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RCOphth EPONYMOUS LECTURE

What does the future hold for the treatment of Fuchs endothelial dystrophy; will 'keratoplasty' still be a valid procedure?

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Abstract

Fuchs endothelial corneal dystrophy (FECD) is a well recognized corneal disorder characterized by the presence of collagenous warts extending from Descemet membrane (guttae) and endothelial cellular dysfunction due to cell loss and/or degeneration. Because of the characteristic abnormal cell morphology as seen with specular microscopy as well as the limited regenerative capacity *in vivo*, the endothelial cells were considered to be 'dystrophic'. Hence, FECD is commonly managed by replacement of the endothelium with donor tissue by means of a penetrating or endothelial keratoplasty. The latter procedure has now been refined to the isolated transplantation of a donor Descemet membrane and its endothelium, referred to as Descemet membrane endothelial keratoplasty (DMEK). Unexpectedly, clinical observation made after DMEK seemed to challenge the current concept of the state of the endothelium in FECD; we actually observed an important role for the 'dystrophic' host endothelium in re-endothelialization of the denuded DM, and subsequent corneal clearance. In addition, recent studies regarding the pathophysiology of FECD made us realize that the endothelial cells are not 'dystrophic' *per se*, but in the course of time may have acquired a dysfunction instead. This paper describes the rationale behind this new concept and based on this, discusses the possibilities for future, less invasive treatment modalities for FECD.

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Introduction

Fuchs endothelial corneal dystrophy (FECD) is an endothelial cellular dysfunction due to cell loss and/or degeneration.[1,2] The disorder has four distinctive stages. In the early stages, visual acuity is reduced by 'guttae', that is, collagenous warts extending from the Descemet membrane that compromise the optical performance of the cornea.[1,2] In the advanced stages, the cornea shows edema as a result of reduced endothelial pump function.[2–6] This entity was first recognized by Dr Ernst Fuchs in the early 1900s, with the sole availability of a candle light illuminated slit-lamp. The function of the endothelium was not yet known, and as the condition seemed irreversible, the cornea was considered to be 'dystrophic.' The condition was first described to be an 'epithelial dystrophy', and the term later evolved to a 'combined dystrophy' and 'endothelial dystrophy'. The overall clinical picture seemed to fit into the definition of the term 'corneal dystrophy', first used to refer to 'a group of inherited corneal diseases that are typical bilateral, symmetric, slowly progressive, and without relationship to environmental or systemic factors'.[7]

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However, we would like to challenge this concept; in our opinion, the endothelial cells are not 'dystrophic' *per se*, but in most cases may have acquired a dysfunction instead. This idea is based on recent clinical observations showing the important role of the 'dystrophic' host endothelium in corneal clearance after endothelial keratoplasty,[8–10] and current knowledge regarding the pathophysiology of FECD.[11,12] More detailed knowledge of FECD etiology might even open the door to the development of new, less invasive treatment modalities, and have far reaching consequences for patients, corneal surgeons, and eye banks.

Methodology: PubMed and Google Scholar were used to search the literature. The main search terms used were: 'Corneal dystrophy', 'Fuchs endothelial dystrophy', 'Pathophysiology AND Fuchs endothelial dystrophy', 'Gene AND Fuchs endothelial dystrophy', 'Oxidative stress AND Fuchs endothelial dystrophy', 'Keratoplasty', 'Endothelial keratoplasty', 'Descemet Membrane Endothelial Keratoplasty', 'Wound healing AND corneal endothelium', 'Regeneration AND corneal endothelium', 'Migration AND corneal endothelium', 'Culture AND corneal endothelium', Stem cells AND corneal endothelium', 'Spontaneous clearance AND keratoplasty', 'Donor endothelial cells AND keratoplasty', 'Recipient AND Host AND endothelial cells AND keratoplasty'.

