Advances in endothelial keratoplasty

Birbal, R.S.

Chapter 9

Summary and Future Directions

SUMMARY

Over the past two decades, lamellar keratoplasty has revolutionized the field of corneal transplantation and largely replaced penetrating keratoplasty (PK) as the preferred surgical treatment option for corneal endothelial disorders.[1] Since its introduction in 1998, endothelial keratoplasty (EK) has evolved from Deep lamellar endothelial keratoplasty (DLEK) via Descemet stripping (automated) endothelial keratoplasty (DS(A)EK) to Descemet membrane endothelial keratoplasty (DMEK).[2] Global scarcity of corneal donor tissue inspired further refinement of conventional DMEK and led to the development of Hemi- and Quarter-DMEK.[3,4] These EK-techniques may potentially increase the availability of endothelial donor grafts.

For this thesis, donor tissue preparation for DMEK and the feasibility and clinical outcomes of DMEK and modified DMEK-techniques were evaluated in the management of corneal endothelial disorders.

Part I - Donor tissue preparation

Adequate knowledge of the currently available DMEK graft harvesting techniques may benefit corneal surgeons and eye banks in choosing the best approach for each specific user (**Chapter 2**).[5] Current and evolving techniques to harvest donor tissue show a trend towards increased utilization of a 'no-touch' technique, an approach in which there is no direct physical graft handling to minimize endothelial cell loss.[6] Harvesting techniques may broadly be classified into those based on manual peeling and those based on air- or liquid-assisted detachment at the stroma-Descemet membrane interface.[5] While these techniques are diverse and feature different strengths and weaknesses, different approaches may all provide excellent results.[5]

Part II - Selective, minimally-invasive and potentially tissue-sparing surgical treatment modalities for corneal endothelial disorders

DMEK

Since its clinical introduction in 2006, DMEK has emerged as an increasingly popular surgical treatment option for corneal endothelial disorders.[1,2] Multiple studies have substantiated initial reports on excellent clinical outcomes and have reliably shown that the first 6 months after DMEK appear to be the most critical time period, after which the results mostly stabilize.[7-15] Our expanding DMEK-cohort and simultaneously growing dataset allowed us to perform

in-depth analyses on subgroups. As such, we did not only evaluate overall clinical outcome in our six-month assessment of 1000 DMEKs but also analyzed how surgical indication (Fuchs endothelial corneal dystrophy (FECD) versus bullous keratopathy (BK)) and preoperative lens status (phakic versus pseudophakic) in FECD eyes affected the results (**Chapter 3**).[16]

Our study showed similar high visual acuity levels for both FECD and BK eyes when correcting for preoperative visual acuity and patient age. Hence, it may be important to emphasize that most BK eyes without visual acuity-limiting comorbidities may also expect a good visual outcome, even early after DMEK. While preoperative lens status did not influence DMEK outcomes, preservation of the crystalline lens may be preferred in a select group of younger patients with FECD and a relatively clear lens, as they may still benefit from their residual accommodative capacity and a better overall optical quality of the eye. In addition, the 5-year rate of visually-significant cataract formation after DMEK is relatively low (16%).[7]

DMEK continued to provide excellent clinical outcomes and high graft survival rates up to 5 years postoperatively (**Chapter 4**).[7] In this series of the first 500 DMEK eyes, eyes with FECD demonstrated better survival probabilities at 5 years postoperatively compared to eyes with other surgical indications (93% for isolated FECD versus 72% for other indications). A technique learning curve may also have been involved in attaining higher graft survival rates. This was reflected by the higher survival probability of the second 250 DMEK cases (94%) versus the first 250 cases (88%) and substantiated by the significantly lower survival probabilities of eyes with a graft detachment of >1/3 of the graft surface area (27%) compared to eyes with completely attached grafts (95%) or only small detachments (91%). Major graft detachments occurred less frequently in the second 250 cases (2.4%) compared to the first 250 cases (4.4%). These outcomes support the beneficial effect of an early re-bubbling procedure.

This study confirmed that the excellent visual outcomes achieved at 6 months after DMEK may be maintained up to at least 5 years postoperatively. The overall postoperative complication rate remained relatively low throughout the 5-year study period. Partial graft detachment was the main early postoperative complication, whereas allograft rejection and secondary graft failure constituted the more severe complications in the later postoperative period. Repeat keratoplasty was required at a relatively low rate (8.8%).

DMEK in challenging cases

With specific surgical modifications, DMEK proved feasible in eyes with a glaucoma drainage device (GDD) and provided acceptable clinical outcomes (**Chapter 5**).[17,18] Our data show thatthe presence of a GDD may reduce graft longevity and pose a risk for more frequent re-grafting, as we noticed that DMEK graft survival was lower in eyes with a GDD compared to our standard DMEK cohort: the survival probability was 89% at 1 year after DMEK, and decreased to 67% at 2 years after DMEK. The presence of a GDD negatively affected donor endothelial cell density (ECD). At 1 year after DMEK, ECD decline was 71%, which is almost twice as high as for our standard DMEK cohort. The incidence of secondary graft failure (8.7%) was also higher compared to after standard DMEK. The underlying cause of the faster drop in graft survival and the steeper ECD decline in the presence of a GDD may be multifactorial. It may be due to changes in aqueous humor circulation patterns owing to a GDD, which may adversely affect endothelial cell viability, and/or the GDD itself that may induce a breach in the blood-aqueous barrier caused by heavily rubbing or forcefully blinking, resulting in an increase of influx of oxidative, apoptotic, and inflammatory proteins, which may potentially damage corneal endothelial cells.[19-24] In addition, eyes with glaucoma necessitating a GDD may be more prone to immune reactions, as glaucomatous ganglion cell damage may be related to immune responses as well.[25]

Graft detachment was the main early postoperative complication, with 22% of eyes requiring a re-bubbling procedure. This may reflect that eyes with a GDD are more prone to surgical complications, which is possibly related to the added difficulty of pressurizing these eyes with air at the conclusion of the operation.

