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Long-Term Results after DMEK (Descemet's Membrane Endothelial Keratoplasty)

Inaugural-Dissertation zur Erlangung des Doktorgrades der gesamten Humanmedizin

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vorgelegt von

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Abbreviations:

AMD: age related maculopathy

Anti-VEGF: anti-vascular endothelial growth factor

BSS: balanced salt solution

BCVA: best corrected visual acuity

CCT: central corneal thickness

CME: cystoid macular edema

CV: corneal volume

D: Dioptre

DALK: Deep anterior lamellar keratoplasty

DSAEK: Descemet's stripping automated endothelial keratoplasty

ECD: endothelial cell density

ES: endothelial cells' score

FED: Fuchs endothelial dystrophy

i. e.: this means

iol: intraocular lens

IOP: intraocular pressure

mmHg: millimeter of mercury

ND:YAG-Laser: Yttrium-Aluminium-Garnet-Laser

OCT: optical coherence tomography

POAG: primary open angle glaucoma

PK: penetrating keratoplasty

SD: standard deviation

SF6: sulfur hexafluoride

TCF4: transcription factor 4

Abstract

Purpose: To evaluate the long-term clinical outcomes and rate of complication after Descemet's membrane endothelial keratoplasty (DMEK)

Design: a cross-sectional, case series study

Methods: 142 patients who underwent DMEK in the University Eye Clinic in Marburg, Germany between 2010 and 2014 were included (230 eyes). We evaluated the best corrected visual acuity (BCVA), refraction, central corneal thickness (CCT), corneal volume (CV) and endothelial cells density (ECD) and compared them to the preoperative values. The graft survival and rate of post-operative complications were also evaluated.

Results: Mean follow-up time was 47 ± 13.3 months. The BCVA improved from 0.60 ± 0.32 logMAR preoperatively to 0.10 ± 0.22 logMAR in patients with no other ocular comorbidities (201 eyes). 71.1% of the patients with no ocular comorbidities had a BCVA of 0.11 logMAR or better (≥ 0.8 decimal), whereas 49.2% of them had a full BCVA of 0.00 logMAR or better. The CCT decreased from 675 ± 112µm preoperatively to 547 ± 52 µm 4-7 years after DMEK and the CV decreased from 65.2 ± 8.4 mm^2 preoperatively to 61.9 ± 5.4 mm^2. The endothelial cells loss was 1392 ± 455 cells/mm^2, which corresponds to a total loss of 54.7% of the graft cells on average. The graft survival rate was 92% with an average survival time of 76.6 ± 1.3 months.

Conclusion: DMEK provides high visual outcomes that may remain stable 4-7 years after the operation. DMEK has a high graft survival rate and a relatively low rate of postoperative complications. This renders DMEK a first-line treatment of endothelial cells diseases nowadays.

1- Introduction:

1.1 The Human Eye [71, 82]:

The human eye is located in the orbital cavity, protected within its rigid bony walls. The average antero-posterior diameter of the eyeball is about 24mm. The vertical diameter is about 23mm and the horizontal one is about 23.5mm in average. The circumference of the eyeball is around 74.91mm at the equator. Its volume is approximately 6.5 cm^3 and its weight is about 7.5 gram in adults.

The eyeball does not actually have the shape of a ball. It consists of two parts, or spheres, of different sizes placed in front of each other. The anterior part, the clear cornea, has the radius of curvature of 8mm. It is transparent and constitutes one-sixth of the eyeball. The other part, the sclera, has the radius of curvature of 12mm. It is opaque and forms about five-sixth of the eyeball.

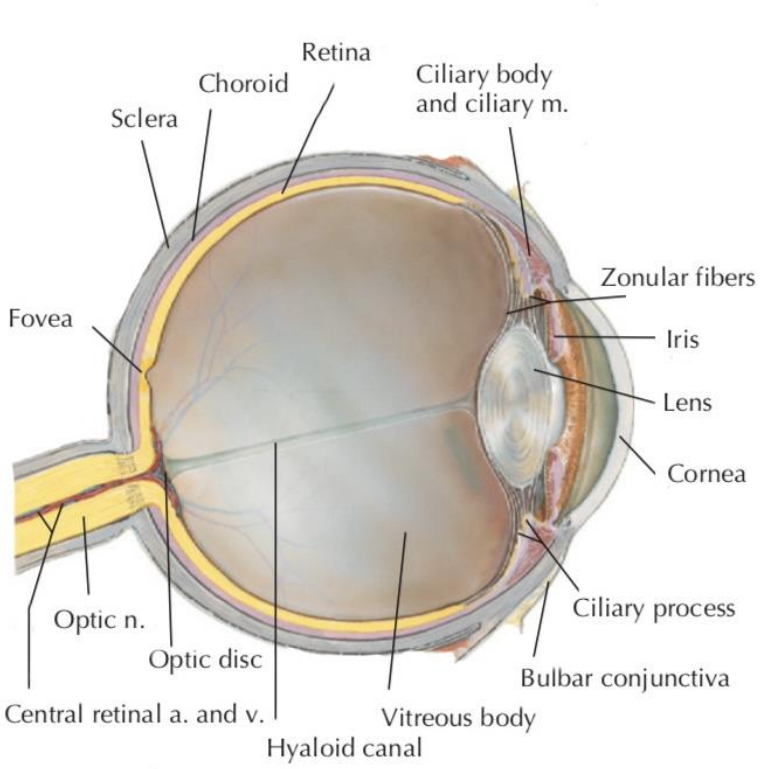

Figure 1: sagittal cut showing the anatomy of the human eye (Modified from source: Netter's clinical Anatomy. John T. Hansen. 3.rd Edition. 2014 by Saunders, Elsevier)

1.2. **Anatomy of the Cornea [71, 82]:**

The transparent cornea constitutes the most anterior one-sixth of the human eye. It is the main organ responsible for vision through its function in refracting the light that enters the eye. It separates the air, which has a refractive index of 1.00, from the aqueous humor, which has a refractive index of 1.33. With its refractive index of 1.376, the cornea has a total central refractive power of 43.1 D.

The cornea is a convex structure but has an elliptical shape. Its diameters are approximately 10.6mm vertically and 11.7mm horizontally in adults. The anterior surface and the posterior surface of the cornea have a radius of curvature of about 7.7mm and 6.9mm, respectively. The thinnest part of the cornea is at its center, where it is 540µm thick on average, whereas the thickest part is at the periphery, where it can be 700µm thick. The weight of the cornea is about 10mg. It has a surface area of 1.3cm^2 approximately, which constitutes about 1/14 of the surface of the human eye.

The normal cornea is free of blood vessels. It is supplied by the ends of the first deviation of the trigeminal nerve (cranial V). About 50-450 sensory neurons end in the cornea, making it the most densely innervated structure of the human body [69].

The cornea consists of five layers. From front to back are:

1.2.1. The Epithelium [43, 71]:

It is a layer of stratified squamous non-keratinized cells. It is about 50-100 µm thick and constitutes 5-10% of the total corneal thickness. It consists of 5-6 layers of cells. The superficial layer consists of 2-3 layers of flat cells with horizontal nuclei. They are attached to each other by desmosomes. These cells do not keratinize in the normal cornea. The middle layer consists of wing cells, which have a polyhedral shape, convex anterior surfaces and concave posterior surfaces. The deep layer consists of basal cells, which are columnar tall cells forming a single layer resting on the basal membrane. The epithelium does not have melanocytes, except in dark skinned people where they

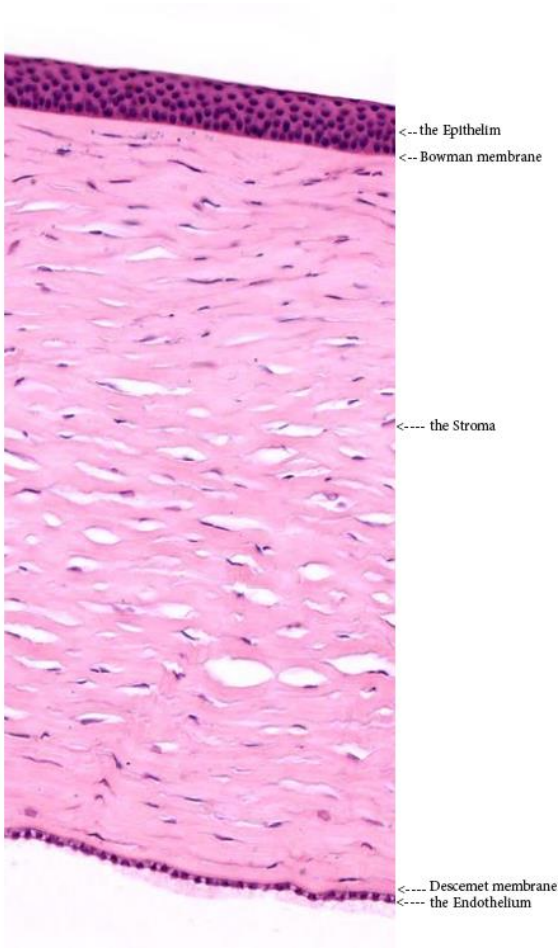

Figure 2: Histology image showing the five layers of the cornea (Modified from Histology guide, virtual histology laboratory. http://160.94.138.53/index.html)

could be found at the limbus. Langerhans cells, which are immunocompetent cells, are present in the periphery of the epithelium. The epithelium does not have melanocytes, except in dark skinned people where they could be found at the limbus. Langerhans cells, which are immunocompetent cells, are present in the periphery of the epithelium. The epithelium contains stem cells, which are responsible for maintaining a healthy surface of the cornea. These cells are mostly located at the superior and inferior limbus, especially in the palisades of Vogt. The epithelial cells need 7 days to complete the turnover of the corneal surface. The epithelium has the function of a barrier layer beside its role in the optical system of the eye as a reflective surface.

1.2.2. Bowman's Membrane (the anterior limiting lamina) [8, 71, 82]:

The Bowman's membrane lies immediately posterior to the epithelial basal membrane. It is 8-14 µm thick. It consists of acellular collagen fibers, which are randomly packed. The Bowman membrane does not regenerate when damaged but it is rather replaced with scar tissue. Its function is thought to be preventing the stromal keratocytes from exposure to growth factors secreted by epithelial cells.

1.2.3. The Stroma [8, 71, 82]:

90% of the corneal thickness is formed by the stroma. It consists of about 200 layers of collagen fibers, which are narrow and uniform in diameter. These layers are parallel to the corneal surface. Each layer is formed by parallel collagen fibers that run from limbus to limbus. Adjacent layers are positioned in a way in which the parallel collagen fibers form a 90° angle with the parallel fibers of the adjacent layer. This arrangement is important to give the cornea its transparency. These layers of collagen are embedded in glycosaminoglycans. Between these collagen layers, stromal cells called keratocytes are found. The cornea has about 2.4 million keratocytes, which synthesize collagen and proteoglycan. In addition, macrophages, lymphocytes and polymorphonuclear leukocytes are sometimes found in the stroma. The stroma cannot regenerate after damage.

1.2.4. Descemet's membrane (the posterior limiting lamina) [8, 71, 82]:

Descemet's membrane is the basal membrane of the endothelium. It is a strong homogeneous membrane consisting of type IV collagen fibers. It is about 3-4 µm thick at birth and 10-12µm thick at adulthood. Descemet's membrane is easily separated from the stroma and the endothelium. However, it can regenerate after damage. Decsemet's membrane forms protrusions into the anterior chamber at the periphery of the cornea, which are called Hassel-Hanle bodies.

1.2.5. The endothelium[8, 71, 82]:

It consists of a single layer of flattened hexagonal cells 20 µm in diameter and 5µm thick. The young adults have an average of 500,000 endothelial cells, with a density of about 3000/mm². With age, the number of cells decreases at around 0.6% per year and the remaining cells spread and get thinner. The endothelial cells cannot regenerate. If injured, healing occurs by migration, rearrangement and enlargement of the residual cells [8, 71, 82]. This layer acts as a barrier between the aqueous humor and the stroma. It is also very important to control the hydration of the cornea as well as nutrition. This is achieved by the leaky barrier that the endothelium forms through the apical gap and macula occludens junctions. The ATPase-dependent metabolic pump located in the lateral plasma membrane also plays an important role in this function, which is important to maintain the corneal clarity [83].

1.3. Pathology of the cornea:

The corneal pathology is very variable. Some of the corneal diseases with an inflammatory nature are caused by different forms of injury such as trauma, chemical or physical injury of infections caused by bacteria, viruses or fungi. Non-inflammatory diseases include many groups of corneal diseases such as corneal degenerations, corneal ectasias and corneal dystrophies[43].

Corneal dystrophies describe a group of non-inflammatory diseases. They are bilateral, progressive hereditary disorders [84]. The corneal dystrophies have been historically classified depending on their histological location. The classification of ICD3 in 2005 divided the corneal dystrophies into 4 categories, depending on the knowledge of their clinical findings and genetic analysis [84]:

Category 1: clinically and histologically well-defined corneal dystrophies with specifically defined mutations and well known gene-mapping

Category 2: clinically and histologically well-defined corneal dystrophies with one or more known chromosomal loci, but yet unidentified genes

Category 3: clinically and histologically well-defined corneal dystrophies with yet unmapped chromosomal loci

Category 4: new, yet undefined corneal dystrophies

Corneal dystrophies are disorders that require a corneal transplantation when the sight is affected.

Table 1: the ICD3 classification of the corneal dystrophies (C = Category) [84]

Epithelial and subepithelial dystrophies	
1. Epithelial basement membrane dystrophy (EBMD)	Usually degenerative, rarely C1
2. Epithelial recurrent erosion dystrophies (EREDs)—Franceschetti corneal dystrophy (FRCD), Dystrophia Smolandiensis (DS), and Dystrophia Helsinglandica (DH)	C3
3. Subepithelial mucinous corneal dystrophy (SMCD)	C4
4. Meesmann corneal dystrophy (MECD)	C1
5. Lisch epithelial corneal dystrophy (LECD)	C2
6. Gelatinous drop-like corneal dystrophy (GDLD)	C1
Epithelial–stromal TGFBI dystrophies	
1. Reis–Bücklers corneal dystrophy (RBCD)	C1
2. Thiel-Behnke corneal dystrophy (TBCD)	C1
3. Lattice corneal dystrophy, type 1 (LCD1): variants (III, IIIA, I/IIIA, IV)	C1

4. Granular corneal dystrophy, type 1 (GCD1)	C1
5. Granular corneal dystrophy, type 2 (GCD2)	C1
Stromal dystrophies	
1. Macular corneal dystrophy (MCD)	C1
2. Schnyder corneal dystrophy (SCD)	C1
3. Congenital stromal corneal dystrophy (CSCD)	C1
4. Fleck corneal dystrophy (FCD)	C1
5. Posterior amorphous corneal dystrophy (PACD)	C1
6. Central cloudy dystrophy of François (CCDF)	C4
7. Pre-Descemet corneal dystrophy (PDCD)	C1 or C4
Endothelial dystrophies	
1. Fuchs endothelial corneal dystrophy (FECD)	C1, C2, or C3
2. Posterior polymorphous corneal dystrophy (PPCD)	C1 or C2
3. Congenital hereditary endothelial dystrophy (CHED)	C1
4. X-linked endothelial corneal dystrophy (XECD)	C2
Removed dystrophies	
Grayson-Wilbrandt corneal dystrophy (GWCD)	C4

1.3.1. Fuchs endothelial dystrophy:

Fuchs endothelial dystrophy (FED) is a common endothelial dystrophy, first described by Ernst Fuchs in 1910 [57]. It is a bilateral slowly progressive irreversible disease, sometimes asymmetrical. FED affects women 3 times more than men. It seems to affect patients with open angle glaucoma more often [43]. This disease is more common in old patients in the fifth or sixth decade, but there are cases of early onset. It affects about 4% of the people over the age of forty [63]. Most cases of FED are sporadic, whereas some cases are autosomal dominant. It is thought that following genes play a role in its inheritance: Locus 13p Tel-13q12.13, 15q, chromosome 18, 18q21.2-q21.32; early-onset variant: 1p43.3-p32. A mutation in COL8A2 gene was found to be associated with early-onset FED [26]. In addition, the transcription factor 4 (TCF4) on chromosome 18 is thought to be related to the genetics of FED [7, 26]. A meta-analysis study suggested a relation between the four variations of TCF4 (rs613872, rs2286812, rs17595731, and rs9954153) and the risk of FED [46].

As mentioned above, FED can be classified under Category 1, 2 or 3. Category 1 are cases with early onset, category 2 are cases with known genetic loci by which the gene is not localized yet. In these cases, transcription factor 4 (TCF4) may be involved. Category 3 are cases with no known inheritance [7].

FED is characterized by accelerated loss and dysfunction of the endothelial cells. Microscopically, these cells seem to be larger (polymegathism) and more polymorphic (pleomorphism) than normal endothelial cells. The dysfunction of these cells causes deposition of collagen and extracellular matrix in the Descemet's membrane, which results in the thickening of this membrane. The number of the Na^+-K^+ -ATPase pump sites is reduced, which causes the dysfunction of this pump. In this stage, the endothelial cells lose their function as a barrier, which causes corneal swelling or corneal edema [7].

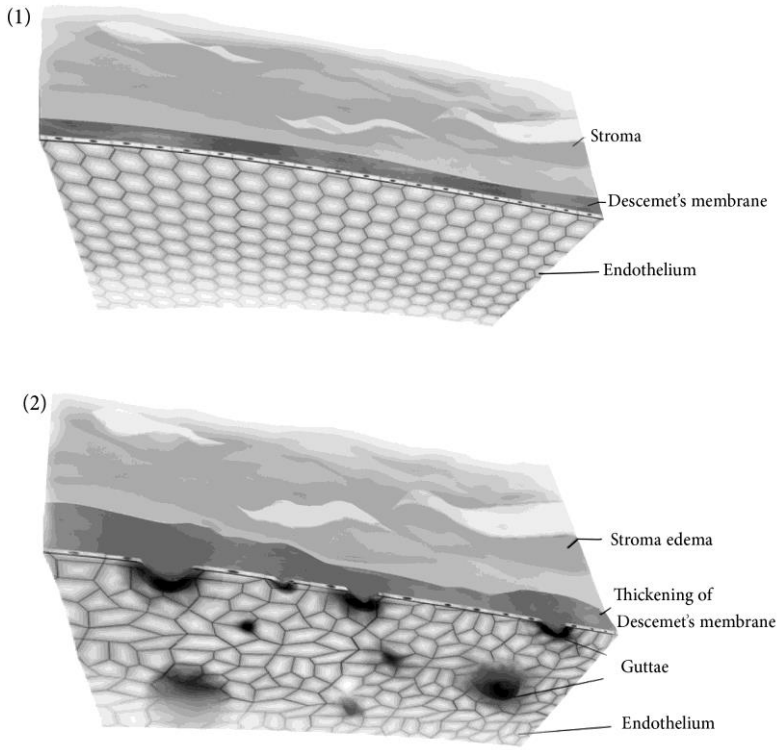

Figure 3 (1) presentation of the normal cornea, (2) presentation showing the pathology of FED

(modified after KA. Wojcik)

In FED, anvil- or mushroom-shaped excrescences of Descemet's membrane are found. These are called guttae and are formed from dystrophic endothelial cells. They may be protruding in the anterior chamber or buried in the Descemet's membrane [57] . FED and endothelial dystrophy without endothelial decompensation are often termed "cornea guttata" (drop-like cornea) [63]. The guttae can be well recognized on slit lamp biomicroscopy as small drop-like protrusions on the posterior surface of the cornea. It may progress causing "beaten metal" appearance (particularly on retroillumination), which can be combined with melanin depositions [43]. The progression of this disease leads to endothelial cells decompensation, which causes stromal edema. The thickness of the central cornea may progress up to 1 mm [7]. Epithelial edema develops in more advanced stages when the stromal thickness increases by about 30%, causing the

progression of epithelial bullae. Later stages may be combined with subepithelial fibrosis[7].

