Clinical outcomes of modern lamellar keratoplasty techniques

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Chapter 1

General introduction

THE HUMAN CORNEA

The cornea, the dome-shaped ´window of the eye`, covers the anterior chamber, iris and pupil. It has a diameter of approximately 11.5 millimeters and is normally about 500 to 600 micrometers (µm) thick.[1] Its function is to protect the inner ocular structures as well as to provide about 2/3 of the eye's refractive power. It is one of the most innervated and sensitive tissues of the body, with unmyelinated nerve endings derived from the ophthalmic branch of the trigeminal nerve. At the same time, the absence of blood and lymphatic vessels contributes to corneal clarity and optimal optical performance, while contributing to the cornea's immunologic privilege.[1,2] The human cornea consists of five layers: epithelium, Bowman layer (BL), stroma, Descemet membrane (DM) and endothelium (Figure 1).

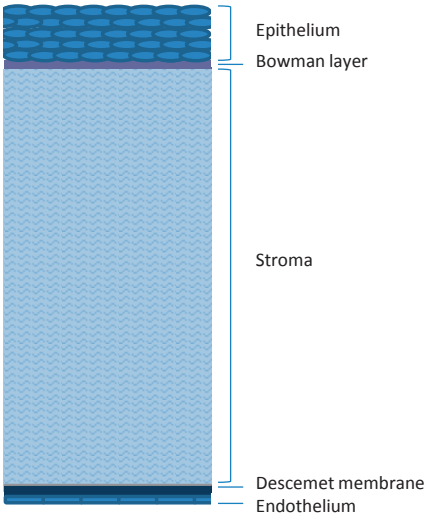

Figure 1. Schematic representation of the human cornea.

Epithelium

The epithelium is the outermost part of the cornea, comprising about 10 percent (around 53µm in thickness) of total corneal thickness.[3] It is a stratified, non-keratinized structure characterized by extreme uniformity from limbus to limbus, composed of 5 to 6 layers of cells, tightly and orderly arranged without intercellular spaces, and increasingly flattened as it reaches the surface. Corneal epithelial cells have an average lifespan of 7 to 10 days, and routinely undergo orderly involution, apoptosis, and desquamation,[1] in which deeper cells replace the desquamating superficial cells in an orderly, apically directed fashion.[4] An important source of new corneal epithelial cells are the epithelial stem cells, localized at the corneal limbus. As these cells migrate to the central cornea, they differentiate into basal cells, forming the deepest epithelial layer, which is connected to the underlying epithelial basement membrane by hemi-desmosomes. Just anterior to the basal cell layer are 2 to 3 layers of wing cells, which are covered by desquamating superficial cells. These desquamating superficial cells are tightly connected to their neighboring cells through tight junctional complexes, which prohibits tears, toxins and microbes from entering the deeper corneal layers.[1,5] Despite the high turnover rate of the epithelial cells, a highly controlled, steady thickness profile, and a tight arrangement of cells is maintained to guarantee a constant corneal power, with

a good optical quality.[3,6] Furthermore, the superficial flattened epithelial cells interact with the mucous layer of the tear film to ensure a smooth, stable optical surface that resists bacterial adherence.[5,7]

Bowman Layer

The BL, situated directly underneath the epithelial basement membrane, is an 8 to 14 µm thin acellular layer with the front being smoother than the more irregular posterior part; it thins with age.[8,9] The BL contains randomly-organized tightly-woven collagen fibrils (mostly types I and V collagen), which differ from the thicker collagen fibrils of the underlying stroma that run in alignment across the cornea in characteristic lamellae.[10,11] Thus far, the function of this firm dense corneal layer remains unclear. It has been hypothesized that the BL may act as a biological barrier against pathogen infiltration, especially viruses.[1,11,12] The BL may also have a role in modulating epithelial-stromal wound-healing, since subepithelial-stromal scarring seems not to occur in the presence of an intact BL.[13,14] Additionally, given its strong rigidity, it may be valuable for maintaining the corneal shape or strength.[8,11,15]

Stroma

About 90% of the total cornea is made up by stroma. It consists primarily of water (78%), collagen (15%), and non-collagenous proteins (7%) which are supported by scattered keratocytes,[16] predominantly situated in the anterior stroma. Collagen fibers (mainly types I and V) are arranged in parallel bundles, i.e. fibrils, which are packed in parallel arranged layers or lamellae. Each lamella is positioned at right angles relative to fibers in adjacent lamellae.[17] In the central cornea, the stroma comprises about 200 lamellae, which are more densely packed in the anterior region.[18] The anterior lamellae are highly interlaced,[19] inserting into the BL.[20] Also the mid-stromal lamellae are considerably interwoven,[21] whereas the posterior lamellae seem to have less interlacing. As such, the posterior stroma swells easily while the more interwoven anterior stroma does not.[22] The peripheral stroma is thicker than the central, and the collagen fibrils run more circumferentially as they approach the limbus.[23] Furthermore, stromal interweaving seems to extend to the deeper posterior lamellae in this region of the cornea.[19]

The lamellar arrangement within the stroma helps to maintain the cornea's overall shape, while the unique structure and organization of the collagen fibrils within the lamellae allow corneal transparency.[24] Specifically, uniformity in the collagen fibrils' diameters and the restriction in the range of distances between adjacent collagen fibrils, maintained by the interfibrillar proteoglycans, may be important determinants.[25-27] Additionally, cytoplasmic molecules, the so-called 'corneal crystallins', within the keratocytes seem to be responsible for reducing backscatter of light from the keratocytes, supporting corneal transparency by matching the refractive index of the cell cytoplasm

to the extracellular matrix.[28] Dysfunction in any of these components can reduce corneal clarity, and consequently may result in functional loss.

Descemet membrane

Beneath the stroma lies the DM, an extracellular matrix, mainly composed of type IV collagen fibrils and laminin that serves as a basement membrane for the corneal endothelium. Apparently, endothelial cells create this membrane by secreting the different components.[29,30] Three different zones can be distinguished: a 0.3 µm thin anterior non-banded zone, adjacent to the posterior stroma, an anterior banded zone (2-4 µm) and a posterior amorphous non-banded zone (>4 µm), which thickens with age.[30] Just anterior to the DM, a very thin, strong pre-DM layer, may exist.[31]

Endothelium

The endothelium is the corneal innermost monolayer of approximately 4 µm thin in adulthood. The endothelial layer consists of closely-packed hexagonal cells that are essential in keeping the cornea transparent, by regulating fluid and solute transport between the aqueous humor and stroma. The cells serve as selective barriers allowing leakage of solutes and nutrients from the aqueous humor to the cornea. On the other hand, a net flux of ions from the stroma to the aqueous humor is ensured through Na-K ATPase pumps, found in the basolateral endothelial cell membranes, and intracellular carbonic anhydrase, which create an osmotic gradient that causes passive diffusion of fluid in the same direction.[1,32-34] In a healthy cornea, the endothelium maintains a perfect balance between fluid moving into and being pumped out of the cornea,[1,32] maintaining the stroma in a relative state of deturgescence, while dysfunction of either the barrier or the pumps results in corneal edema.