Most of the observed postoperative complications seem to be inherent to the presence of a GDD, and may partially be mitigated by special surgical considerations.[17,18] For this select group of patients it is imperative to do appropriate patient counseling.

Modified DMEK-techniques

Descemet membrane endothelial transfer

In the early years of EK, it was generally believed that, for grafted endothelial tissue to restore corneal transparency, a complete apposition between donor and host tissue was mandatory; i.e. without a fully, centrally-attached graft, corneal clearance could not be obtained and visual rehabilitation would not occur. Over the past decade, a growing number of studies have described spontaneous corneal clearance in the presence of a detached endothelial graft after DS(A)EK or DMEK, or in the absence of an endothelial graft, that is 'descemetorhexis only', thereby challenging this concept and questioning the necessity of grafting after descemetorhexis.[26-40] Descemet membrane endothelial transfer (DMET), in which descemetorhexis is followed by insertion of an almost completely free-floating Descemet roll (i.e. with the graft fixated within a corneoscleral incision to ensure contact with the posterior cornea) aims to obtain corneal clearance by endothelial cell migration.[41]

Our initial evaluation of DMET comprised a cohort of 12 eyes from 12 patients, seven operated on for FECD and five for BK, and showed repopulation of the denuded recipient stroma and corneal clearance in all eyes operated on for FECD, but not in those operated on for BK.[41,42] This suggests that the underlying pathology may be the main determinant of the clinical outcome and that recipient endothelial cells rather than donor endothelial cells contribute to corneal clearance.

While DMET initially showed promising results for FECD cases, our study on the long-term outcome of these 16 DMET cases showed that, regardless of the etiology of endothelial dysfunction, all corneas ultimately decompensated and required repeat EK (**Chapter 6**).[43] Hence, the regenerative capacity of endothelial cells in eyes with FECD may not be sufficient to ensure complete and durable corneal deturgescence after DMET. In order to obtain complete and lasting corneal rehabilitation, a (nearly) fully, centrally-attached Descemet graft may be mandatory.

Hemi-DMEK

In 2014, Hemi-DMEK was introduced. This technique allowed for the utilization of a single donor cornea for two endothelial keratoplasty procedures in two recipient eyes with FECD, thereby potentially doubling the availability of endothelial donor tissue.[44]

Our initial cohort of ten Hemi-DMEK eyes showed that the same level of visual rehabilitation may be acquired with Hemi-DMEK as with conventional DMEK (**Chapter 7**).[45-49] While delayed corneal clearance may occur in the periphery of the cornea due to bare stromal areas resulting from the mismatch of the circular descemetorhexis and the semicircular shape of the Hemi-DMEK graft, the central cornea was not negatively affected as the Hemi-DMEK graft was positioned to cover the central cornea, thereby resulting in fast visual clearance.

In the first 6 months after surgery, a higher decline in ECD was observed than after conventional DMEK (65% vs 34%). This may be explained by different patterns of endothelial cell redistribution and migration after Hemi-DMEK compared to conventional DMEK that may be due to larger denuded stromal areas. In addition, ECD measurements at different graft areas (centrally for conventional DMEK and more peripheral or at the graft edge for Hemi-DMEK) may produce this difference in the ECD decrease. After the initial drop in ECD, an annual decline of 6-7% was observed, which is comparable to that after conventional DMEK. Hence, the ECD decrease after this early drop may be caused by similar mechanisms in both techniques. As with conventional DMEK, the main complication after Hemi-DMEK was graft detachment (40%), which may be associated with the learning curve for this modified DMEK technique; another factor may be the different graft shape, as the Hemi-DMEK graft has one shorter axis. A higher number of re-bubbling procedures was performed for graft detachments after Hemi-DMEK, as minor graft detachments more often affected the visual axis.

Quarter-DMEK

Given the initial success of Hemi-DMEK and our goal to utilize corneal donor tissue even more efficiently, Quarter-DMEK was introduced.[50] Quarter-DMEK offers the theoretical benefit of reduced donor antigen load and of potentially quadrupling the amount of donor tissue available for transplantation as four endothelial grafts may be obtained from one donor cornea and transplanted into four recipient eyes.[50]

In our initial series of 19 Quarter-DMEK eyes, BCVA values equaled BCVA outcomes after conventional and Hemi-DMEK (**Chapter 8**).[51,52] Quarter-DMEK provided fast visual rehabilitation, but corneal deturgescence was slower than after conventional DMEK and, in particular, lagged behind along the round limbal edge of the Quarter-DMEK graft and in the adjacent bare stromal areas.[51,52]

In vitro evaluation of organ-cultured Quarter-DMEK grafts revealed that endothelial cell migration is asymmetrical and primarily occurs along the radial cut edges of the graft, and not at the round edge of the graft, i.e. the far, limbal periphery of the graft.[53] This asymmetrical migration of corneal endothelial cells may be attributed to the different molecular structure of the peripheral DM.[53] With (initial) corneal clearance and endothelial cell migration primarily occurring along the radial cut edges, it may be worthwhile to position the graft eccentrically, with its radial cut edges near the pupillary area and the peripheral round edge near the corneal periphery, to avoid slowly-resolving corneal edema in the visual axis.