Clinical observations after Descemet membrane endothelial keratoplasty suggesting a role for the 'dystrophic' recipient endothelium in corneal clearance

Corneal endothelial cells are considered to be 'dystrophic' and have limited to no regenerative capacity *in vivo*.[3,6,13] Therefore, replacing the diseased endothelium with healthy donor tissue through a penetrating or endothelial keratoplasty is generally accepted as the only effective treatment option for FECD.[14] With the evolution of endothelial keratoplasty, the procedure has been refined to isolated transplantation of a donor Descemet membrane and its endothelium. This procedure is known as 'Descemet membrane endothelial keratoplasty' (DMEK).[14–16] Clinical observations made after DMEK[8–10] quite unexpectedly led us to reconsider the presumption that FECD is an irreversible pathological condition owing to 'dystrophic' endothelial cells. In DMEK eyes, a more rapid clearance was seen in the corneal periphery than in the central cornea overlying the transplant.[9] Also, in DMEK eyes with an eccentrically positioned or a partially detached transplant, the area between the edge of the descemetorhexis and the edge of the transplant often showed faster clearance than the area over the transplant. This observation held true even if the transplant edge showed an outward roll, potentially 'blocking' donor endothelial cell migration toward the peripheral host cornea.[8,9] The most striking observation was seen in DMEK eyes complicated by complete detachment of the donor Descemet graft, where the cornea still cleared in the majority of cases.[9] In other words, the recipient bare posterior stroma (denuded after descemetorhexis) was somehow re-endothelialized.[10]

This 'spontaneous clearance' of the host cornea was observed only when the indication for surgery was FECD, whereas no improvement was seen in eyes operated on for bullous keratopathy.[10] This suggests that corneal clearance depended on the underlying pathogenesis responsible for the endothelial dysfunction, which may be explained by the host peripheral endothelium having a significant role in the early repopulation of the denuded recipient posterior stroma. However, the presence of a donor graft may be mandatory for the migration of a recipient peripheral endothelium over denuded posterior stroma, as it appeared to be completely absent in a patient in whom a DMEK procedure was interrupted after making the descemetorhexis (ie, without graft implantation),[9] and only partly successful in a nonrandomized, prospective clinical study, where patients underwent a descemetorhexis without endothelial keratoplasty.[17] These data suggest that the term 'dystrophy' may in fact be misleading, because it defines an irreversible cellular pathology, whereas the clinically observed endothelial wound healing patterns seems to prove the opposite; this is a reversible condition, where the host corneal endothelium is capable of restoring its normal mosaic on the bare stroma after removal of the pathologically altered Descemet membrane.

How can these findings be explained? To answer this question we need to look into what is currently known about the pathophysiology of FECD and wound healing patterns of the normal and FECD corneal endothelium (Figure 1).

Pathophysiology of FECD

Although not much is known about the pathological mechanism behind FECD, it is suspected that genetic mutations and environmental factors, and possibly their interactions,[11,12] lie at the root of the disease. When we take a look at what is currently known about the genetic background of FECD, mutated genes have only been found in inherited cases and some sporadic cases. For instance, FECD has been associated with rare mutations in *SLC4A11* (solute carrier family 4, sodium borate transporter, member 11),[18,19] *TCF4* (transcription factor 4),[20–25] *TCF8* (transcription factor 8),[26,27] *CLU* (clusterin),[24] and *LOXHD1* (lipoxygenase homology

