Elucidation of the migratory behaviour of the corneal endothelium

Miron, A.

CHAPTER 1

Introduction and outline of the thesis

Alina Miron

Netherlands Institute for Innovative Ocular Surgery, Rotterdam, The Netherlands

GENERAL INTRODUCTION

Posterior lamellar keratoplasty techniques have gained increasing acceptance over the past two decades to become the leading form of corneal transplantation. Initially described by Melles et al in 1998 , there have been multiple technical modifications, culminating in Descemet membrane endothelial keratoplasty (DMEK).[1,2] These technical refinements have allowed endothelial keratoplasty to gain not only widespread acceptance but to make it the treatment of choice for endothelial diseases.[3,4] The selective transplantation of the endothelium through a small incision allows for faster and more complete visual rehabilitation, with minimized interface haze and antigen load, less astigmatism and fewer suture-related complications.[5,6] Fuchs endothelial corneal dystrophy is the most common indication for corneal transplantation.[7,8] It is characterized by deterioration of endothelial cells, from the center towards the corneal periphery, and the development of characteristic basement membrane excrescences known as guttae. Interestingly, the diseased endothelial cells have demonstrated an increased migration speed compared to normal corneal endothelial cells, which may impact the healing response to surgical procedures under pathological conditions.[9]

The research presented in this thesis focusses on *in vivo* and *in vitro* corneal endothelial cell migration from shape-adapted corneal grafts for the treatment of corneal endothelial disorders. A greater understanding of the cell migration mechanisms from phenotypically distinct regions of the endothelium may assist in selecting the optimal donor tissue and predicting *in vivo* cell behavior. Understanding cell migration *in vivo* has clinical implications for corneal transplantation, for instance when pharmaceutical cell modulation may further improve the patient outcomes. In this introduction, we will discuss the corneal anatomy, the basic physiological function of the corneal endothelium, surgical approaches, and available treatment options for mitigating endothelial cell dysfunction.

THE HUMAN EYE

The eye consists of three basic structural layers, which enclose the optically clear aqueous humour, lens, and vitreous body. The outermost layer is a tough collagenous structure comprised of the cornea and the sclera. The middle layer is highly vascular and is known as the uvea. This layer contains the main blood supply to the eye and consists, from posterior to anterior, of the choroid, the ciliary body, and the iris. The innermost layer is the retina, which rests on the choroid and lines the inside of the posterior segment. The retina is one of the most metabolically active tissues in the body, receives most of its nourishment from the vessels within the choroid, and is responsible for the perception of images (Figure 1). To reach the retina, light must pass through and be refracted by the cornea which is responsible for two thirds of the refractive power of the eye.

The Cornea

The cornea is a transparent avascular tissue about 520 µm thick which acts as a primary barrier against infection and mechanical damage to the internal structure of the eye.[10] The role of the cornea in the refraction of light requires it to be both optically transparent and sufficiently curved to bend light rays with minimal light scattering. Normally, more than 90% of the incident light is transmitted through the cornea.[11] Maintaining corneal transparency is of prime importance for visual function. Since the cornea lacks blood vessels, the anterior surface receives nutrients via diffusion from the tear fluid, the periphery from scleral vessels while the posterior side is supplied by the aqueous humor. The cornea is also supplied by neurotrophins via the nerve fibers that innervate it. Its anatomic structure is relatively simple consisting of three cellular layers, namely the epithelium, the stroma and the endothelium which contain epithelial cells, keratocytes and endothelial cells, respectively. In addition, there are two important acellular interfaces: the Bowman layer between the

epithelium and the stroma, and the Descemet membrane (DM) between the stroma and endothelium (Figure 2).

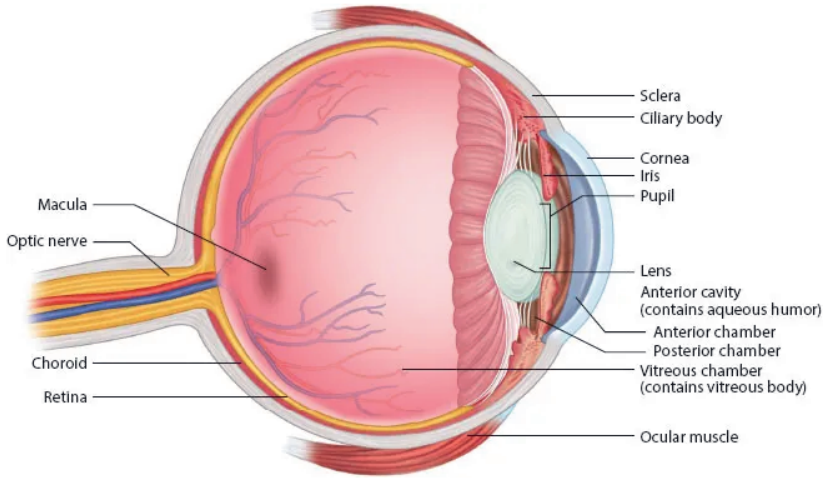

Figure 1| Anatomy of the eye – sagittal section.

Source: https://basicmedicalkey.com/ophthalmic-drug-delivery/

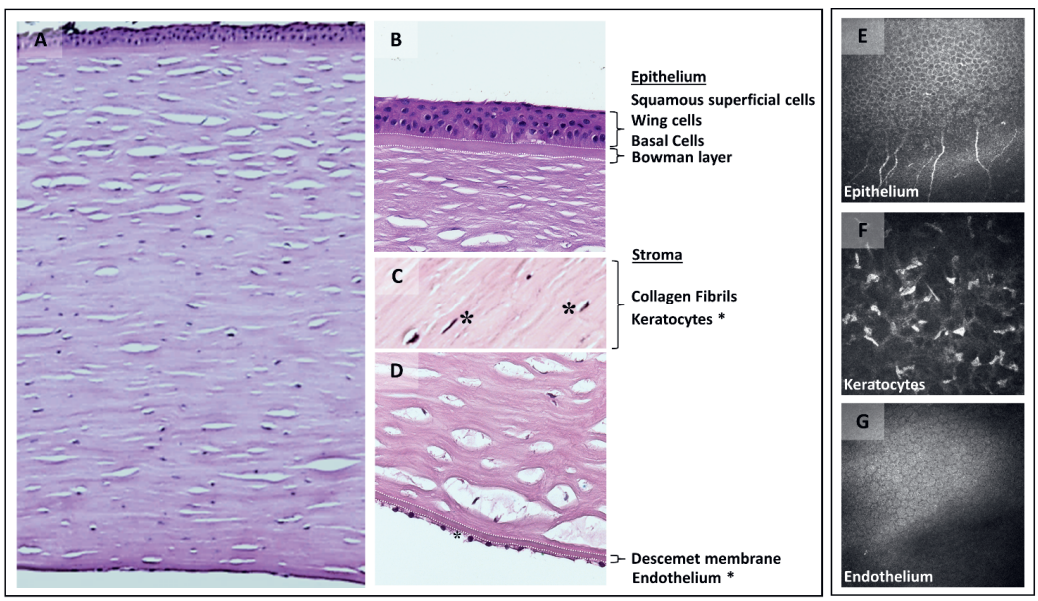

Figure 2| The human cornea. (A) Photomicrograph of a cornea stained with hematoxylin-eosin (H&E) with emphasis on (B) epithelium – formed by superficial squamous cells, wing cells and a single layer of inner columnar basal cells, Bowman layer – an acellular tough membrane situated between corneal epithelium and stroma, (C) stroma – the layer that gives the cornea strength and gives it its curved shape, (D) Descemet membrane – the innermost surface of the cornea that acts as a basement membrane for the inner endothelium – a monolayer of homogeneous, closely packed, hexagonal cells; original magnification: (A) 100 µm, (B-D) 400 µm. (E-G) confocal microscopy images of epithelial cells, stromal keratocytes and endothelial cells.