Endothelial cells do not regenerate, but instead stretch to compensate for lost cells. As this process occurs, the remaining cells grow in size (polymegathism) and lose their hexagonal shape (pleomorphism).[1] The endothelial cell density is around 6000 cell/mm^2 at birth and 3500 cells/mm^2 by the age of 5 years.[32] This number decreases gradually throughout life at an average rate of 0.6% per year.[1] Accelerated cell loss may occur after ocular surgery, such as cataract extraction and corneal transplantation, probably due to surgical trauma and postoperative inflammation.[1,33,35] Corneal transparency may be maintained with an endothelial cell density as low as 500 cells/mm^2 (range 750-250 cells/mm^2), after which a proper corneal fluid balance may no longer be preserved and stromal swelling with subsequent loss of corneal transparency may occur.[33]

Corneal optical properties

The visual performance of the cornea depends on its shape, transparency and surface regularity. As described, a highly-organized matrix of corneal collagen fibrils maintains

corneal clarity by minimizing light scatter. Anything that interferes with this matrix or affects the corneal surface may compromise the corneal optical quality.

In an average human eye, the cornea forms a positive prolate-shaped (steepest curvature at the corneal apex, progressively flatter curvature toward the periphery) meniscus lens.[7] The anterior corneal surface has an approximate refractive power of +48 D, while the refractive power of the posterior corneal surface is -6 D. To convert radii of curvature into corneal power it is often assumed that the entire cornea has a uniform refractive index of 1.376, whereas refractive indices of 1.3375 (keratometric index) and 1.0 (air) are normally used to determine the anterior keratometric corneal power. In young individuals, the anterior corneal surface tends to have with-the-rule-astigmatism (steepest meridian is vertical), but astigmatism becomes more against-the-rule (steepest meridian is horizontal) with increasing age.[36-38] The posterior cornea tends to be steeper in the vertical meridian, inducing against-the-rule astigmatism, that shows only a minimal variation with age.[39] The relationship between the anterior and posterior corneal surface is important, and normally the posterior surface compensates approximately 30% of the anterior corneal astigmatism.[40]

Together with the lens, the cornea plays a major role in the shape and amount of the total human eye optical aberrations,[41] i.e. any light rays misdirected from their intended image point. In the normal population, the dominant optical aberrations include the ordinary second-order sphero-cylindrical errors, which are called lower-order aberrations or refractive errors (myopia, hyperopia, and regular astigmatism). Lower-order aberrations are typically correctable with spectacles, whereas higher-order aberrations (HOAs), which normally comprise about 10% of total aberrations, cannot.[42] Total eye aberrations are shown to be less than those of the anterior cornea alone, which may indicate that aberrations of both the posterior cornea and crystalline lens partly compensate for the anterior corneal surface aberrations, although this compensation mechanism seems to decrease with age.[40]

A useful representation to describe total eye aberrations as well as the aberrations of both corneal surfaces is the use of Zernike Polynomials (Figure 2). The polynomials describe typical optical properties or imperfections, using varying radial orders and angular frequencies, demonstrated as different wavefront error maps.[41,43-45] Each polynomial is named according to the image defects it represents (e.g. astigmatism, coma or spherical aberration), affects the optical quality in a specific way, and can be positive or negative (Figure 2). Generally, the magnitude of contribution decreases with increasing Zernike order, and polynomials near the center of the Zernike tree (e.g. coma, spherical aberration) tend to affect vision more than those near the edge of the tree (e.g. trefoil) (Figure 2).[43] Furthermore, the different polynomials interact to either increase or decrease visual function, depending on their relative contributions and how they are combined.[41]

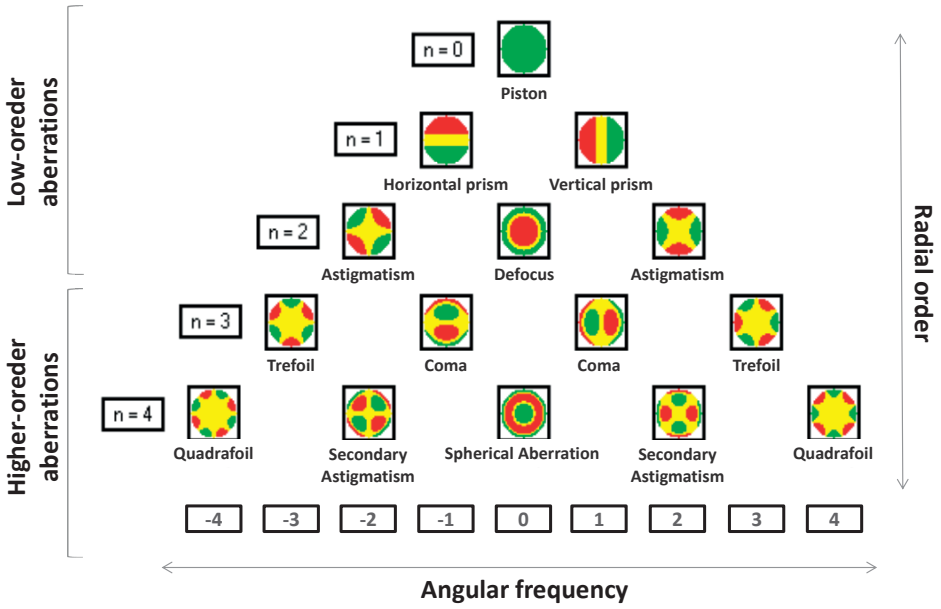

Figure 2. Chart showing the Zernike tree with the common Zernike polynomials. An aberration-free flat plane, known as piston, represents a theoretically perfect eye.

A healthy clear cornea is able to transmit almost all incident light in the visible part of the spectrum.[24,25] Loss of clarity, or increased haziness, indicates a degradation of the corneal tissue,[46,47] and may be a sign of a pathological process, such as infection, corneal dystrophy or degeneration. Corneal haze (back-scattered light) most likely represents a mixture of light scattered from small particles and specular reflections from adjacent tissues with different refractive indices.[46] Back-scattered light itself cannot compromise the vision, but is associated with corneal changes that typically increase forward-scattered light, which degrades the periphery of the point-spread function. As such, disability glare (reduced visibility due to light sources in the visual field), impaired contrast sensitivity and decreased visual performance in unfavorable ambient light conditions may arise.[48-50]

Corneal imaging

Various diagnostic imaging devices are currently available to analyze the cornea, including corneal topography, corneal tomography, anterior segment optical coherence tomography, specular microscopy and confocal microscopy.

With corneal topography, the anterior corneal surface can be evaluated, most commonly by reflecting a series of concentric rings, known as Placido rings, on the cornea. A digital camera then captures the reflected pattern, after which the shape of the cornea is processed and calculated by the device's software. The principle of corneal topography

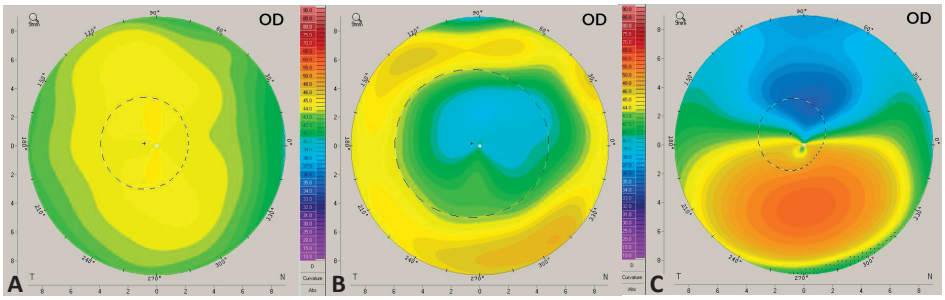

Figure 3. Corneal topography maps showing (A) a prolate-shaped cornea with the steepest curvature (warmer colors) at the corneal apex, (B) an oblate-shaped cornea after Laser in-situ keratomileusis with a flatter central curvature (cooler colors) compared to a steeper peripheral shape, and (C) an irregular-shaped cornea with the steepest curvature in the inferior corneal meridian as in keratoconus.

