoidance of these pitfalls has been advocated by the International Workshop on Dry Eye (DEWS) 2007 report [2] as well as the TFOS Workshop on Meibomian Gland Dysfunction [38]. Recommendations to avoid bias in subject selection and statistical analysis in clinical trials have been detailed in the TFOS International Workshop on Contact Lens Discomfort [39]. The most recent recommendations and guidelines for design and anal-

ysis of clinical trials in dry eye disease were published in 2017 in the TFOS DEWS II Clinical Trial Design Report [40].

Probably the most important feature in a clinical trial of dry eye therapy is recognizing the mode of action (MOA) of the proposed therapy and designing the trial to test parameters that are likely to be improved in the expression of the dry eye state. Obviously, for an intervention that stimulates aqueous secretion, the endpoints of tear volume and tear production would be important as well as features of composition and the protection of the ocular surface. For treatments that reduce inflammation, the more likely parameters of interest would be conjunctival vascular dilation and reduction of biomarkers of inflammation such as cytokines or metalloproteinases in the tear film as well as characteristics of cellular infiltration in the conjunctiva. Typically signs of damage to the ocular surface would also be important outcomes.

Table 29.1 Pitfalls in clinical trials of treatments for dry eye

Pitfall	Possible cause
Placebo response	Desire to have a beneficial response
	Better compliance with treatment schedule
	Control (vehicle) has inherent beneficial lubricant effect
Inclusion criteria	
Definition of dry eye	
Symptoms	Confounding symptoms due to blepharitis or non-dry eye diseases
Signs	Confounding staining due to intercurrent disease or prior therapy (e.g., preservative effect)
Severity of disease	Mean severity of disease not great enough to allow 25% improvement
	Restriction of study population to one severity level reduces generalizability
Outcome measures	
Inappropriate outcome	Outcome parameters that do not measure the likely effect of the therapy (e.g., Schirmer test when treatment does not involve tear stimulation)
Single measurement in time	Repeated measures analysis may be needed in measuring chronic disease
Subjective measures	
Symptom grading inadequate	Verbal descriptor too limiting, cultural language disparity
Objective measures	
Wide variability, center effect	Lack of standardization of techniques across investigative sites
Surface staining variability	
Fluorescein	Concentration too great or inadequate mixing with tear fluid
	Measuring too quickly or after inappropriate delay
Lissamine green	Inadequate amount of stain
	Measuring too quickly or after inappropriate delay
	Illumination too bright
Surface staining grading	Lack of standardization
	Truncated scale on extremes that do not allow resolution of response

Modified from Foulks [30]

Appropriate inclusion and exclusion criteria are also essential for a successful clinical trial. If subjects are recruited solely upon the basis of symptoms, there can occur a selection bias that may compromise the trial, particularly since DED shares symptoms with other ocular surface diseases such as allergic conjunctivitis and blepharitis which should be excluded from the trial [3, 41]. Likewise, if potential subjects have been using topical or systemic treatments that would affect subjective or objective parameters that are being evaluated in the clinical trial such treatments should be discontinued well in advance of entering the trial (e.g., topical cyclosporine, lifitegrast, corticosteroids, autologous serum).

Selection of primary endpoints and outcome parameters can be crucial since the subjects often describe symptoms of DED in various terms such as burning, itching, grittiness, sensation of something in the eye, or dryness. It is often helpful to use a very broad descriptor such as discomfort as one parameter and then use an additional more specific descriptor such as dryness, as has been successfully done in at least three recent successful trials [42–44]. Questionnaires have been validated to quantify levels of discomfort or interference with activities of daily life or environmental exposures [45, 46], and a valuable approach has been to capture both severity and frequency of discomfort [47, 48]. Many previous trials have used Likert categorical scales to evaluate symptoms, but more recent success has been achieved with visual analog scales (VAS) [46, 47]. Although disturbance of vision is recognized in the description of DED [2], the advanced instruments to capture this outcome parameter are usually not readily available at clinical sites [49, 50].

The primary outcome parameters for objective signs of tear film function have classically been assessment of tear film volume in the inferior tear meniscus, measurement of tear film instability by the tear breakup time (TBUT), and change in volume with time by the Schirmer test. All of these have some inherent variability in their accuracy. Measurements of composition of the tear film are now possible with the determination of tear film osmolarity and the level of matrix metalloproteinase (MMP9) [51, 52]. As noted previously,

elevation and variability of tear film osmolarity in DED is a very specific diagnostic marker of disease, whereas non-dry eye patients have very stable osmolarity [53]. Evaluation of osmolarity in a clinical trial requires multiple measurements to assess not only a specific level of osmolarity but also the variability of measurements both in time and between eyes and how that variability reduces with time and treatment [28].

Assessment of damage to the ocular surface relies upon the use of vital stains, most often fluorescein and lissamine green solutions. It is important to have a standardized technique across clinical trial sites for both preparation and delivery of the stains, as well as the method of observation/recording of the staining of both corneal and conjunctival portions of the ocular surface [30, 35]. The various grading systems that have been used in the past have measured interpalpebral staining [54, 55] and total corneal and conjunctival staining [35, 56, 57], but newly advocated systems concentrate more on the central and inferior zones of the cornea due to greater responsiveness of the inferior zone and the visual importance of the central zone [43].

Recent clinical trials in both environmental and CAE situations have demonstrated the importance of the inferior area of the cornea as perhaps the most demonstrably responsive to therapy [42, 43]. A grading system often preferred in Sjögren's disease trials is that recommended by the Sjögren's International Collaborative Clinical Alliance Research Group [58].

Determining the appropriate severity of disease both for symptoms and signs is also important. It has long been recognized that subjects should show a level of severity at entry sufficient to allow demonstration of an effect on both symptoms and signs at statistically significant levels. With regard to symptoms, it has recently been shown that subjects who have been consistently using daily artificial tears are more likely to demonstrate an improvement in symptoms [42, 43]. Likewise, recent clinical trials have shown that results are often better for those subjects with more significant levels of discomfort [42, 43].

As mentioned earlier, the placebo response which is so often observed in clinical trials in

DED is a major obstacle to overcome in clinical trial design. The true placebo effect of participation in a clinical trial can occur from the subject’s desire to improve symptoms on therapy, and this effect is often seen in regard to symptoms [31]. Improvement in signs of DED, however, is also frequently seen with the control placebo or vehicle. This may partially be explained by the subject being motivated to greater compliance in use of whichever medication is assigned, and hence vehicle use may improve the lubrication for the ocular surface [30, 59]. Evidence from an analysis of pooled participants in three clinical trials suggests that the amount of placebo response is related to the level of baseline for parameters being studied and that age greater than 40 years increases the placebo response [60].

One attempt to reduce this placebo effect is to use a vehicle run-in period of 2 weeks prior to randomization to active therapy. Such run-in has three potential benefits: The first benefit is that discontinuation of prior topical medications containing preservatives or excipients that damage the ocular surface are removed [61]. The second benefit is that the lubrication effect of the vehicle may be achieved prior to beginning active therapy. The third benefit is a possible reduction of regression-to-the-mean effect that can statistically complicate data analysis [39, 62].

Recommendations for improvement in clinical trial design are summarized in Table 29.2. These recommendations are based upon lessons learned from a better understanding of dry eye disease and results of prior clinical trials.

Evolving Clinical Trial Issues

In the past, the requirement of the US Food and Drug Administration for approval of a new drug was the demonstration of meaningful improvement in both a sign and a symptom of dry eye disease in two replicated clinical trials. Recently, however, the agency has allowed approval based upon clinical trials that demonstrated statistically significant improvement in a sign in two replicated trials and a symptom in two replicated trials but not necessarily the same trials [42, 43]. This is

an encouraging development that could facilitate more approvals. It is still critically important to design the clinical trials in dry eye with regard to prospective approved clinical guidelines and a priori statistical accuracy [63–65].

A broader assessment of symptomatic response will be required in future clinical trials with more attention paid to patient reported outcomes (PRO) [66]. Better assessment of both severity and frequency of symptoms is desirable as is attempted through the SANDE questionnaire [47]. The guidelines for PRO are still being fully developed [66]. We will need to refine quality of life (QOL) assessments that are specific to dry eye disease and meet the expectations of PRO guidelines [67, 68].

Table 29.2 Summary recommendations for clinical trials in dry eye disease

1. Recognize the potential disparity between symptoms and signs; do not couple responses but rather analyze for separate effects upon symptoms and signs
2. Select patients for inclusion based upon the type of dry eye being treated and the mechanism of the drug action
3. When evaluating a small population of subjects with clinical characteristics that suggest greater responsiveness in one subgroup relative to another, pre-stratify subgroups that might be expected to have differential responsiveness to treatment. While this reduces the overall number of patients in each group with implications to statistical power, such an approach preserves the power of randomization to therapy
4. Select the outcome variable based upon the pharmacological effect of the drug to be tested
5. Avoid confounding treatment variables such as ancillary or supplemental therapy that may have an effect on tear function. Control for concurrent HRT, nutritional supplement, or anti-inflammatory therapy
6. Standardize testing procedures across all investigators and investigative sites, paying particular attention to the limitations or necessary caveats of testing methods
7. Select a grading scale that is appropriate for the severity and breadth of change anticipated in response to a given therapy
8. Use visual analog scales (VAS) for symptom evaluation and allow patient to see previous response when providing present response
9. Don’t be surprised by a control response and be alert to possible salutary effects of the selected control, particularly if it is a novel delivery system
10. Consider the use of a controlled adverse environment, particularly when an acute and short-acting pharmacological effect is expected

The increasing recognition of the neurological changes that can produce symptoms similar to the discomfort of dry eye disease (corneal neuropathic pain) [69–73] and the fact that more severe DED impairs sensation such that symptoms can diminish in the face of increasing ocular surface damage require attention. Strategies will need to be developed to control inclusion and exclusion criteria for entry into trials as well as developing analytical methods to detect and resolve such paradox.

