Descemet membrane endothelial keratoplasty: graft rejection, failure and survival

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CHAPTER 1

General Introduction and Thesis Outline

GENERAL INTRODUCTION

Common indications for corneal transplantation include Fuchs Endothelial Corneal Dystrophy, bullous keratopathy, and failed transplants. These endothelial disorders used to be treated with a full thickness (penetrating) graft for more than a century, until the successful replacement of the host posterior stroma with the diseased Descemet membrane and the endothelial monolayer by a similar donor graft was introduced by Melles in 1998. In the following decade, this posterior lamellar (endothelial) keratoplasty approach underwent continuous anatomical refinement by eliminating the stroma from the graft and herewith achieving a selective replacement of only the diseased Descemet membrane with its endothelium. This approach was called Descemet Membrane Endothelial Keratoplasty (DMEK). DMEK convinces with excellent clinical outcomes and lower complication rates compared to earlier keratoplasty techniques.

This thesis investigates two important complications after DMEK that may have an impact on graft survival, namely allograft rejection and graft failure. By understanding reasons for failed or unsuccessful DMEK grafts, certain complications may be avoided, more quickly recognized, or even prevented in the future. To understand the subtle differences in corneal transplantation techniques, this introduction will provide an overview of the corneal anatomy and surgical approaches and their evolution.

THE HUMAN EYE

The eye is surrounded and shielded by the eyelids. From anterior to posterior, the eye consists of the cornea, conjunctiva and sclera, the anterior chamber with aqueous humor, iris and pupil, the crystalline lens, the vitreous and retina plus choroid, and the optic nerve (Figure 1). The cornea will be the focus of this thesis.

The cornea

The human cornea is the transparent, avascular, and usually invisible structure in the very front of the eye. In adults, the cornea's diameter measures about 11–12 mm, horizontally about 1–2 mm more than vertically, while the corneal thickness is about 500 micrometers (μm) centrally and increases towards the periphery.[1–3] The cornea is a highly innervated tissue with a density of nerve endings that is about 300 to 400 times higher than in the skin.[2,4] The innervation is required for tissue repair and for pain perception.[2] Already minor damage to the cornea or a

Figure 1. Cross-section of the human eye.
Source: https://www.outlanderanatomy.com/a-real-eye-opener-the-eye-part.

very tiny foreign body on its surface can cause colossal pain and irritation. This can be a real nightmare, but in fact it is a blessing for the eye because ensuing permanent damage or inflammation of the cornea may cause irreversible visual impairment or even blindness. Besides, the cornea is the main refractive component in the eye´s optical system that contributes to a sharp image and has a major responsibility in protecting the eye from infections.[1-3] Five layers can be distinguished within the cornea, of which three are cellular layers and two serve as interfaces. From the front to the back those layers are the epithelium, Bowman layer, stroma, Descemet membrane and endothelium (Figure 2).[1-3]

The *epithelium* is about 40-50 μm thick and, due to the junctional complexes between the epithelial cells, it forms a strong barrier to germs, toxins, dust, and other substances that may harm the inner eye. The epithelium comprises five to six cell sheets of three sorts of cell types: the outer multilayer of superficial cells which is covered by the tear film is followed by two to three layers of wing cells

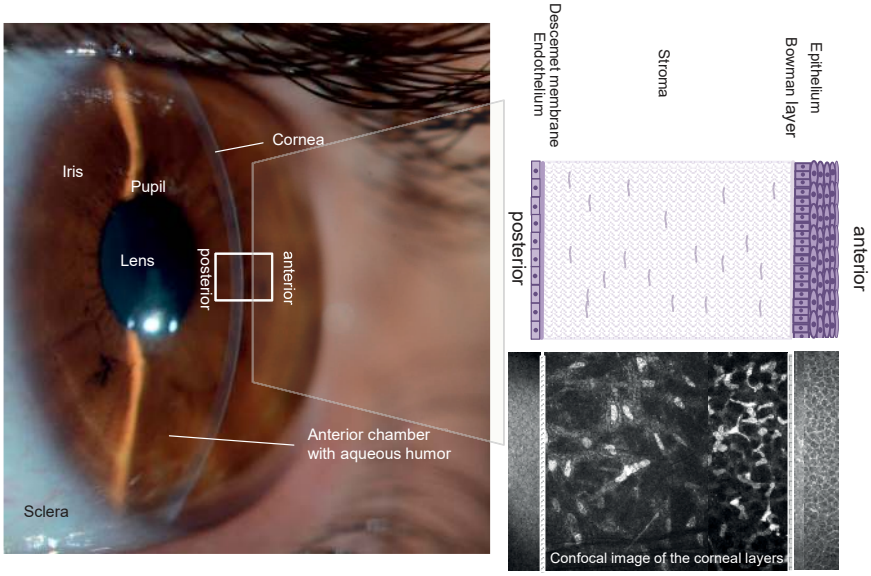

Figure 2. Slit lamp image of the author´s anterior eye (left) and a cartoon image (right, middle) and confocal microscopy image (right, bottom) of the corneal layers from anterior to posterior (right to left).

and the deepest single columnar basal cell layer. The latter adheres posteriorly to the epithelial basement membrane.[1,2]

Directly adjacent to the epithelial basement membrane is the *Bowman layer*, named after William Bowman (1816-1892), who was an English ophthalmologist and anatomist. He discovered this 8-15 μm thin membrane-like layer that becomes thinner with age and consists of interwoven collagen fibers. The collagen is secreted by stromal keratocytes, and the Bowman layer appears to merge with the stromal fibers, which could explain why it is histologically looked upon as the acellular superficial layer of the anterior stroma.[1,2] An anatomical characteristic of the Bowman layer that probably contributes to its stiffness and aids in sustaining the corneal shape is that, in contrast to the fibers of the stroma, those of the Bowman layer are smaller in diameter and randomly arranged.[1-3,5,6]