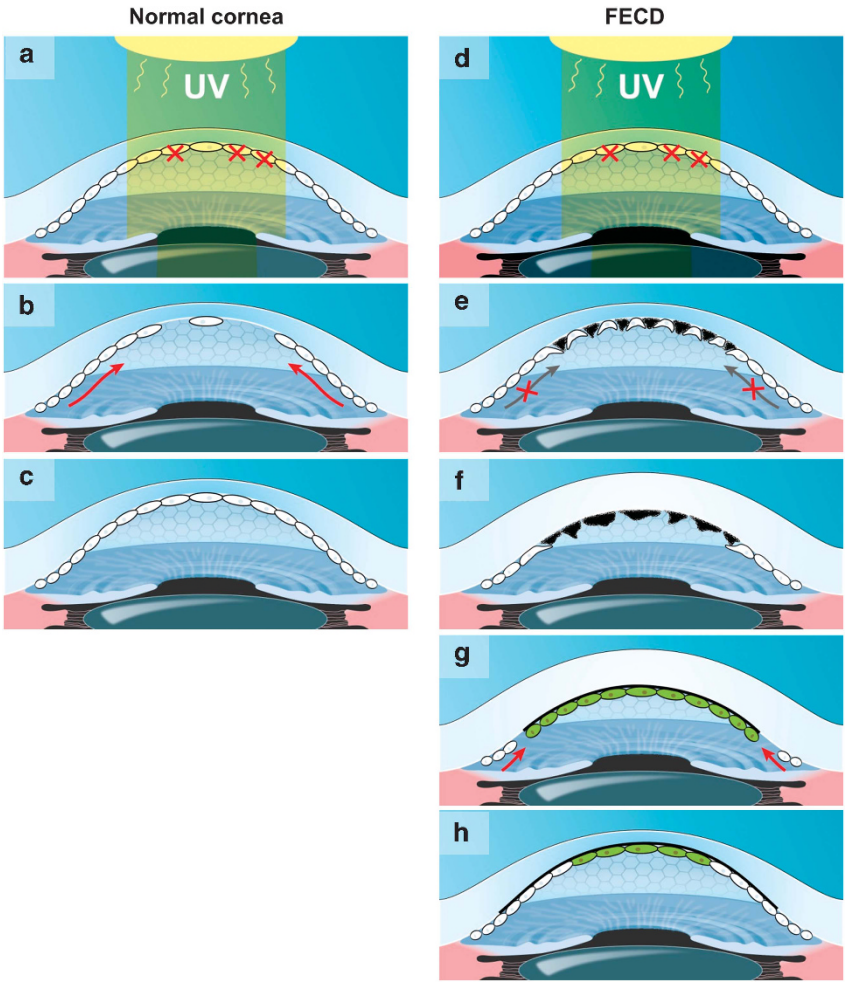

Figure 1 Wound healing response and regenerative potential of recipient endothelial cells of normal and FECD corneas. Here, ultraviolet (UV) radiation is taken as an example to demonstrate the wound healing response of normal and FECD corneal endothelium to oxidative stress-induced damage and apoptosis. As the central cornea is thinner in comparison with the peripheral cornea, it is suggested that UV radiation has its most damaging effects (in the form of oxidative stress) on the central endothelial cells, whereas the peripheral endothelial cells are protected from oxidative stress-induced damage (a, d). Although cells have several defense mechanisms against short-time exposure to oxidative stress, during prolonged exposure, the damage which cannot be repaired will accumulate, and consequently, the cell dies by apoptosis. In the case of corneal endothelium, this leaves a gap between the rest of the endothelial cells, which might compromise the endothelial pump and cause stromal swelling. In the normal cornea (b, c), this defect is supposedly covered by the centripetal migration of stem-like cells in the periphery (arrows in b) which, during migration to the center of the cornea, become mature endothelial cells, dedicated to keep the cornea thin and transparent. However, in FECD corneas (e), the central endothelial cells are even more susceptible to UV-induced damage, resulting in a higher number of apoptotic cells and more gaps between cells. Here, the defect cannot be covered by the peripheral stem-like cells because of the physical barrier in the form of guttae (black structures). Therefore, the adjacent endothelial cells need to stretch to cover the gap the lost cell has created. Eventually, as the guttae progress and coalesce, these cells are also lost, and the cornea decompensates and requires a keratoplasty procedure to restore normal vision (f). When DMEK is performed, the barrier in the form of guttae is removed by the descemetorhexis, and when the graft (green) is implanted, the peripheral stem-like cells are again able to migrate to the center (arrows) (g). In the end, the endothelial pump is restored and the cornea becomes thin and transparent again. In due time, as the centripetal migration of recipient stem-like cells continues, they may mix among the donor cells (green) (h).