Recent restrictions on compounding pharmacies in the preparation and transport of diagnostic solutions such as fluorescein and lissamine green will complicate the operational aspect of clinical trials in DED [74]. Options have been developed to maintain access to standardized diagnostic liquids [74].

Moving Forward

Future clinical trial designs for evaluating treatments for DED should maintain appropriate selection criteria for patient enrollment including documentation of the diagnosis and severity of disease with inclusion criteria based upon demonstrated need and use of topical lubricants prior to entry. It will also be important to enforce high-quality standardization for evaluation of procedures across clinical sites with precise documentation of assessments.

It is important to solve logistical challenges to previously accepted evaluation techniques and to improve upon the accuracy of measurement of features such as TBUT and tear film osmolarity, in order to verify precision and comparability between investigative sites. Continued use of trial design to minimize placebo response with use of vehicle run-in control is advisable.

Development of improved methods to separate and analyze the complex neurobiological changes that occur in DED will be required, particularly in identifying subjects with neuropathic corneal pain versus those with DED. One very helpful step forward would be to accurately identify those subjects with DED whose ocular surface sensation is so impaired that appreciation of pain and discomfort is reduced due to disease and

whose symptom identification is dissociated from the severity of the disease.

There is a need better to identify and validate objective biomarkers of disease activity [25, 75, 76]. Numerous molecules have been identified as being altered in DED, but difficulties in sampling or analysis have prevented validation of such biomarkers for effective and practicable use as outcomes in clinical trials for dry eye disease. Validation of biomarkers and preferably demonstration of an association with symptoms will be essential for obtaining regulatory approval of new therapies.

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Engaging the Next Generation in Cornea: Insights from Cornea Society University

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Jessica Ciralsky

Introduction

A chapter dedicated to engaging the next generation of cornea specialists is very fitting in a book dedicated to Dr. Claes Dohlman, who is often referred to as the father of modern corneal science. His contributions are innumerable and touch all aspects of ophthalmology. One of his most remarkable achievements is in ophthalmic education. He established the first formal cornea fellowship program in the United States and personally trained over 200 cornea fellows. This number grossly underestimates his impact since he has touched the lives and careers of so many other cornea specialists through his protégés, who have gone on to train second- and third-generation cornea specialists who can trace their lineage back to him.

Background

The field of cornea is constantly evolving and progressing at a rapid pace. Engaging the next generation of cornea specialists in research, scholarship, innovation, and teaching will ensure the continued growth and advancement of our field and the emergence of next-generation lead-

ers. This idea spurred the formation of Cornea Society University (CSU), an initiative dedicated to career development for young cornea specialists.

A few years ago, the Cornea Society leadership, under the direction of Dr. Kathryn Colby, a Dohlman fellow, launched an initiative to increase its relevance to young cornea specialists. Through discussions with cornea fellowship program directors, cornea fellows, and young cornea specialists in their first few years of practice, it became apparent that large educational gaps exist in the guidance of young career development. Thus, Cornea Society University was created to address these needs and fill in the gaps for the next generation of cornea specialists.

Medical education is taught in a very traditional way. We impart knowledge that we have learned to trainees through formal lectures, textbooks, and journal articles. In clinic, knowledge is also passed on during hands-on patient care, with the attending often discussing a case and transferring specific knowledge about the clinical findings, differential diagnoses, assessments, and plans. Professional development and career guidance, on the other hand, has typically been approached with a “figure it out on your own” mentality. Cornea Society University was created to help develop a more robust career development curriculum. Younger generations should be able to learn from previous generations’ successes

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and failures. The wheel should not need to be recreated with every graduate.

During the 2015 Cornea Fellows Educational Summit, a meeting that is attended by most US cornea fellows each year, the first Cornea Society University programming was launched. Several interactive sessions were held dedicated to different topics on career development including choosing a career path, work-life balance, and negotiating contracts. Due to positive feedback received during these sessions, Cornea Society University became a permanent part of the curriculum for the Cornea Fellows Educational Summit each year. In 2016, the program was expanded into a yearlong educational platform dedicated to cornea fellows and young cornea specialists to provide ongoing education throughout the academic year. This initially involved monthly e-newsletters that featured articles addressing both clinical education and career development and videos of previously recorded Cornea Fellows Educational Summit lectures.

In 2017, further expansion of Cornea Society University led to the launch of a dedicated interactive website corneasocietyuniversity.org. On the website, there are several unique features including a dedicated young keranet, past issues of Cornea Society University, and a calendar of events. There is a robust video education section that includes past videos of Cornea Fellows Educational Summit lectures, Cornea Society University webinars, and professional development interviews. There is also a forum to submit challenging cases to solicit feedback and advice from colleagues, showcase a new technique, or show a complication and the resultant teaching pearl. The most recent expansion has included the addition of networking events that are held twice a year, one at the American Academy of Ophthalmology meeting and the other at the American Society of Cataract and Refractive Surgery meeting. These events were developed to provide young cornea specialists the opportunity to interact and network with colleagues, expand their peer group, and learn about a specific professional development or practice-building topic.

Understanding and Adapting

In order to engage the next generation, we first must understand them and ourselves. Although every person is unique, there are generational differences with distinct characteristics associated with each generation. In medicine, Traditionalists, Baby Boomers, Generation Xers, and Millennials comprise the four generations encountered in the workplace. Traditionalists, the generation born between 1925 and 1945, are often referred to as the “silent generation.” Many senior faculty members and patients fall into this generation, known for their strong work ethic and all-work-and-no-play attitude [1, 2].

Baby Boomers, those who were born between 1946 and 1964, are often in senior roles and have leadership positions in the workplace. They are known as workaholics who live to work and define themselves through their work. Generation Xers were born between 1965 and 1979 and often hold junior or mid-level roles at work. They are often described as cynical, skeptical, and technology savvy. They believe in work-life balance and commonly make family a priority. Many Millennials are children of Baby Boomers or Generation Xers [1, 2].

Millennials, also referred to as Generation Y, were born between 1980 and 2000. Our current medical students, residents, fellows, and young ophthalmologists in their first 5 years of practice are Millennials. Millennials are the next generation of ophthalmologists, the generation we are trying to engage. Understanding their characteristics, needs, and priorities can help all generations work together more seamlessly. Millennials are realistic, globally conscious, team oriented, inclusive, and safety conscious. They have shorter attention spans and are accustomed to getting knowledge instantly through technology. Often referred to as “digital natives,” they grew up with technology and are technology experts who expect to constantly be connected to technology. They are active learners and flourish in an interactive group setting as opposed to a traditional lecture. Additionally, mentorship is important to them and they enjoy frequent communication, guidance, and encouragement [1, 2].

Armed with this knowledge about the typical characteristics of Millennials, we should adapt and modify traditional engagement efforts. Strategies that have worked well for other generations will not work well for this generation. Cornea Society University's experience with engaging the Millennial generation has taught me that our efforts should be focused on (1) mentorship; (2) work-life balance; (3) technology-driven strategies; and (4) community building.

Mentorship

The origin of the word *mentor* dates back to Homer's *The Odyssey*. In the poem, "Mentor" was a friend of Odysseus and advisor of Telemachus, his son. The modern English definition reflects this origin with mentor defined as a "wise and trusted counselor or teacher." Although mentorship is extremely important throughout one's career, it is even more important during one's training and in the first few years out of training. Millennials crave mentorship and when queried about unmet needs in career development, they repeatedly request more consistent, meaningful mentorship opportunities.

The mentor-mentee relationship is an incredible opportunity for both the mentor and the mentee. Ophthalmology is known for innovation and progress, and the next generation will continue to advance our field through the development of new techniques, new diagnostic tests, and new treatments. For the mentor, you have the privilege of fostering this next generation of ophthalmologists and playing a role in shaping and advancing our field. Your mentees' successes and accomplishments will be your legacy, your imprint on our field. Many mentors are motivated by the desire to give back to the next generation as our mentors once did for us. For the mentee, you gain wisdom and insight from colleagues who have already traversed a similar path. Mentors often have invaluable advice they have learned through mistakes and achievements in their own career. Mentors can also help you build a larger network and community within ophthalmology.

Finding a mentor is the initial step and often the most difficult step. Although we would all love to find one mentor who could guide us throughout our career and help every step of the way, this is often unattainable. Our needs are dynamic throughout our career, and we often need different mentors at different stages. Finding a mentor starts with defining one's current needs or concerns that would benefit from the guidance of a mentor. At the beginning of one's career, advice may focus on finding a job and all of the questions that arise in that arena: academics vs private practice, solo vs group practice, location, etc. At later times in our career, a desirable mentor may be one with specialized knowledge related to a novel technology or surgical procedure you are hoping to adopt in your practice. And at other times, a desirable mentor may be a local ophthalmologist who can advise one on the local opportunities and job market in a given location.

Once you have defined the scope and focus of the mentorship needed, you next need to actually find a mentor. Many times, the mentor-mentee relationship forms naturally through residency or fellowship opportunities. Other times, however, despite a great deal of effort on the mentee's part, a "natural" mentor-mentee relationship does not form. In these cases, potential mentors may need to be sought out more formally. At ophthalmology meetings, you may approach a potential mentor, someone you respect and whose career you would like to emulate, and ask for advice. You may also approach potential mentors through e-mail introductions or through mutual colleagues.

Cornea Society University is launching a new program, which is being spearheaded by Dr. Peter Veldman, dedicated to mentorship. The Defined Scope Mentoring Program is a formal track to find a mentor, a program hoping to fill the gap for mentees who have been unsuccessful in finding a mentor on their own. This program pairs junior and more senior members of the Cornea Society together for a targeted mentorship arrangement that lasts a defined amount of time, typically around 4 months. The mentor-mentee relationship will focus on a specific need or concern for the mentee for which the mentor has expertise or experience.