The *stroma*, the major component of the cornea, makes up approximately 80-90% of the entire corneal thickness.[1,3] Just like the Bowman layer, its anatomic structure plays an essential role in sustaining the characteristic corneal shape and rigidity.[7] The stroma predominantly contains water and non-aqueous components, i.e. collagen fibers, proteoglycans and cells.[1,2,8] The collagen fibers are

regularly organized in parallel bunches forming so-called fibrils with a characteristically small and uniform diameter.[1,2,8,9] The interfibril spaces are relatively homogenous and constant, which is maintained by proteoglycans. Fibrils are united in lamellae which are surrounded by keratocytes that form a three-dimensional network and vary in density throughout the stroma. Keratocytes continuously process (digest and produce) stromal components. The central cornea comprises about 200 lamellae with a packing density decreasing from anterior to posterior.[1,2,8-10] Because the lamellae show considerably more interweavements anteriorly than posteriorly, the posterior stroma swells more easily.[7,8] In contrast to other collagenous tissues, the cornea is transparent and clear, allowing optimal optical performance. This clarity is achieved by the unique delicate arrangement and organization of the fibrils and their constant and balanced turnover, as well as the lack of blood and lymphatic vessels.[2,8,9,11] In addition, corneal transparency strongly depends on the integrity of the epithelial and endothelial layers but also on the stable stromal water content of about 78%.[2,9] The deturgescence of the cornea is controlled by the endothelium and its basement membrane, the Descemet membrane.

The *Descemet membrane*, named after the French physician Jean Descemet (1732–1810), adheres anteriorly to the stroma and posteriorly to the corneal endothelial monolayer that secretes Descemet membrane. The latter is composed of a fine collagen meshwork that thickens after birth until adulthood from 3 μm to about 10 μm, respectively.[12] Histologically, three layers can be identified: a thin non-banded layer (0.3 μm) adjacent to the posterior stroma, an anterior banded zone (2–4 μm), and a posterior non-banded zone (>4 μm), which thickens with age.[12,13] The existence of a distinctive noncellular pre-Descemet stromal layer in the human cornea is controversial.[14,15]

The *endothelium* is directly adjacent to the posterior side of Descemet membrane and lines the inner surface of the cornea that is in contact with the aqueous humor in the anterior chamber. The cells have mostly a hexagonal shape, are interlaced and contain various junctions to enable intercellular exchange of molecules and electrolytes.[2] When examined from posteriorly, this monolayer appears very 'relaxed' and 'peaceful' while resembling a honeycomb mosaic (Figure 3).[1,2] However, when looking more closely, the endothelial cells have a large nucleus and plenty of cytoplasmic organelles (e.g., mitochondria, Golgi apparatus, etc.) that imply a high metabolic activity.[2] This activity is essential to maintain corneal transparency to preserve vision. The cells accomplish this by providing a fluid pump and a leaky barrier that together form a so-called pump-

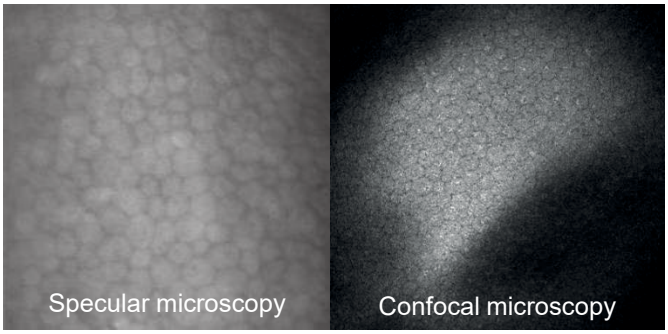

Figure 3. Specular (left) and confocal (right) microscopy images displaying the honeycomb mosaic of the corneal endothelial cell layer.

leak mechanism.[9,16] Consequently, a relative corneal dehydration is realized by passive (energy-free) fluid diffusion along an osmotic gradient from the relatively hypotonic stroma towards the relatively hypertonic aqueous humor. This stream is promoted by active (energy-requiring) pumps in the endothelial cell walls that transport ions from the stroma to the aqueous humor to maintain the osmotic gradient. Simultaneously, the endothelium possesses a barrier function allowing a selective fluid/nutrients drift from the anterior chamber into the avascular stroma through leaky intercellular junctions.[1,2,16–19] Given that the endothelial cells are healthy and sufficient, fluid inflow and outflow is in equilibrium. An imbalance due to endothelial dysfunction or major cell depletion caused, for example, by ocular surgery, inflammation, or trauma, can lead to corneal edema and thickening with subsequent loss of clarity.[1,2]

So far, the main treatment to restore corneal clarity in opaque corneas is corneal transplantation, also known as keratoplasty. Descemet Membrane Endothelial Keratoplasty (DMEK) is the most recent surgical innovation among endothelial keratoplasty techniques that selectively replaces the diseased endothelium with its Descemet membrane by a healthy donor endothelial Descemet sheet.

ADVANCES IN CORNEAL TRANSPLANTATION

Corneal grafting is the oldest, most frequently performed and most successful form of transplantation in medicine and was developed to restore corneal transparency in eyes with diseased and opaque corneas by replacing the affected tissue by a healthy donor graft. There is notable controversy in the literature about who first introduced the idea and concept of corneal transplantation. Fact is,

