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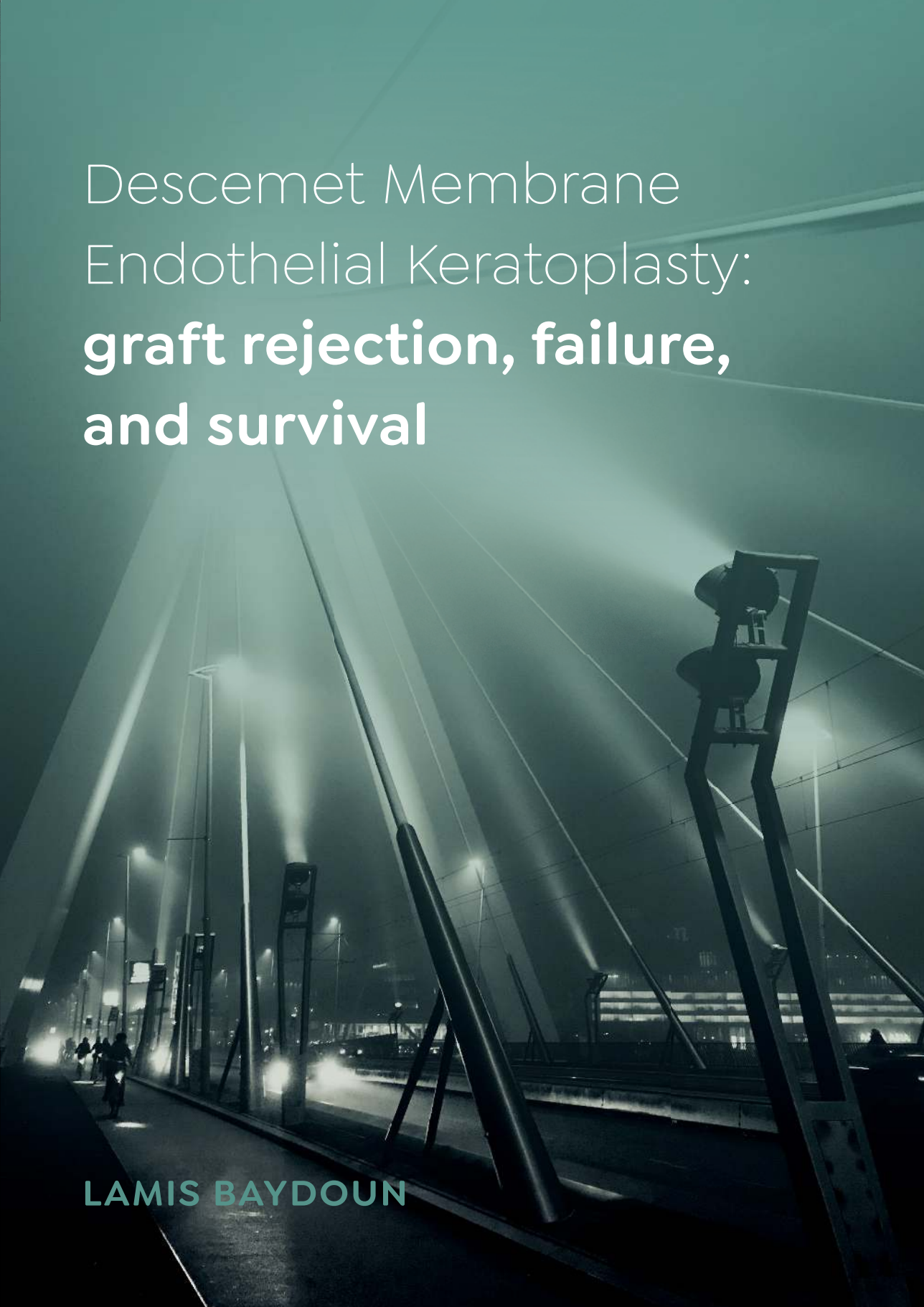

LAMIS BAYDOUN

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Thesis, Leiden University Medical Center, The Netherlands

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Descemet Membrane Endothelial Keratoplasty: graft rejection, failure, and survival

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For my Family

CONTENT

PREFACE

PREFACE

The *eye* is a beautiful and precious sensory organ that collects information from our environment to analyze and process our surroundings. In metaphors, such as 'I will guard it as my eyeball' or 'the eye is the mirror of our soul', we express the eyes´ value and importance and describe their ability to reflect emotions, our mood, and feelings in a non-verbal manner. Our eyes are pivotal for human interactions, especially in an increasingly digital and 'viral' world where 'real-world' interactions are diminishing or limited by face masks. Further, our 'eyes cannot lie' and may reveal health conditions and extraocular diseases to an ophthalmologist long before they become visible to doctors from other specialties. Ophthalmologists save eyes and only sporadically lives; it is fortunate that with medical and surgical skills, many eyes can be saved from blindness, though visual impairment caused by diseases of the lens or cornea can more often be reversed or treated than those of the macula and optic nerve.

The *cornea* will be the focus of this thesis. It is the transparent and most anterior structure of the eyeball, also referred to as 'the window of the eye' because it is clear as glass to allow rays of light to enter and produce an image on the retina, our ocular 'film'. The cornea consists of five layers, and when diseased, it often loses transparency and becomes cloudy as does the image that is transferred. To restore vision in such cases, for more than 100 years we have replaced all layers by a full thickness transplant, referred to as penetrating keratoplasty (PK), regardless of which layers were still healthy and functioning. In medicine, this unselective approach was the earliest and, so far, most successful form of transplantation, owing to the unique corneal immune privilege, that, in contrast to other organs, lack vessels to maintain transparency and to allow acceptance of the foreign donor tissue.

I vividly remember my fascination while assisting my first PK during my early residency at the University of Bonn, Germany. Back then, I was not aware of the evolving, even more fascinating innovative lamellar keratoplasty techniques that targeted the isolated replacement of only the diseased corneal layer(s). About a decade later I moved to Rotterdam, the Netherlands to stay for almost 7 years - instead of the originally intended 6 months - to specialize in these techniques with emphasis on Descemet membrane endothelial keratoplasty (DMEK), that selectively replaces the two posterior corneal layers (Descemet membrane and endothelium) with the same healthy layers from a donor cornea. In the past years, DMEK has revolutionized corneal transplantation all over the globe.

However, new keratoplasty techniques will only be established in the long-term if complications can be reduced and clinical outcomes improved, while graft longevity is equal or better than in earlier techniques.

In this thesis, I will investigate rejection, failure, and survival of the DMEK graft, topics that could not be closer linked to our daily life.

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

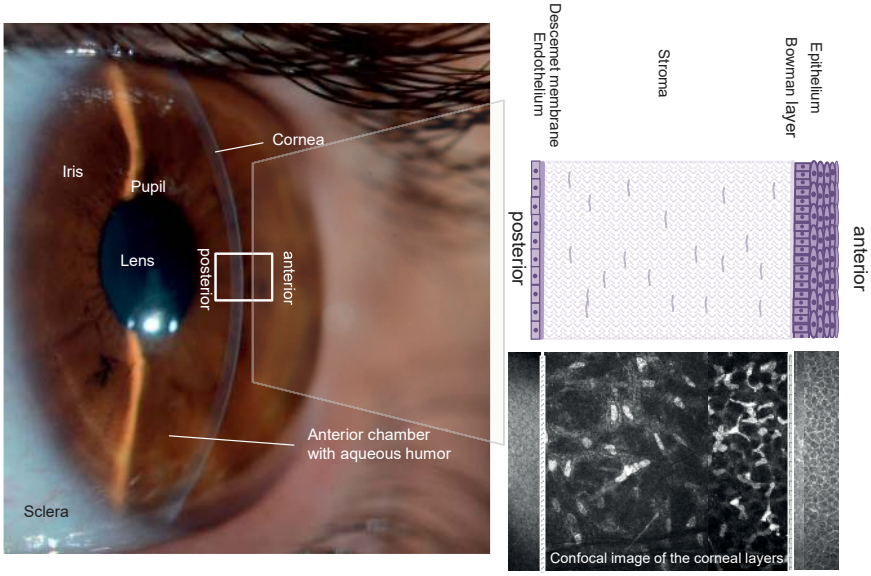

Figure 2. Slit lamp image of the author´s anterior eye (left) and a cartoon image (right, middle) and confocal microscopy image (right, bottom) of the corneal layers from anterior to posterior (right to left).

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-

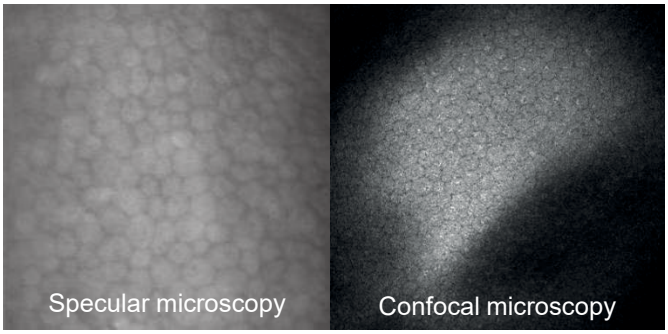

Figure 3. Specular (left) and confocal (right) microscopy images displaying the honeycomb mosaic of the corneal endothelial cell layer.

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,

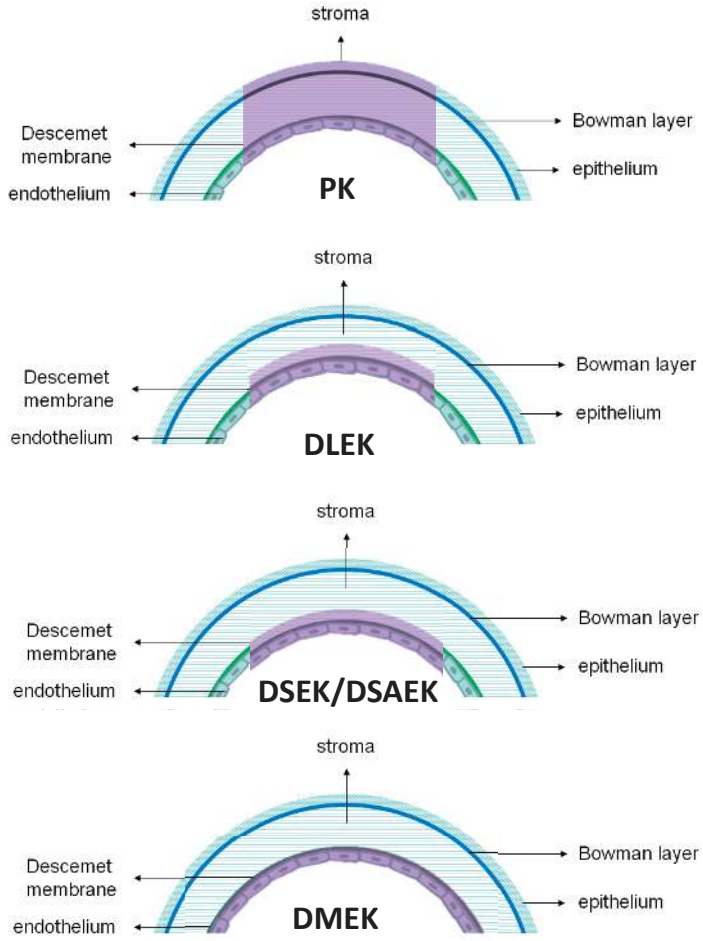

Figure 4. Evolution of Endothelial Keratoplasty.
PK = Penetrating Keratoplasty; DLEK = Deep Lamellar Endothelial Keratoplasty; DSEK/DSAEK = Descemet Stripping (Automated) Endothelial Keratoplasty; DMEK = Descemet Membrane Endothelial Keratoplasty.

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

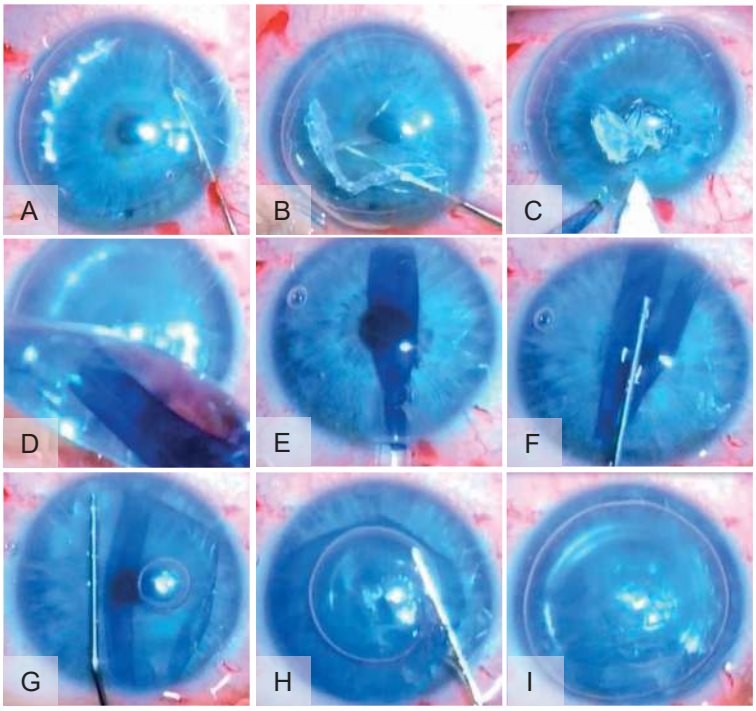

Figure 6. Steps of standardized 'no-touch' DMEK: (A, B) Scoring and stripping of the recipient's Descemet membrane from the posterior stroma, under an air-filled anterior chamber. (C) Creation of a 3.0mm limbal tunnel incision at the 12:00 o'clock position. (D, E) DMEK roll placed in an injector after 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.

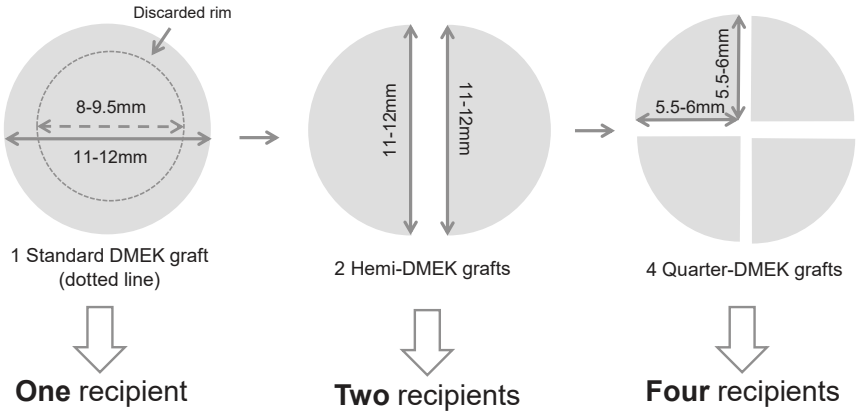

Figure 7. Different graft shapes of available DMEK techniques. (Left) standard DMEK: a central 8.5-9.5mm circular DMEK-graft is trephinated and transplanted into one recipient; the outer Descemet membrane rim is discarded. (Middle) Hemi-DMEK: the entire 11-12mm diameter Descemet membrane sheet is utilized 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]

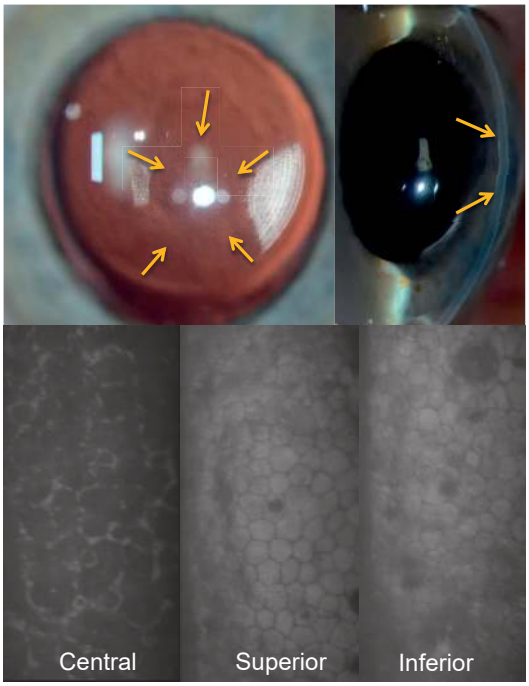

Figure 8. Slit lamp images (upper row) of a cornea in reflecting light (left) and with a slit from the side (right) showing centrally localized guttae (arrows). Specular microscopy images (lower row) show that in the same eye central endothelial cells are not visible whereas in the peripheral areas (superior,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

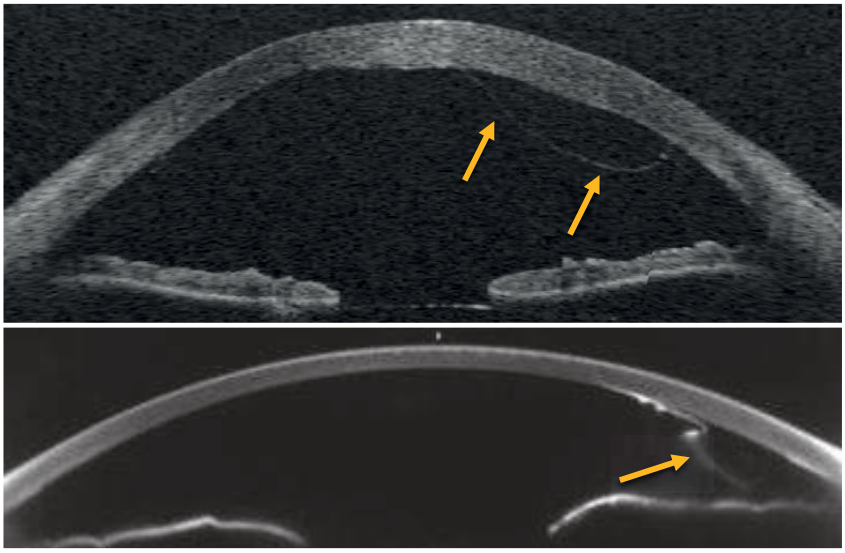

Figure 9. DMEK graft detachments (arrows) visible on Anterior Segment Optical Coherence Tomography (upper picture, notice the curling of the graft towards the stroma, indicating correct orientation) and on a Scheimpflug image (lower picture).

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

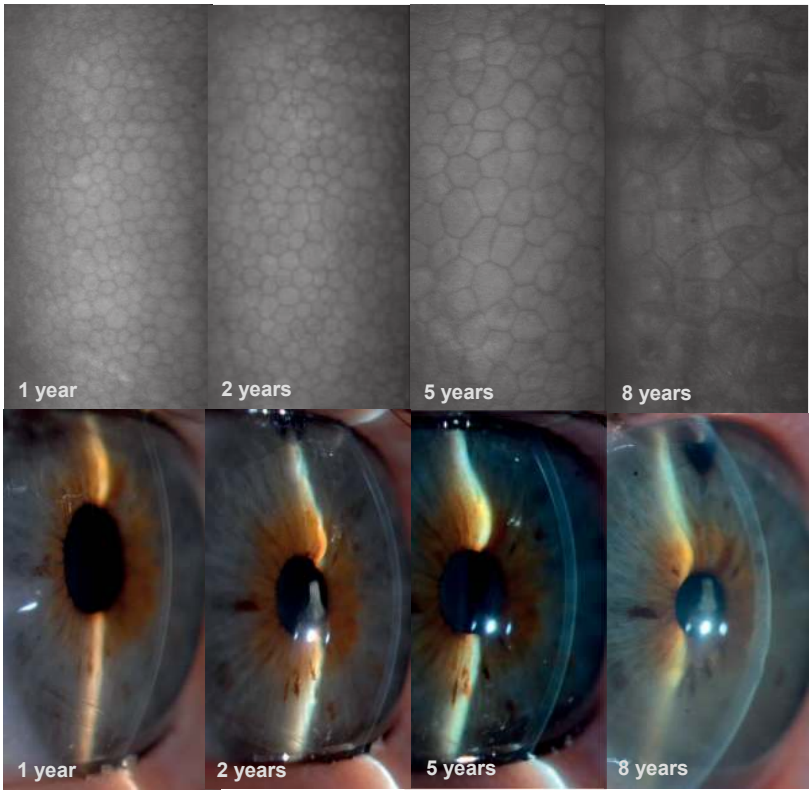

Figure 10. Specular Microscopy (upper row) and slit lamp images (lower row) of an eye eventually developing graft failure at 8 years after Descemet Membrane Endothelial Keratoplasty.

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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PART 1

Graft failure, Graft Survival and Repeat DMEK

CHAPTER 2

10-Year Clinical Outcome of the First Patient Undergoing Descemet Membrane Endothelial Keratoplasty

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ABSTRACT

Purpose: To describe the 10-year clinical outcome of the first patient worldwide who underwent Descemet membrane endothelial keratoplasty (DMEK).

Methods: In 2006, a 63-year-old man presented at the Melles Cornea Clinic, Rotterdam, with bilateral Fuchs endothelial dystrophy and cataract. After phacoemulsification, in vivo DMEK was performed in the left eye and 10 months later in the right eye. Best spectacle-corrected visual acuity (BSCVA), endothelial cell density, pachymetry, and complications were recorded every 6 months over a 10-year period.

Results: BSCVA in the left eye improved from 20/60 (0.3) before surgery to 20/17 (1.2) at 1 month, and remained stable over 10 years, ranging from 20/20 (1.0) to 20/13 (1.5). BSCVA in the right eye improved from 20/50 (0.4) preoperative and 20/60 (0.3) at 1 month to 20/25 (0.8) at 3 months and 20/17 (1.2) at 6 months, ranging from 20/25 (0.8) to 20/17 (1.2) over 9 years. Both eyes underwent YAG-laser-capsulotomy to manage posterior capsule opacification at 5 and 4 years after DMEK, respectively. Endothelial cell density in the right and left eyes, respectively, decreased by 43% and 45% at 1 year, 52% and 59% at 5 years, and 72% and 68% at 10/9 years, respectively. No intraoperative or postoperative complications occurred; at the last follow-up, both corneas were clear.

Conclusions: The first DMEK patient worldwide may show all short and long-term characteristics of this endothelial keratoplasty technique: outstanding patient satisfaction, quick visual recovery, low incidence of complications, and graft longevity. Published studies in the past decade would suggest that this case was the start of a new era in corneal transplantation.

INTRODUCTION

In the past decades, the Netherlands Institute for Innovative Ocular Surgery (NIIOS) has introduced several surgical techniques for the treatment of corneal endothelial disease, now referred to as "deep lamellar endothelial keratoplasty," "Descemet stripping (automated) endothelial keratoplasty," and most recently "Descemet membrane endothelial keratoplasty" (DMEK), that is, the selective replacement of Descemet membrane (DM) and its endothelium.[1] The concept of DMEK was first introduced in 1998, and the first patient was operated in 2006.[2] Since then, DMEK has shown clinical outcomes that may surpass all earlier (endothelial) keratoplasty techniques, with unprecedented visual outcomes and acceptable donor endothelial cell survival.[3,4] Today, the number of DMEK procedures performed may increase every year,[5] and the technique may have the potential to soon become the preferred treatment option for endothelial disorders.

The aim of this study was to evaluate the first DMEK case performed, now reaching its 10-year follow-up.

CASE REPORT

In 2006, a 63-year-old Dutch man was referred to the Melles Cornea Clinic Rotterdam because of bilateral cataract and Fuchs endothelial dystrophy with decreasing best spectacle-corrected visual acuity (BSCVA) to 20/50 (0.4) in the right eye and 20/60 (0.3) in the left eye. DMEK was performed in the left eye 6 weeks after phacoemulsification. Ten months after initial DMEK, the same procedures were performed in the right eye. The study was approved by the Institutional Review Board, and the patient signed an Institutional Review Board–approved informed consent form for research participation. The study adhered to the Declaration of Helsinki.

Donor age was 54 and 59 years for the grafts in the patient's left and right eyes, respectively. From donor globes obtained less than 36 hours postmortem, corneoscleral buttons were excised and stored in organ culture at 31°C. Preoperative donor endothelial cell density (ECD) and viability were evaluated with an inverted light microscope (Axiovert 40; Zeiss, Göttingen, Germany). After 2 weeks of culture, the endothelial cell morphology and viability were evaluated, and the corneoscleral buttons were mounted endothelial side up on a custom-made

holder. After trephination, the DM was stripped from the posterior stroma with microforceps, so that a 9.0-mm diameter DM sheet with its endothelium was obtained.[6] The DM formed a roll spontaneously, with the endothelium on the outside and was stored in organ culture medium until the time of transplantation.

DMEK was performed as previously described.[2] With a custom-made scraper (Melles scraper; DORC International, Zuidland, the Netherlands) and/or a reversed Sinskey hook (DORC International), a 9.0-mm descemetorhexis was created under air. After staining with 0.06% trypan blue solution (VisionBlue; DORC International), the DMEK graft was sucked into a Pasteur pipette (Hippocratech, Rotterdam, the Netherlands) and injected through a 3.5-mm limbal tunnel incision into the recipient anterior chamber. The graft was oriented with the endothelial side facing the recipient iris before it was unfolded over the iris and lifted against the recipient posterior stroma by injecting an air bubble underneath the graft. Then, the anterior chamber was filled completely with air for 30 minutes followed by air/fluid exchange.[2] Peripheral iridotomy was performed before DMEK. Postoperative medication included topical antibiotics and steroids; 1 year after surgery, fluorometholone drops were used twice a week.

The DMEK procedures in both eyes were uneventful and corneas cleared quickly (Figure 1). In the first eye (left eye), BSCVA improved from 20/60 (0.3) before surgery to 20/17 (1.2) at 1 month, and remained stable over 10 years, ranging from 20/20 (1.0) to 20/13 (1.5). In the second eye (right eye), BSCVA improved from 20/50 (0.4) preoperative and 20/60 (0.3) at 1 month to 20/25 (0.8) at 3 months and 20/17 (1.2) at 6 months, ranging from 20/25 (0.8) to 20/17 (1.2) over 9 years. At 5 and 4 years after DMEK, respectively, both eyes underwent YAG-laser-capsulotomy for posterior capsule opacification.

Postoperative ECD was evaluated using a Topcon SP2000p/SP3000p noncontact autofocus specular microscope (Topcon, Tokyo, Japan). ECD of the left eye decreased from 3000 cells/mm2 before surgery, to 1680 cells per square millimeter at 1 year, 1450 cells per square millimeter at 5 years, and 820 cells/mm2 at 10 years (compared with preoperative values, a decrease of 43%, 52%, and 72%); ECD of the right eye decreased from 2800 cells per square millimeter to 1550 cells/mm2 at 1 year, 1150 cells/mm2 at 5 years, and 900 cells/mm2 at 9 years (a decrease of 45%, 59%, and 68%) (Figure 2). Throughout the follow-up period, central pachymetry values varied within 544 to 567 μm in both eyes. At the last follow-up, pachymetry measured 550 μm in the left eye and 553 μm in the right

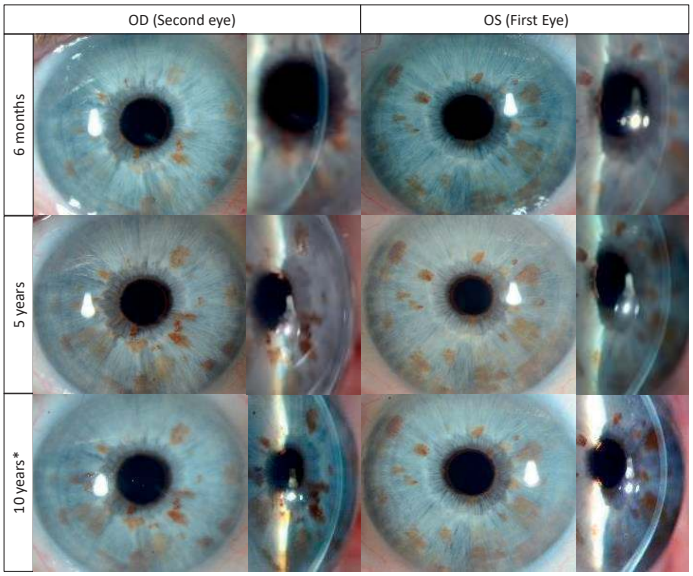

Figure 1. Preoperative and postoperative slit lamp images of the first eye (left eye) and second fellow eye (right eye) operated on with DMEK, throughout the 10-year follow-up period. *Follow-up period for the second eye (right eye) is 9 years.