Regardless of how the mentor-mentee relationship forms, both parties need to be dedicated to making the relationship impactful. Mentees must often take the initiative to set up meeting times, either by telephone or in person. They should establish early on the best form of communication with the mentor to ensure good correspondence. Mentors are often overextended with commitments and should therefore only agree to take on a mentee when they can allot the required time and effort needed to make the experience valuable for both parties.

Work-Life Balance

Our next generation of ophthalmologists, those currently in ophthalmic training or recent graduates, is of the Millennial generation. Work-life balance is an important priority for Millennials, and many of them will evaluate a career or job opportunity based on the ability to achieve good work-life balance. Engaging the next generation requires one to be cognizant of these priorities.

A national survey conducted several years ago evaluated burnout and work-life balance in physicians. Doctors were more likely to experience burnout symptoms and were more likely to be dissatisfied with their work-life balance when compared with other working US adults. Although ophthalmologists fared slightly better than most physicians, there was still a sizable percentage of ophthalmologists who reported burnout symptoms (43.4%) and dissatisfaction with work-life balance (43.2%) [3].

Finding work-life balance should be a priority for all ophthalmologists. Achieving this balance takes time and effort. It does not just happen naturally for anyone. It starts with self-reflection. You must know yourself and your personal goals and then actively manage your time and set priorities. It is important to recognize that this is a dynamic process that needs to be reassessed often as competing priorities only seem to multiply as you get older. Take the time to reflect on what is and is not working and reprioritize to make sure you are coming closer to achieving the balance you need.

Engaging the next generation requires us to help them achieve the work-life balance they desire through mentorship, flexibility, and work-life blend activities. As a mentor, you can help guide Millennials through the process of finding their work-life balance. Although you may not have all of the answers, your support and guidance can lay the groundwork for their journey. Flexibility is one of the most coveted characteristics of a desirable job for Millennials. In medicine, there are certain restraints that will lend some rigidity to our schedules, but there are still opportunities for some flexibility. Some practices may allot time off for ophthalmologists to take part in medical missions, which is extremely desirable for Millennials who are civic-minded and globally conscious. Flexibility may also be as simple as adjusting start times back 30 minutes to allow parents to drop their children off at school in the morning. Work-life blend activities include family-friendly meetings or meetings that blend education with a favorite hobby. When you attend a meeting, you take time away from the office and your family, and this may limit the number of meetings you can attend in a given year. By combining family time with a meeting or combining a favorite activity like skiing with a meeting, you may encourage Millennials to participate in more meetings.

Technology-Driven Strategies

Engagement strategies for Millennials need to cater to their values and needs. Technology is an integral part of a Millennial's life. They grew up with technology and expect to be connected to up-to-date technology at all times. They are also very open to change and do not accept that things should be done a certain way just because that is how they have always been done. This applies particularly to communication and education.

For many older ophthalmologists, participating in social media may seem unnecessary for the practice of medicine or medical organizations. Why would patients and other physicians need to connect through Facebook or Twitter or Snapchat? But for Millennials, social media is

often a preferred form of communication. If you want to reach Millennials, both patients and younger physicians, you have to embrace social media and use it to everyone's advantage. Upcoming meetings or organizations should have a social media presence where potential attendees or members can interact and participate. This builds traction for a meeting or buzz for an organization and keeps members engaged. Social media sites need to be actively managed and updated to hold Millennial's attention.

Education has always been linked to technology for Millennials. When they want to answer a particular question, they do not try to look it up in the index of a textbook; they simply ask Siri or Google the answer. This should not be looked at as negative. The Internet provides up-to-date information that is available at any time with the click of a button. We should try to learn from Millennials and harness this technology-based learning to advance the practice of medicine. We should also take time to teach our next generation how to assess the validity and accuracy of Internet resources on which they rely to ensure they are learning the correct material [1, 2].

Educators need to understand Millennials' learning preferences in order to design teaching plans that appeal to the next generation. Traditional lectures with slides full of text or long prose-filled textbooks are outdated and unappealing to most Millennials. They want interactive lectures with question response or audience participation, case-based lectures where diagnostic dilemmas or treatment questions are presented, or video-based lectures to hold their attention. Millennials are often referred to as good multitaskers with short attention spans which is probably a result of the hyperconnected world they grew up in. For this reason, many Millennials learn best when information is created in short bite-sized units which are easily retained and absorbed. Design your lectures as if they are a string of 150-character tweets or 2-minute high-quality high-content videos rather than a long soliloquy on your favorite subject. Group learning is another strong preference among Millennials. Educators should

encourage collaborative learning and design projects that take advantage of group learning.

E-learning is often a preferred method of obtaining information. YouTube and Eyetube are commonly referenced and used in surgical education. These sites allow you to see different techniques in real time. Many Millennials learn new techniques or modify known techniques through rigorous study of surgical videos posted online. Discussion forums are another great e-learning tool as they allow one to post videos or case presentations to a group of colleagues to stimulate discussion or ask for advice on a particularly challenging case. It can often be intimidating for the younger generation to post cases on a larger forum where some of the experts in the field may chime in with comments. Cornea Society University created a young keranet as an outlet for Millennials to post complex cases to their own peer group. Webinars and previously recorded lectures that can be accessed at any time are also desirable ways of learning. Millennials want instant access to information in real time. They often want to search for answers or learn more about a disease state right after seeing an interesting patient in the office. Ensuring that high-yield information is available online in a digestible format will help our next generation learn in a fashion in to which they are accustomed [1, 2].

Community Building

Millennials thrive on communication and connectedness and often learn best in group settings. Once they leave residency or fellowship and the built-in camaraderie among trainees, their practices may feel lonely. Building a community of ophthalmologists, in particular of ophthalmology peers, can help Millennials thrive. For many, community building will take place online through social media or chat groups; however, there is still a need for in-person community building.

With all of the available e-learning options and live streaming, it may seem that actually attending ophthalmology meetings for education purposes is unnecessary. Although education is still an

important reason for attendance, community building is probably the bigger draw for our Millennial generation. Ophthalmology meetings are a great way to network, both with your peer group and with other ophthalmologists in your field. It is important to find and cultivate your peer network as these are the people you will grow up with. As you mature in your practice, you will experience successes and hit roadblocks. Many of your peers will be experiencing similar challenges as you try to navigate balancing a new family with work obligations or juggling teaching, research, and clinical duties. Tapping into this collective wisdom and asking for advice can be invaluable. Cornea Society University hosts two networking events each year for young cornea specialists. The main purpose of these events is to have a dedicated time and space away from the meeting to interact with peers. I have witnessed the growth of this community and the bonds among members.

Joining and participating in ophthalmic organizations can also widen your community of ophthalmologists. Many people join ophthalmic societies as a way to give back to our great field of ophthalmology through teaching or advocacy. Others may join to meet peers with similar interests as group projects and teamwork are the backbone of society work. Millennials are known for their love of collaborative learning and civic-mindedness, and thus, joining an ophthalmic society would be a natural fit. Organizations should look to engage our next generation early in their careers. Earlier engagement can benefit

all generations as Millennials will add fresh ideas and strategies that will in turn attract more Millennials to participate. Building your ophthalmic community takes effort, and you must set aside time to prioritize this important step in your career development.

Conclusions

Engaging the next generation of ophthalmologists begins with understanding the Millennial generation and generational differences and biases. Using this knowledge to adapt and modify traditional engagement efforts will lead to better outcomes. Efforts should be focused on fostering mentorship relationships, allowing better work-life balance, employing technology-driven strategies, and building communities of peers. With these tools focused on addressing the needs and concerns of Millennials, engagement should come easily.

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Corneal Surgery in Children: Past, Present, and Future

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Kevin Z. Xin and Christina Rapp Prescott

Introduction

Pediatric keratoplasty, also referred to as pediatric corneal transplant, is regarded as a high-risk surgical procedure [1]. Prior to the 1970s, corneal transplants were considered to be contraindicated in children, except in those with severe bilateral visual pathology [2–4]. Poor outcomes, difficulties in postoperative management, and high probability of developing amblyopia even after successful surgery were reasons cited by experts at the time for not operating on pediatric patients with unilateral visual problems. Over the past 50 years, substantial improvements have been made in pediatric anesthesia, ophthalmic microsurgery, and improved suture material and techniques, which have increased the success rates of pediatric corneal transplants.

In both adults and children, corneal transplantation has evolved from a single type of surgery (penetrating keratoplasty) to four general categories of corneal transplant procedures: penetrating keratoplasty, endothelial keratoplasty, anterior lamellar keratoplasty, and keratoprosthesis. Penetrating keratoplasty involves transplanting a full-thickness cornea. Endothelial keratoplasty involves transplanting either just the Descemet's membrane and

endothelial cell layers (DMEK) or the Descemet's membrane and endothelial cell layers on a thin layer of posterior stroma (DSEK or DSAEK). This technique is useful for patients with isolated endothelial dysfunction, which is rare in children [1, 5]. Anterior lamellar keratoplasty involves removing the anterior corneal stroma and replacing it with donor tissue without Descemet's membrane or endothelial cells. This technique preserves the host's endothelium and is especially beneficial in children due to their increased risk of graft rejection and trauma and their high endothelial cell count. Lastly, the artificial cornea (keratoprosthesis) contains a central optic that consists of clear inert material that allows transmission of images through an otherwise opaque cornea, but the success rate of these complex transplants is especially poor in children [1].

Despite progress in techniques and an increased number of options for transplantation, children who undergo corneal surgery still face failure rates much higher than their adult counterparts. The surgery itself remains more technically challenging; postoperative management requires numerous examinations under anesthesia; and amblyopia may undermine even an anatomically successful graft. For these reasons, pediatric corneal transplants are still performed infrequently, with one infant corneal transplant performed for every 24,000 live births in the United States, and there is much room for innovation and improvement [6].