domains 1).[28] These mutations, however, are not identified in every cohort of FECD patients.[29,30] In addition to mutations in known genes, linkage studies have identified mutations in chromosomal loci such as in chromosome 5 (FCD3),[31] 9 (FCD4),[27] 13 (FCD1),[32] 18 (FCD 2),[33] and potential linkages at chromosome 1, 7, 15, 17, and X,[20] of which the specific mutated genes have not been identified yet. Some of these mutations have also been identified in other types of endothelial dystrophy; the *SLC4A11* mutation has also been identified in congenital hereditary endothelial dystrophy[34,35] and perceptive deafness (Harboyan syndrome),[36] and

mutations in *TCF8* have also been identified in posterior polymorphous corneal dystrophy.[37,38] This suggests that corneal endothelial dystrophies may not be separate diseases, but may represent a phenotypic continuum with a significant genetic overlap, despite discrete clinical manifestations.[27]

However, in most cases, FECD is not associated with a specific genetic mutation, but may rather be due to an impaired defense to environmental factors, such as oxidative stress.[39] The major inducers of oxidative stress in the cornea are ultraviolet radiation, temperature changes, and aqueous humor soluble factors (Figure 1).[40–42] Corneal endothelial cells are thought to be especially vulnerable to oxidative stress-induced damage because they do not proliferate and have a high level of metabolic activity owing to their pump function.[39,43] Fortunately, these cells have several defense mechanisms against the detrimental effects of oxidative stress,[44,45] thereby reducing damage and promoting cell survival. However, if oxidative stress persists, the defense mechanisms fail, thereby allowing the cell to accumulate damage. As a preventive measure, the cell becomes senescent or, in cases of severe damage, dies by apoptosis.[44] Interestingly, several authors have found evidence of increased apoptosis having a role in endothelial cell loss, as observed in FECD.[46,47] Furthermore, it was shown that the defense mechanism of FECD endothelium against oxidative stress-induced damage is impaired when compared with normal cells,[39,45,48,49] which is supported by the observed significant higher levels of (baseline) oxidative stress-induced damage,[43,45,48] and changes in endothelial cell morphology characteristic for oxidative stress.[50] These data suggest that chronic oxidative stress contributes to the pathophysiology of FECD, and that the decreased defense system renders FECD endothelial cells even more susceptible to oxidative stress-induced damage than normal cells already are. It is to be noted that the corneal center is the site where oxidative stress is probably most prominent.[40] This might explain why FECD initiates in the corneal center, as endothelial cells in this area are exposed to the highest levels of oxidative stress-induced damage. Whether or not oxidative stress is also responsible for the early induction of guttae remains to be determined. However, this theory would agree with aberrant protein expression, which is one of the consequences of oxidative stress-induced damage.[51]

Wound healing patterns of the normal and FECD corneal endothelium

Until recently it was thought (as endothelial cells were believed to have no regenerative potential) that when a corneal cell is 'lost', it induces a wound healing response where the defect of the lost cell is covered by an adjacent endothelial cell through migration and elongation.[13] However, a recent study by He *et al*,[40] suggested that the corneal periphery contains a reservoir of stem-like cells that replace damaged or dead endothelium by continuous centripetal migration patterns (Figures 1b and c). These stem-like cells are supposedly shielded from oxidative stress-induced damage because of their specific location at the very edge of the posterior cornea.[40] However, upon migration to the center of the cornea, the stem-like cells are exposed to oxidative stress, which induces their maturation to end-stage corneal endothelial cells, dedicated to keep the cornea in a deturgescent state.[40] This wound healing mechanism could be impaired in FECD due to the presence of guttae, which act as a physical barrier to the centripetal migration of these peripheral stem-like cells (Figure 1e). Consequently, as in FECD the central cornea is depleted of endothelial cells due to an accumulation of oxidative stress-induced damage, the only wound healing response comes from the adjacent endothelial cells, which have to stretch and elongate to cover the defect and maintain the endothelial pump. As guttae develop, these attempts at wound healing are even further impaired. This may lead to a vicious cycle, where the cells covering the bare area are also damaged and lost. Eventually, too few cells are left to maintain the endothelial pump. This results in corneal decompensation and requires a keratoplasty procedure to restore normal vision (Figure 1f).