Visually-significant graft detachment requiring re-bubbling procedures (42%) occurred at a rate comparable for Hemi-DMEK (40%), but at a slightly higher rate compared with the first 25 cases of the initial conventional DMEK case series (36%). This may be related to more difficult graft handling during surgery, to curvature incongruence, considering the central recipient cornea is aligned with the paracentral donor cornea, and/or the fact that graft detachments in Quarter-DMEK almost always involve the visual axis, prompting re-bubbling procedures more quickly. As with Hemi-DMEK, a steep initial decline in ECD was observed in the first 6 months postoperatively (68%), which was followed by a slower decline thereafter. This may be explained by increased surgical manipulation and endothelial cell migration as the mismatch between the larger descemetorhexis and the smaller triangular-shaped Quarter-DMEK graft may contribute to larger areas of bare stroma that need to be colonized by migrating donor cells.

Quarter-DMEK may benefit from a smaller descemetorhexis (diameter) aiming to reduce the surface of the bare areas that need to be repopulated by endothelial cells, adapted graft preparation protocols to reduce the endothelial cell loss along the radial cut edges of the graft and/or by eliminating the round peripheral edge of the Quarter-DMEK graft to promote cell migration toward the adjacent bare area in the corneal periphery. While Quarter-DMEK may induce sufficient corneal deturgescence, topical administration of Rho-associated kinase (ROCK)-inhibitors, as also applied in 'Descemet stripping only' and endothelial cell injection therapy, may potentially enhance endothelial cell migration and corneal clearance.[54,55]

CONCLUDING REMARKS

DMEK graft dissection techniques are diverse and feature different strengths and weaknesses. While the type of utilized DMEK-graft dissection technique may influence clinical outcomes after DMEK, a single technique does not need to be universally adopted. It is, however, imperative for those preparing DMEK tissue to know the different techniques available, so they can choose the best approach for them individually and for their given setting.

DMEK has shown to provide excellent short- as well as mid-term clinical outcomes for various surgical indications such as FECD and BK. In addition, DMEK proved feasible in challenging cases such as glaucomatous eyes with a glaucoma drainage device. While DMET initially showed promising results for FECD cases, it ultimately failed to provide complete and durable corneal rehabilitation, highlighting the importance of a well-attached endothelial graft to achieve durable corneal clearance. Therefore, conventional DMEK may remain the preferred treatment option for long-term management of corneal endothelial disorders.

Hemi-DMEK and Quarter-DMEK may be encouraging because the procedures may allow for clinical outcomes similar to conventional DMEK and may potentially increase the availability of endothelial donor tissue. If longer-term studies show that outcomes remain stable, these techniques may become an alternative to conventional DMEK. Quarter-DMEK, however, may benefit from some further modifications in order to obtain improved clinical outcomes in terms of cell density decrease and additional studies are warranted to further evaluate this.

FUTURE DIRECTIONS

Modern lamellar keratoplasty techniques have significantly improved clinical outcomes of corneal transplantation and reduced the rates of postoperative complications such as graft rejection and graft failure. Nonetheless, postoperative complications remain a major cause of repeat transplantation, while at the same time, global shortage of corneal donor tissue persists. Evolution in the field of corneal endothelial regeneration is therefore targeted towards overcoming these obstacles.

In recent years, Descemet stripping without endothelial keratoplasty (DWEK), also known as Descemet stripping only (DSO), bioengineered corneal endothelium, pharmaceutical agents such as Rho kinase (ROCK)-inhibitors, and gene therapy have been proposed as alternative or complementary treatment options in the management of corneal endothelial dysfunction.

DWEK was introduced for the treatment of early FECD stages following numerous observations of spontaneous corneal clearance in eyes with an endothelial defect in the absence of an endothelial graft.[26,27,36] As the name suggests, this technique entails removal of the diseased central DM and endothelium without insertion of an endothelial donor graft.[29-35,37-40] DWEK intends to stimulate centripetal migration of healthy, peripheral endothelial cells to replace the central endothelium. Early case series on the clinical outcomes of DWEK generated mixed results, with better clearance rates reported in cases where a smaller 3-4 diameter descemetorhexis was employed.[54] This may be explained by the limited and transitory capacity of recipient endothelium to self-repair in eyes with FECD, as observed after DMET. Drawbacks of DWEK include unpredictability of corneal clearance and suboptimal vision despite corneal clearance.[54] Fast, slow and non-responders have been described. As no donor tissue is used, outcomes are most likely determined by either patient or surgical factors. While no patient factors of significance have yet been described, the presence of posterior stromal scarring, related to stromal scoring, is more often observed in slow to non-responders.[54] Consequently, recommendations have been made to strip DM without scoring it, thereby aiming to maximize cell preservation and migration. Further recommendations included placing emphasis on symmetry and centration of the descemetorhexis during surgery to minimize ghosting and irregular astigmatism. Pharmacological adjuncts such as ROCK-inhibitors have been described to significantly speed up visual recovery and induce higher central endothelial cell counts. In addition,

ROCK-inhibitors have been described as salvage therapy in initially unsuccessful DWEK cases.[56,57] If DWEK does not induce corneal clearance, subsequent EK may still be performed with favorable outcomes. Although DWEK may represent a cost-effective and time-efficient procedure, worldwide adoption has been reasonably limited by its inconsistent outcomes. Larger studies with longer follow-up are required to further determine the potential of this technique.