The progression of FED was described in four stages [1, 83]:

Stage 1: Cornea guttata is seen through biomicroscopy. The guttae here are central and non-confluent. In this stage, patients are asymptomatic.

Stage 2: Cornea guttata starts to spread to the periphery of the cornea and begins to confluence. The endothelial cells enlarge and start losing their hexagonal shape. Because of the increased corneal edema in this stage, the patients start experiencing painless decrease in vision.

Stage 3: This stage is characterized by epithelial edema and the formation of bullae, which are small separations between the epithelium and the Bowman layer. The rupture of these bullae causes episodes of pain. In this stage, the vision decreases further.

Stage 4: This stage is characterized by a chronic edema with subepithelial fibrosis. Corneal vascularization may be seen.

FED presents with decrease in vision, which is rare before the age of fifty. The blurry vision is usually worse in the morning, because of the decreased evaporation of the corneal surface while sleeping [7]. The rupture of the bullae causes exposure of the naked endings of the nerves, which causes patients to experience episodes of pain and discomfort [43]. These painful episodes may decrease after developing subepithelial fibrosis [7].

FED can be diagnosed with the slit lamp examination, where the corneal changes can be observed. The early changes of the endothelial cells can be detected with endothelial cells microscope.

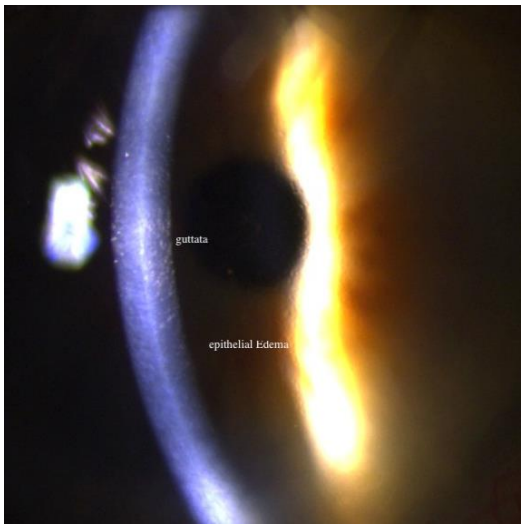

Figure 4: appearance of the cornea with slit lamp biometry in FED: cornea guttata with cornea edema

(with kind permission, Dr. Ibrahim Wardeh, Emsland-Augenzentrum)

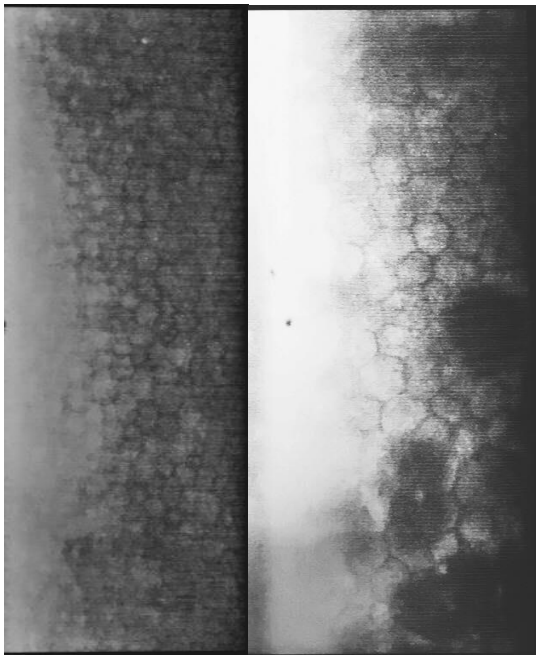

Figure 5: View of the corneal cells with a specular microscope: to the left normal endothelial cells, to the right guttae in FED (Own examination in University eye clinic Marburg, Germany)

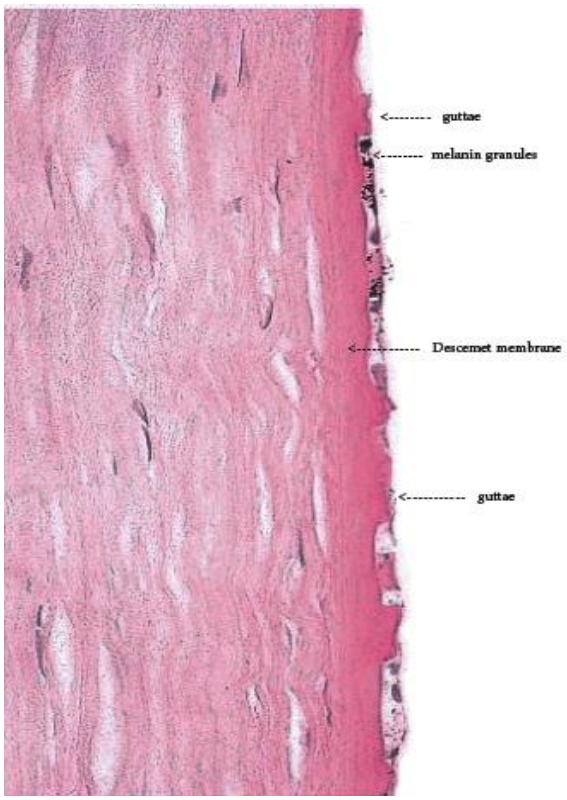

Figure 6: Histology of cornea with FED: Arrows showing a thickened and irregular Descemet's membrane, guttae excrescences and melanin granules in some endothelial cells (modified from source: https://basicmedicalkey.com/eye-and-ocular-adnexa-2/)

1.3.2. Bullous keratopathy:

It is a decompensation of the cornea, which occurs postoperatively early or years later. In many cases, the cause is endothelial cells loss through intraocular operations, most commonly cataract operations. It can also be caused by primary endothelial disease (Fuch's endothelial dystrophy, congenital hereditary endothelial dystrophy or posterior polymorphous dystrophy) [63]. The decompensation of the endothelial cells results in Decsemet's folds and stromal edema, followed by epithelial edema. In more advanced stages, small separations between the epithelium and Bowman layer occur, resulting in micro- cysts called bullae[9].

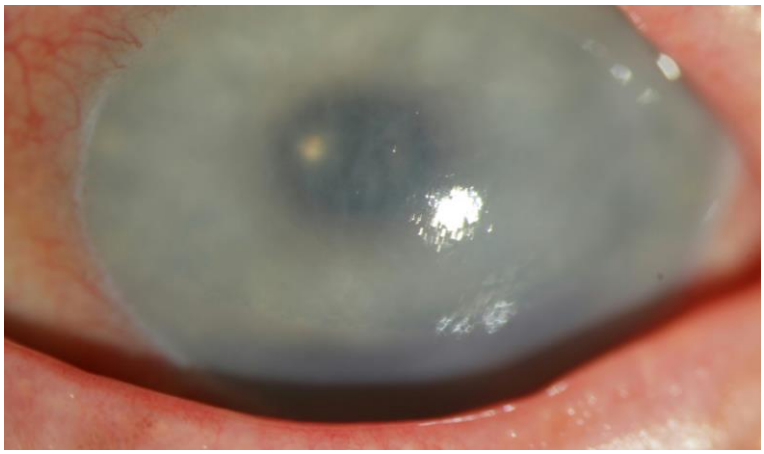

Figure 7: bullous keratopathy, clinical appearance (with kind permission, Dr. Ibrahim Wardeh, Emsland-Augenzentrum)

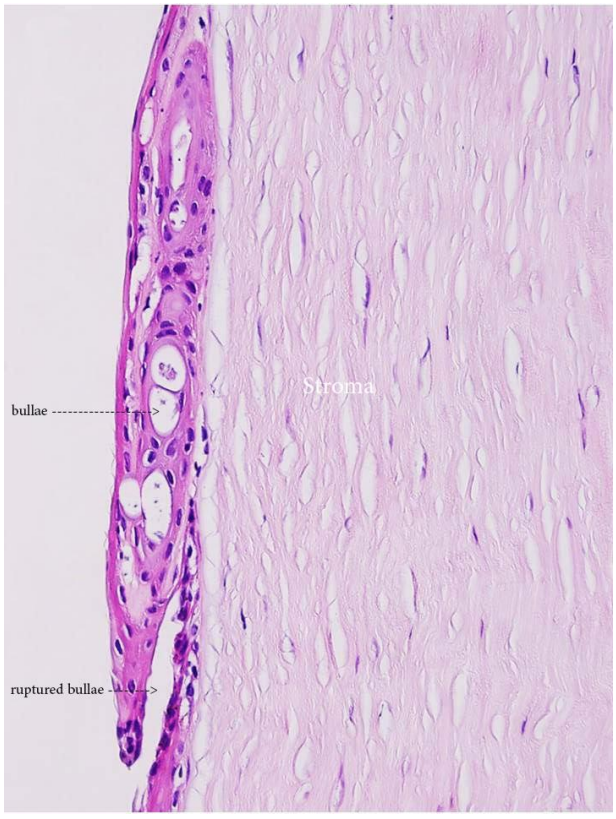

Figure 8: Histology of bullous kertopathy. the thickened corneal stroma can be seen. Arrows showing the bullae that can rupture leading to painful erosions (modified from Qiao's pathology. Source: http://picssr.com/photos/jian-hua_qiao_md/interesting/page122?nsid=42574434@N03)

1.3.3. Treatment:

In the early stages of FED, symptomatic therapy can help in reducing the corneal edema. Using sodium chloride eye drops and ointments (5%) makes the tear film hypertonic, which will extract water out of the cornea and thus reduces the corneal edema. The eye drops are usually used four times a day, most effectively in the morning after waking up. The ointment is usually used at bedtime[7].

Lowering the intraocular pressure can also be helpful, especially in cases with corneal decompensation. It is very important to remember that carbonic anhydrase inhibitors eye drops have to be avoided in these patients. The carbonic anhydrase inhibitors work through inhibiting the bicarbonate pump, which negatively influence the endothelial cells. This causes an increase in corneal edema and cornea guttata[85].

In cases of bullous keratopathy with ruptured bullae, using bandage soft contact lenses can be helpful to relieve pain.

Advanced cases of FED and bullous keratopathy require surgical treatment. This is achieved through replacing the ill corneal tissue with a healthy tissue of a donor's cornea.

1.4. Keratoplasty:

1.4.1. Penetrating keratoplasty (PK) [2, 5, 37]:

PK is an operation, in which the ill cornea of the host is replaced with a donor's full-thickness corneal graft. It is the first method of keratoplasty performed. Since the 18th century, there were attempts to perform corneal transplantation on the eyes of animals and humans. The first successful corneal transplantation was performed in 1905. Since then, the results improved a lot through the development of microscopes and surgical instruments. The indication of PK has been changed throughout the years. FED and bullous keratopathy are two of the major indications of PK. Nevertheless, these

indications have been changed in many countries after the development of lamellar keratoplasty.

1.4.2. Deep anterior lamellar keratoplasty (DALK) [81]:

DALK is a transplantation of a partial-thickness graft, which contains the Bowman membrane and the anterior stroma of the donor. This preserves the endothelium and Descemet's membrane of the host. DALK is indicated in patients with a healthy endothelium and is not performed in patients with FED.

1.4.3. Descemet's stripping automated endothelial keratoplasty (DSAEK) [11]:

In DSAEK, the donor graft consists of posterior corneal stroma, Descemet's membrane and the endothelium. It is one of the techniques of the posterior lamellar keratoplasty, where the thickness of the graft is about 150-250 µm. DSAEK has been widely used to treat patients with FED.

1.4.4. Descemet's membrane endothelial keratoplasty (DMEK):

DMEK is a posterior lamellar keratoplasty, modified from DSAEK. It was first described in 2002 by Gerrid Melles, who laid the foundation of posterior Keratoplasty[51]. The graft in DMEK consists only of Descemet's membrane and the endothelium, which makes the graft about 15 µm thick. Because the graft does not contain a stroma, there is no stroma-to-stroma interface[18]. This makes DMEK superior to DSAEK when posterior lamellar keratoplasty is indicated.

1.4.4.1. Advantages of DMEK:

DMEK is achieved through small incisions, keeping the eye closed. This minimizes the possible complications of an "open-sky" surgery in PK, such as choroidal hemorrhage[13]. Through its sutureless technique, the postoperative astigmatism is much less than PK. Hence, DMEK provides the fastest recovery of vision and the best visual outcomes compared to other methods of keratoplasty. It was shown that DMEK patients have

better visual acuity and faster recovery compared to DSAEK patients 3 and 6 months after the operation[78]. In a comparative study of 15 patients, one eye was treated with DMEK and the other with DSEAK. After 12 months, most of the patients (85%) were happier with the DMEK eye, which had the faster and better visual recovery[36].

Another advantage of DMEK is the long-time graft survival. The risk of graft rejection in DMEK eyes is 15 times lower than DSAEK eyes and 20 times lower than PK eyes[13].

1.4.4.2. Indications of DMEK:

DMEK is indicated in all diseases that cause endothelial cells dysfunction, such as Fuchs endothelial dystrophy, posterior polymorphous dystrophy, congenital hereditary endothelial dystrophy, bullous keratopathy, iridocorneal endothelial syndrome and graft failure after PK or DSAEK. An important condition to perform DMEK is that the other layers of the cornea are clear. If corneal scars or corneal vascularization exist, DMEK shall not be performed. In these cases, a full-thickness keratoplasty can be the alternative. It is not recommended to perform DMEK in aphakic eyes, eyes with anterior chamber lenses or eyes with iris abnormalities because in these cases, the very thin graft may be easily displaced into the posterior chamber[13].

1.4.4.3. Technique of DMEK:

A peripheral iridotomy is performed preoperative with ND:YAG-laser (Neodym-dotierter Yttrium-Aluminium-Granat-Laser). It is usually performed inferiorly at 6 o'clock. The aim of this procedure is to reduce the risk of pupillary block, which can be caused by the intracameral gas postoperatively. An intraoperative iridectomy is an alternative.

1.4.4.3.1. Preparing the donor tissue:

Preparing the 15 µm thick graft from the donor tissue is one of the challenges in this operation. It has a learning curve and needs experience. Most of the surgeons in Germany prepare the graft themselves shortly before the operation[41]. It is also possible

to get grafts that are prepared in the eye bank. Eye-bank-prepared grafts help to minimize the time needed for the operation as well as the stress on the surgeon. These eye bank prepared grafts can be stored in an organ culture medium for about three weeks with an acceptable loss of the endothelial cells[47]. During the preparation, there is a loss of about 0.02% of the endothelial cells. After four to six days, the loss of the endothelial cells is about 8%, which is acceptable[41]. It has been shown that the clinical outcomes and the rate of complications are the same in the DMEK eyes that received an eye-bank-prepared graft compared to the DMEK eyes that received a surgeon-prepared graft[64].

It is important to measure the white-to-white (limbus-to-limbus) in the eyes where DMEK will be performed. This helps in choosing the size of the graft. If the eye is too small and the graft is too big, it is difficult to see the graft edges and the paracentesis may become overlapped by the graft[75]. The grafts from older donors are easier for preparation as they are thicker and more resistible. This minimizes the possible tearing during preparation. Older grafts are also easier to manipulate during the operation[75]. Tearing can occur in about five percent of the grafts during the learning curve. Therefore it is recommended to have a backup tissue when performing the operation[47].

There are many techniques for preparing the graft. Most of the surgeons do this manually. However, it can also be performed with assistance of the femtosecond laser. One of the most used techniques is the one described by Gerrit Melles. The corneoscleral donor tissue should be replaced on a custom-made fixation device with the endothelium on the upper side. A free edge should be created by cutting the Descemet's membrane anterior to the trabecular meshwork and scleral spur for example with a hockey stick. This process should be done for 180° to 360°. After this, the Descemet's membrane is gently stripped from the posterior stroma by holding the edge of the graft with a tying forceps. This is done until two-thirds of the graft are separated from the stroma. Then the graft is brought to its original position by submerging it with saline. A central trephination is then made. Thereafter, the graft is

completely separated from the posterior stroma with forceps. When the graft is prepared, it rolls up with the endothelium being at the outside[30, 47]. Staining the graft with 0.06% trypan blue solution (Vision blue®, DORC, Niederlande) during the preparation helps to recognize any tears or defects in the graft. This can be repeated as much as needed[75]. Another method to prepare the graft is the SCUBA (Submerged Cornea Using Backgrouds Away), which was described by Art Giebel. In this technique, the corneal periphery is marked inside the trabecular meshwork for 360° with a blunt instrument (e. g. Y-hook). During this, the rim should be stabilized with toothed forceps and the stromal fibers should not be torn. A partial thickness trephination is performed centrally. After staining with trypan blue, the tissue is put in a chamber containing corneal storage solution (Optisol®, Bausch & Lomb/USA). The tissue is here suspended with fluid above and under it, which makes visualizing and handling of the graft easier. The Descemet's membrane is then stripped from the posterior stroma with non-toothed forceps[30, 75]. Finally, the graft is either stored in a tissue storage solution, or put in trypan blue if it is going to be used immediately. Many modifications and other methods were described for preparing the graft for DMEK, which will not be discussed here.

1.4.4.3.2. Steps of DMEK:

DMEK can be performed under topical anesthesia, subtenon, peribulbar, retrobulber anesthesia as well as general anesthesia[75].

An incision is created in the clear cornea, usually at 12 o'clock, it ranges between 2.7-3 mm depending on the devices used. A decemetorhexis is then performed under complete air fill in the anterior chamber. In this step, the ill membrane of the recipient is stripped off the stroma and pulled toward the incision at 12 o'clock with Sinskey-hook, Y-hook or other stripper. This can easily be done without any damage to the posterior stroma[52]. The deseased Descemet's membrane is then removed through the incision outside of the anterior chamber.

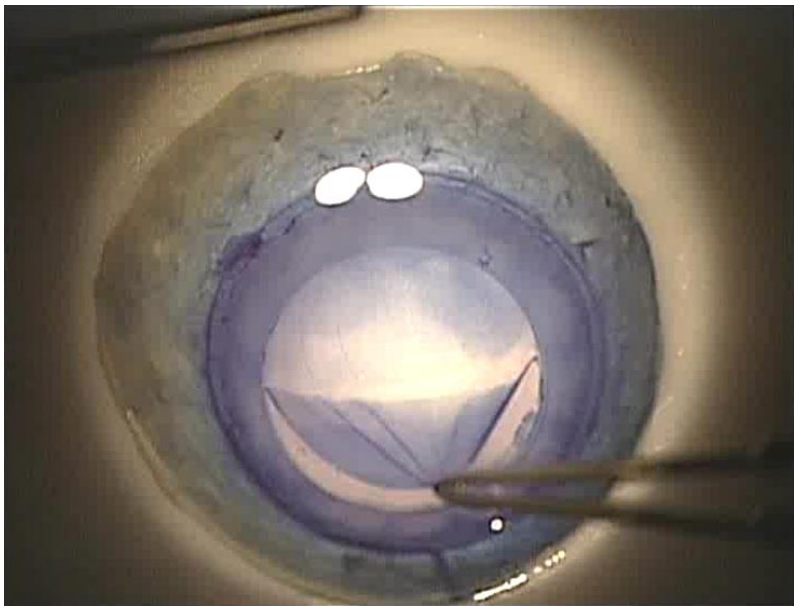

Figure 9: Preparing the graft by gently stripping the Descemet's membrane to separate it from the stroma

(Own presentation in University eye clinic Marburg, Germany)

There are many possible devices to insert the graft into the eye. One of them is a special glass pipette with an attached bulb, which was developed by Melles. Intraocular lens injectors with BSS can also be used. Another option is the modified Jones tube, which was developed by Streiko[13, 75]. It is recommended to stain the graft with trypan blue for at least 60 seconds to make it more visible[75].