Possible mechanisms behind corneal clearance after DMEK

How does the above explain the before-mentioned clinical observations that suggest recipient endothelial migration after DMEK? Given the above, removing the physical barrier (guttae) by means of a penetrating or endothelial keratoplasty may enable the peripheral stem-like cells to freely migrate again and to mix among the donor cells (Figures 1g and h).[52–54] This might also explain the longer survival of corneal grafts in patients with high ECD in the corneal periphery,[55,56] the more rapid clearance in the corneal periphery than in the central cornea after DMEK,[9] and the faster clearance over the gap between the edge of the descemetorhexis and the edge of the transplant than over the transplant itself.[8,9] In case of 'spontaneous clearance', a similar mechanism might have taken place where by removing the barrier (guttae), the peripheral stem-like cells are once again able to cover the denuded stroma by migrating to the corneal center (Figures 2a–c). Thus, the above mentioned studies indicate that in FECD, the endothelial cells are not necessarily 'dystrophic'. This is further substantiated

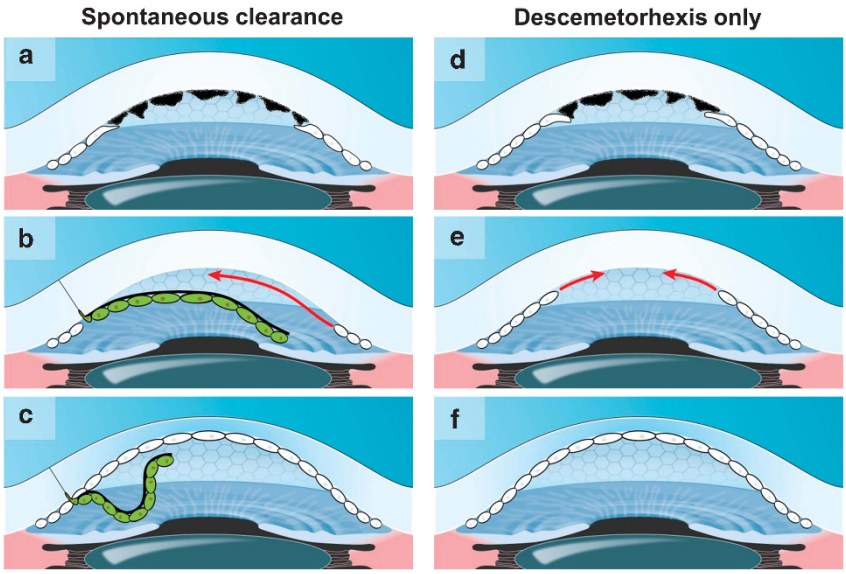

Figure 2 Mechanisms behind spontaneous clearance and a sole descemetorhexis in the management of FECD. In the case of spontaneous clearance, it is thought that the donor graft somehow induces recipient endothelial migration. By removing the guttae that act as barriers to the migration of peripheral stem-like cells (a) and inserting a free-floating donor tissue (green) (only attached to the incision for stable positioning), clinical observations indicate that migration of the recipient stem-like cells is induced (b). Consequently, the bare stroma is covered by recipient endothelial cells (c), the endothelial pump is restored, and the cornea is returned to its deturgescent state. Alternatively, in some cases, a descemetorhexis, without the concomitant transplantation of donor tissue, might be sufficient to induce recipient endothelial migration (d–f). One of the prerequisites for success is the early detection of the disease, when the guttae have not progressed to the far periphery yet, and sufficient peripheral stem-like cells remain to cover the bare stroma (d). After removal of the physical barrier by means of the descemetorhexis, proliferation and migration of the peripheral stem-like cells may be initiated (e), resulting in a restoration of the normal endothelial mosaic and pump function, thus a thin and transparent cornea.

