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Ex vivo excimer laser ablation of cornea guttata and ROCK inhibitor-aided endothelial recolonization of ablated central cornea

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ABSTRACT.

Purpose: **To determine whether excimer laser ablation of guttae is a viable strategy for removal of diseased tissue in Fuchs' endothelial corneal dystrophy (FECD) on excised human Descemet membranes and whether an excimer laser-created wound on healthy human corneas *ex vivo* is recolonized with corneal endothelial cells.**

Methods: **Descemet membranes of FECD patients and corneal endothelium of normal human corneas were ablated *ex vivo* using an excimer laser licensed for glaucoma surgery. Specimens were kept in cell culture medium supplemented with 10 µM of rho-kinase inhibitor ripasudil. Corneal endothelial cell regeneration was observed using light and electron scanning microscopy. Furthermore, the whole corneal samples were evaluated by haematoxylin/eosin staining and immunohistochemical analysis using antibodies against Na^+/K^+-ATPase.**

Results: **Guttae and corneal endothelium could be ablated with an excimer laser without total ultrastructural damage to the Descemet membrane or stroma. Nearly complete endothelial wound closure was accomplished after 26–38 days in treated corneas. Light and electron scanning microscopy suggested the establishment of a layer of flat endothelial cells. Additionally, Na^+/K^+-ATPase expression could only be observed on the inner side of the Descemet membrane.**

Conclusion: **Our proof of concept study demonstrated that excimer lasers can be used to ablate diseased tissue from excised FECD Descemet membranes *ex vivo*. Additionally, corneal endothelial cells recolonize a previously ablated endothelial area in healthy human corneas *ex vivo* under treatment with ripasudil. Thus, our results are the first experimental basis to further investigate the feasibility of an excimer laser ablation as a graftless FECD treatment option.**

Key words: corneal endothelium – corneal graft – Descemet membrane – endothelial keratoplasty – excimer laser – Fuchs' endothelial corneal dystrophy

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Introduction

Fuchs' endothelial corneal dystrophy (FECD) is the most common dystrophy of the corneal endothelium leading to a significant decline in visual acuity. A gradual loss of endothelial cells leads to reduced corneal deturgescence via loss of Na^+/K^+-ATPase density (McCartney et al. 1987; McCartney et al. 1989) with subsequent corneal oedema (Elhalis et al. 2010) and opacity (Borboli & Colby 2002).

As Fuchs' endothelial corneal dystrophy develops, the characteristic proteinaceous lesions known as guttae accumulate as excrescences on the Descemet membrane (Abbott et al. 1981). In early stages, they may produce light scatter and visual blur, when confluent and enlarged, they are not compatible with endothelial cell homeostasis and lead to cell death. This effect may be size related, with large guttae presenting an insurmountable challenge to cell monolayer formation. Guttae typically follow a centrifugal distribution pattern with peripheral involvement in advanced disease stage (Elhalis et al. 2010). By electron microscopy, Descemet membranes in FECD show a typical additional posterior banded layer (PBL) consisting of dense fibrillary components and collagen compared with normal Descemet membranes (Xia et al. 2016). Furthermore, electron microscopy reveals a banded structure within guttae. With regard to the

ultrastructure of guttae, there are many hypotheses, but the exact mechanisms of PBL and gutta formation are unclear. One hypothesis suggests unknown processes may lead to a transformation of endothelial cells to a fibroblast-like phenotype, secreting collagen fibrils and thus forming the PBL. Focal accelerations of those transformation processes are suggested to lead to guttae (Iwamoto & DeVoe 1971; Adamis et al. 1993).

State-of-the-art treatment of FECD is the endothelial keratoplasty. The predominant forms remain Descemet stripping endothelial keratoplasty (DSEK), where the diseased host Descemet membrane and endothelium are removed and replaced through donor endothelial cells and an additional stromal layer (Wacker et al. 2016), and Descemet membrane endothelial keratoplasty (DMEK; Dapena et al. 2013). First described by Melles et al. (2006), DMEK differs from DSEK by replacing the abnormal Descemet membrane and endothelium without any stromal graft leading to a significantly faster recovery of visual acuity (Price et al. 2009).