After dislodging the reciepient's Descemet's membrane outside of the eye, the graft should be gently aspirated into one of the devices. Then the graft is injected into the anterior chamber, which is filled with BSS. The orientation of the graft is checked once it is in the anterior chamber. One of the techniques to insure that the endothelium is facing the iris' side is Moutsouris sign or ''blue cannula''. Here, the curls of the double roll should show upwards. A tip of a cannula is inserted inside a peripheral curl. If the cannula's tip appears blue, this indicates that it is inside the curl. In this case, Moutsouris sign is positive and the graft is correctly oriented. However, if the tip of the cannula does not change in color, then it is outside the curl. Here, Moutsours sign is negative and the

graft is upside down[80]. Another way to check the orientation is to observe the fluttering of the edges of the roll while tapping the outer surface of the cornea[75]. If the graft is upside down, a right orientation can be achieved with several techniques with the help of BSS waves or by gently tapping the cornea.

After it is assured that the graft is correctly oriented, it should be unfolded. Many techniques are used to unfold the graft, the most used being the standardized no-touch DMEK[22]. The rolls are separated through gentle taps on the corneal surface. They are then completely unfolded through injecting an air bubble inside the rolls on top of the graft. When the graft is unrolled, the air is removed from the interface with the same cannula. It is also possible to tap the cornea with a cannula while manipulating the air bubble inside the eye. This technique is known as Dapena-maneuver. If the roll is tight and the graft is asymmetrical, Dirisamer technique can be used. In the latter technique, the cornea is pressed against the iris with a cannula and the graft is unrolled through taps on the cornea with another cannula. Another technique to unfold the graft is the single sliding cannula maneuver, which can be used when the roll is loose. Here, using the cannula, the graft is unfolded through repetitive downward movements applied on the outer surface of the cornea. Direct touching of the graft should be avoided. It has been shown that these four intraoperative techniques used to unfold the graft do not affect the clinical outcomes. The rate of postoperative complication was also not related to the technique used to unfold the graft[22, 80].

When the graft is unfolded, it should be positioned onto the posterior stroma of the host. This is achieved through injecting air or sulfur hexafluoride (SF6) 20% or 5% underneath the graft. The anterior chamber is 100% filled with air or sulfur hexafluoride tamponade. It is recommended to use sulfur hexafluoride 5% as tamponade instead of air. The incidence of graft detachment requiring re-bubbling, which is a re-injection of air bubble in the anterior chamber, is significantly lower when sulfur hexafluoride 5% is used. No additional complications were described with the use of sulfur hexafluoride 5%[10].

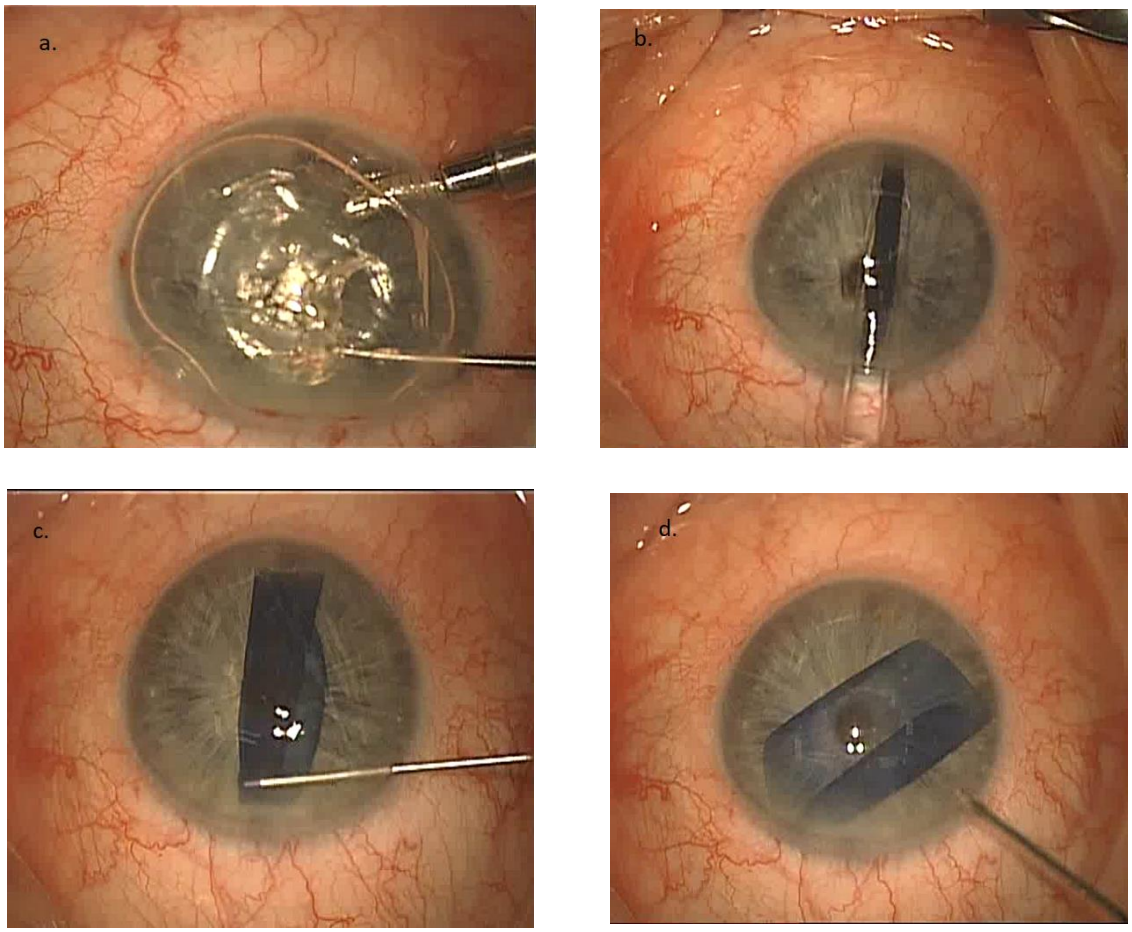

Figure 10: steps of DMEK, Part 1: a. Descemetorhexis with sinskey-hook under complete air fill. b. injecting the graft into the anterior chamber. c. controlling the position of the graft in the anterior chamber. d. correctly positioning the graft in the anterior chamber (own presentation in the University eye clinic Marburg, Germany)

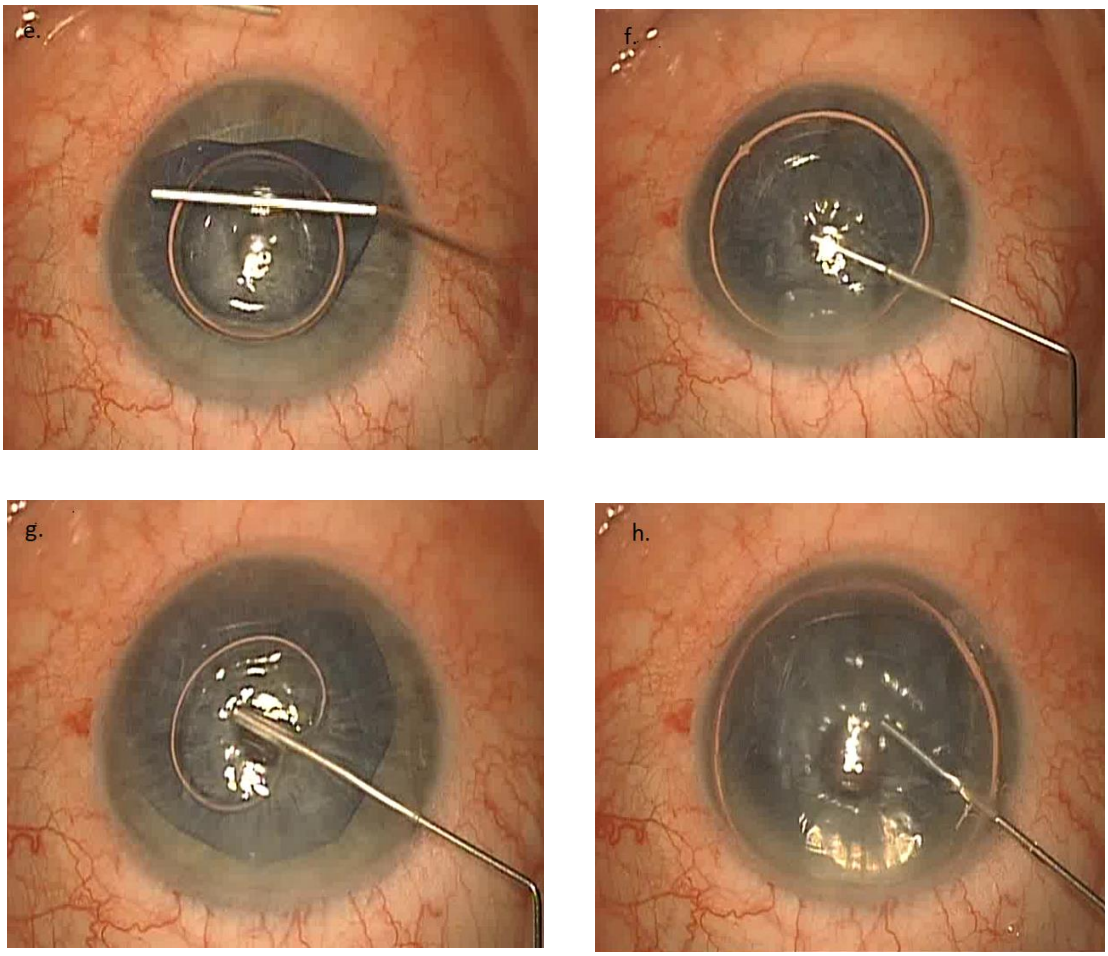

Figure 11: steps of DMEK, Part 2: e. unfolding the graft through injecting air on top of it. f. the graft is completely unfolded. g. removing the air bubble. h. injecting air or gas underneath the graft at the end of the operation to achieve contact between the graft and the anterior stroma of the host (own presentation in the University eye clinic Marburg, Germany)

Intraoperative optical coherence tomography (iOCT) can also be used. It is useful in all steps of DMEK. IOCT helps to visualize the remnants of the recipient's Descemet's membrane as well as the rolling and orientation of the graft. This may reduce the manipulation needed and save time[73].

The vast majority of surgeons reduce the size of the bubble 1-2 hours postoperative to avoid pupillary block.

Postoperatively, the patients should lie on their backs to keep the bubble on the lower part of the cornea. This should be done as long as the gas bubble remains in the eye (usually 2-3 days).

Graft detachment is the most common complications after DMEK. If the attachment is in the periphery and the cornea is clear, they can be observed. Otherwise, re-bubbling should be performed[75].

2- Aim of the study:

The aim of this study is to analyze the clinical outcomes 4-7 years after DMEK. The patients who underwent DMEK at the University Eye Clinic of Marburg, Germany between 2010-2014 were included. We evaluated the visual acuity, refraction, corneal volume and central corneal thickness. These measured values were compared to the documented values before the operation. The endothelial cells density was also measured and compared with the endothelial cell density of the transplanted graft. The rate of the complications as well as the rate of re-operation and graft survival was reviewed.

3- Patients and methods:

3.1. Design of the study:

This study is a cross-sectional, case series study. The study was conducted in accordance with the institution's Good Clinical Practice and the Declaration of Helsinki. An application for ethical approval was applied to the Ethics Committee, Faculty of Medicine, Philipps University of Marburg in May 2017. The study was approved by the Ethics committee on 03.08.2017, approval number: Studie 80/17. After the approval was obtained, all patients who underwent DMEK in the university eye clinic of Marburg, Germany between 2010 - 2014 were contacted and asked to attend a follow-up examination. The invitation to participate in this study was sent to the patients by post. The aim of this study and the examinations that will be performed were described in the

invitation. The patients who had interest to participate contacted us via email or telephone and appointments were arranged. A single presentation for a follow-up examination for every patient was requested. At this presentation, all the required examinations were performed. These examinations were performed in the University Eye Clinic of Marburg, Germany between August 2017 and December 2017 by three examiners. All patients were fully informed about the study and the examinations that will be undertaken.

All patients included in this study signed an information sheet, which proved that they have received and understood the content of the study and that they are willing to participate in it.

3.2. Patients:

The total number of patients who underwent DMEK at the University Eye Clinic of Marburg, Germany between 2010 and 2014 was 265. 142 of them attended to our follow-up examination and were included in this study (230 eyes). The other patients (125 patients, 165 eyes) were not able to attend.

3.3. Surgical protocol:

DMEK was performed in all patients between 2010 and 2014 at the University Eye Clinic, Marburg, Germany by three different surgeons. All the donor corneas were supplied by external certified corneal banks. The preparation of the graft from the donor tissue was performed by the surgeon immediately before surgery. The Descemet's membrane was carefully stripped off the stroma as previously described (see 1.4.4.3.1.). 95.7% of the operations were performed under general anesthesia (220 eyes) and 4,3% (10 eyes) were performed under subtenon's anesthesia. As described above (1.4.4.3.2), the host's Descemet's membrane was removed with a reverse Sinskey hook. The graft was stained with 0.06% trypan blue and then injected in the anterior chamber with a DMEK injector (DMEK surgical disposable set, D. O. R. C., the Netherlands). The graft was unfolded with

the standard non-touch technique as described by Dapena et al[22]. At the end of the operation, the anterior chamber was completely filled with 5% SF6 in 34.3% of the eyes (79 eyes) of with air in 65,7% of them (151 eyes). Cyclopentolate hydrochloride 1% eye drops were applied at the end of the surgery. The gas bubble in the anterior chamber was reduced to 60%-80% by the surgeon 90 minutes after the operation to avoid acute angle closure. The efficiency of the peripheral iridotomy, that was performed at 6 o'clock with ND:YAG a few days prior to surgery, was also checked. No complications were reported during the bubble reduction. The patients were instructed to maintain a supine face-up position for the first two to three days after surgery to keep the gas bubble near the graft. The standard postoperative local treatment included: Ofloxacine eye drops 0.3% (Floxal; Bausch & Lomb GmbH, Berlin, Germany) four times a day for the first two weeks after surgery, Cyclopentolate hydrochloride 1% eye drops (Zyklolat EDO; Dr. Gerhard Mann, Chem-pharm, Fabrik GmbH, Berlin Germany) twice a day for the first week after surgery and dexamethasone 0.1% (Dexa-EDO; Dr. Gerhard Mann, Chem-pharm, Fabrik GmbH, Berlin Germany) six times a day for the first month after surgery then with a reduction to one drop a day every two months until once a day. Dexamethasone was replaced by Loteprednol etabonate 0.5% (Lotemax, Dr. Gerhard Mann, Chem-pharm, Fabrik GmbH, Berlin Germany) in patients who developed a steroid-induced IOP-elevation.

3.4. Examinations:

Medical history was obtained from all patients included in the study. They were asked about their satisfaction after the surgery, their medical history, continuation of the steroid eye drops and if any additional treatment or operation were performed in the

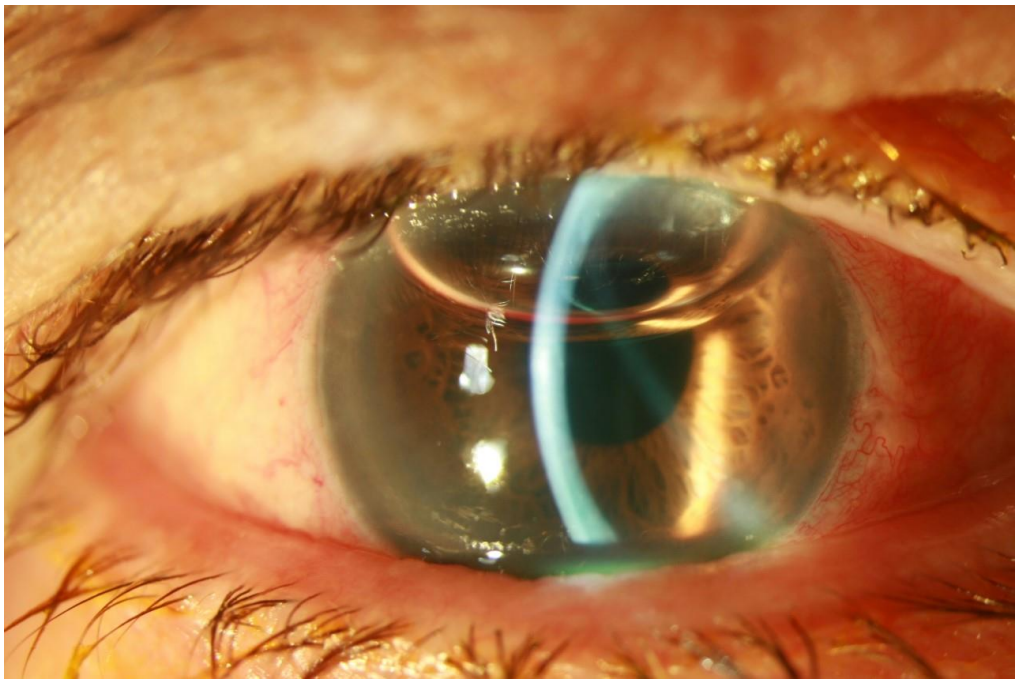

Figure 12: a slit lamp photo 3 days after DMEK (own presentation in University eye clinic Marburg)

time between the last visit and our follow-up examination. Then we performed a subjective refraction to determine the refractive status of the eye and the best corrected visual acuity BCVA. The grafts were evaluated with the slit lamp. The slit lamp examination also included the measurement of the intraocular pressure with Goldmann applanation tonometry and the examination of other eye structures including the retina. Thereafter, a corneal topography was performed with the Pentacam® (Oculus GmbH, Wetzlar, Germany) and the endothelial cells were evaluated with specular microscope (Topcon SP-2000, Japan).

3.4.1. Refraction and visual acuity:

The visual acuity refers to the minimum legible threshold of the eye. It is measured by the point, at which the eye can distinguish letters or figures at a specific distance.There are many systems to measure the visual acuity using charts of letters (optotyes) that progressively get smaller. One of the most used methods is Snellen visual acuity[6]. We

used the decimal notation (Visus), which is another method to express the visual acuity as a decimal number. We tested the visual acuity at a distance of 6 meters (20 ft) with charts containing numbers. Using the conversion charts of visual acuity, we converted the visual acuity to logMAR method (base-10 logarithm of the minimum angle of resolution), where the charts are related to each other with a logarithmic system. We performed a subjective refraction of each patient to determine the refractive status of the eye and to determine the best corrected visual acuity (BCVA). In the subjective refraction, we relied on the patients' response to determine the refractive errors, if present. These errors may contain spherical and/or cylindrical portions. The refractive errors were compared with the preoperative refractive errors to determine the refractive shift after DMEK.

Table 2: Visual acuity converting chart. Data taken from Clinical Optics. Section 3. American academy of ophthalmolog , the eye M. D. association. 2014-2015

Feet (20)	**Meters (6)**	**Decimal notation (Visus)**	**LogMAR**
20/10	6/3	2.00	-0.30
20/15	6/4.5	1.33	-0.12
20/20	6/6	1.00	0.00
20/25	6/7.5	0.80	0.10
20/30	6/9	0.67	0.18
20/40	6/12	0.50	0.30
20/50	6/15	0.40	0.40
20/60	6/18	0.33	0.48
20/80	6/24	0.25	0.60
20/100	6/30	0.20	0.70
20/120	6/36	0.17	0.78
20/150	6/45	0.13	0.88

20/200	6/60	0.10	1.00
20/400	6/120	0.05	1.30

3.4.2. Slit lamp examination:

The slit lamp is the basic and most used device that enables the examination of all segments of the eye. With a possible magnification until x32, the status of the cornea and graft can be precisely evaluated. Any evidence of cornea guttata, pigment depositions on the graft or signs of graft rejection can be determined. We examined all the segments of the eye, including the retina to rule out any possible complications or any reasons that may cause a reduction in the visual acuity. The retina was examined with the slit lamp using the +90 D non-contact lens (Volk – Ltd/USA). Furthermore, the measurement of the intraocular pressure (IOP) with the use of Goldmann applanation tonometry was also included in our examination to detect any unknown steroid response.