Expanding on the concept of DWEK, *primary descemetorhexis followed by acellular Descemet membrane transplantation* (DMT) was introduced after *in vitro* tests showed that endothelial cell migration after descemetorhexis might be facilitated by the presence of a Descemet membrane.[58,59] A first *in vivo* human study demonstrated the potential of this technique in achieving repopulation of the transplanted acellular DM graft with healthy, peripheral host endothelial cells and corneal clearance.[60] Further series are warranted to determine the clinical (additional) merit of this technique.

Human corneal endothelial cells can enlarge and migrate but are believed not to proliferate *in vivo*, whereas they do proliferate *in vitro*.[61-64] Currently, the only way to replace diseased corneal endothelial cells (CECs) is by EK. The global shortage of endothelial grafts inspired the development of 'tissue-engineered endothelial grafts' that can subsequently be transplanted into humans. Usage of *bioengineered corneal endothelium* basically comprises two primary approaches: scaffold-based and cell-based. The concept of transplanting CECs was first suggested by Jumblatt and associates in 1978.[65] In an animal study on rabbit eyes, full-thickness transplantation of a rabbit cornea seeded with cultured endothelial rabbit cells was shown to restore corneal transparency.[66] Since then, several studies have demonstrated the feasibility of transplanting CECs to restore corneal clarity not only *in vitro* but also *in vivo* with both non-human and human CECs.[67-70] All initially reported procedures, however, required the use of a human donor cornea as a carrier for the CECs, which hampered the merits of cultured CEC transplantation as the same number of donor corneas would still be required to treat patients.[71] The introduction and success of lamellar keratoplasty techniques such as DS(A)EK and DMEK, inspired scientists to develop bioengineered corneal endothelial cell sheets that could subsequently be implanted like a DS(A)EK/DMEK graft by a DSAEK/DMEK procedure. Previous *in vitro* studies have evaluated the use of denuded DM, human anterior lens capsules (HALC) and bioengineered matrices consisting of silk-fibroin, collagen, gelatin or a combination of biopolymers, as

potential carriers for cultured CECs.[68,72-80] Subsequent *in vivo* animal studies tested the use of (cross-linked) collagen sheets, plastic compressed collagen type I 'REAL architecture for 3D tissues' (RAFT), and biological carriers such as DM, HALC and amniotic membrane.[72,78,79,81-84] However, none of the carriers reported in the literature to date have been an adequate replacement of standard endothelial grafts and therefore, bioengineered cell-carrier constructs have not yet progressed into clinical practice.

To avoid carrier-related challenges, alternative methods to transplant cultured CECs were trialed, such as injecting free-floating corneal endothelial cells into the anterior chamber. In 2018, a proof-of-concept clinical study by Kinoshita and associates demonstrated that injection of human CECs restored the corneal endothelium in 11 human eyes with BK.[55] After removal of an approximately 8 mm diameter portion of the diseased corneal endothelium with a silicon tip needle, ex vivo cultured CECs, supplemented with a ROCK-inhibitor, were injected into the recipients anterior chamber. All eyes demonstrated regeneration of a monolayer sheet-like structure and achieved restoration of corneal transparency. At 24 weeks after cell injection, ECD was more than 500 cells/mm^2 (range, 947 to 2833 cells/mm^2).

While injecting cultured CECs into the anterior chamber is a minimally invasive approach that shows great promise, larger, prospective, randomized controlled trials are required to refine this technique and to ensure long term efficacy and safety. These might include studies to evaluate potential adverse effects (for example, unattached donor cells entering the systemic circulation and their effect), host immune response (or lack thereof) to cultivated injected endothelial cells, the role of HLA matching, and the potential role of ROCK-inhibitors.[85] Finally, it is possible that adoption of this technique may be slow, despite successful results, as the protocols need to be carefully standardized and need to comply with high regulatory demands including good manufacturing practice (GMP) for cell production, which currently results in very high costs as compared to standard endothelial grafts.

The use of *Rho kinase or rho-associated protein kinase (ROCK)-inhibitors,* as pharmaceutical therapeutic agents or adjuncts for the treatment of corneal endothelial dysfunction, has been a topic of great interest. ROCK is a serine/threonine kinase that serves as an essential downstream effector of Rho-GTPase, and ultimately affects cell adhesion, motility, proliferation, differentiation and apoptosis.[86-89] While the most commonly known ROCK-inhibitor 'Y-27632'

has shown promising results in promoting corneal endothelial regeneration in *in vitro* experiments and in *in vivo* animal models, it may be premature to assume that all the beneficial effects of ROCK inhibitors observed in animal models will be similarly reproduced in humans since animal CECs possess stronger regenerative potential.[89-98] ROCK-inhibitors have also been described as salvage therapy after DWEK and as complementary therapy in DWEK and cell-based therapies.[54,84,97] While ROCK-inhibitors show potential, their efficacy and safety on corneal regeneration *in vivo* needs to be further determined in adequately powered human clinical trials.

Gene therapy is also being explored as a potential avenue for management of corneal endothelial diseases. Although FECD is genetically heterogeneous, many cases are associated with expanded trinucleotide cytosine-thymine-guanin (CTG) repeats in the TCF4 gene.[99] Emerging therapies utilizing anti-sense oligonucleotides (AON) and prokaryotic clustered regularly interspaced palindromic repeat (CRISPR) endonucleases aim to target this sequence and functionally knock down its gene expression.[100] While *ex vivo* human studies have shown that gene therapy is a potentially viable treatment option in the management of FECD, further research has yet to show whether this also holds true for *in vivo* human clinical trials.[101-105]

Exciting novel treatment modalities such as regenerative therapy, bio-engineered corneal grafts, cell therapy and gene therapy have emerged and show promising preliminary results. Further research is warranted to refine the current techniques and to investigate the therapeutic relevance of each of them. Until then, endothelial keratoplasty will remain the standard of care for the management of corneal endothelial dysfunction.