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Pediatric Corneal Pathology: Indications for Surgery

The differential diagnosis of corneal opacifications of childhood is extensive, and surgical options as well as outcomes depend greatly on indication. In 1966 Feigin and Caplan suggested that the corneal opacities may be divided into four main groups: (1) those appearing at or near the time of birth, (2) those associated with systemic illnesses, (3) those related to infections, (4) and the hereditary corneal dystrophies of various types [7]. Stulting et al. divided corneal opacifications into three groups: congenital, acquired non-traumatic, and acquired traumatic [8]. Indications for Pediatric Corneal Transplants According to the classification system described by Dr. Ken Nischal in 2012, corneal opacities in children can be classified into two categories: primary corneal disease and secondary corneal disease [9]. Primary corneal diseases are all congenital and include corneal dystrophies, corneal dermoid, peripheral sclerocornea, and CYP1B1 cytopathy. Secondary corneal disease can be divided into congenital causes (due to maldevelopment of the anterior segment) and acquired causes (including infection, trauma, and metabolic causes) [9]. The prevalence of congenital corneal opacities interfering with vision is approximately 2.2/100,000 and represents the most common indication for pediatric corneal transplantation [10]. Peter's anomaly, part of the anterior segment dysgenesis spectrum of developmental anomalies, is the most common cause of congenital corneal opacities in developed countries. Additional indications for pediatric corneal transplantation include sclerocornea, dermoid, microphthalmia, as well as metabolic diseases. Most of the published papers on pediatric corneal transplantation are from the United States of America, where the most common indication for transplantation overall is congenital opacity, followed by acquired non-traumatic and acquired traumatic [11–15]. Most Asian studies show anterior segment dysgenesis to be the most common indication – up to 80% of patients. In African studies, acquired indications are more prevalent, including corneal scar, after infectious keratitis

or trauma, and keratomalacia, associated with poverty. In contrast to congenital indications, some of the acquired corneal pathologies may be preventable [16–23]. In the Asia-Pacific region and developing world, infectious causes are more prevalent, and indications for pediatric keratoplasty include infectious keratitis, corneal scars, and ulceration secondary to HSV or bacterial infection [24]. Opacities secondary to acquired traumatic causes and non-traumatic causes are additional indications for pediatric keratoplasty. Causes of acquired trauma to the cornea include forceps trauma to the eye during delivery, corneal keloids, and trauma from amniocentesis. There are approximately 160,000 cases of eye trauma in children per year in the United States, and acquired traumatic corneal scars account for 8–26% of the pediatric penetrating keratoplasties performed [24]. Acquired non-traumatic causes of corneal scarring include keratoconus and other ectasias, corneal ulcers, congenital/childhood glaucoma, and Stevens-Johnson syndrome (SJS) and toxic epidermal necrosis (TEN). Congenital or infantile glaucoma can cause the cornea to become edematous, cloudy, and enlarged. The incidence of congenital and childhood glaucoma is 2.29 per 100,000 [25].

Section 1: Past

History of Corneal Transplantation

Significant progress in corneal transplantation did not occur until the nineteenth century, when an epidemic of blinding “Egyptian ophthalmia”, most often caused by *Chlamydia trachomatis* infection, spread across Europe. British and French soldiers engaged in the Egyptian campaigns of the Napoleonic wars (1789–1899) contracted the disease and subsequently introduced it to Europe [26, 27]. Experimentation with corneal transplantation began around this time with both human and animal donor tissue [26]. In 1837, an Irish surgeon, Samuel Bigger, reported performing a successful corneal transplantation in a gazelle. The first recorded therapeutic corneal xenograft on a human was performed in 1838 by

New York-based ophthalmologist Richard Kissam [26]. The procedure involved transplanting a pig xenograft into a human eye. While there was “increased light perception” initially, the cornea quickly opacified and was absorbed in less than a month [28]. Subsequent attempts at corneal transplantation were made using both full-thickness and partial-thickness corneas. Yet these attempts at human corneal transplantation performed in the nineteenth century using either animal or human donor tissue had little or no success, due to limited understanding of immunology, surgical technique, antiseptic principles, and anesthesiology at the time [26].

The first successful full-thickness corneal transplant eventually occurred in 1905. It was performed by Dr. Eduard Zirm, an ophthalmologist practicing in Olomouc (located in the present-day Czech Republic). This historical procedure involved obtaining two full-thickness corneal grafts from the enucleated eye of an 11-year-old donor and transplanting them to the patient, a 45-year-old man who had sustained bilateral alkali burns to the eye. Although the graft in the patient’s right eye failed, the graft in the left eye survived and visual acuity in that eye improved from counting fingers to 6/36 at 6 months. The left eye graft remained clear up until the patient’s death 3 years later [28]. Factors contributing to the success of Zirm’s transplant included the use of exclusively human corneas (prior surgeons have attempted using animal corneas), placement of the grafts between moist gauze for protection prior to transplantation, and strict adherence to antiseptic techniques [26].

Since Zirm’s surgery, innumerable ophthalmologists have contributed to improvements in surgical methodology, technology, and instrumentation; better understanding of corneal biology; and decreased rates of rejection through use of anti-inflammatory agents [26]. The Russian ophthalmologist Vladimir Filatov (1875–1956) first suggested the use of cadaveric corneas since live donors were in short supply, and corneal tissue obtained from a live or deceased donor was time sensitive and required immediate transplantation [29]. This phenomenon was observed by the American ophthalmologist Richard Townley

Paton who established the first eye bank in New York in 1944 [30, 31]. The Spanish ophthalmologist Ramon Castroviejo (1904–1989) conducted detailed studies of graft technique in the United States, popularized the use of direct sutures, and was innovative in surgical instrumentation [32].

Obstacles to Pediatric Corneal Transplantation

Despite advances in adult corneal transplant surgery during the first half of the twentieth century, pediatric corneal transplants were still performed infrequently prior to the mid-1970s [3]. Pediatric corneal transplantation offers its own unique set of challenges that can be divided into preoperative, intraoperative, and postoperative [1].

Preoperatively, children, and in particular infants, may lack communication skills. This makes surgical evaluation, especially visual acuity testing, difficult. Furthermore, many children requiring corneal transplantation have other ocular comorbidities including cataract, glaucoma, anterior segment dysgenesis, optic nerve damage, and retinopathy, which may increase surgical complexity and limit final visual potential. As a result, a complete preoperative ocular evaluation, often including an exam under anesthesia, is necessary to assess visual potential and identify comorbidities that may also need to be addressed [1]. Intraoperatively, the pediatric eye is smaller and the sclera and cornea are less rigid. This combined with the positive posterior vitreous pressure encountered in pediatric eyes makes tissue manipulation challenging [1, 4]. Postoperatively, children tend to have higher rates of rejection and infection than adults. They may require multiple exams and suture removal under anesthesia. Postoperative management should involve coordination across multiple ophthalmic subspecialties to minimize the risk of developing amblyopia and optimize long-term visual prognosis [1].

Given the aforementioned challenges, corneal transplantation in children has been a controversial topic. Opponents of performing the proce-

ture in children cited poor results, difficulties in postoperative management, and problems of amblyopia as reasons for not operating [33]. In 1958, Leigh advised against performing penetrating keratoplasty in patients younger than 16 years old [33, 34]. Likewise, Castroviejo was concerned that unruly behavior of children in the postoperative period may jeopardize corneal graft outcomes [33, 35]. There are still some ophthalmologists who feel that corneal transplant surgery is generally indicated in only pediatric patients with bilateral corneal involvement, though they are now in the minority [10, 36].

History of Corneal Transplantation in Children

Penetrating keratoplasty, also known as full-thickness corneal transplant, is one of the oldest corneal transplant techniques. This was the technique used by Dr. Eduard Zirm in his landmark 1905 surgery. Since the 1960s, several reports of successful penetrating keratoplasties in the pediatric age group have been published [33].

In 1965, Picetti and Fine published a study involving 40 penetrating keratoplasties in patients under the age of 16. This study demonstrated that the visual results of pediatric patients were similar to those obtained in adults. Likewise, rates of adverse reactions such as homograft reaction or wound leak were similar to those observed in adult patients. This study concluded that in a child with a corneal opacity that may lead to amblyopia, corneal transplantation should be performed as early as possible [37].

Similarly, in 1976, Stone et al. published a paper discussing three cases of penetrating keratoplasty for treatment of patients with Peter's anomaly. Among the three patients, two had clear corneas more than a year after surgery. The third patient had opacification of the cornea at the 6 weeks postoperative visit. However, the third patient had missed follow-up appointments after surgery, and this factor may have contributed to the patient's adverse outcome [38, 39].

The above studies demonstrated that with contemporary surgical techniques, clear grafts could be maintained in children with corneal opacities.

Factors contributing to the improvement in pediatric surgery outcomes in the 1960s and 1970s included better surgical techniques as well as the introduction of direct suturing with fine silk sutures which could be left in place for several weeks without causing serious discomfort to the patient. The latter may have also helped prevent attempts by children to inadvertently traumatize the graft by rubbing or scratching their eyes [8, 37].

Yet despite these improvements, the effectiveness of penetrating keratoplasty is limited by high rates of graft failure and surgical complication, including incision-site infection. The reported percentages of graft rejection in pediatric patients who have undergone penetrating keratoplasty range from 20% to 50% [12, 17, 40]. A significant cause of graft failure in children is bacterial keratitis, which is in part caused by the presence of sutures at the graft/host interface that serve as a potential nidus for bacterial growth [41].

History of Selective Transplantation in Children

Because of the relatively high rate of graft failure in children, as well as the increased risk of trauma and infection, the potential benefits of selective transplantation are even greater in children than in their adult counterparts. However, despite the potential benefits, endothelial and lamellar keratoplasties have not routinely been performed in children. This is slowly changing, though the adoption of these techniques in pediatric patients continues to lag behind, compared to their adult counterparts.