3.4.3. Corneal topography:

Many devices with different systems are available to measure the corneal topography. In this study, we used the Pentacam® HR (Oculus Optik GmbH, Wetzlar, Germany). The Pentacam HR is a non-contact device that uses the Schleimpflug camera to quantify the corneal topography. Using a slit illumination and a camera, sectional images are generated. The Schleimpflug principle allows the rotation of the slit-camera system around 360° to create about 50 radially oriented images that provide approximately 138,000 recognizable elevation values. The analysis of these images allows the creation of a three-dimensional image of the anterior parts of the eyes, including the anterior and posterior faces of the cornea, the iris, the anterior chamber and the lens[58]. A computer software analyzes these images and creates various maps, which enables the evaluation of the anterior segments of the eye. The sagittal curvature map, the refractive power map of the anterior surface, the EKR power map and the pachymetry

map are some of the important charts in the Pentacam analysis[59]. Based on the Pentacam HR figures, we evaluated the central corneal thickness CCT as well as the corneal volume CV. These parameters are indicators of the endothelial cells' function. When this function is decreased, a corneal decompensation results in abnormal water content in the cornea leading to an increased corneal volume[76]. Since the corneal topography with Pentacam HR is one of the routine preoperative examinations before DMEK, we were able to compare these values before and after the surgery.

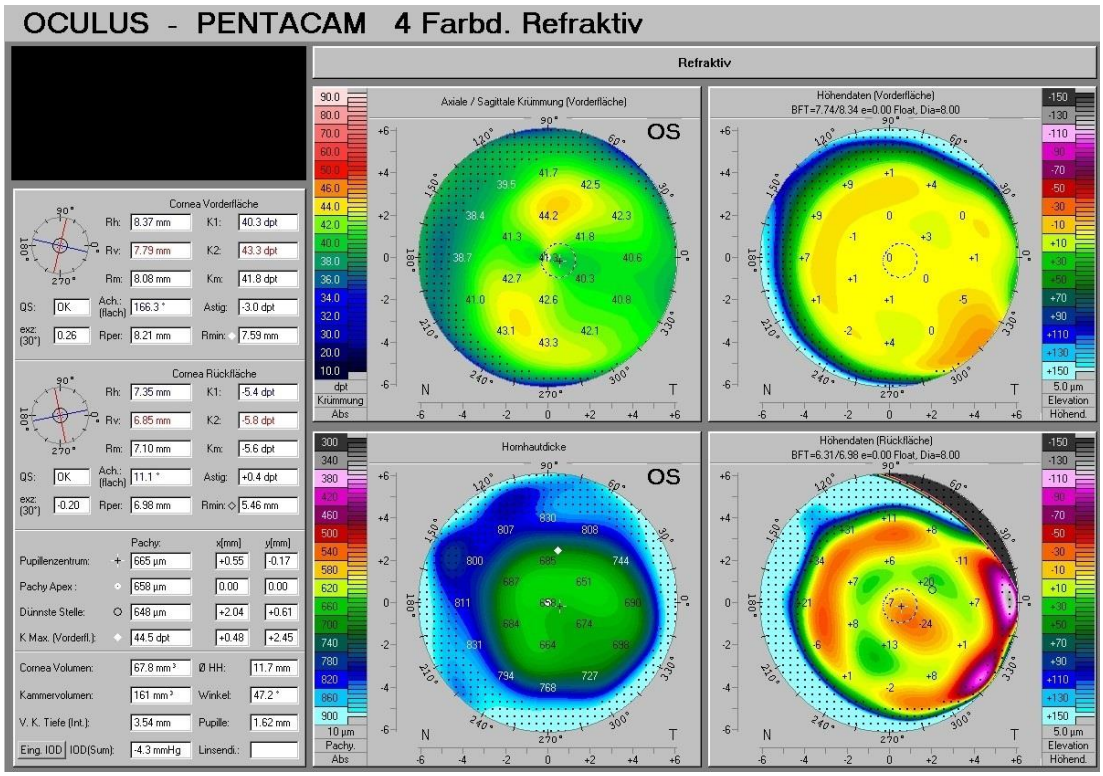

Figure 13: presentation showing Pentacam examination of a patient with corneal edema in FED (own presentation in University eye clinic in Marburg, Germany)

3.4.4. Measurement of the endothelial cells:

The count of the endothelial cells was examined with the specular microscope of Topcon SP2000P (Topcon, Tokyo, Japan). It uses near infrared light for observation and Xenon flash max. 60 W/sec. for photography. This non-contact device uses auto alignment and auto capture system to capture images of endothelial cells. Fixation targets are set up at 12, 2, 6 and 10 o'clock[77]. These images are automatically analyzed through the device to determine the count of the endothelial cells and the standard deviation.

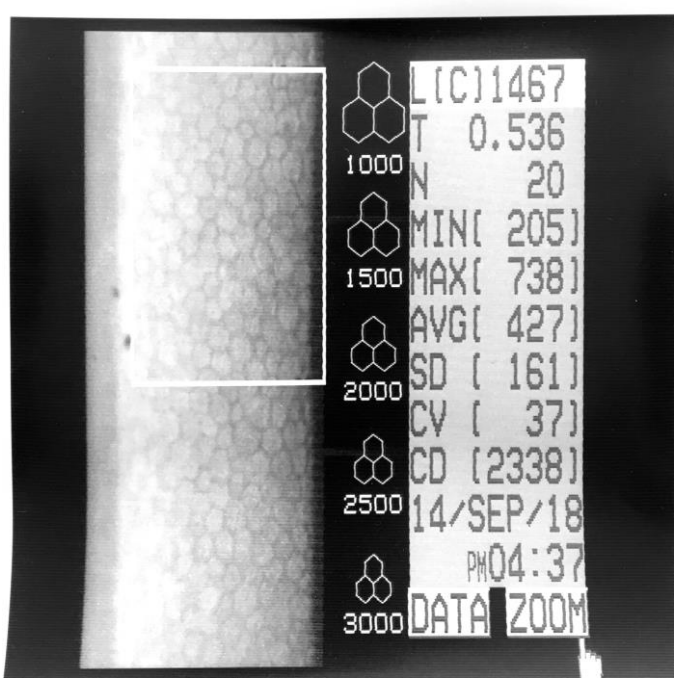

Figure 14: normal ECD with Topcon specular microscope

3.5. Statistical analysis:

We used Microsoft Excel 97-2003 to collect the preoperative and postoperative data. Main measures were BCVA, refraction, CCT, CV, ECD and the complications. The preoperative data was taken from the electronic files of our patients in the University eye clinic in Marburg. The preoperative and postoperative CCT and CV was measured

using the Pentacam® HR (Oculus GmbH, Wetzlar, Germany). Variables were described with an average, a standard deviation, a median, a maximum and a minimum. The data was analyzed using SPSS software (IBM® SPSS® Statistics Version 24). To compare the preoperative data with the postoperative one, we used the two sample t-test. We presented the difference between the variables using Box-Plot graphs. We analyzed the graft survival using the Kaplan-Meier curves. Log Rank Test was also used to analyze the factors that could be related to graft survival. P less than 0.05 was considered statistically significant.

4- Results:

4.1. Patients:

We examined 142 patients who underwent DMEK between 2010 and 2014 at the University Eye Clinic in Marburg, Germany (230 eyes). The mean follow-up time was 47 months (SD 13.3) (maximum 82 months, minimum 20 months). In 38% of the patients, one eye was operated on (54 patients), whereas both eyes underwent surgery in 62% (88 patients). 59.9% of the patients were females (85 patients) and 40.1% were males (57 patients). The average age of our patients at the time of operation was 69.24 (SD 9.09) (minimum 44.09, maximum 94.03). One of our male patients was at the age of 100 when he came to our follow up examination. 50.9% of the eyes were right eyes (117 eyes) and 49.1% left eyes (113 eyes). The indication of DMEK was FED in 94.3% of the eyes (217 eyes) and bullous keratopathy in 5.7% of the eyes (13 eyes) (one case of graft decompensation after PK, two cases of graft decompensation after DSAEK, one case of trauma during birth, one case of bullous keratopathy after the implantation of aphakic anterior chamber intraocular lens and eight cases of bullous keratopathy after cataract operation). 96.1% of the eyes were pseudophakic at the time of the operation (220 eyes), whereas 4.3% were phakic (9 eyes) and 0,4% were aphakic (one eye). Other known ocular comorbidities were: glaucoma in 6% of the eyes (14 eyes; seven with primary open angle glaucoma POAG, four with PEX-glaucoma and three with pigment

dispersion glaucoma. Two of these cases underwent a trabeculectomy with mitomycin-C (5 years and approximately 20 years ago), age related maculopathy AMD in 5.6% of the eyes (13 eyes), known amblyopia in 3.4% (eight eyes), epiretinal gliosis in 1.7% (4 eyes), 1,7% with map-dot-fingerprint dystrophy (four eyes), 1,3% with asteroid hyalosis (three eyes), choroidal vascularization in myopia in 0.8% (two eyes), 0.8% of the eyes underwent retinal detachment operation longer than 20 years ago (two eyes), 0,8% with known keratokonus (two eyes, both underwent crosslinking many years ago), vitreoretinal traction in 0.4% (one eye), 0,4% with known central retinal occlusion (one eye) and 0,4% with known optic nerve atrophy (one eye).

4.2. Grafts and operations:

As mentioned above (see 3.3), the operations were performed by three different surgeons; 27.6% of the operations by W. S., 22.0% by K. D. and 50.4% by F. M. S. All the donor corneas were supplied by external certified corneal banks. The grafts were prepared by the surgeon immediately before the operation. The size of the graft was adapted to the eye of the donor based on the white-to-white measurement of patient's eye. The size of the grafts ranged from 7.0 to 9.5mm. The average count of the endothelial cells in the graft was 2559 cells/mm² (SD 316) with a median of 2500 cells/mm² (minimum 2032 cells/mm², maximum 4655 cells/mm²).

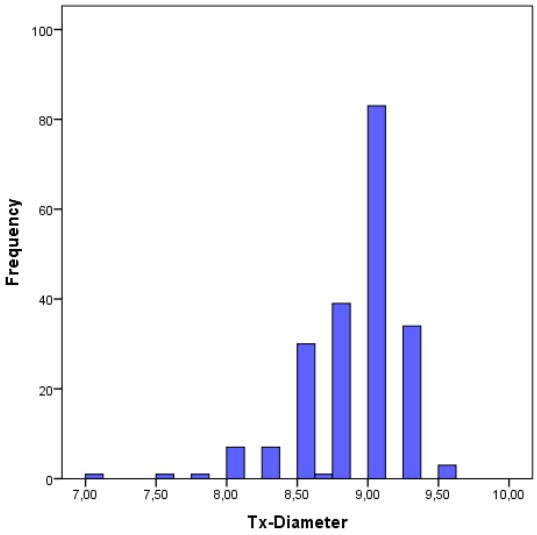

Figure 15: Graph showing the size of the grafts used in our patients (own presentation in the University eye clinic Marburg, Germany)

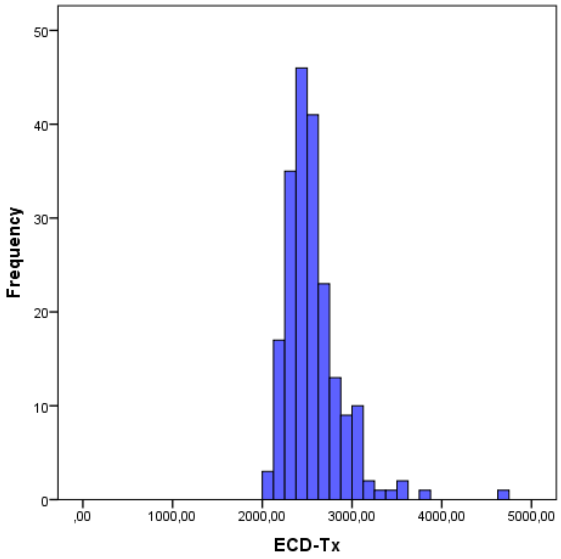

Figure 16: Graph showing the endothelial cells density of the grafts

In 0.8% of the eyes (two eyes), DMEK was combined with phacoemulsification of the lens.

4.3 Preoperative examination:

4.3.1. BCVA and Refraction**:**

In evaluating the BCVA, we excluded the patients with low visual potential, i.e. all patients who have other ocular comorbidities affecting the visual outcome. This includes all patients with AMD, macular pucker, amblyopia, choroidal neovascularization in myopia and optic nerve atrophy.

The average preoperative BCVA in these patients (n=201) was 0,60 logMAR (= 0.25 decimal) (SD 0,32).

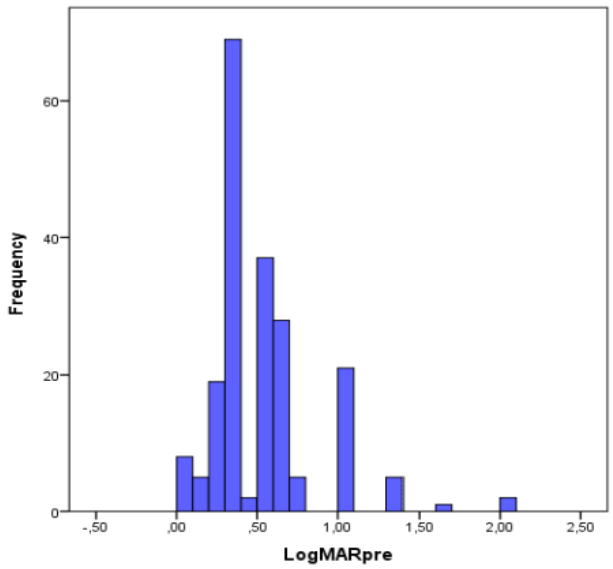

Figure17: Graph showing BCVA with logMAR preoperatively

We analyzed the refraction in both its components, spherical and cylindrical. The average spherical component of the preoperative refraction was 0,09 D (SD 1.0), whereas the average of the cylindrical component was -0.45 D (SD 0.70).

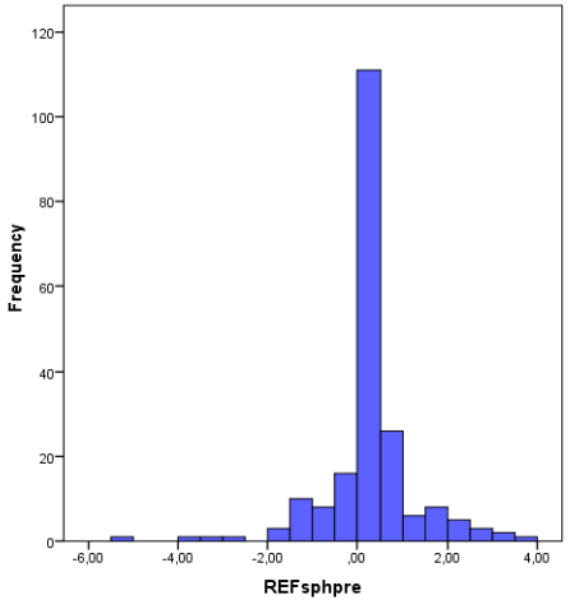

Figure18: Graph of the spherical component of the preoperative refraction

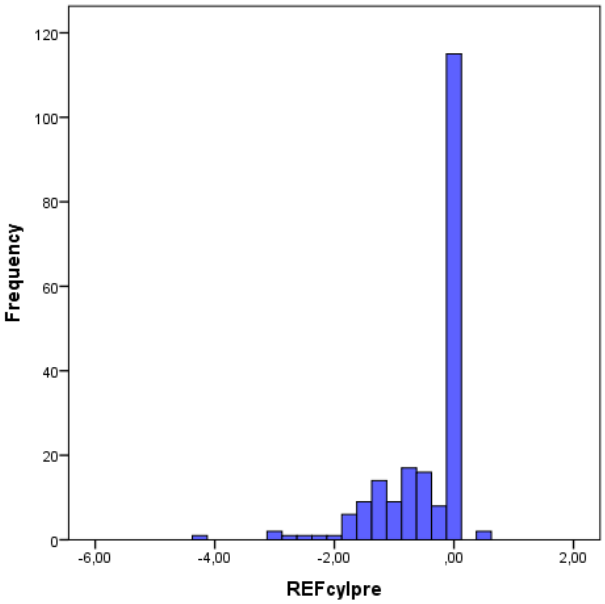

Figure19: Graph of the cylindrical component of the preoperative refraction

4.3.2. Central Corneal Thickness and Corneal Volume:

We evaluated the central corneal thickness CCT and the corneal volume CV from the Pentacam HR examination. The preoperative CCT was on average 675µm (SD 112µm), with a median of 649µm (minimum 447µm, maximum 1211µm). The preoperative CV was on average 65.2mm^2 (SD 8.4mm^2), with a median of 63.9mm^2 (minimum 52.2mm^2, maximum 104.0mm^2).

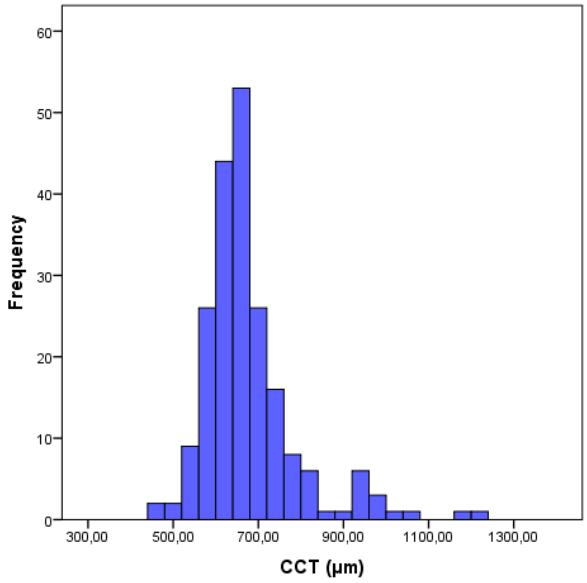

Figure20: the preoperative central corneal thickness

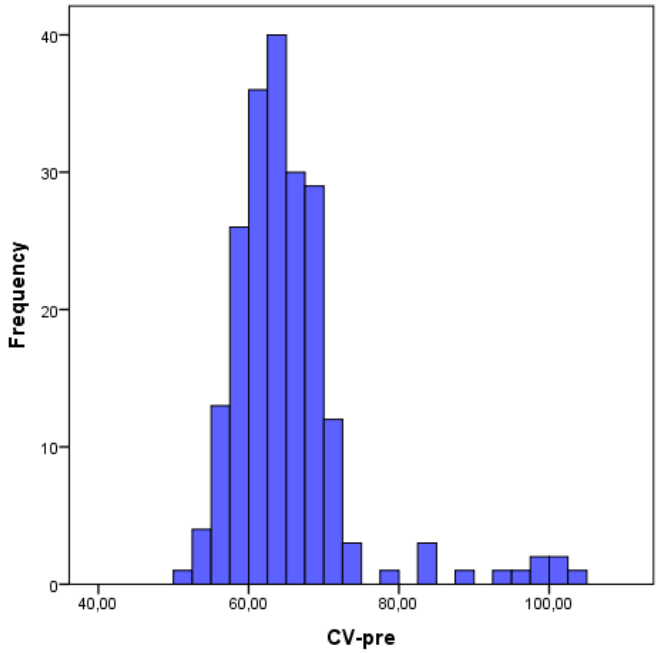

Figure 21: the preoperative corneal volume

4.4. Postoperative Examination:

Steroid eye drops were discontinued 12-18 months after the operation in 77.3% of the eyes (177 eyes). In 22.7% of them, these eye drops were continued once a day or once every other day until the time of our examination (15.3% continued Dexamethasone dihydrogenphosphat 0.1% eye drops, 4.4% used Loteprednol etabonat 0.5% eye drops and 3.0% of the patients used Fluorometholone eye drops).

4.4.1. BCVA and Refraction:

The average BCVA postoperatively in our patient (the group with no other ocular comorbidity) was 0.10 logMAR (= 0.8 decimal) (SD 0.22).

The paired sample t-test showed a difference of 0.42 (sd 0.33) between the preoperative and post-opertive BCVA, which is highly significant ($p<0.0005$).