The epithelium

The epithelium is a five-to-seven layered cell sheet that serves a number of functions. It helps to keep the corneal surface optically smooth and provides a barrier to external biological agents and chemical damage.[12] It represents about 10% (~ 50 µm) of the total corneal thickness and it is constantly sloughed off and regenerated, which helps the eye to heal itself from mild trauma or abrasions. The epithelium consists of flat superficial differentiated cells, deeper winged cells (daughter cells of the basal layer which are pushed anteriorly), and an underlying monolayer of columnar basal cells. The differentiated squamous cells have surface microvilli and occupy the outer 1 – 3 cell layers of the epithelium (Figure 2, A, B, and E). The function of the microvilli is to increase the cell surface area allowing a close association with the tear film. The underlying basal cells have wing-like extensions, rarely undergo division and migrate superficially to differentiate into squamous cells. The innermost basal cell layer consists of a single layer of columnar cells with important functions including the generation of new wing cells and maintenance of the epithelial organization, and acting as a scaffold on which cells can migrate. The transparency of the normal epithelium is the result of the homogeneity of the refractive index of cells throughout this cellular layer.[13] When an excessive accumulation of fluid (edema) occurs, the epithelium loses its homogeneity and the corneal surface becomes irregular. Any surface irregularity can cause a reduction in vision along with symptoms of glare, photophobia, and halos around lights due to light scatter.

The Bowman layer

The Bowman layer forms the anterior boundary between the epithelium and the stroma and consists of randomly oriented collagen type I fibrils supported within a proteoglycan matrix. This interconnecting network of the anchoring fibrils in the anterior cornea confers considerable strength and resistance to trauma and helps the cornea to maintain its shape (Figure 2, A and B). However, once damaged, it cannot be regenerated.[14]

The stroma

The stroma is a structured lattice of collagens and proteoglycans deposited in sheets known as lamellae and maintained by specialized keratocytes (Figure 2, A, C, and F).[15] The keratocytes are scattered throughout the stroma and are linked to one another via dendritic processes.[16] Keratocytes are typically dormant in the quiescent stroma but can become active and then produce and turnover crystalline proteins to maintain corneal transparency.[17] The unique arrangement of evenly-spaced collagen fibrils provides structural strength, shape, stability, and transparency to the cornea.[18]

The Descemet membrane

The Descemet membrane (DM) is a thin acellular layer that acts as the basement membrane of the endothelial cells. It is continuously produced and deposited by the endothelial cells, resulting in a thickness increase throughout life at a rate of about 1 to 2 µm per decade reaching about 10 µm in older adults (Figure 2, A and D).[19,20] In adults, the DM consists of two ultrastructurally distinct layers: an anterior, highly organized banded layer of collagen lamellae and proteoglycans formed during gestation, and a posterior, more amorphous layer produced by the extracellular matrix deposition of the endothelial cells.[21] The gradual increase in thickness of the posterior layer suggests that either there is no degradation of its constituents or the rate of synthesis of constituents is higher than the degradation rate.[22] The wide-spaced collagen fibers found in the DM do not adhere strongly to the stroma, and so a surgical cleavage plane can be created allowing the DM to be peeled and dissected as a sheet.[23]

The endothelium

The endothelium is a thin monolayer of hexagonal cells covering the posterior surface of DM, lining the inner surface of the cornea, and maintained by nutrients from the aqueous humor (Figure 2, A, D, and G). Despite their simple hexagonal appearance, endothelial cells are quite complex in function. Adjacent cells communicate through intracellular junctions[24–27] that mediate electrical and chemical coupling between neighboring cells, whereas the basal cell margins adjacent to DM are ruffled, with tightly-joined interdigitating foot processes, some of which appear to insert into neighboring cells.[28,29] The whole assembly allows the endothelium to function as a "leaky" barrier, forming resistance to the permeability of solutes and fluid through paracellular transport routes,[27,30] but allowing the passage of nutrients from the aqueous humor to feed the avascular cornea.[31] The corneal endothelium (CE) counteracts the osmotic tendency of the corneal stroma to swell by removing excess stromal fluid via the activity of proton pumps, which are located mainly on the basolateral side of the membrane.[32,33] The dynamic balance between the barrier and active pump of the endothelium is essential for maintaining the relatively dehydrated state of the stroma required for transparency (i.e., stromal deturgescence).[34] Once the endothelial monolayer is compromised, the relative balance between the leak rate and metabolic pump rate is lost.

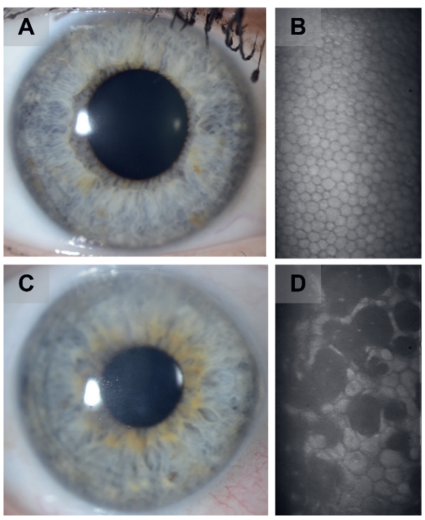

Figure 3| Slit-lamp photograph showing a transparent cornea (A, slit lamp image overview) optimally hydrated by a healthy endothelium (B, specular microscopy image showing an endothelium characterized by hexagonal cells with highly uniform polygonal morphology). Corneal endothelial decompensation (D, specular microscopy image showing empty spaces in the endothelial mosaic) leading to overhydration of the cornea (C, slit lamp image overview).

Endothelial cell density (ECD) begins at about 5000-6000 cells/mm^2 at birth, but as the cornea grows the cells spread out and the density declines to about 3500 cells/mm^2 in the young adult. From the second to the eighth decade, the cell density further decreases to about 2600 cells/mm^2 with an attrition rate of approximately 0.6% per year.[35–37] Human corneal endothelial cells (hCEC) are not thought to have a significant capacity for *in vivo* regeneration, thus making them unable to replace significant numbers of dead or damaged cells.[38] While the cells do not appear to have the capacity to proliferate, hCEC can respond to minor damage by stretching and centripetal migration into the injured area, to maintain proper structure and function.[26,35] The phenomenon of cell spreading can be associated with the variability in cell size (polymegathism) and cell shape

(pleomorphism) observed in older individuals. Given the importance of its function, damage to the endothelium is potentially more serious than that to the other corneal layers and can result in cell loss and loss of vision.[40] However, the age-associated decline of the CE does not usually affect the critical barrier and pump function.[41] In contrast, acute hCEC loss due to conditions such as endothelial dystrophies, chemical burn, or previous refractive or intraocular surgeries may lead to corneal decompensation.[42–48] When the hCEC density decreases below the critical threshold range of 500 to 1000 cells/mm^2 the pump function may be compromised, and the cornea can become edematous and cloudy (Figure 3).[49] In such cases, corneal transplantation is currently the only effective option to improve vision and reduce pain. The gold standard treatment is to replace the ineffective endothelium selectively with healthy, functional donor CE through a corneal transplant.