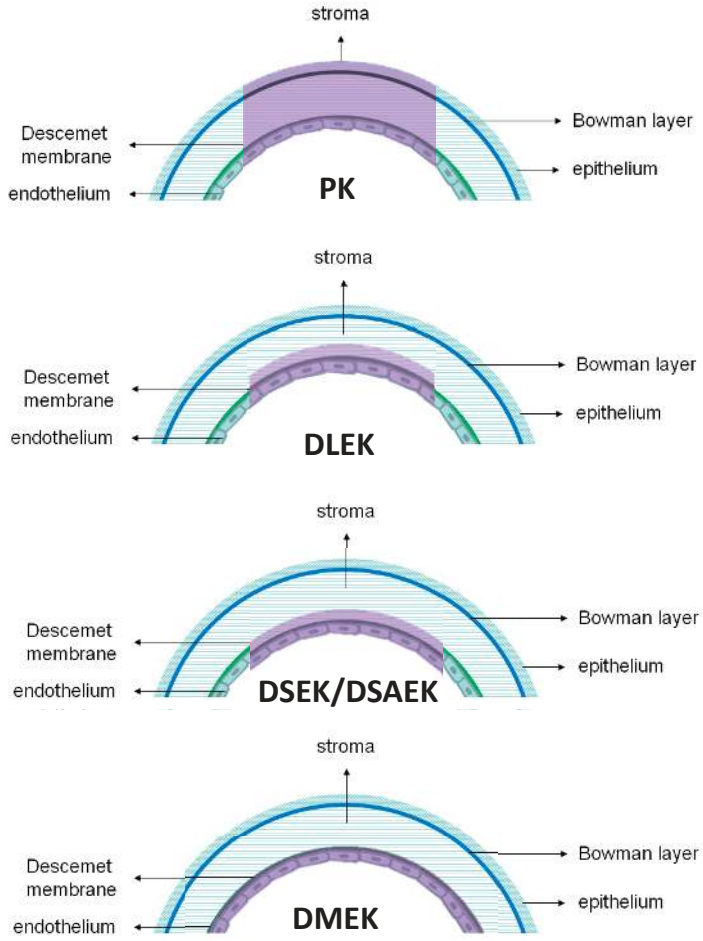

Figure 4. Evolution of Endothelial Keratoplasty.
PK = Penetrating Keratoplasty; DLEK = Deep Lamellar Endothelial Keratoplasty; DSEK/DSAEK = Descemet Stripping (Automated) Endothelial Keratoplasty; DMEK = Descemet Membrane Endothelial Keratoplasty.

that the early efforts can be traced back more than 200 years and, interestingly, the evolution of exchanging the entire corneal tissue (full-thickness or penetrating keratoplasty) or only selected anterior or posterior corneal layers (lamellar keratoplasty) took place side by side from the very beginning (Figure 4).[20]

Penetrating keratoplasty

In 1789, the French surgeon Guillaume Pellier de Quengsy first suggested to replace corneal scars by glass framed in silver that was then attached to the sclera.[20-22] However, experiments in corneal transplantation did not begin until the early 19th century. In 1813, a German ophthalmologist from Braunschweig, Karl

Himly, was the first to suggest that opaque animal corneas could be replaced by a healthy cornea of another animal.[23,24] Later, his student Franz Reisinger initiated experimental animal full thickness corneal transplantations and in 1818 introduced the term 'keratoplasty'.[20,25] Still, it required 70 more years until the first heterologous graft, a lamellar anterior graft, was successfully transplanted in 1888 by Arthur von Hippel, another German ophthalmologist, who already at that time believed that corneal transparency depended on the integrity of the endothelium and Descemet membrane.[20,26]

In 1905, the Austrian ophthalmologist Eduard Zirm performed the first homologous penetrating procedure in a human.[27] Subsequently, many contemporary ophthalmologists furthered and refined the penetrating approach which appeared less demanding and back then resulted in better clinical outcomes compared to the lamellar approach.[28,29] Undoubtedly, the advent of general anesthesia, microscopes, 'aseptic' surgical conditions, antibiotics and corticosteroids to treat allograft rejection, as well as the development and improvement of new instruments, trephines, suture material and donor tissue preparation and preservation played an important role in increasing the technique´s popularity and success.

Penetrating keratoplasty (PK) remained for many decades the standard of care for a variety of corneal disorders affecting any corneal layer. However, although PK could, to some degree, provide acceptable functional improvement, there were obvious limitations, which included: 'open-sky surgery', suture-related delayed healing and inflammation, secondary ulcers, globe instability, corneal vascularization, higher risk of graft rejection, unpredictable refractive errors with unsatisfactory visual outcomes and graft failure due to endothelial cell decay.[30-42] Attempts in reducing complications and enhancing outcomes by applying laser-guided donor and recipient corneal trephination or secondary artificial lens implantation provided only some relief.[43-46] In addition, strikingly, 30-50% of indications for PK affected only the corneal endothelium, which practically meant the unnecessary removal of also healthy anterior and central corneal tissue.[47]

Posterior lamellar keratoplasty

In the 1950s, the ophthalmologists, José Barraquer from Spain, and Charles Tillet from the United States, were the first to introduce a technique for lamellar endothelial replacement by suturing the transplant underneath a manually dissected stromal flap.[48,49] This was a promising concept; however, the technique

was challenging and difficult to adopt. Later modifications by diverse scientists were not yet successful.[50,51] In 1998, the Dutch ophthalmologist, Gerrit Melles, introduced a concept in which the adherence of the endothelial transplant to the recipient posterior stroma was achieved by injecting an air bubble into the anterior chamber underneath the graft while the patient remained in a supine position.[52,53] Posterior Lamellar Keratoplasty (PLK) proved to be clinically successful and was the beginning of an exciting era of successional developments in new techniques (Figure 4).[54]

Deep Lamellar Endothelial keratoplasty (DLEK)

In the primary PLK procedure, a 7-7.5mm recipient stromal disc with the diseased endothelium was excised and a similar-sized donor tissue of stroma and endothelium was inserted through a 9mm and later a 5mm sclero-corneal incision.[54,55] The latter was technically possible because the graft was folded like a 'taco' and then unfolded inside the recipient's anterior chamber.[55] Melles` PLK technique was soon popularized in the United States by Mark Terry as 'Deep Lamellar Endothelial Keratoplasty' (DLEK).[56,57] Although the preliminary results of the procedure were encouraging, the manual dissection of the recipient and donor lenticules were quite demanding which inhibited broad adoption.

