Advances in endothelial keratoplasty

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Chapter 1

General Introduction and Thesis Outline

GENERAL INTRODUCTION

The human cornea is the most anterior, transparent structure of the globe. It serves as a barrier to protect intraocular structures and provides about two-thirds of the entire refractive power of the eye.[1] The cornea measures 11-12 mm horizontally, and 10-11 mm vertically, with a central radius of curvature of approximately 8 mm. It has an average thickness of 500 to 600 μm.[1-3] With a high degree of innervation by the ophthalmic branch of the trigeminal nerve (approximately 300-400x that of the epidermis), it is one of the most sensitive tissues in the human body.[1] The cornea is uniquely avascular, and acquires its nutrients from the tear film or aqueous humor.[1,3,4] The lack of vascularization contributes to corneal clarity, optical performance, and relative immune privilege.[1,3] The cornea is amenable to transplantation and eye banks play an important role in procurement, storage, and allocation of corneal tissue for transplantation.

ANATOMY AND PHYSIOLOGY OF THE HUMAN CORNEA

The human cornea is a transparent tissue with a high degree of spatial organization and a strong correlation between structure and function. It consists of five histologic layers, from anterior to posterior: epithelium with its basement membrane, Bowman layer, stroma, Descemet membrane (DM) and endothelium (Fig. 1).[1-4] In order to optimize corneal optics and refractive power, a healthy tear film-cornea interface is required to provide a smooth and regular surface.[1,3,4] The tear film forms the primary biodefense system for the anterior surface of the eye.[1,3,4] It supplies nutrients and growth factors, which are essential for corneal homeostasis.[1,3,4]

The *epithelium* is the outermost anterior layer of the cornea. It is about 50 μm thick, and is composed of 5-7 layers of non-keratinized, stratified, squamous epithelial cells.[1,4] The epithelium is highly uniform from limbus to limbus to maintain a smooth refractive surface. It contributes to corneal transparency by having few intracellular organelles, and high concentrations of the intracytoplasmic enzyme crystalline.[1] The epithelium forms an effective corneal barrier and consists of several layers of superficial, flat, polygonal cells, two or three layers of suprabasal or wing cells, and a single cell layer of columnar basal cells.[1,3] Corneal epithelial cells have an average lifespan of 7-10 days and

complete epithelial turnover takes place on a weekly basis.[1,3,4]The epithelial basement membrane is 40-60 nm thick, and is composed of type IV collagen and laminin secreted by basal cells.[1,3,4]

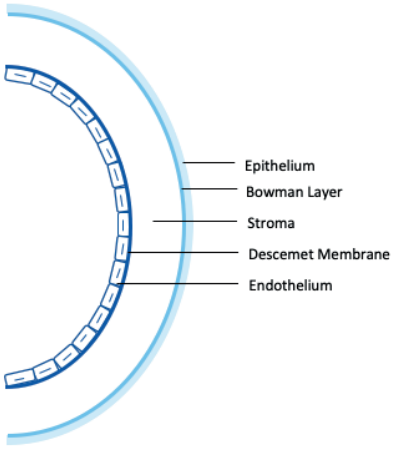

Figure 1. Schematic representation of the anatomical layers of the human cornea.

Bowman layer (BL) is an acellular layer positioned just beneath the epithelial basement membrane.[1-4] The anterior surface is very smooth, while the posterior surface extends into the anterior stroma.[1-4] It is approximately 8-14 µm thick, and thins with age.[1,5] In contrast to the underlying stromal collagen fibrils (diameter 32-36 nm) that run uniformly parallel across the corneal to form characteristic lamellae, BL consists of smaller, randomly interwoven collagen fibrils (24-27 nm).[6] These fibrils are primarily composed of collagen types I and III and form a dense, felt-like sheet.[7]BL does not regenerate after injury and to date, the physiologic function of BL remains to be elucidated.[1,3]

The *stroma* provides the largest portion of the structural framework of the cornea. It accounts for nearly 90% of the total corneal thickness and measures an average of 500 µm in humans.[1,3,4] The stroma contributes to corneal transparency, mechanical strength, and tectonic stability. It is made up of collagen fibers embedded in an extracellular matrix (ECM) composed of mainly water, inorganic salts, proteoglycans, and glycoproteins.[8] Keratocytes are the major cell type of the stroma and are scattered among the stromal lamellae.[1,3,4] They are involved in maintaining stromal homeostasis and hold the potential to create collagen molecules and glycosaminoglycans, while also creating matrix metalloproteases (MMPs).[1,3,4] Most of the keratocytes reside in the an-

terior stroma and contain corneal crystallins that are responsible for reducing backscatter.[9] In a healthy cornea, keratocytes remain dormant. They transform into myofibroblasts in response to various types of injury and participate in wound repair by producing ECM, secreting cytokines and collagen-degrading enzymes, and by contracting the edges of the wound. The collagen fibers (mainly types I and V) are structured in parallel bundles and organized in parallel-arranged lamellae.[1,3,4] Human stroma consists of 200-250 distinct lamella.[1,3,4] Each of them is aligned at right angles relative to fibers in adjacent lamellae.[10] The stroma is thicker peripherally than centrally, and as the collagen fibrils approach the limbus they may change direction to run circumferentially.[11] The ultrastructure of the lamellae varies, based on the stromal depth: deeper layers are more strictly organized than superficial layers.[3] The high degree of spatial organization of stromal fibers and extracellular matrix contributes to corneal transparency and rigidity. The posterior lamellae in the central cornea are more hydrated than the anterior lamellae and are believed to have less interlacing, resulting in easier swelling of the posterior stroma compared with the anterior stroma.[3] Stromal collagen fibrils are surrounded by specialized proteoglycan, consisting of keratan sulfate or chondroitin sulfate/dermatan sulfate side chains, which help regulate hydration and structural properties.[3]