71.1% of the patients had a BCVA of o.o1 logMAR or better, whereas 49.2% of all patients had a full BCVA of 0.00 logMAR (= 1.0 decimal) or better.

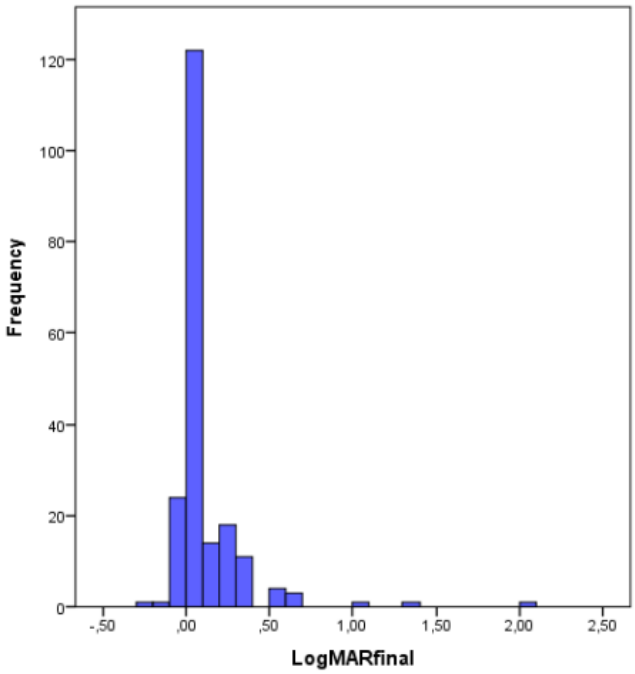

Figure 22: Graph showing BCVA with logMAR postoperativley

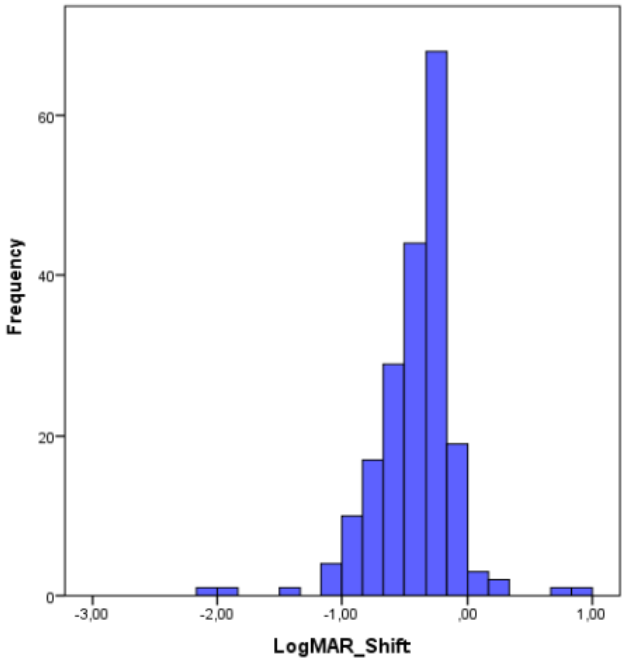

Figure 23: Graph showing the difference between preoperative and postoperative BCVA with logMAR

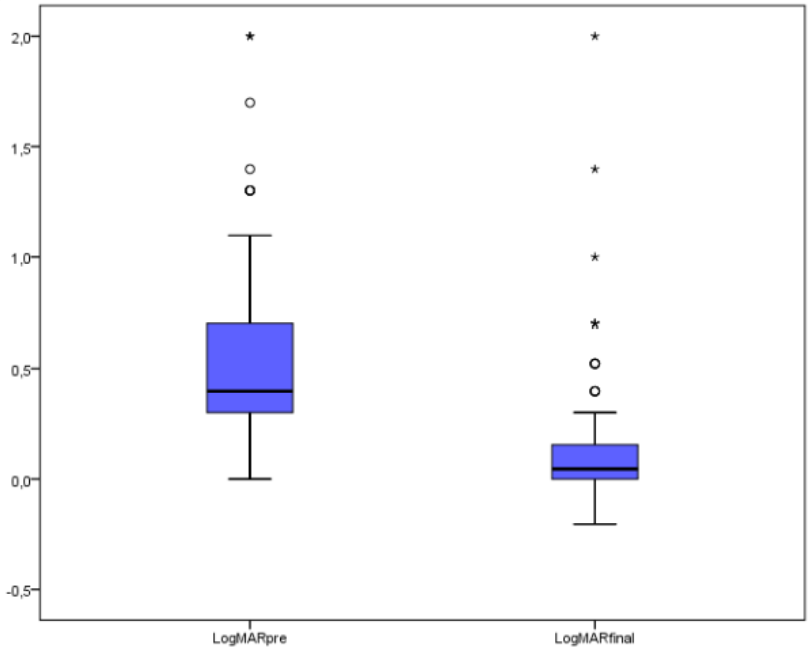

Figure24: Box-Plot graph showing the difference between the preoperative and postoperative BCVA with logMAR

The subjective refraction at our examination showed an average of spherical component of 0.35 D (SD 1.05) and cylindrical component of -1.18 D (SD 1.07).

Comparing these results with the preoperative refraction showed a shift of +0.25 D In the spherical component (SD 1,10) and a shift of -0.73 D in the cylindrical component (SD 1.21).

Analyzing these values with paired samples test shows that there is a significant difference between the preoperative and postoperative spherical and cylindrical component ($p<0.0005$).

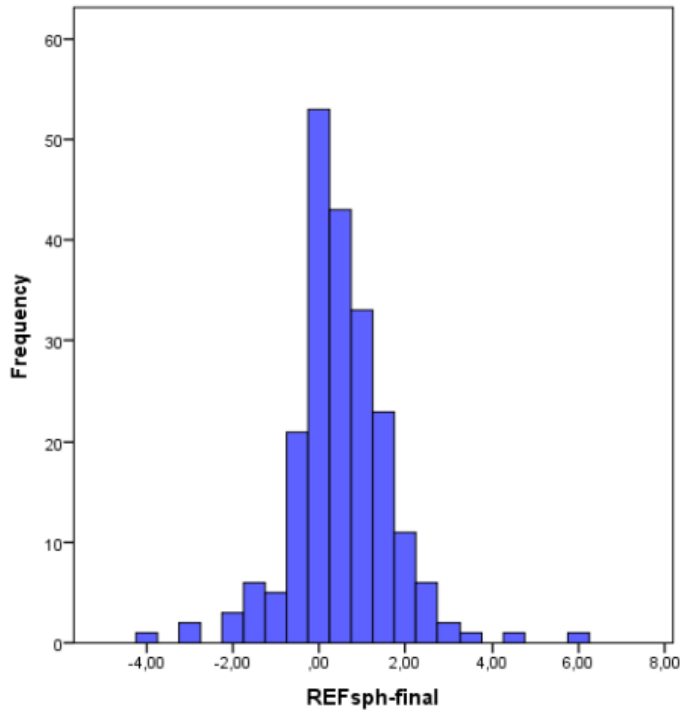

Figure25: Graph of the spherical component of the postoperative refraction

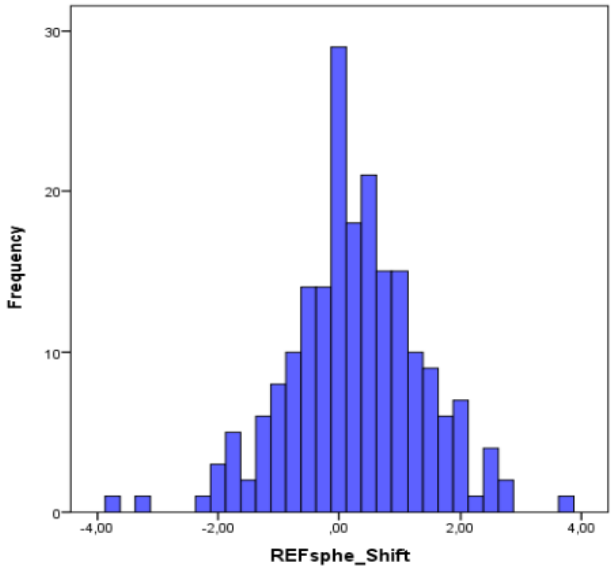

Figure26: Graph showing the difference between the preoperative and postoperative spherical component of refraction

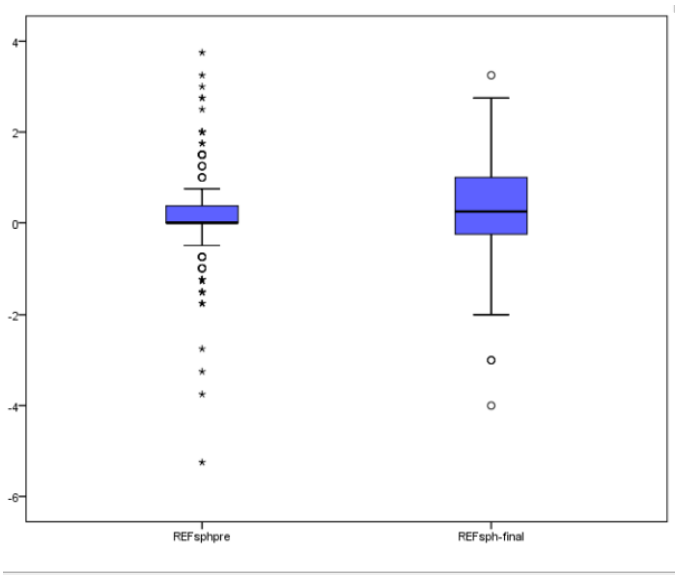

Figure27: Box-Plot graph showing the difference between the preoperative and postoperative spherical component of refraction

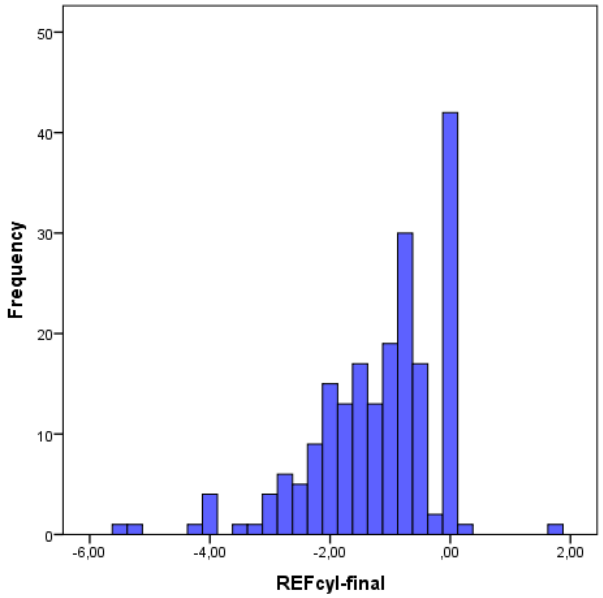

Figure28: Graph of the cylindrical component of the postoperative refraction

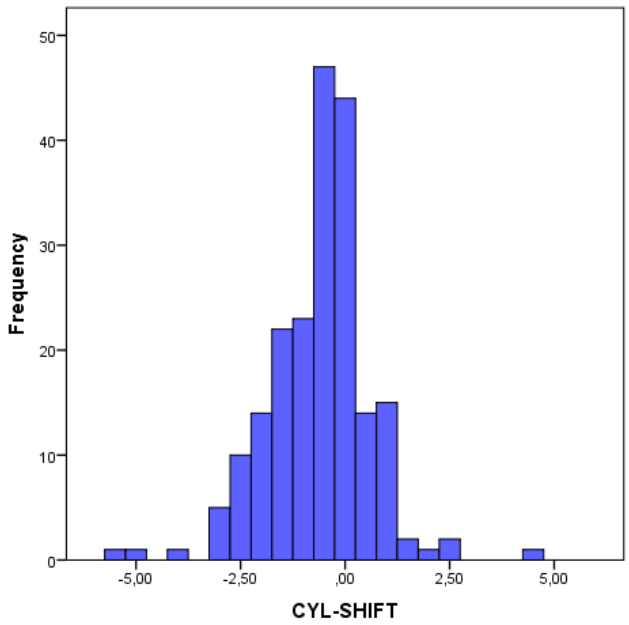

Figure29: Graph showing the difference between the peroperative and postoperative cylindrical component of refraction

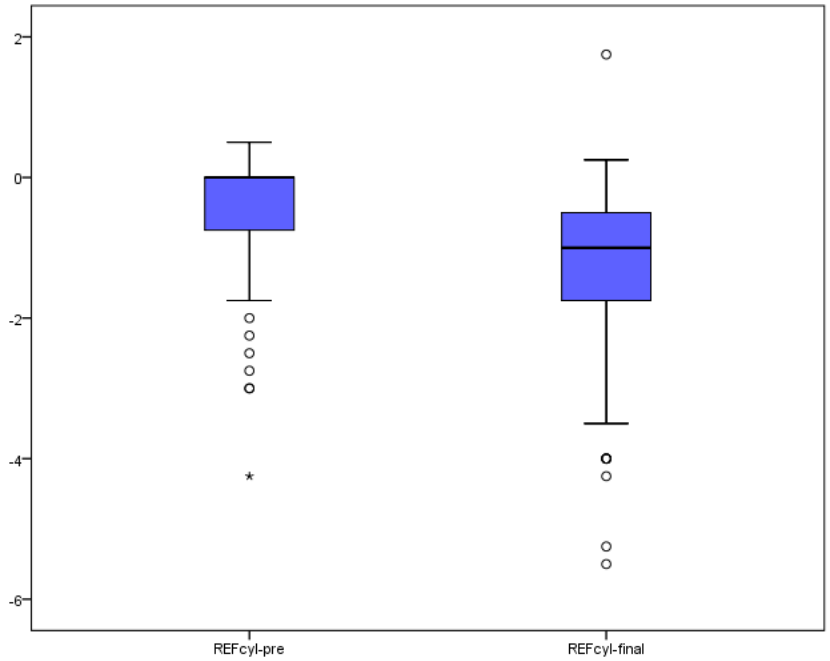

Figure30: Box-Plot graph showing the difference between the preoperative and postoperative cylindrical component of refraction

4.4.2. Slit lamp examination:

We performed an ophthalmic evaluation with a slit lamp to detect any sign of early graft rejection, including pigment deposits and guttata on the grafts. 45.7% of the eyes did not show any pigment deposits on the graft, 40.4% of the eyes showed isolated pigment deposits, which have no clinical significance, 11.3% of the eyes showed groups of pigment deposits and 5% showed diffuse scattered pigment deposits. We instructed the last two groups of these patients to take a low dose of steroid eye drops over a long time (1-2 times daily), unless they already do.

64.4% of the eyes were totally free of any guttae on the graft. We were able to detect less than five guttaa on the graft in 26.1% of the eyes, between five and ten guttea in 8.6% of the eyes, and more than ten in 0.9% of them. The last two groups of these patients were also instructed to take a low dose of steroid eye drops as mentioned above.

4.4.3. Central Corneal Thickness and Corneal Volume:

The postoperative CCT was on average 547µm (SD 52µm), Median 544µm (minimum 373µm, maximum 870µm). The reduction in the CCT at the last follow-up after DMEK was on average 128µm (SD 107µm), Median 108µm (minimum is gain of 56µm, maximum reduction 614µm), which is highly significant ($p<0.0005$).

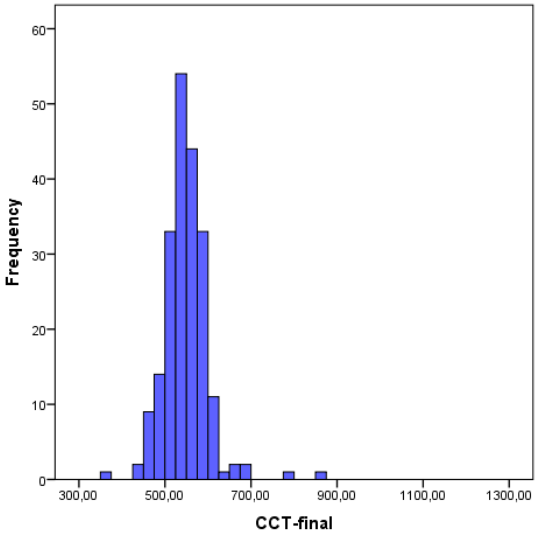

Figure31: Graph showing the postoperative central corneal thickness

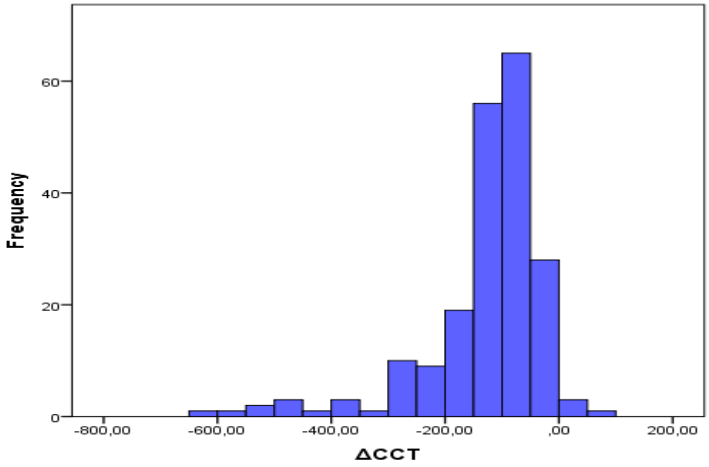

Figure 32: Graph showing the difference between preoperative and postoperative central corneal thickness

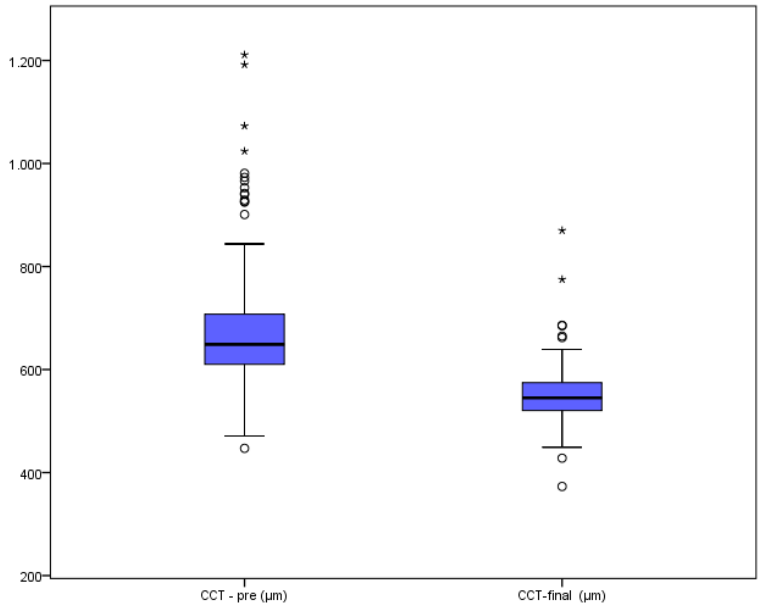

Figure 33: Box-Plot graph showing the significant difference in between CCT before and after DMEK

The postoperative CV was on average 61.9mm^2 (SD 5.4mm^2), Median 62.1mm^2 (minimum 48.0mm^2, maximum 88.0mm^2). The reduction of the CV at the last follow-up was on average 3mm^2 (SD 7.5mm^2), Median -1.8mm^2 (minimum gain of 18.2mm^2, maximum loss of 35.2mm^2), which is highly significant ($p<0.0005$).