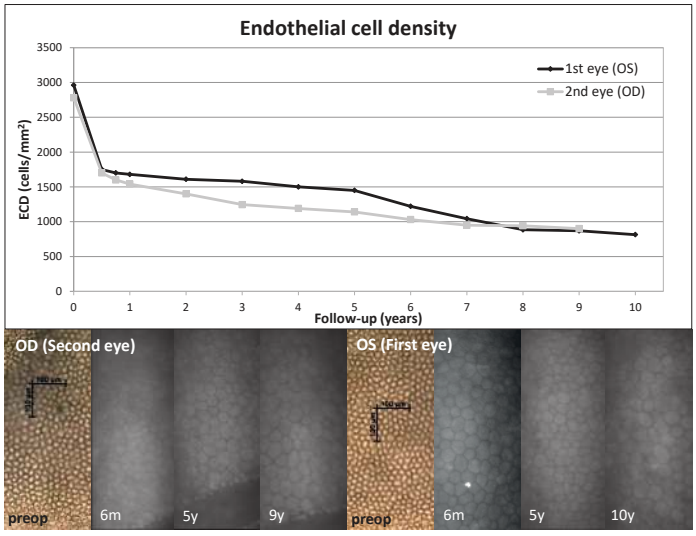

Figure 2. Graph (upper row) displaying the endothelial cell density decrease and preoperative (preop) and postoperative specular microscopy images (lower row) of the first eye (left eye) and second eye (right eye) operated on with DMEK, throughout the 10-year follow-up period.

eye, and both corneas were clear (Figure 1). No intraoperative or postoperative complications occurred throughout the follow-up period.

DISCUSSION

The first patient worldwide operated on with DMEK showed excellent clinical outcomes up to 10 years postoperatively, which may reflect the potential of the technique for the entire DMEK cohort so far. In both eyes, BSCVA quickly recovered to its full visual potential within the first months, which agrees with the majority of DMEK cases reaching ≥20/25 (≥0.8) at 6 months.3 ECD showed a decrease of about 70% compared with preoperative values, similar to 10-year Descemet stripping endothelial keratoplasty eyes.[7]

This first DMEK case may also be indicative for the long-term graft survival after DMEK exceeding 90% at 8 years,4 which may in part be explained by the lack of complications associated with the procedure: suture-related and wound-healing problems were eliminated, the incidence of allograft rejection may be reduced to 1% to 2%, and the risk of glaucoma and/or other concurrent pathology may be minimized.[1–4,8,9]

Given the unprecedented clinical outcomes and extraordinary patient satisfaction, DMEK may have the potential to be adopted as the next preferred treatment option for corneal endothelial disorders. Techniques for DMEK graft preparation and surgery have evolved into standardized “no-touch” procedures allowing step-by-step performance to shorten the learning curve. Furthermore, the possibility of obtaining precut tissue from specialized eye banks may have made it easier for surgeons to start out with this new surgical technique.

After 10 years of performing DMEK, we may have entered a new era of corneal transplantation. Modifications of the DMEK technique such as hemi-DMEK and quarter-DMEK, by which multiple grafts could be recovered from one single donor cornea, may soon allow for far more efficient use of donor corneal tissue to balance the increasing demand for corneal transplants worldwide.[10]

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CHAPTER 3

Endothelial Survival after Descemet Membrane Endothelial Keratoplasty Effect of Surgical Indication and Graft Adherence Status

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ABSTRACT

Objective: To determine endothelial survival and its association with the indication for surgery and/or partial graft detachment in DMEK.

Design, Setting, Participants: Retrospective cross-sectional study of data collected from August 8, 2006, until June 17, 2015, at a tertiary referral center. A total of 352 eyes were evaluated up to 8 years after DMEK for Fuchs endothelial corneal dystrophy (FECD; n = 314), bullous keratopathy (BK; n = 31), and failed previous endothelial graft (n=7), of which 314 eyes had complete graft attachment and 38 eyes had partial graft detachment (one-third of the graft surface area or less). Endothelial cell density was measured with specular microscopy, and Kaplan-Meier survival estimates were based on eyes with endothelial failure. Endothelial survival was followed up to 8 years after DMEK.

Main outcomes and Measures: Endothelial cell density, endothelial failure, and endothelial survival.

Results: Endothelial cell density decreased to a mean (SD) of 952 (366) and 771 (321) cells/mm^2 at 7 and 8 years postoperatively, respectively. Higher endothelial cell densities were found in eyes with FECD compared with those with BK (estimated mean difference, 261 cells/mm^2; 95% CI, 118-404; P = .003) and in eyes with attached grafts compared with those with partially detached grafts (estimated mean difference, 330 cells/mm^2; 95% CI, 208-452; P < .001), until 8 years. In 11 eyes (3.1%) that had concomitant ocular pathology, endothelial failure occurred within 4 years after DMEK. The overall graft survival probability was 0.96 at 5 and 8 years (95% CI, 0.94-0.99). At 8 years, better survival rates were found in eyes with FECD than in those with BK (survival probability, 0.97 [95% CI, 0.95-0.99] vs 0.84 [95% CI, 0.70-0.99], respectively); until the same follow-up, survival probabilities in eyes with attached and partially detached grafts were 0.97 (95% CI, 0.95-0.99) and 0.91 (95% CI, 0.82-0.99), respectively.

Conclusions and Relevance: Endothelial decay was higher in eyes with a partial graft detachment than in those with attached grafts and lower in eyes with FECD than in those with BK. Endothelial failure only occurred in eyes with concomitant ocular pathology. These results suggest that eyes with DMEK that have undergone surgery for FECD with a completely attached graft may have an excellent prognosis.

INTRODUCTION

Since its introduction in 1998, endothelial keratoplasty has become increasingly popular and evolved from deep lamellar endothelial keratoplasty to Descemet stripping endothelial keratoplasty (DSEK) and Descemet stripping automated endothelial keratoplasty (DSAEK), and most recently to Descemet membrane endothelial keratoplasty (DMEK).[1]

Although DMEK provides excellent visual acuity recovery of 20/25 or even better in about three-quarters of the eyes,[2-4] there is not yet any indication of long-term graft survival (i.e., ≥10 years) in DMEK or earlier endothelial keratoplasty techniques. We recently reported an 84% graft survival rate at 10 years in our first deep lamellar endothelial keratoplasty cohort.[5] For DSEK/DSAEK, survival rates up to 5 years postoperatively seem to resemble midterm graft survival rates after penetrating keratoplasty (PK).[6-14]

Midterm evaluation of endothelial cell density (ECD) after DMEK showed a 7% annual decrease that may mimic that of earlier endothelial keratoplasty techniques, while the decrease appears to be slower than after PK.[15-17] This may hint toward a higher endothelial survival probability after DMEK. If so, not only faster visual rehabilitation but also higher long term endothelial survival would be important considerations for surgeons to choose DMEK over PK as a preferred treatment method in corneal endothelial disease.

The aim of this study was to assess midterm endothelial survival by evaluating ECD decay and endothelial graft failures in the first DMEK cohort worldwide and to evaluate its association with the indication for surgery (Fuchs endothelial corneal dystrophy [FECD] vs bullous keratopathy [BK]) and the presence of a partial graft detachment.

METHODS

This was a retrospective cross-sectional study of data that had been collected from August 8, 2006, until June 17, 2015, of 500 consecutive eyes that underwent DMEK in 395 patients (including the learning curve of the first 25 DMEK procedures).

Table 1. Demographic Characteristics and Exclusions

	Characteristic	Value
Included DMEK eyes	Included Eyes after DMEK / Patients, No.	352 / 352
	Sex, No. (%)	
	Male	154 (43.8)
	Female	198 (56.2)
	Recipient´s age, mean (SD) [range], y	68 (13) [20-96]
	Participation time, mean (SD) [range], mo[a]	42 (22) [0-96]
	Lens status, No. (%)	
	Phakic	91 (25.8)
	Pseudophakic	259 (73.6)
	Aphakic	2 (0.6)
	Indication, No. (%)	
	Fuchs endothelial corneal dystrophy[b]	314 (89.2)
	Bullous keratopathy	31 (8.8)
	Pseudophakic bullous keratopathy	14
	Aphakic bullous keratopathy	1
	Congenital glaucoma	4
	Phakic intraocular lens[c]	11
	After trauma	1
	Regraft after DSEK/DSAEK	7 (2.0)
	Graft adherence status at 6 mo postoperatively, No. (%)	
	Attached	314 (89.2)
	Partially detached[d]	38 (10.8)
Included Donors for DMEK	Donors	
	No.	352
	Age, mean (SD) [range], y	65 (10) [41-85]
	Sex, No. (%)	
	Male	219 (62.2)
	Female	133 (37.8)
	Cause of death, No. (%)	
	Cerebrovascular, cardiac/stroke	176 (50.0)
	Cancer	97 (27.6)
	Respiratory	57 (16.2)
	Trauma	6 (1.7)
	Other	16 (4.5)
	Time from death to preservation, mean (SD) [range], h	22 (7) [7-39]
	Time from preservation to surgery, mean (SD) [range], d	13 (4) [6-25]
	Preoperative endothelial cell density, mean (SD), cells/mm^2	2533 (216)

Table 1. Demographic Characteristics and Exclusions *(continued)*

	Characteristic	Value
Excluded DMEK eyes	Excuded eyes, (n = 148)	
	Second fellow eyes, No.	106
	DMEK after penetrating keratoplasty, No.	2
	Graft detachment greater than one-third of surface area, No.[e]	40
	Cases 1-25, learning curve	9
	Cases 26-100	13
	Cases 101-500	18

DMEK=Descemet membrane endothelial keratoplasty; DSAEK=Descemet stripping automated endothelial keratoplasty; DSEK=Descemet stripping endothelial keratoplasty.
[a]Time from surgery until the last available visit with a successful graft or a failed graft necessitating regrafting.
[b]Including 1 eye with a posterior polymorphous endothelial dystrophy and 1 aphakic eye.
[c]Phakic intraocular lens was removed in 6 eyes.
[d]One-third of the graft surface area or less.
[e] Eyes in which reliable endothelial cell density measurements could not always be obtained.

Of each patient with bilateral DMEK, the second eye that underwent surgery was excluded from the analysis (n = 106). Because reliable ECD measurements could not always be obtained in eyes with a larger graft detachment (more than one-third of the graft surface area), only eyes with a detachment of one-third of the graft surface area or less (partially detached) were determined as a cutoff point for inclusion in the study. Hence, 40 eyes with a larger detachment were excluded, as were 2 eyes with DMEK performed as a secondary procedure after PK. Thus, 352 unilateral eyes that underwent DMEK in 352 patients were included in our study (Table 1).

Of these 352 eyes, 314 underwent DMEK for FECD, 31 underwent DMEK for BK (pseudophakic BK, aphakic BK, congenital glaucoma, phakic intraocular lens, or trauma), and 7 underwent DMEK as a secondary procedure to manage low visual outcome or graft failure after DSEK/DSAEK (Table 1). Sixteen eyes (4.5%) had preexisting glaucoma (FECD, n = 7; BK, n = 8; failed DSEK/DSAEK, n = 1), of which 4 had congenital glaucoma. In total, 314 eyes had an attached graft and 38 had a partially detached graft (Table 1). The mean (SD) participation time after DMEK was 42 (22) months (range, 0-96 months) (Table 1).

This study was approved by the institutional review board of the Netherlands Institute for Innovative Ocular Surgery as a retrospective data review. All patients signed an institutional review board–approved informed consent form. The study was conducted according to the Declaration of Helsinki.[18]

Donor Tissue

Harvesting of the Descemet membrane graft was performed as previously described.[19,20] In short, corneoscleral buttons from donor globes were obtained post-mortem and stored in organ culture medium at 31°C.In the eye bank, endothelial cell morphology and viability were evaluated and the corneoscleral buttons were mounted endothelial side up on a custom-made holder so that a 9.5-mm-diameter Descemet membrane sheet with its endothelium could be stripped from the posterior stroma. Due to the elastic tissue properties, a Descemet roll formed spontaneously with the endothelium on the outside. Descemet rolls were then stored in organ culture medium until the day of transplantation (Table 1).

Surgery

A circular 9.0-mm-diameter descemetorhexis was performed under air by scoring and stripping the Descemet membrane from the posterior stroma with a reversed Sinskey hook (D.O.R.C.International).In eyes that underwent DMEK as a secondary procedure, the primary DSEK/DSAEK graft was carefully removed from the recipient posterior stroma with a reversed Sinskey hook.

The donor Descemet roll was stained with 0.06% Trypan blue solution (VisionBlue; D.O.R.C. International), sucked into a custom-made injector (DMEK inserter; D.O.R.C. International), and injected through a 3.0-mm limbal tunnel incision into the recipient anterior chamber. The graft was oriented with the endothelial side facing the recipient iris and with the donor Descemet membrane facing the recipient stroma. After complete graft unfolding over the iris through indirect manipulation by an air bubble, by flushing with balanced salt solution, and by gentle strokes on the corneal surface, an air bubble was injected under the graft to attach and fixate it onto the recipient posterior stroma. The anterior chamber was then completely filled with air for 60 minutes followed by an air/liquid exchange, leaving a 30% to 50% air bubble.[21]

Postoperative medication included topical antibiotics for 2 weeks and a steroid regimen of dexamethasone sodium phosphate, 0.1%, eyedrops 4 times daily for 4 weeks, followed by fluorometholone eyedrops 4 times daily, tapered to once daily until 1 year postoperatively and thereafter once daily or once every other day.[22]

Data Collection

Donor ECD was measured preoperatively in vitro using an inverted light microscope Axiovert 40; Zeiss) and postoperatively every 6 months up to 8 years with an SP3000p noncontact autofocus specular microscope (Topcon Medical Europe BV). At the same time intervals, all eyes had routine examinations, including biomicroscopy, anterior segment optical coherence tomography (Heidelberg Engineering GmbH), and Scheimpflug imaging (Pentacam; Oculus).

Analysis of ECD was done by multiple trained technicians. For every image, the automatically delineated cell borders were carefully checked. If they were not correctly assigned by the program, a manual correction was applied to correctly assign the cell borders. Three central images were analyzed per eye and follow-up point and results were averaged. For every analysis, the largest possible part of the image was used.

Endothelial graft failure was diagnosed with slit-lamp biomicroscopy, revealing corneal edema that necessitated repeat keratoplasty. Primary graft failure was defined as absent corneal clearance after surgery despite full graft attachment; secondary graft failure was defined as a corneal decompensation after a postoperative interval with a clear cornea.

To determine whether the indication for surgery affected the outcomes, the FECD subgroup was compared with the BK subgroup (Figure 1B and Figure 2B).

For each eye, graft adherence status was categorized as either completely attached or partially detached. These 2 subgroups were compared with each other to determine whether partial graft detachment affected ECD decay and endothelial survival (Figure 1C and Figure 2C).

Statistical Analysis

Participation time of an eye was defined as the time from surgery until the last visit with a successful graft or a failed graft necessitating repeat keratoplasty.

Linear mixed models were used to identify possible differences in ECD outcomes over 8 years between the different subgroups, FECD vs BK and attached vs partially detached grafts, while controlling for possible confounders of the patient (age, sex, lens status, preoperative glaucoma), the donor (cause of death, sex, age), and the donor and graft processing times (times from death to preservation, preservation to preparation, preparation to surgery). Examination of the

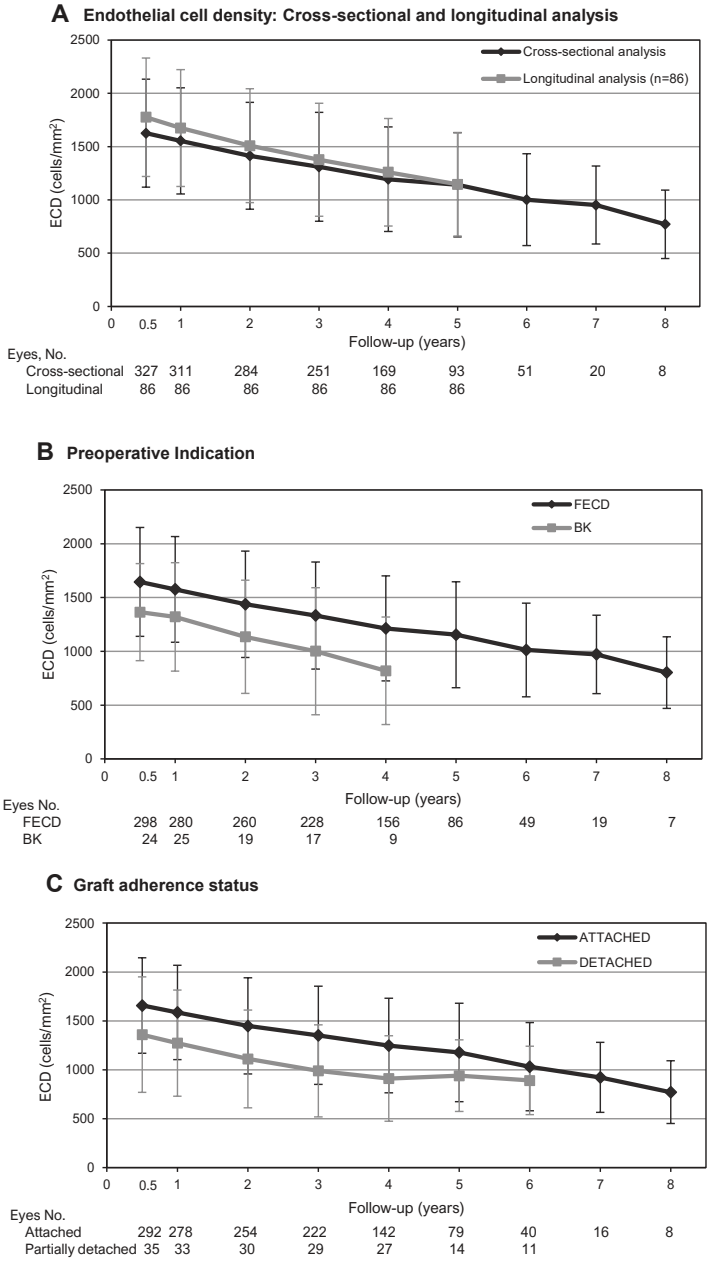

Figure 1. Mean Endothelial Cell Density (ECD) up to 8 years after Descemet Membrane Endothelial Keratoplasty. (A) Mean ECD by cross-sectional analysis of the entire cohort for each follow-up point and longitudinal analysis from 6 months until 5-year follow-up. (B) Mean ECD by preoperative indication of Fuchs endothelial corneal dystrophy (FECD) vs bullous keratopathy (BK). (C) Mean ECD by graft adherence status of attached grafts vs partially detached grafts. Error bars indicate standard deviation.

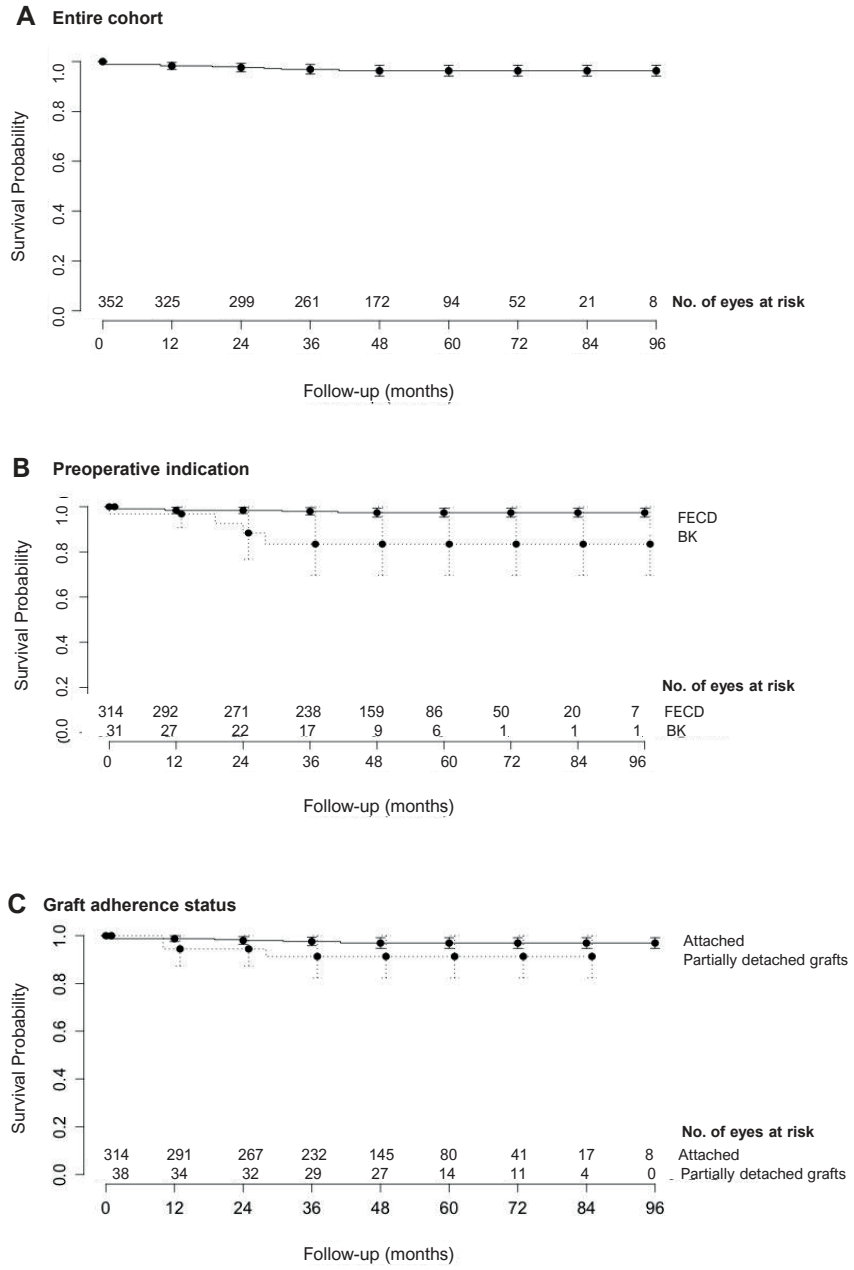

Figure 2. Kaplan-Meier survival curves up to 8 Years after Descemet Membrane Endothelial Keratoplasty (A,B,C) Survival probabilities and 95% confidence intervals for the overall group (A), for those with preoperative indication of Fuchs endothelial corneal dystrophy (FECD) vs bullous keratopathy (BK; B), and for those with attached grafts vs partially detached grafts (C).

residuals did not reveal violations of the assumptions (normality, homoscedasticity, outliers). There were a few eyes with high standardized residuals (between 3.5 and 4.0), for which the model does not fit well. Because 17 of 352 eyes did not have any follow-up ECD value at 6 months and onward, only the remaining 335 eyes could be included in the linear mixed model ECD analysis.

Based on the observed survival times of all eyes up to 8 years, survival distributions were estimated using the Kaplan Meier estimator. Survival times were assessed with Cox regression while taking possible risk factors (age, preoperative ECD, etc.) into account, to evaluate whether preoperative indication (FECD vs BK) and graft adherence status (attached vs partially detached) have an effect on survival time. Survival analysis for the entire group comprised 352 eyes; survival analysis for the subgroups comprised 345 eyes because the small regraft group (n = 7) was not included.

Statistical analyses were performed with R version 3.1.3 statistical software (R Foundation for Statistical Computing) using the package "survival," "rms," and "nlme."

RESULTS

Endothelial Survival in Terms of Endothelial Decay

In the cross-sectional analysis of 352 eyes, the mean (SD) ECD was 1626 (507) cells/mm^2 at 6 months (n = 327), 1554 (498) cells/mm^2 at 12 months (n = 311), 1414 (502) cells/mm^2 at 24 months (n = 284), 1310 (511) cells/mm^2 at 36 months (n = 251), 1194 (491) cells/mm^2 at 48 months (n = 169), 1142 (490) cells/mm^2 at 60 months (n = 93), 1002 (431) cells/mm^2 at 72 months (n = 51), 952 (366) cells/mm^2 at 84 months (n = 20), and 771 (321) cells/mm^2 at 96 months (n = 8) after DMEK (Figure 1A). Of the available eyes with survived (clear) grafts 7 years after DMEK, 5.0% had an ECD of less than 500 cells/mm^2 and 45.0% had an ECD of 1000 cells/mm^2 or more (Table 2).

In the longitudinal ECD analysis of 86 eyes with available ECD at each point from 6 months until 5-year follow-up, the mean (SD) ECDs at 6, 12, 24, 36, 48, and 60 months after DMEK were 1776 (555), 1674 (549), 1508 (534), 1377 (530), 1259 (504), and 1145 (483) cells/mm^2, respectively (Figure 1A).

Table 2. Availability of Endothelial Cell Density at each follow-up point after DMEK

Variable	Follow-up time (years)									
	Pre-operative	0.5	1	2	3	4	5	6	7	8
DMEK eyes, No.	352	352	352	352	349	252	159	79	36	15
Eyes with missing data, No.	0	9	14	16	21	15	10	3	5	--
Eyes lost-to follow-up, No. (%)	NA	12 (3.4)	21 (6.0)	45 (12.8)	68 (19.5)	57 (22.6)	52 (33.3)	21 (22.7)	7 (19.4)	4 (26.7)
Failure or reoperation,No.	NA	4	6	7	9	11	4	4	4	3
Failures per interval, No.	NA	4	2	1	2	2	0	0	0	0
Eyes available for ECD analysis, No.	352	327	311	284	251	169	93	51	20	8
Eyes with ECD <500 cells/mm^2, No. (%)	0	4 (1.2)	5 (1.6)	9 (3.2)	12 (3.4)	6 (2.4)	4 (4.3)	4 (7.8)	1 (5.0)	1 (6.7)
Eyes with ECD >1000 cells/mm^2, No. (%)	352 (100)	283 (86.6)	258 (82.3)	217 (76.4)	170 (67.7)	99 (57.9)	51 (54.8)	23 (45.1)	9 (45.0)	1 (6.7)

DMEK=Descemet membrane endothelial keratoplasty; ECD=Endothelial cell density; NA=not applicable

Up to 8 years, a significantly higher ECD was found in eyes with FECD than in those with BK (estimated mean difference, 261 cells/mm^2; 95% CI, 118-404; P = .003) (Figure 1B),

and eyes with attached grafts had a significantly higher ECD than those with partially detached grafts (estimated mean difference, 330 cells/mm^2; 95% CI, 208-452; P < .001) (Figure 1C). The graft was partially detached in 35 of 314 eyes with FECD (11.1%) and in 3 of 31 eyes with BK (9.7%).

The risk factor preoperative ECD had an effect on the outcome ECD: for each additional 100 cells/mm^2 before DMEK, the final ECD outcome at 8 years post-operatively increased by an average of 86 cells/mm^2 (95% CI, 69-104; P < .001). Among the donor death causes (cancer, cardiac or stroke, respiratory, and trauma or other), cancer was associated with the highest ECD outcome until 8 years. When compared with the baseline category cardiac or stroke, the estimated mean difference was 133 cells/mm^2 (95% CI, 43-224; P = .01).

Endothelial Survival in Terms of Primary and Secondary Graft Failures

Endothelial failure occurred in 11 of 352 eyes (3.1%) within 4 years after DMEK; 4 eyes were diagnosed as having primary graft failure and 7 were diagnosed as having secondary graft failure. All of these eyes had concomitant ocular pathology, including partial graft detachment.

Based on the number of primary and secondary graft failures in the entire cohort, the estimated survival probability was 0.97 (95% CI, 0.95-0.99) at 3 years and 0.96 (95% CI, 0.94-0.99) at 5 and 8 years (Figure 2A). Survival probabilities were higher in eyes with FECD than in those with BK at 3 years (0.98 [95% CI, 0.96-0.99] vs 0.84 [95% CI, 0.70-0.99], respectively) as well as at 5 and 8 years (0.97 [95% CI, 0.95-0.99] vs 0.84 [95% CI, 0.70-0.99], respectively) (Figure 2B). In eyes with attached grafts and partially detached grafts, survival probabilities at 8 years were 0.97 (95% CI, 0.95-0.99) and 0.91 (95% CI, 0.82-0.99), respectively (Figure 2C). The baseline hazard risk for failure was 0.02 (average number of expected failures per eye per 12-month interval). Preoperative indication BK significantly increased the (baseline) hazard risk of failure by a factor of 5 (hazard ratio = 5.09 [95% CI, 1.24-20.83]; P = .02). The graft adherence status of detached increased the hazard risk of failure by a factor of approximately 3, but not significantly (hazard ratio = 2.79 [95% CI, 0.73-10.68]; P = .13). The possible risk factors such as baseline ECD did not have a significant effect on the hazard risk of failure.

Graft Failure and Other Postoperative Complications

Of the 4 eyes with a primary graft failure, 3 were within the learning curve. The remaining eye developed BK after ocular trauma with corneal perforation.

Of the 7 eyes with a secondary graft failure, 2 had a DMEK performed for BK after phakic intraocular lens removal associated with glaucoma episodes necessitating filtering surgery. One eye that had DMEK for corneal decompensation due to congenital glaucoma in the presence of a brunescent cataract and a Baerveldt shunt developed graft failure after phacoemulsification 9 months after DMEK. Two eyes developed secondary graft failure after allograft rejection, and 2 eyes had an ECD less than 500 cells/mm^2 in the presence of a partial graft detachment at 6 months.