In 2013, Dua studied the effect on corneal biomechanics and cleavage planes of injecting air into the posterior stroma as is done in deep anterior lamellar keratoplasty (DALK) with the big bubble (BB) technique. He proposed that there exists another, distinct, well-defined layer between the posterior stroma and Descemet membrane.[12] This acellular, 6-12 µm thick tissue was coined "Dua's layer", later renamed the "Dua-Fine layer".[13] It has been the source of much controversy and debate. Other groups have postulated that while this layer has a unique cohesiveness and configuration, it does not represent a distinct and separate corneal layer. Rather, the BB technique helps to describe the mechanical posterior stromal response to non-physiologic stress.[14,15]

Descemet membrane (DM) is located directly behind the posterior stroma and is the basement membrane of the corneal endothelium. DM gradually increases in thickness from 3 µm at birth to 10-12 µm in adulthood.[3] It is continually secreted by the corneal endothelium. Three distinct zones may be distinguished: a thin non-banded zone adjacent to the stroma (0.3 µm), an anterior banded zone (2-4 µm) and a posterior, amorphous, non-banded zone (>4 µm), that thickens with age. DM primarily consists of collagen types IV and VIII, laminin, and fibronectin.[16,17] DM, with its adjacent endothelium, can be peeled off from

the posterior stroma as a single sheet. Once completely detached, DM will spontaneously curl into a single or double roll.[18,19]

The *endothelium* is the innermost posterior layer of the human cornea and measures 4 μm in thickness in adulthood. This monolayer consists of tightly-packed hexagonal cells and appears as a honeycomb mosaic when viewed posteriorly.[3] The endothelium plays a key role in preserving corneal transparency by maintaining the cornea in a relative state of deturgescence.[1] The 'pump-leak' hypothesis proposes that the endothelium in a healthy cornea achieves corneal clarity by maintaining a state of equilibrium between two fluid transport pathways. A low-resistance apical junction between the endothelial cells allows fluid from the anterior chamber to 'leak' into the stroma (passive diffusion), whereas Na^+/K^+- and bicarbonate-dependent Mg^{2+}-ATPase pumps create local osmotic gradients, thereby actively returning fluid from the stroma to the anterior chamber.[1] Dysfunction of either of these pathways can result in corneal edema and reduced corneal transparency. The endothelial cell density (ECD) is approximately 6000 cells/mm^2 at birth and gradually decreases to about 3500 cells/mm^2 by the age of 5 years as the eyes grow.[20,21] During adulthood, ECD decrease slows down to an annual decrease of approximately 0.6%.[22,23] Apart from aging, accelerated cell loss may be caused by a genetic predisposition, prior intraocular surgery, trauma, elevated intraocular pressure, diabetes mellitus, and chronic anterior chamber inflammation.[24] Endothelial cells do not regenerate *in vivo*. When cells are lost, an endothelial defect will be restored by expansion (polymegathism) and active migration of adjacent cells. During this process, loss of hexagonality of the cells may occur (pleomorphism).[3,25,26] When the ECD count decreases to the extent that the overall remaining endothelial pumping capacity fails to maintain the equilibrium between the beforementioned pathways, endothelial decompensation may occur, resulting in irreversible corneal edema, reduced corneal clarity, pain and vision loss.[27]

Common indications for endothelial keratoplasty

Fuchs Endothelial Corneal Dystrophy

Fuchs endothelial corneal dystrophy (FECD) is the most common corneal dystrophy and currently one of the leading indications for corneal transplantation.[28] It was first described in 1910 by the Austrian ophthalmologist Ernst Fuchs and is a slowly progressive, bilateral corneal disease. Hallmark features of FECD include accumulation of wart-like excrescences of DM better known as 'guttae', thickening of DM, endothelial cell pleomorphism and polymegathism and loss of endothelial cells (Figs. 2,3).[29-32] With advancing disease, stromal edema may compromise visual function, with vision being worse in the morning and improving during the day. In end-stage disease, epithelial bullae may develop, evolving into subepithelial fibrosis and corneal vascularization.

Based on the time of onset of disease, two clinical subtypes of FECD may be distinguished: early-onset FECD (3-40 years) and late-onset FECD (>40 years), with the late-onset form being more common.[33] The early-onset form of FECD has been associated with autosomal dominant Q455K, Q455V and L450W mutations in the gene encoding the alpha 2 subunit collagen 8 (COL8A2). Men and women are equally affected. In contrast to early-onset FECD, a female predominance of 3:1 has been reported for late-onset FECD. Currently, 5 causal genes (TCF4, AGBL1, LOXHD1, SLC4A11 and ZEB1) and 4 causal loci on chromosomes 5, 9, 13, and 18 have been identified in individuals with late-onset FECD. Expanded repeats of the trinucleotide cytosine-thymine-guanine (CTG repeats) in the 3rd intron of TCF4 within chromosome 18q21.1 may be the most commonly identified genetic contributor to FECD.[34] Despite the identification of some genetic factors, the exact pathophysiology of FECD remains unclear and is thought to be a combination of both environmental and genetic factors. Both subtypes display a similar linear rate of disease progression.