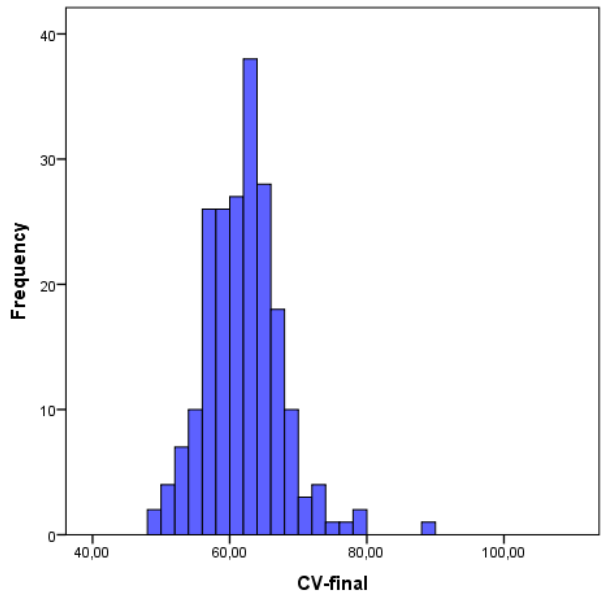

Figure34: Graph showing the postoperative corneal volume

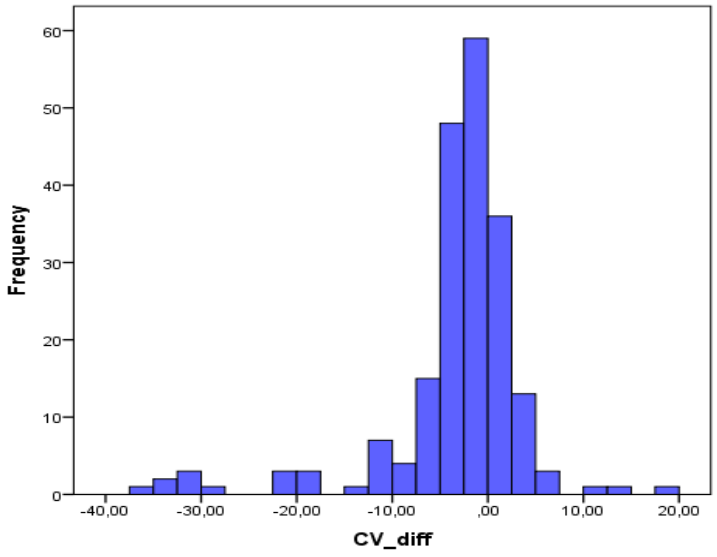

Figure 35: Graph showing the difference between preoperative and postoperative corneal volume

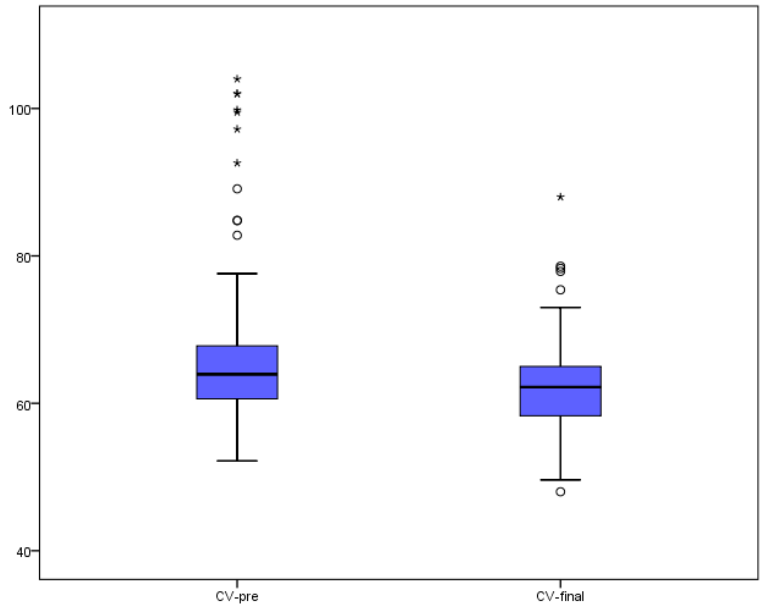

Figure36: Box-Plot graph showing that there is a significant difference in CV before and after DMEK ($p<0.0005$)

4.4.4. Count of endothelial cells:

The endothelial cells were evaluated by the specular microscope (Topcon SP-2000). The postoperative count of the endothelial cells was on average 1166 cells/mm² (SD 490) with a median of 1075 cells/mm² (minimum 292 cells/mm², maximum 3195 cells/mm²).

The loss of the endothelial cells at the last follow-up after DMEK was on average 1392 cells/mm² (SD 455) with a median of 1432 cells/mm². This corresponds to an average loss of 54.7% in 4-7 years (SD 16.8).

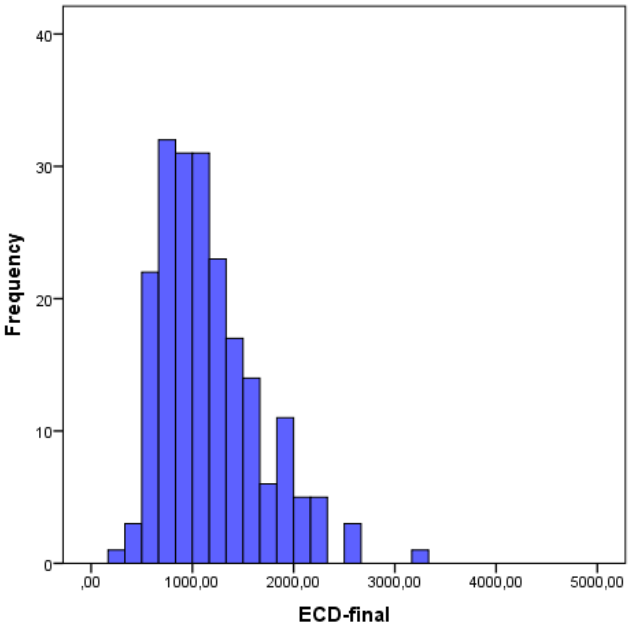

Figure37: Graph showing the postoperative endothelial cells density

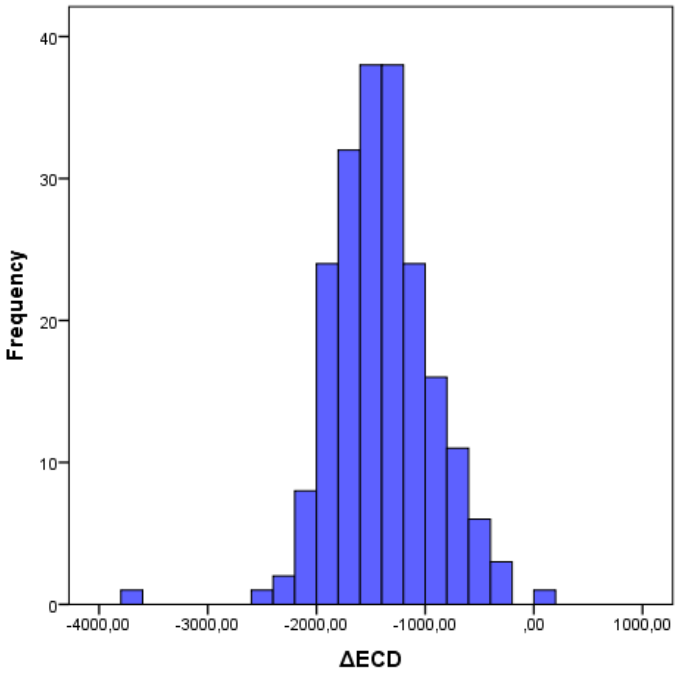

Figure38: Graph showing the loss of the endothelial cells density 4-7 years after DMEK

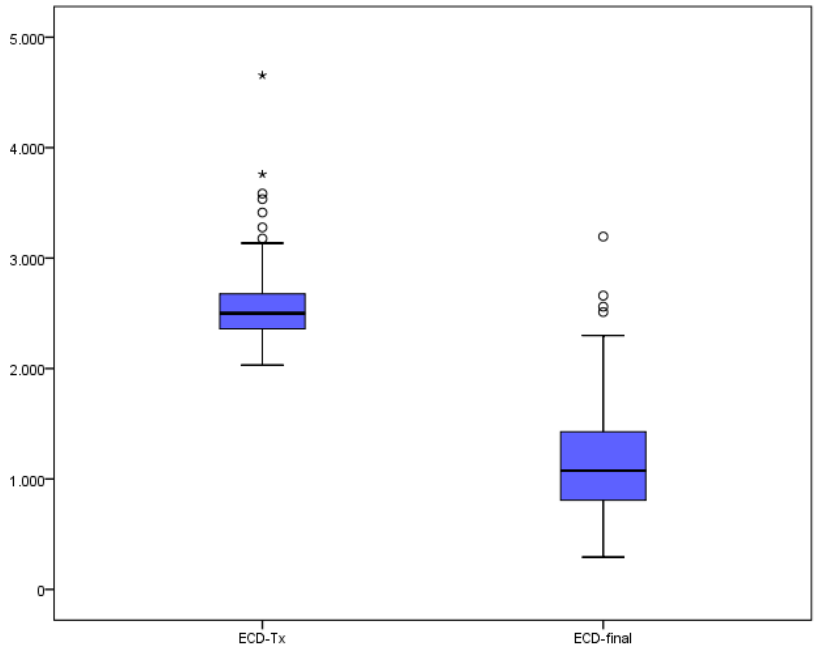

Figure39: Box-Plot graph showing the difference between the ECD of the grafts and the postoperative ECD

The morphology of the endothelial cells was subjectively evaluated by an observer (K. D.), who has a good experience in evaluating the images of the specular microscopy. The morphology of the endothelial cells was graded and the endothelial cells were given a score from 1 to 5 as described by Melles et al[53]:

1. Regular hexagonal cells with regular disturbance. No signs of cellular activity, i.e. no visible cellular nuclei and no increased cellular reflectivity.

2. The cells are a bit irregular in morphology and/or in disturbance. No signs of cellular activity.

3. Mild to moderate irregularity in the morphology and/or disturbance with mild to moderate cellular activity.

4. Severe irregularity in the morphology and/or disturbance with obvious cellular activity as well as the enlargement of the cells nuclei.

5. Extreme irregularity in the morphology and/or disturbance with high cellular activity.

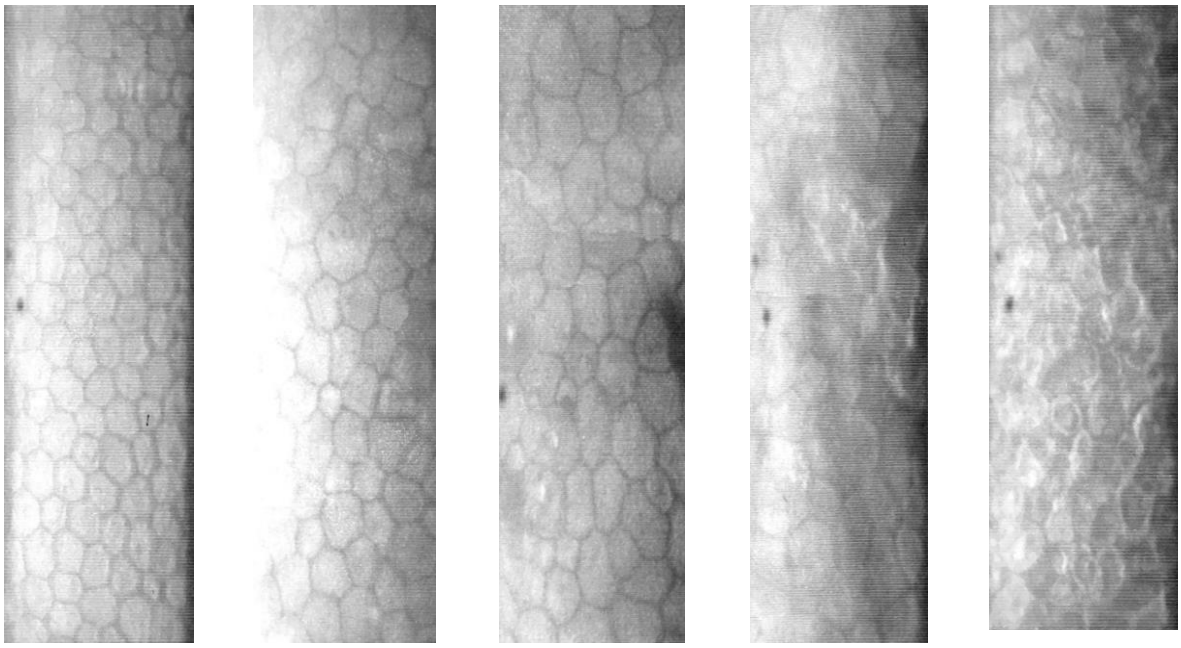

Figure 40: showing the endothelial cells' score (Own examination in University eye clinic Marburg, Germany. Classification taken from: Monnereau C., Bruinsma M., Ham L., Baydoun L., Oellerich S., Melles G., Endothelial Cell Changes as an Indicator for Upcoming Allograft Rejection Following Descemet's Membrane Endothelial Keratoplasty., 10.1016/j.ajo.2014.05.030)

The results of our endothelial cells evaluation were the following: score 1 in 35.6% of the eyes, score 2 in 43.2% of the eyes, score 3 in 17.1% of the eyes, score 4 in 3.6% of the eyes, and score 5 in 0.5% of the eyes.

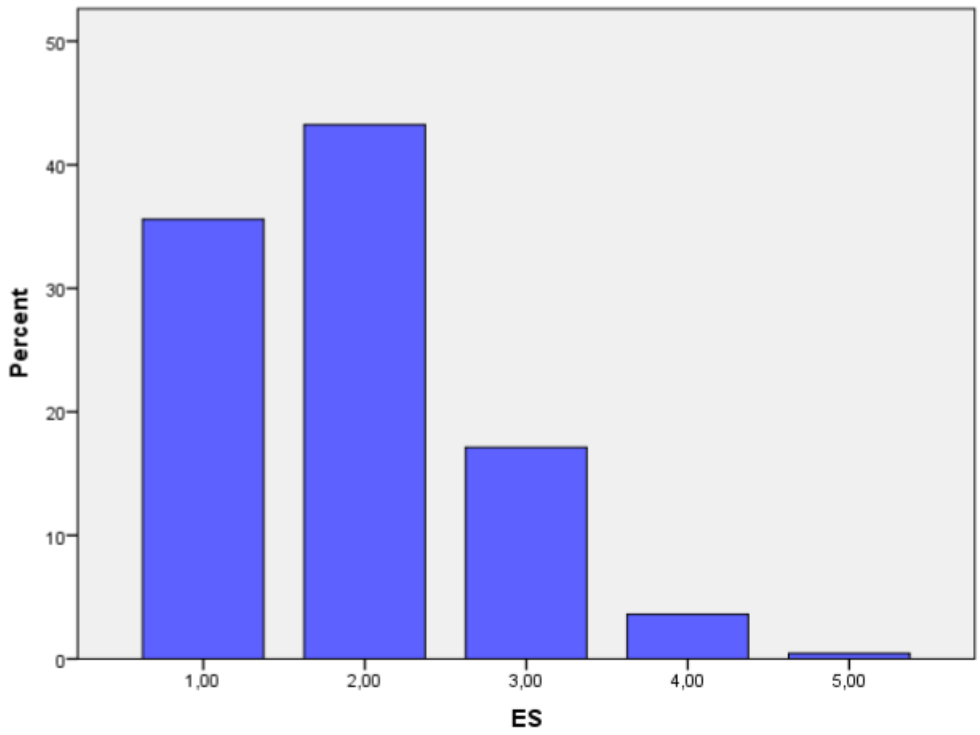

Figure41: Graph showing the endothelial cells score of our patients

4.4.5. Rate of re-bubbling:

In 80.4% of the cases (185 eyes), no graft detachment occurred and no re-bubbling was needed, whereas in 19.6% of the cases (45 eyes) a graft detachment occurred and re-bubbling was performed. A graft detachment requiring re-bubbling occurred in 7.9% of the eyes filled with 5% SF6 Gas at the end of the operation (6 out of 76 eyes) and in 26% of the eyes filled with air (40 out of 151 eyes). Re-bubbling was performed once in 95.5% of these cases (43 eyes) and twice in 4.5% of them (two eyes).

The time point at which re-bubbling was performed was on average 23.8 days after DMEK with a standard deviation of 12 (Median 21 days, minimum 2 days, maximum 59 days).

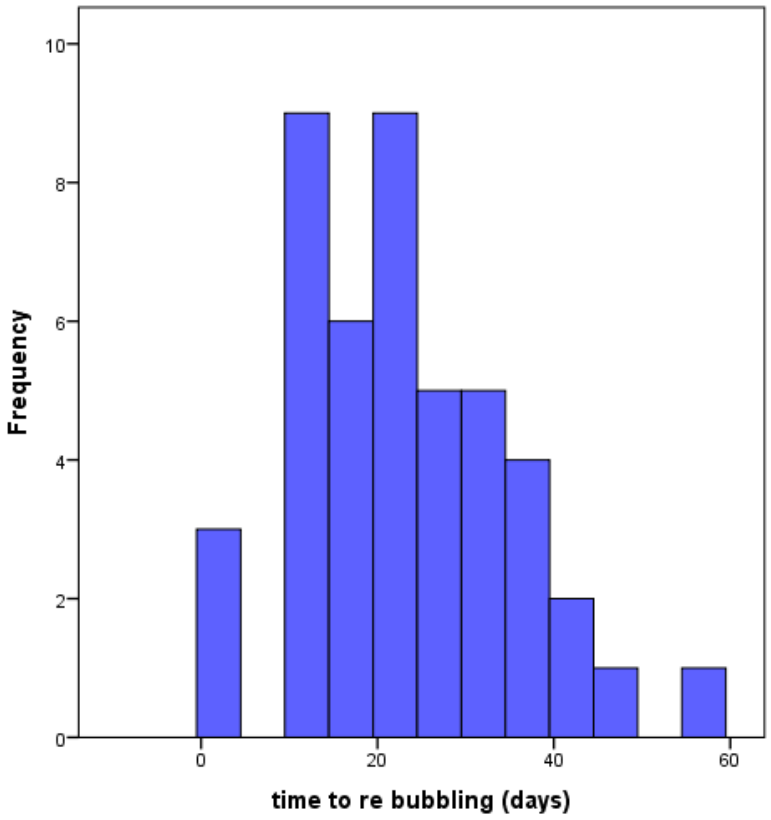

Figure 42: Graph showing the time to re-bubbling after DMEK

4.4.6. Rate of complications and re-operation:

Steroid response developed in 11.7% of the eyes (27 eyes). The IOP was under 25 mmHg in 81.5% of them (22 eyes) and between 25 and 35 mmHg in 18.5% (5 eyes) of them. Secondary glaucoma (non-steroid induced) developed in 1.7% of the eyes (4 eyes). The IOP was medically controlled in all of these patients, except in one case (0.4%), where a trabeculectomy with mitomycin-C had to be performed one year after DMEK.

Cyctoid macular edema developed in 2.6% of the eyes (6 eyes). One of them (16.6%) received intravitreal Dexamethazone implant (Ozurdex® (Allergan)), while another eye (16.6%) received intravitreal anti-vascular endothelial growth factor (anti-VEGF)

injection. The other cases were observed and underwent a spontaneous recovery. 0.9% of the eyes (2 eyes) developed an exudative AMD, which had to be treated with intravitreal Anti-VEGF.

Vitrectomy had to be performed in 0.9% of the eyes (2 eyes) due to retinal detachment and in 0.4% of the eyes (one eye) due to macular pucker.

Band keratopathy developed in 0.9% of the eyes (2 eyes). In both cases, the visual outcome was affected, thus epithelium debridement had to be performed. It is notable that both of these cases underwent re-DMEK after a graft rejection.

Posterior vitreous detachment with disturbing floaters rose in 0.4% of the eyes (one eye). In one case (0.4% of the eyes), central retinal vein occlusion occurred 2 years after DMEK. Urrets-Zavalia syndrome (dilated and fixed pupil after PK) was observed in 0,4% of the eyes (one eye).

We observed deposits on the anterior surface of the intraocular lenses in four of the pseudophacic eyes. It is important to point out that 0.9% of the eyes (two cases) underwent an extraction of the iol and a secondary implantation of iris-fixatd iol because of vision-affecting IOL-opacities. This operation was performed 18 months after DMEK in one eye and 36 months after DMEK in the other.