All other corneas with postoperative complications potentially affecting endothelial cell survival remained clear throughout the study period: reversible allograft rejection (n = 6), rebubbling (n = 4), postoperative glaucoma (n = 14), pars plana vitrectomy (n = 1), and phacoemulsification (n = 10).

DISCUSSION

In PK and DSEK/DSAEK, graft survival has been described to vary with factors such as the indication for surgery, re-transplantation, comorbidity (e.g., glaucoma), complications (e.g., allograft rejection), and donor characteristics.[10,13,14,23,24] To determine the causes associated with graft longevity in DMEK, we evaluated endothelial survival in terms of ECD decay and endothelial failure in a first DMEK cohort up to 8 years postoperatively.

However, comparisons between studies require caution because graft survival may vary per region, demographic characteristics, and surgical setting and because various studies used different inclusion and exclusion criteria, causing varying survival outcomes.[25,26] A complicating factor is the terminology used: graft survival may not mirror graft failure because technical failures may not provide information on graft viability (e.g., grafts positioned upside down have been shown to carry healthy endothelial cells).[27] Similarly, a common indication for repeat DMEK is graft detachment, but microscopic analysis of explanted grafts showed a normal and viable endothelial cell layer.[27] For that reason, we did not define our outcome measurements in terms of success rate or graft survival

but instead based our analysis on ECD decay and on eyes with endothelial graft failure.

Endothelial Survival in Terms of Primary and Secondary Graft Failure

For DSAEK, a 3-year graft survival rate of 87% to 97% has been reported[6,7]; for DSEK, a 5-year survival rate of 93% has been reported.[8] After PK, survival rates may vary from 75% to 95% at 3 and 5 years.[6,7,9,10] The overall DMEK survival probability in our cohort was 0.96 at 5 and 8 years postoperatively.

Interestingly, all 11 endothelial graft failures in our study seemed to only be associated with surgical error, comorbidity, or postoperative complications. Of the 4 eyes that showed a primary graft failure, 3 were within the first 25 DMEK operations (learning curve), and these eyes may have undergone reoperation too early when the cornea failed to clear within 3 weeks. We later learned that in the presence of a completely attached graft, some transplanted corneas may need a longer time to clear.[28] The remaining eye with primary graft failure had a history of BK after penetrating ocular trauma. Eyes that developed a secondary graft failure had a partial graft detachment with a low ECD, BK after phakic intraocular lens implantation (and removal) complicated by glaucoma episodes, congenital glaucoma, or allograft rejection preceding the transplant failure. These findings would suggest that, overall, mainly eyes with comorbidity are at risk for graft failure or, in other words, that endothelial survival probability would be high in eyes that have undergone DMEK without complication.

Compared with PK, survival probabilities with DMEK may have improved owing to elimination of suture-related complications (suture loosening, sterile inflammation, stromal melt), lower incidence of allograft rejection, better preservation of the anterior chamber angle anatomy, and faster tapering of steroids (reducing the risk of glaucoma and cataract formation).[22,29,30]

When stratified by the indication for surgery, graft survival probability in terms of endothelial failure until 8 years after DMEK was better in eyes with FECD than in those with BK (0.97 vs 0.84, respectively). This finding may agree with studies on DSEK/DSAEK and PK, in which eyes with FECD consistently showed better graft longevity.[7–10,17,23]

When analyzed for graft adherence status, the graft survival probability in eyes with a completely attached graft was higher than in those with a partial detach-

ment (0.97 vs 0.91, respectively). Still, partial graft detachment did not seem to be significantly associated with higher risk of endothelial graft failure. This could be attributed to the relatively low number of eyes with endothelial failure in our cohort. Larger series in longer follow-up studies may be required to evaluate whether partial graft detachment is associated with a higher risk of endothelial failure.

It stands to reason that in both BK and partial graft detachment, a relative depletion of cells and/or the underlying pathology relates to lower endothelial survival rates. If so, partial graft detachment - albeit visually insignificant – could benefit from (earlier) surgical intervention, although repeat rebubbling has also been associated with lower ECDs.[31]

Furthermore, the distribution of endothelial graft failures over time may be of interest: 6 of the 11 failures occurred within the first postoperative year. This may suggest that if the early postoperative course after DMEK is uneventful, the graft may have an excellent prognosis on long-term survival, especially in eyes with FECD, because late-onset secondary graft failure was consistently associated with comorbidity unrelated to the transplant itself. However, identification of risk factors was limited by the relatively small number of failures in our cohort combined with the amount of censored observations.

Endothelial Survival in Terms of ECD Decay

In addition to visual outcomes surpassing those of PK and DSEK/DSAEK, the relatively low number of endothelial failures in our study may suggest that DMEK also has the advantage of longer graft longevity. To further substantiate this hypothesis, we evaluated the decay in ECD during the first 8 years in an attempt to calculate how many eyes that underwent DMEK would have an ECD less than 500 cells/mm^2, an ECD that may be associated with impending graft failure.[17,32] Within the entire cohort of survived clear grafts, fewer than 10% of eyes had an ECD less than 500 cells/mm^2 at each follow-up point. Any predictions of a time at which low ECD may result in graft failure and whether a certain ECD constitutes a threshold related to graft failure seem unreliable because the long-term sample size was relatively small. A larger data set may be necessary to allow a reliable prediction on long-term endothelial survival after DMEK.[33]

CONCLUSIONS

This study shows that until 8 years after DMEK, endothelial survival may be promising. In particular eyes with FECD and a completely attached graft may have an excellent prognosis in the longer term.

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CHAPTER 4

Refining the Terminology of Graft Failure in Reports of Endothelial Keratoplasty Outcomes

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VIEWPOINT

Graft survival studies are important to evaluate the longevity of transplanted donor corneal tissue. Since 1998, corneal transplantation has undergone an evolution from conventional techniques such as penetrating keratoplasty to minimally invasive techniques such as Descemet stripping (automated) endothelial keratoplasty, and Descemet membrane endothelial keratoplasty for the treatment of corneal endothelial diseases.[1]

To assess the "graft survival" rate in a cohort of patients, the eyes that developed irreversible corneal edema for "endothelial graft failure" need to be determined. In the literature on penetrating keratoplasty, the terms *primary* and *secondary graft failure* are used to describe early endothelial failure (primary: cornea did not clear after surgery) as a result of endothelial damage during surgery and late endothelial failure (secondary: cornea cleared initially but showed decompensation at a later time point) resulting from endothelial decay owing to postsurgical inflammation, allograft rejection, suture-related inflammation, or glaucoma (surgery), among other things.[2] Therefore, *graft failure* and *graft survival* may have been used as mirroring terms pointing in opposite directions; both terms reflect the "graft success rate" in terms of endothelial survival, either from a positive angle (survival) or a negative angle (failure). In other words, in the penetrating keratoplasty literature, "graft failure" equaled "endothelial failure" and indirectly reflected the graft survival rate in a cohort. As a result, "graft survival" became synonymous with "endothelial survival".

In endothelial keratoplasty, these definitions may no longer be interchangeable for 2 reasons *(Figure)*. First, owing to the techniques available, the endothelium may be functional but simply not able to function depending on the attachment status of the Descemet membrane endothelial keratoplasty graft. As an extreme example, in an eye with a completely detached graft floating in the recipient's anterior chamber, the graft may be perceived as having "failed" because the cornea is edematous, but a detached graft has also been shown to carry a viable and potent endothelium, but no corneal clearance can be anticipated owing to its anatomically false position.[3] The same holds for eyes with a graft positioned "upside down," where the cornea is also most often edematous owing to a detached and anatomically false oriented graft (endothelium facing recipient stroma). Referring to such grafts as having failed is a rather colloquial term used for a "clinically or technically unsuccessful" graft, but it is misleading in terms of actual endothelial survival. It may be important in an eye with corneal edema

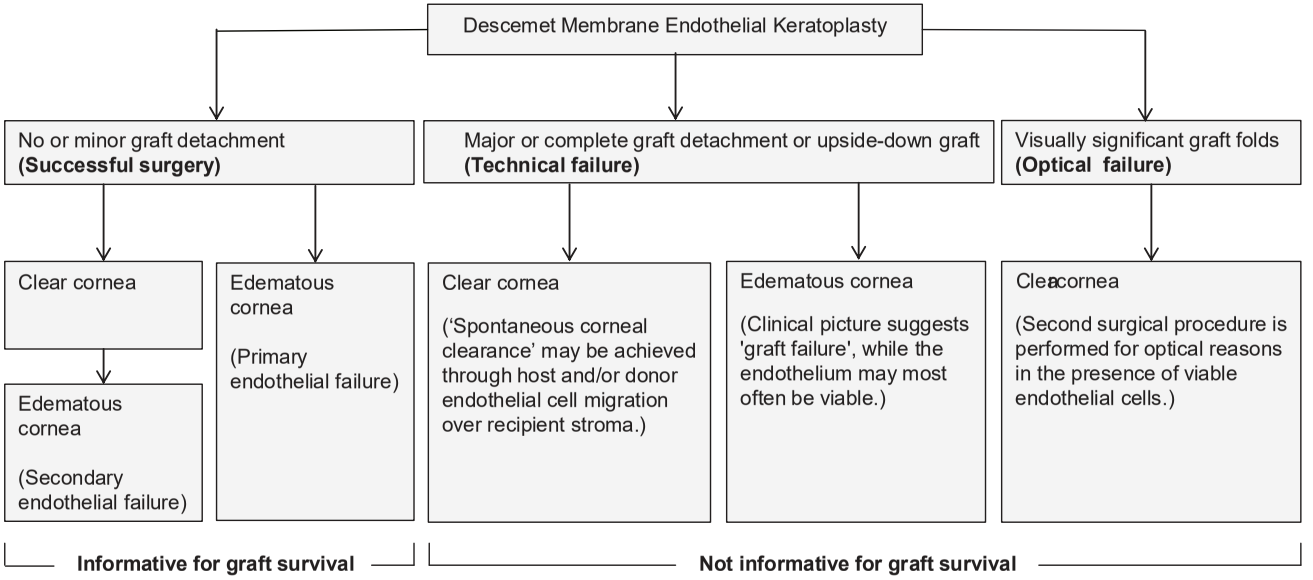

Figure. Schematic representation of the relationship between graft attachment status and endothelial viability (i.e., graft survival).

after Descemet membrane endothelial keratoplasty to differentiate between a "technical failure" and a real endothelial dysfunction because after complete apposition by a rebubbling procedure or after correct orientation, these grafts frequently show good viability (i.e., corneal deturgescence and clearing). Clearly, a graft cannot "fail" and be viable at the same time. Therefore, the definition for "graft failure" and "graft survival" should be reconsidered for endothelial keratoplasty studies.

The second reason for fine-tuning definitions is that the currently used terms may also be misleading the other way around in endothelial keratoplasty. In cases with a partial or complete graft detachment after Descemet membrane endothelial keratoplasty, the cornea may also show "spontaneous corneal clearance."[4] Hence, in these cases, the cornea may also clear despite the graft dehiscence, requiring no further surgical intervention. Naming this condition, a graft failure (because of the graft detachment) also seems to be a misnomer, especially when considering that, in these eyes, the endothelial cell density measurements may reflect endothelial cell migration (from the donor and/or recipient) over the recipient's posterior stroma, which means that these cases may also show a different long-term endothelial cell survival than grafts with complete apposition.[5]

Similarly, despite a clear cornea with a functional endothelium after Descemet stripping (automated) endothelial keratoplasty, visual rehabilitation may be limited by graft folds or interface scarring. Referring to these grafts as "failed" rather than "optically unsuccessful" seems to be a misnomer as well.[6] The same applies to penetrating keratoplasty when replaced for other reasons than endothelial failure[7] (e.g., unsatisfactory refractive outcomes). Not only from a surgical and clinical point of view but also considering the efficacy of eye banking, "graft survival" should only refer to (donor) endothelial cell survival, and these evaluations should not be diluted with false-positive ("spontaneous corneal clearance") and false-negative (surgically unsuccessful) cases.

In our opinion, therefore, the terminology used for graft survival studies needs to be further refined because the term graft failure is no longer synonymous with endothelial failure and, therefore, has become less informative (e.g., completely detached endothelial graft carrying viable endothelium) and because the term graft success rate does not mimic graft viability (e.g., spontaneous clearance after host endothelial cell migration). To enable the comparison of future corneal transplant survival studies, it would seem more accurate to evaluate graft attachment status, corneal clearance status, and the presence of visually significant

graft folds when a second surgical procedure is indicated to distinguish actual "endothelial graft failure" from "technical failure" and "optical failure" (Figure).

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CHAPTER 5

Repeat Descemet Membrane Endothelial Keratoplasty after Complicated Primary Descemet Membrane Endothelial Keratoplasty

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ABSTRACT

Purpose: To describe the clinical outcome and complications of repeat Descemet membrane endothelial keratoplasty (re-DMEK).

Design: Retrospective case series study at a tertiary referral center.

Participants: From a series of 550 consecutive DMEK surgeries with ≥6 months follow-up, 17 eyes underwent re-DMEK for graft detachment after initial DMEK (n = 14) and/or endothelial graft failure (n = 3). The outcomes were compared with an age-matched control group of uncomplicated primary DMEK surgeries.

Methods: The re-DMEK eyes were evaluated for best-corrected visual acuity (BCVA), densitometry, endothelial cell density (ECD), pachymetry, and intraoperative and postoperative complications.

Main Outcome Measures: Feasibility and clinical outcome of re-DMEK.

Results: In all eyes, re-DMEK was uneventful. At 12 months, 12 of 14 eyes (86%) achieved a BCVA of ≥20/40 (≥0.5); 8 of 14 eyes (57%) achieved ≥20/25 (≥0.8), 3 of 14 eyes (21%) achieved ≥20/20 (≥1.0), and 1 eye (7%) achieved 20/17 (1.2); 5 eyes were fitted with a contact lens. Average donor ECD decreased from 2580±173 cells/mm^2 before to 1390±466 cells/mm^2 at 6 months after surgery, and pachymetry from 703±126 mm to 515±39 mm, respectively. No difference in densitometry could be detected between re-DMEK and control eyes (P = 0.99). Complications after re-DMEK included primary graft failure (n = 1), secondary graft failure (n = 2), graft detachment requiring rebubbling (n = 1), secondary glaucoma (n = 2), cataract (n = 1), and corneal ulcer (n = 1). One eye received tertiary DMEK.

Conclusions: In the management of persistent graft detachment and graft failure after primary DMEK, re-DMEK proved a feasible procedure. Acceptable BCVA may be achieved, albeit lower than after DMEK in virgin eyes, and some cases may benefit from contact lens fitting. Complications after re-DMEK may be better anticipated than after primary DMEK because graft detachment and graft failure tended to recur, suggesting that intrinsic properties of the host eye play a role in graft adherence and graft failure.

INTRODUCTION

In recent years, endothelial keratoplasty (EK) techniques such as Descemet stripping EK (DSEK) and Descemet stripping automated EK (DSAEK) have evolved and gained wide acceptance, progressively replacing penetrating keratoplasty (PK) as a primary treatment for endothelial disease. The most recent development in EK is Descemet membrane EK (DMEK), which selectively replaces the Descemet membrane (DM) and the endothelium, providing a near-anatomic restoration of the cornea with fast and unprecedented visual results.[1-4] In addition, DMEK has been shown to give good visual outcome when performed as a secondary procedure after "failed" DSEK/DSAEK.[5,6] In addition, DMEK may be performed for secondary graft failure in PK, as an alternative to repeat PK.[7]

The outcomes of re-keratoplasty have been well documented and include a higher risk of corneal scars and astigmatism and in particular allograft rejection.[8-12] Limited reports or case series are available for repeat EK,[13-17] and in particular for repeat DMEK (re-DMEK).[18,19] With growing numbers of EK surgeries, and with DMEK becoming accepted worldwide, it may be important to determine further treatment options in the event of DMEK transplant failure, the technical feasibility and clinical outcome of re-DMEK, and whether re-DMEK is associated with specific complications.

The aim of our study therefore was to identify causes of unsuccessful primary DMEK, describe the surgical modifications of re-DMEK compared with primary DMEK, and report the clinical outcome of re-DMEK in a series of eyes that previously underwent DMEK compared with DMEK control eyes.

METHODS

From a total of 550 consecutive DMEK cases, 17 eyes of 17 patients (8 male, 9 female; 3 phakic, 14 pseudophakic) with an average age of 69±14 years (range, 47-90 years) underwent re-DMEK after unsuccessful primary DMEK. The initial preoperative diagnoses included Fuchs endothelial dystrophy (n = 15), pseudophakic bullous keratopathy (n = 1), and bullous keratopathy after corneal perforation (n = 1). Primary DMEK grafts were removed and replaced by a secondary DMEK graft in a second operative procedure, and the postoperative course of the re-DMEK was followed for ≤12 months. All re-DMEK surgeries were performed by 2 experienced corneal surgeons (I.D., G.M.; Table 1).

Table 1. Demographics of the Study Group and an Age-matched Control Group of Primary DMEK eyes

Variable	Study Group (Secondary DMEK eyes)	Age-matched control group (Primary DMEK eyes)
Patients / Eyes (n)	17/17	17/17
Age (years), mean ± SD (range)		
Patients	69±14 (47-90)	68±13 (48-88)
Donor 2nd DMEK	66±14 (43-85)	--
Gender (male/female)	8/9	9/8
Pseudophakic/phakic (n)	14/3	14/3
Time (months) between 1st and 2nd DMEK (Mean ± SD, (range))	16±9 (4-33)	--

SD = Standard Deviation; DMEK = Descemet membrane endothelial keratoplasty

All patients signed an institutional review board-approved informed consent; the study was conducted according to the Declaration of Helsinki and registered at www.clinicaltrials.gov (study registration no. NCT00521898).

Donor Tissue Protocol

The procedure for harvesting a DMEK graft has been described previously.[20,21] In short, corneoscleral buttons were excised from donor globes ≤36 hours post-mortem and stored in organ culture at 31°C (CorneaMax; Eurobio, Courtaboeuf, France). After 1 week of culture, endothelial cell morphology and viability were evaluated and the corneoscleral buttons were mounted endothelial side up on a custom-made holder. A 9.5-mm-diameter sheet of DM with its endothelium was removed from the posterior stroma with the corneoscleral rim immersed in balanced salt solution. Owing to the elastic tissue properties, a Descemet roll formed spontaneously, with the endothelium on the outer side. Each Descemet roll was then stored for 5 to 10 days in organ culture medium until the time of transplantation.

Repeat DMEK Operative Procedure

All re-DMEK eyes were operated under local anesthesia (4 ml 1% ropivacain hydrochloride with 1 ml 150 IE Hyason), followed by an ocular massage and a Honan's balloon for 10 minutes; the patient was positioned in the anti-Trendelenburg position. Surgeries were performed as described previously,[22] with a few adjustments (Table 2). Instead of performing a descemetorhexis, the primary DMEK graft was carefully removed from the recipient posterior stroma with a reversed Sinskey hook (D.O.R.C. International, Zuidland, The Netherlands) under air. A 3-mm limbal

Table 2. Surgical Tips for Repeat Descemet Membrane Endothelial Keratoplasty (DMEK)

1.	If possible, identify and remove the cause of graft failure for the initial DMEK graft (control Intraocular pressure, reposition glaucoma tube, remove anterior chamber intraocular lens, etc. before re-DMEK).
2.	If possible, re-open old corneal tunnel incision and side ports, to avoid 'double' entry wounds that may interfere with instrument insertion.
3.	With a reversed Sinskey hook, remove the primary DMEK graft that commonly shows more stickiness to the host stroma than the Descemet Membrane in a virgin eye during descemetorhexis.
4.	Carefully remove 'sticky' graft remnants by additional scraping while monitoring completeness of graft removal 'under air,' but avoid damage to the host posterior stroma. The application of trypan blue into the host anterior chamber may additionally aid to visualize Descemet Membrane remnants.
5.	Particularly when re-DMEK is performed to manage graft detachment with or without extensive corneal edema after initial DMEK, remove all endothelial cells that have migrated over the stroma underneath the detached area, and leave the host anterior chamber filled with air for up to 60-120 minutes, to avoid detachment from recurring in the same quadrant(s).

tunnel incision was made (or reopened) for insertion of the new DMEK graft. After graft removal, the posterior stromal surface was meticulously checked, and any graft remnants were carefully removed with a custom-made scraper (D.O.R.C. International).

The donor Descemet roll was stained with a 0.06% Trypan blue solution (VisionBlue, D.O.R.C. International), configured as a "double roll," and sucked into an injector (DMEK-inserter; D.O.R.C. International) to inject it into the recipient anterior chamber. The graft was oriented endothelial side down (donor DM facing recipient posterior stroma). By indirect manipulation with air and balanced salt solution, the graft was then gently unfolded over the iris and positioned onto the recipient posterior stroma by injecting an air bubble underneath the graft. The anterior chamber was left completely filled with air for ≥60 minutes (average bubble time, 64±8 minutes), followed by an air-liquid exchange to pressurize the eye while leaving a 30% to 50% air bubble in situ. Each operative procedure was recorded on DVD (Pioneer DVR-RT601H-S, Tokyo, Japan). The postoperative medication regime included antibiotics and steroid similar as for primary DMEK.[23]

Data Collection

All eyes were examined before and at 1 week and 1, 3, 6, and up to 12 months after re-DMEK. The clinical outcome was evaluated by comparing the preoperative with postoperative best-corrected visual acuity (BCVA), pachymetry, Pentacam imaging (Oculus, Wetzlar, Germany) and anterior segment optical coherence tomography (Heidelberg Engineering GmbH, Heidelberg, Germany), as well as

slit-lamp biomicroscopy images (Topcon Medical Europe BV, Capelle a/d IJssel, The Netherlands).

For corneal densitometry (backscattered light) analysis (Pentacam; Oculus), 3 different fixed corneal layers - the anterior layer (anterior 120 μm), central layer, and posterior layer (posterior 60 μm) - as well as fixed corneal concentric rings around the apex (central 0-2, 2-6, 6-10, and 10-12 mm) as provided by the software, were examined.[24] Values at 6 months after re-DMEK were compared with those 6 months after uneventful primary DMEK using an age- and lens status-matched control group (Table 1). Corneal density was quantified on a scale from 0 (clear) to 100 (completely opaque).

Donor endothelial cell density (ECD) was evaluated in vitro with light microscopy in the eye bank (Axiovert 40 inverted light microscope; Zeiss, Göttingen, Germany) and photographed (PixeLINK PL-A662; Zeiss). Postoperative ECD was evaluated using a Topcon SP3000p noncontact autofocus specular microscope (Topcon Medical Europe BV).

Eyes with low visual potential (glaucomatous optic neuropathy, age-related macular degeneration) were excluded from BCVA analysis and the eye with primary graft failure after re-DMEK was excluded from BCVA and densitometry analysis.

Statistical Analysis

Intraoperative and postoperative complications, BCVA, and ECD, were recorded in a SQL database. Paired t tests were performed to identify significant differences in outcomes between the study and control group. $P < 0.05$ was considered significant.

RESULTS

Indications for Repeat DMEK

We performed re-DMEK in a series of 17 eyes that showed unsatisfactory visual outcomes after primary DMEK and for which improvement could be expected by a transplant replacement. Low visual outcome after primary DMEK was attributed to clinically significant graft detachment (n = 14) and endothelial graft failure (n = 3; Table 3 [available at www.aaojournal.org]; Figure 1).

In eyes with graft detachment, 3 eyes had a detachment of at least one third and 8 eyes of more than one third of the graft surface area, and 3 eyes had the graft positioned upside down (Figure 2). In these eyes, BCVA ranged from counting fingers (1/60) to 20/25 (0.8). In the 3 eyes with an endothelial graft failure, 1 eye showed a primary graft failure (graft attached, but cornea did not clear after surgery) and 2 eyes had a secondary graft failure (graft attached, cornea initially cleared but decompensated later in the postoperative course) associated with allograft rejection (n = 1) or late endothelial failure without rejection (n = 1; Figure 3).

The average time between the initial and secondary DMEK was 16±9 months (range, 4–33 months; Table 3, available at www.aaojournal.org). The large variation in postoperative time could be attributed to the fact that in some eyes the cornea initially cleared despite graft detachment ("spontaneous corneal clearance") but decompensated later (n = 5; Table 3, available at www.aaojournal.org).

Repeat DMEK Operative Procedure

All re-DMEK surgeries were uneventful and could be performed with minor modification to the standard DMEK protocol (Table 2). In most eyes, the removal of the primary DMEK graft proved more difficult than a descemetorhexis in a virgin eye because of stronger adherence of the graft to the recipient stroma. The DM remnants could best be visualized "under air" using a complete airfill of the recipient anterior chamber. In 6 cases, the posterior corneal stroma was additionally scraped to remove DM remnants.

Visual Acuity

At six months after re-DMEK, 10 of 13 eyes (77%) attained a BCVA of ≥20/40 (≥0.5); 5 of 13 eyes (38%) attained ≥20/25 (≥0.8), and 2 of 13 eyes (15%) attained ≥20/20 (≥1.0). At 12 months, 12 of 14 eyes (86%) attained ≥20/40 (≥0.5); 8 of 14 eyes (57%) attained ≥20/25 (≥0.8), 3 of 14 eyes (21%) attained ≥20/20 (≥1.0) and 1 eye (7%) attained 20/17 (1.2). At both follow-up intervals, 5 eyes had been fitted with a contact lens (Figure 4).