Descemet Stripping (Automated) Endothelial Keratoplasty (DS(A)EK)

In 2002, Melles and colleagues modified the DLEK technique by facilitating the removal ('stripping') of the host's diseased corneal Descemet membrane and endothelium. This so-called 'descemetorhexis' was combined with the insertion of the same graft as used earlier in DLEK and was then redefined as 'Descemet-Stripping Endothelial Keratoplasty' (DSEK).[58-60] To simplify donor tissue preparation, Mark Gorovoy popularized the use of a microkeratome and adjusted the nomenclature by an additional ´A`: 'Descemet-stripping *Automated* Endothelial Keratoplasty' (DSAEK).[61] Standardizing graft preparation was a decisive step in making this technique accessible to a broad range of surgeons because it enabled eye banks to provide pre-cut tissue and surgeons to prepare the tissue on their own. Subsequent adoption of endothelial keratoplasty could no longer be halted since, in contrast to its precursor PK, the lamellar techniques provided better and faster visual recovery with more predictable refractive errors due to the preserved ocular anterior surface. Furthermore, these novel techniques were associated with less complications and minimized the risk of intraoperative expulsive bleeding due to the 'closed-globe' surgery, of traumatic wound dehiscence due to the avoidance of large penetrating incisions, caused less wound

healing problems, less corneal vascularization due to the sutureless surgery, and a remarkably lower risk of allograft rejection.[62,63]

After almost 100 years, in which PK had been the only available form of (unselective) corneal transplantation, PLK had its breakthrough and was implemented as the new 'gold standard' for the treatment of endothelial pathologies within 10 years of its creation.[64]

Descemet Membrane Endothelial Keratoplasty (DMEK)

Still, also these innovative lamellar procedures showed functional limitations with undesired fluctuations in visual outcome and optical aberrations which seemed attributed to graft thickness and graft irregularity. The additional posterior stroma in the endothelial transplant could induce interface haze and scarring and the preparation of the graft both manually and with a microkeratome could result in an irregular graft thickness. The latter may produce posterior surface irregularities and aberrations that may be even higher than after PK.[65-73]

One might expect better visual outcomes after restoration of the human corneal anatomy. Consequently, Melles further refined endothelial keratoplasty by eliminating the stroma from the donor graft and hereby achieving a selective replacement of only the diseased Descemet membrane with its endothelium.[74-75] In 2006, Melles performed the first surgery on a Dutch patient (Figure 5) with Fuchs Endothelial Corneal Dystrophy (FECD) using this new approach and named this innovation Descemet Membrane Endothelial Keratoplasty (DMEK).[76-77]

Figure 5. Photograph showing Dr. Gerrit Melles (left) and the first patient (right) who underwent a Descemet Membrane Endothelial Keratoplasty in August 2006 at the Melles Cornea Clinic, Rotterdam, The Netherlands in 2016. Patient´s consent received for publication.

Simultaneously, the Melles group developed a technique for DMEK graft preparation that preserved the anterior stroma for transplantation into another eye that suffered from stromal disease.[78,79] Despite the convincing preliminary results of the thinner DMEK with exceptional visual acuity levels and a considerably lower incidence of allograft rejections, surgeons were hesitant to start with this new technique since preoperative graft preparation, intraoperative graft handling (unfolding, orientation), as well as postoperative graft dehiscence, i.e. the (in) complete DMEK graft detachment from the posterior stroma, were difficult steps and hurdles.[80-91] In the following years, technique standardization and refinement of preparation and surgery (Figure 6) steepened the surgeon´s learning curve and lowered the threshold to offer DMEK to patients.[92-102] Aside from increased

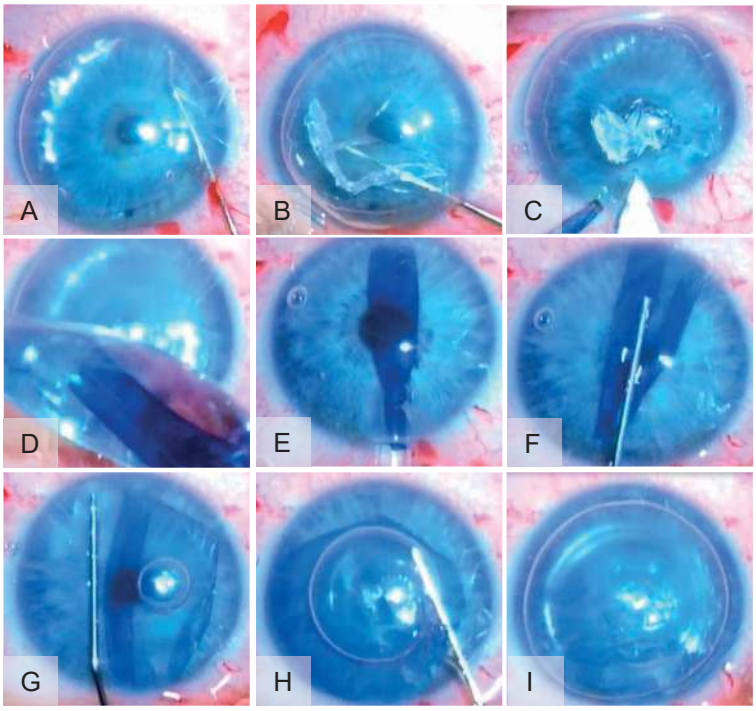

Figure 6. Steps of standardized 'no-touch' DMEK: (A, B) Scoring and stripping of the recipient's Descemet membrane from the posterior stroma, under an air-filled anterior chamber. (C) Creation of a 3.0mm limbal tunnel incision at the 12:00 o'clock position. (D, E) DMEK roll placed in an injector after staining with trypan blue solution before injection into the recipient anterior chamber. (F, G, H) Orientation of the graft (donor endothelial side down, donor Descemet membrane side up facing posterior stroma) and unfolding over the iris. (I) Injection of an air-bubble underneath the graft to position it onto the recipient posterior stroma. At the end of the surgery, the anterior chamber is left completely filled with air for about 30-60 minutes, followed by an air-liquid exchange, leaving a 30% to 50% air-bubble in the anterior chamber.

surgeon´s experience, adoption was further encouraged by the decreasing detachment rates thanks to either air or gas tamponade.[103-108]

Since the introduction, DSEK/DSAEK as well as DMEK have evolved into generally established techniques for the treatment of endothelial disorders. Both techniques are still being modified: DSEK/DSAEK grafts are becoming thinner to reach DMEK outcomes in terms of visual acuity levels, whereas DMEK modifications aim at utilizing endothelial donor tissue more efficiently by transplanting different graft shapes or sizes.[108-119] Accordingly, in 2014 and 2016, respectively, the Melles group introduced 'Hemi-DMEK' and 'Quarter-DMEK' techniques in which from one donor cornea two semi-circular or four quarter-shaped DMEK grafts can be prepared that can potentially be transplanted into two or four recipients (Figure 7).[112-119] This approach would then allow doubling and quadrupling the number of endothelial grafts recovered from the same donor pool. Although standard DMEK and modified DMEK techniques may be similar surgically, the indications for each surgery type may differ.