The demand for corneal grafts worldwide is continuously rising, in particular for the treatment of Fuchs' endothelial corneal dystrophy. According to recent figures, FECD is the number one indication for corneal transplantation worldwide. In 2012, 39% of all corneal grafts were used for the treatment of FECD (Gain et al. 2016). In light of anecdotal evidence suggesting some regenerative capacity of the native endothelium (Dirisamer et al. 2011), and in need of a graft free alternative, many groups came up with a novel procedure: Descemet stripping without endothelial keratoplasty (DWEK; Moloney et al. 2015). In brief, corneal clearance is achieved following a manual descemetorhexis without using any donor tissue (Shah et al. 2012; Bleyen et al. 2013). This approach is further encouraged by recent studies reporting a positive effect of rho-associated kinase (ROCK) inhibitor ripasudil on corneal wound healing. In rabbits, Okumura et al. (2015) showed a significant increase in corneal clearance after damage of the corneal endothelium following topical treatment with the ROCK inhibitor Y-27632. Those findings are in line with Moloney et al. (2017), who observed an acceleration of corneal clearance in FECD patients treated with topical ripasudil following manual descemetorhexis. Overall, there is strong evidence that corneal endothelial cells (CEC) have the distinct potential to regenerate or migrate after removal of the Descemet membrane in FECD.

At present, therefore, it has been demonstrated that surgical removal of guttae along with their basement membrane improves visual acuity in FECD (Shah et al. 2012; Bleyen et al. 2013; Okumura et al. 2015; Moloney et al. 2017). Once this wound is created, however, cells in the peripheral cornea must migrate across to restore a functional cell layer, with or without the encouragement of ROCK inhibition. It has also been demonstrated *in vitro* that this process is faster and more reliable if the underlying basement membrane (Descemet membrane) is left intact (Okumura et al. 2018; Soh & Mehta 2018). In addition, *in vivo* experience of DWEK surgery has demonstrated that injury to underlying stromal tissue may result in unfavourable healing response (Garcerant et al. 2019).

The surgical challenge presenting itself therefore is to determine whether it is possible to remove guttae from the posterior cornea while preserving the underlying Descemet membrane. The guttae themselves are outgrowths of protein, tightly bound to the membrane and not easily removed. An excimer laser which photoablates tissue is an ideal tool to smooth the posterior cornea profile and allow uninterrupted migration and monolayer formation of endothelial cells.

Excimer lasers are gas lasers emitting ultraviolet light, which are used in corneal surgery since 1983 (Trokel et al. 1983). Commonly a pulsed laser beam with a very short pulse duration is applied to the desired corneal tissue. Nevertheless, the focal energy output is high. Different parameters contribute to the outcome of laser ablation, especially the wavelength, pulse duration and spot diameter (Verma et al. 2017). The pulse energy essentially influences the area and depth of corneal ablation by a single pulse (Arba Mosquera & Verma 2015). Besides refractive surgery, excimer lasers are nowadays used in a broad field of ophthalmic surgical procedures, including minimally invasive glaucoma surgery (Wilmsmeyer et al. 2006).

The aim of our current *ex vivo* study was to investigate whether the ablation of guttae by an excimer laser is possible and whether an ablation of the corneal endothelium of healthy human corneas is followed by a recolonization with endothelial cells.

Materials and Methods

Corneal tissue culture

Sclerocorneal rings not suitable for corneal transplantation due to lack of sufficient endothelial cell count or positive microbiological testings were obtained from the Bavarian tissue bank (Munich, Germany). None of the donors had any previous eye disease. Human tissue was secured in a humane fashion compliant with the Declaration of Helsinki following approval by the local ethics committee (approval ID: 73416). Descemet membranes were received with consent from FECD patients undergoing Descemet membrane endothelial keratoplasty.

Both types of tissue were stored in uncoated cell culture plastic (NUNC, Langenselbold, Germany) and kept in culture medium (Dulbecco's MEM; Biochrom GmbH, Berlin, Germany) supplemented with 10% fetal calf serum (Biochrom GmbH), 50 IU penicillin/ml and 50 µg streptomycin/ml at 37°C and 5% carbon dioxide.