is based upon the assumption that the cornea is prolate, and therefore non-prolate corneas or irregular corneal surfaces are often misdiagnosed (Figure 3).[51] Furthermore, to obtain a clear image, the epithelial surface as well as the tear film must be intact. The newest color light-emitting diode (LED) corneal topography devices, in which a color-coded checkerboard is projected on the cornea instead of Placido rings, may allow for higher predictability in central irregular corneas as compared to Placido-based topographers.[52,53]

Whereas corneal topographers can only measure the anterior corneal surface, corneal tomography devices can evaluate both the anterior and posterior cornea, as well as determine corneal thickness (pachymetry), anterior chamber depth and lens parameters.[54] Three types of corneal tomographers are currently available: slit scanning devices (i.e. Orbscan II), Scheimpflug imaging devices (i.e. Pentacam), and devices that combine Scheimpflug cameras with Placido based topography (i.e. Galilei, Sirius and TMS-5/Tomey). A slit scanning device projects optical slits at a fixed 45-degree angle along multiple points across the cornea. A digital video camera captures the reflections of the slits, after which the reflections can be analyzed to construct representations of the anterior and posterior cornea.[54] With Scheimpflug-based corneal imaging, a rotating Scheimpflug camera captures multiple cross-sectional slits in a two-second scan across the cornea, while a stationary camera, centered at the pupil, aligns the images and monitors ocular fixation. The three-dimensional images are then analyzed to compose anterior and posterior corneal topographies based on height data.[54,55] Some Scheimpflug-based tomographers (e.g. Pentacam) also provide corneal wavefront and densitometry analysis (Figure 4).

Anterior segment optical coherence tomography (OCT) uses optical interferometry to produce high-resolution, cross-sectional images of the cornea and other anterior segment structures. It is being employed more and more often to picture anterior segment pathologies, and for anatomical imaging for surgical purposes.[56] Two main OCT

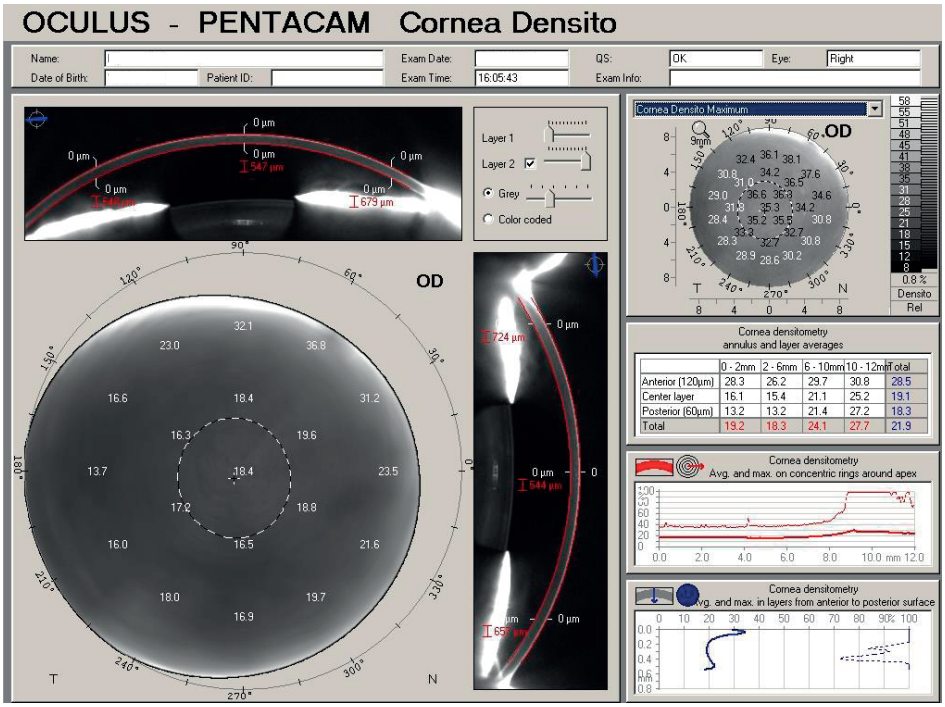

Figure 4. Screen display of Scheimpflug-based (Pentacam) corneal densitometry assessment.

categories can be distinguished; time-domain (TD) vs. spectral-domain (SD, also called Fourier-domain). TD-OCT's utilize a moveable reference mirror, which moves for each scan to determine the ocular structure depth. SD-OCT's use a fixed reference mirror and a Fourier transformation algorithm of the interferogram to measure depth, resulting in faster acquisition and better image quality as compared to TD-OCT's.[57]

Specular microscopy allows for visualization of the corneal endothelium, by projecting light onto the cornea, and capturing the image reflected from the optical interface between the endothelium and the aqueous humor. Modern specular microscopes allow for analysis of the endothelial cell size, shape and density.[58]

Corneal confocal microscopy is a relatively novel and rapidly evolving clinical technique enabling real-time, in vivo, microstructural analysis of every layer of the cornea. In confocal microscopy, a single point of tissue is both optically illuminated (using white light or a focused laser beam) and simultaneously imaged by a point detector, both having the same focal point, or being "confocal". To optimize the optical resolution, the light source and the detector use pinholes that work 'in tandem'. To increase the field of view, the instrument instantaneously illuminates and synchronously images a small corneal region with numerous tiny light spots or slits, which can be reconstructed to create a functional field of view with high resolution and magnification.[59-60]

CORNEAL TRANSPLANTATION

Corneal transplantation, also known as corneal grafting or keratoplasty, is a surgical procedure in which a damaged or diseased cornea is replaced by corneal donor tissue (the graft). It remains the main method to restore vision in eyes with irreversible affection of corneal clarity. In addition, globe preservation, pain reduction and cosmetic appearance of the eye may be reasons for keratoplasty. While indications differ between locations and institutes, in developed western countries, corneal transplantation is most often performed for disorders in which the endothelium is impaired, such as Fuchs endothelial dystrophy, bullous keratopathy, and failed previous corneal grafts, or ectatic disorders, which are mostly due to keratoconus.[61]

History of corneal transplantation

The first suggestion to replace an opaque cornea for manufactured transparent material in order to restore vision originated from the French surgeon Guillame Pellier de Quengsy in 1789.[62] This was actually the first inspiration for what is nowadays known as keratoprosthesis. However, it lasted until 1818 when Franz Reisinger initiated experimental corneal transplantation in animals. Reisinger also introduced the term 'keratoplasty'.[63] At the end of the 19th century, around 1890, professor Arthur von Hippel promoted anterior lamellar corneal transplants, since he understood that corneal transparency mainly depended on the integrity of the endothelium and DM.[64]

In 1905, Eduard Zirm performed the first successful human corneal transplantation, a full-thickness keratoplasty on a 45-year-old male with severe bilateral alkali burns. The operation was done under general anesthesia and 'strict' aseptic conditions.[65] Vladimir Filatov, a Russian eye surgeon, followed with his first attempts in full-thickness corneal transplantation in 1912, which eventually resulted in a successful transplantation using corneal tissue from a deceased human donor in 1931. Filatov is considered the father of eye banking and is credited for popularizing the use of cadaveric human donor corneas for transplantation.[66] Another pioneer was Ramon Castroviejo from Spain, who did his first penetrating transplantation in 1936 in an advanced keratoconus patient, achieving significant improvement in vision. He also devised numerous instruments, which were very important for further improvement of corneal surgery in general.[67,68]

Further development and standardization of transplantation techniques followed, while research concentrated on tissue preservation and preparation. In 1944, a first eye bank was established in New York by Townley Paton and, in 1961, the Eye Bank Association of America (EBAA) was founded.[69] This organization developed the standards for obtaining, preserving, storing and using corneal donor tissue.