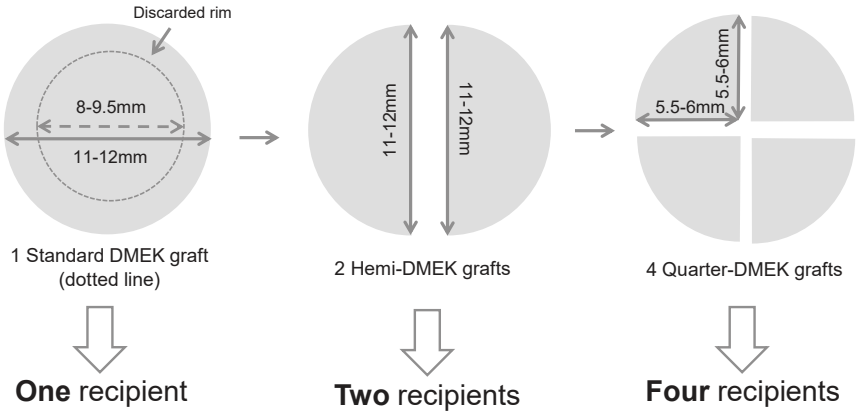

Figure 7. Different graft shapes of available DMEK techniques. (Left) standard DMEK: a central 8.5-9.5mm circular DMEK-graft is trephinated and transplanted into one recipient; the outer Descemet membrane rim is discarded. (Middle) Hemi-DMEK: the entire 11-12mm diameter Descemet membrane sheet is utilized to obtain two semi-circular grafts to be transplanted into two recipients. Each Hemi-DMEK graft has a similar surface area as a circular standard DMEK graft. (Right) Quarter DMEK: each Hemi-DMEK graft is further divided into two quarters, providing in total four Quarter-DMEK grafts to be transplanted into four recipients.

INDICATIONS FOR DMEK

Patients with visual impairment from endothelial dysfunction are the target group for endothelial keratoplasty. DMEK has gained broad popularity over the past years. Along with the rising numbers of procedures, also the surgical experience with this technique has expanded and with it the ease for surgeons to perform DMEK on more patients with diverse endothelial diseases.[6,103,120,121]

Fuchs Endothelial Corneal Dystrophy (FECD)

FECD, the most common indication for DMEK, was first described in 1910 by the Austrian ophthalmologist, Ernst Fuchs, who initially termed it 'Dystrophia epithelialis corneae' since he assumed an epithelial pathology due to the morphologic changes in the corneal surface with reduced corneal sensitivity. Despite lacking a slit lamp, Fuchs assumed a defective endothelial layer, but since he could not judge the distinct anatomic changes, he suggested to redefine etiology and nomenclature later again if necessary.[122] One typical characteristic of FECD is the presence of corneal 'guttae' which were first observed and termed by the Swiss ophthalmologist Alfred Vogt in 1921.[123] 'Guttae' result from focal thickening of the Descemet membrane forming excrescences due to accumulations of collagen that are produced by abnormal endothelial cells.[2] They are often scattered more horizontally and inferiorly over the posterior corneal surface than vertically and superiorly.[124,125] Disease progression with continuous loss of endothelial cells ultimately results in corneal edema with subsequent fibrotic changes of stroma and epithelium. Patients complain of fluctuating or gradually deteriorating vision, glare (especially at night), but also of ocular pain from ruptured epithelial bullae due to uncovered nerve endings.[2,126–128]

FECD is a slowly progressive, usually bilateral disease of the posterior corneal layers.[2] An early-onset and a late-onset phenotype can be distinguished. The early-onset form occurs in only 1% of FECD cases, is equally present in males and females and can manifest already in the first decade of life to progress further before the age of 50. The late-onset, more common form occurs predominantly in women and develops in the second or third decade, to become symptomatic in the fifth to sixth decade.[2,127–129] Clinical progression of FECD is graded in different stages depending on the extension and confluence of guttae and the presence of corneal edema.[2,127,130,131] While mild edema may not necessarily cause visual deterioration, the presence of guttae without edema can significantly induce light scattering and photophobia, as well as reduce contrast sensitivity that may be very bothersome for the patient.[132]

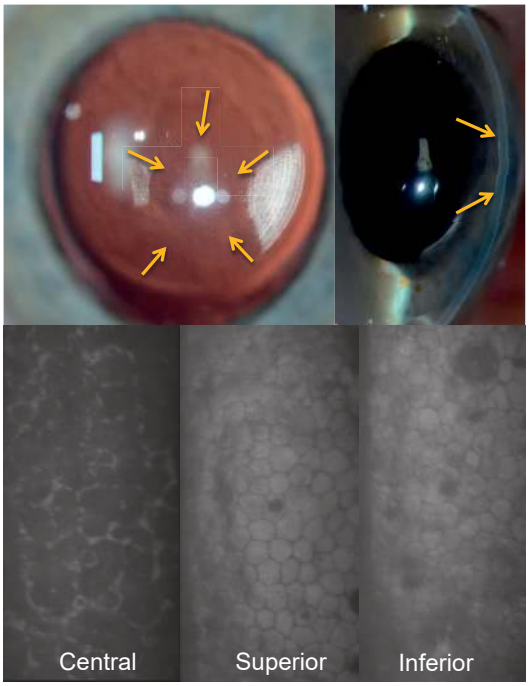

Figure 8. Slit lamp images (upper row) of a cornea in reflecting light (left) and with a slit from the side (right) showing centrally localized guttae (arrows). Specular microscopy images (lower row) show that in the same eye central endothelial cells are not visible whereas in the peripheral areas (superior, inferior) cells can be visualized with only few guttae (dark spots).