Densitometry

Densitometry values 6 months after re-DMEK were compared with those of control eyes 6 months after uneventful primary DMEK. At 6 months, there was a tendency toward higher densitometry values in the total central concentric ring around the apex (central 0–2 mm) in all layers (P = 0.33), and there was no detectable difference in total densitometry between re-DMEK and control eyes

Table 3: Clinical outcome after initial and repeat Descemet membrane endothelial keratoplasty detailed for the whole study group

No.	STUDY GROUP				PREOP	AFTER INITIAL DMEK							INTER-VAL	AFTER RE-DMEK						
						Last follow-up (months) before re-DMEK								Follow-up 6 months after re-DMEK						
Case/ (DMEK)	Age/ Gender	Indication for initial DMEK	Eye	Lens status	BSCVA	CCT (µm)	Last FU (m)	BSCVA	ECD (cells/mm²)	CCT (µm)	Remarks	Indication for re-DMEK	Time (m) after initial DMEK	BSCVA	BCLVA	Irregular Surface (Pentacam)	ECD (cells/mm²) Preop./ 6m FU	CCT (µm)	Graft status (Pentacam, AS-OCT)	Remarks
1 (26)	74 M	FED	OD	Pseudo-phakic	CF	n.a.	10	20/40 (0.5)	1530	607	Re-bubbling at 3m 'Sponta-neous clearance'	Graft detached >1/3, folds in optical axis	11	20/60 (0.3)	20/23 (0.9)	Yes	2800/ 2020	526	Graft detached ≤1/3, same area as in 1st graft	--
2 (94)	75 M	FED	OD	Pseudo-phakic	20/50 (0.4)	637	6	20/100 (0.2)	n. m.	743	'Sponta-neous clearance'	Graft upside-down	7	20/50 (0.4)	--	No	2500/ 1140	556	Attached	Secondary IOP elevation requiring glaucoma surgery
3 (38)	48 F	FED	OS	Phakic	20/50 (0.4)	678	19	CF*	350 (12m)	650	Re-bubbling at 1m & 6w 'Sponta-neous clearance'	Graft upside-down	20	20/25 (0.8)	--	No	2550/ 1690	566	Attached	Phacoemul-sification at 10m
4 (79)	64 F	FED	OS	Pseudo-phakic	20/60 (0.3)	686	18	20/400 (0.05)	n. m.	834	Re-bubbling at 3m	SGF	21	20/17 (1.2)	--	Mild	2400/ 350	526	Attached	SGF at 12m Tertiary DMEK
5 (36)	71 F	FED	OD	Pseudo-phakic	20/133 (0.15)	643	30	20/60 (0.3)	360 (12m)	663	'Sponta-neous clearance	Graft upside-down	31	20/33 (0.6)	20/28 (0.7)	Yes	2400/ 1110	478	Attached	--

Table 3: Clinical outcome after initial and repeat Descemet membrane endothelial keratoplasty detailed for the whole study group *(continued)*

No.		STUDY GROUP			PREOP		AFTER INITIAL DMEK					INTER-VAL	AFTER RE-DMEK						
							Last follow-up (months) before re-DMEK						Follow-up 6 months after re-DMEK						
Case/ (DMEK)	Age/ Gender	Indication for initial DMEK	Eye	Lens status	BSCVA	CCT (µm)	Last FU (m)	BSCVA	ECD (cells/mm²)	CCT (µm)	Remarks	Indication for re-DMEK	Time (m) after initial DMEK	BSCVA	BCLVA	Irregular Surface (Pentacam)	ECD (cells/mm²) Preop/ 6m FU	CCT (µm)	Graft status (Pentacam, AS-OCT)	Remarks
6 (135)	64 M	FED	OD	Pseudo-phakic	20/60 (0.3)	631	12	20/60 (0.3) 20/40# (0.5)#	940 (6m)	637	Allograft rejection at 10m	SGF	16	20/60 (0.3)	CL induced edema	Mild	2400/ 970	544	Small para-central detach-ment ≤1/3	Paracentral remnant, chronic rejection?
7 (243)	50 M	BK	OD	Phakic	20/80 (0.25)	728	3	20/50 (0.4)	490	719	--	PGF	4	20/17 (1.2) 3m	--	No	2500/ 1500	509	Small peripheral detach-ment ≤1/3, same area as in 1st graft	SGF at 12m
8 (224)	75 F	FED	OS	Pseudo-phakic	20/100 (0.2)	556	9	20/100 (0.2)	n. m.	875	--	Graft detached >1/3, folds in optical axis	10	20/40 (0.5)	--	Yes	2755/ 1410	544	Small peripheral detach-ment ≤1/3	--
9 (225)	86 M	PPBK	OD	Pseudo-phakic	20/50 (0.4)	645	6	20/100 (0.2)	n. m.	735	--	Graft >1/3 detached	11	20/50 (0.4)	--	Yes	2875/ n.m.	446	Attached	--
10 (80)	59 M	FED	OD	Pseudo-phakic	20/40 (0.5)	743	27	20/25 (0.8)	700 (12m)	751	--	Graft ≤1/3 detached	30	20/33 (0.6)	20/20 (1.0)	Yes	2785/ 1360 (12m)	435	Graft detached ≤1/3, same area as in 1st graft	Corneal ulcer at 2.5m

Table 3: Clinical outcome after initial and repeat Descemet membrane endothelial keratoplasty detailed for the whole study group *(continued)*

No.		STUDY GROUP			PREOP	AFTER INITIAL DMEK							INTER-VAL	AFTER RE-DMEK							
						Last follow-up (months) before re-DMEK								Follow-up 6 months after re-DMEK							
Case/ (DMEK)	Age/ Gender	Indication for initial DMEK	Eye	Lens status	BSCVA	CCT (µm)	Last FU (m)	BSCVA	ECD (cells/mm^2)	CCT (µm)	Remarks	Indication for re-DMEK	Time (m) after initial DMEK	BSCVA	BCLVA	Irregular Surface (Pentacam)	ECD (cells/mm^2) Preop/ 6m FU	CCT (µm)	Graft status (Pentacam, AS-OCT)	Remarks
11 (335)	47 F	FED	OS	Phakic	20/28 (0.7)	811	3	20/40 (0.5)	1270	793	Paired donor of case#17	Graft >1/3 detached	4	20/28 (0.7)	--	No	2900/ 2000	563	Attached, folds	--
12 (249)	67 F	FED	OD	Pseudo-phakic	20/50 (0.4)	685	9	20/100 (0.2)	n. m.	809	Central flat ulcer at 8m	Graft >1/3 detached	12	20/50 (0.4)	--	Yes	2500/ 1370	535	Attached	--
13 (107)	85 F	FED	OS	Pseudo-phakic	20/60 (0.3)	680	30	20/133 (0.15)	320 (24m)	680	'Sponta-neous clearance'	Graft >1/3 detached	33	20/23 (0.7)	--	Yes	2600/ 1820	498	Attached	--
14 (329)	87 F	FED	OS	Pseudo-phakic	20/40 (0.5)	614	11	20/80 (0.25)	340	689	--	Graft ≤1/3 detached	12	20/40 (0.5)	--	Yes	2500/ 720	491	Attached	ARMD
15 (319)	90 F	FED	OD	Pseudo-phakic	20/100 (0.2)	724	12	20/133 (0.15)	490 (6m)	564	--	Graft ≤1/3 detached	13	HM	--	Yes	2400/ n.m.	884	Attached	Air-bubble induced IOP elevation PGF
16 (233)	61 M	FED	OD	Pseudo-phakic	20/25 (0.8)	539	15	20/133 (0.15)	n. m.	688	--	Graft >1/3 detached	17	20/33 (0.6)	20/25 (0.8)	No	2500/ 1490	526	Graft detached >1/3, attached after one re-bubbling at 1w	YAG-CT at 4m

Table 3: Clinical outcome after initial and repeat Descemet membrane endothelial keratoplasty detailed for the whole study group *(continued)*

No.	STUDY GROUP			PREOP			AFTER INITIAL DMEK						INTER-VAL	AFTER RE-DMEK							
							Last follow-up (months) before re-DMEK							Follow-up 6 months after re-DMEK							
Case/ (DMEK)	Age/ Gender	Indication for initial DMEK	Eye	Lens status	BSCVA	CCT (µm)	Last FU (m)	BSCVA	ECD (cells/mm²)	CCT (µm)	Remarks	Indication for re-DMEK	Time (m) after initial DMEK	BSCVA	BCLVA	Irregular Surface (Pentacam)	ECD (cells/mm²) Preop/ 6m FU	CCT (µm)	Graft status (Pentacam, AS-OCT)	Remarks
17 (334)	61 M	FED	OD	Pseudo-phakic	20/25 (0.8)	566	14	20/28 (0.7)	n. m.	519	Paired donor of case #11	Graft >1/3 detached	14	20/23 (0.9)	--	Mild	2500/ 1675	490	Attached	--

DM = Descemet membrane
DMEK = Descemet membrane endothelial keratoplasty
FED = Fuchs endothelial dystrophy
BK = Bullous Keratopathy
PPBK = Pseudophakic Bullous Keratopathy
CCT = central corneal thickness
BSCVA = Best spectacle corrected visual acuity
BCLVA = Best contact lens visual acuity
CF = Counting fingers
HM =hand movements
YAG-CT = YAG capsulotomy
ARMD= Age related macular degeneration
n. a. = not available
n. m. = not measurable
w = weeks
m = months
*after YAG laser treatment
#fitted with a contact lens after primary DMEK

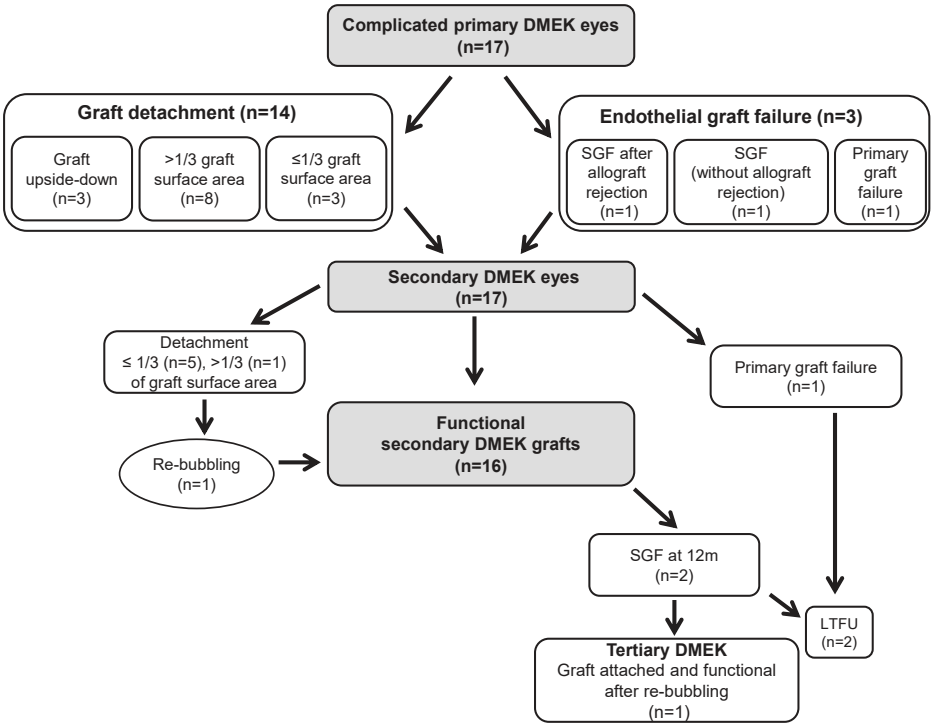

Figure 1. Indications for repeat Descemet Membrane Endothelial Keratoplasty (DMEK) and its postoperative course. LTFU = lost to follow-up; SGF = secondary graft failure.

for any central-to-peripheral optical zone or at any anterior-to-posterior stromal level (P = 0.99; Table 4).

ECD and Pachymetry

Donor ECD (of the second graft) averaged 2580 (±173) cells/mm^2 before (n=17), and 1390 (±466) cells/mm^2 (- 46.1%) at 6 months (n=15) and 1294 (±459) cells/mm^2 (- 49.8%) at 12 months after re-DMEK (n=13). Pachymetry values decreased from 703 (±126) μm before (n=17), to 515 (±39) μm at 6 months after re-DMEK (n=16) (Table 4).

Graft detachment and rebubbling after Repeat DMEK

Significant graft detachment after re-DMEK (more than one third of the graft surface area) was observed in 1 eye (case 16) that was managed with a rebubbling procedure (120-minutes airfill of the host anterior chamber) at 1 week postoperatively. Small peripheral or partial detachments of not more than one third of the graft surface area were detected in 5 eyes (cases 1, 6, 7, 8, and 10) but did

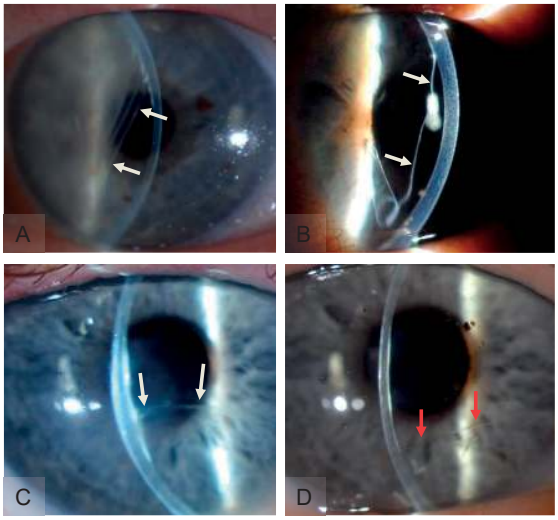

Figure 2. Slit lamp images of graft detachments after primary Descemet Membrane Endothelial Keratoplasty (DMEK), which comprised the main indication for repeat DMEK. (A) Case 8, graft detachment of greater than one third of the graft surface area after primary DMEK (white arrows). (B) Case 3, the graft was positioned upside down and detached (white arrows). (C, D) Case 1, in an eye that had a graft detachment after the initial DMEK (white arrows), the graft detached again in the same area after re-DMEK (red arrows).

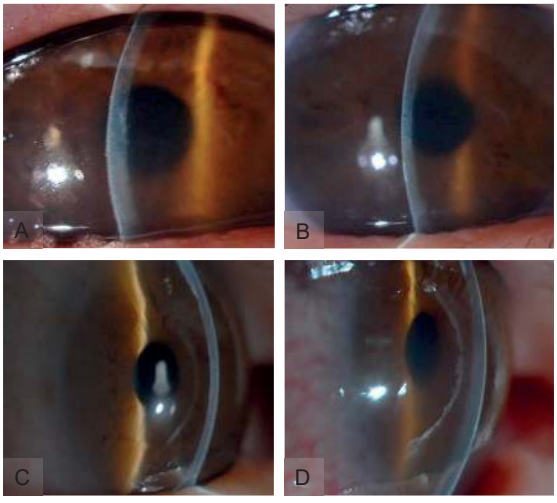

Figure 3. Slit lamp images of an eye that underwent repeat Descemet Membrane Endothelial Keratoplasty (DMEK) for primary graft failure after DMEK that again developed graft failure after repeat DMEK. (A) Case 7, corneal decompensation after traumatic corneal perforation and (B) primary graft failure after initial DMEK. (C) One month after repeat DMEK the cornea cleared, but (D) secondary graft failure was observed at 12 months.

not require further treatment. The remaining 11 re-DMEK eyes showed full graft attachment (Table 3, available at www.aaojournal.org).

In 3 eyes (cases 1, 7, and 10) that developed a partial graft detachment after primary DMEK showed again a detachment in the same corneal quadrant after re-DMEK (Figure 2; Table 3, available at www.aaojournal.org).

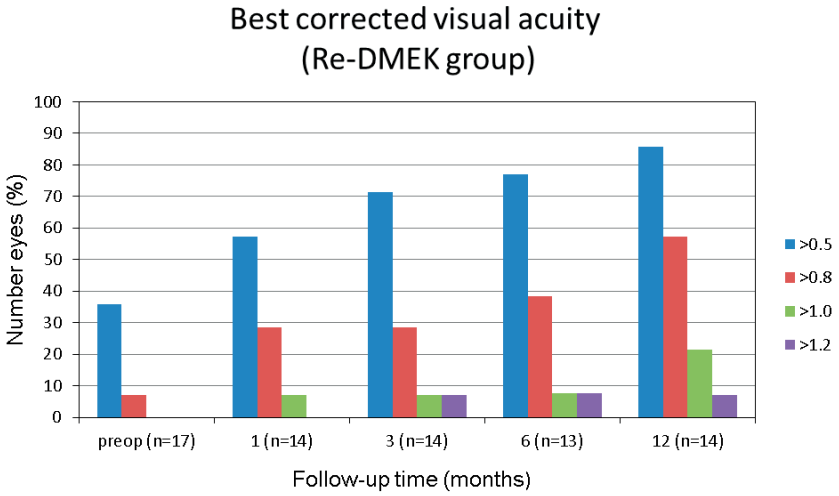

Figure 4. Best-corrected visual acuity ≤12 months after repeat Descemet Membrane Endothelial Keratoplasty. Five eyes were fitted with a contact lens at 6 and 12 months postoperatively. The legend represents the decimal visual acuity levels.

Table 4. Outcome measures Secondary Descemet Membrane Endothelial Keratoplasty (study group) and Primary Descemet Membrane Endothelial Keratoplasty (control group)

Variable	Study group (n=17)	Control group (n=17)	*P*-value
Densitometry*			
Total (ant/central/post & central to peripheral zone)	26.8±3.7 (n=15)	26.1±5.1	*P*=0.99
Total (anterior/central/posterior) 0-2mm zone	25.1±8.7 (n=15)	20.0±3.9	*P*=0.33
Anterior 0-2mm zone	35.3±16.2 (n=15)	28.0±5.4	*P*=0.60
Central 0-2mm zone	19.8±8.1 (n=15)	16.7±3.4	*P*=0.60
Posterior 0-2mm zone	20.3±7.3 (n=15)	15.5±3.5	*P*=0.20
Endothelial Cell Density (cells/mm^2)			
Preoperative	2580±173 (n=17)	2596±244	*P*=0.85
Postoperative 6 months	1390±466 (n=15)	1813±606	*P*=0.04
Postoperative 12 month	1294±459 (n=13)	1728±607	*P*=0.07
Pachymetry (μm)			
Preoperative	703±126 (n=17)	663 (±77)	*P*=0.28
Postoperative at 6 months	515±39 (n=16)	520 (±35)	*P*=0.40

Values are presented as means ± standard deviation unless otherwise noted
*two eyes excluded with a central corneal scar due to an ulcer after DMEK (case 12) and after repeat DMEK (case 10)

Other Postoperative Complications after Repeat DMEK

One eye (case 15) showed primary and 2 eyes (cases 4 and 7; Figure 5) secondary graft failure 12 months after re-DMEK (Table 5). Two of these eyes had also shown secondary graft failure of the initial DMEK graft (Table 3, available at www.aaojournal.org; Figure 3). One eye had a tertiary DMEK that was performed in the same manner as in re-DMEK, and the interface was meticulously scraped to remove all remnants from previous grafts. A postoperative graft detachment was managed by an uneventful rebubbling procedure with 120-minutes airfill of the host anterior chamber. The visual acuity improved from counting fingers (1/60) before surgery to 20/20 (1.0) at 6 months and 20/17 (1.2) at 9 months postoperatively.

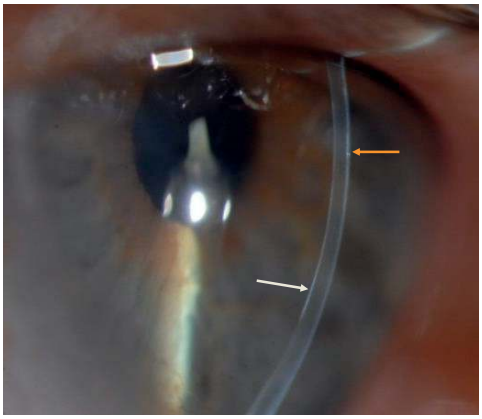

Figure 5. Six months after repeat Descemet Membrane Endothelial Keratoplasty. The eye (case 4) shows typical scarring patterns across the interface between the donor Descemet graft and the host stroma (presumably induced by removal of the first Descemet graft; white arrow) and a subepithelial haze (which may have resulted from prolonged corneal edema; orange arrow).

Table 5. Complications after Repeat Descemet Membrane Endothelial Keratoplasty

Complication	No.
Graft detachment	6
One third or less of graft surface area	5
More than one third of graft surface area	1
Graft failure (primary*/secondary)	3
Suspect of recurrent allograft rejection	1
IOP elevation	2
Air-bubble induced*	1
Secondary glaucoma requiring glaucoma surgery	1
Cataract formation (out of 3 phakic eyes)	1
Corneal ulcer	1
Descemet membrane remnant of primary DMEK graft	1

IOP = intraocular pressure; DMEK = Descemet Membrane Endothelial Keratoplasty;
*This complication occurred in the same eye.

In the 1 eye that underwent re-DMEK for graft failure after allograft rejection (case 6), 2 episodes of suspected recurrent allograft rejection were observed after re-DMEK and were managed by an intensified topical steroid regime.

Two eyes showed postoperative glaucoma (Table 3, available at www.aaojournal.org). In case 15, the intraocular pressure elevation was induced by the air bubble and, although reversed by removal of the air from the host anterior chamber, the cornea of this eye did not clear. The other eye (case 2) had secondary open-angle glaucoma that required filtering surgery.

One of the 3 phakic eyes (cases 3, 7, and 11) developed a cataract for which phacoemulsification was performed 10 months after re-DMEK (case 3).

One eye (case 10) developed a corneal ulcer 2.5 months after re-DMEK that could be managed with systemic and topical antibiotics and steroids.

In 1 eye (case 6), a remnant of the primary DMEK graft was observed at the interface between the secondary graft and the host posterior stroma.

DISCUSSION

Feasibility of Repeat DMEK

Our study showed that re-DMEK was technically feasible in all eyes that showed graft detachment or DMEK transplant failure. Compared with primary DMEK, some modifications in the operative protocol may be considered in re-DMEK to avoid intraoperative and postoperative complications (Table 2). Unlike a virgin DM during descemetorhexis, a DMEK graft was found to show relatively strong adherence to the host posterior stroma, with a higher risk of graft remnants. Performing a "normal" descemetorhexis "under air" to better visualize DM to enable its complete removal in routine DMEK has been advocated, so that in re-DMEK it may be even more critical to monitor previous graft removal "under air." As an additional check, Trypan blue may be applied to stain the primary DMEK graft to identify remnants left in situ.[18] When re-DMEK is performed in eyes that developed a detachment of the initial DMEK graft, it may be especially important to meticulously scrape the recipient posterior stroma in the area of the detachment and remove all (migrated) endothelial cells covering the stromal defect to enable better graft adherence. In these cases, it may also be recommended to increase the air bubble time at termination of the surgery to 90 to 120 minutes

because secondary grafts may show a tendency to detach in the same area as the initial DMEK graft.

Clinical outcome

After repeat PK, visual outcomes have been reported to be worse than in primary PK.[25,26] To manage low visual outcome after primary DSAEK or DSEK, repeat DSAEK or repeat DSEK has been reported to be effective with visual acuity outcomes of ≥20/40 (0.5)[13,16] In our group of re-DMEK eyes, that had reduced visual acuity owing to corneal edema, BCVA improved in all eyes in which re-DMEK was successful. Overall, however, a smaller number of eyes may achieve a final BCVA level that compares to uneventful primary DMEK, with about 40% to 50% of eyes reaching ≥20/25 (≥0.8) after re-DMEK at 6 to 12 months, whereas 80% to 90% may reach this level after primary DMEK.[2,4] In addition, about one third of re-DMEK eyes required contact lens fitting to further improve BCVA.

We recently reported that corneal surface irregularities could result from superficial corneal scarring after long-standing corneal edema.[27] If so, this would be an argument for earlier operative reintervention after failed primary DMEK. Higher paracentral densitometry values in eyes after repeat DSAEK after failed primary DMEK, than after primary DSAEK have been reported,[28] and may relate to diffuse scarring of the interface between the graft and the host posterior stroma and/or subepithelial scarring. However, these findings could not be substantiated in our study because no difference in densitometry values were found between repeat DMEK and control DMEK eyes (Figure 5).

In re-DMEK, ECD decrease seemed to be higher compared with primary DMEK at 6 months (-46% vs. -34%) and at 12 months (-50% vs. -37%).[19,29,30] These results could (in part) be explained by negative selection bias, because eyes with a greater tendency toward lower ECD are more likely to require re-DMEK. Nonetheless, all corneas cleared and pachymetry values returned to normal in all but 1 case that showed primary graft failure, presumably associated with air bubble-induced IOP elevation in the immediate postoperative phase.

Complications

The spectrum of complications after re-DMEK resembled that after primary DMEK. However, some complications may be anticipated when reviewing the postoperative course after the initial DMEK and/or the indication for reintervention. Three eyes (cases 1, 7, and 10) had a graft detachment after initial DMEK and the secondary DMEK graft showed a tendency toward graft detachment in the

same corneal quadrant(s). Two eyes (cases 4 and 7) that showed graft failure of the primary DMEK again developed late graft failure after re-DMEK. Both observations may suggest that host intrinsic properties, like the eye's anatomy and/or comorbidities, may aid or interfere with graft adherence and may influence the risk of graft failure.

Other complications seemed incidental and larger series with longer follow-up may be needed to reveal any difference in complications between primary and secondary DMEK, for example, the risk of allograft rejection, which is known to increase with the number of re-keratoplasty procedures in PK.[12]

Indications for and Timing of Re-DMEK

In a recent case series, the main indication for re-DMEK was upside-down graft positioning.[18] In the current study, re-DMEK was largely performed to manage significant graft detachment (n = 14; of which only 3 grafts were positioned upside down) and primary or secondary graft failure (n = 3).

Compared with other studies that reported reintervention 1 to 6 months after the initial DMEK,[18] in our series re-DMEK was performed at later postoperative time intervals, on average at 16±9 months (range, 4-33 months). Our conservative approach may have resulted from the observation that corneas with partially detached grafts still cleared ("spontaneous corneal clearance").[31,32] As a result, our study may be negatively biased because DMEK eyes with a graft detachment that reached an acceptable BCVA after spontaneous clearance never became eligible for re-DMEK. Also, postponing reintervention may have resulted in longer episodes of corneal edema and secondary superficial and stromal scarring, requiring contact lens fitting to reach the eye's maximal potential.[27] To avoid secondary stromal changes induced by persistent corneal edema owing to a larger graft detachment, it could therefore also be argued to rebubble the graft (or to perform a re-DMEK) in the early postoperative phase.

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PART 2

Prediction of DMEK Rejection and Transplantation of Smaller Endothelial Grafts

CHAPTER 6

Quantitative Assessment of Aqueous Flare after Descemet Membrane Endothelial Keratoplasty for Fuchs Endothelial Dystrophy

Cornea 2018;37:848–53

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ABSTRACT

Purpose: To assess aqueous flare as a measure of subclinical inflammation after Descemet membrane endothelial keratoplasty (DMEK) for Fuchs endothelial dystrophy.

Methods: In this prospective cross-sectional and longitudinal case series at a tertiary referral center, 173 DMEK eyes of 169 patients and 19 age-matched healthy control eyes were included. Aqueous flare [photon count per millisecond (ph/ms)] was assessed by laser flare photometry at 1 day, 1 week, and 1 month after DMEK in group I (evaluation of postsurgical blood–aqueous barrier recovery; n = 25) and on average 28 (±19) months (range, 3–86 months) after DMEK in group II (evaluation of long-term inflammation; n=148).

Results: In group I, flare levels decreased from 1 day to 1 week [25.1 (±9.1) ph/ms vs. 13.4 (±4.8) ph/ms; P=0.003] and remained stable up to 1 month after DMEK [12.1 (±3.2) ph/ms; P=0.387]. However, average flare at 1 month was higher than that in healthy controls (P<0.001). The long-term flare value after DMEK (group II) was 9.6 (±4.2) ph/ms and was higher in eyes associated with allograft rejection (n=6) versus those without rejection [16.7 (±7.8) ph/ms vs. 9.3 (±3.8) ph/ms, respectively, P<0.001]. All eyes associated with rejection had flare values above 10 ph/ms.

Conclusions: Aqueous flare after DMEK quickly decreased within the first postoperative month, indicating fast recovery of the blood–aqueous barrier. Long-term flare levels were higher in eyes associated with rejection, suggesting persistent subclinical inflammation. A flare level above 10 ph/ms may be used as a threshold for identifying eyes associated with or at risk of allograft rejection after DMEK.

INTRODUCTION

Surgical trauma and associated inflammation cause breakdown of the blood-aqueous barrier, leading to leakage of proteins and inflammatory cells into the anterior chamber, recognized as "flare" with biomicroscopy.[1–5] Laser flare photometry allows for objective and noninvasive assessment of flare levels, so that the device is used in monitoring uveitic eyes for disease progression/remission or recurrence.[4–8] Various studies evaluated aqueous flare levels in ocular diseases (e.g., retinal detachment) or after ocular surgery (phacoemulsification, trabeculectomy, or vitrectomy) to investigate recovery of the blood–aqueous barrier.[9–12] Higher flare levels have also been observed after penetrating keratoplasty, and remarkably higher levels have been associated with allograft reactions.[13–15]

In the past decade, we have introduced several techniques for endothelial keratoplasty including Descemet membrane endothelial keratoplasty (DMEK).[16] DMEK allows for selective replacement of the diseased corneal endothelial cell layer, thus possibly allowing significant reduction in surgical trauma and complications.[16–20] Although the technique is minimally invasive and a much lower antigen load is being transplanted, the DMEK transplant can still induce (subclinical) inflammation.[21] Clinical observation suggests that DMEK eyes hardly show intraocular inflammation after surgery, and even in the event of allograft rejection, flare often seems negligible on slit-lamp examination.[21–23]

However, we recently described signs of endothelial cell activation that may occur up to 18 months before post-DMEK allograft rejection becomes clinically manifest.[24] Therefore, the aim of this study was to evaluate the presence of inflammation in post-DMEK eyes using laser flare photometry and to assess its potential in detecting eyes that may be at risk of developing allograft rejection.

MATERIALS AND METHODS

Aqueous flare was prospectively measured in 173 eyes of 169 patients [mean age: 67 (±10) years] after DMEK for Fuchs endothelial dystrophy during regular follow-up examinations at the Melles Cornea Clinic, Rotterdam, the Netherlands. Exclusion criteria were central corneal edema beyond 3 months after DMEK, scarring, or graft detachment in the central cornea that may influence flare. Because corneal edema,[25] epithelial bullae, and guttae may influence preoperative flare measurements, only postoperative flare values were evaluated.

Two DMEK groups were evaluated: group I comprised 25 DMEK eyes of 25 patients that were measured prospectively at 1 day, 1 week, and 1 month after DMEK (longitudinal analysis) to evaluate flare levels in the immediate postoperative phase as a measure of recovery of the blood–aqueous barrier (i.e., surgical trauma) (Table 1). Group II comprised 148 primary eyes of 148 patients that were assessed during routine follow-up visits at different time points in a cross-sectional analysis. Only eyes that had reached 3-month follow-up were included in this group to assess

Table 1. Demographics and topical medication regimen of study and control eyes

	Eyes after DMEK (173 eyes, 169 patients)		**Eyes without corneal transplantation**
	Group I (Early flare evaluation)	**Group II (Late flare evaluation)**	**Control eyes**
Number of eyes	25	148	19
Number of patients	25[1]	148[1]	19
Average FU time (±SD), months	--	28 (±19)	--
Evaluated time points	1 day, 1 week, 1 month	--	--
Median FU time (range), months	--	24 (3-86)	--
Patient age, years			
Mean (±SD)	68 (11)[2]	67 (10)[2]	68 (8)[2]
Gender			
Female	16	79	9
Male	9	69	10
Preoperative indication			
Fuchs endothelial dystrophy	25	148	--
Lens status			
Phakic	4	46	18
Pseudophakic	21	102	1
Diabetes	1	13	0
Topical medication			--
None	0	2[3]	
Fluorometholone[4]	0	139[4]	
Dexamethasone	25	5[5]	
Loteprednol/ Rimexolone	0	2	
Antiglaucoma medication	0	5[6]	0

DMEK = Descemet Membrane Endothelial Keratoplasty; FU = Follow-up; SD = Standard Deviation

[1] in 4 patients, contralateral eye in other group

[2] matched (P>0.05)

[3] Medication has been stopped by patient

[4] Frequency according to the standard protocol once every other day and up to 4 times per day

[5] Two of those applied Dexamethasone after an episode of allograft rejection

[6] Two of those were eyes following rejection and 3 without rejection

flare in the later postoperative phase. Four patients had both eyes included: 1 eye in group I and the contralateral eye in group II.