REFERENCES

1. Eye Bank Association of America. *2019 Eye Banking Statistical Report* Washington, DC: Eye Bank Association of America; 2020
2. Melles GR. Posterior lamellar keratoplasty: DLEK to DSEK to DMEK. *Cornea* 2006;25:879-81
3. Lam FC, Baydoun L, Dirisamer M, et al. Hemi-Descemet membrane endothelial keratoplasty transplantation: a potential method for increasing the pool of endothelial graft tissue. *JAMA Ophthalmol* 2014;132:1469–73
4. Müller TM, Lavy I, Baydoun L, et al. Case report of Quarter-Descemet membrane endothelial keratoplasty for fuchs endothelial dystrophy. *Cornea* 2017;36:104–7
5. Birbal RS, Sikder S, Lie JT, et al. Donor tissue preparation for Descemet membrane endothelial keratoplasty: An updated review. *Cornea* 2018;37:128-35
6. Terry MA. Endothelial keratoplasty: why aren't we all doing Descemet membrane endothelial keratoplasty? *Cornea* 2012;31:469–71
7. Birbal RS, Ni Dhubhghaill S, Bourgonje VJA, et al. Five-year graft survival and clinical outcomes of 500 consecutive cases after Descemet membrane endothelial keratoplasty. *Cornea* 2020;39:290-7
8. Rodríguez-Calvo-de-Mora M, Quilendrino R, Ham L, et al. Clinical outcome of 500 consecutive cases undergoing Descemet's membrane endothelial keratoplasty. *Ophthalmology* 2015;122:464–70
9. Hamzaoglu EC, Straiko MD, Mayko ZM, et al. The first 100 eyes of standardized Descemet stripping automated endothelial keratoplasty versus standardized Descemet membrane endothelial keratoplasty. *Ophthalmology* 2015;122:2193–9
10. Deng SX, Lee WB, Hammersmith KM, et al. Descemet membrane endothelial keratoplasty: safety and outcomes: a report by the American Academy of Ophthalmology. *Ophthalmology* 2018;125:295–310
11. Guerra FP, Anshu A, Price MO, et al. Descemet's membrane endothelial keratoplasty: prospective study of 1-year visual outcomes, graft survival, and endothelial cell loss. *Ophthalmology* 2011;118:2368–73
12. Feng MT, Price MO, Miller JM, et al. Air reinjection and endothelial cell density in Descemet membrane endothelial keratoplasty: five-year follow-up. *J Cataract Refract Surg* 2014;40:1116–21
13. Schlögl A, Tourtas T, Kruse FE, et al. Long-term clinical outcome after Descemet membrane endothelial keratoplasty. *Am J Ophthalmol* 2016;169:218–26
14. Price DA, Kelley M, Price FW Jr, et al. Five-year graft survival of Descemet membrane endothelial keratoplasty (EK) versus Descemet stripping EK and the effect of donor sex matching. *Ophthalmology* 2018;125:1508–14
15. Schrittenlocher S, Schaub F, Hos D, et al. Evolution of consecutive Descemet membrane endothelial keratoplasty outcomes throughout a 5-year period performed by two experienced surgeons. *Am J Ophthalmol* 2018;190:171–8
16. Birbal RS, Baydoun L, Ham L, et al. Effect of surgical indication and preoperative lens status on Descemet membrane endothelial keratoplasty outcomes. *Am J Ophthalmol* 2020;212:79-87