Excimer laser

All experiments were conducted with a modified ExTra ELT excimer laser (MLase AG, Germering, Germany) with a pulsed beam emitting light of 308 nm and a pulse duration of 60–120 ns. The laser was previously licensed for minimally invasive glaucoma surgery. Laser beam output at the desired destination was maintained by a fibre optical laser applicator (FIDO, MLase AG).

Corneal endothelium and guttae ablation procedure

Following epithelial abrasion, sclerocorneal rings were mounted onto a cell culture plastic with the corneal endothelium facing up. Staining of the wounded corneal endothelial area was conducted with trypan blue (VisionBlue; D.O.R.C. Dutch Ophthalmic Research Center, Zuidland, the Netherlands).

Descemet membranes were flat mounted and pinned onto cell culture plastic dishes with entomological pins (Ento Sphinx s.r.o., Pardubice, Czech Republic). To simulate physiological conditions, sodium hyaluronate (Healon® PRO; Johnson & Johnson, New Brunswick, NJ, USA) was injected between the Descemet membrane and the cell culture plastic followed by staining with trypan blue.

Excimer laser treatment included step-by-step circular guttae ablation involving the central 4 mm of the corneal endothelium and the Descemet membrane, avoiding impact on the basal membrane of the same and without direct tissue contact. In addition, in sclerocorneal rings a corridor heading to the limbus was formed to facilitate migration of corneal endothelial cells out from the peripheral endothelium. Centrally, besides a superficial ablation, a deep wound to stromal tissue was created intentionally to investigate the effect of an accidental damage to the corneal stroma. The whole procedure was conducted on three human corneas.

Light and electron scanning microscopy

Sclerocorneal rings and Descemet membranes were analysed and photodocumented weekly using a stereo microscope (Stemi 508; Carl Zeiss AG, Jena, Germany) following staining with trypan blue.

Before electron scanning microscopy, corneas were fixed in a cacodylate-buffered solution containing 4% paraformaldehyde (PFA) and 4% glutaraldehyde for 24 hr. Subsequent to washing with cacodylate buffer and dehydration in ascending ethanol and acetone series, the samples were critical point dried and sputter coated with gold-palladium. Images were captured with an Auriga scanning electron microscope (Carl Zeiss AG).

To assess whether recolonized corneal endothelial cells expand on wounded area, the cell area of 60 CEC of three corneas were measured in ImageJ (ImageJ 1.50c4; National Institute of Health, Bethesda, MD, USA). Comparison was made between regular endothelial cells in unaffected areas of the cornea and those cells colonized on the previously ablated area (for both groups: $n = 30$) using Student's t-test.

Immunohistochemistry

For immunohistochemistry, corneas were sliced into center-involving eighth and fixed with 4% PFA for 4 hr, followed by embedding in paraffin according to standard procedures. Meridional sections of the corneas were stained with haematoxylin and eosin and analysed by light microscopy on an Axiovision microscope (Carl Zeiss AG).

For immunostaining against the endothelial Na^+/K^+-ATPase, sections were hydrated and washed with 0.1 M phosphate buffer at room temperature for 10 min and blocked with 3% bovine serum albumin (BSA) and 0.1% Triton X-100 in 0.1 M phosphate buffer for 60 min followed by an incubation with anti-mouse Na^+/K^+-ATPase antibodies (1:50; Novus Biologicals, Wiesbaden, Germany) in 0.3% BSA and 0.01% Triton X-100 in 0.1 M phosphate buffer for 24 hr at 4°C. Following three washing cycles with 0.1 M phosphate buffer, Alexa488-coupled anti-mouse antibodies (1:1000; ThermoFisher Scientific, Waltham, MA, USA) were added for 60 min. After final incubation, samples were washed three times with 0.1 M phosphate buffer 10 min each and were mounted with fluorescent mounting medium containing 1:50 4',6-diamidino-2-phenylendole (DAPI; Vector Laboratories Inc., Burlingame, CA, USA). Analysis was performed on an Axiovision fluorescent microscope (Carl Zeiss AG).