Penetrating keratoplasty

The full-thickness replacement of the cornea by donor tissue (penetrating keratoplasty, PK), regardless of the healthy layers, was the preferred surgical treatment for all corneal disorders. Although initial poor graft survival rates after PK resulted in renewed interest in lamellar corneal transplants (lamellar keratoplasty, LK) around the 1950's,[70-73] the development of corticosteroids for the treatment of allograft rejection, the introduction of antibiotics and operating microscopes, and the evolution of microsurgical instruments and newer suture materials all contributed to the increased popularity of PK. Since its introduction, different suturing techniques and graft-sizing practices have been described, but the largest advance in PK has been the recent introduction of the femtosecond laser to trephine the recipient and donor tissues, theoretically providing better apposition and faster healing.[74-76]

Technically, PK is often successful, initially providing a clear cornea. However, the postoperative course may be compromised due to complications derived from corneal sutures, ocular surface problems or wound dehiscence associated with incomplete wound healing.[77-81] As a result, PK is often accompanied by slow, insufficient, and unsatisfactory visual rehabilitation, which may be even further complicated by refractive problems such as anisometropia and high (irregular) astigmatism.[77,80,82,83] Furthermore, complications such as continuous and increased endothelial cell decline and allograft rejection, which may both be provoked by other complications (e.g. sutures, corneal vascularization, glaucoma), may eventually result in graft failure.[80,84-88]

The long-term graft survival rate after PK ranges from around 50% to 80% at 10 years postoperatively, depending on indication and complications.[84-86] For uncomplicated Fuchs endothelial dystrophy or keratoconus, the likelihood of having a functional graft in the long run is relatively good,[84,85] whereas corneas transplanted for pseudophakic or aphakic bullous keratopathy, infectious corneal ulcers and re-grafts may have a lower graft survival rate.[80,86,88-90]

Lamellar keratoplasty

Problems frequently encountered with PK eventually led to renewed interest, further developments, and popularization of LK (Figure 5). In contrast to PK, the fundamental hypothesis behind LK was to replace only the diseased part of the cornea, leaving the recipient's healthy corneal layers intact and to resect the least amount of tissue, with less risk of rejection and more success. Anterior LK potentially improves graft survival rates by selectively replacing the diseased anterior corneal layers and retaining the healthy endothelium.[87,91] The development of posterior LK procedures has dramatically enhanced the predictability and speed of visual rehabilitation in endothelial disorders.[77,92] Posterior and anterior LK procedures will be separately discussed in part 1 and 2 of this thesis.

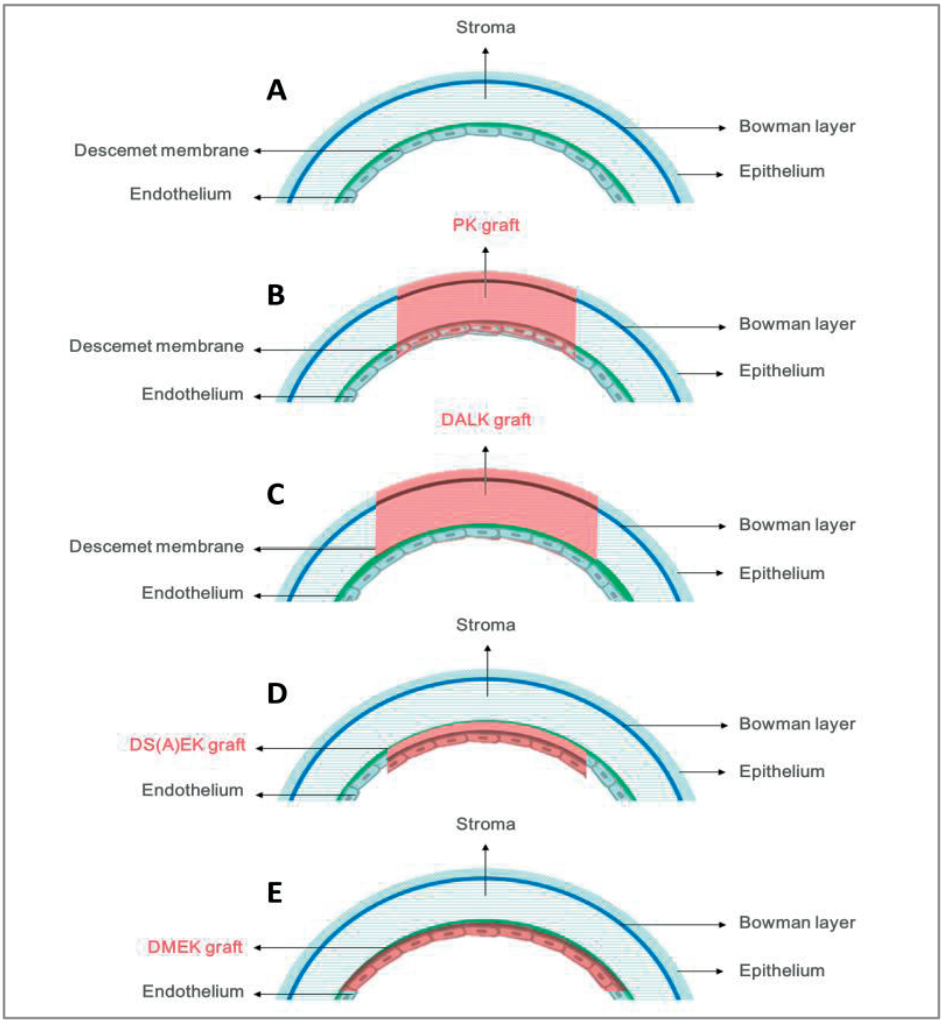

Figure 5. Schematic overview displaying (A) a virgin cornea, (B) penetrating keratoplasty (PK), and (C-E) the different lamellar keratoplasty techniques, i.e. (C) Deep anterior lamellar keratoplasty (DALK), (D) Descemet stripping (automated) endothelial keratoplasty (DS(A)EK), and (E) Descemet membrane endothelial keratoplasty (DMEK). (Source: van Dijk K, et al. Contact lenses after keratoplasty what to expect and what to look for with contact lens management in post-keratoplasty corneas. Contact Lens Spectrum 2014, August Issue).

PART I: SELECTIVE, MINIMAL INVASIVE TREATMENT OF ENDOTHELIAL DISORDERS

Endothelial disorders

The various types of endothelial disorders share the presence of posterior corneal alterations, i.e. in the endothelium and DM, which may eventually cause corneal swelling and

opacification and impair visual acuity. Different corneal endothelial dystrophies with their own unique pathophysiologic mechanism may be distinguished. In addition to the endothelial dystrophies, various insults to the endothelium may affect the function of the endothelium. In this thesis, the most common "endothelial" indications for corneal transplantation will be described.

Fuchs endothelial dystrophy

Fuchs endothelial dystrophy is the most common corneal dystrophy, frequently resulting in visual loss. It is a slowly progressive, bilateral, corneal disease and was first described a century ago by the Austrian ophthalmologist Ernst Fuchs.[93] Primarily, it is a disorder of the posterior cornea with the formation of focal excrescences of DM, termed 'guttae', which appear as round dark areas within the cellular monolayer on specular microscopy (Figure 6). This is accompanied by endothelial cell density decrease and abnormally enlarged, polymorphic cells surrounding the guttae.