ADVANCES IN ENDOTHELIUM TRANSPLANTATION AND REGENERATION

Cornea transplantation is an operation used to remove all or only the damaged part of the cornea to replace it with healthy cornea tissue (the transplant) of a suitable deceased donor. Fuchs endothelial corneal dystrophy (FECD) is a common form of corneal endothelial dystrophy and the most common indication for cornea transplantation worldwide.[50] FECD dystrophy progresses slowly and is characterized by a progressive loss of central corneal endothelial cells, thickening of DM and deposition of basement membrane excrescences in the form of guttae (Figure 3, C and D).[51]

Before cornea transplantation can be performed, the donor tissue should be harvested, disinfected, assessed, prepared and stored, a highly regulated process most often performed by an eye bank. Since the first successful transplantation in 1905 by Zirm,[52] for many years, the procedure of choice to manage corneal disorders was penetrating keratoplasty (PK), i.e., a full-thickness graft in which all corneal layers are replaced (Figure 4, top). Although improvements in the visual acuity were achieved with PK, frequent complications were reported: (i) denervation, (ii) suture-related complications such as astigmatism, infection and increased risk of immune-mediated graft rejection, (ii) and a significant increase in the prevalence of glaucoma following transplantation.[53] Moreover, since visual acuity outcomes after PK were not predictable, the procedure was usually only performed after the patient's visual acuity level had dropped to levels below 0.3.[54,55] These drawbacks of PK led to the development of posterior lamellar keratoplasty techniques to replace PK with less invasive surgical interventions for the treatment of endothelium-related corneal diseases.[56–59]

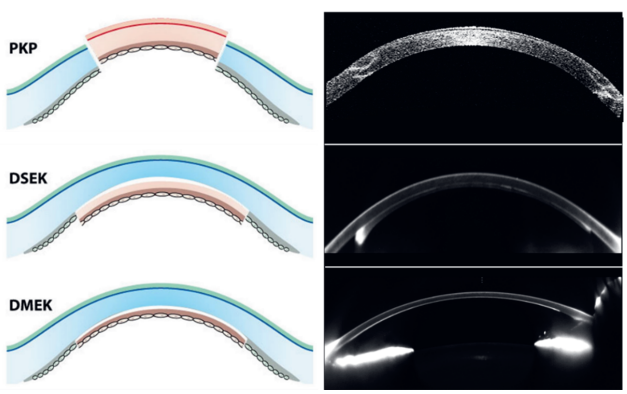

Figure 4| Fundamental developments in endothelial keratoplasty. Cartoon images of the PK, DS(A)EK, and DMEK principles (Left) and the post operative view of the cornea after the three procedures (Scheimpflug image – Pentacam), respectively (Right).[60-62]

One of the keystones of modern endothelial keratoplasty (EK) was laid by Melles et al.[63] in 1998, where he described the posterior lamellar keratoplasty (PLK), an initially mid-stromal approach to replace the posterior cornea. Since the removal of the diseased tissue for PLK was technically still challenging, in 2002, Melles introduced the 'descemetorhexis' procedure, during which the diseased host Descemet membrane with its endothelium is selectively removed ('stripping').[64] This enabled the introduction of Descemet stripping (automated) endothelial keratoplasty (DS(A)EK), a procedure that became adopted by corneal surgeons all over the world (Figure 4, middle).[65] Compared to PK, DS(A)EK resulted in a faster visual rehabilitation and more predictable refractive outcome, while suture and wound-related problems could be avoided. DS(A)EK allows the retention of a better structural integrity of the eye and allograft rejection rates were reduced.[66,67] However, thickness irregularities of the donor graft or stromal interface haze causing optical aberrations could sometimes result in variable visual acuity outcomes.[67] Following the widespread adoption of DS(A)EK, the Melles group further revised its approach and reported the results of a newer procedure that eliminated the variability of the stroma by producing a stroma-less graft in the form of Descemet Membrane Endothelial Keratoplasty (DMEK).[68–70] In DMEK, the normal corneal anatomy is restored by selective replacement of the diseased corneal layer only (Figure 4, bottom). Although initially challenging, recent developments have facilitated tissue handling in a "no-touch" manner. As a result, the procedure is designed with reproducible, standardized steps, that can be implemented by most corneal surgeons in any clinical settings and with relatively low costs.[71]

Compared to PK, lamellar keratoplasty procedures provide faster recovery, better visual results, with less scarring and fewer optical stromal aberrations. In addition, lower rates of post-operative endothelial cell loss have been reported in long-term outcome studies. [72]

Globally, the main barrier to patients receiving these treatments is the lack of suitable donor tissue. To cope with global shortage of donor corneas, the concept of using one donor cornea for the treatment of multiple patients was realized with the introduction of the 'Hemi-DMEK'[73–75] and 'Quarter-DMEK'.[76–79] These approaches use one donor cornea to prepare two semi-circular or four quadrant-shaped DMEK grafts with the potential to double or quadruple the availability of endothelial grafts. These techniques are surgically similar to standard DMEK and achieved comparable visual acuity outcomes, however, the initial case series showed higher rates of graft detachment and relatively low postoperative endothelial cell density.[80,81] Another limitation of these techniques, particularly for Quarter-DMEK, is that the surgical indication is mostly restricted to cases of Fuchs endothelial corneal dystrophy with central corneal guttata (Figure 5B) and without peripheral corneal edema on slit-lamp examination.

Clinical reports of patients who achieved corneal clearing despite failure of graft adhesion after endothelial keratoplasty[82,83] stimulated research into an entirely different concept – the possibility of cell free treatments. The initial cases with corneal clearance despite graft detachment were followed by reports of endothelial regeneration after Descemet's Stripping Only (DSO) procedures, also known as Descemetorhexis without endothelial keratoplasty (DWEK), that has emerged as a new tissue-free treatment option for patients with central FECD.[84–86] Like any surgical technique, DSO has also evolved over time. Currently, only a small area of diseased DM with a diameter of about 4–6mm, along with its endothelial cells and guttae in the visual axis, is removed. The bare area is then left to clear by the migration of peripheral endothelial cells. The presence of healthy peripheral cells is therefore a pre-requisite for successful DSO treatment but despite improvements in high resolution imaging technologies to determine the peripheral cell density reserve, some patients still do not clear after DSO.[87] The complexity of determining not only the density but the migration capacity and quality of the peripheral endothelial cell reserve in order to define the ideal candidate for DSO, may be the major limiting factor on the widespread use of this approach[88] which shows a 12-month failure rate of 8% - 18%.[89–94] Recently, studies have reported on the topical administration of Rho-kinase inhibitors (ROCK-inhibitors) in combination with DSO.[95–96] It has been shown that the use of ROCK-inhibitors promotes the

recovery of ECD, of visual quality, and supports the concept of peripheral endothelial cell proliferation and/or migration by decreasing peripheral ECD loss. Therefore, by combining DSO with ROCK inhibitors an important economical saving for society could take place as it does not require donor tissue nor expensive post-operative care.

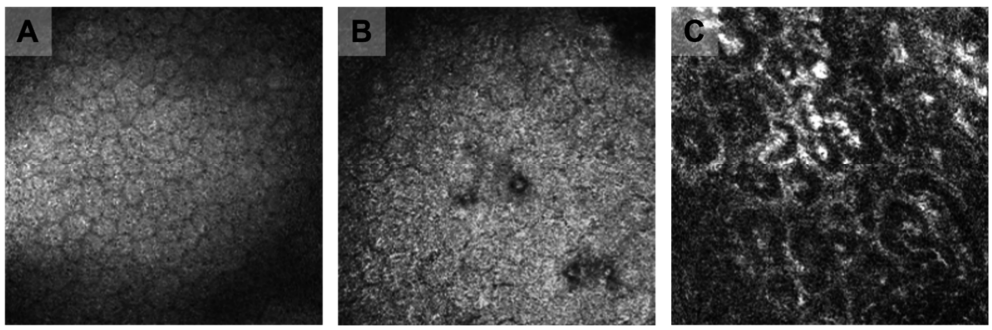

Figure 5| *In vivo* confocal microscopy images: (A) healthy corneal endothelium (B) mild FECD: guttae appear rather scattered and appear in only a small portion of the endothelial area, and (C) severe FECD: confluent guttae visible across the entire endothelium.