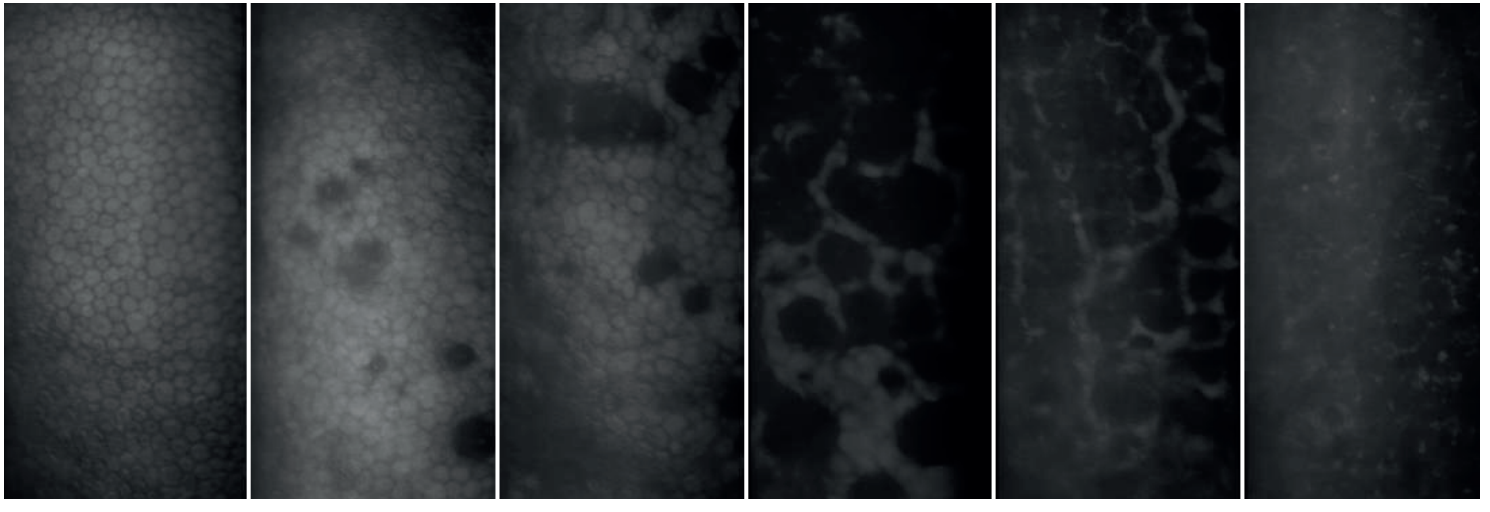

Figure 2. Specular microscopy images displaying healthy endothelium (left image) and different stages, from moderate to advanced, of Fuchs endothelial corneal dystrophy (images from left to right).

Bullous keratopathy

Bullous keratopathy develops as a result of endothelial decompensation due to endothelial injury caused by various conditions or events such as birth injury or intraocular surgery, including complicated cataract surgeries, glaucoma surgeries, or vitreoretinal surgeries. Symptoms may present in the immediate post-traumatic period or years after the injury. With advancing corneal edema, patients often manifest with (sub)epithelial bullae resulting in painful corneal micro-defects when they rupture.[35] In advanced stages, subepithelial fibrosis, with or without BL disruption, may develop.[35]

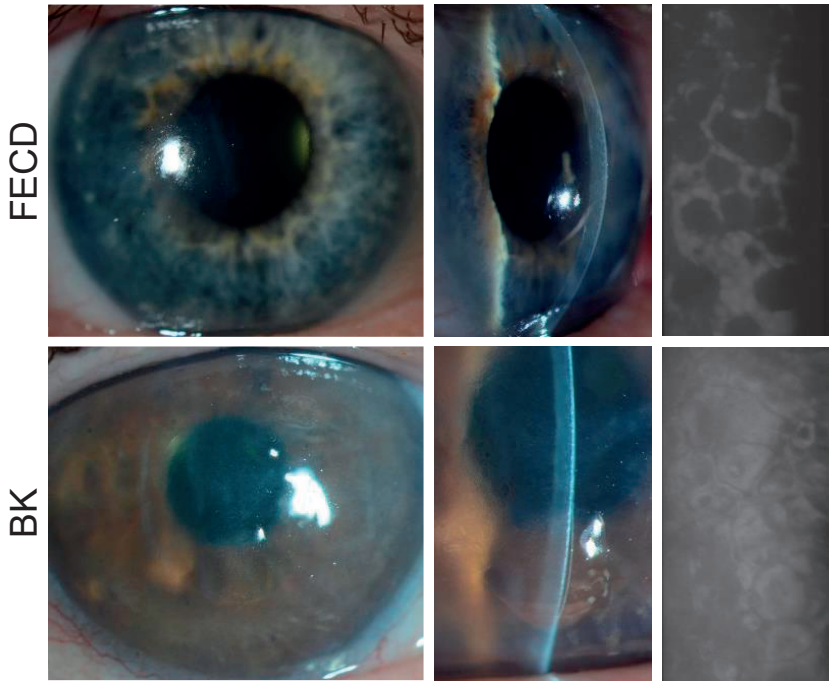

Figure 3. Slit-lamp and specular microscopy images of eyes with Fuchs endothelial corneal dystrophy (FECD) and bullous keratopathy (BK).