With advancing disease, loss of endothelial cells results in progressive stromal edema and subsequent loss of vision, especially in the morning, as an initial symptom. In end-stage disease, the cornea will slowly generate painful epithelial bullae, evolving into subepithelial fibrosis and finally ending up with corneal vascularization.[94,95]

Two clinical subtypes have been identified: early-onset Fuchs endothelial dystrophy, which is rare, presents within the first decade of life and progresses through the second to third decade; and the more common late-onset Fuchs endothelial dystrophy that

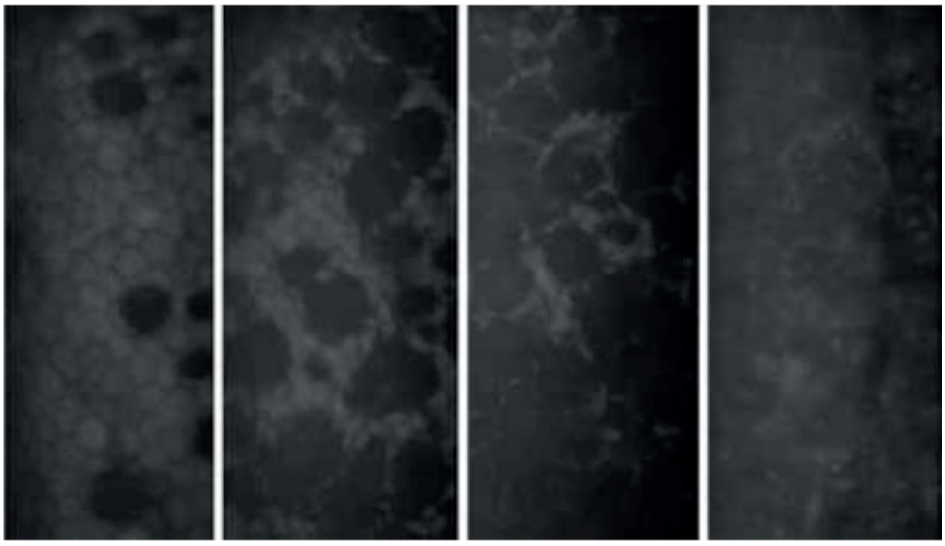

Figure 6. Specular microscopy images of advancing Fuchs endothelial dystrophy (left to right). (Source: Ham L. Descemet Membrane Endothelial Keratoplasty: Donor Tissue Preparation and Clinical Outcomes. Optima Grafische Communicatie Rotterdam, 2011).

starts at the second to third decade and becomes symptomatic during the fifth to sixth decade of life.[94,96,97] Early-onset Fuchs endothelial dystrophy is accompanied by a thickened anterior banded DM zone. In late-onset Fuchs endothelial dystrophy, multiple layers of basement membrane–like material have been found on the posterior nonbanded part of DM.[97] Both subtypes appear to have a similar progression from disease onset until corneal decompensation, and have a female predominance at a ratio of around 3:1.[97]

The pathological mechanism behind Fuchs endothelial dystrophy is not yet completely understood. The genetic basis appears complex and heterogeneous, demonstrating variable expressivity and incomplete penetrance. Autosomal dominant inheritance of Fuchs endothelial dystrophy occurs; however, in most cases, Fuchs endothelial dystrophy has not yet been found to be associated with a specific genetic mutation.[97] Additionally, environmental factors such as oxidative stress may play a role in the development or progression of the disorder by accelerating cell damage and apoptosis.[98-102]

Bullous keratopathy

Bullous keratopathy refers to the development of irreversible corneal edema due to endothelial decompensation as a complication secondary to trauma (most often surgical trauma, such as complicated cataract extraction), glaucoma or congenital abnormalities.[103] It may manifest in the immediate post-traumatic period, or symptoms may not present for many years. With increasing corneal edema, (sub)epithelial bullae may form and rupture, causing painful micro-defects in the corneal surface. Moreover, in advanced bullous keratopathy, subepithelial fibrosis, with or without BL-disruption and superficial vascularization, may frequently be observed.[104-106]

Corneal graft failure

Corneal graft failure can be defined as an irreversible loss of corneal clarity caused by a directly dysfunctional corneal graft (primary graft failure), or occurring years after corneal transplantation (secondary graft failure).[107] Risk factors include continuous and increased postoperative endothelial cell decline and allograft rejection episodes, which in turn can be provoked by complications such as (secondary) glaucoma, ocular surface problems, suture-related problems and corneal vascularization.[80,81,84-86,88,108] Furthermore, transplantation indication, socio-economic and geographical factors play a role in the possible development of graft failure.[108-110]

Management of endothelial dysfunction

Although the management of endothelial dysfunction may be somewhat different between entities and individual cases, in general, endothelial disorders may initially be treated with topical sodium chloride solutions, topical corticosteroids and reduction of

intraocular pressure.[111,112] Furthermore, soft bandage lenses may be useful to relieve the pain in case of recurrent erosions caused by epithelial bullae.[113] However, since corneal endothelial cells show minimal or no regeneration,[1] most cases ultimately require corneal transplant surgery. Traditionally, the standard surgical approach was to replace the full thickness of the cornea with donor tissue, as in PK. More recently, various types of posterior LK techniques have been introduced, among which is Descemet membrane endothelial keratoplasty (DMEK), the latest innovation in that field, [114,115] and the subject of this thesis (part 1).

Posterior lamellar keratoplasty

The concept of posterior LK, also called endothelial keratoplasty (EK), was established in the 1950s and 1960s by Charles Tillet and Jose Barraquer.[73,116] However, their attempts of posterior lamellar exchange underneath a manually-dissected stromal flap were often unsuccessful and were at that time perceived as technically more difficult than a PK. In 1998, Gerrit Melles came up with a breakthrough idea, as he demonstrated that a posterior donor lamella could adhere to the posterior recipient stroma with only an air bubble in the anterior chamber as support. The procedure, called posterior LK, required excision of a 7.0–7.5-mm diameter recipient posterior stromal button with attached endothelium, after which a similar size posterior donor button - containing the same tissue layers (stroma and endothelium) - could be inserted through a 9-mm limbal incision.[117] The initial technique was modified in 2000 by Melles et al, using a 5-mm incision and folding the donor tissue like a 'taco' to enable insertion.[118] Meanwhile, Mark Terry popularized both techniques as deep lamellar endothelial keratoplasty (DLEK).[119,120] Promising results however were tempered by technical difficulty to manually dissect both donor and host stromal beds.