DSO/DWEK is likely to only be an option for milder FECD cases and will not eliminate the demand for corneal tissue. With this in mind, other alternatives for donor graft material are being investigated. Several clinical trials are underway evaluating approaches to using corneal endothelial cell replacement therapy through the injection of cultivated cells from a donor in the presence of mitogens[97,98] or even loaded with magnetic nanoparticles.[99] Expanding cells in culture allows far more patients to be treated by a single corneal donor. The longest-running trial using cell injection delivery has enrolled more than 60 participants in Japan[100] and recently reported its 5-year follow-up of the first 11 patients.[101] The most recently reported clinical trial with cultured endothelial cells was conducted from November 2020 to May 2021 at the Quesada Clinic in San Salvador, El Salvador, and the investigators injected cultured cells of two donor corneas into 50 affected eyes with the goals of reducing corneal edema and restoring vision. Although official data is not yet available, the investigators have confidence in the procedure's efficiency.[102] Figure 6 summarizes the current and developing regenerative medicine therapies to treat corneal endothelial disease.

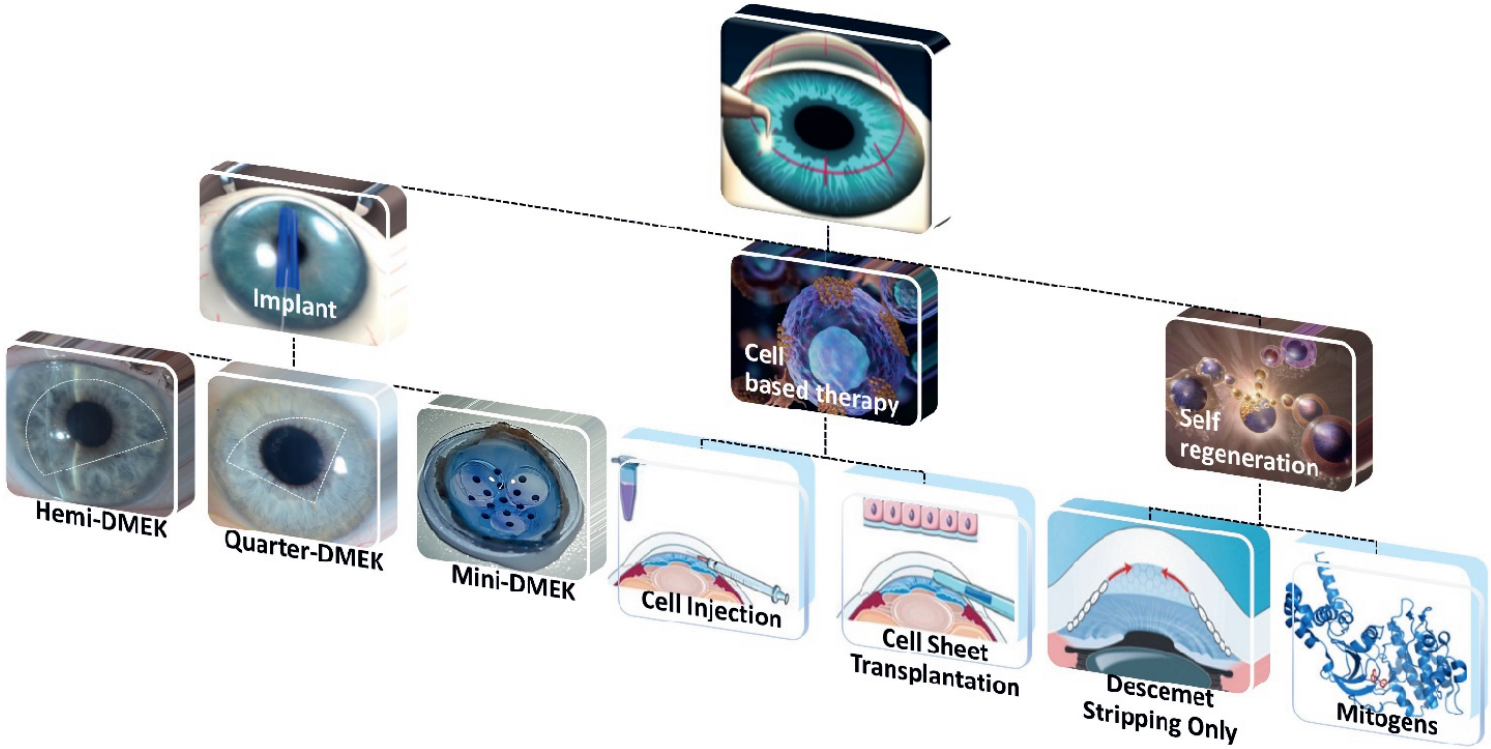

Figure 6|Strategies to counteract the global shortage of donor material. Implant approach: efficient use of donor cornea with Hemi-DMEK, Quarter-DMEK, Mini-DMEK. Cell based therapy: cell injection using cultured cells of mature differentiated cells or cell sheet transplantation. Self regeneration through Descemet stripping only in the presence or absence of mitogens.

AIM AND THESIS OUTLINE

The thesis focuses on the *in vivo* and *in vitro* behavior of corneal endothelial cells before and after endothelial keratoplasty. The first part of the project concentrates on the ECD decrease after DMEK and DMEK graft viability prior to transplantation. The second part focusses on regenerative strategies for the treatment of FECD by developing and applying *in vitro* cell migration assays. *In vitro* cell migration from DMEK grafts of various sizes and shapes are investigated in a 3D cell culture system aiming to identify critical parameters for the successful clinical application of corneal endothelial therapies.

The first two chapters focus on the pre- and postoperative ECD measurements of DMEK grafts. In **Chapter 2**, we analyse ECD decrease after DMEK and demonstrate that about half of the observed ECD decrease at 6 months after DMEK is an *in vivo* decline from 1 day to 6 months postoperatively. The remaining decrease between preoperative and 1 day postoperative ECD values may be attributed to measurement error in the eye bank. In **Chapter 3**, we further examine the high ECD drop in the early postoperative phase after DMEK by analyzing the effect of graft preparation and organ-culture storage on ECD and viability of DMEK grafts prior to their release for transplantation.

The next six chapters focus on obtaining a better understanding of the regenerative capacity of the corneal endothelium by collecting the latest updates on the corneal endothelial wound process and performing explant outgrowth assays using a novel 3D culture technique. In **Chapter 4**, we summarize what is currently known about the wound healing characteristics from a biochemical level in the lab to the regenerative features seen in the clinic. In **Chapter 5**, cell migration of shape-adapted DMEK (Quarter-DMEK) grafts is replicated in an *in vitro* culture system and possible reasons for the lack of endothelial cell migration from the peripheral round edge of Quarter-DMEK grafts are examined. In **Chapter 6**, a novel 3D culture technique for an improved studying *in vitro* cell migration from explant tissue is tested. The technical 'ins and outs' of the proposed culture system and the cell ability to remodel the artificial matrix during migration while the explant tissue is maintained and fixed on a rigid position are analysed. In **Chapter 7,** we use the 3D culture system to explain the migration capacity of the peripheral endothelium, to provide more effective graft modifications prior to clinical implementation. In **Chapter 8**, we determine the migration of peripheral corneal endothelial cells in the presence and absence of mitogens to better understand the cell migration mechanism from phenotypically-distinct regions of the endothelium. In **Chapter 9**, a new preparation process and surgical testing of small diameter DMEK grafts are investigated. Additionally, by engaging the 3D hydrogel system, the surgical effect on endothelial cell density, viability, and migration capacity is evaluated.