17. Birbal RS, Tong CM, Dapena I, et al. Clinical outcomes of Descemet membrane endothelial keratoplasty in eyes with a glaucoma drainage device. *Am J Ophthalmol* 2019;199:150-8
18. Birbal RS, Tong CM, Dapena I, et al. Reply to a comment: Clinical outcomes of Descemet membrane endothelial keratoplasty in eyes with a glaucoma drainage device. *Am J Ophthalmol* 2019;208:440-1
19. Kim P, Amiran MD, Lichtinger A, et al. Outcomes of Descemet stripping automated endothelial keratoplasty in patients with previous glaucoma drainage device insertion. *Cornea* 2012;31:172–5
20. Schoenberg ED, Levin KH, Savetsky MJ, et al. Surgical outcomes of DSAEK in patients with prior Ahmed glaucoma drainage device placement. *Eur J Ophthalmol* 2013;23:807–13
21. Topouzis F, Coleman AL, Choplin N, et al. Follow-up of the original cohort with the Ahmed glaucoma valve implant. *Am J Ophthalmol* 1999;128:198–204
22. Williams KA, Lowe M, Bartlett C, et al. Risk factors for human corneal graft failure within the Australian corneal graft registry. *Transplantation* 2008;86:1720–4
23. Anshu A, Price MO, Richardson MR, et al. Alterations in the aqueous humor proteome in patients with a glaucoma shunt device. *Mol Vis* 2011;17:1891–1900
24. Rosenfeld C, Price MO, Lai X, et al. Distinctive and pervasive alterations in aqueous humor protein composition following different types of glaucoma surgery. *Mol Vis* 2015;21:911–18
25. Chen H, Cho KS, Vu THK, et al. Commensal microflora-induced T cell responses mediate progressive neurodegeneration in glaucoma. *Nat Commun* 2018;10;9:3209
26. Balachandran C, Ham L, Verschoor CA, et al. Spontaneous corneal clearance despite graft detachment in Descemet membrane endothelial keratoplasty (DMEK). *Am J Ophthalmol* 2009;148:227–34
27. Price FW Jr, Price MO. Comment on "Spontaneous corneal clearance despite graft detachment after Descemet membrane endothelial keratoplasty." *Am J Ophthalmol* 2010;149:173–4; author reply 174–5
28. Zafirakis P, Kymionis GD, Grentzelos MA, et al. Corneal graft detachment without corneal edema after Descemet stripping automated endothelial keratoplasty. *Cornea* 2010;29:456–8
29. Moloney G, Petsoglou C, Ball M, et al. Descemetorhexis without grafting for Fuchs endothelial dystrophy—supplementation with topical ripasudil. *Cornea* 2017;36:642–8
30. Iovieno A, Neri A, Soldani AM, et al. Descemetorhexis without graft placement for the treatment of Fuchs endothelial dystrophy: pre-liminary results and review of the literature. *Cornea* 2017;36:637–41
31. Galvis V, Tello A, Berrospi RD, et al. Descemetorhexis without endothelial graft in Fuchs dystrophy. *Cornea* 2016;35:e26–8
32. Borkar DS, Veldman P, Colby KA. Treatment of Fuchs endothelial dystrophy by Descemet stripping without endothelial keratoplasty. *Cornea* 2016;35:1267–73
33. Moloney G, Chan UT, Hamilton A, et al. Descemetorhexis for Fuchs' dystrophy. *Can J Ophthalmol* 2015;50:68–72
34. Koenig SB. Planned Descemetorhexis without endothelial keratoplasty in eyes with Fuchs corneal endothelial dystrophy. *Cornea* 2015;34:1149–51

35. Satué Palacián M, Sánchez Pérez A, Idoipe Corta M, et al. Descemetorhexis and corneal clearing: a new perspective on the treatment of endothelial diseases. *Arch Soc Esp Oftalmol* 2014;89:1–3
36. Koenig SB. Long-term corneal clarity after spontaneous repair of an iatrogenic Descemetorhexis in a patient with Fuchs dystrophy. *Cornea* 2013;32:886–8
37. Shah RD, Randleman JB, Grossniklaus HE. Spontaneous corneal clearing after Descemet's stripping without endothelial replacement. *Ophthalmology* 2012;119:256–60
38. Bleyen I, Saelens IE, van Dooren BT, et al. Spontaneous corneal clearing after Descemet's stripping. *Ophthalmology* 2013;120:215
39. Braunstein RE, Airiani S, Chang MA, et al. Corneal edema resolution after "Descemetorhexis." *J Cataract Refract Surg* 2003;29:1436–9
40. Ziaei M, Barsam A, Mearza AA. Spontaneous corneal clearance despite graft removal in Descemet stripping endothelial keratoplasty in Fuchs endothelial dystrophy. *Cornea* 2013;32:e164–6
41. Dirisamer M, Ham L, Dapena I, et al. Descemet membrane endothelial transfer: "Free-floating" donor Descemet implantation as a potential alternative to "keratoplasty." *Cornea* 2012;31:194–7
42. Dirisamer M, Yeh RY, van Dijk K, et al. Recipient endothelium may relate to corneal clearance in Descemet membrane endothelial transfer. *Am J Ophthalmol* 2012;154:290–6
43. Birbal RS, Parker J, Dirisamer M, et al. Descemet Membrane Endothelial Transfer: Ultimate Outcome. *Cornea* 2018;37:141-4
44. Lam FC, Baydoun L, Dirisamer M, et al. Hemi-Descemet membrane endothelial keratoplasty transplantation: a potential method for increasing the pool of endothelial graft tissue. *JAMA Ophthalmol* 2014;132:1469–73
45. Lam FC, Baydoun L, Satué M, et al. One year outcome of Hemi-Descemet membrane endothelial keratoplasty. *Graefes Arch Clin Exp Ophthalmol* 2015;253:1955–8
46. Lie JT, Lam FC, Groeneveld-van Beek EA, et al. Graft preparation for Hemi-Descemet membrane endothelial keratoplasty (hemi-DMEK). *Br J Ophthalmol* 2016;100:420–4
47. Gerber-Hollbach N, Parker J, Baydoun L, et al. Preliminary outcome of Hemi-Descemet membrane endothelial keratoplasty for Fuchs endothelial dystrophy. *Br J Ophthalmol* 2016;100:1564–8
48. Müller TM, Baydoun L, Melles GR. 3-Year update on the first case series of Hemi-Descemet membrane endothelial keratoplasty. *Graefes Arch Clin Exp Ophthalmol* 2017;255:213–5
49. Birbal RS, Hsien S, Zygoura V, et al. Outcomes of Hemi-Descemet Membrane Endothelial Keratoplasty for Fuchs Endothelial Corneal Dystrophy. *Cornea* 2018;37:854-8
50. Müller TM, Lavy I, Baydoun L, et al. Case report of Quarter-Descemet membrane endothelial keratoplasty for Fuchs endothelial dystrophy. *Cornea* 2017;36:104–7
51. Zygoura V, Baydoun L, Ham L, et al. Quarter-Descemet membrane endothelial keratoplasty (Quarter-DMEK) for Fuchs endothelial corneal dystrophy: 6 months clinical outcome. *Br J Ophthalmol* 2018;102:1425–30
52. Birbal RS, Ni Dhubhghaill S, Baydoun L, et al. Quarter-Descemet Membrane Endothelial Keratoplasty: One- to Two-Year Clinical Outcomes. *Cornea* 2020;39:277-82
53. Miron A, Spinozzi D, Bruinsma M, et al. Asymmetrical endothelial cell migration from in vitro Quarter-Descemet membrane endothelial keratoplasty grafts. *Acta Ophthalmol* 2018;96:828–33