History of Endothelial Keratoplasty in Children

Endothelial keratoplasty involves the selective transplantation of components of the cornea rather than the full-thickness corneal transplantation performed in penetrating keratoplasties. The first successful endothelial keratoplasty was performed by Dr. Charles Tillett in 1956 [42]. However, endothelial keratoplasty techniques were initially hindered by problems including

interface haze, scarring, and epithelial ingrowth. This explains why Tillett's technique was not repeated until the late 1990s when Dr. Gerrit Melles developed the modern technique of sutureless endothelial replacement, which he named the posterior lamellar keratoplasty (PLK) [43, 44]. In 2001, Terry and Ousley reported successful results in patients using a similar procedure, which they named deep lamellar endothelial keratoplasty (DLEK) [45]. The DLEK technique gave rise to two modified endothelial keratoplasty techniques – the Descemet stripping automated endothelial keratoplasty (DSAEK) and Descemet membrane endothelial keratoplasty (DMEK). The DSAEK and DMEK techniques share similarities and differences. Both involve replacing the patient's endothelium and Descemet membrane with a transplanted disk. The difference is that while DMEK transplants donor corneal endothelium and Descemet membrane, DSAEK transplants contain donor corneal stroma, along with donor corneal endothelium and Descemet membrane.

Historically, penetrating keratoplasty (PK) has been the gold standard for the treatment of endothelial failure in children [46]. In contrast, endothelial keratoplasty is a relatively new technique in the field of pediatric corneal transplant, with the first pediatric DSAEK being performed in 2008 [47, 48]. DSAEK has the advantage of being a closed-sky technique, with a smaller wound, requiring fewer sutures and a faster visual and refractive recovery. This unique set of benefits makes DSAEK especially suitable for the pediatric age group, who are prone to traumatic wound dehiscence, PKP-related suture complications, and amblyopia. Furthermore, DSAEK/DSEK grafts in general clear faster than penetrating keratoplasty grafts, with some DSAEK grafts clearing as early as one week postoperatively. As a result, patients with DSAEK can sooner proceed to amblyopia therapy with refractive correction and patching [46, 49].

Since 2008, the number of reported cases of pediatric endothelial keratoplasty has increased significantly, but nonetheless remains proportionally lower than that of adult endothelial keratoplasty. The reasons for this are both technical challenges in carrying out the procedure in pedi-

atric eyes and differing indications for corneal transplantation between children and adults. First, in terms of surgical challenges, the anterior chambers of pediatric eyes are shallower than that of adult eyes due to positive vitreous pressure and a relatively anterior position of the crystalline lens. Furthermore, there is also a tendency for pediatric eyes to collapse more frequently [49]. In terms of differing indications, pediatric DSAEK procedures are performed most often for corneal opacities and Peter's anomaly, whereas in adults, the procedure is most commonly performed for treatment of Fuchs endothelial dystrophy or pseudophakic corneal edema [49]. Congenital corneal opacities interfering with vision occurred at 2.2 per 100,000, and among the congenital causes, Peter's anomaly has the highest incidence rate of 1.5 per 100,000 [6]. By comparison, the incidence of adult indications for corneal endothelial transplantation is much higher. The incidence for Fuchs endothelial dystrophy is 4% for individuals over the age of 40, and the incidence of pseudophakic corneal edema is 1–2% in the adult population [50, 51].

History of Anterior Lamellar Keratoplasty in Children

Deep anterior lamellar keratoplasty (DALK) is a partial-thickness cornea transplant procedure involving transplantation of the corneal stroma while leaving the host's corneal endothelium and Descemet's membrane intact [52]. The first successful lamellar keratoplasty (LK) for visual improvement was reported by German ophthalmologist Dr. Arthur von Hippel in 1888. It involved transplanting a rabbit cornea into a human patient. During the first half of the twentieth century, before the advent of corticosteroids and modern eye baking, anterior lamellar keratoplasty was commonly performed due to lower risks of graft rejection. However, at the time, LK was a tedious and time-consuming process that left patients with a best expected visual acuity of 20/30 or worse [53]. As a result, with improvements in penetrating keratoplasty technique, lamellar keratoplasty became relegated to a

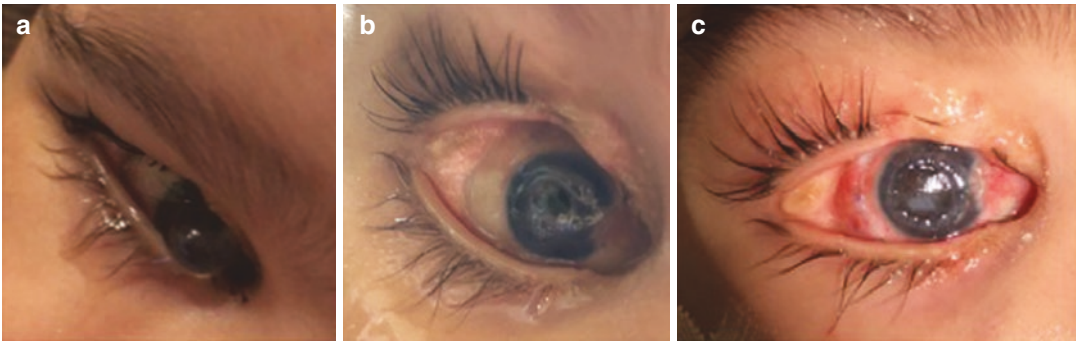


Fig. 31.1 (a) An 18-month-old child with Goldenhar's syndrome who presented with a perforated cornea with his crystalline lens protruding from the eye. (b) Temporal

dermoid, lid malformation, and perforated cornea. (c) Post full-thickness corneal transplantation

second-line procedure. Until recently, anterior lamellar keratoplasties were performed mainly for tectonic indications including pterygium and peripheral ulcerative diseases such as Mooren's ulcer or rheumatoid arthritis [52, 53].

Despite DALK's superior safety and stability, its visual results still do not match those of PK, though the difference is getting less significant as DALK techniques improve [53]. With the development of newer surgical techniques that bare the Descemet membrane, such as the "big bubble," visual outcomes with DALK have improved. However, the surgery remains technically challenging and has not been as widely adopted as DSAEK. As surgical techniques improve and intraoperative OCT becomes more widely available, hopefully this will become the standard surgery for anterior corneal pathology, including ectasias and visually debilitating scars.

The appeal of the anterior lamellar keratoplasty is greater in pediatric patients since this technique circumvents the problem of graft endothelial rejection, which accounts for over 40% of corneal graft failures in pediatric patients [16]. This is simply because in DALK, the patient's own endothelium is left intact. Given this unique property of DALK, it is an excellent surgical option for children with anterior corneal pathology and a healthy endothelium [2]. Prior studies have shown a dramatically decreased rate of graft rejection when compared to traditional PKP, which has been reported between 20% and 50% [12, 17, 40].

However, DALK is more challenging in pediatric eyes than adult eyes. This is since the pediatric patients have reduced scleral rigidity, which makes manipulation more difficult, increased the risk of intraoperative corneal perforation, and prolonged surgical time [1, 2]. These operative challenges, along with the steep learning curve, have limited the popularity of this technique among corneal surgeons (Fig. 31.1).

History of Keratoprosthesis in Children

The idea of an artificial cornea was first proposed by the French ophthalmologist Dr. Guillaume Pellier de Quengsy in 1789. De Quengsy was also the first to propose the creation of a porous skirt in the prosthesis "in order to maintain the circulation of lubricating humors." [54] Thus, although for the wrong reasons, de Quengsy came up with a concept that is still incorporated into modern keratoprosthesis to allow for the free passage of metabolites [55].

The first pediatric keratoprosthesis surgeries were performed in 2006 to treat congenital corneal opacities in two infants. Both patients had undergone several surgical procedures prior to placement of the keratoprosthesis. One of the patients had postoperative choroidal and retinal detachment, whereas the other patient had no postoperative complications [56]. The following year, a study involving 21 patients with Boston

keratoprosthetics placed and two patients with AlphaCor keratoprosthetics placed was carried out, making it one of the largest pediatric keratoprosthetics studies to date. Among the Boston keratoprosthetics cases, all prostheses were retained without dislocation or extrusion. Furthermore, the visual axis remained clear in 100% of these patients [55]. In contrast, among the AlphaCor cases, neither implant was retained. One of the prostheses dislocated secondary to trauma, while the other simply extruded by itself.

Keratoprosthetics offer several benefits when compared to penetrating keratoplasty. These include no risk of rejection and a rapid visual recovery leading to a decreased risk of amblyopia [55, 57, 58]. Since the synthetic optic remains clear even if the donor cornea is rejected, rejection is far less of a concern (although extrusion is and currently limits the longevity of keratoprosthetics in children). Other benefits include absence of astigmatism, due to the synthetic optical center of the keratoprosthetics, which leads to improved vision and easy correction of any residual refractive error with a bandage contact lens, the power of which can be easily determined by retinoscopy.

Although Aquavella et al. demonstrated successful use of keratoprosthetics in pediatric patients, subsequent studies have not echoed the same level of optimism [55]. These more recent studies consisted of a small number of case reports and reviews on the topic of pediatric keratoprosthetics, which have been summarized by Fung et al. [9, 59]. Fung et al. performed a retrospective multicenter case series looking at pediatric keratoprosthetics placed in Canada from 2010 to 2014 and noticed that although traditional rejection is not an issue, multiple other complications can occur, some of which are specific to the keratoprosthetics. The most common complication is formation of a retroprosthetic membrane, which can usually be treated with a YAG (neodymium-doped yttrium aluminum garnet) laser in adults, but must be surgically removed in children, due to both the density of the membrane and the inability of most children to tolerate a laser procedure without anesthesia. In addition, the donor carrier corneal tissue can melt in the

setting of a dense retroprosthetic membrane, causing wound leakage and extrusion of the keratoprosthesis. The presence of a synthetic device, coupled with the need for long-term contact lens wear, increases the risk of infection. These infections can be severe and include corneal ulceration and endophthalmitis. As a result, given the substantially higher rates of complication, higher chance of device failure, and worse visual outcomes than those observed in adults, the authors of this study have discouraged the use of keratoprosthetics in pediatric patients [59, 60].