References

1. Melles GRJ, Ong TS, Ververs B, van der Wees J. Descemet membrane endothelial keratoplasty (DMEK). Cornea. 2006 Sep;25(8):987-90. https:// doi.org/10.1097/01.ico.0000248385.16896.34 PMID: 17102683

2. Melles GR, Dapena I. How to get started with standardized 'no-touch' Descemet endothelial keratoplasty (DMEK). Netherlands Institute for Innovative Ocular Surgery. 2014. ISBN: 978-90-8890-277-2

3. Flockerzi E, Maier P, Böhringer D, Reinshagen H, Kruse F, Cursiefen C, et al. Trends in corneal transplantation from 2001 to 2016 in Germany: a report of the DOG-section cornea and its keratoplasty registry. Am J Ophthalmol. 2018 Apr;188:91-98. https://doi.org/10.1016/j.ajo.2018.01.018 PMID: 29410297

4. Eye Bank Association of America. The 2021 Eye Banking Statistical Report includes data from all 56 U.S. and 11 international member eye banks, plus one EBAA-accredited entity for the calendar year 2021 and represents a complete picture of eye banking activity among eye banks. Available from: https://restoresight.org/members/publications/statistical-report/

5. Waldrop WH, Gillings MJ, Robertson DM, Petroll WM, Mootha VV. Lower corneal haze and aberrations in Descemet membrane endothelial keratoplasty versus Descemet stripping automated endothelial keratoplasty in fellow eyes for Fuchs endothelial corneal dystrophy. Cornea. 2020 Oct;39(10):1227-1234. https://doi.org/10.1097/ICO.0000000000002416 PMID:32639312

6. Woo JH, Ang M, Htoon HM, Tan D. Descemet membrane endothelial keratoplasty versus Descemet stripping automated endothelial keratoplasty and penetrating keratoplasty. Am J Ophthalmol. 2019 Nov;207:288-303. https://doi.org/10.1016/j.ajo.2019.06.012 PMID: 31228467

7. Sarnicola C, Farooq AV, Colby K. Fuchs Endothelial Corneal Dystrophy: Update on pathogenesis and future directions. Eye Contact Lens. 2019 Jan;45(1):1-10. https://doi.org/10.1097/ICL.0000000000000469 PMID: 30005051

8. Lin F, Zhang L, Wang Y, Fu D, Wang Y, Zhou X. A 20-year bibliometric analysis of Fuchs endothelial corneal dystrophy: from 2001 to 2020. BMC Ophthalmol. 2022 Jun 8;22(1):255. https://doi.org/10.1186/s12886-022-02468-x PMID: 35676652

9. Tone SO, Wylegala A, Böhm M, Melangath G, Deshpande N, Jurkunas UV. Increased corneal endothelial cell migration in Fuchs endothelial corneal dystrophy. Am J Ophthalmol. 2021 March1;1:100006 https://doi.org/10.1016/j.xops.2021.100006

10. Harayama K, Amemiya T, Nishimura H. Development of the cornea during fetal life: comparison of corneal and bulbar diameter. Anat Rec. 1980 Nov;198(3):531-5. https://doi.org/10.1002/ar.1091980313 PMID: 7457942

11. Mishima S, Hedbys BO. Physiology of the cornea. Int Ophthalmol Clin. 1968 Fall;8(3):527 PMID: 4243831

12. Kinoshita S, Adachi W, Sotozono C, Nishida K, Yokoi N, Quantock AJ, et al. Characteristics of the human ocular surface epithelium. Prog Retin Eye Res. 2001 Sep;20(5):639-73. https://doi.org/10.1016/s1350-9462(01)00007-6 PMID: 11470454

13. Dohlman CH. The function of the corneal epithelium in health and disease. The Jonas S. Friedenwald Memorial Lecture. Invest Ophthalmol. 1971 Jun;10(6):383-407. PMID: 4325305

14. Gipson IK, Spurr-Michaud SJ, Tisdale AS. Anchoring fibrils form a complex network in human and rabbit cornea. Invest Ophthalmol Vis Sci. 1987 Feb;28(2):212-20. PMID: 8591898

15. Hay ED, Linsenmayer TF, Trelstad RL, von der Mark K. Origin and distribution of collagens in the developing avian cornea. Curr Top Eye Res. 1979;1:1-35. PMID: 400678

16. Müller LJ, Pels L, Vrensen GF. Novel aspects of the ultrastructural organization of human corneal keratocytes. Invest Ophthalmol Vis Sci. 1995 Dec;36(13):2557-67. PMID: 7499078

17. Jester JV, Moller-Pedersen T, Huang J, Sax CM, Kays WT, Cavangh HD, et al. The cellular basis of corneal transparency: evidence for 'corneal crystallins'. J Cell Sci. 1999 Mar;112 (Pt 5):613-22. https://doi.org/10.1242/jcs.112.5.613 PMID: 9973596

18. Newsome DA, Gross J, Hassell JR. Human corneal stroma contains three distinct collagens. Invest Ophthalmol Vis Sci. 1982 Mar;22(3):376-81. PMID: 6277819

19. Johnson DH, Bourne WM, Campbell RJ. The ultrastructure of Descemet's membrane. I. Changes with age in normal corneas. Arch Ophthalmol. 1982 Dec;100(12):1942-7. https://doi.org/10.1001/archopht.1982.01030040922011 PMID: 7150061

20. Yee RW, Matsuda M, Schultz RO, Edelhauser HF. Changes in the normal corneal endothelial cellular pattern as a function of age. Curr Eye Res. 1985 Jun;4(6):671-8. https://doi.org/10.3109/02713688509017661 PMID: 4028790

21. Wulle KG, Lerche W. Electron microscopic observations of the early development of the human corneal endothelium and Descemet's membrane. Ophthalmologica. 1969;157(6):451-61. https://doi.org/10.1159/000305709 PMID: 5796012

22. Gilbert Smolin and Richard A. Thoft, The Cornea: Scientific Foundations and Clinical Practice. 4 ed, ed. C.S. Foster, D.T. Azar, and C.H. Dohlman. 2005, 530 Walnur Street, Philadelphia, PA 19106 USA: Lippincott Williams & Wilkins 1323

23. Johnson DH, Bourne WM, Campbell RJ. The ultrastructure of Descemet's membrane. I. Changes with age in normal corneas. Arch Ophthalmol. 1982 Dec;100(12):1942-7. https://doi.org/10.1001/archopht.1982.01030040922011 PMID: 7150061

24 Rae JL, Lewno AW, Cooper K, Gates P. Dye and electrical coupling between cells of the rabbit corneal endothelium. Curr Eye Res. 1989 Aug;8(8):859-69. https://doi.org/10.3109/02713688909000876 PMID: 2791630

25 Joyce NC, Harris DL, Zieske JD. Mitotic inhibition of corneal endothelium in neonatal rats. Invest Ophthalmol Vis Sci. 1998 Dec;39(13):2572-83. PMID: 9856767