54. Garcerant D, Hirnschall N, Toalster N, et al. Descemet's stripping without endothelial keratoplasty. *Curr Opin Ophthalmol* 2019;30:275-85
55. Kinoshita S, Koizumi N, Ueno M, et al. Injection of cultured cells with a ROCK inhibitor for bullous keratopathy. *N Engl J Med* 2018; 378: 995-1003
56. Huang MJ, Kaine S, Dhaliwal DK. Descemetorhexis without endothelial keratoplasty verses DMEK for treatment for Fuchs endothelial corneal dystrophy. *Cornea* 2018; 37: 1479-83
57. Macsai MS, Shiloach M. Use of topical rho kinase inhibitors in the treatment of Fuchs dystrophy after Descemet stripping only. *Cornea* 2019; 38: 529-534
58. Bhogal M, Lwin CN, Seah XY, et al. Allogeneic Descemet's Membrane Transplantation Enhances Corneal Endothelial Monolayer Formation and Restores Functional Integrity Following Descemet's Stripping. *Invest Ophthalmol Vis Sci* 2017 1;58:4249-4260
59. Soh YQ, Peh G, George BL, et al. Predicative Factors for Corneal Endothelial Cell Migration. *Invest Ophthalmol Vis Sci* 2016;57:338-48
60. Soh YQ, Mehta JS. Regenerative Therapy for Fuchs Endothelial Corneal Dystrophy. *Cornea* 2018;37:523-27
61. Joyce NC, Meklir B. Joyce SJ, et al. Cell cycle protein expression and proliferative status in human corneal cells. *Invest Ophthalmol Vis Sci* 1996; 37: 645-55
62. Joyce N. Proliferative capacity of corneal endothelial cells. *Exp Eye Res* 2012; 95: 16-23.
63. Baum JL, Niedra R, Davis C, et al. Mass culture of human corneal endothelial cell. *Arch Ophthalmol* 1979; 97: 1136-40
64. Soh YQ, Peh GS, Mehta JS. Translational tissues for human corneal endothelial tissue engineering. *J Tissue Eng Regen Med* 2017; 11: 2425-42
65. Jumblatt MM, Maurice DM, McCulley JP. Transplantation of tissue-cultured corneal endothelium. *Invest Ophthalmol Vis Sci* 1978;17:1135-41
66. Jumblatt MM, Maurice DM, McCulley JP. Transplantation of tissue-cultured corneal endothelium. *Invest Ophthalmol Vis Sci* 1978;17:1135–41
67. Gospodarowicz D, Greenburg G, Alvarado J. Transplantation of cultured bovine corneal endothelial cells to rabbit cornea: clinical implications for human studies. *Proc Natl Acad Sci U S A* 1979;76:464–8
68. Ishino Y, Sano Y, Nakamura T, et al. Amniotic membrane as a carrier for cultivated human corneal endothelial cell transplantation. *Invest Ophthalmol Vis Sci* 2004;45:800–6
69. Sumide T, Nishida K, Yamato M, et al. Functional human corneal endothelial cell sheets harvested from temperature-responsive culture surfaces. *Faseb J* 2006;20:392–4
70. Hitani K, Yokoo S, Honda N, et al. Transplantation of a sheet of human corneal endothelial cell in a rabbit model. *Mol Vis* 2008;14:1–9
71. Okumura N, Koizumi N. Regeneration of the Corneal Endothelium. *Curr Eye Res* 2020;45:303-12
72. Mimura T, Yamagami S, Amano S. Corneal endothelial regeneration and tissue engineering. *Progr Retin Eye Res* 2013;35:1–17
73. Mimura T, Yamagami S, Yokoo S, et al. Cultured human corneal endothelial cell transplantation with a collagen sheet in a rabbit model. *Invest Ophthalmol Vis Sci* 2004;45:2992–7
74. Watanabe R, Hayashi R, Kimura Y, et al. A novel gelatin hydrogel carrier sheet for corneal endothelial transplantation. *Tissue Eng Part A* 2011;17:2213–19