Flare values of group I (at 1-month follow-up) and group II were compared with those of 19 eyes of 19 age-matched healthy subjects with no corneal disease or history of recent ocular surgery (Table 1). None of the included subjects had a history of (non)infectious uveitis or immune disease, and none of the control eyes received topical anti-inflammatory treatment.

All patients signed an institutional review board–approved informed consent form for research participation. The study adhered to the tenets of the Declaration of Helsinki.

Donor Tissue Recovery and DMEK Surgery

DMEK grafts were prepared as has been previously described.[26,27] Corneoscleral buttons were excised from donor globes and stored in organ culture medium (CorneaMax, Eurobio, Courtaboeuf, and France). After evaluation of endothelial cell morphology and viability, corneoscleral buttons were mounted endothelial side up on a custom-made holder to remove a 9- to 10-mm diameter Descemet sheet with its endothelium from the posterior stroma. A "Descemet-roll" formed spontaneously, with the endothelium on the outer side.

All eyes received YAG-laser iridotomy about 2 weeks before DMEK, followed by fluorometholone 3 times daily over 1 week. DMEK surgery was performed as has been previously described.[28] A circular 9.0-mm "descemetorhexis" was performed with a reversed Sinskey hook (DORC International, Zuidland, the Netherlands) under an air-filled anterior chamber. The donor "Descemet-roll" was then stained (0.06% Trypan blue solution, Vision Blue; DORC International), aspirated into a custom-made injector (Melles DMEK injector; DORC International), and inserted through a 3.0-mm limbal tunnel incision into the recipient anterior chamber. The graft, oriented with the endothelium facing the recipient iris and Descemet membrane facing recipient posterior stroma, was then completely unfolded over the iris before an air bubble was injected underneath the graft to position it onto the recipient posterior stroma. The anterior chamber was then completely filled with air for 60 minutes, followed by an air–liquid exchange leaving up to 50% air.

At the end of surgery, subconjunctival dexamethasone and gentamicin were injected. Postoperative medication included antibiotic eye drops for 2 weeks and a steroid regimen of dexamethasone 0.1% drops 4 times daily for 4 weeks,

followed by fluorometholone 0.1% drops 4 times daily, tapered to once daily at 1 year postoperatively, and once daily or once every other day thereafter. In the case of steroid-induced ocular hypertension/glaucoma or ocular discomfort from preservatives, or a previous episode of allograft rejection after DMEK, the patient received an alternative antiinflammatory medication (rimexolone 1%, loteprednol 0.5%, or dexamethasone 0.1% without preservatives) (Table 1). Topical medication was defined as the current medication that was applied for the last 3 to 6 months until the follow-up visit of flare measurement (Table 1).

Aqueous Flare Measurement

All eyes were examined with slit-lamp biomicroscopy before laser flare photometry (KOWA FM-700 laser flare meter; Kowa Company, Chofu, Tokyo, Japan). Laser flare readings were performed by 2 examiners (L.B. and F.C.L.) according to a recommended protocol (Kowa Laser Flare-Cell Photometry Medical Advisory Board 1994).6 Without dilating the pupil, the emitted scanning laser beam (laser diode, 640 nm, 35 μW) was focused at the anterior chamber, and the amount of light scattering (proteins in the anterior chamber) from the beam in a window of 0.3 x 0.5 mm in the anterior chamber was detected by a photomultiplier, where it was converted into electrical signals and analyzed to determine the "flare value" in photon count per millisecond (ph/ms). For each eye, 10 consecutive flare readings with a background scatter of ≤15% were taken, while the highest and lowest measurements were deducted; the remaining 8 measurements were averaged to obtain the flare value.

Endothelial Cell Density Measurement

Endothelial cell density was evaluated with an SP3000p noncontact autofocus specular microscope (Topcon Medical Europe BV, Capelle a/d IJssel, the Netherlands). The automatically delineated cell borders of every image were carefully checked for accuracy and redefined manually if required. Three central images were analyzed and averaged per eye.

Statistics

Paired *t* tests were applied to assess differences in age between all 3 groups and in flare quantity between the 3 follow-up points (1 day, 1 week, and 1 month) of group I and in comparison with the control group. P values of less than 0.05 were considered to be statistically significant.

Multiple linear regression analyses were performed while correcting for covariates (age, sex, lens status, and diabetes) to assess differences in flare between

group II and the control group and to assess whether flare within group II was associated with any of the covariates, the follow-up time or endothelial cell density.[29] Sensitivity analysis evaluated whether eyes with (previous) rejection influenced the results.[29] All multiple tests were controlled for false discovery rate.[30] The relative importance of each predictor (effect sizes) was provided to estimate the relevance of the effect, representing a small (r^2 = 0.01), medium (r^2 = 0.09), or large effect (r^2 = 0.25).[31]

RESULTS

Group I: Flare Levels ≤1 Month After DMEK (Longitudinal Analysis)

In group I (short-term flare measurement within the first month after DMEK), the mean flare value was 25.1 (±9.1), 13.4 (±4.8), and 12.1 (±3.2) ph/ms at 1 day, 1 week, and 1 month, respectively, with a significant decrease in flare levels within the first week (P=0.003), which remained stable up to 1 month (P=0.387) (Figure 1; Table 2). Average flare at 1 month was higher than that in healthy controls (P < 0.001). None of the eyes had subjective or objective signs of allograft rejection within this early postoperative period.

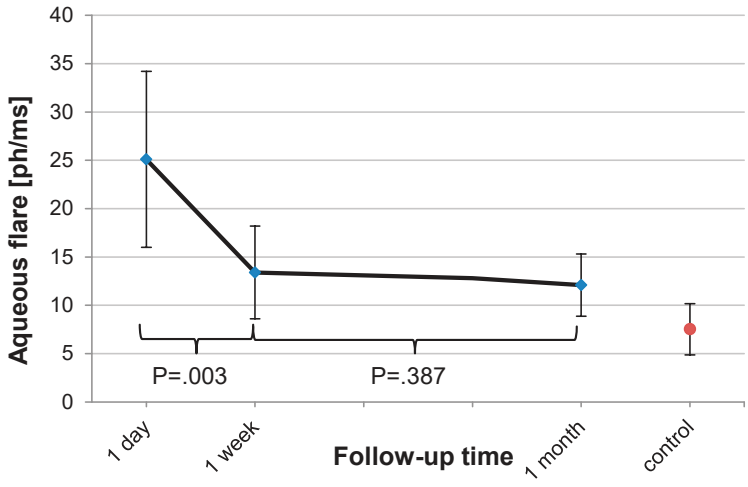

Figure 1. Short-term aqueous flare after Descemet Membrane Endothelial Keratoplasty (DMEK). The figure shows aqueous flare evolution in group I (blue diamonds) at 1 day, 1 week, and 1 month after DMEK. Vertical bars represent standard deviations. The average flare value for the control group of healthy eyes is displayed for comparison (red circle).

Table 2. Aqueous flare in eyes with previous Descemet membrane endothelial keratoplasty and in healthy control eyes

	DMEK eyes (Group I)			DMEK eyes (Group II)			Control group
				Entire Group	Subgroups		
					Eyes without allograft rejection	Eyes associated with allograft rejection	
Follow-up							
Time-point or interval	1 day	1 week	1 month	3 - 86 months	3 - 86 months	7 - 67 months	--
Mean (SD), months				28 (±19)	28 (±19)	25 (±21)	--
Median, months				24	24	19	--
No. of eyes	10	19	12	148	142	6	19
Flare (ph/ms)							
Average (±SD)	25.1 (±9.1)	13.4 (±4.8)	12.1 (3.2)^^	9.6 (±4.2)	9.3 (± 3.8)	16.7 (± 7.8)	7.5 (±2.6)
Median	29.4	12.5	13.2	8.7	8.6	14.1	7.8
Range: Min - Max	7.3 - 35.6	6.5 - 22.7	6.8 - 18.1	3.0 - 31.3	3.0 - 26.6	10.3 - 31.3	2.5 - 14.1
Estimated mean [95%CI]	--	--	--	9.6 [9.0, 10.3]^^	9.2 [8.6, 9.9]^^	16.7 [13.5, 20.0]†	7.3 [5.3, 9.3]
ECD (cells/mm^2)							
Average (±SD)	n.m.	n.m.	n.m.	1360 (±490)	1370 (±490)	1060 (±520)	n.m.
No. of measurements				137	131	6	

SD = Standard deviation; CI = Confidence interval; n.m. = not measured

^^p<0.05 compared to control group

†P<o.o5 compared with DMEK eyes without rejection

Group II: Flare Levels ≥ 3 Months After DMEK (Cross-Sectional Analysis)

In group II (longer-term flare measurements), flare levels were assessed on average 28(±19) months after DMEK (median: 24 months, range: 3–86 months) (Table 1). Except for 2 eyes (flare 7.5 ph/ms and 11.5 ph/ms), all eyes were under low-dose topical anti-inflammatory treatment (Table 1). Three eyes had a history of reversible allograft rejection 5, 13, and 63 months before flare measurement, and 2 eyes were rejection suspects on slit-lamp examination (asymptomatic suspect with suspicious endothelial deposits, flare 31.3 ph/ms, and symptomatic suspect with conjunctival injection/ subjective pain/ keratic precipitates, flare 11.4 ph/ms), and 1 eye was diagnosed with allograft rejection 6 months after flare measurement (flare 12.1 ph/ms). All 6 eyes associated with allograft rejection had flare values above 10 ph/ms, and flare values were higher [estimated mean 16.7 ph/ms, 95% confidence interval (CI) (13.5–20.0)] than eyes without an allograft rejection episode [estimated mean 9.2 ph/ms, CI (8.6–9.9)] ($P < 0.001$, $r^2 = 0.1$). None of the 6 eyes associated with rejection discontinued topical medication before the rejection episode, and none of those eyes developed secondary graft failure.

In total, 102/142 (72%) of DMEK eyes not associated with rejection had a flare value below 10 ph/ms (Table 2; Figure 2).

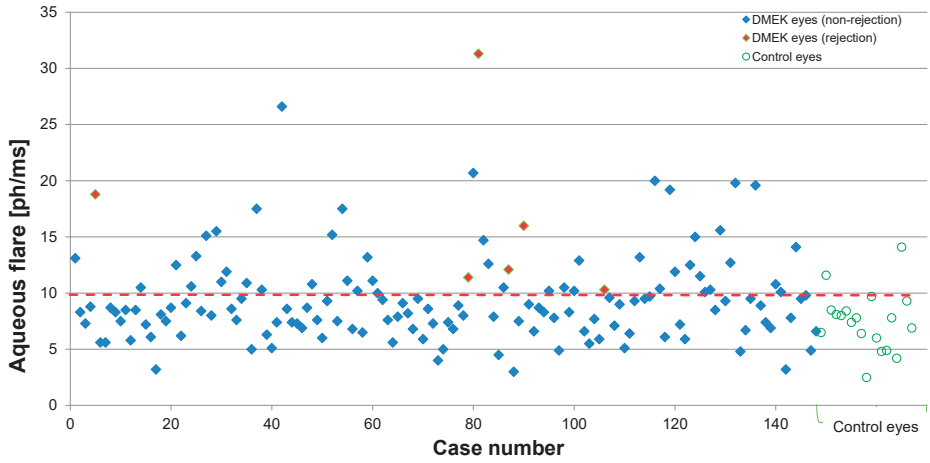

Figure 2. Aqueous flare values after Descemet Membrane Endothelial Keratoplasty (DMEK) of the cross-sectional group. The figure displays all flare values of post-DMEK eyes of group II (blue and red squares) and control eyes (green circles). Note that eyes associated with rejection (red squares) all have flare values above 10 ph/ms (red dashed line) and most post-DMEK eyes without rejection (blue squares) had flare values below 10 ph/ms.

The mean flare value was higher in group II than in healthy controls {9.6 ph/ms [95% CI (9.0–10.3)] vs. 7.3 ph/ms [CI (5.3–9.3)]}, respectively (P=0.03), estimated mean difference: -2.35 ph/ms [Ci (-4.5-0.19)], r^2 = 0.03] (Table 2). Sensitivity analysis showed that this result did not change after discarding the rejection/suspect eyes (n = 6).

Within group II, age was associated with flare (r^2 = 0.04, P = 0.009), while flare quantity was not associated with the follow-up time or endothelial cell density, sex, lens status, or presence of diabetes (P > 0.800 for all parameters).

DISCUSSION

Laser flare photometry has become a routine diagnostic tool in monitoring patients with uveitis and intraocular inflammation.[6,8,25,32,33] There have been various studies assessing aqueous flare also after penetrating keratoplasty;[13–15] however, to date, there is no study measuring flare values after endothelial keratoplasty, and in particular, after DMEK, probably because eyes after DMEK look so intriguingly "quiet" and "safe," even during a rejection episode. Hence, although inflammation may be less and a lower rejection incidence with milder forms of rejection has been reported, a clinically "invisible" immune reaction may lead to a faster endothelial cell density decrease, a main risk factor for secondary graft failure.[34,35] It may, therefore, be important to monitor the "inflammatory status" in post-DMEK eyes, especially while patients tend to be "asymptomatic."[21,22]

Interestingly, even some post-DMEK eyes that were not associated with rejection still showed on average higher flare values than virgin control eyes, although all but 2 eyes were still on a topical steroid medication (mostly low dose fluorometholone) (Table 1). This may suggest that DMEK eyes suffer from persistent inflammation, in other words, seemingly uncomplicated DMEK eyes may subclinically still be subject to long-term activation. If so, it may be hypothesized that such persistent upregulation results from a chronic immune response to the allograft or incomplete restoration of the blood-aqueous barrier after DMEK surgery. Still, the chronic immune response seems to be "controlled" by low-dose topical steroids, which is supported by the higher incidence of rejection in uncomplicated DMEK eyes after steroid discontinuation.[21] This would underline the importance of a long-term or even indefinite steroid regimen after DMEK.

Notably, the 3 post-rejection eyes showed an elevated flare level, despite the "clinically" reversed rejection episode. Hence, these eyes may have persistent breakdown in their blood-aqueous barrier because of long-term subclinical inflammation, which could explain enhanced endothelial cell density decrease and higher risk of graft failure in post-rejection eyes.

To further evaluate repair of the blood-aqueous barrier after surgery, we studied the flare curves of post-DMEK eyes within the first postoperative month. Measurements were difficult to perform on day 1 (because of residual corneal edema). At 1 week, more reliable measurements could be obtained, showing a quick decrease in flare levels stabilizing at 1 month postoperatively. However, flare levels at 1 month were on average still higher than those in non-DMEK control eyes. This observation could explain the presence of cystoid macular edema observed by some authors after DMEK or triple-DMEK that could be prevented by intensified steroid medication in the immediate postoperative phase after DMEK.[36,37] Earlier studies also reported that recovery of the blood-aqueous barrier can be related to the type of surgery, incision size, and intraoperative trauma to uveal tissues.[1–3,10–12,38,39] Although with DMEK, endothelial disease is nowadays treated in a minimally invasive manner, the presence of an allograft may still have a (persistent) effect on flare levels after DMEK.

We have recently described specular microscopy and Scheimpflug imaging as possible ophthalmic tools to identify eyes at risk of developing allograft rejection.[22,24] In the current study, we used laser flare photometry as a tool for screening post-DMEK eyes. Remarkably, all DMEK eyes associated with allograft rejection at some point (either suspects or post-rejection) showed a flare level above 10 ph/ms, whereas about 1/3 of DMEK eyes not associated with rejection showed flare values above 10 ph/ms. The 2 rejection-suspect eyes had flare levels of 11.4 and 31.3 ph/ms. Hence, flare assessment may aid in deciding on treatment (yes or no) in specific cases. Also, the eye that was 6 months later diagnosed with allograft rejection had a flare level of 12.4 ph/ms. Although it may be difficult to draw conclusions from these limited cases, if considered "at risk," eyes with flare levels above 10 ph/ms may be monitored more closely, whereas those with lower flare values approximating virgin control eyes may be managed with routine follow-up examinations (Figure 2). Further long-term studies need to evaluate the potential of laser flare photometry as an additional device or as a stand-alone diagnostic in this important field.

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CHAPTER 7

360-Degree Scheimpflug Imaging to Predict Allograft Rejection after Descemet Membrane Endothelial Keratoplasty

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ABSTRACT

Purpose: To describe the use of 360-degree Scheimpflug imaging as a diagnostic tool for detection and documentation of subtle corneal changes preceding upcoming allograft rejection after Descemet membrane endothelial keratoplasty (DMEK).

Methods: A total of 17 eyes (16 patients) were diagnosed with clinically manifest allograft rejection 2 to 42 months after DMEK. 360-degree Scheimpflug images of consecutive follow-up examinations (from 3–60 months) of "asymptomatic" eyes before, during, and after rejection were retrospectively analyzed, to determine which abnormalities could be detected before allograft rejection became clinically manifest. The images were compared with DMEK control eyes (without rejection episode).

Results: Scheimpflug images at the time of rejection showed keratic precipitates as distinct retrocorneal nodular elevations and/or a significant increase in pachymetry of ≥7%. More subtle changes could be identified retrospectively in 9/17 eyes (53%) on an average at 8 (±5) months before rejection became clinically manifest; in all eyes, these subtle changes were not recognized at routine slit-lamp examinations by various ophthalmologists as inflammatory changes heralding allograft rejection. Secondary graft failure occurred in 4/17 eyes (24%). None of the control eyes showed relevant abnormalities with Scheimpflug imaging.

Conclusions: By screening the posterior corneal surface with 360-degree Scheimpflug imaging, subtle inflammatory retrocorneal deposits can be detected and recorded during consecutive follow-up visits. Hence, Scheimpflug imaging may have the potential to become a diagnostic tool for early detection of upcoming allograft rejection in asymptomatic DMEK eyes, that is, before the immune response becomes clinically manifest and before substantial endothelial cell damage occurs.

INTRODUCTION

Allograft rejection is one of the main complications after keratoplasty, potentially leading to secondary graft failure.[1,2] With the transition from full-thickness to lamellar grafting, the incidence of rejection has been reported to decline from 10% to 15% in penetrating keratoplasty (PK), to 5%–10% in Descemet-stripping (automated) endothelial keratoplasty (DSEK/DSAEK), to 1%–2% in Descemet membrane endothelial keratoplasty (DMEK).[1,3–11]

Recently, we described that specular microscopy image analysis may allow for detection of upcoming allograft rejection after DMEK because specific changes in endothelial cell morphology may precede allograft rejection.[12] Because these endothelial cell changes may be expected to be associated with other morphologic changes of the cornea, we hypothesized that Scheimpflug imaging and pachymetry could potentially reveal additional corneal changes that herald allograft rejection after DMEK.

Hence, the aim of the study was to assess corneal changes in 360-degree Scheimpflug images and pachymetry readings of consecutive follow-up visits taken from asymptomatic DMEK eyes that later developed a clinically manifest, that is, proven, allograft rejection and to compare the findings with those of DMEK control eyes (without a later rejection episode), to define additional screening parameters next to endothelial cell morphology changes.

MATERIALS AND METHODS

From a series of 750 consecutive DMEK eyes (570 patients operated on between 2006 and 2014, with a mean follow-up time of 51 [±23] months [range: 12–111 months]), 17 eyes of 16 patients (mean age 65 [±14] yrs) that developed a clinically manifest allograft reaction were enrolled in our retrospective study. These eyes underwent surgery for Fuchs endothelial corneal dystrophy (n = 12), pseudophakic bullous keratopathy (n = 1), bullous keratopathy for phakic intraocular lens removal (n = 2) and in the presence of a glaucoma drainage device (n = 1), or for failed DSEK (n = 1) and developed clinically manifest allograft rejection 2 to 42 months after surgery (mean follow-up after DMEK 35 (±15) months, range 9–60 months) (Table 1). None of these eyes had a history of infectious or noninfectious uveitis before DMEK and/or systemic immune disease.

Table 1. Demographics of rejection and control eyes after Descemet Membrane Endothelial Keratoplasty

	Study eyes (allograft rejection)	Control eyes (no allograft rejection)
Eyes / Patients (n)*	17/16	34/34
Mean age (years) ± SD (range)	65±14 (31-80)	66±12 (38-83)
Gender (male/female)	10/6	20/14
Pseudophakic / phakic	12/5	24/10
Preoperative diagnosis		
Fuchs endothelial corneal dystrophy	12	27
Bullous keratopathy	4**	5†
Re-graft (Failed DSEK)	1	2
Mean donor age (years) ± SD (range)*	63±10 (48-81)	63±7 (48-74)
Mean follow-up time (months) ± SD, (range)	35±15 (9-60)	47±12 (18-60)

* The study group and control group did not differ significantly ($P > .05$).
**Bullous keratopathy in the presence of a glaucoma tube (n=1), after phakic intraocular lens removal (n=2) and pseudophakia (n=1).
†Bullous keratopathy for radial keratotomy/laser in situ keratomileusis (n=1), for pseudophakia with an anterior (n=1) or posterior chamber lens (n=1) and for phakic intraocular lens removal (n=2).
SD = Standard deviation; DSEK = Descemet stripping endothelial keratoplasty.

From the same cohort, 34 asymptomatic eyes of 34 patients (matched for age, sex, lens status, and surgical indication) with a mean age of 66 (±12) years served as a control group; none of these had a history of allograft rejection or uveitis (Table 1).

The study was approved by the institutional review board of the Netherlands Institute for Innovative Ocular Surgery. All patients signed institutional review board–approved informed consent for research participation. The study adhered to the Declaration of Helsinki.

Rejection Episode

Graft rejection was defined as an event at which objective clinical findings were observed on slit-lamp examination (with or without subjective complaints). These included an endothelial rejection line, keratic precipitates with or without an increase in corneal thickness, anterior uveitis, and/or ciliary injection.

Donor Tissue

The procedure for harvesting a DMEK graft has previously been described.[13,14] In short, corneo-scleral buttons were excised from donor globes ≤36 hours post-mortem and stored in organ culture medium (CorneaMax; Eurobio, Courtaboeuf,

France) at 31°C. Endothelial cell morphology and viability were evaluated, and corneo-scleral buttons were mounted endothelial side up on a custom-made holder. A 9- to 10-mm diameter Descemet sheet with its endothelium was removed from the posterior stroma with the corneo-scleral rim immersed in balanced salt solution. Owing to elastic tissue properties, a "Descemet roll" formed spontaneously, with the endothelium on the outer side. Average donor age was 63 (±10) years for the study group and 63 (±7) years for the control group (Table 1).

Surgery

The DMEK surgical procedure has previously been described.[15] In short, a circular 9.0-mm diameter "descemetorhexis" was performed with complete air fill of the recipient's anterior chamber, by scoring and stripping off Descemet membrane from the posterior stroma with a reversed Sinskey hook (DORC International, Zuidland, the Netherlands). In the eye that underwent DMEK as a secondary procedure, the primary DSEK graft was carefully removed from the recipient's posterior stroma using a reversed Sinskey hook in an anterior chamber filled with air.

The donor Descemet roll was then stained (0.06% Trypan blue solution, VisionBlue, DORC International), sucked into a custom-made injector (Melles DMEK injector, DORC International) and injected through a 3.0-mm limbal tunnel incision into the recipient's anterior chamber. The graft was oriented with the endothelial side facing the recipient's iris, and Descemet membrane facing recipient's posterior stroma. After complete graft unfolding over the iris, a large air bubble was injected underneath the graft to position it onto the recipient's posterior stroma. The anterior chamber was then completely filled with air for 60 minutes followed by an air–liquid exchange leaving a 30%–50% air bubble in the anterior chamber.

At the end of surgery, subconjunctival dexamethasone and gentamicin were injected. Postoperative medication included antibiotic eye drops for 2 weeks, and a steroid regimen of dexamethasone 0.1% drops, 4 times daily for 4 weeks, followed by fluorometholone drops, 4 times daily tapered to once daily at 1 year postoperatively, and once daily or once every other day thereafter.[16]

Measurements

All study and control eyes were routinely examined before surgery, and postoperatively at 1, 3, 6, 9, and 12 months, and at 6-month intervals thereafter. Sequential images of pachymetry and high-resolution rotating Scheimpflug imaging (Pentacam; Oculus, Wetzlar, Germany) and slit-lamp photography (Topcon Medi-

cal Europe BV, Capelle a/d IJssel, the Netherlands) were evaluated retrospectively between 3 and 60 months postoperatively, in an attempt to detect corneal changes previously unrecognized.

For each measurement, the Scheimpflug camera generates 25 images over 360 degrees to produce a 3-dimensional image. For each study and control eye, all 25 Scheimpflug scans taken along the same meridian were compared between consecutive follow-up points to evaluate changes in the corneal posterior surface by 2 masked observers.

Changes in central corneal thickness were evaluated using differential pachymetry maps generated by Pentacam software. For all 17 eyes, Scheimpflug images and central corneal thickness before and after rejection were available, and in 11 eyes, reliable Scheimpflug images were also available at the time of rejection.

Statistical Analysis

An unpaired t test was performed for comparison of the study and control groups. $P < 0.05$ was considered statistically significant.

RESULTS

From a total of 750 DMEK eyes, 17 eyes developed allograft rejection on an average of 18 (±13) months (range: 2–42 months, median: 18 months) after surgery. One patient had allograft rejection in both eyes.

Scheimpflug Images and Pachymetry Findings Before Allograft Rejection Became Clinically Manifest

In retrospect, corneal abnormalities (retrocorneal spots varying in size and density and/or an increase in central pachymetry) could be identified on Scheimpflug images before clinical manifestation of the allograft rejection in 9/17 eyes (53%) (Figures 1, 2). In all 9 eyes, retrocorneal spots were observed, and 2 eyes also had a >7% increase in pachymetry. In these 9 eyes, allograft rejection was diagnosed at 22 (±9) months (median: 24 months) after DMEK, although early changes could be detected retrospectively at 8 (±5) months before this time point, at which the eyes were consistently described asymptomatic in slit-lamp examination reports (Figure 2).

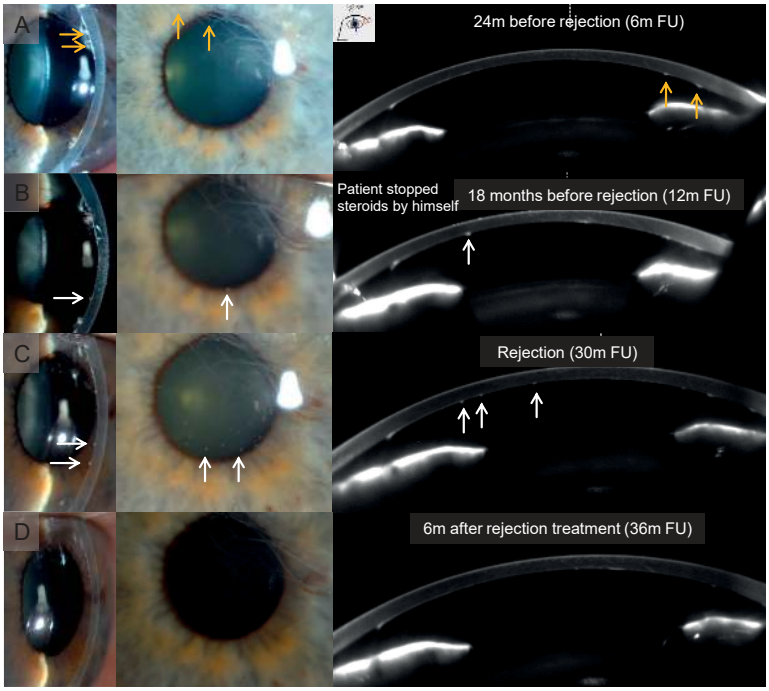

Figure 1. Slit lamp and Scheimpflug images of an eye after Descemet Membrane Endothelial Keratoplasty (DMEK). Images at (A) 6 months, (B) 12 months, (C) 30 months, and (D) 36 months follow-up (FU) after DMEK. Clinical allograft rejection manifested at 30 months postoperatively. In retrospect, keratic precipitates (white arrows) could already be detected 18 months before rejection (B and C) but were not regarded as abnormal during sequential follow-up visits because the eye had a good visual acuity and was completely quiet. Orange arrows outline wrinkles in the graft after partial graft detachment in that area. Six months after treatment (36 months after DMEK), the corneal changes have disappeared (D).