26 Joyce NC. Proliferative capacity of corneal endothelial cells. Exp Eye Res. 2012 Feb;95(1):16-23. https://doi.org/10.1016/j.exer.2011.08.014 PMID: 21906590

27 Srinivas SA. Dynamic regulation of barrier integrity of the corneal endothelium. Optom Vis Sci. 2010 Apr;87(4):E239-54. https://doi.org/10.1097/OPX.0b013e3181d39464 PMID: 20142793

28 Sherrard ES, Ng YL. The other side of the corneal endothelium. Cornea. 1990 Jan;9(1):48-54. PMID: 2297995

29. He Z, Forest F, Gain P, Rageade D, Bernard A, Acquart S, et al. 3D map of the human corneal endothelial cell. Sci Rep. 2016 Jul 6;6:29047. https://doi.org/10.1038/srep29047 PMID: 27381832

30. Joyce NC. Proliferative capacity of corneal endothelium. Prog Retin Eye Res. 2003 May;22(3):359-89. https://doi.org/10.1016/s1350-9462(02)00065-4 PMID: 12852491

31. Bonanno JA. Identity and regulation of ion transport mechanisms in the corneal endothelium. Prog Retin Eye Res. 2003 Jan;22(1):69-94. https://doi.org/10.1016/s1350-9462(02)00059-9 PMID: 12597924

32. Tervo T, Palkama A. Electron microscopic localization of adenosine triphosphatase (NaK-ATPase) activity in the rat cornea. Exp Eye Res. 1975 Sep;21(3):269-79. https://doi.org/10.1016/0014-4835(75)90098-6 PMID: 126867

33. Mergler S, Pleyer U. The human corneal endothelium: new insights into electrophysiology and ion channels. Prog Retin Eye Res. 2007 Jul;26(4):359-78. https://doi.or/10.1016/j.preteyeres.2007.02.001 PMID: 17446115

34. Geroski DH, Matsuda M, Yee RW, Edelhauser HF. Pump function of the human corneal endothelium. Effects of age and cornea guttata. Ophthalmology. 1985 Jun;92(6):759-63. https://doi.org/10.1016/s0161-6420(85)33973-8 PMID: 2412197

35. Murphy C, Alvarado J, Juster R, Maglio M. Prenatal and postnatal cellularity of the human corneal endothelium. A quantitative histologic study. Invest Ophthalmol Vis Sci. 1984 Mar;25(3):312-22. PMID: 6698749

36. Bourne WM, Nelson LR, Hodge DO. Central corneal endothelial cell changes over a ten-year period. Invest Ophthalmol Vis Sci. 1997 Mar;38(3):779-82. PMID: 9071233

37. Williams KK, Noe RL, Grossniklaus HE, Drews-Botsch C, Edelhauser HF. Correlation of histologic corneal endothelial cell counts with specular microscopic cell density. Arch Ophthalmol. 1992 Aug;110(8):1146-9. https://doi.org/10.1001/archopht.1992.01080200126039 PMID: 1497530

38. Laule A, Cable MK, Hoffman CE, Hanna C. Endothelial cell population changes of human cornea during life. Arch Ophthalmol. 1978 Nov;96(11):2031-5. https://doi.org/10.1001/archopht.1978.03910060419003 PMID: 718491

39. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. Physiol Rev. 2006 Jan;86(1):279-367. https://doi.org/10.1152/physrev.00012.2005 PMID: 16371600

40. Laing RA, Sanstrom MM, Berrospi AR, Leibowitz HM. Changes in the corneal endothelium as a function of age. Exp Eye Res. 1976 Jun;22(6):587-94. https://doi.org/10.1016/0014-4835(76)90003-8 PMID: 776638

41. Laing RA, Sanstrom MM, Berrospi AR, Leibowitz HM. Changes in the corneal endothelium as a function of age. Exp Eye Res. 1976 Jun;22(6):587-94. https://doi.org/10.1016/0014-4835(76)90003-8 PMID: 776638

42. Hecht F. Letter: Partial trisomy 15 (dup15q) syndrome and the need for precision in clinical cytogenetics. J Pediatr. 1975 Nov;87(5):842-3. https://doi.org/10.1016/s0022-3476(75)80340-4 PMID: 1185371

43. Burns RR, Bourne WM, Brubaker RF. Endothelial function in patients with cornea guttata. Invest Ophthalmol Vis Sci. 1981 Jan;20(1):77-85. PMID: 6969713

44. Ventura AC, Wälti R, Böhnke M. Corneal thickness and endothelial density before and after cataract surgery. Br J Ophthalmol. 2001 Jan;85(1):18-20. https://doi.org/10.1136/bjo.85.1.18 PMID: 11133705

45. Erickson P, Doughty MJ, Comstock TL, Cullen AP. Endothelial cell density and contact lens-induced corneal swelling. Cornea. 1998 Mar;17(2):152-7. https://doi.org/10.1097/00003226-199803000-00006 PMID: 9520190

46. Amon M, Menapace R, Radax U, Papapanos P. Endothelial cell density and corneal pachymetry after no-stitch, small-incision cataract surgery. Doc Ophthalmol. 1992;81(3):301-7. https://doi.org/10.1007/BF00161768 PMID: 1483372

47. Olsen T. Corneal thickness and endothelial damage after intracapsular cataract extraction. Acta Ophthalmol (Copenh). 1980 Jun;58(3):424-33. https://doi.org/10.1111/j.1755-3768.1980.tb05743.x PMID: 7415829

48. Ravalico G, Tognetto D, Palomba MA, Lovisato A, Baccara F. Corneal endothelial function after extracapsular cataract extraction and phacoemulsification. J Cataract Refract Surg. 1997 Sep;23(7):1000-5. https://doi.org/10.1016/s0886-3350(97)80071-5 PMID: 9379368

49. Edelhauser HF. The balance between corneal transparency and edema: the Proctor Lecture. Invest Ophthalmol Vis Sci. 2006 May;47(5):1754-67. https://doi.org/10.1167/iovs.05-1139 PMID: 16638979

50. Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F, et al. Global Survey of Corneal Transplantation and Eye Banking. JAMA Ophthalmol. 2016 Feb;134(2):167-73. https://doi.org/10.1001/jamaophthalmol.2015.4776 PMID: 26633035

51. Moshirfar M, Somani AN, Vaidyanathan U, Patel BC. Fuchs Endothelial Dystrophy. StatPearls Publishing; 2022 Jan. 2022 May 24. PMID: 31424832

52. Moffatt SL, Cartwright VA, Stumpf TH. Centennial review of corneal transplantation. Clin Exp Ophthalmol. 2005 Dec;33(6):642-57. https://doi.org/10.1111/j.1442-9071.2005.01134.x PMID: 16402960

53. Power BJ, Power WJ (2022). Penetrating keratoplasty and complications management. In: Albert DM, Miller JW, Azar DT, Young LH (eds) Albert and Jakobiec's Principles and Practice of Ophthalmology. Springer, Cham. https://doi.org/10.1007/978-3-030-42634-7_220

54. Thompson RWJ, Price MO, Bowers PJ, Price FWJ. Long-term graft survival after penetrating keratoplasty. Ophthalmology. 2003 Jul;110(7):1396-402. https://doi.org/10.1016/S0161-6420(03)00463-9 PMID: 12867398

55. Patel SV. Graft survival after penetrating keratoplasty. Am J Ophthalmol. 2011 Mar;151(3):397-8. https://doi.org/10.1016/j.ajo.2010.10.006 PMID: 21335107