Section 2: Present

Indications for Pediatric Corneal Transplants

According to the classification system described by Dr. Ken Nischal in 2012, corneal opacities in children can be classified into two categories: primary corneal disease and secondary corneal disease [9]. Primary corneal diseases are all congenital and include corneal dystrophies, corneal dermoid, peripheral sclerocornea, and CYP1B1 cytopathy. Secondary corneal disease can be divided into congenital causes (due to maldevelopment of the anterior segment) and acquired causes (including infection, trauma, and metabolic causes) [9]. The prevalence of congenital corneal opacities interfering with vision is approximately 2.2/100,000 and represents the most common indication for pediatric corneal transplantation [10]. Peter's anomaly, part of the anterior segment dysgenesis spectrum of developmental anomalies, is the most common cause of congenital corneal opacities in developed countries. Additional indications for pediatric corneal transplantation include sclerocornea, dermoid, microphthalmia, as well as metabolic diseases. In the Asia-Pacific region and developing world, infectious causes are more prevalent, and indications for pediatric keratoplasty include infectious keratitis, corneal scars, and ulceration secondary to HSV or bacterial infection [24].

Opacities secondary to acquired traumatic causes and non-traumatic causes are additional

indications for pediatric keratoplasty. Causes of acquired trauma to the cornea include forceps trauma to the eye during delivery, corneal keloids, and trauma from amniocentesis. There are approximately 160,000 cases of eye trauma in children per year in the United States, and acquired traumatic corneal scars account for 8–26% of the pediatric penetrating keratoplasties performed [24].

Acquired non-traumatic causes of corneal scarring include keratoconus and other ectasias, corneal ulcers, congenital/childhood glaucoma, and Stevens-Johnson syndrome (SJS) and toxic epidermal necrosis (TEN). Congenital or infantile glaucoma can cause the cornea to become edematous, cloudy, and enlarged. The incidence of congenital and childhood glaucoma is 2.29 per 100,000 [25].

Current Corneal Issues: Myopia and Ectasia

Penetrating keratoplasties are often complicated by myopia, with rates as high as 69%. This has been attributed to an axial myopic shift related to normal growth patterns of the eye [61]. The rate of developing myopia in the postoperative period is higher if donor tissue is less than 2 years of age since it can lead to steeper postoperative transplant curvature [1, 62]. Similarly, oversizing donor buttons to achieve easier wound closure and reduce the incidence of post-penetrating keratoplasty glaucoma can also increase the patient's risk of developing myopia [1]. If unaddressed, unilateral high myopia can lead to anisometropia with aniseikonia. Although contact lenses can reduce the severity of aniseikonia, fitting and maintaining contact lenses in pediatric patients is often difficult [62].

Corneal ectasia is defined as a progressive thinning, bulging, or distortion of the cornea and is the leading indication for corneal transplantation in pediatric patients. It can significantly impact both uncorrected and spectacle-corrected visual acuities. Corneal ectasia occurs both spontaneously (primarily keratoconus) and in the setting of either a relatively predisposed cornea undergo-

ing surgery or a normal cornea that becomes weakened below a well-tolerated threshold during surgery [63]. Postoperative ectasia, due to LASIK surgery, was first described by Seiler et al. in 1998, and rates of ectasia are higher in younger patients undergoing LASIK [63–65]. Penetrating keratoplasty has been demonstrated to have high rates of success in treating corneal ectasia. However, there is the risk of recurrence after surgery, with some instances occurring several decades after the penetrating keratoplasty [66]. The exact incidence of recurrent ectasia in penetrating grafts is unclear, with some reports suggesting 11% while other reports claiming it as a rare occurrence [67–69]. Fortunately, there are other methods to manage ectasia including contact lenses, intracorneal ring placement, and corneal collagen cross-linking. Collagen cross-linking is a technique developed by Wollensak et al. that uses riboflavin as a photosensitizer and UV light to induce cross-linking to strengthen the cornea. In Wollensak et al.'s study, keratoconus progression was stopped in all eyes, and 15 of the 23 eyes demonstrated mild improvement in VA [70, 71].

New Developments in Pediatric Corneal Surgery

Recent developments in pediatric corneal surgery have manifested in the form of laser-assisted procedures, incorporation of real-time optical coherence tomography (OCT), and use of gamma-irradiated grafts. The incorporation of laser-assisted techniques and OCT into the field has come hand in hand in the form of the femto-second laser-assisted lamellar keratoplasty (FALK) procedure. In FALK, anterior segment OCT is first performed to determine the depth of the pathology. During the surgery, OCT provides the surgeon with real-time dynamic feedback about tissue alterations that have been made [2, 72, 73]. The use of OCT in both the preoperative and intraoperative setting is different from the technology's original role, which was limited to imaging the retina and anterior segment in clinic. After the pathological areas of the cornea have

been identified, a femtosecond laser makes the excision necessary to remove the pathological tissue, cut an identical donor corneal lenticulae, and use it to replace the excised tissue. The femtosecond laser technology, developed in the 1990s, is capable of emitting light pulses of short duration that can achieve the desired incision in the tissue with minimal collateral damage. This ultimately allows FALK to produce a smoother interface, yield better surgical reproducibility, and potentially reduce the graft-rejection rate [74]. In cases where the corneal pathology spares a residual bed of 300 μm or more, the surgeon can place the donor corneal lenticulae without a suture [2, 75].

The safety of FALK in pediatric patients was demonstrated through a series of case reports. One of the first such reports was published in 2008 by Yoo et al. and described the use of FALK to treat 12 adult and pediatric patients who had developed anterior corneal scarring [73, 76]. In this study, the mean improvement in visual acuity was a gain of 2.5 lines. Furthermore, none of the patients had graft rejection, infection, or epithelial ingrowth. Likewise, in 2009, Agarwal et al. described the use of FALK to treat a case of Avellino corneal dystrophy in a child, with the patient achieving a best corrected visual acuity of 20/40 at the 19 months follow-up [77].

Another recent development in pediatric corneal transplantation is the use of gamma-irradiated corneas. These donor corneas have had keratocytes and endothelial cells eliminated by radiation. Gamma-irradiated corneas convey several advantages. First, it reduces the rates of an immunogenic reaction [78–80]. Second, gamma irradiation provides a use for donor cornea that has an unhealthy endothelium and would otherwise have to be discarded. This is since irradiated corneas with unhealthy endothelium but uncompromised stroma can be used for transplant procedures such as DALK wherein a viable donor endothelium is not required. Third, irradiation prolongs the shelf life of the sterile corneal to more than a year at room temperature [78].

Currently, there is limited data regarding the safety and effectiveness of irradiated corneal

tissue in pediatric corneal transplants. However, the results available so far have appeared promising. For instance, Utine et al. described in 2011 the use of irradiated corneal lenticules in three pediatric patients undergoing lamellar keratoplasty. All three patients had clear epithelialized grafts at almost a year out from surgery [80].

Refractive Surgery in Children

When compared to adult refractive surgery, the field of pediatric refractive surgery is a relatively young field, with the first report of a laser-assisted refractive surgery performed in a pediatric patient published in 1995 by Singh et al. [61, 81] In 2002, Astle et al. reported performing photorefractive keratectomy on 40 eyes of pediatric patients between 1 and 6 years old for treatment of anisometropic amblyopia or high myopia that had failed more conservative treatment methods. Patients in this study demonstrated an average improvement in best corrected visual acuity from 20/70 to 20/40 during the first year after surgery [82]. In addition to these studies, there have also been many case reports covering the topic of pediatric refractive surgery. These studies have reported mild to moderate improvements in VA and stable refractive results during the intermediate follow-up period [83–85]. Based on currently available data, pediatric refractive surgery appears safe and effective [61].

When a patient presents with a refractive problem, spectacles are the first-line treatment option. They are currently the most commonly used method to treat pediatric refractive errors and amblyopia [83]. However, there are some problems that spectacles cannot resolve. These include anisometropia amblyopia, bilateral high ametropia, and accommodative esotropia. In these conditions, spectacle correction may contribute to prismatically induced optical aberrations. This in turn can narrow the patient's field of vision. Furthermore, treatment with spectacles may result in aniseikonia and ultimately impede the development of binocular vision in children [83].

Current pediatric refractive surgery techniques can be grouped into three categories – laser vision correction, phakic intraocular lens (IOL) implants, and lensectomy. The first category of laser vision correction includes techniques such as photorefractive keratectomy (PRK), laser-assisted subepithelial keratectomy (LASEK), and laser-assisted in situ keratomileusis (LASIK). In contrast to adult laser vision correction surgeries, the goal of pediatric laser corrective surgery is not to make the patient spectacle independent. Instead, it is to ensure the patient obtains a visual acuity that is corrected to an extent such that amblyopia or visual isolation of one or both eyes does not occur. Complications of laser-based techniques include corneal haze and ectasia [86]. The second category of phakic IOL implants are indicated for children with high refractive error or amblyopia that may not be successfully treated with laser-based techniques. This technique has the advantage of being able to correct a wider range of defects than laser surgery. Furthermore, there is no risk of corneal haze, corneal thinning, or corneal ectasia. However, phakic IOL placement has its own set of unique side effects including increased risk of corneal endothelial damage, IOL displacement, and increased risk of cataract formation [87]. Third, lensectomy with or without IOL placement, similar to phakic IOL placement, is suited for children with high ametropia or anisometropia that cannot be resolved with laser-based techniques. This technique has numerous postoperative complications including retinal detachment and posterior capsule fibrosis [88]. Furthermore, should a lensectomy with IOL placement be performed, then the patient is also at risk for the postoperative complications associated with IOLs.