In 0.4% of the eyes (one eye), the postoperative astigmatism was troublesome, thus Astigmatic keratectomy had to be performed.

Graft failure occurred in 7.8% of the eyes (18 eyes). Re-operation was performed in these cases (17 eyes re-DMEK and one eye DSAEK). Rejection occurred again in one of the eyes that underwent re-DMEK and in the eye that underwent DSAEK. Re-re-DMEK and PK were performed, respectively. We analyzed the reports of the operations and the documentations of these cases to find out the cause of DMEK's failure (see table 3).

In addition, we observed one case of recurrence (0.4% of the eyes), where more than 10 guttae could be seen with the slit lamp (See 4.4.4, ES 5).

Table 3: the 18 cases of graft failure with the suspected reason of failure

	Diameter (mm)	Complications (intra- or postoperative)	re-bubbling	Time of re-operation (Months)	notes	Suspected reason for failure
Case 1	9.50	no	no	48	sudden corneal edema 3 years after DMEK (no steroid)	Presumed graft rejection
Case 2	9.25	Graft detachment	2x (days 14, 23)	11	Graft detachment persisted, rejection after a few months	Typical graft rejection
Case 3	9.00	Difficulties with graft preparation, more manipulation	2x (days 4, 13)	3	Iatrogenic cause	Primary graft failure
Case 4	9.00	Postoperative subluxation of iris-claw iol	no	46	Operative re-positioning of the iol one day after DMEK	Related to ocular comorbidity
Case 5	9.25	Intraoperative graft could not be positioned correctly	no	3	Failed DMEK	Primary graft failure

Case 6	8.25	Tear at the graft edge during preperation. More manipulation was needed to position the graft	no	2	Iatrogenic cause	Primary graft failure
Case 7	9.00	Graft detachment	1x (30 days)	2	Persistence of graft detachment, cornea did not clear up	Primary graft failure
Case 8	8.50	Steroid responder, IOP less than 25 mmHg	no	29	Sudden decompensation 1.5 years after DMEK	Presumed graft failure
Case 9	8.75	no	no	53	Sudden decompensation 4 years after DMEK	Presumed graft failure
Case 10	9.00	More manipulation intra-operatively; graft had to be taken out many times through the injector	1x (day 34)	2	Iatrogenic cause	primary graft failure
Case 11	8.50	Secondary glaucoma (not steroid induced)	1x (day 30)	36	Trabeculectomy was needed, graft rejection occurred a few months later	Related to ocular comorbidity

Case 12	8.75	none	no	7	Cornea did not clear up, no graft detachment	Primary graft failure
Case 13	7.75	none	no	52	Donor graft with a low ECD	Primary graft detachment
Case 14	9.00	Graft detachment	1x (day27)	4	Persistence of Graft detachment	Primary graft failure
Case 15	9.00	none	no	42	Sudden decompensation after 3 years, no steroids	Presumed graft rejection
Case 16	9.25	Graft detachment	no	2	No re-bubbling performed because of corneal edema and Descemet's folds	Primary graft failure
Case 17	9.00	Secondary glaucoma	no	2	Cornea did not clear up, no graft detachment	Primary graft failure
Case 18	9.00	none	no	30	Donor graft with low ECD	Primary graft failure

4.4.7. Graft survival after DMEK:

We analyzed the graft survival after DMEK depending on the number of patients who developed a graft rejection and the time until re-operation. The majority of the patients

(92%) did not experience a rejection or a re-operation, this is marked as censored survival time.

18 patients experienced a graft rejection. The mean survival time of the graft in our patients is 76.6 months (SD 1.3).

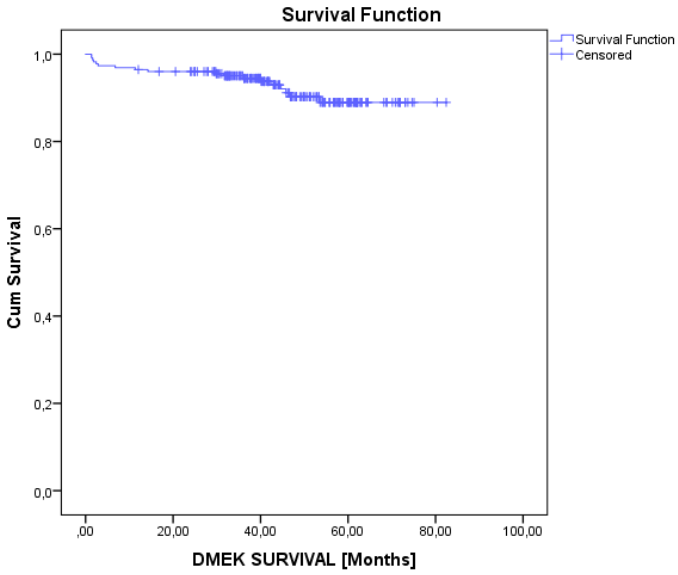

Figure 43: Caplan-Meier curve showing the graft survival time

4.4.7.1. Graft survival related to long time use of steroid eye drops:

We compared the survival time between the patients who stopped the steroid eye drops 12-18 months after the operation and the patients who were still taking steroid eye drops at the time of our examination. 76.5% of the cases (176 eyes) have discontinued cortisone eye drops 12-18 months after the operation. 6.1% of these eyes (12 eyes) needed a re-operation after a graft failure. The other 23.5% (54 eyes) were still receiving cortisone eye drops at the time of our examination. 9.6% of these cases (5 eyes) underwent a re-operation after a graft rejection.

Our analysis showed a graft survival time of 75.4 months in the group of patients who discontinued the steroid eye drops vs. 75.6 months in the group of patients who were

still taking it. The difference is very small and not clinically significant. Analysis of this difference with Log Rank Test (p=0.477) indicated that the survival curves do not differ significantly.

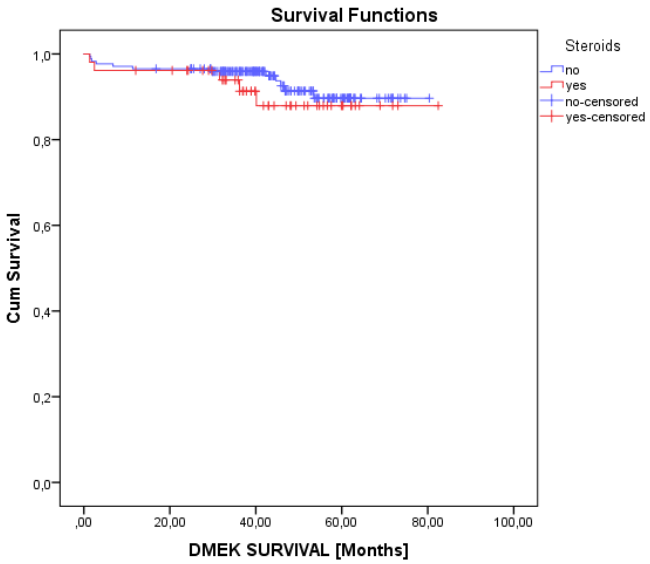

Figure44: Kaplan-Meier curves of the graft survival of two groups of patients, with and without steroid eye drops

4.4.7.2. Graft survival related to re-bubbling:

We analyzed the graft survival time between the cases who did not under-go re-bubbling in graft detachment (80.4% of the cases: 185 eyes) and the cases where re-bubbling was performed (19.6%: 45 eyes). 6.5% of the eyes who did not undergo re-bubbling needed a re-operation because of graft failure, whereas in 13.4% of the eyes who underwent re-bubbling a re-operation was needed. Analysis of the graft survival time showed a survival time of 66.8 months in the eyes that underwent re-bubbling vs. 77.4 months in the eyes where re-bubbling was not performed. Regardless, the Log Rank Test was not significant (p=0.196).

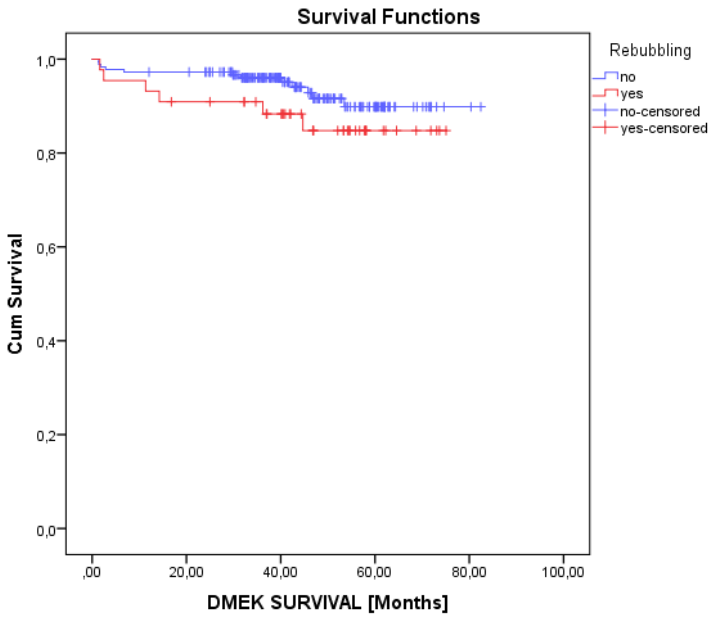

Figure45: Kaplan-Meier curves of the graft survival of two groups of patients, with and without re-bubbling

5- Discussion:

Over the years, PK was widely replaced with the posterior lamellar keratoplasty to treat patients with endothelial cell disorders, including Fuchs endothelial dystrophy, posterior polymorphous dystrophy, congenital hereditary endothelial dystrophy, bullous keratopathy, iridocorneal endothelial syndrome and graft failure after PK or DSAEK. Nowadays, DMEK is considered the golden standard to treat these cases. DMEK was first described by Melles in the Netherlands Institute for Innovative Ocular Surgery (NIIOS). Descemet's membrane and the endothelium of the recipients are replaced with a graft containing these parts, maintaining the outer part of the recipient's cornea[51]. This most targeted technique available for endothelial cells transplantation has many advantages. DMEK is a relatively new surgical technique. Hence, it has been widely studied in the last few years. Many studies compared DMEK to other types of keratoplasty, which will be

briefly discussed in 5.1. Furthermore, a short review of the short-term studies after DMEK will be mentioned before presenting the results of this study in 5.2.

5.1. DMEK compared with other types of keratoplasty:

A review study from Nanavaty et al from 2014 included all randomized controlled trials, which compared patients with FED who underwent PK with patients who underwent endothelial keratoplasty (DSAEK, femtosecons-assosiated DSAEK, DMEK). This study showed that the visual recovery was fastest after the endothelial keratoplasty; the surgically induced astigmatism was less and the rate of the graft rejection was less[55]. Maier et al proved that DMEK results in a better uncorrected visual acuity, a better BCVA, a shorter time of visual recovery and less surgical induced astigmatism compared to PK[48]. These advantages of DMEK led to the consideration that endothelial keratoplasty is the treatment of choice in patients with endothelial cell disorders[18, 28].

DSAEK has also been widely used in the treatment of endothelial cells disorders[11]. However, it has been replaced by DMEK in many centers. DMEK is considered to be a modified technique of DSAEK. The clinical outcomes of DSAEK and DMEK have been compared in many studies. Goldich et al and Guerra et al compared the clinical outcome in patients with FED, where DMEK was performed on one eye and DSAEK in the fellow eye. They both reported a faster visual recovery, higher patients' satisfaction and better visual outcomes in the DMEK-eyes 6 months and 12 months postoperatively, respectively[34, 36].

Droutsas et al and Bhandari et al retrospectively compared the visual rehabilitation and the endothelial cells count 1 year after DMEK and DSAEK and both found DMEK superior to DSAEK in the visual outcome and the speed of visual rehabilitation[16, 25].

A systemic review and meta-analysis by Singh et al showed that DMEK is superior to DSAEK in clinical outcomes including BCVA, although the rate of re-bubbling was higher after DMEK[70].

5.2. Clinical Outcomes after DMEK:

DMEK is considered to be a challenging operation, including the preparation of the thin graft from the donor tissue. The learning curve of the surgeons and the short-term results has been evaluated in many studies.

Dapena et al evaluated the learning curve in the first group of 135 consecutive eyes, where DMEK was first performed worldwide by the Netherlands Institute for Innovative Ocular Surgery (NIIOS). The clinical outcomes showed that 93% of the eyes reached a BCVA of 0.30 logMAR (≥ 0.5) and 73% of them reached the BCVA of 0.10 logMAR (≥ 0.8) 6 months after the operation. The average of the endothelial cells count was 1747 ± 527 cells/mm² 6 months post-operatively. It has been shown that the rate of graft detachment was reduced with experience. The rate of other complications was uncommon[20].

Similar results were published by Droutsas et al, who evaluated the learning curve in the first 25 eyes operated on by a single surgeon, in the absence of an in-house eye bank facility. The 6 month evaluation showed similar good visual outcomes. Graft detachment requiring re-bubbling occurred in 36% of the cases, whereas primary graft failure was reported once in this series[24]. It has been suggested that the postoperative BCVA and the postoperative endothelial cells count is not related to the surgical experience, but rather to the function and adherence of the graft to the donor tissue[20].

This was also proposed by Rodríguez-Calvo-de-Mora et al, who suggested that the clarity and the optical quality of the cornea after DMEK could be as good as a virgin cornea[65].

On the contrary to these short-term studies, we aimed to evaluate the long-term results after DMEK. We did not only analyze the main parameters that indicate a good function of the endothelial cells, but also scanned for all short-term and long-term complications that occurred within our follow-up time. We evaluated the long-term graft survival and

tried to find relating factors that may affect it. In addition, we analysed the reasons that led to graft failure in some cases in our follow-up time.

In our follow-up, we observed an increase in the BCVA of 0.42 ± 0.33 between preoperative and post-opertive BCVA, which is highly significant ($p<0.0005$). It is interesting to mention that 71.1% of these patients had a BCVA of 0.11 logMAR or better (≥ 0.8 decimal), whereas 49.2% of them had a full BCVA of 0.00 logMAR or better (≥ 1.0 decimal).

As mentioned before (see 3.4.3), the central corneal thickness CCT and the corneal volume CV are very important parameters used as indicators of the endothelial cells' function. The increase in these parameters demonstrates an abnormal water content in the cornea, which means that the function of the endothelial cells is decreased[76]. The reduction of CCT after the follow-up time in our patients was 128 ± 107 µm; corresponding to a reduction of CV of 3 ± 7.5mm². Both of these parameters were highly significant ($p<0.0005$) and indicated a significant improvement in the function of the cornea in the long-term follow-up.

Similar results were published by Ham et al, who reported the midterm results 4-7 years after DMEK (n = 250 eyes). This study suggested, that the improvement of the visual acuity and the reduction of the CCT were significant after the first six postoperative months but remained relatively stable in the postoperative seven years[38]. In addition, Schloegl et al reported similar clinical outcomes in his study of 97 eyes 5 years after DMEK[67].

We analysed the preoperative and postoperative refraction in both its components, spherical and cylindrical, depending on the subjective refraction of our patients. With a spherical shift of +0.25 ± 1.10 D and cylindrical shift of -0.73 ± 1.21 D, we found that the change of refraction is very small and that the refraction could remain stable many years after DMEK.

Other short-term studies by Ham et al (n=50)[39] and Roeck et al (n=139) [66] showed also a small refraction shift. However, they studied these changes in a follow up of 6 months and 1 year after DMEK, respectively.

It has been shown that the main change in the corneal astigmatism after DMEK happens on the posterior surface of the cornea[4]. This is due to the sutureless microinvasive technique, which enables preservation of the anterior cornea. It is very important to emphasize, that the refraction shift and the operation-induced astigmatism after DMEK is very minimal compared to the astigmatism after PK[48].

Analyzing the endothelial cell count in our study showed an average loss of 1392 ± 455 cells/mm² in the last follow-up. This corresponds to a total loss of 54.7% of the graft cells on average (SD 16.8). This loss rate was similar to other studies such as the study of Schloegl et al (n=79) who reported a loss of 44.5% of the endothelial cells 5 years postoperatively[67]and Ham et al (n=250) who reported a decrease of 33.9% of the endothelial cells in the first 6 postoperative months, followed by a stable decrease of 9% yearly in the first 4-7 years after DMEK. This decrease is considered acceptable and similar to the decrease in the EZD after DSAEK and preferable to the decrease after PK[38].

This annual analysis could not be performed in our cross-sectional study but was also confirmed by Baydoun et al, who proved that there is a decrease of 35% in the ECD after 6 months, 38% after 1 year, 43% after 2 years, 52% after 4 years and 55% after 5 years. This points out to a rapid loss of endothelial cells in the first 6 months after DMEK followed by a stable annual loss of 7%. Although the loss of the ECD is similar 5 years after DMEK and DSAEK, it It was suggested that the short term damage of the endothelial cells could be less in DMEK compared to DSAEK due to the non-touch surgical techniques[15].

Feng et al also assumed that the loss of endothelial cells after DMEK is mainly due to the surgery itself. They found the rate of endothelial cell loss in DMEK superior to this rate in DSAEK and PK reported in other studies[27].

5.3. Rate of complications:

5.3.1. Graft Detachment and re-bubbling

Graft detachment refers to the lack of adherence between the graft and the stroma of the recipient. It is the most common complication after DMEK. The graft detachment may be total, including the whole graft, or partial detachment[20].

A peripheral partial detachment, which does not affect the visual outcome, can be monitored. On the other hand, a partial detachment disturbing the vision or a total detachment has to be managed by injecting air or gas in the anterior chamber, which is called re-bubbling. A graft detachment can be well recognized in the clear cornea with slit lamp bio-microscopy. If corneal edema presents and there is difficulty recognizing the detachment, the anterior chamber optical coherence tomography (OCT) can be a very useful device.

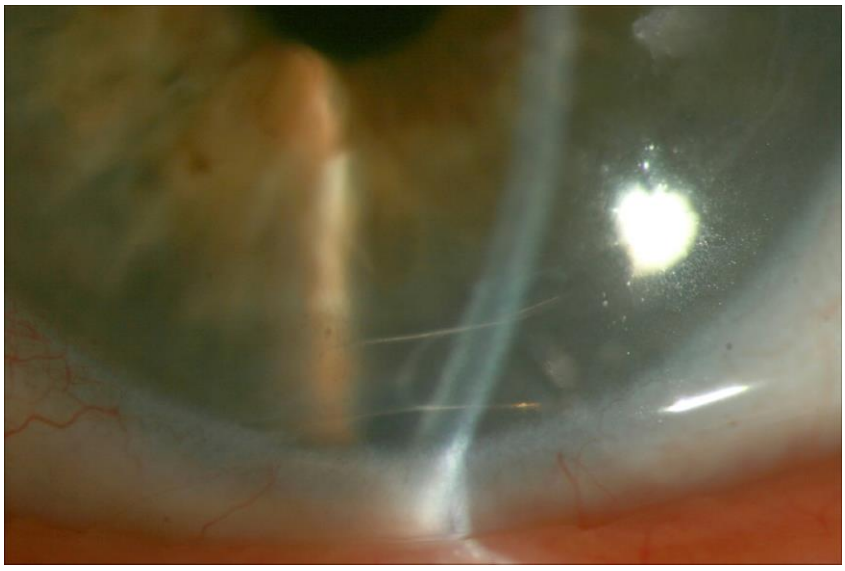

Figure 46: showing a slit lamp photo of peripheral graft detachment (own examination in the University eye clinic, Marburg, Germany)

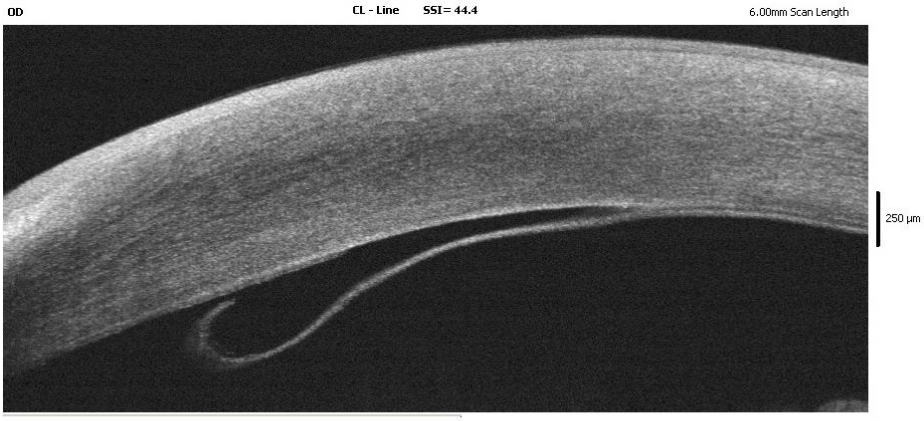

Figure 47: cornea-OCT of a graft detachment (own examination in the University eye clinic, Marburg, Germany)

None of our patients had a total graft detachment. A partial graft attachment involving the center of the cornea occurred in 19.6% of the eyes (45 eyes). These cases were managed with air-re-bubbling. It was performed once in 95.5% of these cases (43 eyes) and had to be performed twice in 4.5% of them (two eyes). Re-bubbling was performed at a range of 23.8 ± 12 days after DMEK.