Scheimpflug Images and Pachymetry Findings at the Time of Clinically Manifest Allograft Rejection

When allograft rejection was clinically diagnosed, of the 16 patients (17 eyes), 12 reported either typical subjective complaints (e.g., ocular pain and redness, fluctuating or decreased visual acuity, n = 10) or nonspecific mild ocular discomfort (n = 2), and 4 had no subjective complaints. Objective clinical findings included corneal edema (n = 10) with various degrees of keratic precipitates (n = 17). Except for 2 patients who had discontinued topical steroids on their own initiative, 14 patients still were on a medication regimen of fluorometholone, once every other day and up to 4 times daily, according to the postoperative standard protocol after DMEK.

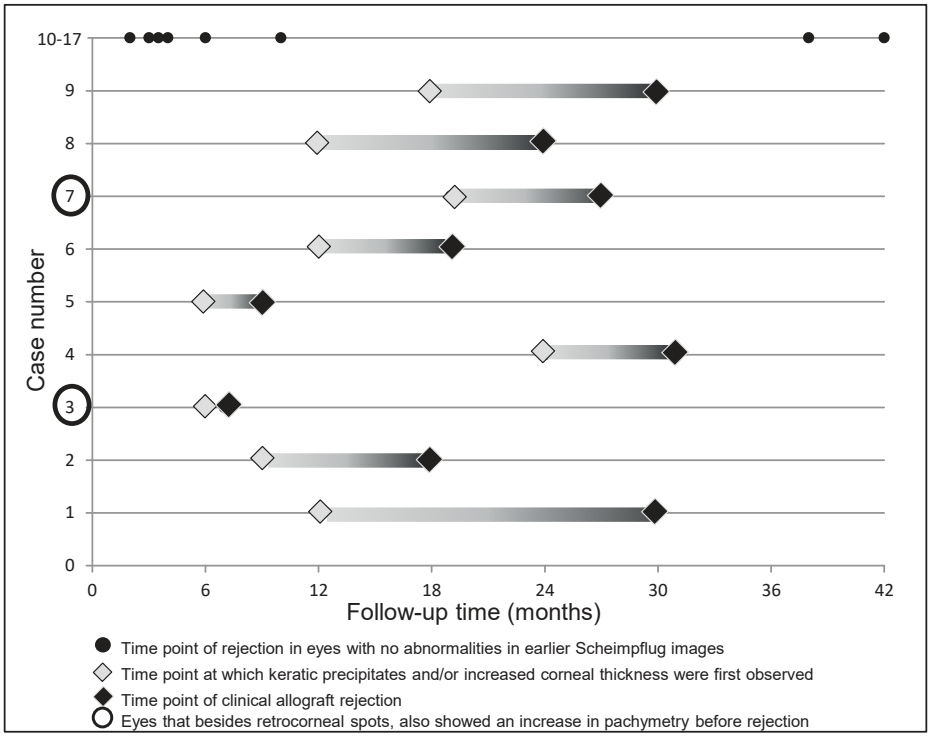

Figure 2. Graph displaying the time points of rejection after Descemet Membrane Endothelial Keratoplasty and the time points of earlier changes as retrospectively detected with Scheimpflug imaging. Time point of rejection (black diamonds) is displayed along with the earlier time points at which in retrospect corneal changes could be found in 9 eyes with Scheimpflug imaging (gray diamonds). Time points of rejection in eyes with no abnormalities in earlier Scheimpflug images (black points) are displayed in the upper line.

At the time of allograft rejection, in 11/17 eyes, Scheimpflug images were made. In 5 of these 11 eyes, the pachymetry differential map showed increased central pachymetry by >7% compared with the previous follow-up point. Furthermore, in 10 of these 11 eyes, retrocorneal (hyper) reflective elevated spots were seen across the endothelium. These spots corresponded to keratic precipitates seen on slit-lamp examination (Figure 1). After intensified topical steroid treatment, pachymetry returned to normal and/or the keratic precipitates disappeared (Figure 1). Four eyes (24%) improved, only for a short time, and later developed graft failure. All eyes had normal intraocular pressure at the time of rejection.

Control Eyes

Throughout the study period, none of the 34 DMEK control eyes matched for patient and donor age (P = 0.86 and P = 0.94, respectively) and baseline charac-

teristics (indication, sex, and lens status) showed any subjective or objective signs indicative of allograft rejection. The average increase in central pachymetry for control eyes ranged from 0.0% to 6.6%, that is, it did not exceed 7% between consecutive postoperative follow-up intervals from 3 to 60 months, and no keratic precipitates were observed.

DISCUSSION

Allograft rejection after DMEK has been reported to occur in 1% to 2% of cases,[10,11] as compared with 5% to 15% in DSEK/DSAEK and PK.[1,3–9] Recently, we reported that endothelial cell changes on specular microscopy may proceed to allograft rejection.[12] To further validate these findings, we commenced the current retrospective study to determine whether changes on Scheimpflug imaging had predictive value for development of allograft rejection.

This study shows that 3 to 6 monthly screening of post-DMEK eyes by Scheimpflug imaging may allow early detection of subtle corneal changes that herald upcoming allograft rejection. In a series of 17 DMEK eyes that developed allograft rejection, more than half of them retrospectively showed corneal changes without subjective symptoms 8 (±5) months before rejection became clinically evident. With Scheimpflug imaging, (hyper)reflective retrocorneal spots corresponding to keratic precipitates varying over time in size and number and/or significant increase in central pachymetry could be found in 9/17 eyes (53%) compared with the previous follow-up examinations. All of these eyes were examined by various ophthalmologists and before allograft rejection became clinically manifest, the relatively subtle changes were not recognized as being abnormal, and no targeted treatment was given.

Postkeratoplasty allograft rejection is typically diagnosed when the patient expresses subjective complaints such as ocular discomfort and/or a drop in visual acuity, which correlate with anterior uveitis and a “red eye.” Compared with PK, “milder” forms of rejection have been described for DSEK/DSAEK and DMEK with patients often lacking subjective complaints as described in about 30% and 80% in DSEK/DSAEK and DMEK, respectively.[6,8,17,18] In our study, about 25% (4/17) of eyes had no subjective complaints, whereas objective clinical signs could be seen on slit-lamp examination.

Our findings concerning the changes in the Scheimpflug imaging before rejection may be surprising for 2 reasons. First, our study may show that more than half of the eyes that were at risk of developing allograft rejection could have been recognized much earlier with Scheimpflug imaging and pachymetry in a "prodromal phase," a phase in which the still (subjectively) asymptomatic eye heralded allograft rejection, months before an immune response became clinically manifest. Second, from the first follow-up point with corneal changes, it took an average of 8 months until rejection manifested. However, rejection is known to be a T-cell–mediated immune response, but the timeline may not agree with the typical time lag (<1 month) for such a reaction to develop full-blown allograft rejection.[19,20]

Early detection of upcoming allograft rejection in eyes that are still asymptomatic may be important to potentially avoid irreversible damage to the graft, because the early keratic precipitates and increased central pachymetry values may be accompanied by significant changes in endothelial cell morphology and a progressive decrease in endothelial cell density.[12,21] Early recognition of these abnormalities could potentially allow for much more effective intervention. That is, in contrast to high-dose steroids after allograft rejection becomes clinically manifest, treatment may now be started long before the damage is done to the endothelium by a massive immune response (Figure 3).

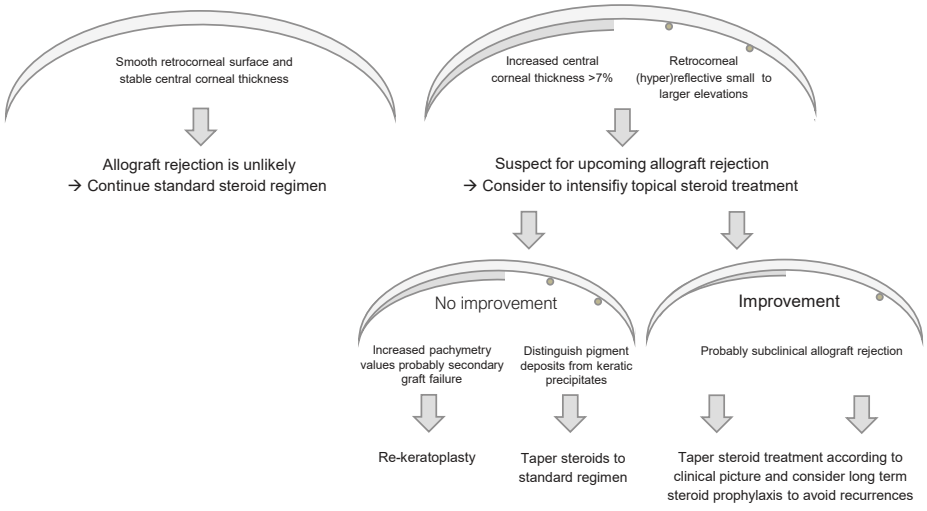

Figure 3. Decision tree for corneal changes (increase in central pachymetry and/or keratic precipitates) preceding allograft rejection, as visualized with Scheimpflug imaging.

There may be 2 reasons why these signs have not been recognized before on slit-lamp examination. First, most eyes that eventually developed full-blown rejection appeared intriguingly "normal" with stable visual acuity, a "white" conjunctiva, a "quiet" anterior chamber, normal pachymetry, and a lack of subjective complaints. Second, against this background, inflammatory deposits were generally overlooked or considered harmless, or misinterpreted as retrocorneal pigment. However, with increasing experience, pigmentary depositions may be better distinguished from keratic precipitates by their finer and crisper appearance, their more abundant location at graft edges, and the fact that they do not change in sequential Scheimpflug images, whereas keratic precipitates would typically increase in number over time, change in size and location, and are often also found centrally. Because the increase in central pachymetry did not exceed 7% in the control eyes, another alarm sign could be a >7% increase in central pachymetry in sequential differential maps.

Furthermore, the time point of the early changes may be of interest, that is, in 9 of the 17 eyes, the immune response may have started at 13 (±6) months (median: 12 months) after surgery, whereas allograft rejection became clinically manifest at 22 (±9) months. This would agree with the observation that most rejections may occur within the first 24 months after keratoplasty.[4,8,22] Thus, Scheimpflug screening may be especially useful within this time frame.

A limitation of our study may be that the predictive value of Scheimpflug imaging in the detection of upcoming allograft rejection would require at least 2 images with a sufficient time interval in between. In our study, 3 eyes showed rejection within the first 3 months, which may not have been detected because the 1-month image did not show a detectable change.

In conclusion, 360-degree Scheimpflug imaging may aid in detecting and documenting minute inflammatory retrocorneal deposits, which may be indicative of upcoming allograft rejection. The possibility to recognize upcoming allograft rejection may benefit treatment in these eyes because it would allow for earlier treatment with steroids to avoid a clinically manifest immune response with substantial endothelial damage. Scheimpflug imaging may have the potential for early detection of eyes that may be at risk to develop allograft rejection. This diagnostic tool could complement specular microscopy for the evaluation of associated corneal endothelial changes.

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CHAPTER 8

Combined Specular Microscopy and Scheimpflug Imaging to Improve Detection of an Upcoming Allograft Rejection after DMEK

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ABSTRACT

Purpose: To assess whether combined analysis of specular microscopy and Scheimpflug imaging improves detection of an upcoming allograft rejection following Descemet membrane endothelial keratoplasty (DMEK).

Methods: Retrospective analysis of 22 eyes that had developed a clinical proven allograft rejection 28 (±22) months (range: 4–84 months) after DMEK. Specular microscopy and Scheimpflug images routinely made after DMEK were retrospectively analysed for changes in endothelial cell morphology (e.g., nuclear activation), cell density (>10%) and pachymetry (>7%), and/or the presence of subclinical keratic precipitates. The same parameters were evaluated for 22 control eyes matched for age, gender, and surgery indication.

Results: A total of 20/22 eyes (91%) showed detectable changes 0.25–75 months before allograft rejection became clinically manifest: 13/22 (59%) showed both specular microscopy and Scheimpflug imaging changes; 5/22 (23%) only had changes on Scheimpflug imaging; and 2/22 (9%) only had specular microscopy changes. In 18/22 (82%) and 14/22 (64%) eyes, subclinical keratic precipitates and endothelial cell morphology changes could be detected, respectively. A total of 11/22 (50%) eyes concurrently showed a >10% drop in endothelial cell density and 4/22 (18%) a >7% pachymetry increase. Of the control eyes, 7/22 (32%) showed changes with specular microscopy but not with Scheimpflug imaging.

Conclusions: Combined analysis of specular microscopy and Scheimpflug imaging may allow recognizing an upcoming allograft rejection in over 90% of eyes and up to 6 years before rejection becomes clinically manifest. Early recognition of eyes at risk may allow for targeted intensified steroid treatment to prevent endothelial cell damage associated with rejection.

INTRODUCTION

Descemet membrane endothelial keratoplasty (DMEK), the selective replacement of the diseased endothelial cell layer, offers several advantages, including relatively quick and near complete visual recovery (Rodríguez-Calvo-de-Mora et al. 2015; Oellerich et al. 2017; Price et al. 2017; Deng et al. 2018). DMEK may also be associated with a lower risk of allograft rejection of 1–2%, compared to that in penetrating keratoplasty (10–15%) and Descemet stripping (automated) endothelial keratoplasty (DSEK/DSAEK) (5–10%) (Thompson et al. 2003; Allan et al. 2007; Claesson & Armitage 2009; Jordan et al. 2009; Lee et al. 2009; Dapena et al. 2011a; Anshu et al. 2012; Li et al. 2012; Wu et al. 2012).

Still allograft rejection is one of the more severe complications that may result in endothelial cell damage and secondary graft failure (Anshu et al. 2012; Williams et al. 2012). Hence, to be able to recognize and treat early signs of an immune reaction to possibly prevent manifestation of rejection would be desirable. We have recently shown that specific changes in endothelial cell morphology with specular microscopy may precede rejection (Monnereau et al. 2014). Likewise, Scheimpflug imaging may allow for detection of subtle immune reactions (keratic precipitates not seen on biomicroscopy) and pachymetric deviations may be expected to occur before rejection becomes clinically manifest (Baydoun et al. 2016). Therefore, to increase the power of our method to recognize upcoming allograft rejections, the various measurements and displaying techniques may be combined to work towards an improved algorithm for early allograft detection in post-DMEK eyes.

The aim of the current study was to correlate the findings of specular microscopy and Scheimpflug imaging in order to assess whether combining these diagnostic devices has added value in detecting eyes at risk of rejection after DMEK.

MATERIALS AND METHODS

From a series of 1077 consecutive DMEK eyes of 741 patients with a mean follow-up time of 64 (±33) months (range: 3–139 months), 27 eyes (2.5%) of 26 patients developed a clinical proven allograft rejection 2–84 months (median 19 months) after surgery. Five eyes were excluded from analysis owing to poor image quality (two eyes with congenital glaucoma) or the occurrence of a rejection ≤3 months after DMEK (so that no baseline images were available) (Table 1). Hence, 22 eyes

Table 1. Demographics of rejection and control eyes after Descemet membrane endothelial keratoplasty

	Study group rejection eyes	Control group non-rejection eyes
Eyes / Patients (n)	22/21	22/22
Mean age (years) ± SD (range)	66 ± 13 (30-80)	67± 11 (44-79)
Average time-point of rejection (months), (median, range)	28 ± 22 (25, 4-84)	--
Gender (male/female)	12/9	13/9
Pseudophakic/phakic	17/5	18/4
Preoperative diagnosis		
FECD	17	18
Pseudophakic BK	--	1
BK		
(removed) phakic IOL	1	--
In the presence of a glaucoma tube	2	--
Pseudophakic eye with radial keratotomy, extra incisions, LASIK	--	1
Failed DSEK (presence of glaucoma tube and AC-IOL)	2 (1)	2 (0)
Mean donor age (years) ± SD (range)	66 ± 11 (48-84)	67 ± 11 (48-85)
Excluded eyes (5 out of 27 rejection cases)		
Eyes with rejection ≤ 3 months after DMEK (FED, PPBK, removed phakic IOL)	3	--
Eyes with poor image quality (congenital glaucoma)	2	0

AC-IOL: Anterior chamber intraocular lens
DSEK: Descemet stripping endothelial keratoplasty
FECD: Fuchs endothelial corneal dystrophy
PPBK: Pseudophakic bullous keratopathy
SD: Standard deviation

of 21 patients (9 female/12 male; 17 pseudophakic/5 phakic; aged 66 (±13) years) were included in our retrospective study (Table 1). DMEK was performed for Fuchs endothelial corneal dystrophy (n = 17), bullous keratopathy owing to phakic intraocular lens removal (n = 1) or a glaucoma drainage device (n = 2), and for failed DSEK (n = 2) (Table 1). All included rejection eyes have been operated between January 2008 and January 2016. In addition, 22 eyes matched for age, gender, and surgery indication without a rejection episode after DMEK were included as normal controls and were evaluated until the last available follow-up point (Table 1). None of the rejection or control eyes that received DMEK after failed DSAEK had a rejection episode in the earlier DSAEK graft.

All patients had signed an IRB-approved informed consent for research participation prior to surgery. The study adhered to the Declaration of Helsinki.

Rejection episode

Graft rejection was defined as an event with objective clinical findings (rejection line, keratic precipitates with or without an increase in corneal thickness, anterior uveitis and/or ciliary injection) on slit-lamp examination, with or without subjective complaints.

Allograft rejection after DMEK was treated with topical steroids while the frequency of the steroid application (four times per day to hourly) depended on the clinical appearance of rejection (mild, only keratic precipitates/severe, corneal decompensation). Four eyes of the study group developed endothelial graft failure after allograft rejection and needed regrafting.

Donor tissue processing and DMEK surgery

Descemet membrane endothelial keratoplasty (DMEK) grafts were prepared as described previously and then stored in organ culture medium until the time of transplantation (Lie et al. 2008; Groeneveld-van Beek et al. 2013). Average donor age for the study and control group was 66 (±11) and 67 (±11) years, respectively (Table 1).

'No-touch' DMEK surgery was performed in all cases in a standardized manner as described in detail earlier (Dapena et al. 2011b). In eyes that underwent DMEK as a secondary procedure, the primary DSEK graft was removed from the recipient posterior stroma using a reversed Sinskey hook in an anterior chamber filled with air.

At the end of surgery, subconjunctival dexamethasone and gentamicin were injected. Postoperative treatment consisted of topical antibiotics for two weeks, and a steroid regime of dexamethasone 0.1% four times daily for four weeks, followed by fluorometholone 0.1% four times daily, tapered to once daily at 1 year postoperatively and once daily or once every other day thereafter (Dapena et al. 2011b).

Measurements and analysis

All eyes were evaluated before surgery, and postoperatively at 3, 6, 9 and 12 months and at 6-month intervals thereafter, with specular microscopy to moni-

tor endothelial cell morphology and density (Topcon SP3000, Topcon Europe Medical, The Netherlands).

Central endothelial cell morphology was graded by three masked observers (LB, MB and SO) on a scale from 1 to 5 as previously described (Figure 1, top row) (Monnereau et al. 2014): (1) 'quiet' endothelial cell layer with a regular cell morphology and distribution, that is a cell layer of inactivated cells with no nuclei visible and a hexagonal cell pattern without exhibiting polymorphism and polymegathism; (2)

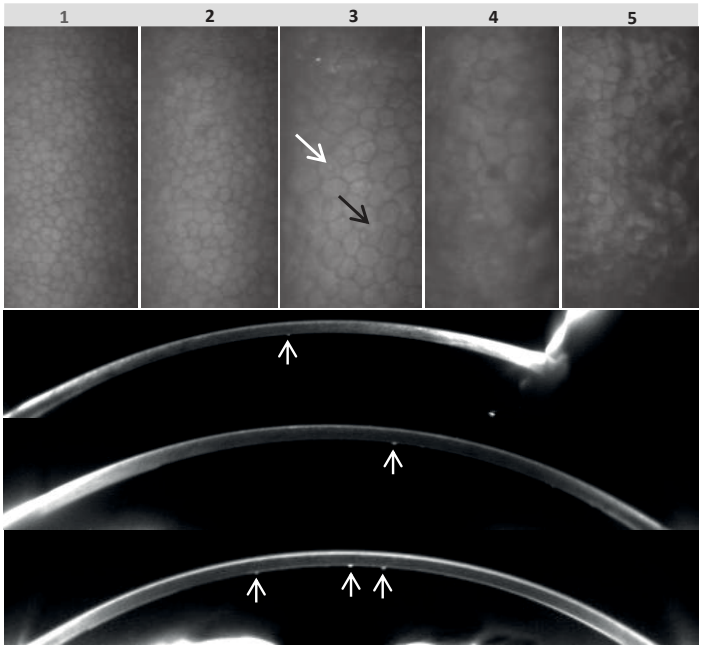

Figure 1. Examples of specular microscopy and Scheimpflug images in eyes following rejection after Descemet Membrane Endothelial Keratoplasty (DMEK). Specular microscopy images (top row) and Scheimpflug images (bottom rows) displaying the sequential stages used to subjectively score endothelial cell morphology on a scale from 1 to 5 (top row, left to right): quiet endothelial cell layer with a regular cell morphology and distribution, without any sign of cellular activation (1); slightly irregular endothelial cell morphology and/or distribution, without any sign of cellular activation (2); mild to moderate irregular endothelial cell morphology and/or distribution, with mild to moderate appearance of cellular activation. Note the increased cellular reflectivity (black arrow) with detectable cell nuclei (white arrow) (3); severe irregular endothelial cell morphology and/or distribution, and clear presence of cellular activation with enlarged cell nuclei (4); extreme irregular endothelial cell morphology and/or distribution, and presence of highly activated cells (5). (bottom rows, top to bottom) Scheimpflug images of three DMEK eyes showing different densities of keratic precipitates (arrows) from localized small keratic precipitates one month before rejection to more prominent keratic precipitates 5.5 years before rejection and to multiple keratic precipitates 12 months, respectively, before rejection was diagnosed.

slightly irregular endothelial cell morphology and/or distribution, but without any sign of cellular activation, that is visibility of cellular nuclei and/or increased cellular reflectivity; (3) mild to moderate irregular endothelial cell morphology and/or distribution, and mild to moderate appearance of cellular activation; (4) severe irregular endothelial cell morphology and/or distribution, and clear presence of cellular activation with enlarged cell nuclei; (5) extreme irregular endothelial cell morphology and/or distribution, and presence of highly activated cells (Figure 1, top row). An average score of >2.5 was considered significant at any of the follow-up points (Monnereau et al. 2014).

For endothelial cell density analysis, the automatically delineated cell borders were manually corrected. Three central images of each eye were analysed per follow-up point, and the results were averaged. A drop in endothelial cell density of >10% between follow-up points (≤6 months) was considered significant.

At corresponding follow-up points, Scheimpflug images (Pentacam, Oculus, Wetzlar Germany) were evaluated by the same observers for keratic precipitates, seen as hyperreflective retrocorneal deposits varying in size and density as previously described (Figure 1, bottom rows) (Baydoun et al. 2016). For each measurement, the Scheimpflug camera generates 25 images over 360° to produce the three-dimensional image; for each eye, all 25 scans taken were analysed up to the time-point before rejection. Scheimpflug-based central corneal thickness was measured and the differences between consecutive visits calculated. A pachymetry change of >7% between follow-up points (≤6 months) was considered a significant change (Baydoun et al. 2016).

RESULTS

From the 22 eyes in our study with an average rejection time-point of 28 (±22) months (median: 25 months, range: 4–84 months) after DMEK, 20 eyes (91%) retrospectively showed detectable changes on specular microscopy (endothelial cell morphology change and/or endothelial cell density decrease) and/or with Scheimpflug imaging (subclinical keratic precipitates and/or pachymetry increase) up to 75 months (median: 10 months, range: 0.25–75 months) before allograft rejection became clinically manifest (Figure 2; Table 2) and only two eyes showed no alterations throughout the entire studied period (Figure 2, Table 2). In total, 13 out of the 22 eyes (59%) showed concomitant alterations with specular microscopy and Scheimpflug imaging (Figure 2, Table 2). In five eyes

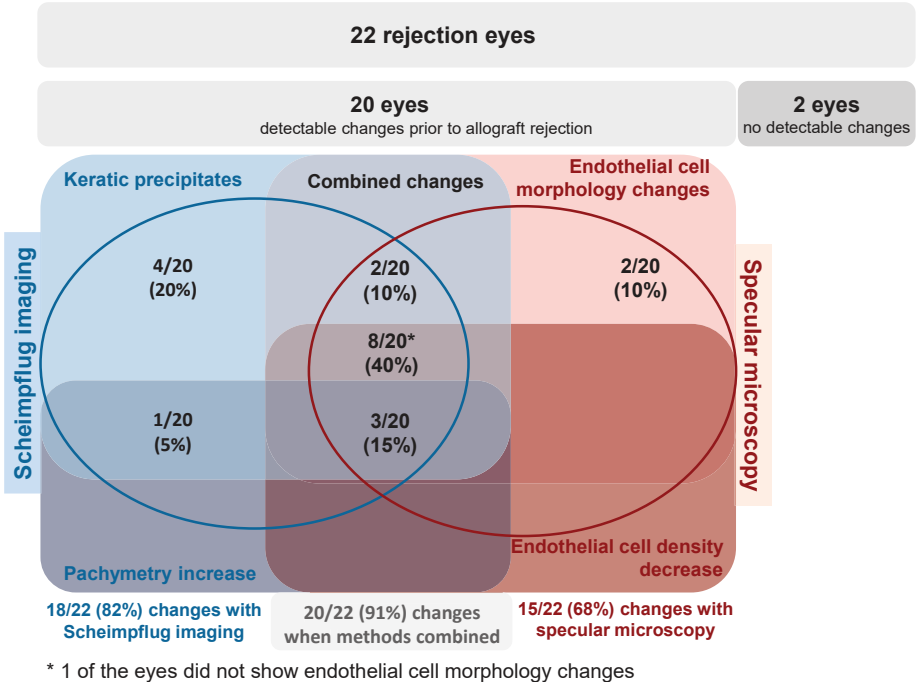

Figure 2. Diagram illustrating all study eyes and the distribution of subclinical changes recognized in those eyes prior to clinical manifestation of allograft rejection after Descemet Membrane Endothelial Keratoplasty: with Scheimpflug imaging (subclinical keratic precipitates and increase in pachymetry) and specular microscopy (changes in endothelial cell morphology and decrease in cell density). Asterisk indicates that one of those eyes did not show endothelial cell morphology changes.

(23%), changes were only seen with Scheimpflug imaging (keratic precipitates n = 4, pachymetry increase n = 1) and in two eyes (9%) only on specular microscopy (endothelial cell morphology changes n = 2) (Figure 2, Table 2). Changes with specular microscopy were observed in 15/22 eyes (68%) (Figure 2, Table 2): 14/22 eyes (64%) had endothelial cell morphology changes 1–50 months before rejection (on average 17 (±13) months, median 17 months, average scores between 2.6 and 5) and 11/22 eyes (50%) had at least once a >10% (range 11–44%) endothelial cell density decrease between the follow-up points 1–26 months before rejection (on average 13 (±8) months, median 13 months). Both parameters concurred in 10 eyes (Table 2).

Changes with Scheimpflug imaging were observed in 18/22 eyes (82%) (Figure 2, Table 2): All 18/22 eyes (82%) showed keratic precipitates 0.25–75 months before rejection (on average 18 (±20) months, median 11 months), and four of these eyes

(4/22, 18%) also had a pachymetry increase of >7% 1–23 months before rejection (on average 13 (±11) months, median 14 months).

The combination of keratic precipitates and endothelial cell morphology changes concurred in 12 eyes of which 10 eyes also showed endothelial cell decrease and three eyes a pachymetry increase (Figure 2, Table 2). Hence, in three eyes all four parameters concurred.

During the follow-up period of up to 8.5 years, 7/22 control eyes (32%) retrospectively showed detectable changes with specular microscopy but not with Scheimpflug imaging. Six eyes either had endothelial cell morphology changes (n = 2, average scores between 2.6 and 2.9) or a high endothelial cell density decrease (n = 4) while in one eye both changes concurred. In the latter eye, a continuous endothelial cell density decay was noted postoperatively from 3 years onwards which resulted in endothelial graft failure 7 years after DMEK; intraocular inflammation was absent at all follow-up time-points (Table 2).