56. Melles GRJ. Posterior lamellar keratoplasty: DLEK to DSEK to DMEK. Cornea. 2006 Sep;25(8):879-81. https://doi.org/10.1097/01.ico.0000243962.60392.4f PMID: 17102659

57. Borderie VM, Boëlle PY, Touzeau O, Allouch C, Boutboul S, Laroche L. Predicted long-term outcome of corneal transplantation. Ophthalmology. 2009 Dec;116(12):2354-60. https://doi.org/10.1016/j.ophtha.2009.05.009 PMID: 19815285

58. Fernandez MM, Afshari NA. Endothelial keratoplasty: from DLEK to DMEK. Middle East Afr J Ophthalmol. 2010 Jan;17(1):5-8. https://doi.org/10.4103/0974-9233.61210 PMID: 20543930

59. Price MO, Feng MT, Price FWJ. Endothelial Keratoplasty Update 2020. Cornea. 2021 May 1;40(5):541-547. https://doi.org/10.1097/ICO.0000000000002565 PMID: 33252380

60. Farid M. Future Directions in Corneal Transplantation. A look at surgeries, donor tissue use, laser advances and more. Available from https://www.cornealphysician.com/issues/2020/april-2020/future-directions-in-corneal-transplantation (accessed on 3 August 2022)

61. Muijzer MB, van Luijk CM, van den Bogaerdt AJ, Kruit PJ, van Beek EG, Melles GRJ. Prospective evaluation of clinical outcomes between pre-cut corneal grafts prepared using a manual or automated technique: with one-year follow-up. Acta Ophthalmol. 2019 Nov;97(7):714-720. https://doi.org/10.1111/aos.14074 PMID: 30803143

62. Moutsouris K, Ham L, Dapena I, van der Wees J, Melles GRJ. Radial graft contraction may relate to subnormal visual acuity in Descemet stripping (automated) endothelial keratoplasty. Br J Ophthalmol. 2010 Jul;94(7):951-3. https://doi.org/10.1136/bjo.2009.167718 PMID: 20606030

63. Melles GR, Eggink FA, Lander F, Pels E, Rietveld FJ, Beekhuis WH, Binder PS. A surgical technique for posterior lamellar keratoplasty. Cornea. 1998 Nov;17(6):618-26. https://doi.org/10.1097/00003226-199811000-00010 PMID: 9820943

64. Melles GRJ, Wijdh RHJ, Nieuwendaal CP. A technique to excise the Descemet membrane from a recipient cornea (descemetorhexis). Cornea. 2004 Apr;23(3):286-8. https://doi.org/10.1097/00003226-200404000-00011 PMID: 15084862

65. Price FWJ, Price MO. Descemet's stripping with endothelial keratoplasty in 50 eyes: a refractive neutral corneal transplant. J Refract Surg. 2005 Jul-Aug;21(4):339-45. https://doi.org/10.3928/1081-597X-20050701-07 PMID: 16128330

66. Guilbert E, Bullet J, Sandali O, Basli EB, Laroche L, Borderie VM. Long-term rejection incidence and reversibility after penetrating and lamellar keratoplasty. Am J Ophthalmol. 2013 Mar;155(3):560-569.e2. https://doi.org/10.1016/j.ajo.2012.09.027 PMID: 23218931

67. Lee PWB, Jacobs DS, Musch DC, Kaufman SC, Reinhart WJ, Shtein RM. Descemet's stripping endothelial keratoplasty: safety and outcomes: a report by the American Academy of Ophthalmology. Ophthalmology. 2009 Sep;116(9):1818-30. https://doi.org/10.1016/j.ophtha.2009.06.021 PMID: 19643492

68. Melles GRJ, Lander F, Rietveld FJR. Transplantation of Descemet's membrane carrying viable endothelium through a small scleral incision. Cornea. 2002 May;21(4):415-8. https://doi.org/10.1097/00003226-200205000-00016 PMID: 11973393

69. Melles GRJ, Ong TS, Ververs B, van der Wees J. Descemet membrane endothelial keratoplasty (DMEK). Cornea. 2006 Sep;25(8):987-90. https://doi.org/10.1097/01.ico.0000248385.16896.34 PMID: 17102683

70. Melles GRJ, Ong TS, Ververs B, van der Wees J. Preliminary clinical results of Descemet membrane endothelial keratoplasty. Am J Ophthalmol. 2008 Feb;145(2):222-227. https://doi.org/10.1016/j.ajo.2007.09.021 PMID: 18061137

71. Dapena I, Moutsouris K, Droutsas K, Ham L, van Dijk K, Melles GRJ. Standardized "no-touch" technique for Descemet membrane endothelial keratoplasty. Arch Ophthalmol. 2011 Jan;129(1):88-94. https://doi.org/10.1001/archophthalmol.2010.334 PMID: 21220634

72. Maier, P., Reinhard, T. (2017). Long-term clear graft survival and chronic endothelial cell loss following posterior lamellar keratoplasty. In: Cursiefen, C., Jun, A. (eds) Current Treatment Options for Fuchs Endothelial Dystrophy. Springer, Cham. https://doi.org/10.1007/978-3-319-43021-8_14

73. Lam FK, Baydoun L, Dirisamer M, Lie J, Dapena I, Melles GRJ. Hemi-Descemet membrane endothelial keratoplasty transplantation: a potential method for increasing the pool of endothelial graft tissue. JAMA Ophthalmol. 2014 Dec;132(12):1469-73. https://doi.org/10.1001/jamaophthalmol.2014.3328 PMID: 25211529

74. Birbal RS, Hsien S, Zygoura V, Parker JS, Ham L, van Dijk K et al. Outcomes of Hemi-Descemet membrane endothelial keratoplasty for Fuchs endothelial corneal dystrophy. Cornea. 2018 Jul;37(7):854-858. https://doi.org/10.1097/ICO.0000000000001578 PMID: 29557816

75. Müller TM, Baydoun L, Melles GRJ. 3-Year update on the first case series of Hemi-Descemet membrane endothelial keratoplasty. Graefes Arch Clin Exp Ophthalmol. 2017 Jan;255(1):213-215. https://doi.org/10.1007/s00417-016-3517-5 PMID: 27783157

76. Baydoun L, Zygoura V, Hsien S, Birbal RS, Spinozzi D, Lie JT, et al. Clinical feasibility of using multiple grafts from a single donor for Quarter-DMEK. Acta Ophthalmol. 2018 Aug;96(5):e656-e658. https://doi.org/10.1111/aos.13720 PMID: 29498213

77. Zygoura V, Baydoun L, Ham L, Bourgonje VJA, van Dijk K, Lie JT et al. Quarter-Descemet membrane endothelial keratoplasty (Quarter-DMEK) for Fuchs endothelial corneal dystrophy: 6 months clinical outcome. Br J Ophthalmol. 2018 Oct;102(10):1425-1430. https://doi.org/10.1136/bjophthalmol-2017-311398 PMID: 29343529

78. Müller TM, Lavy I, Baydoun L, Lie JT, Dapena I, Melles GRJ. Case report of Quarter-Descemet membrane endothelial keratoplasty for Fuchs endothelial dystrophy. Cornea. 2017 Jan;36(1):104-107. https://doi.org/10.1097/ICO.0000000000001008 PMID: 27583798

79. Birbal RS, Ni Dhubhghaill S, Baydoun L, Ham L, Bourgonje VJA, Dapena I, et al. Quarter-Descemet membrane endothelial keratoplasty (Quarter-DMEK) for Fuchs endothelial corneal dystrophy: 6 months clinical outcome. Br J Ophthalmol. 2018 Oct;102(10):1425-1430. https://doi.org/10.1136/bjophthalmol-2017-311398 PMID: 31490274