CORNEAL TRANSPLANTATION

History of corneal transplantation

Replacing diseased corneal tissue has been under consideration for a long time, with major changes occurring in recent years. The first description of keratoprosthesis originates from the French surgeon Guillaume Pellier de Quengsy.[36] During the French revolution in 1789, he hypothesized that a transparent material could be used to replace an opaque cornea in order to restore vision. In 1796, Erasmus Darwin proposed the first corneal trephine and postulated that the cornea might heal secondary to forming a transparent scar.[37] In 1813, Karl Himley proposed replacing opaque animal corneas with corneas from other animals, but it was not until 1818 that his student Franz Reisinger initiated these experimental animal corneal transplants.[38]In 1824, Reisinger coined the term 'keratoplasty' and proposed using animal tissue to replace human corneas. His animal experiments, however, failed to produce clear grafts. In 1837, the Irish surgeon Samuel Bigger reported his first successful penetrating graft on a pet gazelle blinded by extensive corneal scarring.[39] In 1838, inspired by Bigger, New York-based ophthalmologist Richard Kissam performed the first recorded corneal xenograft, from a 6-month old pig, on a young Irishman in 1838.[40] While increased light perception occurred immediately after the operation, the cornea opacified within the first fortnight and was absorbed within one month after the operation. For the remainder of the 19th century, the pioneers of corneal transplantation could be divided into two main groups: those who favored full-thickness allografts (Henry Powers) and those who favored partial-thickness lamellar xenografts (Arthur von Hippel).[38,41] In 1905, the first successful human allograft was performed by Eduard Zirm.[42] The recipient was a farmer who had sustained bilateral alkali burns while cleaning out a chicken coop with lime 16 months earlier. Zirm used donor tissue from the enucleated eye of an 11-year old boy whose eye had been blinded by a penetrating injury to the sclera. The eye was enucleated and the one donor cornea was used to procure two full-thickness grafts of 5 mm in diameter. While the graft in the right eye failed, the graft in the left eye remained clear and improved the visual acuity of the recipient from counting fingers preoperatively to 6/36 at 6 months after the operation. Since then, innumerable ophthalmologists and scientists have contributed to improving the technique, and in the century thereafter, penetrating keratoplasty (PK) became the mainstay of care in the treatment of all corneal disorders regardless of which layer was diseased.

History and evolution of endothelial keratoplasty

While the lamellar approach was already described with xenografts by Arthur von Hippel in 1888, it was not pursued in the decades thereafter. This was possibly because lamellar transplants were perceived to be technically more challenging than full-thickness transplants. While PK can yield an optically transparent cornea, it is also prone to potential complications such as poor wound healing, suture-related problems, high astigmatism, allograft rejection, graft failure, and unsatisfying visual outcomes, with many patients requiring contact lenses to reach their full visual potential after keratoplasty.[43,44]

Nevertheless, Charles Tillett performed the first posterior lamellar endothelial transplant underneath a manually dissected stromal flap in a patient with FECD in 1956.[45] In the 1960s, Barraquer et al. applied a similar technique which unfortunately also proved relatively unsuccessful.[46] These early attempts may have failed due to lack of suitable instrumentation to dissect thin corneal layers and limited understanding of endothelial cell physiology, resulting in early complications, and/or insufficient visual outcomes. As a result, the concept of endothelial keratoplasty was, once again, abandoned.

It was not until 1998, that Melles et al. introduced a technique for *posterior lamellar keratoplasty (PLK)*, currently known as *endothelial keratoplasty (EK)*, in which a posterior lamellar disc was excised from the recipient cornea and a same-size donor disc, consisting of posterior stroma, DM and endothelium, was implanted through a limbal scleral incision.[47] Although technically challenging, this technique provided clinical outcomes surpassing PK and circumvented many PK-associated complications.[48] In 2001, this technique was popularized as *deep lamellar endothelial keratoplasty (DLEK)* in the United States by Terry et al. (Fig. 4). In the initial PLK/DLEK technique, a donor disc was implanted into the recipient cornea through a 9-mm sclerocorneal incision and positioned against the recipient posterior cornea by means of an air-bubble.[47] In 2000, the initial technique was modified by Melles et al., folding the donor disc like a 'taco' to enable insertion through a self-sealing 5-mm tunnel incision.[49] This technique was popularized as *small incision DLEK*. Worldwide adoption was tempered by the technical difficulty of the procedure, which necessitated manual dissection of both donor and host tissue.

To simplify the technique, Melles et al. abandoned recipient stromal dissection and introduced 'descemetorhexis', a new approach in which only recipient DM and endothelium were stripped, using a reversed Sinskey hook.[50] Des-

cemetorhexis was followed by implantation of a taco-folded donor disc, which was subsequently positioned onto the denuded host posterior stroma with an air-bubble. This approach was first performed clinically in 2001 and was later popularized by Price et al. as *Descemet stripping endothelial keratoplasty (DSEK)*.[50-52] Gorovoy et al. further simplified the technique by introducing an automated microkeratome to dissect the donor graft from a corneoscleral button mounted on an artificial anterior chamber. [53] This modification changed the nomenclature to *Descemet stripping automated endothelial keratoplasty (DSAEK)* (Figs. 4, 5). After these refinements in technique, the worldwide adoption of DSEK/DSAEK grew exponentially and it became the preferred treatment option for corneal endothelial disorders.[54]

Although DSEK/DSAEK represents a massive improvement compared to its predecessors, it still has some drawbacks. Even after technically successful transplantations, final visual acuity is variable and occasionally unsatisfyingly low. This has among others been ascribed to the presence of varying thickness of posterior stroma within the donor graft.[54-60]

In 2002, Melles et al. further refined the concept of endothelial keratoplasty by completely eliminating the posterior stroma from the donor graft, allowing selective replacement of bare DM with its endothelial layer. [49] This technique was coined *Descemet membrane endothelial keratoplasty (DMEK)* and was first performed successfully in a patient in 2006 (Figs. 4, 5).[61]

Descemet Membrane Endothelial Keratoplasty

After its introduction in 2006, the surgical procedure was further refined and standardized and as a result, a standardized 'no-touch' DMEK-technique was introduced in 2011.[62] The technique entailed scoring and descemetorhexis under air followed by an air-fluid exchange and implantation of a DMEK graft, ideally folded into a double roll with the curls facing upward, into the recipient anterior chamber. The DMEK graft was then unfolded over the iris by means of an air bubble injected in between the two curls and corneal tapping, and lifted against the recipient posterior stroma by inserting an air bubble underneath the DMEK graft. At the end of the surgery, a complete air fill of the anterior chamber was maintained for 60 minutes, after which an air-liquid exchange was performed to pressurize the eye and promote graft adherence.