Data analysis

Quantification of endothelialized area after trypan blue staining was conducted by a semi-automated colour threshold function in ImageJ. Data analysis was conducted with Microsoft Excel (Microsoft Office 365, Microsoft, Redmond, WA, USA). Graphs were plotted with Prism 8 (GraphPad Software, San Diego, CA, USA).

Results

Corneal endothelial cells migrate following descemetorhexis

To investigate migratory capacity of corneal endothelium, we conducted manual descemetorhexis only on human corneas *ex vivo*. Keeping them in cell culture medium supplemented with 10 μM of ripasudil for 2 days, migration of endothelial cells from the edges of the descemetorhexis into the wounded area was observed by electron microscopy (Fig. 1A,B). The corneal endothelial cells seemed to migrate across the step formed by the edge of the descemetorhexis onto corneal stroma to re-establish corneal endothelium.

Laser ablation of guttae and Descemet membranes additional posterior banded layer

To analyse whether the pulsed excimer laser beam can ablate guttae and the additional posterior banded layer of the Descemet membrane of patients suffering from FECD, preparations of patients undergoing Descemet membrane endothelial keratoplasty were whole mounted on a cell culture dish and stained with trypan blue prior to *ex vivo* treatment. To test whether a layer-by-layer removal ablation of both diseased structures is possible, the tip of the fibre optical laser

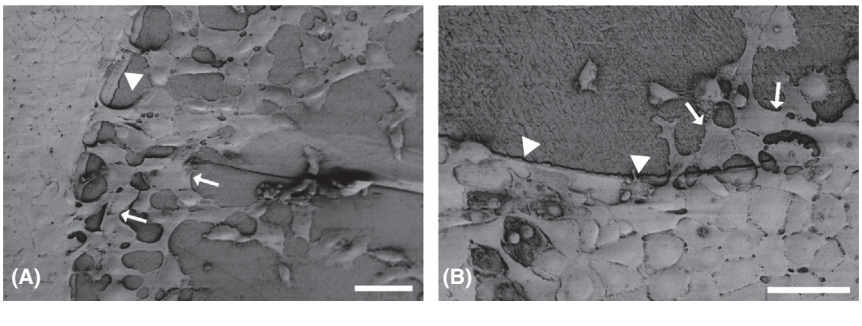

Fig. 1. Electron scanning microscopy of regular human corneas following manual descemetorhexis. (A and B) Representative images of the corneal endothelium following manual descemetorhexis. The arrows point at grouped corneal endothelial cells, appearing to span migrating networks towards the stroma, the triangles show the edge of the descemetorhexis. Scale bar: 50 μm.