In 2002, Melles et al. further simplified the procedure by removing only the host DM and its endothelial cells by a scraping movement with a reversed Sinskey hook, called descemetorhexis. Descemetorhexis combined with a DLEK-graft is referred to as Descemet stripping endothelial keratoplasty (DSEK).[121] When the donor tissue then was dissected with a microkeratome, as developed by Mark Gorovoy, the procedure was called Descemet stripping automated endothelial keratoplasty (DSAEK).[122]

Adoption of EK accelerated after eye banks began to prepare the donor tissue for surgeons,[123] and in the last decennium, DSEK/DSAEK has replaced PK as the standard surgical technique for the treatment of corneal endothelial disorders. Compared to PK, EK resulted in a faster visual rehabilitation, with more predictable refractive outcomes and less postoperative astigmatism, while suture and wound-related problems could be avoided. At the same time, EK provided a better retained structural integrity of the eye, reducing the risk of catastrophic eye loss from suprachoroidal haemorrhage, both intra- and postoperatively.[92,115]

Variability in visual outcomes after DSEK/DSAEK, however, weakened these advantages to some extent. Full visual recovery was not always achieved, despite having otherwise healthy eyes and clear corneas.[92,114,115,124,125] Possible explanations include stromal scarring and fibrosis secondary to the underlying pathology and increased light scatter.[48-50,126-129] In addition, optical degradation at the graft-recipient interface, increased corneal thickness and increased HOAs have been studied as causes for suboptimal visual outcomes after DSEK/DSAEK.[130-136] While the anterior corneal surface is left uncompromised, posterior corneal HOAs after DSEK/DSAEK seem to be comparable to or even larger than post-PK.[137,138] This may be secondary to graft decentration, curvature-mismatch between the recipient and the graft, graft wrinkling or uneven graft thickness from asymmetric trephination.[132,133,139] It has been theorized that thinner posterior lamellar grafts would result in a more regular posterior graft surface, while better conforming to the recipient posterior corneal curvature and, consequently, their use could improve the visual outcome.[132,134,139]

Descemet membrane endothelial keratoplasty

Melles et al. realized that EK could be further refined by selectively implanting a donor DM and endothelium without any stroma (such as in a DSEK/DSAEK graft).[140] The first successful case report of a technique, nowadays referred to as DMEK, was presented in 2006.[141]

As with other keratoplasty techniques, DMEK-graft preparation may be performed by the surgeon at the time of surgery or in an eye bank setting 1 to 2 weeks prior to the surgery. Precut tissue may save surgical time, avoids surgical postponement caused by unsuccessful tissue preparation and requires fewer investments in equipment and preparation skills. Furthermore, eye banks can perform all routine quality checks, such as endothelial cell layer evaluation and sterility testing before shipping the tissue.

The DM can be relatively easily stripped off due to the fragile interconnections with the posterior stroma, making it feasible to prepare isolated grafts of DM and its endothelium.[142,143] When the DM is peeled from a donor corneo-scleral rim, it rolls up with the endothelium on its outer surface, probably owing to the elastic properties of the tissue.[142,143]

The initially-described DMEK-graft preparation technique consisted of stripping a 9.5-mm diameter DM from a corneo-scleral rim submerged in saline.[142,143] The method proved-safe and reproducible, with less than 5% tissue loss and an acceptable endothelial cell decrease of 4% to 7%.[142] In 2012, the preparation process became safer and easier with the introduction of the so called 'no-touch' DMEK-graft preparation procedure, in which a rim of trabecular meshwork tissue is left in-situ, and the DMEK-graft is trephined on an underlying soft contact lens.[144]

A standardized 'no-touch' surgical technique for DMEK was published in 2011 by Dapena et al.[145] The technique comprised the creation of three side-ports. The recipient's DM was then scored and stripped from the posterior stroma, performing a 9.0-mm diameter descemetorhexis, with the anterior chamber filled with air. Subsequently, the DMEK-roll was thoroughly rinsed with balanced salt solution, stained with 0.06% trypan blue solution, and drawn into a custom-made injector for injection into the recipient anterior chamber through a 3.0-mm limbal tunnel incision at the 12:00 o'clock position. The graft was oriented with the endothelial side down (donor DM facing the recipient posterior stroma) by indirect manipulation with air and BSS, then gently spread out over the iris, after which it was positioned onto the recipient posterior stroma with an air bubble injected underneath.[145] At the end of the surgery, the anterior chamber was left completely filled with air for a period of one hour, followed by an air-liquid exchange to pressurize the eye, leaving a 30% to 50% air-bubble in the anterior chamber. Patients were asked to remain supine for 48 to 72 hours after surgery.[145]

DMEK aims to restore the normal corneal anatomy by selective replacement of the diseased corneal layers only and, as such, is a truly disease-specific form of keratoplasty (Figure 7). Initial DMEK outcomes were promising with a relatively low complication rate (early graft detachment being the main complication), good visual results in the majority of patients, and endothelial cell survival similar to earlier keratoplasty techniques.[115,146-151]

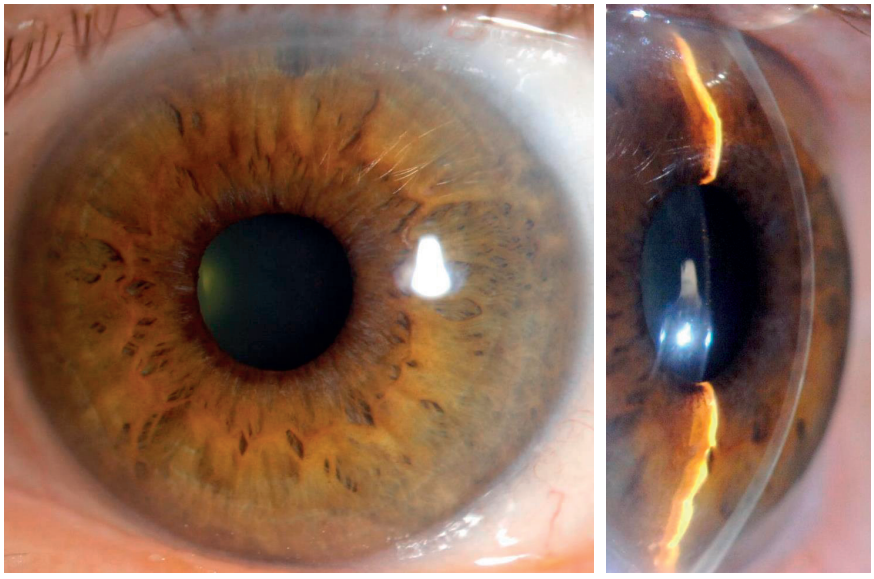

Figure 7. Slit-lamp photographs of an eye at 3 months after Descemet membrane endothelial keratoplasty for Fuchs endothelial dystrophy. Note the 'normal' anatomical restoration of the transplanted cornea as well as its clarity.

PART II: NEW TREATMENT OPTION FOR ADVANCED KERATOCONUS

Keratoconus

Keratoconus is the most common ectatic corneal disorder, often described as a bilateral, often asymmetrical, non-inflammatory, progressive disorder characterized by thinning and protrusion of the cornea, causing a compromised optical performance. The disorder generally becomes apparent during the second decade of life, during puberty, and progresses until the fourth decade, when it usually stabilizes.[152,153] The established prevalence of keratoconus among the general population is approximately 1/2000,[154] but much higher rates have been mentioned in some parts of the world.[155,156]

The pathogenesis of keratoconus, although not well understood, may include genetic, environmental and mechanical factors. Possibly, a genetic predisposition requires an environmental event to provoke progressive disease.[157-159] Different associations have been described; specifically atopy, eye rubbing, positive family history, and several syndromes and diseases, such as Down syndrome, Ehlers-Danlos syndrome and Leber congenital amaurosis.[152,153,157,158,160-164] Furthermore, new evidence suggests an overexpression of inflammatory mediators, including cytokines and interleukin 6 (IL-6), in cases with keratoconus.[165-167] In addition, oxidative stress may play an important role in the pathogenesis of keratoconus.[98,167]