Recently, it has been suggested that in FECD eyes with only centrally localized guttae and no or only mild corneal edema (Figure 8), transplantation of a smaller Quarter-DMEK graft may be sufficient to enhance corneal clearance in the stripped area and diminish visual distortions.[118,119] Removing only the affected central Descemet membrane and endothelium with a small central descemetorhexis may help to sustain viable peripheral recipient endothelial cells, potentially even eliminating the need for a transplant as in Descemetorhexis only (DSO).[133–137]

Bullous keratopathy

Bullous keratopathy stands for an entity of conditions that may show the same clinical picture but that differ in their underlying cause. These eyes are often characterized by pronounced and quickly progressing corneal edema that is associated with endothelial cell depletion. It often follows surgical trauma, as in 'pseudophakic bullous keratopathy' after (complicated) cataract surgery or after glaucoma (tube) surgery, but can also occur in eyes with congenital glaucoma, aphakia or rarely after ocular trauma.[138,139] In contrast to patients with FECD, patients with bullous keratopathy often experience a faster non-fluctuating decrease in visual acuity, while visual distortions and pain are similar.

Failed transplants

Another indication for DMEK consists of eyes with corneal decompensation following corneal transplantation, so-called graft failure. Corneal edema results from the inability of the (remaining) graft endothelial cells to maintain corneal clarity. When irreversible and accompanied by major visual impairment, only repeat transplantation can restore vision. Graft failure can occur after any form of transplantation, i.e., PK, DSEK/DSAEK and DMEK. From long-term studies we know that certain eyes are more prone to develop failure.[140–142] Risk factors for graft failure include an enhanced cell decline and low cell count early after keratoplasty, high risk recipient preoperative indications (e.g., herpetic eye disease), concomitant glaucoma, repeat grafting, prior rejection episodes, atopic ocular surface disease and corneal vascularization.[38–42,140–145] In early keratoplasty studies, the term graft failure traditionally referred to endothelial failure, i.e., a non-functioning graft. More recent studies 'confusingly' also use this term to characterize any graft that requires repeat transplantation, like for unsatisfying refractive or visual outcomes after PK or DSEK/DSAEK, despite a clear graft with functioning endothelium.[41,146]

DMEK COMPLICATIONS

Complications can either occur intraoperatively and/or postoperatively after any surgery. With the novel PLK techniques, but especially with DMEK, the type, incidence and severity of complications has changed considerably. Still, prevention and improved management of complications will further enhance clinical outcomes and patient satisfaction. In the following part, DMEK complications that are relevant for this thesis will be described.

Graft dehiscence

Following the introduction of endothelial keratoplasty, graft detachment evolved as a new complication in the early postoperative period.[89–91] It describes the partial or complete non-adherence of the endothelial graft to the recipient posterior stroma. Compared to DSEK/DSAEK grafts, DMEK grafts detach more easily and tend to curl up, which often hinders spontaneous re-attachment.[147] Although it was initially assumed that complete apposition of the graft is required to achieve corneal clearance, we now know from clinical observations that corneas with smaller detachments, for example involving only graft edges (≤ 1/3 of the graft surface area), can clear without secondary intervention.[148–151] Spontaneous clearance was also observed in eyes with larger or complete detachments with

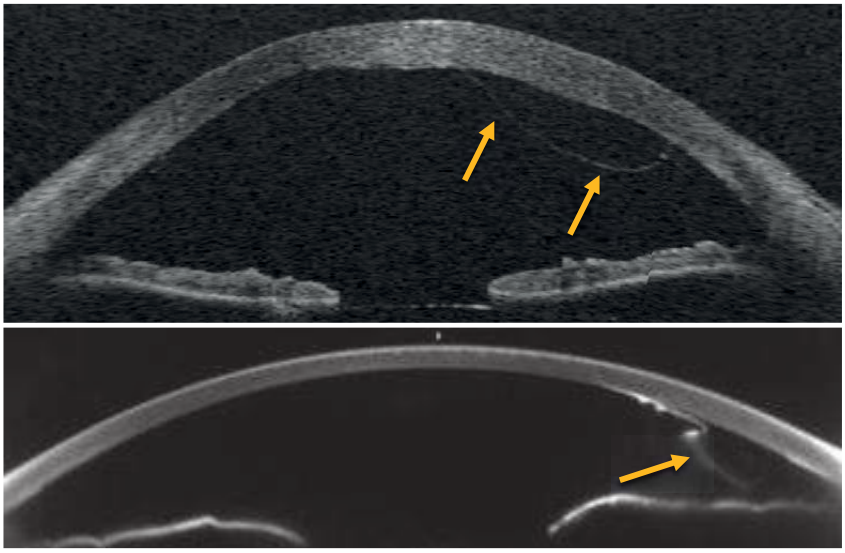

Figure 9. DMEK graft detachments (arrows) visible on Anterior Segment Optical Coherence Tomography (upper picture, notice the curling of the graft towards the stroma, indicating correct orientation) and on a Scheimpflug image (lower picture).

or without involvement of the optical axis (> 1/3 of the graft surface area).[150,151] Because clearance would often take longer (> 3 months) and may not be long-lasting, nowadays a re-injection of air/gas (so-called re-bubbling) or direct re-grafting is recommended in larger detachments.[147,152] Diverse causes for DMEK detachments have been identified, among which are: insufficient pressurization of the eye with wound leakage at the end of the surgery, residual Descemet remnants, eye rubbing and inward graft folds.[153] A preventable reason for DMEK detachment is an inverted graft.[147] After stripping the Descemet membrane from the posterior stroma, it usually forms a roll with the endothelium on the outer side. This knowledge is essential to decide on proper graft orientation during surgery.[78,92] For identifying graft dehiscence, anterior segment Optical Coherence Tomography (OCT), that allows cross-sectional imaging of the anterior eye, has become indispensable (Figure 9).[154] Some surgeons use this tool also intraoperatively (intraoperative OCT) to determine and manage graft orientation and unfolding.[102,155] In contrast to an edematous cornea, in reasonably clear corneas, detached graft areas can also be visualized on a Scheimpflug image (Figure 9).[154]