Similar rate of re-bubbling was reported by Ham et al, 15.6% once and 2% twice in their four to seven years follow-up study of 250 eyes[38]. Rodriguez et al also reported a rate of 15.8% in a study consisting of 500 eyes[65]. The rate of re-bubbling has a relatively big range in other studies. This can be because some surgeons prefer to perform re-bubbling in any small graft detachment, whereas others tend to observe the cases where the detachment is small and does not affect the vision[60].

Many factors could play a role in the risk of graft detachment. These factors were not analyzed in our study, but are worth mentioning. Learning curve is one of the important factors. It was reported, that the rate of graft detachment was reduced with time in which DMEK was performed by a single surgeon[20, 38].

The support of the gas/air tamponade is very important in the attachment of the graft to the stroma of the recipient. The rate of re-bubbling increases if the support of the tamponade is insufficient or if the filling of the anterior chamber is not adequate[23]. In addition, the amount of air/gas available in the anterior chamber in the first 2-3 postoperative hours and the IOP after the operation plays a role in the rate of graft detachment[45, 60].

The material used as a tamponade also plays a role in the rate of graft detachment requiring re-bubbling. We analyzed the surgical protocol of our patients and found out that re-bubbling was performed in 26.5% of the eyes with air tamponade, whereas a graft detachment requiring re-bubbling occurred only in 7.9% of the eyes with 5% SF6 tamponade. It is interesting to mention that the patients in our study are a part of the 400 eyes group studied retrospectively by Ampazas et al from Marburg University Eye Clinic, who reported a significant difference in the rate of re-bubbling in eyes with air tamponade (20.4%) compared to eyes with SF6 5% tamponade (6.8%)[10]. In addition,

Güell et al reported a lower rate of re-bubbling when using Sulfur Hexafluoride 20% as a tamponade compared to air tamponade[35]. This has to be supported with a supine face-up position of the patient postoperatively[10, 35]. The fact that only 34.3% of our patients had a 5% SF6 tamponade may be a cause of the relatively high rate of re-bubbling in our study.

The size of the descemetorhexis seems to be important in the rate of graft detachment. It is recommended to perform a bigger descemetorhexis to avoid the overlap between the graft and the peripheral remnants of the recipient's Descemet's membrane[79]. Furthermore, the presence of any remnants of the recipient's Descemet's membrane in the interface between the graft and the stroma may increase the rate of re-bubbling[79]. Combining DMEK with cataract extraction may increase the risk of graft detachment[45].

The age of the recipient and the donor do not play a role in the rate of graft detachment[23]. The ocular comorbidity of the recipient has not been proved to be a risk factor of graft detachment[45].

5.3.2. Glaucoma and IOP-elevation:

Glaucoma is one of the possible complications after keratoplasty. The rate of glaucoma after DMEK is less than the rate after PK and DSAEK[49, 56]. We observed a post-operative exacerbation of glaucoma in 21.5% (3 cases) of the patients with previously known glaucoma. IOP could be conservatively controlled in two of our cases and surgically in the last one. Naveiras et al reported a similar rate, with IOP-elevation in 25% of the patients with known glaucoma[56].

In the presence of the peripheral iridotomy by 6 o'clock, none of our patients developed a bubble-induced angle closure.

The most common cause of IOP-elevation after DMEK is steroid-induced one[49]. Steroid-induced IOP-elevation occurred in 11.7% of the eyes in our study (27 eyes). In these

patients, dexamethasone was replaced by Loteprednol etabonate 0.5% and glaucoma eye drops. IOP could be medically controlled in all of these patients and they all had a clear cornea at the time of our follow-up. The incidence of steroid-induced IOP-elevation was 8.0% in a 12 month study by Maier et al[49] and 0.7% in 22 month study by Naveiras, who used a different post-DMEK regimen where dexamethasone was replaced with fluorometholone after the first postoperative month in all patients[56].

Another possible cause of delayed glaucoma after DMEK is peripheral anterior synechiae caused by adhesions between the edge of the graft and the iris of the patient[56].

5.3.3. IOL-Opacities:

We observed opacities on the anterior surface of the iol in 2.6% in our pseudophakic patients. These opacities may cause blurred vision and glare, making the exchange of the IOL necessary.

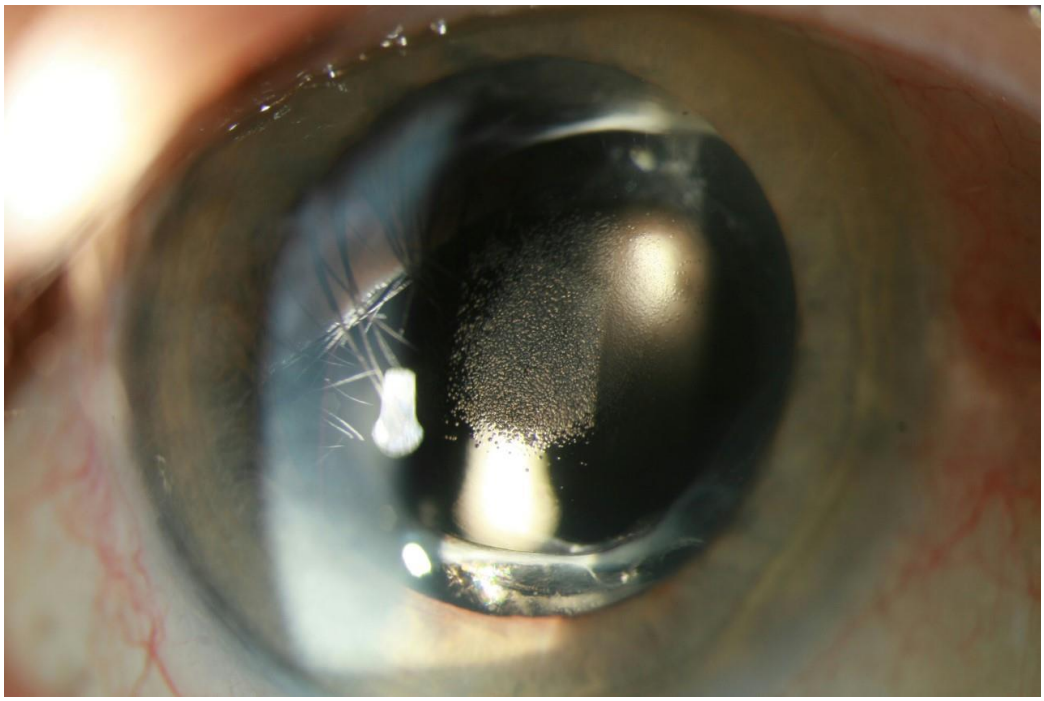

Figure 48: a slit lamp photo of iol opacities (own examination, University eye clinic, Marburg, Germany)

These opacities were also observed in 2.5% of the eyes in a study by Schrittenlocher et al 2-4 years after DMEK, who suggested that these opacities could occur in hydrophilic

as well as hydrophobic IOLs. These opacifications occurred in eyes where SF6 gas was used as a tamponade as well as eyes where air was used. In this study, 87.5% of the affected eyes underwent re-bubbling at least once. Hence, the rate of re-bubbling appears to be the most important factor leading to the formation of these opacities[68].

Only in two of our cases, where iol opacities occurred, re-bubbling was performed (33.3%).

Another study found IOL-calcifications in five out of 153 DSAEK and in two out of 450 DMEK eyes. Five out of of these seven eyes underwent re-bubbling. All of these eyes had hydrophilic acrylic IOLs[50]. Similar opacities were reported after DSAEK in eyes with hydrophilic IOLs where re-bubbling was performed[54].

Analysis of our patients' medical history revealed that all cataract operations were performed in other clinics many years before the patients attended our hospital. Hence, we were not able to figure out what material were these IOLs made of.

The analysis of 13 explanted hydrophilic iols after DSAEK and DMEK at the David J Apple International Laboratory in the University of Heidelberg revealed that these opacities are crystalline deposits located underneath the anterior surface of the IOL. It has been proven by scanning electron microscopy and energy dispersive X-ray spectroscopy that these deposits consist of calcium phosphate[33].

It has been suggested that the reason for these calcification was the contact of the lens surface with air as well as the breakdown of the blood-aqueous barrier due to air contact[50]. The high exposure to the ultraviolet radiation may also be a factor causing these deposits. This was supported by the fact that the opacities were more concentrated in the center of the iol[68]. Another factor may be the dehydration of the hydrophilic acrylate material of the iol when it comes in contact with the exogenous tamponade[33].

These calcifications are not reversible and cannot be treated with ND:YAG[33, 68]. The only treatment option is the IOL exchange.

The above mentioned calcifications should be distinguished from IOL glistening, which consists of microvacuoles within the IOL material. Glistening occurs mostly in hydrophobic IOLs and rarely affects the visual acuity[33]. Iol-glistening was observed in 11.4% of our patients.

5.3.4. Other rare Complications:

- Postoperative cystoid macula edema occurred in 2.6% of the eyes with no other retinal comorbidities (6 eyes). Spontaneous regression occurred in 4 of these eyes, whereas intravitreal dexamethasone (Ozurdex) had to be injected in one eye and intravitreal anti-vascular endothelial growth factor (anti-VEGF) in the other. A higher incidence of 7.5% and 13.8% was reported by Flanary et al[29] and Kocaba et al[44], respectively. However, both of these studies evaluated the incidence of CME only 6 months after DMEK. In most cases, CME after DMEK resolves with medical therapy and does not affect the long-term visual outcomes[44].

It has been suggested that DMEK may be a risk factor for developing CME, because the incidence of CME after DMEK and triple-DMEK is higher than the incidence of CME after a cataract operation (1-2%)[44]. DMEK was not combined with phacoemulsification of the lens in any of our patients who developed a CME.

- Urrets-Zavalia syndrome, which refers to a fixed, dilated pupil due to iris-atrophy after keratoplasty, is one of the very rare complications after DMEK. It occurred in 0.4% of the eyes in our study. An incidence of 1% was reported by Foroutan et al after PK, DALK and DSAEK[31]. A case of Urrets-Zavalia syndrome after DMEK was reported by Holtmann et al[42]. Beside the iris-atrophy and the possible mechanical damage of the innervation system of the iris, the air or gas tamponade leading to IOP-elevation through pupillary block could be a cause of fixed dilated pupil due to iris ischemia[31].

- Central artery occlusion occurred in 0.4% of the eyes. Since this occurred three years after DMEK, we believe that it is not a complication of the operation itself, but rather caused by systemic comorbidities of this patient.

- Retinal detachment occurred in 0.8% of the eyes (two eyes), 12 and 15 months after DMEK. Vitrectomy was performed in these two cases. Vitrectomy was also performed in 0.04% of the eyes due to epiretinal gliosis affecting the visual outcome seven months after DMEK. It is not certain if DMEK is a risk factor to cause retinal detachment or epiretinal gliosis.

5.4. Recurrence of FED after DMEK:

Some studies mentioned guttae-like changes on the corneal grafts many years after PK[17, 19]. It has been suggested that the recurrence of FED after PK is possible in some patients with genetic tendency[3]. Clinically, we observed less than five guttata on the graft in 26.1% of the eyes, between five and ten guttata in 8.6% of the eyes and more than ten

in 0.9% of the eyes. Zygoura et al reviewed ‘’dark spots’’ on the grafts in 40% of the eyes 7 years after DMEK (n = 83). It was not combined with morphological changes of the endothelial cells in all of the cases. Hence, they did not refer to it as recurrence of FED after DMEK[86].

It has been suspected that these changes may be early signs of graft rejection[19, 86]. Therefore, we instructed these patients to take cortisone eye drops and increase the monitoring rate.

5.5. Rate of graft failure and re-operation and graft survival:

Graft failure occurred in 7.8% of the eyes (18 eyes). Re-DMEK was perfumed in 17 of these eyes, whereas DSAEK was performed in the remaining eye.

We analyzed the operations’ reports and the documentations of these patients to find out the cause of DMEK failure. A primary graft failure, which is the failure of the cornea to clear up in the first 3-4 postoperative weeks[40], occurred in 11 of these eyes (61.1%). In three cases, the cause was iatrogenic due to damage of the graft during the preparation or the surgery. In these cases, the graft was attached but non-functional. In one case, intra-operative attachment of the graft was unsuccessful. In three cases, a graft detachment persisted despite re-bubbling. In four cases, no reason of primary graft failure could be found. No intra-operative complications were reported and the graft was attached but the cornea did not clear up.

Ham et al analyzed the reasons of primary graft failure in 11 eyes after DMEK. These reasons were classified in three groups: partial graft detachment, total graft detachment and failure without graft detachment. They showed that the failure was not related to an insufficiency of the endothelial cells density[40].

In seven of our cases, a graft failure occurred later. The cornea was clear in the interval between DMEK and graft failure. One of these cases (5.6%) developed a typical graft rejection a few months after DMEK in the form of pigment participates on the graft with

endothelial decompensation. In four of the cases (22.2%), we referred to the failure as ''presumed graft rejection''. In these cases, an acute endothelial decompensation with corneal edema occurred without typical signs of graft rejection. These manifestations occurred 18 to 36 months after DMEK. In two cases (11.1%), we assumed the cause of failure to be related to ocular comorbidity.

The rate of re-operation after DMEK was 7.3% in our patients (17 eyes re-DMEK and one eye DSAEK). The time to re-operation was on average 20.7 months after DMEK (SD 20.5) (maximum 53 months, minimum 2 months). Our graft survival rate was 92.2% with an average survival time of 76.6 ± 1.3 months.

In their midterm study 4-7 years after DMEK (n =250), Ham et al reported a graft failure rate of 15.2%. A repeated transplantation (DMEK or DSAEK) had to be performed in most of them within the first postoperative year. The midterm graft survival rate was 96%[38]. A similar survival rate of 95% five years after DMEK was reported by Schloegl et al (n=79)[67]. It has been proven that the incidence of graft rejection in DMEK is less than other types of keratoplasty[12, 21, 72].

The longer graft survival rate may be due to the minor invasive surgical technique in DMEK. Baydoun et al supposed that the survival in patients with FED is higher than in patients with bullous keratopathy (97% and 84%, respectively)[14].

It is not yet clear, if the long term use of steroid drops has a protective effect against a late graft rejection. We did not find a significant difference in the graft survival rate between the patients who discontinued cortisone eye drops one year after DMEK and the patients who were still using these drops (75.4 vs. 75.6 months, respectively).

Price et al found a difference in the survival rate in an early control-study (2 years after DMEK) between patients who discontinued the steroid drops one year after DMEK and the patients who used the drops once a day (rejection 6% vs. 0.27%) (n = 400)[62]. Further

studies are needed to analyze the relationship between continuing cortisone eye drops and the long-term graft survival.

It is also not certain if re-bubbling is a risk factor for late graft failure. We did not find a significant difference in the graft survival rate between the eyes that underwent re-bubbling and the eyes that did not. Some studies found that re-bubbling does not cause a higher loss of ECD[27, 61]. On the other hand, it was suggested that re-bubbling causes extra trauma on the tissues, which causes a higher loss of endothelial cells. This means that re-bubbling may be a risk factor for late graft failure[76]. Further studies are needed to find out the relation between re-bubbling and graft failure.

5.6 Limitations of our study:

In our cross-sectional study, we tried to contact 265 patients who underwent DMEK between 2010 and 2014 in our hospital. Due to this long time period, we could not reach all of them. The contact database of some patients was not up to date. In addition, some patients were not able to attend because of a general illness, a long travelling distance, lack of free time or other reasons. As a result, our study only included 142 out of 265 patients. In addition, we assumed that some patients were not willing to participate due to absence of any problems with their eyes. This may have influenced the postoperative outcomes evaluated in this study.

In some of the study patients, the fellow eye was operated on after 2014. These eyes were also included in this study, which resulted in a wider range of follow-up time.

6- Conclusion:

DMEK has widely replaced PK in the treatment of endothelial cell diseases. It is known that DMEK provides faster visual rehabilitation compared to PK and DSAEK. In this study, we proved that these high visual outcomes remain stable many years after the operation. The corneal parameters indicate that the endothelial cells could still have a very good function years after the transplantation. DMEK has a high rate long-term graft survival (92%) and a relatively low graft failure rate. The postoperative complications after DMEK are few, treatable and rarely affect the long-term visual outcomes. Only eyes that developed serious complications such as IOL-opacification or graft rejection were prone to failure within our long-term study. In conclusion, this study confirmed DMEK to be currently the first choice in the treatment of endothelial cells diseases.

7. Summary in German:

Ziel der Arbeit: Evaluation der langfristigen Ergebnisse sowie der Komplikationsrate nach Descemet's Membran Endothelialen Keratoplastik (DMEK)

Methoden: Eine cross-sectional, Fall-Serien Studie. Insgesamt wurden 230 Augen von 142 Patienten, die zwischen 2010 und 2014 eine DMEK an der Universitäts-Augenklinik Marburg bekommen haben, untersucht. Die best-korrigierte Sehschärfe (BCVA), die Refraktion, die zentrale Hornhautdicke, das Hornhautvolumen sowie die Endothelialzelldichte wurden als Parameter herangezogen und mit den präoperativen Befunden verglichen. Die Transplantat-Überlebensrate sowie die postoperativen Komplikationen wurden ebenfalls betrachtet.

Ergebnisse: Die Nachbeobachtungszeit betrug 47 ± 13.3 Monate. Bei den Patienten die keine anderen okuläre Erkrankungen hatten hat sich die BCVA von 0.60 ± 0.32 logMAR präoperativ auf bis zu 0.10 ± 0.22 logMAR verbessert (201 Augen). 71.1% dieser Patienten hatten eine BCVA von 0.11 logMAR oder besser (≥ 0.8 dezimal), wobei 49.2% dieser Patienten eine volle BCVA von 0.00 logMAR oder besser erreicht haben. Die zentrale Hornhautdicke hat von 675 ± 112µm präoperativ auf 547 ± 52 µm in der letzten Follow-up Untersuchung abgenommen, und das Hornhautvolumen hat von 65.2 ± 8.4 mm^2 präoperativ auf 61.9 ± 5.4 mm^2 abgenommen. Der Endothelzellverlust lag bei 1392 ± 455 Zellen/mm^2, was einem durchschnittlichen Verlust von 54.7% der Transplantatzellen entspricht. Die Transplantat-Überlebensrate lag bei 92% mit einer durchschnittlichen Überlebenszeit von 76.6 ± 1.3 Monaten.

Schlussfolgerung: DMEK bietet hohe visuelle Ergebnisse und sehr gute klinische Befunde, die mehrere Jahre nach der Operation stabil bleiben können. Durch die hohe Transplantat-Überlebensrate und die niedrige postoperative Komplikationsrate wird DMEK derzeit als erste Wahl zur Behandlung von Endothelzellerkrankungen eingesetzt.

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