Despite significant advances in refractive surgery technique over the years, the current body of literature regarding pediatric refractive surgery is relatively limited and has been studied much less than its adult counterpart. Much of the research performed in this field is in the form of case reports that often lack a control group [83]. Prospective controlled clinical trials will be necessary to better evaluate the long-term safety and

effectiveness of these techniques as well as how surgical outcomes compare with those of more conservative treatment approaches.

Conclusion

Postoperative care is intensive for the surgeon, patient, and family since care requires involvement of multiple ophthalmologic subspecialties and frequent examinations under anesthesia. Focused efforts to correct refractive error and treat amblyopia require long-term therapy to optimize visual outcomes [10]. This multitude of modifiable factors likely explains the vast differences in the reported success rates of pediatric keratoplasty even for similar indications. For instance, the success rate has been estimated to be between 15% and 100% for patients with Peter's anomaly and between 55% and 100% for patients with acquired traumatic conditions [3, 24]. Despite significant variability in the reported statistics, two trends are worthy of mention and have been demonstrated in multiple studies. First, the rates of graft survival in children are lower than those of adults, and should a graft rejection occur, it is much more difficult to reverse the process in children. For instance, Alldredge and Krachmer reported that graft rejection was reversible in only 28% of pediatric patients, whereas it was reversible in 50–78% of adult patients [24, 89]. Second, the rates of graft survival are higher for patients with acquired corneal opacities than patients with congenital corneal opacities [2, 90].

The success rate of pediatric corneal transplantation has been improving over the years. To maximize success after keratoplasty, children must follow intensive perioperative and postoperative regimens with frequent follow-up visits, high-dose topical corticosteroids, and early suture removal [1]. With advances in ophthalmology, including newer keratoplasty techniques of endothelial keratoplasty, DALK, and improvements in the keratoprosthesis, we have more options in providing targeted therapies to pediatric patients.

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Part VI

Closing



Future Directions in the Field of Cornea

32

Reza Dana, Afsaneh Amouzegar,
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Regenerative Medicine

The field of regenerative medicine has undergone substantial advancement in the past 15 years. The development and use of novel methods for harnessing different stem cell populations (adult stem cells including mesenchymal stem cells, embryonic and induced pluripotent stem cells), growth factors and pharmaceuticals that can enhance tissue regeneration have led to a veritable revolution in biomedicine.

In the context of the cornea, there are several interrelated areas that we believe deserve particular attention in the context of regenerative medicine, as detailed below.

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Cultivation of Endothelial Cells

Although thought to be non-dividing in vivo and replaceable only with corneal transplantation, the latest studies on corneal endothelium (CE) have defied previous notions and have led the breakthrough new data on the endothelial cell plasticity and its clinical applications. The introduction of Rho kinase (ROCK) inhibitors has shown promising results in reconstitution of the endothelial monolayer in human studies. The seminal study by Shigeru Kinoshita et al. on the treatment of endothelial dysfunction with injection of cultured cells with ROCK inhibitor has started a new era of cell therapy in endothelial diseases [1]. Moreover, the regenerative capacity of the endothelium in Fuchs endothelial corneal dystrophy (FECD) patients has been demonstrated by reports on successful re-endothelialization after Descemet's stripping without endothelial keratoplasty (DWEK) [2].

The notion that the corneal endothelium harbors stem cells has been verified by the findings of neural crest-derived progenitor cells (NCDPC) interspersed in the endothelial monolayer [3]. Both normal and FECD corneas harbor NCDPCs, indicating that not all cells in FECD are diseased or have acquired epigenetic modifications causing the degenerative attrition. The main questions that still need to be addressed are (i) can we identify the location of these CE stem cells and (ii) what are the specific factors that could stimulate the differentiation and propagation of these seemingly

dormant progenitors, in order to “heal” the defective monolayer at the times of injury and disease.

From a slightly different standpoint, several groups have reported generation of CE from iPS, embryonic stem, and mesenchymal stem cells and have greatly advanced the field in endothelial cell regeneration. The shortage of transplantable allogeneic corneas poses the challenge of developing novel approaches to treating endothelial dysfunction worldwide; indeed, finding alternate sources for endothelial transplantation is an important future goal that appears, at least, theoretically feasible.

Novel Methods for Replacing the Corneal Epithelium Including Use of Cultivated Cells

At the forefront of regenerative medicine is regeneration of epithelium and endothelium of the cornea. Cultivated limbal epithelial cell transplantation, jump-started by G. Pellegrini in 1997, has evolved and been optimized by employing various manufacturing techniques [4]. In order to circumvent the risks of allograft rejection (as can be seen in limbal allografts) and damage to the donor eye (as is sometimes seen in limbal autografts, such as CLAU), ex vivo-cultivated limbal epithelial cell (CLEC) has been developed. This technique is advantageous due to the ability to utilize a small amount of the patient’s own tissue. The risk of damage to the donor eye is bypassed by expanding limbal epithelial (stem) cells from a small biopsy (2–3 mm²) onto the substrate, such as amniotic membrane, in culture before transplantation. The success of using such a new surgical approach has been reported in several human studies [5–13]. Generally, human studies on autologous CLEC transplantation demonstrate an overall low risk for use in patients, rendering the technique safe and effective to treat LSCD.

Taking into consideration prior human experience with CLEC from other countries, our group has set a goal to optimize and standardize the techniques of cultivated autologous limbal epithelial cell (CALEC) preparation for the clinical trial in the USA. By using completely defined reagents, performing additional testing on reagents used, using autologous starting material, and removing antibiotics and murine feeder cells, we have set to improve

upon the quality of the cell therapy product and process development. Similarly, the upsurge of GMP-compliant and animal product-free methods has been important in streamlining the epithelial cell manufacturing process throughout the world. The standardization of processes and protocols as well as delineation of endpoints that evaluate the efficacy of treatments in clinical trials will be important for the future of this technology, with the ultimate goal to maximize safety and predictability of the patients’ clinical outcomes.

Use of Mesenchymal Stem Cells, Stromal Stem Cells, or Derivatives to Promote Stromal Regeneration and Prevent Scar Formation

Ever since the discovery of mesenchymal stem cells (MSCs) by Arnold Caplan in the early 1990s [14], studies have unraveled the regenerative potential of these cells to a great extent in different clinical conditions, including in ocular pathologies. While a bulk of the studies conducted thus far focus on the regenerative capacity of MSCs due to their ability to engraft and differentiate into different cell types, a major shift in our understanding of the full potential of these cells has led to the discovery that the immunomodulatory potential of MSCs plays a central role in their regenerative function. Studies in animal models have shown that MSCs regulate the pathogenic function of immune cells during ocular inflammation and suppress the influx of inflammatory cells to the injured cornea [15–17]. These studies have shown that MSCs interact directly with precursor myeloid cells and inhibit their differentiation into mature pathogenic cells during inflammation [16] and regulate neutrophil effector functions through suppressing their expression of myeloperoxidase and N-elastase [15]. Corneal stromal stem cells (CSSC), which are mostly localized to the anterior limbal stroma, have recently been recognized for their potential application in stromal replacement and restoration of corneal transparency [18]. Application of CSSC engrafted in fibrin gel to corneal wounds has been shown to suppress neutrophil infiltration to the cornea and prevent the formation of stromal scar tissue in mice, a finding which has been attributed to the CSSC expression

of tumor necrosis factor α -stimulated gene 6 protein (TSG-6) [19]. A recent investigation on the application of CSSC in bioengineering corneal stromal tissue has shown that scaffold-free tissue sheets formed by CSSC closely mimic the structure of native corneal stroma and incorporate into the corneal stroma of mice without losing their transparency [20], suggesting their potential application in replacing scarred corneas.

MSC-derived exosomes and soluble factors have been increasingly recognized for their role in mediating the immunomodulatory and tissue-regenerative properties of MSCs [21]. Recent work has shown that MSCs secrete high levels of hepatocyte growth factor (HGF) and suppress the development of corneal opacity in animals with corneal stromal injury upon systemic administration [22]. This effect is mediated by suppressing the conversion of corneal fibroblasts into α -smooth muscle-actin expressing myofibroblasts, the major mediators of stromal scarring. Notably, expression of HGF by MSCs is central to their ability to restore corneal opacity and promote wound repair after stromal injury [22]. A recent investigation evaluating the function of human corneal MSC-derived exosomes on corneal epithelial regeneration has shown exosomes to significantly improve epithelial wound closure in vitro and accelerate epithelial wound healing in a mouse model of corneal injury. Larger studies are required to assess the efficacy of MSC-derived factors in preclinical studies; however the superior biosafety and stability of these factors over direct delivery of MSCs coupled with promising results of initial studies make these approaches attractive novel tools in management of corneal scarring and nonhealing wounds.

Use of Neuropeptides and Neurotrophic Factors that Promote Regeneration of Corneal Cells and Provide Cytoprotection Against Noxious and Cytotoxic Signals Which Prevent Tissue Growth and Development of Factors that Promote Nerve Regeneration

Corneal nerves play a critical role in the normal physiologic functioning of the cornea, as well as

corneal response to stress and injury. The trigeminal nerve provides the cornea with an array of different sensory (mechanical, pain) fibers that respond to mechanical, chemical, thermal, and inflammatory stimuli [23, 24]. Corneal nerves also provide trophic supply to the ocular surface, stimulate wound healing, and maintain anatomic integrity by releasing growth factors and neuro-mediators such as substance P (SP), neuropeptide Y, calcitonin gene-related peptide (CGRP), acetylcholine, cholecystokinin, noradrenaline, serotonin, met-enkephalin, brain natriuretic peptide, vasopressin, and neurotensin [25, 26]. Specifically, several lines of evidence show that SP and CGRP modulate corneal epithelial cell proliferation, migration, and adhesion [26, 27]. Conversely, studies in animal models demonstrated that corneal denervation causes morphologic and metabolic changes of corneal epithelial cells, characterized by loss of microvilli, cell swelling, decreased epithelial thickness, increased epithelial permeability, and decreased cell proliferation [26].