Allograft rejection

The immune system is essential to fight (life)threatening pathogens and agents. We can differentiate two forms of immune reactions: a non-specific quick 'in-

nate (natural) immune system' that attracts immune cells to the required sites by excreting cytokines and that triggers the specific 'adaptive (acquired) immune system' by presenting specific antigens via the lymph nodes.[2] Although the adaptive immune system reacts initially with a delay, it can be more efficient because it is antigen-specific and develops a memory to react quicker in case of a second 'attack' by the same pathogen. The adaptive immune system utilizes two specific immune cells: B cells that direct the humoral (antibody) system and T cells, the cellular arm of the adaptive system; both are equipped with specific receptors. The B-cell receptor is an antibody that directly recognizes specific pathogens whereas the T-cell receptor can only recognize processed pathogens that are presented via HLA (human leukocyte antigen) molecules, which are cell-surface proteins on other immune cells. There are two classes of HLA antigens: 1. HLA Class I molecules, which present intracellular peptides to cytotoxic T cells (CD8 positive), and 2. HLA Class II molecules which present extracellular antigens (from outside a cell) to helper T cells (CD4 positive). Antigen presentation on HLA Class II molecules triggers the development of an immune response. HLA Class II molecules are expressed on a variety of immune cells, such as antigen presenting cells (APCs, e.g., dendritic cells, macrophages, monocytes), but also on B cells and activated T cells.[2]

The eye consists of vulnerable tissues with limited capabilities to regenerate: not only corneal endothelial cells but also retinal cells cannot proliferate. Consequently, an immune-mediated inflammation can lead to cell damage with functional loss or even blindness. To protect the eye, nature has provided it with a unique 'immune privilege'.[156,157]

The Dutchman, Jacobus van Dooremaal, a student of F.C. Donders in Utrecht, already showed in 1873 that he could implant living tissue in the anterior chamber of the eye of dogs and rabbits without getting the normal severe immune response.[158]

In 1948, the British zoologist Peter Medawar and colleagues were the first who discovered that the eye was an immune privilege site. Their conclusions were based on observations of prolonged survival of an allogeneic tissue graft that was placed in the anterior chamber of the eye.[159] Since this work was groundbreaking for tissue and organ transplantation, Medewar is often referred to as the 'father of transplantation'.[160]

To date, the immune privilege of the cornea and anterior chamber is explained by the following mechanisms: 1. anatomical, cellular, and molecular barriers, 2. eye-derived immunological tolerance, and 3. an active systemic ocular immuno-suppressive component.[156,157,159,161] The blood-aqueous barrier and the avascular nature (lacking blood and lymphatic vessels) of the cornea hinder inflammatory cells from entering the eye and antigens to be presented to the immune-competent sites.[162,163] Tolerance is achieved by different mechanisms, such as a relative absence of APCs and the moderate expression of HLA antigens in stroma and endothelium.[164] In addition, an active suppression is realized by the presence of immunosuppressive cytokines (e.g., transforming growth factor-β, α-melanocyte stimulating hormone, vasoactive intestinal peptide), and anterior chamber-associated immune deviation (ACAID), that specifically helps to suppress cellular delayed type hypersensitivity and antibody-medicated immune responses against antigens introduced into the anterior chamber.[165-167]

This immune privilege is thought to be the reason why corneal transplantation is among the most successful tissue transplantations even without tissue typing or systemic immunosuppression.[168] This relates only to low-risk keratoplasties.[38,169] Some transplanted corneas still experience allograft rejection: a major risk factor is the extent of corneal vascularization and the state of the recipient bed.[2] Consequently, a breakdown in the immune privilege can be observed when a graft is placed in an inflamed eye, in cases with heavy recipient vascularization, after pre-sensitization by a previously rejected graft, in case of large eccentric grafts or application of pro-inflammatory medication.[2,166,169-173]

Since the introduction of endothelial keratoplasty, rejection rates have decreased with each technical refinement, possibly due to an increasing experience of surgeons, leading to less surgical trauma, and by transplantation of less donor tissue.[174,175] Until two years after surgery, rejection rates with PK, DSEK/DS(A)EK, and DMEK were 20%, 12%, and 1%, respectively, in a study with similar indications for grafting, comparable patient demographics and the same topical corticosteroid treatment.[85] This low rejection rate, but also the occurrence of milder rejection forms, were observed by several scientific groups.[84,85,175-179] With DMEK, HLA matching is practically not performed anymore and rejection rates did not increase after lowering the steroid potency one month after surgery but did increase when steroids were discontinued in the second year after DMEK.[178,179] Recently, the cumulative rejection rate 10 years post-DMEK was 4%.[180] Risk factors for rejection in DMEK are still unclear; however, in a study on DSEK, Afro-American race was found to be a significant risk factor for rejection.[181] Interestingly, mor-

phologic endothelial cell changes were observed in eyes that later developed DMEK rejection.[182] Although graft failure in eyes following DMEK-rejection in the first five years after surgery was rare,[175] it would be useful to avoid any allograft rejection and subsequent endothelial cell decline by recognizing eyes at risk of developing rejection.