Depending on the severity of keratoconus, all layers of the cornea may become involved in the pathological process.[152,153] The corneal epithelial basal cells may degenerate and grow towards the BL. The BL often shows breakages, which are then filled with stromal collagen. In the stroma, a decrease in the number of lamellae and keratocytes, degradation of fibroblasts, changes in lamellar organization, and uneven distribution of the collagen fibrils have been observed.[152,153,168,169] The DM and endothelium are usually unaffected. However, breakage of DM may occur in severe cases and elongation of endothelial cells pointing towards the cone has been reported.[152,153]

The clinical presentation of keratoconus varies depending on disease severity. While early keratoconus can go unnoticed, keratoconus progression will cause subjective visual loss and photophobia, caused by corneal deformation and astigmatism. In moderate to advanced keratoconus cases, stromal thinning may accompany corneal ectasia, which is greatest at the apex of the cone (Figure 8). Vogt striae, fine vertical parallel lines in the posterior stroma and DM, and a Fleischer ring, iron deposits on the epithelial basal membrane, may be visible on slit-lamp examination. Furthermore, BL breakage may cause corneal scarring. In severe cases, a so-called 'Munson sign', a V-shaped distortion of the lower eyelid in down gaze, may be visible and corneal hydrops, causing excessive corneal edema, may occur due to sudden breaks of DM.[152,153,170]

To grade keratoconus, the Amsler-Krumeich classification system is one of the oldest and still the most commonly used.[171] With this classification, which is often used for

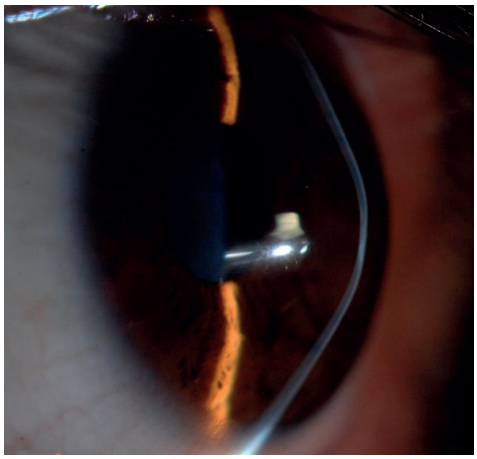

Figure 8. Slit-lamp photograph of an eye with advanced keratoconus. Note the deformation of the corneal curvature, as well as the thinning at the apex of the cone

research purposes and is integrated in the Pentacam software, the severity of keratoconus is graded from stage I to IV using spectacle refraction, central keratometry and pachymetry, and the presence or absence of corneal scarring.[55,171] However, the Amsler-Krumeich scale relies on relatively 'outdated' parameters and it fails to address currently available information and technological advances. Furthermore, it does not deal with objective and subjective visual function. Therefore, various modifications to better diagnose or characterize the severity of keratoconus have been suggested, such as the use of corneal topometric irregularity indices, corneal HOAs, corneal thickness maps, anterior and posterior corneal elevation, and corneal biomechanical properties.[172-178] Preferably, a classification system should combine clinical findings such as visual performance with corneal topometric and tomographic parameters.[179]

Management of keratoconus

The first step in the management of keratoconus comprises optimizing patient's vision by means of optical correction. In early stages of the disorder, spectacles or soft contact lenses are a viable option, while in moderate to advanced cases, specially designed soft lenses, hybrid and rigid gas-permeable lenses are indicated. Scleral lenses become desirable to achieve visual rehabilitation in cases in which wearing the more traditional contact lens designs is not possible anymore (e.g. poor centration, instability, or low tolerability).[180]

For patients with unacceptable vision, also while wearing a contact lens, in which the vision cannot be adequately restored by other means, corneal transplantation remains the standard of care. Two main types of corneal transplantation are available for the treatment of keratoconus: PK, and deep anterior lamellar keratoplasty (DALK). In the latter only the anterior corneal layers are replaced, preserving the patient's own endothelial cell layer.[181]

Anterior lamellar keratoplasty

Since the renewed interest in lamellar corneal transplants around the 1950's, there has been a group of ophthalmic surgeons, including Paufique,[182] Malbran,[183] and Anwar,[184] who performed anterior LK instead of PK for visual improvement in corneal diseases with normal corneal endothelium, such as keratoconus. However, it took until the mid-1980s before innovative procedures were developed and widely adopted to remove diseased corneal anterior stroma up to the DM,[185-187] as in DALK. Different lamellar dissection techniques to achieve removal of all, or almost all, of the corneal stroma have been described, including intrastromal air injection,[185] big-bubble technique,[186] hydrodissection,[188] viscoelastic dissection,[189] and manual dissection.[190]

Most of the currently used DALK techniques are based on the Anwar big bubble or the Melles manual dissection technique. The Anwar technique comprises a partial-depth trephination of the cornea, followed by forcibly injecting air deep in the stroma (creating a "big-bubble") to detach DM from the stroma.[186] With the Melles manual dissection technique the stroma is manually dissected away from the underlying DM using a series of a different sizes curved spatulas.[190] In the latter technique, the depth of dissection can be determined after filling the anterior chamber with air, by using the air-to-endothelium interface as a reference plane.[191] A drawback of both techniques may be that perforation of DM is relatively frequent (4 – 30%),[91,190,192-195] and, depending on the size of perforation, conversion to PK may be required. Because with the Melles manual DALK technique the dissection is more 'controlled' and, in the event of a perforation, the perforation site tends to be small, the procedure can often be completed. Otherwise, since no corneal surface incisions have been made at the time of the dissection, the operation can be aborted and reattempted at a later date.[190,191]

A major advantage of DALK compared to PK is the maintenance of the recipient endothelium which results in much lower rates of endothelial cell density decrease and practically eliminates endothelial allograft rejection, suggesting a long lifetime for a DALK graft.[196] Furthermore, the retained recipient corneal endothelium and DM allow for better preservation of the ocular integrity, permitting earlier suture removal due to faster wound recovery and, consequently, fewer wound healing-related problems.[77,91] As a consequence, DALK provides a somewhat faster visual rehabilitation, although final visual outcomes may be at best equal to PK, provided that stromal dissection reaches the level of DM.[91,197-200] Similar to PK, DALK grafts require fixation by sutures, which may give rise to several complications such as cheese wiring, neovascularization, and suture loosening, potentially leading to a sequence of adverse events with disappointing visual results and graft failure.

Treatments to avoid corneal transplantation

Clinical observation, supported by recent research studies, suggests that eyes with advanced keratoconus may be prone to a sequence of (inflammatory) ocular surface reactions,[152,201-204] which may render DALK and PK high-risk procedures. Both surgeries tend to worsen any existing ocular surface problems, as both involve surface incisions, corneal denervation, and placement of long-lasting sutures. Furthermore, keratoconus patients are generally young, and may possibly survive their corneal transplant. Consequently, it seems reasonable that many of these patients require more than one corneal graft during their life, with re-grafts having inferior clinical outcomes and worse graft survival than the initial corneal transplant.[205,206] Another limitation of PK/DALK for the treatment of keratoconus may be that these treatment options do not halt progression of keratoconus, possibly due to ongoing ectatic progression of the recipient corneal rim, increasing graft-host interface misalignment, or recurrent disease in the donor button.[207-209]