Since its implementation, DMEK has shown to provide faster visual rehabilitation, improved visual outcomes, and lower graft rejection rates compared with

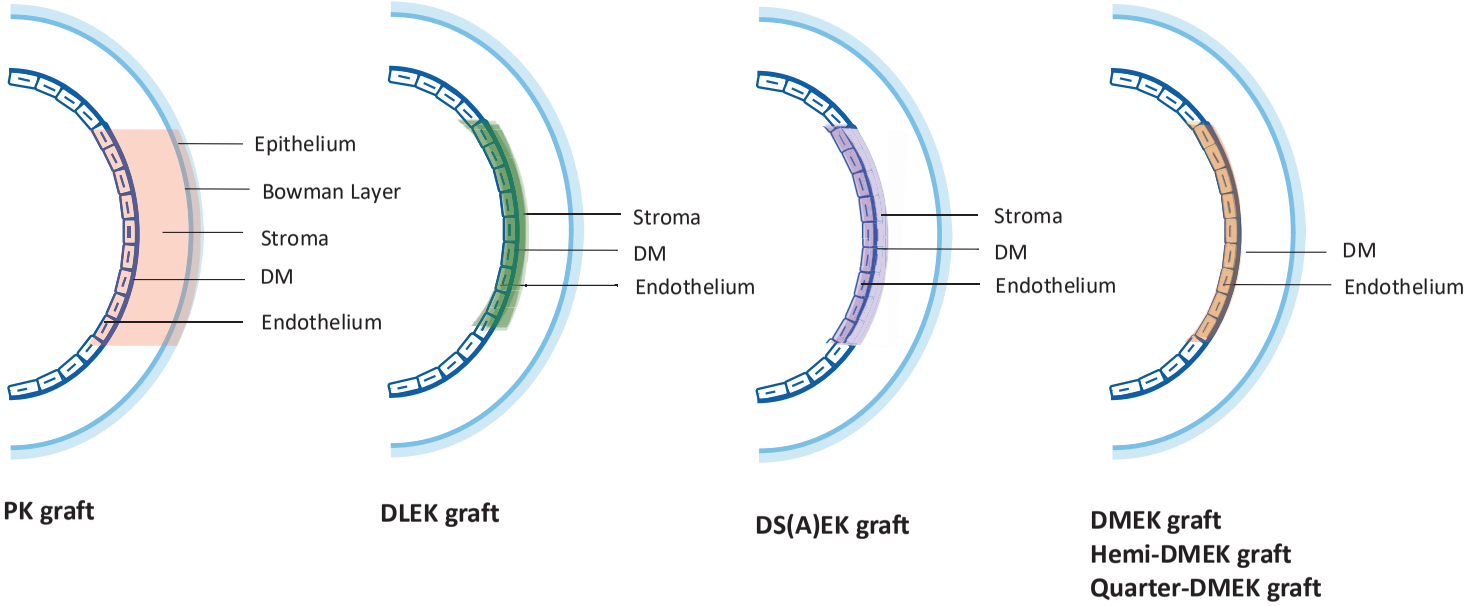

Figure 4. Schematic overview displaying the evolution of posterior keratoplasty techniques, from left to right: Penetrating keratoplasty (PK), Deep lamellar endothelial keratoplasty (DLEK), Descemet stripping (automated) endothelial keratoplasty (DS(A)EK) and conventional, Hemi- and Quarter-Descemet membrane endothelial keratoplasty (DMEK, Hemi-DMEK and Quarter-DMEK). DM= Descemet membrane

earlier EK-techniques.[63-70] In 2015, the American Academy of Ophthalmology evaluated the clinical efficacy, effectiveness and safety of DMEK by means of a systematic review.[71] The assessment revealed that 11 studies with 6-month clinical outcomes after DMEK reported that 32% to 85% of eyes achieved a BCVA of 20/25 or better, and 12 studies reported that 17% to 67% achieved a BCVA of 20/20 or better. Comparison of final visual acuity levels after DMEK and DSEK showed that, after surgery, a higher percentage in the DMEK group achieved a BCVA of 20/25 or better (50% vs 6%, 67% vs 31%, 53% vs 15% and 55% vs 13%) and a BCVA of 20/20 or better (46% vs 13%). Complications of DMEK include graft detachment, graft failure, allograft rejection, and endothelial cell loss. The mean rejection rate of 22 studies was 1.9% (range, 0% - 5.9%) during follow-up periods ranging from 6 months to 8 years. This is lower than the mean rejection rate of 10% (range, 0% - 45.5%) reported after DSEK.

Owing to its excellent results, an increasing number of corneal surgeons are adopting DMEK globally, and with increasing surgical experience complication rates are decreasing.[71] DMEK is nowadays increasingly employed in challenging cases such as eyes with anterior chamber intraocular lens implants and eyes with glaucoma drainage devices.[72-76]

Corneal graft failure

Corneal graft failure is an irreversible loss of corneal transparency due to graft dysfunction and thereby may become an indication for repeat keratoplasty. Graft failure is considered “primary”, if the cornea never cleared to regain satisfactory vision after the transplant surgery, or “secondary”, if the cornea initially cleared, but then decompensated at a later time point.[77] Predisposing risk factors for graft failure include previous graft failure, glaucoma (especially previous tube shunt surgery), peripheral anterior synechiae, corneal vascularization, immunologic allograft rejection, and ocular surface disease, especially lack of tears.[78] Signs of corneal graft failure include increased corneal thickness and corneal edema. Initial treatment consists of topical corticosteroid and hypertonic saline drops. Definitive treatment requires a repeat corneal transplantation.