80. Gerber-Hollbach N, Parker J, Baydoun L, Liarakos VS, Ham L, Dapena I et al. Preliminary outcome of hemi-Descemet membrane endothelial keratoplasty for Fuchs endothelial dystrophy. Br J Ophthalmol. 2016 Nov;100(11):1564-1568. https://doi.org/10.1136/bjophthalmol-2015-307783 PMID: 26837507

81. Birbal RS, Ni Dhubhghaill S, Baydoun L, Ham L, Bourgonje VJA, Dapena I et al. Quarter-Descemet membrane endothelial keratoplasty: one- to two-year clinical outcomes. Cornea. 2020 Mar;39(3):277-282. https://doi.org/10.1097/ICO.0000000000002127 PMID: 31490274

82. Dirisamer M, Yeh RY, van Dijk K, Ham L, Dapena I, Melles GRJ. Recipient endothelium may relate to corneal clearance in Descemet membrane endothelial transfer. Am J Ophthalmol. 2012 Aug;154(2):290-296.e1. https://doi.org/10.1016/j.ajo.2012.02.032 PMID: 22633346

83. Ziaei M, Barsam A, Mearza AA. Spontaneous corneal clearance despite graft removal in Descemet stripping endothelial keratoplasty in Fuchs endothelial dystrophy. Cornea. 2013 Jul;32(7):e164-6. https://doi.org/10.1097/ICO.0b013e31828b75a1 PMID: 23594769

84. Moloney G, Chan UT, Hamilton A, Zahidin AM, Grigg JR, Devasahayam RN. Descemetorhexis for Fuchs' dystrophy. Can J Ophthalmol. 2015 Feb;50(1):68-72. https://doi.org/10.1016/j.jcjo.2014.10.014 PMID: 25677286

85. Borkar DS, Veldman P, Colby KA. Treatment of Fuchs endothelial dystrophy by Descemet stripping without endothelial keratoplasty. Cornea. 2016 Oct;35(10):1267-73. https://doi.org/10.1097/ICO.0000000000000915 PMID: 27310885

86. Iovieno A, Neri A, Soldani AM, Adani C, Fontana L. Descemetorhexis without graft placement for the treatment of Fuchs endothelial dystrophy: preliminary results and review of the Literature. Cornea. 2017 Jun;36(6):637-641. https://doi.org/10.1097/ICO.0000000000001202 PMID: 28410355

87. Davies E, Jurkunas U, Pineda R. Predictive factors for corneal clearance after Descemetorhexis without endothelial keratoplasty. Cornea. 2018 Feb;37(2):137-140. https://doi.org/10.1097/ICO.0000000000001427 PMID: 29053562

88. Blitzer AL, Colby KA. Update on the Surgical management of Fuchs endothelial corneal dystrophy. Ophthalmol Ther. 2020 Dec;9(4):757-765. https://doi.org/10.1007/s40123-020-00293-3 PMID: 32840804

89. Davies E, Jurkunas U, Pineda R. Predictive factors for corneal clearance after Descemetorhexis without endothelial keratoplasty. Cornea. 2018 Feb;37(2):137-140. https://doi.org/10.1097/ICO.0000000000001427 PMID: 29053562

90. Moloney G, Petsoglou C, Ball M, Kerdraon Y, Höllhumer R, Spiteri N et al. Descemetorhexis without grafting for Fuchs endothelial dystrophy-supplementation with topical Ripasudil. Cornea. 2017 Jun;36(6):642-648. https://doi.org/10.1097/ICO.0000000000001209 PMID: 28476048

91. Huang MJ, Kane S, Dhaliwal DK. Descemetorhexis without endothelial keratoplasty versus DMEK for treatment of Fuchs endothelial corneal dystrophy. Cornea. 2018 Dec;37(12):1479-1483. https://doi.org/10.1097/ICO.0000000000001742 PMID: 30222714

92. Macsai MS, Shiloach M. Use of topical Rho Kinase inhibitors in the treatment of Fuchs dystrophy after Descemet stripping only. Cornea. 2019 May;38(5):529-534. https://doi.org/10.1097/ICO.0000000000001883 PMID: 30720541

93. Artaechevarria AJ, Wells M, Devasahayam RN, Moloney G. 5-Year outcomes of Descemet stripping only in Fuchs dystrophy. Cornea. 2020 Aug;39(8):1048-1051. https://doi.org/10.1097/ICO.0000000000002270 PMID: 31990847

94. Davies E, Jurkunas U, Pineda R. Pilot study of corneal clearance with the use of a Rho-kinase inhibitor after Descemetorhexis without endothelial keratoplasty for Fuchs endothelial corneal dystrophy. Cornea. 2021 Jul 1;40(7):899-902. https://doi.org/10.1097/ICO.0000000000002691 PMID: 33758139

95. Koizumi N, Okumura N, Ueno M, Nakagawa H, Hamuro J, Kinoshita S. Rho-associated kinase inhibitor eye drop treatment as a possible medical treatment for Fuchs corneal dystrophy. Cornea. 2013 Aug;32(8):1167-70. https://doi.org/10.1097/ICO.0b013e318285475d PMID: 23715376

96. Macsai MS, Shiloach M. Use of topical Rho kinase inhibitors in the treatment of Fuchs dystrophy after Descemet stripping only. Cornea. 2019 May;38(5):529-534. https://doi.org/10.1097/ICO.0000000000001883 PMID: 30720541

97. Ueno M, Toda M, Numa K, Tanaka H, Imai K, Bush J, et al. Superiority of mature differentiated cultured human corneal endothelial cell injection therapy for corneal endothelial failure. Am J Ophthalmol. 2022 May;237:267-277. https://doi.org/10.1016/j.ajo.2021.11.012 PMID: 34788595

98. Kinoshita S, Koizumi N, Ueno M, Okumura N, Imai K, Tanaka H, et al. Injection of cultured cells with a ROCK inhibitor for bullous keratopathy. N Engl J Med. 2018 Mar 15;378(11):995-1003. https://doi.org/10.1056/NEJMoa1712770 PMID: 29539291

99. Xia X, Atkins M, Dalal R, Kuzmenko O, Chang KC, Sun CB, et al. Magnetic human corneal endothelial cell transplant: delivery, retention, and short-term efficacy. Invest Ophthalmol Vis Sci. 2019 Jun 3;60(7):2438-2448. https://doi.org/10.1167/iovs.18-26001 PMID: 31158276

100. Dermot McGrath. Published: Thursday, December 3, 2020. Cell therapy for Fuchs' dystrophy. Published by EuroTimes for ESCRS. Accessed on August 5, 2022: https://www.escrs.org/eurotimes/cell-therapy-for-fuchs-dystrophy

101. Numa K, Imai K, Ueno M, Kitazawa K, Tanaka H, Bush JD, et al. Five-year follow-up of first 11 patients undergoing injection of cultured corneal endothelial cells for corneal endothelial failure. Ophthalmology. 2021 Apr;128(4):504-514. https://doi.org/10.1016/j.ophtha.2020.09.002 PMID: 32898516

102. CGTLive's Weekly Rewind – July 22, 2022. July 22, 2022 CGTLive Staff. Cell Therapy a Promising Option in Corneal Endothelial Disease. Accessed on August 5, 2022: Cell Therapy a Promising Option in Corneal Endothelial Disease (cgtlive.com)