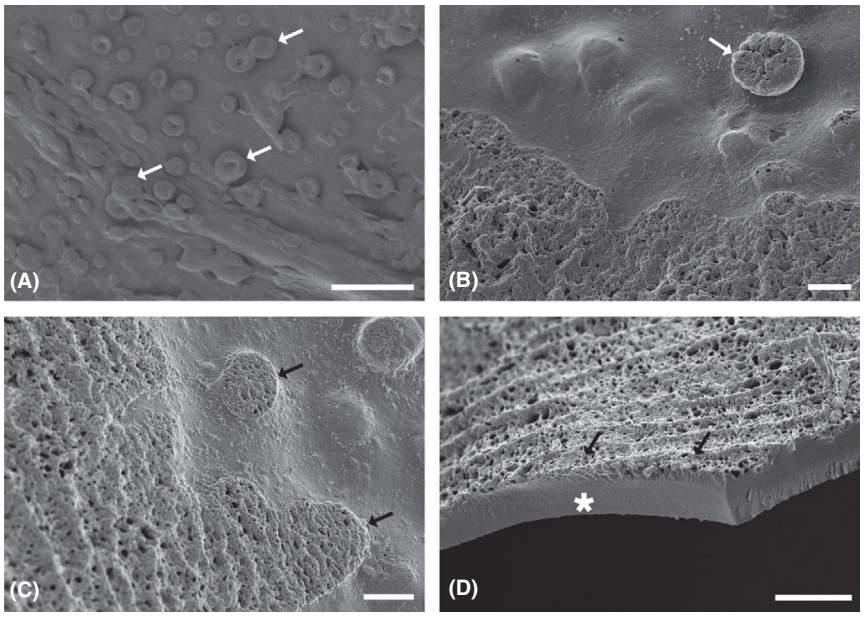

Fig. 2. Electron scanning microscopy of Fuchs' endothelial corneal dystrophy (FECD) patients' Descemet membranes before and after excimer laser ablation. Corneal endothelium and guttae (arrows) could be ablated step-by-step without any microscopic damaging impact on the posterior nonbanded layer (PNBL). Sub-panel A of Fig. 2 shows an electron microscopy image of an untreated FECD patient. In comparison, B–D indicate ablated corneal endothelium and guttae. In cross-section of the guttae and after complete corneal endothelium ablation, a fibrillary spongiform structure became visible. The PNBL underneath seemed smooth and unaffected in electron microscopy (indicated by the asterisk). Scale bars for A and D: 50 μm; B and C: 5 μm.

applicator was carefully approached until first laser effects could be observed. By using this technique, focal excrescences could be removed with a high degree of precision (Fig. 2A,B). In addition, the spongy material of the additional posterior banded layer of the Descemet membrane could be removed in a layer-by-layer manner without affecting the subjacent normal Descemet membrane (Fig. 2C,D).

Laser ablation of corneal endothelial cells and Descemet membrane

To further examine whether the excimer laser can ablate corneal endothelial cells without destruction of the Descemet membrane, *ex vivo* human sclerocorneal rings were stained with trypan blue before laser treatment. By carefully approaching the tip of the fibre optical laser applicator, the corneal endothelium could be cleared without removal of Descemet membrane (Fig. 3A). Only a few ruptures in the Descemet membrane were detected. In areas where a targeted ablation was wanted, the Descemet membrane was removed completely and highly organized collagen bundles of the corneal stroma were observed by scanning electron microscopy (Fig. 3B).

Restoration of the corneal endothelium following ablation

To test whether corneal endothelial cells can migrate onto a laser wounded area the endothelium and in part the Descemet membrane of human *ex vivo* corneas from healthy donors was laser-ablated following trypan blue staining. To further analyse whether a corridor towards the trabecular meshwork enhances migration of endothelial cells from the periphery to the corneal centre the circumscribed wound area was extended to the periphery at the 12 o'clock position (Fig. 4A,B). Six days after treatment, several small lighter areas, which were located in a spot-like manner adjacent to the unaffected endothelium within the trypan blue stained wound, were observed indicating an initial recolonization by corneal endothelial cells under cell culture conditions (Fig. 4C). In subsequent weeks, the lighter areas extended, fused with each other and nearly completely closed after a maximum of 38 days (Figs 4D–F and 5). Intriguingly, 2 weeks after laser ablation the broadest recolonized area was observed at the 12 o'clock position suggesting that the corridor might enhance migration of corneal endothelial cells and hence recovery of the corneal endothelium (Fig. 4D).

Figure 5 indicates a re-endothelialization of the ablated area starting promptly postsurgery. Already after 6 days, corneal endothelial cells migrated towards the wounded surface. Migration continued steadily until days 13–19. Subsequently, a strong decline of the exposed ablated surface could be observed. A nearly complete closure of the wounded area was achieved after 26–38 days (Fig. 5).

As we observed a wound closure of the laser-ablated area in our *ex vivo* experiments, we investigated morphology and specificity of migrated cells by light as well as electron scanning microscopy and immunohistochemistry. On meridional sections of the