Auxiliary techniques

As DMEK may still be perceived as relatively challenging in preparing and handling of the delicate donor graft, alternative keratoplasty techniques such as *Ultra-thin DSAEK* (in which a thin layer of posterior stroma (<100 µm) is transplanted as part of the donor lenticule), *pre-Descemet endothelial keratoplasty (PDEK) (*in which an even thinner layer of posterior stroma ‘the

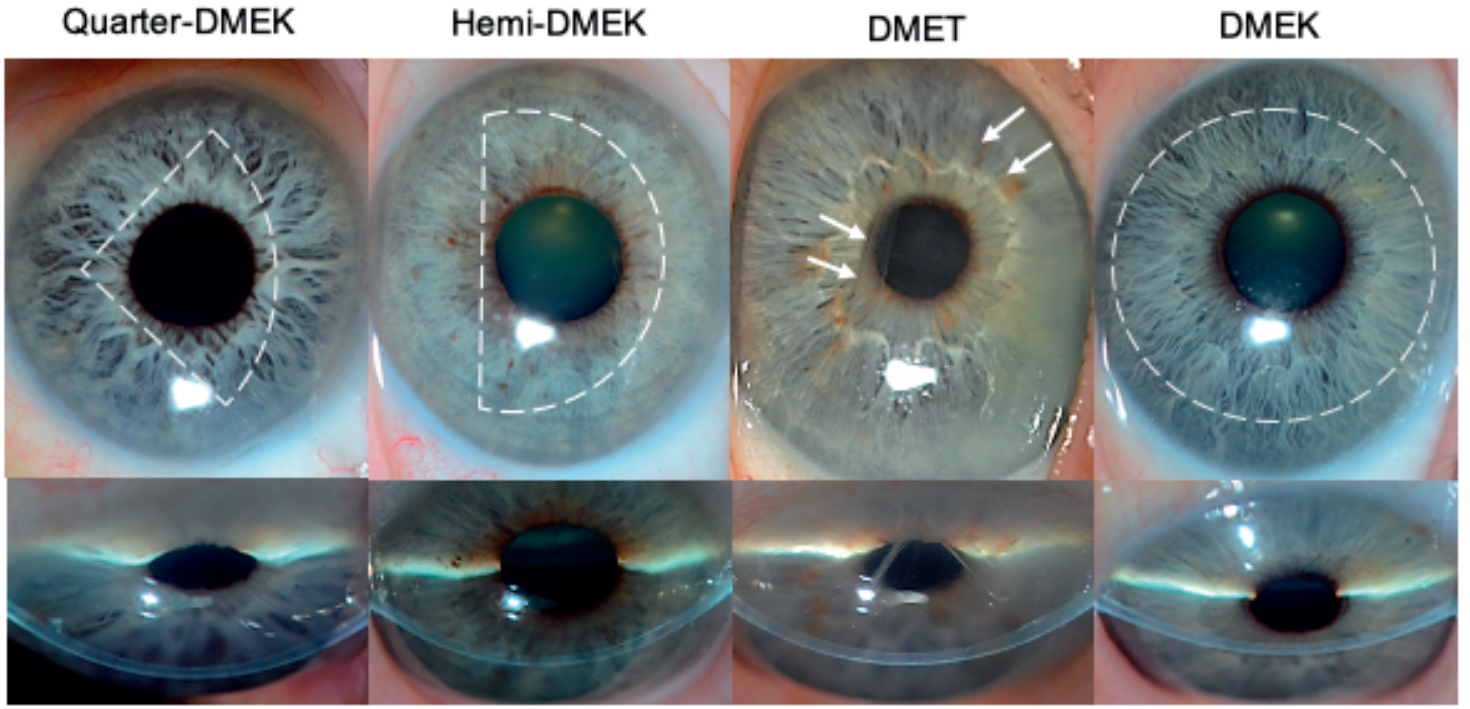

Figure 5. Slit-lamp images of eyes that underwent Descemet membrane endothelial keratoplasty (DMEK), Descemet membrane endothelial transfer (DMET), Hemi-DMEK and Quarter-DMEK. White dashed lines indicate the graft outline.

pre-Descemet layer' (<20 μm) is transplanted with the donor lenticule), and *DMEK with a stromal rim (DMEK-S)* were introduced as a middle way to allow for easier preparation and handling of the DMEK graft combined with visual outcomes possibly equaling those of DMEK.[79-81]

MODIFIED DMEK TECHNIQUES

Descemet Membrane Endothelial Transfer

The clinical observation that corneas showed resolution of corneal edema in the first few weeks after DMEK/DSAEK, despite (partial) graft detachment or in the absence of a DMEK graft, led to the introduction of *Descemet membrane endothelial transfer (DMET),* which consists of a descemetorhexis followed by insertion of the almost completely free-floating Descemet roll (i.e., with the graft contacting the posterior cornea only at the corneal incision) in 2008 (Fig. 5).[82-98] While preliminary results showed that DMET was effective in the management of eyes with FECD, it was not in eyes with BK.[85,98] This prompted the hypothesis that host endothelial cells in eyes with FECD still had some regenerative capacity and had retained the potential to migrate to bare stromal areas to repopulate them. This hypothesis was reinforced by case reports which reported corneal clearance after 'descemetorhexis only'.[86,87,89,93,99,100] However, mixed results have been reported for the latter technique, with a significant number of corneas failing to clear. A major drawback of DMET and 'descemetorhexis only' is that host peripheral endothelial cell migration is a relatively slow process and that, if corneal clearance occurs at all, it may take up to several months.