While various corneal cell types get support from nerves, they too can secrete factors that are in turn neurotrophic. For example, corneal epithelial cells and keratocytes release growth factors that stimulate nerve survival, maturation, and growth, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins 3 and 4/5, and ciliary neurotrophic factor (CNTF) [24, 28–30]. Thus, corneal nerves, corneal epithelial cells, and keratocytes mutually interact with one another through a complex network of mediators and growth factors [24]. The ability to influence this network for therapeutic purposes will almost undoubtedly play an important role in the coming frontiers of corneal science and disease management.

Among these factors, one of the best characterized is NGF, the founder of the neurotrophin family. NGF plays a key role in corneal nerve survival and regeneration [31]. It has been demonstrated that corneal epithelial cells, keratocytes, and corneal endothelial cells express NGF receptors, the high-affinity NGF receptor, tyrosine kinase A (TrkA), and the low-affinity pan-neurotrophin receptor, the p75 (p75NTR), and are able to produce and release NGF [29, 32]. In addition, NGF is released in both aqueous humor and tear film in

physiologic and pathologic conditions [33, 34]. Experimental studies have demonstrated that NGF is a key factor for corneal sensory nerve survival [35] and that topical administration of NGF is able to induce (i) corneal healing concomitant with recovery of near-normal corneal sensitivity, (ii) increase in tear production, and (iii) stimulation of corneal limbal stem cells [30, 34, 36, 37].

Recently, a recombinant human NGF (rhNGF), named Cenegermin, has been developed for topical use for the treatment of neurotrophic keratitis (NK). NK is characterized by loss of corneal trigeminal innervation, leading to the decrease or absence of corneal sensitivity, and associated with development of persistent epithelial defects and non-healing corneal ulcers [38–40]. The use of recombinant NGF will provide a leap forward in our ability to treat complex ocular surface disorders that have a neurotrophic component.

In addition to the cross-talk between nerves, epithelial cells, and keratocytes, there is increasing evidence for an important cross-talk between nerve-derived factors and the corneal endothelium. It has now been established that surface and stromal keratopathies associated with nerve degeneration (such as dry eye disease) are also associated with loss in the density of endothelial cells [41, 42]. Based on this, we have now established that corneal endothelial cells have ample expression of receptors for a variety of nerve factors including vasoactive intestinal peptide (VIP) and alpha-melanocyte-stimulating hormone (α -MSH), among others. Remarkably, we have shown that administration of these factors can have a profoundly positive influence on the capacity of corneal endothelial cells to withstand the toxic effects of inflammatory cytokines and oxidative stress [43]. The enhancement of endothelial cell survival from these approaches could conceivably have a significant effect on eye banking and transplantation technologies.

Immunity

The immune system is comprised of a complex network of cells and organs that work together to protect the body both in homeostasis and under

“danger” signals, such as infection and tumor growth. While host protection against microbial pathogens is a principal aspect of immunity, the immune response needs to be tightly regulated to prevent damage to normal cells. This is particularly critical in the case of the cornea where unwanted inflammation can lead to scarring and diminished vision.

In the past 10–15 years, our understanding of how the immune system is regulated has blossomed considerably. New findings in this area will undoubtedly continue to influence our understanding of disease pathophysiology and influence development of novel therapeutics.

For the past several decades, corticosteroids have been the primary immunosuppressive strategy employed in managing the immune rejection of corneal allografts, despite their toxic side effects and limited efficacy in those patients who have rejected previous transplants or who have vascularized host beds. Accordingly, there is a pressing unmet clinical need for alternative therapeutics to modulate the immune response following corneal transplantation. The substantial progress that has been made in unraveling the cellular and molecular pathways that drive graft rejection using murine models is encouraging [44]. However, despite a variety of therapeutic approaches being suggested by these murine keratoplasty studies, there remains a distinct lack of therapies that cross the translational gulf to be adopted in clinic. One challenge is that graft opacity and neovascularization in animal keratoplasty models are determined based on clinical scoring systems that are inherently vulnerable to interobserver variability and lack of readout reproducibility. This challenge may be overcome by the application of new technologies to obtain reliable measures of graft rejection and opacity, such as the evaluation of graft thickness by anterior segment spectral domain optical coherence tomography and the assessment of myofibroblast infiltration by *in vivo* confocal microscopy.

The immunomodulatory strategies that have been established in animal models target either the afferent arm of the immune response (APC maturation and migration, lymphangiogenesis), the efferent arm (generation of Th1 cells, hemangiogenesis), or immunosuppression (expansion

of Tregs, tolerogenic DCs, and MSCs) [44]. There is room for optimism that one or more of these strategies will yield a viable approach for amplifying tolerance and suppressing graft rejection in the clinic. Of particular note are efforts to utilize anti-VEGF agents in high-risk transplantation, and there is some evidence from case series and case control studies that bevacizumab is effective in decreasing corneal neovascularization and promoting graft survival [45–47]. Larger multicenter studies are required to evaluate the safety and efficacy of bevacizumab and other candidate molecules, in order to expand the surgeon's toolkit in preventing graft rejection.

Gene Editing, CRISPR, and Gene Therapy

The advent of genome engineering strategies carries significant implications for the future of both the study and treatment of inherited corneal conditions, such as corneal dystrophies. Although at the time of writing there are no ongoing clinical trials to treat corneal conditions with gene therapy, the accessibility, relative immune privilege, and ease of visualization of the cornea renders it an ideal candidate for gene-based treatments.

The major approaches for genome engineering consist of RNAi, genome augmentation, and programmable nucleases [48]. Genome augmentation involves introduction of the functional copy of the gene to treat loss-of-function mutations and is a strategy currently employed in ongoing clinical trials for retinal degenerations. The programmable nucleases are a novel tool that manipulates the genome in a sequence-specific manner by employing the nucleases programmed to cleave and facilitate editing of a precise sequence in a target gene. The ease of nuclease engineering for a specific target renders clustered regulatory interspersed palindromic repeats (CRISPR)-associated nuclease, Cas9, to be the most commonly used genome-editing tool. In research studies, CRISPR-Cas9 has been used to generate a mouse model of Schnyder corneal dystrophy [49] and to aid in specific gene knockout studies in vitro for study of oxidative stress in FECD [50]. Moreover, in vivo intrastromal injection

of CRISPR-Cas9 targeting most prevalent TGFBI corneal dystrophy mutations has shown promising results in dominant negative corneal dystrophies [51].

Gene editing is of particular interest in treating FECD, as it is the most common endothelial dysfunction, necessitating corneal transplantation in many cases, and has a strong genetic component [52]. The discovery of TCF4 trinucleotide repeats in the majority of FECD cases points to a potential target for genome-editing therapies [53]. Several considerations when implementing the gene-editing applications in humans are efficient delivery to the target cells, specificity and efficiency of editing the target gene, and safety; the latter calls for minimization of the off-target effects. In summary, targeting disease-causing mutations, as seen in corneal dystrophies, opens up an era for potential personalized therapeutics in the cornea.

Cornea Replacement

No tissue or organ can be indefinitely immune from dysfunction. In the case of the cornea, when this failure occurs in the context of severe inflammation or structural/anatomical disturbances that can adversely affect the outcome of tissue transplantation, it is important to consider alternatives to conventional tissue grafting approaches. In this regard, and in particular in the field of biomaterials, we have witnessed considerable advances in the recent past which we expect can impact the frontiers of corneal science and medicine in the coming decade.

Multiple biomedical engineering approaches have been proposed for corneal replacement, ranging from keratoprostheses to decellularized xenografts and tissue-engineered corneal substitutes. Since the eighteenth century, when Pellier de Quengsy suggested the restoration of an opaque cornea using a piece of glass surrounded by a silver ring [54], multiple keratoprosthetic technologies have been developed for clinical use. Most are composed of a non-degradable central optical zone made of poly(methyl methacrylate) (PMMA) that is surrounded by a biological material (i.e., tooth, bone, or cornea) to

promote the biointegration of the prosthesis with the host [55, 56]. In order to facilitate it, new approaches based on surface modifications of the PMMA have been described recently, such as coatings with titanium [57, 58] or hydroxyapatite [59, 60]. In an attempt to develop substitutes that are similar to the human cornea, decellularized xenografts and tissue-engineered scaffolds have also been proposed. Corneal xenografts have been used since the nineteenth century showing poor results because of the immediate rejection of the xenotransplant by recipients [61, 62]. Decellularization is one approach to minimize immunogenicity, and thus various methods have been proposed to achieve this [63, 64]. Recently, gamma-irradiated decellularized porcine cornea has also been transplanted in human patients with limited success [65, 66].

In the context of tissue engineering, while numerous models have been described in the literature, very few tissue-engineered corneal substitutes have actually reached patients. Fibrin scaffolds with autologous limbal stem cells cultured on top were implanted in 112 patients with limbal stem cell deficiency, restoring the corneal transparency and the corneal epithelial phenotype in more than 75% of the patients [67]. Acellular scaffolds made of recombinant human collagen have also been used in a small number of patients with keratoconus and stromal scar. These scaffolds promoted a regenerative response from corneal host cells, maintaining the transparency of the cornea, without showing any sign of rejection [68, 69]; however much work still needs to be done to standardize this approach. Recently, this collagen scaffold was modified with the addition of 2-methacryloyloxyethyl phosphorylcholine, in order to avoid or reduce neovascularization of the graft, and implanted in patients at high risk for rejection of their donor grafts [70, 71].

While these are relatively early days in bioengineering applications to the cornea, there is little question that in the coming decade we will see a significant increase in the development of novel engineering solutions to replace the cornea. These new technologies, either alone or in combination with current eye banking and transplantation approaches, may ultimately transform the management of patients with severe corneal diseases.

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