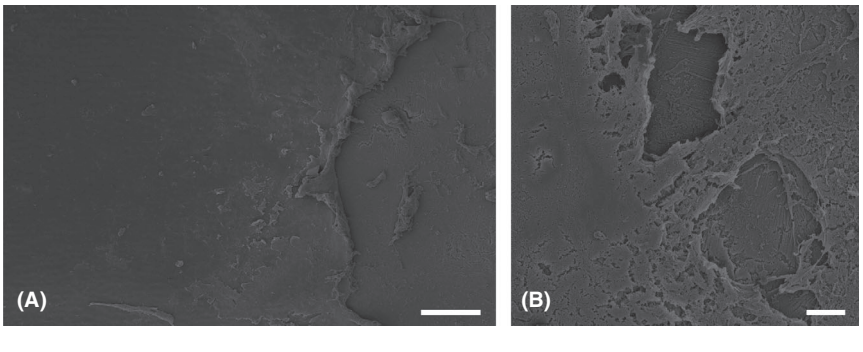

Fig. 3. Ablation of the corneal endothelium and the Descemet membrane in regular human corneas – electron scanning microscopy. (A) Displayed is an area after ablation of the corneal endothelium only. Corneal endothelium seems bumpy and irregular, whereas the subjacent Descemet membrane appears to be smooth after ablation of the endothelium. (B) Underneath the ablated Descemet membrane, properly organized stromal collagen fibres emerge.