DISCUSSION

We have recently shown that specular microscopy and Scheimpflug imaging may be useful diagnostics in the recognition of eyes that may be at risk of allograft rejection following DMEK. Our current study showed that prerejection changes in corneas that underwent DMEK could be detected in 68% of the eyes with specular microscopy and in 82% of the eyes with Scheimpflug imaging. When both these methods were combined, about 90% of the eyes could be recognized of being at risk of developing allograft rejection. If so, a proper algorithm weighing the combined assessment and adequate topical steroid regimens might potentially reduce the incidence of long-term post-DMEK rejections from 1–2.5% to ≤0.25% (Dapena et al. 2011a; Anshu et al. 2012; Monnereau et al. 2014; Baydoun et al. 2016; Hos et al. 2017).

Interestingly, normal control eyes also showed alterations in 32% of the cases. However, these alterations were limited to specular microscopy changes which were in general less pronounced (lower average scores) than the alterations seen in the rejection eyes. In addition, the changes did not continuously deteriorate in time as could be observed in some rejection eyes. One exception was the control eye that developed secondary graft failure (absent of rejection), a condi-

Table 2. Overview of changes in rejection eyes and matched control eyes

Rejection Group					Control Group			
Rejection Case # (Gender, Age)	**Surgery indication**	**Time-point of rejection**	**FU (m) with first changes**	**Overview of changes given in chronological order (first changes (alone or concurrent) = underlined)**	**Matched Control Case # (Gender, Age)**	**Surgery indication**	**FU (m) with first changes**	**Overview of changes given in chronological order**
1 (M,44)	FECD	30	12	ECD decrease, KPs, EC changes	1 (M,47)	FECD	48	ECD decrease, EC changes (endothelial failure)
2 (F,76)	FECD	4	3	EC changes	2 (F,77)	FECD	3	EC changes
3 (M,67)	FECD	84	9	KPs	3 (M,68)	FECD	--	--
4 (M,61)^	FECD	42	18	KPs, EC changes, EC decrease	4 (M,59)	FECD	--	--
5 (M,62)^	FECD	10	3	EC changes	5 (M,63)	FECD	--	--
6 (F,30)	BK*	18	9	KPs	6 (F,48)	FECD	36	ECD decrease
7 (F,57)	FECD	80	24	KPs, EC changes, ECD decrease	7 (F,58)	FECD	--	--
8 (F,76)	FECD	31	24	ECD decrease, KPs	8 (F,79)	FECD	--	--
9 (F,77)	FECD	58	48	KPs	9 (F,77)	FECD	--	--
10 (M,62)	FECD	38	18	KPs, EC changes	10 (M,65)	FECD	--	--
11 (F,78)	FECD	27	3	KPs, EC changes, ECD decrease, Pachy increase	11 (F,79)	FECD	36	ECD decrease
12 (M,77)	Failed DSEK	7	3	KPs, EC changes, ECD decrease, Pachy increase	12 (M,77)	Failed DSEK	--	--
13 (M,40)	FECD	19	13	KPs, EC changes, ECD decrease	13 (M,44)	FECD	--	--
14 (M,71)	FECD	30	9	Pachy increase, KPs	14 (M,72)	FECD	6	EC changes
15 (M,72)	FECD	24	6	ECD decrease, KPs, EC changes	15 (M,73)	FECD	12	ECD decrease
16 (M,78)	FECD	6	5.75	KPs	16 (M,75)	FECD	24	ECD decrease
17 (M,63)	BK†	9	3	EC changes, ECD decrease, KPs	17 (M,62)	BK%	--	--
18 (M,61)	FECD	26	16	KPs, EC changes	18 (M,63)	FECD	--	--

Table 2. Overview of changes in rejection eyes and matched control eyes *(continued)*

Rejection Group					Control Group			
Rejection Case # (Gender, Age)	**Surgery indication**	**Time-point of rejection**	**FU (m) with first changes**	**Overview of changes given in chronological order (first changes (alone or concurrent) = underlined)**	**Matched Control Case # (Gender, Age)**	**Surgery indication**	**FU (m) with first changes**	**Overview of changes given in chronological order**
19 (F,72)	Failed DSEK#	32	9	EC changes, Pachy increase, ECD decrease, KPs	19 (F,73)	Failed DSEK	--	--
20 (F,76)	FECD	24	--	--	20 (F,76)	FECD	--	--
21 (M,80)	BK†	7	4	KPs, EC changes, ECD decrease	21 (M,75)	PPBK	--	--
22 (F,66)	FECD	15	--	--	22 (F,65)	FECD	--	--

* Removed phakic IOL; † Glaucoma tube, # Combined with glaucoma tube & anterior chamber intraocular lens, % Post radial keratotomy, ^ Eyes of the same patient
ECD = Endothelial cell density; Pachy = Pachymetry; FECD = Fuchs endothelial corneal dystrophy, BK= Bullous keratopathy; KPs=Keratic precipitates; DSEK=Descemet stripping endothelial keratoplasty; M/F= Male/Female; PPBK = Pseudophakic bullous keratopathy; FU = Follow-up; m = Months

tion that may also go along with endothelial cell changes as described earlier (Zygoura et al. 2017).

Specular microscopy and Scheimpflug imaging are established methods to monitor post-keratoplasty corneas by assessing a decrease in endothelial cell density (on specular microscopy) and an increase in corneal thickness (with Scheimpflug imaging). This combination of changes may be expected to occur in secondary graft failure not associated with allograft rejection (Lass et al. 2010; Benetz et al. 2013; Baydoun et al. 2015). However, if associated with an upcoming rejection episode, our study showed that these two criteria are virtually always seen in combination with changes in endothelial cell morphology and/or the presence of subclinical keratic precipitates. Notably, the latter two parameters may be directly obtained from the taken measurement, while endothelial cell density decrease and pachymetry increase need to first be calculated.

In addition, within our study group, keratic precipitates and/or endothelial cell morphology changes (82% and 64%, respectively) were observed more often than endothelial cell density decrease or pachymetry increase (50% and 18%, respectively). And the latter two parameters always concurred with keratic precipitates and/or endothelial cell morphology changes. This may agree with the likely pathologic pathway associated with allograft rejection: the immune system may be primarily activated which results in leucocyte (keratic precipitates) and endothelial cell activation (nuclear swelling), which over time results in endothelial cell damage and/or cell death (decrease in endothelial cell density) and consequently corneal decompensation (increase in pachymetry) (Niederkorn & Larkin 2010).

To avoid graft failure due to allograft rejection following DMEK (Claerhout et al. 2003; Baydoun et al. 2015), reversion of a (subclinical) immune reaction at its earliest stage to prevent a full-blown rejection from manifestation should be the main treatment goal. Considering the above observations, as a preliminary algorithm to make the decision to start treatment, we now use the following criteria: 1) progression of subclinical keratic precipitates either isolated or in combination with a continuous degradation in endothelial cell morphology during closer follow-up visits or any other parameter, and 2) a significant decrease in cell density and/or increase in pachymetry, in combination with subclinical keratic precipitates or a degradation in endothelial cell morphology.

Unnecessary or overtreatment with steroids may always be a risk, but if in doubt whether to start intensified steroids, the patient can be monitored more closely by reducing the time until the next follow-up visit. From experience, we learned that reducing the interval between examination often makes the decision on treatment relatively easy without putting the eye at risk.

A limiting factor is that eyes that develop an allograft rejection within the first three to six months may escape detection, because they lack baseline measurements for comparison between follow-up intervals (i.e., it is difficult to determine significant changes if no or just one prior scan is available). In addition, the low rejection rates and the retrospective study design allow only the inclusion of a limited number of eyes and require cautious interpretation of the data. Hence, to determine the benefit of early (prerejection) treatment of eyes at risk of rejection, the rejection group would ideally be compared to a group of eyes at risk that did not receive treatment, which would probably need to be investigated in a prospective multicenter setting.

To prevent 90% of post-DMEK allograft rejections, both specular microscopy and Scheimpflug imaging would need to be performed at 6-month intervals, which is seen as a normal cycle in hospitals and specialized clinics to monitor patients after keratoplasty. Although the additional evaluation of images for keratic precipitates and endothelial cell morphology changes could be seen as a burden for clinicians, in our experience, the screening described in the current study is rather quick, as is the assessment of the images by a trained eye.

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CHAPTER 9

Clinical Feasibility of Using Multiple Grafts from a Single Donor for Quarter-DMEK

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LETTER TO THE EDITOR

Editor, Descemet membrane endothelial keratoplasty (DMEK) may have opened the door towards more efficient use of donor corneal tissue (Lie et al. 2010; Tenkman et al. 2014). Recently, we have introduced Quarter-DMEK for the treatment of Fuchs endothelial corneal dystrophy, which could potentially quadruple the yield of endothelial transplants from the same donor pool (Müller et al. 2017). To deliver on this promise, it would be important to evaluate whether multiple Quarter-DMEK surgeries are feasible in a clinical and eye bank setting, as it may require adapted logistics to distribute eight grafts from one donor within a short time window.

As a first approach, we transplanted Quarter-DMEK grafts in pairs, that is two grafts from each eye of a single 74-year-old male donor (Figure 1), all of which were prepared by the same eye bank technician (JTL), processed in identical fashion at Amnitrans EyeBank Rotterdam and transplanted into four eyes of four patients (mean age 71 (±8) years; Figure 1) on the same day by the same surgeon (LB). Quarter-DMEK graft preparation and surgeries were uneventful, except for Case 1 in which the graft inadvertently was partially flushed out and therefore reinserted. At six months postoperative, all eyes reached a best corrected visual acuity (BCVA) of ≥0.6 (20/30) and three reached ≥0.8 (20/25). For Cases 1 and 2 (grafts obtained from the right donor eye), endothelial cell density decreased by 80% and 79% at six months, and for Cases 3 and 4 (grafts obtained from the left donor eye), 54% and 66%. Both grafts from the right donor eye showed postoperative graft detachment requiring rebubbling.

Our study may be informative in two ways: first, the concept of using multiple endothelial grafts from the same donor cornea proved feasible in a clinical setting. All four eyes obtained an acceptable BCVA and the initial endothelial cell density decrease may be higher than after conventional DMEK, presumably owing to early endothelial cell redistribution over bare areas (Baydoun et al. 2012; Gerber-Hollbach et al. 2016). Second, donor tissue viability may differ between globes obtained from the same donor, even if all tissues were processed in identical manner.

For Quarter-DMEK to get widely adopted, it should not only be clinically successful, but also logistically feasible. Processing eight grafts from a single donor might introduce new challenges, because multiple recipient eyes need to be

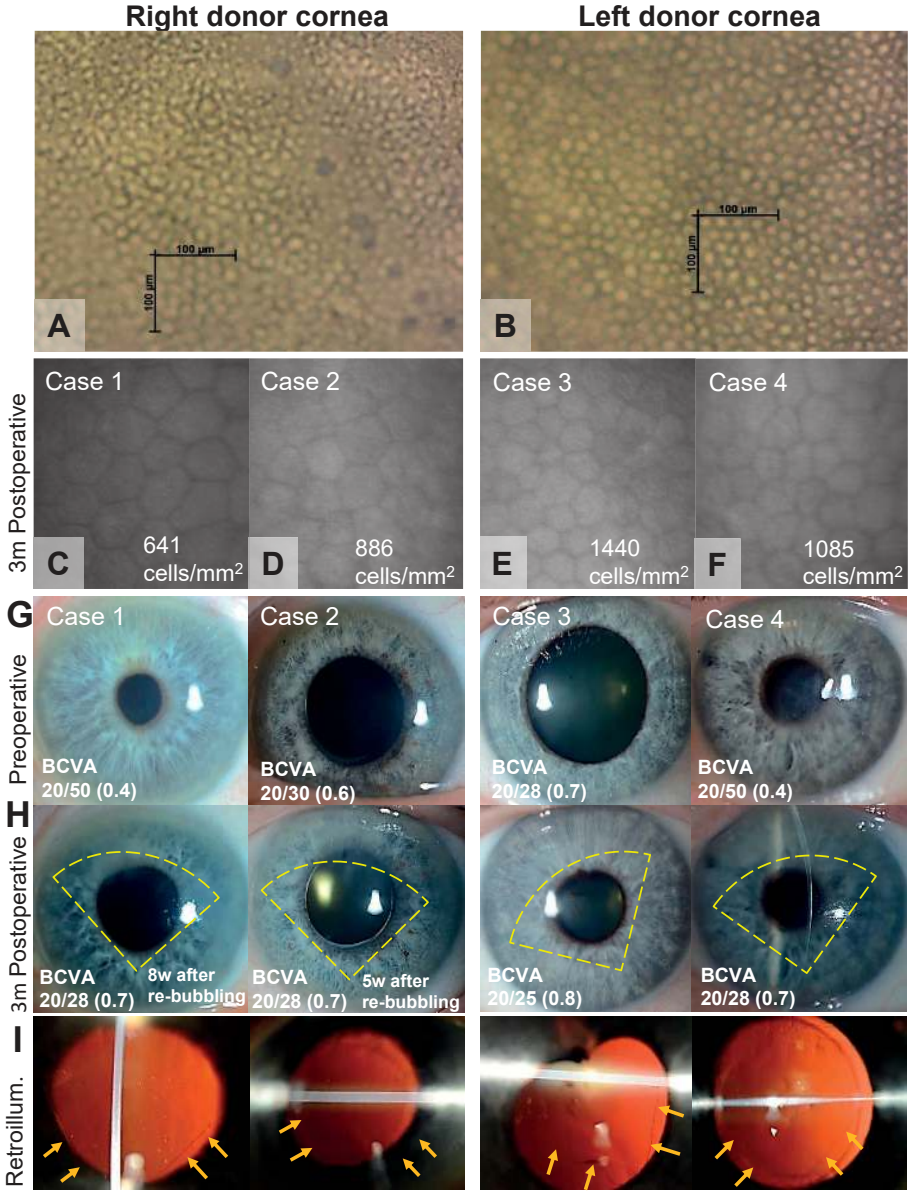

Figure 1. In vitro light microscopy images of the right (A) and left (B) donor cornea procured from the same 74-year-old, male donor, and in vivo specular microscopy images (C-F) at 3 months postoperatively of the four recipient eyes that received Quarter-DMEK grafts from the right donor cornea (C and D) and the left donor cornea (E and F). Endothelial cell density (ECD) at this time-point was 641 cells/mm^2 (Case 1), 886 cells/mm^2 (Case 2), 1440 cells/mm^2 (Case 3) and 1085 cells/mm^2 (Case 4). Slit lamp images of the four Quarter-DMEK eyes preoperatively (G), and 3 months postoperatively (H). The orientation of the Quarter-DMEK grafts is outlined by the yellow dashed lines in the slit lamp (H) and the orange arrows in the retro-illumination images (I). BCVA = best corrected visual acuity at each time-point; retroillum = retro-illumination.

accommodated within a short time window and tissue quality requirements may have to become stricter.

Furthermore, it would be interesting to see whether eye banks and surgeons would prefer transplantation of all grafts from the same donor (eye) in the same clinic. On the one hand, eye banks would then require only one shipment while surgeons may benefit from multiple grafts with similar 'behaviour', enabling some anticipation on surgery and aftercare. However, paired graft dysfunction may occur, potentially resulting in multiple graft-related complications in case of poor tissue viability.

The concept of transplanting multiple grafts from the same donor cornea may require more critical logistics for eye banks/surgeons. In addition, it may allow for unique inner-donor-eye transplant validation to improve surgical outcomes and/or reduce complications by grafting, for example Quarter-DMEK in two phases: if transplantation of a first quadrant shows good graft viability, that is complete attachment and corneal deturgescence, then as a second step, the other quadrants could be released for transplantation.

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CHAPTER 10

Summary, Discussion and Future Directions

SUMMARY AND DISCUSSION

Endothelial Keratoplasty (EK) emerged almost 25 years ago, namely in 1998, and heralded a new era in the field of corneal transplantation.[1-4] Until then, its predecessor, Penetrating Keratoplasty (PK) had been the backbone of any corneal layer replacement for almost 100 years.[5] Within less than a decade, various minimally-invasive EK techniques have been introduced for patients with corneal endothelial disease. A rapid transition from Deep Lamellar Endothelial Keratoplasty (DLEK) to Descemet Stripping (automated) Endothelial Keratoplasty (DS(A)EK) to Descemet Membrane Endothelial Keratoplasty (DMEK) occurred.[6] With each technical refinement, the transplanted graft became thinner and at the same time uncertainty grew concerning endothelial cell density (ECD) decrease and survival due to the more challenging intraoperative graft handling, especially in DMEK.[7] Another concern was the more frequent and seemingly inevitable postoperative graft dehiscence that necessitated repeat intervention in certain cases.[8-11] Despite all these doubts, DMEK has proven to be the culmination of technique `fine-tuning´ as the nearly complete anatomical restoration provided quick postoperative functional `perfection´.[12-17] Another advantage of the thin DMEK graft may contribute to the reduced risk and milder forms of immunologic reactions.[18] The latter could be further reduced by transplanting smaller grafts as in novel modified tissue-sparing DMEK techniques, such as Quarter-DMEK.[19]

This thesis evaluated graft survival and reasons for repeat surgery after primary DMEK and the clinical outcome after repeat DMEK. In addition, diagnostic methods to predict allograft rejection and the feasibility of transplanting multiple Quarter-DMEK grafts from the same donor, that potentially carry a reduced antigen load, were investigated.

DMEK graft survival and repeat DMEK for graft `failure´

DMEK survival: Pioneering in the 21st century

The first in vivo DMEK surgery was performed in 2006 on a Dutch male with Fuchs Endothelial Corneal Dystrophy (FECD).[20] Owing to the outstanding early clinical outcomes that have consistently been confirmed in numerous studies from different transplant centers, this technique has risen worldwide to a popular standard procedure in the treatment of corneal endothelial disorders.[21-23] The initial case report of the first surgery in 2006 showed fast and complete visual recovery within only one week.[20] The same patient underwent DMEK in his contralateral eye 10 months later. Regular follow-up visits enabled us to report the clinical

evolution until 9 and 10 years postoperatively in his right and left eye, respectively (**Chapter 2**).[24] The corneas were clear, lacking signs of endothelial dysfunction or immunologic reaction. For both eyes, we noted excellent long-term outcomes regarding best-corrected visual acuity (BCVA), ECD and lack of complications. This first patient unifies all DMEK advantages, such as rapid visual recovery, sustainable outcomes with superlative patient satisfaction, and graft longevity.

The increasing number of DMEK surgeries at our institute laid the foundation for the first long-term study on graft and endothelial survival in a series of the first 500 DMEK cases (**Chapter 3**).[25] A subgroup analysis was applied to assess an association with surgical indication (FECD versus bullous keratopathy BK), and graft adherence (attached versus partially detached). The results in this large cohort confirmed the outcomes of the first DMEK patient in terms of ECD decay and graft survival up to 8 years after DMEK. In addition, higher ECDs were found in FECD than in BK eyes and in attached compared to partially detached grafts. Likewise, survival probabilities were better in FECD than in BK eyes (97% versus 84%) with the same trend for eyes with attached versus partially detached grafts (97% versus 91%). Graft failure occurred in only 3 % of eyes and was attributed either to the pioneer´s learning curve, concomitant ocular pathologies (e.g., glaucoma) or complications (e.g., allograft rejection, low ECD in the presence of graft detachment). More than 50% of the failures were noted within the first year. Seemingly, only eyes with comorbidities or complications experienced timely graft failure. Hence, this study demonstrated an excellent prognosis on DMEK graft longevity, especially in eyes with FECD that had an attached graft and no concurrent ocular disease or early postoperative DMEK complication.

The survival study triggered a viewpoint on refining the term ´graft failure` in EK outcome reports (**Chapter 4**).[26] To assess donor endothelial viability in DMEK, it appears essential to identify the cause of corneal edema that besides irrevocable endothelial dysfunction may originate from major/complete graft detachment or false (inverted) graft positioning. The latter two should not be misnamed as ´graft failures´ but rather titled as technical failures because corneal edema often resolves after proper graft re-positioning and re-orientation, which proves the presence of viable donor endothelium.[27] Likewise, cases that showed ´spontaneous corneal clearance´ despite major/complete DMEK detachment,[28] will also not reflect donor endothelial viability, since the detected endothelium across the bare stroma may have derived from endothelial cell migration of the donor and/or recipient that both could have an impaired long-term survival compared to donor endothelial cells on an attached DMEK graft. Consequently, accurate

characterization and inclusion criteria with regard to the graft attachment status of the study eyes will benefit the reliability, uniformity and comparability of the data of future survival studies that aim to describe `endothelial viability of DMEK grafts´ and not the ´success rate´ of the individual surgeon or within a cohort.

Reasons and outcomes of repeat DMEK

Based on these considerations, we classified reasons for 'unsuccessful DMEK' in a series of 550 primary DMEK eyes of which 17 received repeat DMEK (**Chapter 5**).[29] Corneal edema for persisting graft detachment was the main indication followed by endothelial graft failure. With minor modifications, repeat DMEK was technically feasible in all eyes and clinical outcomes were overall acceptable. However, less eyes attained the high visual acuity level as in virgin DMEK eyes. The impaired vision could be explained by the formation of corneal surface irregularities and scarring due to long-standing corneal edema, since the postoperative optical quality could be enhanced by contact lens fitting in one third of eyes.[30] To avoid secondary corneal fibrosis, it may therefore be important to not postpone the treatment of persisting DMEK detachments and graft failure for too long.

Complications after repeat DMEK were rare and resembled those of primary DMEK. Still, patients should be counseled about the possibility that certain complications may be expected to recur, as we noticed that detachments of the initial DMEK graft reappeared in the same area of the second DMEK and eyes with graft failure in the first DMEK also developed failure of the second DMEK. Causative factors may be host intrinsic characteristics, such as the eye's anatomy and comorbidities.

Prediction of DMEK rejection and transplantation of smaller endothelial grafts

A continuous ECD decrease after keratoplasty is seen as an indicator for the deterioration of a transplanted graft.[31,32] This decay may be induced and accelerated by different factors that include potential damage during the surgery itself or the presence of concomitant ocular pathologies, such as glaucoma.[33] Another important factor could derive from undetected rejection events, and from subclinical or low-grade rejection and inflammation. Allograft rejection is commonly diagnosed with slit-lamp biomicroscopy showing typical findings, such as conjunctival and/or ciliary injection, anterior uveitis, keratic precipitates, an endothelial rejection line, and corneal edema, while patients describe subjective complaints, such as photophobia, pain, and deterioration of vision that often arise prior to or at the time of the inflammatory event. DMEK eyes often appear

´quiet´, and a lower risk and milder forms of rejection that are often asymptomatic have been described. Still, an ongoing decline in ECD over time is noted that could be caused by invisible inflammation that may lead to an allograft rejection in certain cases.

Detection of subclinical inflammation

We evaluated the intraocular inflammation in 173 FECD eyes post-DMEK by assessing aqueous flare in the early and later postoperative phase with laser flare photometry (**Chapter 6**).[34] Early after DMEK, flare levels decreased quickly but after one month, they were still higher than those in healthy controls suggesting a fast but incomplete recovery of the blood-aqueous barrier. Despite the continuous application of topical steroids, also longer-term flare beyond 3 months postoperatively was higher in DMEK eyes and even higher in those associated with allograft rejection compared to virgin controls (9.2 ph/ms vs. 16.7 ph/ms vs. 7.3 ph/ms, respectively). Hence, although DMEK eyes most often appear ´silent` and uninflamed, a persistent subclinical immune response emitted by the delicate DMEK graft could contribute to a chronic ECD decrease and a higher risk of graft failure in post-rejection eyes. Still, topical steroids appear to be protective since rejection episodes increased in uncomplicated DMEK eyes after steroid cessation.[35] This observation would support the importance of permanent or indefinite steroid application post-DMEK. Interestingly, all DMEK eyes associated with rejection but also 1/3 of eyes not associated with rejection had flare values above 10 ph/ms. This flare level could be used as a threshold to identify eyes associated with or at risk of DMEK rejection. Consequently, patients with lower flare levels may be managed with regular follow-up intervals, whereas those with values above 10 ph/ms could be monitored more frequently to not miss out an upcoming (mild, slowly progressing) rejection episode.

Prediction of graft rejection

To predict DMEK rejection, we theorized that Scheimpflug imaging could be useful in revealing corneal changes that herald rejection by assessing the Scheimpflug pictures and the pachymetry maps. We retrospectively analyzed a cohort of 750 DMEK eyes and identified 17 eyes that had been diagnosed with allograft rejection (**Chapter 7**).[36] Scheimpflug images of the rejection eyes showed distinct retrocorneal elevations and/or a significant increase in pachymetry of ≥7% at the time of rejection. Interestingly, these retrocorneal peculiarities, though more subtle, were recognized in >50% of eyes on average already 8 months before rejection manifested clinically, while those delicate changes escaped detection

at the slit-lamp or simply did not appear alarming since the eyes were asymptomatic and appeared uninflamed.

Our findings suggest, that this diagnostic tool could therefore have potential in identifying DMEK eyes at risk of developing rejection many months in advance and in a phase when the patient may still be asymptomatic. Surprisingly, the inflammatory response, however, seems to be slowly building up to a full-blown rejection which contradicts previous assumptions that rejection is a T-cell–mediated immune response which usually progresses more quickly.[37,38] Since almost 25% of the rejection eyes developed secondary graft failure and because in the majority of eyes these alterations were noted about 12 months after surgery, DMEK patients may be monitored more carefully and within the first two postoperative years.

In an initial pilot study on 7 eyes with proven rejection, we observed that also endothelial cell changes, such as nuclear activation on specular microscopy preceded rejection by 1-18 months.[39] To increase the power of our prediction and potentially reduce the incidence of long-term DMEK rejections, we therefore expanded our analysis to 22 rejection eyes from a cohort of 1077 consecutive DMEK surgeries and combined the evaluation of Scheimpflug imaging (retrocorneal elevations, >7% pachymetry increase) and specular microscopy (nuclear activation and endothelial cell changes, >10% ECD decrease) to improve our algorithm for detecting eyes at risk of rejection (**Chapter 8**).[40] In this follow-up study, our retrospective analysis unveiled that over 90% of rejection eyes showed pre-rejection changes with at least one of the two diagnostics up to 6 years before rejection. Most eyes, that is about 60%, showed changes with both methods, while 25% only had changes on Scheimpflug imaging, and about 10% only on specular microscopy. The presence of subclinical keratic precipitates (retrocorneal elevations) was the most frequent early sign before rejection, followed by endothelial cell morphology changes, >10% drop in ECD and >7% pachymetry increase (82% versus 64% versus 50% versus 20%, respectively). The latter two parameters always concurred with either keratic precipitates and/or endothelial cell changes as logically the activation of the immune system may first provoke leucocyte (keratic precipitates) and endothelial cell activation (nuclear swelling), whereas endothelial cell damage or death (decrease in ECD) and corneal decompensation (increase in pachymetry) would follow thereafter.[41] Interestingly, one third of control eyes also showed changes, that, however, were less prominent, not progressing over time and limited to specular microscopy. This could be because endothelial cell changes were also described in eyes with graft failure without

rejection, whereas keratic precipitates on Scheimpflug imaging were always associated with rejection.

For clinical practice during consecutive follow-up visits of post-DMEK eyes, it could therefore be proposed to screen the posterior corneal surface with 360-degree Scheimpflug imaging to detect and document fine minute inflammatory retrocorneal deposits, to observe endothelial cell morphology, and to monitor deviations in pachymetry and ECD to predict an upcoming rejection, that could be reflected by 1. progression of deposits, 2. deterioration of cell morphology, 3. decrease in ECD and 4. increase in pachymetry over time. In case of proven progression, steroid treatment could be intensified to prevent irreversible endothelial cell damage.

Our prediction studies further illustrate that specular microscopy and Scheimpflug imaging are not only essential diagnostics to monitor the transplanted endothelial cell sheet for decay (cell decrease) and function (corneal transparency and thickness), but that both methods may be actively used to identify eyes at risk of rejection; if rejection is prevented this could benefit the preservation of the endothelial health in post-keratoplasty corneas.

For the described algorithm, however, a basic premise is that rejection occurred only beyond the third postoperative months, so that at least two images with a longer time interval in between are available before the inflammatory event, meaning that eyes with early DMEK rejection will escape detection of pre-rejection signs with this strategy because of missing baseline images.