by a recent publication showing that among the 'dysfunctional' FECD endothelial cells, endothelial cells with regenerative potential exist.[57]

Future perspectives

If this proves correct, it may open the door to also reconsider the current keratoplasty management of FECD, which is the same for all variants. In the future, tailored treatment options may be developed based on the different variants of FECD. However, before we enter this exciting era, a lot of questions need to be answered. First and foremost, the different disorders now all recognized as FECD need to be identified individually, and it should be assessed to what extent genetic mutations and environmental factors are involved in disease onset, severity, and progression. For instance, although the prevalence of the *TCF4* mutation is low, it is associated with a very high risk of developing FECD. Other examples include loss-of-function mutations in *TCF8*, that can interact with FCD4 to modulate FECD severity,[27] and FCD3 mutations presenting clinically with milder phenotypes than FCD1 and FCD2.[31] This implies that genetic mutations, whether alone or in concert with other factors, may be used to identify risk of developing disease or act as prognostic factors to determine disease severity and progression.[58]

Another important question is whether in the different FECD variants, all endothelial cells are affected by the disorder or whether in some a mosaic of non-affected and affected cells exists? Furthermore, the question arises whether a keratoplasty procedure, as it is currently performed, will still be required to restore vision in all of these different variants? Perhaps in some cases, where endothelial cell loss is mainly due to apoptosis, drugs that inhibit apoptosis may be able to delay or even halt FECD progression.[46] One of the biggest challenges in this approach is identifying the disease in a sufficiently early stage, where the intervention still has an effect.

In addition, it needs to be determined what potential contribution the recipient endothelium has in restoring the cellular monolayer across the cornea, and whether the presence of donor tissue is really mandatory for the migration of recipient endothelial cells. If the recipient endothelial cells are able to repair the defect independently of donor tissue, the surgical treatment may be directed toward removing the Descemet membrane and its guttae rather than transplanting donor endothelium (Figures 2d–f). Apparently, just the removal of Descemet membrane could already be effective to

obtain corneal clearance in some cases, however, these results may not be consistent.[9,17]

If, on the other hand, recipient peripheral endothelial cells do need to be stimulated toward migratory and/or regenerative activity by the donor tissue, a central descemetorhexis in combination with free-floating donor tissue, also known as 'Descemet membrane endothelial transfer' (DMET),[10] might be effective in restoring vision (Figures 2a–c). Alternatively, the transplantation of cultured donor-derived corneal endothelial cells or differentiated stem cells[59] may be future options. These cells could be attached to a Descemet membrane-like biomatrix[59] or injected as single cells into the anterior chamber together with an adjuvant, inducing their attachment to the bare stroma.[60–62] The latter strategies could also significantly reduce the need for donor tissue, allowing an even larger group of patients to be treated.

Concluding remarks

Minimizing surgical intervention to treat FECD would greatly benefit patients, corneal surgeons, and eye banks. For patients, the visual prognosis would probably be better, as physiological repopulation and near-perfect anatomical restoration by host cells may prove superior to any currently available keratoplasty procedure. For corneal surgeons, the entire management of corneal endothelial disorders would become less challenging with a reduced risk of graft detachments and other complications. For eye banks, the requests for tissue could shift toward isolated donor Descemet implants and/or cell culture, allowing a far more efficient use of donor tissue. In the end, we may even come to understand that conventional and/or endothelial keratoplasty were just scientific side-steps in the management of FECD. The regenerative capacities of our tissues may yet prove to be the most forgiving compared with all current surgical approaches. In the end, it may be Mother Nature, not the corneal surgeon that does a better repair job.

Conflict of interest

GRJ Melles is a consultant for DORC International BV/Dutch Ophthalmic USA. M Bruinsma and CM Tong declare no conflict of interest.

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