Endothelial cell decay and graft failure

Bowman was the first to describe the appearance of endothelial cells under a microscope. In vivo visualization, however, only became possible in 1920, when Vogt described this mosaic of hexagonal cells visible in the specular reflection of the illuminator in a slit lamp biomicroscope.[19] Nowadays, specular microscopy and meanwhile also confocal microscopy are standard tools to visualize and evaluate the corneal endothelial cells and assess their approximate number (Figure 3).[183,184] At birth, the endothelial cell density measures around 6000 cells/mm^2 and decreases to 3500 cells/mm^2 within only five years and further to 2700 cells/mm^2 before adolescence.[16,185,186] While the cell decrease is accelerated in childhood, after the age of 18 years, the average rate of decrease stabilizes at 0.6% per year.[185,186] Since endothelial cells have no mitotic activity and cannot regenerate, the damage or loss of cells cannot be compensated.[1] Instead, residual neighboring cells migrate and spread out to carpet the denuded space which results in deformation (loss of hexagonality = pleomorphism) and enlargement (polymegathism) of the cells as part of the endothelial wound healing process.[1,2,16-19]

Endothelial cell density decrease can increase after ocular surgery and most importantly in post-keratoplasty corneas.[187-189] Following DMEK, endothelial cell density decreases by approximately 25-40% in the early phase, and after the 6-month follow-up, by 7-9% yearly.[190,191] Endothelial cell density is regularly evaluated after keratoplasty because its decrease is assumed to have impact on the survival of a graft. And although corneal clarity can be maintained with a density as low as 500 cells/mm^2, when a threshold is reached, the cornea may decompensate.[187]

When the cornea fails to clear directly after keratoplasty, this is referred to as 'primary or early failure' mainly due to impaired donor quality but sometimes also due to difficult surgery. In recent DMEK reports, the term primary failure is also used in eyes that required re-grafting for DMEK detachment or upside-down graft positioning. However, in these cases, corneal edema derives rather from a technical failure, i.e., the graft is not positioned/oriented properly, than from endothelial dysfunction. If the graft clears after surgery but fails later, it is referred to

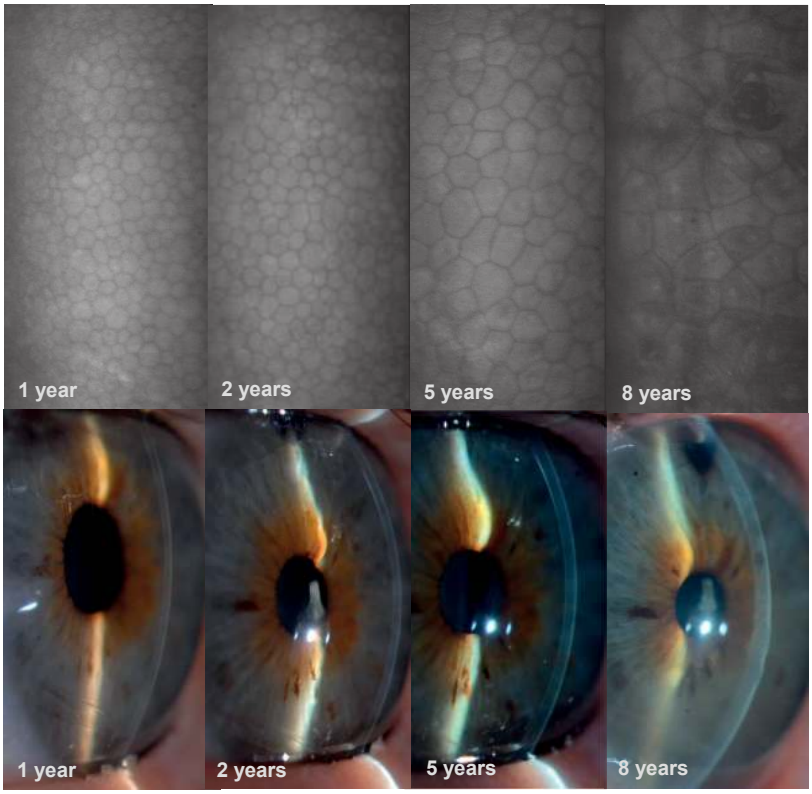

Figure 10. Specular Microscopy (upper row) and slit lamp images (lower row) of an eye eventually developing graft failure at 8 years after Descemet Membrane Endothelial Keratoplasty.

as 'secondary or later failure'. This can, for example, occur after rejection episodes, but also due to comorbidities like glaucoma or the primary indication for surgery (Figure 10). The number of failed grafts in a cohort tells us something about the survival rate of a graft. After PK, survival rates may vary from 75% to 95% at 3 and 5 years and from 50% to 80% at 10 years postoperatively, depending on indication and complications. After DSAEK and DSEK, 3- and 5-year survival rates of respectively 87% to 97% and 93% were reported. Early results after DMEK suggest that survival rates may at least be similar to earlier keratoplasty techniques.[192-198]

AIM AND THESIS OUTLINE

This thesis investigates two complications after DMEK that may have an impact on graft survival, namely graft failure and allograft rejection. Owing to the reduced complication rates in DMEK, we hypothesized that DMEK survival is promising and that outcomes of repeat DMEK to manage ´failed` grafts are acceptable. We further hypothesized that allograft rejection is a rare complication that appears to be more subtle after DMEK and that corneal changes before rejection may manifest themselves by other diagnostic approaches. To test our assumptions, we investigated the following objectives:

The first four chapters focus on graft longevity, graft failure, and repeat DMEK. In **Chapter 2,** the 10-year clinical outcome of the first ever DMEK patient is evaluated. **Chapter 3** depicts the longer-term endothelial survival after successful DMEK surgery in a large DMEK cohort. By identifying eyes with endothelial graft failure, possible reasons, and risk factors for DMEK graft failure are described. **Chapter 4** illustrates the problem of the diverging use of the term graft failure not only for endothelial failure but also for any DMEK graft that needs replacement without considering newly evolved complications such as graft detachment. In **Chapter 5,** the feasibility, and reasons for repeat DMEK along with the clinical outcomes and complications of repeat surgery up to 12 months are investigated.

The next four chapters focus on inflammation and allograft rejection. In **Chapter 6,** we assess whether aqueous flare can be used as a measure of subclinical inflammation in eyes following DMEK. In **Chapter 7,** we identify eyes with allograft rejection in a large consecutive DMEK series and determine the relevance of Scheimpflug imaging as a diagnostic tool for detection of corneal changes preceding allograft rejection. In **Chapter 8,** we correlate the occurrence of corneal changes seen on Scheimpflug images and specular microscopy in eyes before rejection manifests. In addition, we assess the increase in pachymetry and the decrease of the endothelial cell density.
In **Chapter 9,** we analyse the feasibility and outcome of transplanting a DMEK modification in which four smaller Quarter-DMEK grafts, potentially less-antigenic grafts, from the same donor are transplanted into four recipients.

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