As an alternative, UV-crosslinking has recently been introduced as a treatment for progressing early to moderate keratoconus stages, with the aim to strengthen the stromal collagenous corneal matrix and thereby delaying or avoiding further ectasia progression.[210,211] The initially described protocol, the so called Dresden protocol, entails 30 minutes of soaking a de-epithelized cornea with the vitamin and photosensitizer Riboflavin, followed by UV-A irradiation for 30 minutes.[210] Currently, UV-crosslinking is indicated in cases with documented disease progression, a clear central cornea, and a corneal thickness of at least 400 µm after removal of the epithelium.[211] Although techniques are being developed to also treat thinner corneas (such as the use of hypotonic Riboflavin to swell the cornea), at present there have been limited studies of the efficacy and safety of UV-crosslinking in thin corneas, with relatively few included eyes with severe thinning (<350 µm).[212-215] Furthermore, the risk of complications or treatment failure seems higher in steeper keratoconus corneas (>58D).[216,217]

Another possibility to potentially postpone corneal transplantation in ectatic eyes may be by reshaping the cornea using intracorneal ring segments (ICRS), alone or in combination with UV-crosslinking.[218-220] ICRS are made of polymethylmethacrylate plastic and are available in numerous arc-lengths, thicknesses, and designs. The segments are placed into corneal stromal tunnels which may be created manually, or automatically with a femtosecond laser.[220] By normalizing the corneal contour, ICRS may enable a contact lens-intolerant patient to become contact lens-tolerant again.[218,221,222] Beyond this, (un)corrected visual acuity may show a modest improvement.[220,223-225] Still, eyes with severe corneal thinning or steepening seem currently ineligible for ICRS secondary to the relatively higher rate of complications and poorer visual outcomes.[226]

Advanced keratoconus eyes, showing continuous progression, but being ineligible for either UV-crosslinking or ICRS, may still reach satisfactory vision with well fitted contact

lenses, most often scleral lenses. These eyes may therefore similarly benefit from stabilizing the cornea to preserve the vision and to enable continued contact lens wear, and consequently postpone or avoid the need for PK/DALK. However, a procedure that could halt keratoconus progression in these corneas was not available (Figure 9). Prevention of disease (progression) may be the first step in medical care, hence developing a treatment to also halt progression in this group of keratoconus patients was a main objective.

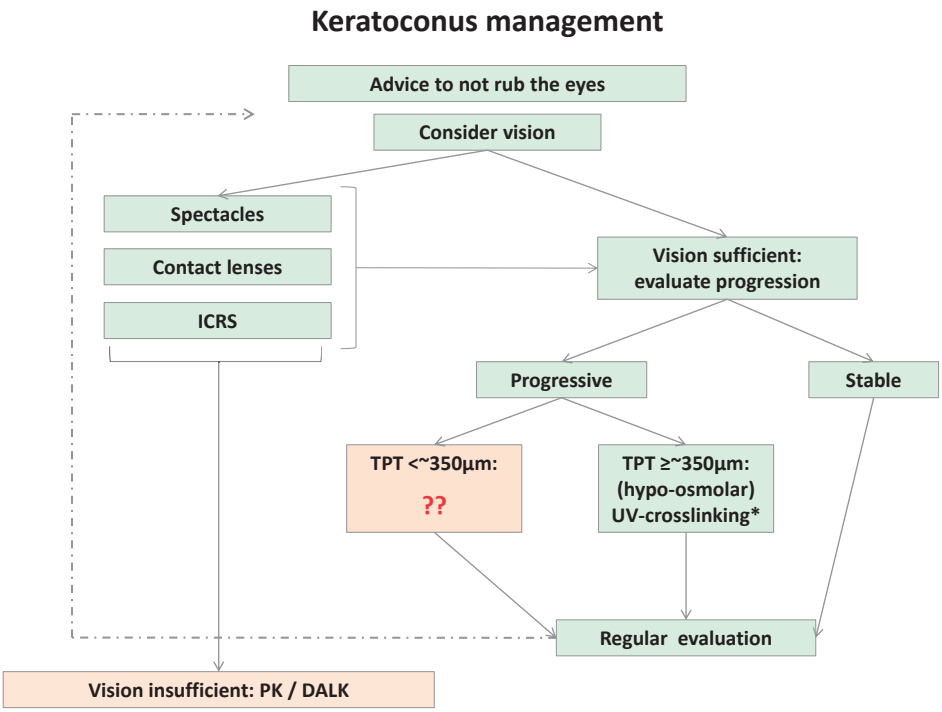

Figure 9. Keratoconus management decision tree. To potentially avoid or postpone the need for penetrating or deep anterior lamellar keratoplasty (PK/DALK), UV-crosslinking may be the preferred treatment for progressive keratoconus in cases with still acceptable vision. Furthermore, intracorneal ring segments (ICRS) seem viable to optimize vision and/or contact lens tolerance. However, very thin corneas seem ineligible for either UV-crosslinking or ICRS and are therefore allowed to continue to progress, with the risk that eventually PK or DALK becomes essential.
(ICRS indicates intracorneal ring segments; PK, penetrating keratoplasty; DALK, deep anterior lamellar keratoplasty; TPT, thinnest point thickness, measured after removal of the epithelium; µm, micrometers; *hypotonic Riboflavin should be used if TPT is between 350 - 400µm.[227])

OUTLINE OF THE THESIS

This thesis evaluates the feasibility and clinical outcomes of DMEK for managing endothelial disorders, and the use of BL grafts, i.e. Bowman layer transplantation, in the management of advanced keratoconus.

The first part concerns the outcomes of DMEK. We hypothesized that selective transplantation of only a DM and its endothelium would provide a fast and unprecedented high rate of optical recovery. In order to test this hypothesis, the first part of this research has been designed to accomplish the following objectives:

In Chapter 2, the feasibility and efficacy of DMEK for the management of Fuchs endothelial dystrophy, bullous keratopathy or previous corneal transplant failure is assessed by evaluating the clinical outcome before and up to 6 months after surgery and by documenting intra- and postoperative complications in a large consecutive series of DMEK eyes.

In Chapter 3, the incidence and causes of anterior corneal surface irregularities associated with visual complaints after successful DMEK surgery are reported and the efficacy of contact lens fitting in these cases is evaluated.

In Chapter 4, corneal higher-order aberrations, backscattered light, and their correlation with visual acuity outcome in a large series of DMEK eyes before and 6 months after surgery for Fuchs endothelial dystrophy are evaluated and compared with an age-matched control group of healthy subjects with no history of ocular disease.

In Chapter 5, the two-year refractive outcomes after DMEK in a larger series of pseudophakic eyes, undergoing DMEK for Fuchs endothelial dystrophy, are monitored. Furthermore, factors influencing the pre- to postoperative refractive changes and the time point of stabilization are determined.

The second part concerns the outcomes of BL transplantation, a newly designed procedure for advanced keratoconus cases, ineligible for UV-crosslinking or ICRS. We hypothesized that partial restoration of the corneal anatomy in an advanced keratoconus cornea might be obtained through implantation of an isolated BL graft, since fragmentation of the recipient's BL is one of the pathognomonic features in advanced keratoconus. BL transplantation aims to stabilize the ectasia and, at the same time, to preserve the patient's vision and enable continued contact lens wear. The procedure should be free from short- and long-term complications that both PK and DALK frequently entail. This part includes the following objectives:

In Chapter 6, the surgical approach for midstromal implantation of an isolated BL graft is described. Furthermore, the ability of this surgical approach to reduce ectasia and to preserve patient's vision is evaluated.

In Chapter 7, the clinical outcome of the first 22 mid-stromal BL transplantations is evaluated and the potential impact of preoperative corneal characteristics on the anatomic effect of the surgery is determined.

In Chapter 8, the clinical outcome up to 5 years after BL transplantation is evaluated to determine whether stabilization of ectasia in advanced keratoconus may be achieved long term.

The different outcomes are discussed in Chapters 9 and 10.

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