75. Liang Y, Liu W, Han B, et al. Fabrication and characters of a corneal endothelial cells scaffold based on chitosan. *J Mater Sci Mater Med* 2011;22:175–83
76. Parikumar P, Haraguchi K, Ohbayashi A, et al. Successful transplantation of in vitro expanded human cadaver corneal endothelial precursor cells on to a cadaver bovine's eye using a nanocomposite gel sheet. *Curr Eye Res* 2014;39:522–6
77. Parikumar P, Haraguchi K, Senthilkumar R, et al. Human corneal endothelial cell transplantation using nanocomposite gel sheet in bullous keratopathy. *Am J Stem Cells* 2018;7:18–24
78. Kopsachilis N, Tsinopoulos I, Tourtas T, et al. Descemet's membrane substrate from human donor lens anterior capsule. *Clin Exp Ophthalmol* 2012;40:187–94
79. Yoeruek E, Saygili O, Spitzer MS, et al. Human anterior lens capsule as carrier matrix for cultivated human corneal endothelial cells. *Cornea* 2009;28:416–20
80. Spinozzi D, Miron A, Bruinsma M, et al. Evaluation of the suitability of biocompatible carriers as artificial transplants using cultured porcine corneal endothelial cells. *Curr Eye Res* 2019;44:243-9
81. Navaratnam J, Utheim TP, Rajasekhar VK, et al. Substrates for expansion of corneal endothelial cells towards bioengineering of human corneal endothelium. *J Funct Biomater* 2015;6:917–45
82. Koizumi N, Sakamoto Y, Okumura N, et al. Cultivated corneal endothelial cell sheet transplantation in a primate model. *Invest Ophthalmol Vis Sci* 2007;48:4519–26
83. Levis HJ, Peh GS, Toh KP, et al. Plastic compressed collagen as a novel carrier for expanded human corneal endothelial cells for transplantation. *PLoS One* 2012;7:e50993
84. Peh GSL, Ang HP, Lwin CN, et al. Regulatory compliant tissue-engineered human corneal endothelial grafts restore corneal function of rabbits with bullous keratopathy. *Sci Rep* 2017;7:14149
85. Dana R. A new frontier in curing corneal blindness. *N Engl J Med* 2018 15;378:1057-8
86. Nakagawa O, Fujisawa F, Ishizaki T, et al. ROCK-I and ROCK-II, two isoforms of Rhoassociated coiled-coil forming protein serine/threonine kinase in mice. *FEBS Lett* 1996 26;392:189-93
87. Riento K and Ridley AJ. Rocks: multifunctional kinases in cell behavior. *Nat Rev Mol Cell Biol* 2003;4:446-56
88. Narumiya S, Tanji M, and Ishizaki T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. *Cancer Metastasis Rev* 2009;28:65-76
89. Okumura N, Ueno M, Koizumi N, et al. Enhancement on primate corneal endothelial cell survival in vitro by a ROCK inhibitor. *Invest Ophthalmol Vis Sci* 2009;50:3680-7
90. Okumura N, Nakano S, Kay EP, et al. Involvement of cyclin D and p27 in cell proliferation mediated by ROCK inhibitors Y-27632 and Y-39983 during corneal endothelium wound healing. *Invest Ophthalmol Vis Sci* 2014 15;55:318-29
91. Peh GS, Adnan K, George BL, et al. The effects of Rho-associated kinase inhibitor Y-27632 on primary human corneal endothelial cells propagated using a dual media approach. *Sci Rep* 2015 16;5:9167
92. Okumura N, Koizumi N, Ueno M, et al. Enhancement of corneal endothelium wound healing by Rho-associated kinase (ROCK) inhibitor eye drops. *Br J Ophthalmol* 2011;95:1006-9
93. Van Horn DL, Hyndiuk RA. Endothelial wound repair in primate cornea. *Exp Eye Res* 1975;21:113-24

94. Van Horn DL, Sendele DD, Seideman S, et al. Regenerative capacity of the corneal endothelium in rabbit and cat. *Invest Ophthalmol Vis Sci* 1977;16:597-613
95. Matsubara M, Tanishima T. Wound-healing of the corneal endothelium in the monkey: a morphometric study. *Jpn J Ophthalmol* 1982;26:264-73
96. Okumura N, Koizumi N, Kay EP, et al. The ROCK inhibitor eye drop accelerates corneal endothelium wound healing. *Invest Ophthalmol Vis Sci* 2013 3;54:2493-502
97. Okumura N, Inoue R., Okazaki R, et al. Effect of the Rho kinase inhibitor Y-27632 on corneal endothelial wound healing. *Invest Ophthalmol Vis Sci* 2015;56:6067-74
98. Okumura N, Okazaki Y, Inoue R, et al. Effect of the Rho-associated kinase inhibitor eye drop (ripasudil) on corneal endothelial wound healing. *Invest Ophthalmol Vis Sci* 2016;57:1284-92
99. Wieben ED, Aleff RA, Tang X, et al. Trinucleotide repeat expansion in the transcription factor 4 (TCF4) gene leads to widespread mRNA splicing changes in Fuchs' endothelial corneal dystrophy. *Invest Ophthalmol Vis Sci* 2017;58: 343–352
100. Karimian A, Azizian K, Parsian H, et al. CRISPR/Cas9 technology as a potent molecular tool for gene therapy. *J Cell Physiol* 2019;234:12267-77
101. Zarouchlioti C, Sanchez-Pintado B, Hafford Tear NJ, et al. Antisense therapy for a common corneal dystrophy ameliorates TCF4 repeat expansion-mediated toxicity. *Am J Hum Genet* 2018;102:528–39
102. Hu J, Rong Z, Gong X, et al. Oligonucleotides targeting TCF4 triplet repeat expansion inhibit RNA foci and mis-splicing in Fuchs' dystrophy. *Hum Mol Genet* 2018;27:1015–26
103. Pinto BS, Saxena T, Oliveira R, et al. Impeding transcription of expanded microsatellite repeats by deactivated Cas9. *Mol Cell* 2017;68:479–90.e5
104. Chang YK, Hwang JS, Chung T-Y, et al. SOX2 activation using CRISPR/dCas9 promotes wound healing in corneal endothelial cells. *Stem Cells* 2018;36:1851–62
105. Zhu AY, Jaskula-Ranga V, Jun AS. Gene Editing as a Potential Therapeutic Solution for Fuchs Endothelial Corneal Dystrophy: The Future Is Clearer. *JAMA Ophthalmol* 2018 1;136:969-70