Hemi- and Quarter-Descemet membrane endothelial keratoplasty

As there is a substantial shortage of donor tissue for endothelial keratoplasty worldwide, which has not yet been met by the implementation of beforementioned techniques, further refinements of DMEK were introduced.[28] In 2014, Hemi-DMEK was introduced aiming to potentially double the availability of endothelial donor tissue (Fig. 5).[101] Hemi-DMEK represents a DMEK modification that differs from conventional DMEK only in graft shape. In Hemi-DMEK, an 'untrephined', full-diameter, semicircular (half-moon shaped) graft is utilized rather than a circular trephined Descemet graft.[102] As a Hemi-DMEK graft is untrephined and a conventional DMEK graft is trephined, both have a comparable graft surface area and a comparable number of endothelial cells is transplanted. Preliminary Hemi-DMEK studies have yielded visual outcomes

similar to those following conventional DMEK.[103-105] Longer-term studies are needed to determine whether the outcomes remain stable.

Mixed clinical outcomes after DMET and Hemi-DMEK and 'descemetorhexis only' led to the development of *Quarter-Descemet membrane endothelial keratoplasty (Quarter-DMEK*).[106] Quarter-DMEK is a hybrid technique that aims to combine the advantages of both DMEK (fast corneal clearance) and 'descemetorhexis only' (host peripheral endothelial cell stimulation). In this relatively new technique, merely one quarter of a full-diameter donor Descemet graft is transplanted into eyes where FECD is limited to the central 6-7 mm optical zone of the cornea (Fig. 5). The first case report of Quarter-DMEK was published in 2016.[106] Quarter-DMEK showed promising visual acuity outcomes, but had a few drawbacks, including a higher rate of postoperative graft detachment, a steeper decline in endothelial cell density in the first 6 months after surgery and prolonged corneal clearance in some parts of the cornea.[107] Additional studies are needed to determine the efficacy of Quarter-DMEK relative to conventional circular DMEK.

EYE BANKING AND CORNEAL TRANSPLANTATION

Since the establishment of the first eye bank by Dr. Townley Paton in 1944, eye banks continue to play a key role in procuring, evaluating and distributing donated ocular tissue for transplantation and research. The evolution of PK to selective, lamellar EK was facilitated by a strong, symbiotic relationship between corneal surgeons and eye banks, especially since dissecting lamellar grafts has been perceived as more challenging than preparing full-thickness PK grafts. A successful outcome after keratoplasty largely depends on viable corneal endothelium.[108] Hence, the morphologic and functional status of the endothelium is the most important determinant for donor cornea suitability for transplantation and maintaining endothelial cell viability from the time of donor tissue retrieval until transplantation. Currently, two preservation methods are being applied by eye banks: hypothermic storage at 2-6ºC and organ culture storage at 30-37ºC.[109] Prolonged storage of donor tissue allows for extensive donor screening and facilitates surgical scheduling.

As with other endothelial keratoplasty techniques, donor tissue for DMEK may be prepared by corneal surgeons prior to surgery (surgeon-cut) or by experienced tissue specialists in an eye bank; this may take place up to 2 weeks

before surgery (pre-cut).[19,20,110,111] Pre-cut tissue may reduce overall intervention costs and surgery time, and allows for post-processing evaluation of the donor graft, providing corneal surgeons with accurate information about the donor tissue prior to surgery.[112]

Various techniques have been described for DMEK graft preparation, which may broadly be classified into those based on manual peeling and those aiming to achieve detachment of Descemet membrane (DM) by either injecting air or liquid between DM and the posterior stroma. Lie et al.[18] described the initial technique for DMEK graft preparation. A donor corneoscleral rim was mounted onto a custom-made fixation device with the endothelial side up. DM was cut anterior to the trabecular meshwork and pushed towards the center of the corneoscleral button. Grasping the outer edge of the graft, DM was loosened over 180 degrees and stripped for two-thirds. By submerging the rim in balanced salt solution (BSS), superficial trephination and complete stripping of DM were facilitated, after which the isolated graft spontaneously formed a roll with the endothelial layer facing outward. Groeneveld-van Beek et al.[19] modified the technique into the standardized "no-touch" technique, in which DM with the adjacent trabecular meshwork is loosened over 360 degrees rather than over 180 degrees and trephined on a soft contact lens instead of on the anterior cornea. The latter technique allows complete stripping of DM and facilitates further handling of the graft. It allows the user to obtain the maximum possible graft size, minimizes endothelial cell damage in the trephination area and leaves the anterior cornea intact and eligible for anterior lamellar keratoplasty. All preparation techniques feature different strengths and weaknesses which will be discussed in this thesis.

CORNEAL IMAGING TECHNIQUES AFTER ENDOTHELIAL KERATOPLASTY

Non-invasive corneal imaging modalities have proven to be useful diagnostic tools for evaluating graft adherence and graft function after EK. While slit-lamp biomicroscopy is the mainstay of corneal evaluation, Scheimpflug imaging and anterior segment optical coherence tomography (AS-OCT) may aid in assessing corneal optics and complications. Additionally, specular microscopy allows for analysis of endothelial cell density (ECD) and morphology.