Quarter-DMEK: utilizing multiple grafts from the same donor cornea

Quarter-DMEK was developed to improve endothelial tissue availability and to further the more efficient tissue use.[19] Theoretically, four smaller Quarter-DMEK grafts can be recovered from one donor cornea and transplanted into four recipients with central FECD. The potentially reduced antigen load placed in an eye, could additionally reduce the inflammatory reaction and graft rejection rate. The first Quarter-DMEK surgery proved feasible resulting in similar visual outcomes as in standard DMEK.[19] We then speculated whether multiple Quarter-DMEK surgeries from the same donor, thus theoretically eight grafts from one donor (four from each donor eye), would be feasible in a clinical and eye bank setting (**Chapter 9**).[42] To begin with, we transplanted two quarters from each globe of a single donor (four quarters from the bilateral pair) into four different patients on the same surgery day. We noticed acceptable visual outcomes in all four eyes,

but similar ECD decrease and complications per transplanted pair that differed from the eyes receiving the pair of the other globe. This suggests that tissue viability may vary between the globes of the same donor though processed within a short time window and in the same fashion. This could pose advantages but also new challenges, that would ask for stricter tissue quality requirements and adjustments of logistics and tissue allocation when processing several grafts from one donor. On the other hand, this concept could offer a vivid in vivo tissue ´quality check´ that to some extend would permit anticipation on the surgery outcomes and complications. Consequently, only if transplantation of the first quadrant of the Descemet sheet from one globe shows favorable graft viability with an uncomplicated course in the first week, then the remaining three quadrants should be released for surgery.

FINAL REMARKS AND FUTURE PERSPECTIVES

After almost 15 years since the first surgery, DMEK has evolved to a standard procedure that also provides excellent long-term clinical outcomes and graft longevity.[20] In the event of corneal edema from graft failure or persistent graft dehiscence, repeat DMEK is a feasible and successful procedure which, when promptly employed, can avoid inferior visual outcomes from anterior corneal surface scarring that may require contact lens correction.

A long-term goal in the management of post-DMEK eyes is to increase graft longevity.[20] This could be targeted by reducing the continuous ECD decrease that may originate from an ongoing inflammation caused by the foreign DMEK tissue. Visualization of the invisible immune reaction to adjust post-DMEK treatment could therefore reduce endothelial cell decay.

In addition, active prediction, and detection instead of passive observation and anticipation of rejection could prevent and reduce long-term rejection by timely treatment to minimize the ´silent` cell damage and subsequent graft failure. Besides the already existing diagnostic devices in ophthalmology, the application of further tools or the development of new technologies could help in making an upcoming rejection diagnostically visible. Ideally, this could be supported by automated recognition and artificial intelligence so that detection would be independent of the examiners experience and would allow a more secure, quick, and standardized follow-up of the rapidly increasing numbers of post-DMEK eyes worldwide.

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CHAPTER 11

Nederlandse Samenvatting

(Dutch Summary)

NEDERLANDSE SAMENVATTING

De technieken met betrekking tot endotheelkeratoplastiek (EK) zijn in 1998, bijna 25 jaar geleden, geïntroduceerd, en luidden een nieuw tijdperk in op het gebied van hoornvliestransplantaties.[1-4] Gedurende de voorgaande 100 jaar was de voorganger van deze technieken, de penetrerende keratoplastiek (PK), dé standaard aanpak voor vervanging van een ziek hoornlvies.[5] In een tijdsbestek van nog geen 10 jaar werden verschillende minimaal-invasieve EK-technieken geïntroduceerd voor patiënten met corneale endotheelaandoeningen. Er vond een snelle overgang plaats van Deep Lamellar Endothelial Keratoplasty (DLEK) naar Descemet Stripping (automated) Endothelial Keratoplasty (DS(A)EK) en Descemet Membrane Endothelial Keratoplasty (DMEK).[6] Bij elke technische verfijning werd het endotheeltransplantaat dunner. Tegelijkertijd nam echter de onzekerheid over de afname van de endotheelceldichtheid (ECD) en de transplantaatoverleving toe. Het transplantaat bleek tijdens de ingreep namelijk steeds moeilijker te manipuleren, vooral bij de DMEK.[7] Een andere zorg vormden de veelvuldige en schijnbaar onvermijdelijke postoperatieve niet-aanliggende transplantaten, aangezien deze aanleiding konden zijn voor een hertransplantatie.[8-11] Ondanks al deze bedenkingen heeft DMEK bewezen een ultieme techniekverfijning te zijn; het bijna volledige herstel van de cornea-anatomie na DMEK levert een ongekend snelle postoperatieve functionele “perfectie” op.[12-17] Andere voordelen van het dunne DMEK-transplantaat zijn het verminderde risico op en de mildere vormen van transplantaatafstoting.[18] Het risico op transplantaatafstoting zou daarbij mogelijk nog verder kunnen worden verlaagd door het transplanteren van kleinere transplantaten.[19]

In dit proefschrift worden de transplantaatoverleving en de redenen voor heroperatie na DMEK geëvalueerd. Ook worden de klinische resultaten van re-DMEK onderzocht. Bovendien werd er onderzoek gedaan naar diagnostische mogelijkheden om transplantaatafstoting te kunnen voorspellen, evenals de haalbaarheid van het transplanteren van meerdere Quarter-DMEK-transplantaten van dezelfde donor, met mogelijk een verminderde antigeenbelasting.

DMEK-transplantaatoverleving en re-DMEK na transplantaatfalen

DMEK-transplantaatoverleving: pionieren in de 21e eeuw

De eerste in vivo DMEK operatie werd in 2006 uitgevoerd bij een Nederlandse man met Fuchs endotheeldystrofie (FECD).[20] Vanwege het snelle herstel en de uitstekende klinische resultaten die consequent werden bevestigd in talrijke

studies van verschillende transplantatiecentra, heeft de DMEK-techniek zich daarna wereldwijd ontwikkeld tot de standaardprocedure voor de behandeling van corneale endotheelaandoeningen.[21-23] Bij de eerste DMEK-operatie in 2006 trad binnen een week een ongekend snel en volledig visueel herstel op.[20] Dit was ongekend. Dezelfde patiënt onderging 10 maanden later een DMEK in zijn andere oog. Regelmatige follow-up bezoeken stelden ons in staat om de klinische resultaten tot 9 en 10 jaar postoperatief voor respectievelijk zijn rechter- en linkeroog te rapporteren (**hoofdstuk 2**).[24] Gedurende de follow-up bleven beide hoornvliezen helder, zonder enig teken van endotheeldysfunctie of immunologische reactie. Ook toonden beide ogen uitstekende langetermijnresultaten voor wat betreft de optimaal gecorrigeerde visus (BCVA), endotheelceldichtheid (ECD) en complicaties. Deze eerste patiënt verenigt dan ook alle voordelen van DMEK: een snel visueel herstel, duurzame resultaten met een uitstekende patiënttevredenheid, en een lange levensduur van het transplantaat.

Het toenemende aantal DMEK-operaties in ons instituut was de basis voor de eerste langetermijnstudie over transplantaat- en endotheeloverleving in de eerste 500 DMEK-ogen (**hoofdstuk 3**).[25] Daarbij werd een subgroepanalyse uitgevoerd om een eventuele associatie met chirurgische indicatie (FECD versus bulleuze keratopathie (BK)) en transplantaatadhesie (aanliggend versus gedeeltelijk afliggend) te onderzoeken. De resultaten van deze grote cohortstudie bevestigden de uitkomsten van de eerste DMEK-patiënt in wat betreft ECD-verlies en transplantaatoverleving tot 8 jaar na DMEK. In deze studie werd verder een hogere ECD gevonden in ogen met FECD dan in bullous keratopathy (BK)ogen. Hetzelfde gold voor volledig aanliggende transplantaten in vergelijking met gedeeltelijk afliggende transplantaten. Ook waren de overlevingskansen beter in FECD- dan in BK-ogen (97% versus 84%), en in volledig aanliggende transplantaten in vergelijking met gedeeltelijk afliggende transplantaten (97% versus 91%). Transplantaatfalen kwam slechts voor in 3% van de onderzochte ogen en had waarschijnlijk te maken met de leercurve van de techniek, comorbiditeiten (zoals glaucoom) of complicaties (zoals transplantaatafstoting of lage ECD bij een afliggend transplantaat). Meer dan 50% van het transplantaatfalen vond plaats binnen het eerste postoperatieve jaar. Daarbij leek het erop dat het transplantaat alleen bij ogen met comorbiditeiten of complicaties snel faalde. Al met al toonde deze studie een uitstekende prognose voor de overleving van een DMEK-transplantaat, vooral in FECD-ogen met een goed aanliggend transplantaat zonder comorbiditeiten en zonder complicaties in de vroege postoperatieve fase.

Deze transplantaatoverlevingsstudie triggerde ons tot het schrijven van een letter-to-the-editor met daarin een standpunt over het verfijnen van de term 'transplantaatfalen' in EK-resultaatuitkomsten (**hoofdstuk 4**).[26] Om de levensvatbaarheid van het donorendotheel in DMEK te beoordelen, blijkt het namelijk essentieel te zijn om de oorzaak van het corneaoedeem te identificeren, dat naast *endotheeldysfunctie* ook door een grote/volledige transplantaatafligging of onjuiste (omgekeerde) plaatsing van het transplantaat kan worden veroorzaakt. De twee laatstgenoemde oorzaken mogen niet foutief "falende transplantaten" worden genoemd, maar moeten worden gezien als *technisch* falen. Dit omdat het hoornvliesoedeem vaak na een juiste herpositionering en heroriëntatie van het transplantaat verdwijnt, wat de aanwezigheid van levensvatbaar donorendotheel aantoont.[27] Ook ogen die 'spontane opheldering van het hoornvlies' vertoonden ondanks grote/complete DMEK-afligging[28] weerspiegelen in die zin niet de levensvatbaarheid van het donorendotheel, omdat het gedetecteerde endotheel over het ontvangende stroma in deze gevallen afkomstig kan zijn van endotheelcelmigratie van zowel donor als ontvanger. Deze kunnen beiden een verminderde overleving op lange termijn hebben in vergelijking met donorendotheelcellen op een goed aanliggend DMEK-transplantaat.

Bijgevolg zullen nauwkeurige karakterisering en inclusiecriteria met betrekking tot de hechtingsstatus van het transplantaat de betrouwbaarheid, uniformiteit en vergelijkbaarheid van de uitkomsten van operaties ten goede komen; overlevingsstudies zijn essentieel en hebben tot doel om de "endotheliale levensvatbaarheid van DMEK-transplantaten" te beschrijven en niet het "succespercentage" van de individuele chirurg of een cohort.

Redenen en uitkomsten van re-DMEK

Op basis van deze overwegingen classificeerden wij vervolgens redenen voor een 'onsuccesvolle DMEK' in een serie van 550 primaire DMEK-ogen, waarvan er 17 een re-DMEK hadden ondergaan (**hoofdstuk 5**).[29] Corneaoedeem bij aanhoudende transplantaatafliggingen was de belangrijkste indicatie, gevolgd door transplantaatfalen als gevolg van endotheeldysfunctie bij een goed aanliggend transplantaat. Met kleine aanpassingen was re-DMEK in alle ogen technisch haalbaar en de klinische resultaten waren over het algemeen acceptabel. Het hoge gezichtsscherpteniveau zoals gezien in 'virgin' DMEK-ogen werd echter in minder ogen bereikt. Dit zou kunnen worden verklaard door onregelmatigheden en littekenvorming aan het hoornvliesoppervlak ten gevolge van langdurig hoornvliesoedeem, aangezien de postoperatieve optische kwaliteit in een derde van deze ogen kon worden verbeterd door een contactlensaanpassing.[30] Om

secundaire fibrose te vermijden, lijkt het dientengevolge belangrijk om herbehandeling bij persisterende DMEK-transplantaatafliggingen en -falen niet te lang uit te stellen.

Complicaties na re-DMEK waren zeldzaam en leken op die van eerste DMEKs. Toch is het goed patiënten te adviseren over de mogelijkheid dat bepaalde complicaties kunnen terugkeren, aangezien gedeeltelijke afliggingen van het initiële DMEK-transplantaat opnieuw verschenen in hetzelfde gebied bij de re-DMEK. Daarnaast vertoonden ogen met transplantaatfalen bij de eerste DMEK ook falen bij de tweede DMEK. De oorzakelijke factoren hiervoor kunnen intrinsieke kenmerken van de patiënt zijn, zoals de anatomie van het oog en comorbiditeiten.

Voorspelling van DMEK-afstoting en transplantatie van kleinere endotheeltransplantaten

Een voortdurende afname van de ECD na keratoplastiek wordt over het algemeen gezien als een indicator voor de achteruitgang van een getransplanteerd transplantaat.[31,32] Verschillende factoren kunnen deze achteruitgang induceren of versnellen, waaronder de operatie zelf of de aanwezigheid van bijkomende oogaandoeningen, zoals glaucoom.[33] Andere belangrijke factoren zijn niet-gedetecteerde transplantaatafstotingen, subklinische of ´low-grade` afstotingen en ontstekingen. Een afstoting wordt gewoonlijk gediagnosticeerd met de spleetlamp en vertoont typische bevindingen, zoals conjunctivale en/of ciliaire roodheid, anterieure uveïtis, beslag op het endotheel, een endotheliale afstotingslijn en corneaoedeem. Patiënten beschrijven daarbij subjectieve klachten als fotofobie, pijn en vermindering van het zicht, die vaak ontstaan net vóór of op het moment van de afstoting. DMEK-ogen zien er vaak 'rustig' uit, en milde vormen van afstoting met een asymptomatisch beloop zijn beschreven. Toch zien we in dit soort gevallen in de loop van de tijd een voortdurende afname van de ECD die mogelijk veroorzaakt wordt door een 'onzichtbare' ontsteking die op zijn beurt in bepaalde gevallen tot afstoting van het transplantaat kan leiden.

Detectie van subklinische ontsteking

Wij onderzochten de mate van intra-oculaire ontsteking in 173 FECD-ogen post-DMEK door middel van het beoordelen van de mate van 'flare' in de voorste oogkamer in zowel de vroege als latere postoperatieve fase, waarbij wij gebruik maakten van laser flare fotometrie (**hoofdstuk 6**).[34] Vroeg na DMEK namen de flareniveaus snel af, maar na een maand bleken deze nog steeds een hogere flare te hebben in vergelijking met gezonde controleogen (7,3 ph/ms). Dit wijst op een snel maar onvolledig herstel van de bloed-oog barrière. Ondanks de

voortdurende toepassing van lokale steroïden was ook op de langere termijn (3 maanden postoperatief) het flareniveau hoger in de DMEK-ogen (9,2 ph/ms), waarbij in ogen die geassocieerd werden met transplantaatafstoting de hoogste waarden werden gemeten (16,7 ph/ms). Hoewel DMEK-ogen meestal 'rustig' en zonder ontsteking lijken te zijn, kan een persisterende subklinische immuunrespons, mogelijk veroorzaakt door het DMEK-transplantaat, dus bijdragen aan een chronische ECD-afname en een hoger risico op transplantaatfalen. Wel lijken lokale steroïden bij te dragen aan bescherming van het transplantaat, aangezien afstotingsepisoden toenamen in ongecompliceerde DMEK-ogen na het stoppen van de steroïden.[35] Deze observatie zou het belang van permanente of onbepaalde toepassing van steroïden na DMEK ondersteunen. Interessant is dat alle DMEK-ogen die geassocieerd waren met afstoting, maar ook 1/3 van de niet met afstoting geassocieerde ogen, flarewaarden boven 10 ph/ms vertoonden. Dit niveau zou kunnen worden gebruikt als een drempel om ogen te identificeren die geassocieerd zijn met of risico lopen op afstoting. Patiënten met lage waarden postoperatief zouden enkel op de standaard follow-up gezien hoeven te worden, terwijl patiënten met waarden boven 10 ph/ms frequenter zouden moeten worden gecontroleerd om niet een opkomende (milde, langzaam voortschrijdende) afstotingsepisode te missen.

Voorspelling van transplantaatafstoting

Om te beoordelen of Scheimpflug-beelden mogelijk van nut konden zijn bij het voorspellen van transplantaatafstoting analyseerden wij retrospectief een cohort van 750 DMEK-ogen en identificeerden daarbij 17 ogen die gediagnosticeerd waren met transplantaatafstoting (**hoofdstuk 7**).[36] Scheimpflug-beelden van de ogen met afstoting toonden op het moment van afstoting duidelijke retrocorneale elevaties en/of een significante toename in pachymetrie van ≥7%. Interessant genoeg waren deze retrocorneale bijzonderheden al maanden (gemiddeld 8 maanden) voordat de afstoting klinisch tot uiting kwam subtiel aanwezig, terwijl deze subtiele veranderingen met de spleetlamp werden gemist of als niet alarmerend werden beschouwd omdat de ogen asymptomatisch waren en niet ontstoken leken.

Onze bevindingen suggereren dat dit diagnostische instrument in potentie zou kunnen helpen bij het identificeren van DMEK-ogen met risico op afstoting, al vele maanden vóór de klinische uiting en in een fase waarin de patiënt nog asymptomatisch is. Verrassend genoeg lijkt de ontstekingsreactie bij transplantaatafstoting zich namelijk langzaam op te bouwen, wat in tegenspraak is met eerdere veronderstellingen dat transplantaatafstoting een T-cel-gemedieerde

immuunrespons is die gewoonlijk sneller verloopt.[37,38] Aangezien bijna 25% van de ogen met afstotingsreactie secundair transplantaatfalen ontwikkelden en omdat in de meerderheid van de ogen deze Scheimpflugveranderingen ongeveer 12 maanden na de operatie werden waargenomen, lijkt het raadzaam DMEK-patiënten vooral binnen de eerste twee postoperatieve jaren zorgvuldig te controleren.

In een eerdere pilotstudie bij 7 ogen met bewezen afstoting stelden wij reeds vast dat ook endotheelcelveranderingen, zoals nucleaire activering op speculaire microscopie, 1 tot 18 maanden aan de afstoting voorafgingen.[39] Om onze voorspelling treffender te maken en daarmee de incidentie van transplantaatafstotingen over de langere termijn te kunnen verminderen, breidden wij onze analyse uit naar 22 ogen waarbij ooit een afstotingsreactie was gediagnostiseerd uit een cohort van 1077 opeenvolgende DMEK-operaties. Tijdens de studie combineerden wij de evaluatie van *Scheimpflug-beelden* (retrocorneale elevaties, >7% pachymetrie toename) en *speculaire microscopie* (nucleaire activering en endotheelcelveranderingen, >10% ECD afname), om zodoende ons algoritme voor het detecteren van ogen met risico op afstoting te verbeteren (**hoofdstuk 8**).[40] Deze retrospectieve analyse onthulde dat meer dan 90% van de afstotingsogen pre-afstotingsveranderingen vertoonden in ten minste één van de twee diagnostische tools tot 6 jaar voor afstoting. De meeste ogen, namelijk ongeveer 60%, vertoonden veranderingen met beide methoden, terwijl 25% alleen veranderingen liet zien op Scheimpflugbeelden en ongeveer 10% alleen met speculaire microscopie. De aanwezigheid van subklinische keratische precipitaten (retrocorneale elevaties) was het meest voorkomende vroege teken voorafgaand aan de afstoting (in 82%), gevolgd door veranderingen in de vorm van de endotheelcellen (in 64%), >10% daling van de ECD (in 50%) en >7% stijging van de pachymetrie (in 20%). De laatste twee parameters vielen steeds samen met keratische precipitaten en/of endotheelcelveranderingen, aangezien logischerwijs de activering van het immuunsysteem eerst leucocyten (keratische precipitaten) en endotheelcelactivering (nucleaire zwelling) kan opwekken, terwijl beschadiging of afsterven van endotheelcellen (daling van ECD) en corneale decompensatie (stijging van pachymetrie) pas daarna volgen.[41] Interessant is dat een derde van de gezonde DMEK controle ogen ook veranderingen vertoonden, die echter minder opvallend waren, niet verergerden in de tijd en beperkt bleven tot speculaire microscopie. De reden hiervoor zou kunnen zijn dat endotheelcelveranderingen ook werden beschreven in ogen met transplantaatfalen zonder afstoting, terwijl keratische precipitaten op Scheimpflugbeelden altijd waren geassocieerd met afstoting.

Voor in de klinische praktijk zou daarom kunnen worden voorgesteld om bij standaard (opeenvolgende) DMEK-controles het posterieure corneaoppervlak routinematig te screenen door middel van 360-graden Scheimpflugbeeldvorming. Op die manier kunnen minuscule inflammatoire retrocorneale afzettingen worden gedetecteerd en gedocumenteerd. Daarnaast zouden de endotheelcelmorfologie en ECD routinematig moeten worden geobserveerd en zouden afwijkingen in pachymetrie moeten worden gemonitored. Bijgevolg zouden opkomende transplantaatafstotingen kunnen worden voorspeld door: 1. progressie van retrocorneale afzettingen, 2. verslechtering van de celmorfologie, 3. afname van ECD en 4. toename van de pachymetrie. In geval van aangetoonde progressie kan de steroïdenbehandeling worden geïntensiveerd om zodoende irreversibele schade aan de endotheelcellen te voorkomen.

Onze voorspellingsstudies illustreren verder dat speculaire microscopie en Scheimpflugbeeldvorming niet alleen essentiële diagnostica zijn om het getransplanteerde endotheelcellaagje te controleren op verval (celafname) en functie (corneale transparantie en dikte), maar dat beide methoden kunnen worden ingezet om ogen die risico lopen op afstoting te identificeren; indien afstoting wordt voorkomen zou dit het behoud van het endotheel van een hoornvliestransplantaat betekenen.

Een uitgangspunt van het beschreven algoritme is echter dat afstoting pas na de derde postoperatieve maand optreedt, zodat ten minste twee beelden met een langer tussenliggend tijdsinterval beschikbaar zijn vóór de afstoting, wat betekent dat ogen met een vroege ontstekingsreactie door het algoritme niet gedetecteerd worden vanwege ontbrekende basisbeelden.

Quarter-DMEK: gebruik van meerdere transplantaten van hetzelfde donorhoornvlies

Quarter-DMEK werd door ons ontwikkeld om de beschikbaarheid van endotheelweefsel te verbeteren en een efficiënter donorweefselgebruik te bevorderen.[19] Theoretisch kunnen vier Quarter-DMEK-transplantaten uit één donorhoornvlies worden geprepareerd en getransplanteerd in vier ontvangers met centrale FECD. Door een mogelijk verminderde antigeenbelasting van het transplantaat zou bovendien de ontstekingsreactie en het uiteindelijke afstotingspercentage kunnen verminderen. De eerste Quarter-DMEK-operatie bleek haalbaar en resulteerde in vergelijkbare visuele resultaten als bij standaard DMEK.[19] Wij speculeerden vervolgens of meerdere Quarter-DMEK-operaties van dezelfde donor, dus theoretisch acht transplantaten van één donor (vier van elk donoroog), haalbaar zouden

zijn in een klinische en oogbanksetting (**hoofdstuk 9**).[42] Daarom transplanteerden wij in het begin twee kwadranten van elke oogbol van één donor (vier kwadranten van het bilaterale paar) in vier verschillende patiënten op dezelfde operatiedag. De visuele resultaten in alle vier ogen waren aanvaardbaar, maar we zagen dat de ECD-afname en de complicaties per getransplanteerd paar (2 kwadranten van 1 oogbol) verschilden van het andere paar. Dit suggereert dat de levensvatbaarheid van het weefsel kan verschillen tussen de oogbollen van dezelfde donor, ook al zijn ze binnen een kort tijdsbestek en op dezelfde manier bewerkt. Dit zou voordelen kunnen opleveren, maar ook nieuwe uitdagingen. Denk hierbij bijvoorbeeld aan strengere kwaliteitseisen voor het weefsel en aanpassingen in de logistiek en weefseltoewijzing bij de verwerking van meerdere transplantaten van één donor. Anderzijds zou dit concept een 'real-life' in vivo "kwaliteitscontrole" van het weefsel mogelijk maken; tot op zekere hoogte kan worden geanticipeerd op de resultaten van een eerste operatie en de complicaties daarvan, waarbij de overgebleven drie kwadranten alleen dan vrijgegeven dienen te worden als transplantatie van het eerste kwadrant van die oogbol een gunstige levensvatbaarheid laat zien met een ongecompliceerd verloop in de eerste week.

SLOTOPMERKINGEN EN TOEKOMSTPERSPECTIEVEN

Na bijna 15 jaar sinds de eerste operatie is DMEK geëvolueerd tot een standaardprocedure die ook uitstekende klinische resultaten op de lange termijn en een lange levensvatbaarheid van het transplantaat biedt.[20] In het geval van hoornvliesoedeem door transplantaatfalen of aanhoudende transplantaataflig-ging, is re-DMEK een haalbare en succesvolle procedure die, indien onmiddellijk uitgevoerd, inferieure visuele resultaten als gevolg van littekenvorming op het voorste hoornvliesoppervlak kan voorkomen.

Een langetermijndoelstelling in de behandeling van post-DMEK ogen is het verlengen van de levensduur van het transplantaat. Dit kan worden nagestreefd door het verminderen van de voortdurende ECD-afname, mogelijk veroorzaakt door een voortdurende ontstekingsreactie vanwege het donor DMEK-weefsel. Visualisatie van de onzichtbare immuunreactie om de post-DMEK behandeling aan te passen zou dientengevolge het verval van endotheelcellen kunnen verminderen.

Bovendien zou preventieve voorspelling en detectie in plaats van passieve observatie en anticipatie op transplantaatafstoting langdurige afstoting kunnen voorkomen en verminderen, door tijdige behandeling om de ´onzichtbare` celschade en het daaropvolgende transplantaatfalen te minimaliseren. Naast de al bestaande diagnostische hulpmiddelen in de oogheelkunde zou de toepassing van andere hulpmiddelen of de ontwikkeling van nieuwe technologieën kunnen helpen bij het zichtbaar maken van een aanstaande afstoting. Idealiter zou dit kunnen worden ondersteund door geautomatiseerde herkenning en kunstmatige intelligentie zodat de detectie onafhankelijk wordt van de ervaring van de onderzoeker. Zodoende kan een veiligere, snellere en meer gestandaardiseerde follow-up van het snel toenemende aantal post-DMEK ogen wereldwijd mogelijk worden gemaakt.

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APPENDICES

List of Publications

Curriculum Vitae

Acknowledgements

LIST OF PUBLICATIONS

1. Vasiliauskaitė I, Quilendrino R, **Baydoun L**, Van Dijk K, Melles GRJ, Oellerich S. Effect of Six-Month Postoperative Endothelial Cell Density on Graft Survival after Descemet Membrane Endothelial Keratoplasty. *Ophthalmology* 2021 - Online ahead of print
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CURRICULUM VITAE

Lamis Baydoun was born on September 25, 1974, in Brunswick (Braunschweig), Germany. After high school graduation, she took a gap year for voluntary work at a local hospital before starting medical school at the University of Magdeburg, Germany, where she received her medical degree in 2001. After a sub-internship in ophthalmology in 2000 at the University Hospital of Zürich, Switzerland, under supervision by Prof. T. Seiler, she did her ophthalmology residency from 2001 until 2004 at the University Hospital of Bonn, Germany, under Prof. M. Spitznas and later Prof. F. Holz, and from 2004 until 2006 at the St.-Johannes-Hospital in Dortmund, Germany, under Prof. J. Kamman and later Prof. M. Kohlhaas. In 2006 she became a Fellow of the European Board (FEBO), finished her Medical Thesis (Dr. med., passed "cum laude") on laboratory research in pediatrics and pathobiochemistry on ´Oxidative Stress in Juvenile Diabetes` (University of Magdeburg, Medical School) and passed her Ophthalmology Board Examination. From 2007 to 2012, she worked as a consultant surgeon in Mülheim/Ruhr, Germany, a teaching hospital of the University of Düsseldorf, Germany, where she directed the departments of Cataract, Cornea, and Uveitis. She got captivated by DMEK while preparing a talk on novel lamellar keratoplasty techniques, which led to the cornea fellowship that she began in 2012 at the Netherlands Institute for Innovative Ocular Surgery (NIIOS) in Rotterdam, The Netherlands, under the guidance of Dr. G. Melles. After a six-month period, she continued her work at NIIOS as a corneal surgeon, research scientist and later as Head of NIIOS Academy. Due to her ongoing fascination with uveitis, she initiated several DMEK rejection research projects and the PROTECT (Prevent Rejection Of The Endothelial Cell Transplant) program. In 2018, she has been associated with the LUMC, Leiden, as a PhD student under the guidance of Prof. Dr. M.J. Jager. In 2019, she returned to Germany (parttime from 2016) as a senior consultant surgeon for Cornea and Cataract at the University Hospital Münster under Prof. Dr. N. Eter where she is currently finalizing her habilitation ('Privatdozentin'). Since 2019, she is also working as a Cornea Specialist with Prof. F. Hafezi at the ELZA Institute Dietikon/Zürich, Switzerland. In December 2021 she will receive the Gold Medal for her work in Ophthalmology from the Intraocular and Refractive Society of India. Due to her passion for education, she is involved in the residency program of all her clinical appointments. During NIIOS Wetlab courses, she trained numerous international ophthalmologists in lamellar keratoplasty techniques, and gives invited lectures internationally on her research. With the NIIOS Academy team, she has since 2015 organized and moderated in person (and online since 2020) scientific meetings ('NIIOS Cornea Evenings') with up to 1000 registrations including renowned international corneal experts in the field.

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