Slit-lamp biomicroscopy is readily available in all ophthalmic clinical settings and aids in the assessment of graft adherence and corneal transparency after endothelial keratoplasty.[113] In the presence of corneal edema, however, it is not always possible to conclusively determine whether the DMEK graft is completely attached or not. 'Flat detachments', i.e. when the DMEK graft is not attached and positioned just parallel to the recipient posterior stroma, may be especially challenging to correctly interpret without the aid of imaging technology.[114] Auxiliary corneal imaging techniques, preferably AS-OCT, can be implemented to ensure that a (partially) detached graft in an eye with severe corneal edema does not go undetected. These techniques may, additionally, help to differentiate between a detached DMEK graft and an attached graft showing delayed corneal clearance, which may occur for instance due to a 'shock to the donor endothelial cells' pumping function.[115]

Corneal tomography analysis of the anterior segment utilizes a camera (based on the rotating Scheimpflug principle) perpendicular to a slit beam which can capture up to 100 images in two seconds (e.g. Pentacam HR). These images are used to create a 3-D model of the anterior segment of the eye and to provide quantitative data such as central radii, corneal asphericity, maps of curvature and elevation, chamber angle, chamber volume and chamber elevation as well as lens transparency.[116] Pentacam Scheimpflug imaging can aid with evaluating corneal astigmatism after keratoplasty and graft adherence after endothelial keratoplasty.[117] A drawback of this technique may be that, particularly in corneas with extensive corneal edema, backscatter may occur, which may impede adequate visualization of the graft and correct interpretation of graft adherence.[114,117] In addition, the Scheimpflug Pentacam uses Zernike polynomials to provide data on corneal wavefront aberrations. This can be valuable in detecting corneal irregularities which may explain unsatisfactory vision after endothelial keratoplasty.[118-120] Densitometry analysis can provide information on stromal opacities possibly affecting the quality of vision and the Pentacam can be applied to analyze the refractive stability of the cornea after endothelial keratoplasty. [121,122]

AS-OCT is a non-invasive imaging modality that provides both quantitative and qualitative information. It has a broad range of clinical applications. It generates two- and -three-dimensional cross-sectional images of tissue by integrating multiple axial scans (A-scans) into a composite lateral beam of light, the B-scan.[123,124] Time domain AS-OCT utilizes a light source emitting at 1310 nm, which offers the advantage of minimized scatter and high penetra-

tion.[123,124] This technique is particularly suited for imaging structural details in optical scattering media such as an edematous cornea, when slit-lamp biomicroscopy and Pentacam may fail to provide conclusive information. Recently, high-speed Fourier domain OCT (FD-OCT) has been introduced, which offers improved spatial resolution compared to time domain OCT. FD-OCT allows in vivo high-speed, high-resolution imaging of weakly backscattering tissues and can detect changes within a 10 μm range in corneal tissue.[125,126] Pre-operatively, AS-OCT may be employed to assess the thickness of the recipient cornea and to estimate the potential size of the graft. Intraoperatively, the OCT may be employed to visualize and assess graft orientation in DMEK surgery; especially in the presence of severe corneal edema, it may lead to faster graft positioning with less graft manipulation.[127] Postoperatively, AS-OCT may aid in detecting complications such as graft dislocation, anterior chamber angle narrowing, and pupillary block.[114,122] In addition, AS-OCT can precisely specify the extent and planarity of graft detachments. In the immediate postoperative period, when there is still an air-bubble in the anterior chamber, AS-OCT images should be interpreted with care as the edges of the air-bubble may reveal themselves as a separate line and may therefore mimic graft detachment. However, the air-bubble commonly presents as a relatively smooth line in comparison to a graft detachment.

Specular microscopy is a non-invasive imaging modality. It is currently the most widely applied diagnostic tool for evaluating the corneal endothelium, as it allows for *in vivo* visualization and analysis of the endothelium.[128-130] It is based on the reflection of the incoming light generated by the difference in refractive index of the endothelial cells and the aqueous humor.[128] As the main objective of endothelial keratoplasty is to regain endothelial function and subsequently corneal transparency, the donor endothelium should be closely monitored during the postoperative course.[128-130] Endothelial cell density is a key quantitative corneal endothelial parameter for evaluating the clinical outcome after keratoplasty, and polymegathism (cell size variability) and pleomorphism (cell shape variability, loss of hexagonal shape) are important qualitative indicators.[131,132] Image quality may be compromised by corneal pathology such as scarring or edema, which can increase light scattering in the stroma from collagen lamellae and keratocytes.[133] Commercial specular microscopes are usually provided with an automatic ECD analysis program. However, sufficient quality of the acquired images, with clearly displayed cell borders, and manual correction, is usually required to ensure reliable ECD measurements.[128,130,134]

AIM AND OUTLINE OF THIS THESIS

This thesis focuses on donor tissue preparation for DMEK and evaluates the feasibility and clinical outcomes of DMEK, DMET, Hemi-DMEK and Quarter-DMEK in the management of corneal endothelial disorders.

The first part of this thesis concerns donor tissue preparation for DMEK.
We tested whether the technique of DMEK graft dissection influences the clinical outcome after DMEK. ***Chapter 2*** provides an overview of the current harvesting techniques available for DMEK and a discussion of these techniques.

The second part of this thesis concerns the clinical outcome of selective, minimally-invasive and potentially tissue-sparing surgical treatment options for corneal endothelial disorders. We hypothesize that complete and lasting corneal rehabilitation may not always require a (nearly) fully, centrally attached large DMEK graft. We evaluated the six-month clinical results of 1000 consecutive DMEK cases and evaluated whether whether outcomes are influenced by surgical indication and preoperative lens status (**Chapter 3**).
Subsequently, we evaluated the five-year graft survival and clinical outcomes of 500 consecutive DMEK cases (**Chapter 4**). The feasibility and clinical outcomes of DMEK in eyes with a glaucoma drainage device are being described in **Chapter 5**. The next three chapters focus on the different endothelial grafting techniques, evaluating subtotal detachment of the DMEK graft after a DMEK procedure or intended DMET (**Chapter 6**), and outcomes of Hemi-DMEK (**Chapter 7**) and of Quarter-DMEK performed for FECD (**Chapter 8**).

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