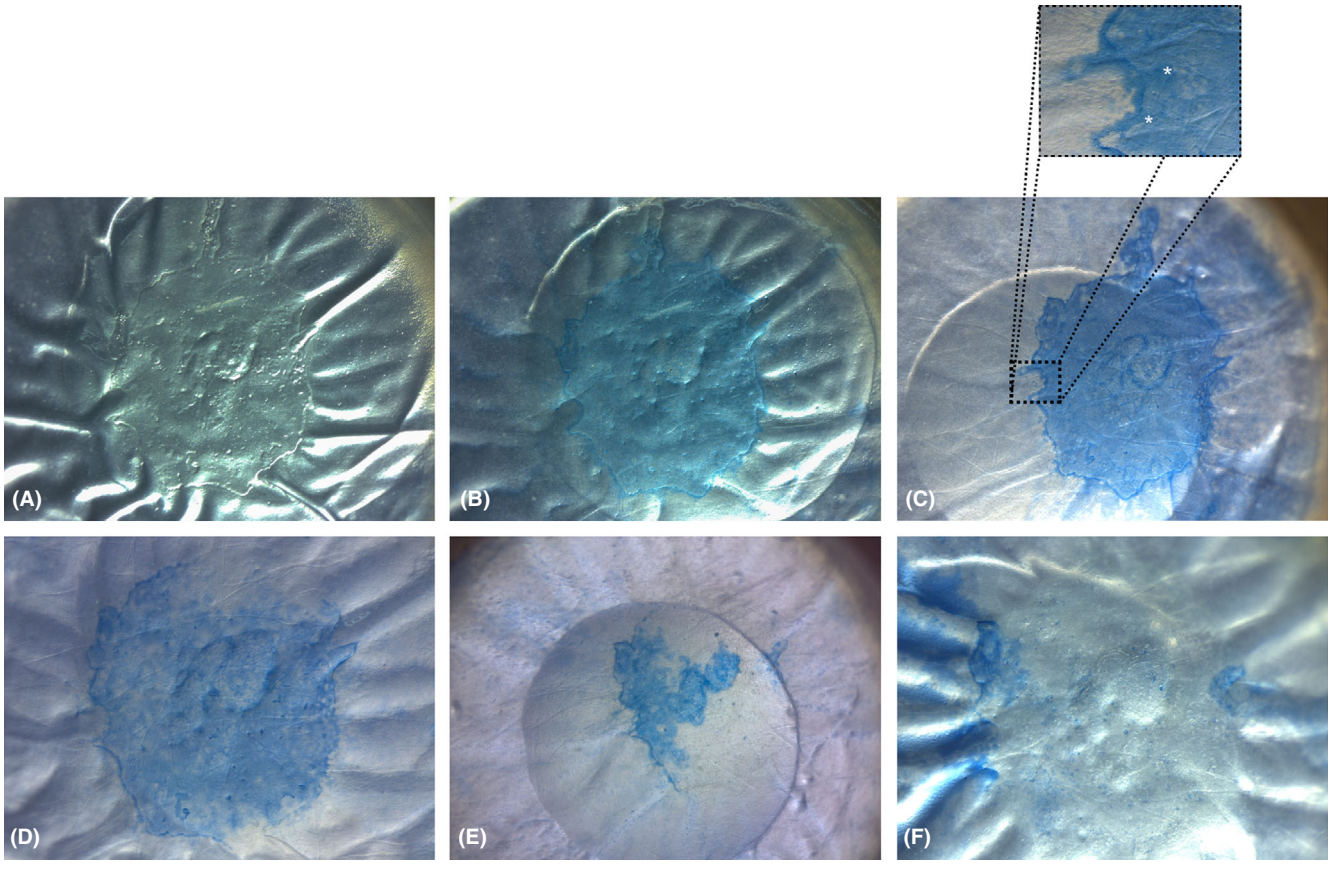

Fig. 4. Light microscopy of a regular human cornea after excimer laser treatment. (A and B) Postoperative images directly after treatment native and stained with trypan blue. There is a clear boundary between intact corneal endothelium and ablated area. On 12 o'clock position a small corridor was formed. (C–F): Displayed are analogous captures on days 6 (C), 13 (D), 20 (E) and 26 (F). The sub-panel of C shows small cell clusters beyond the ablation border (asterisk).

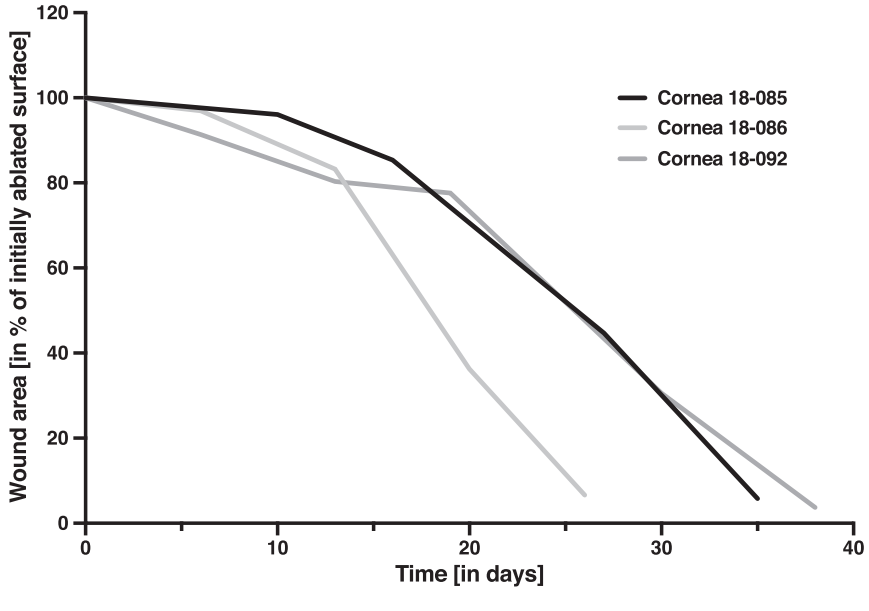

Fig. 5. Re-endothelialization of ablated surface. The graph displays the remaining wound area in percent of the initially ablated surface of human corneas *ex vivo* over time.

cornea, a re-established Descemet membrane, which is lined by flat endothelial cells, was observed (Fig. 6A). No specific signal for Na^+/K^+-ATPases was detected in the corneal stroma and epithelium, whereas adjacent to the inner side of the Descemet membrane specific staining for Na^+/K^+-ATPases was observed (Fig. 6B). Further on, no obvious damage of the corneal stroma following laser treatment was detected. In line, by electron scanning microscopy a dense monolayer of flat cells with a central dome-shaped eversion was seen (Fig. 6C). Quantification of cell size demonstrates a significant 2.8-fold increase of CEC surface in the wounded area ($1122.1 \pm 294.6\ \mu m^2$) when compared to unaffected resident cells ($398.3 \pm 49.6\ \mu m^2$; $p < 0.001$), suggesting that immigrated CEC expand to cover the wounded area. Overall, our data strongly suggest that the inner corneal surface is recolonized by endothelial cells.

Discussion

The challenge in DWEK surgery is encouraging cell migration across a bare stromal defect. ROCK inhibition has been established as a probable aid

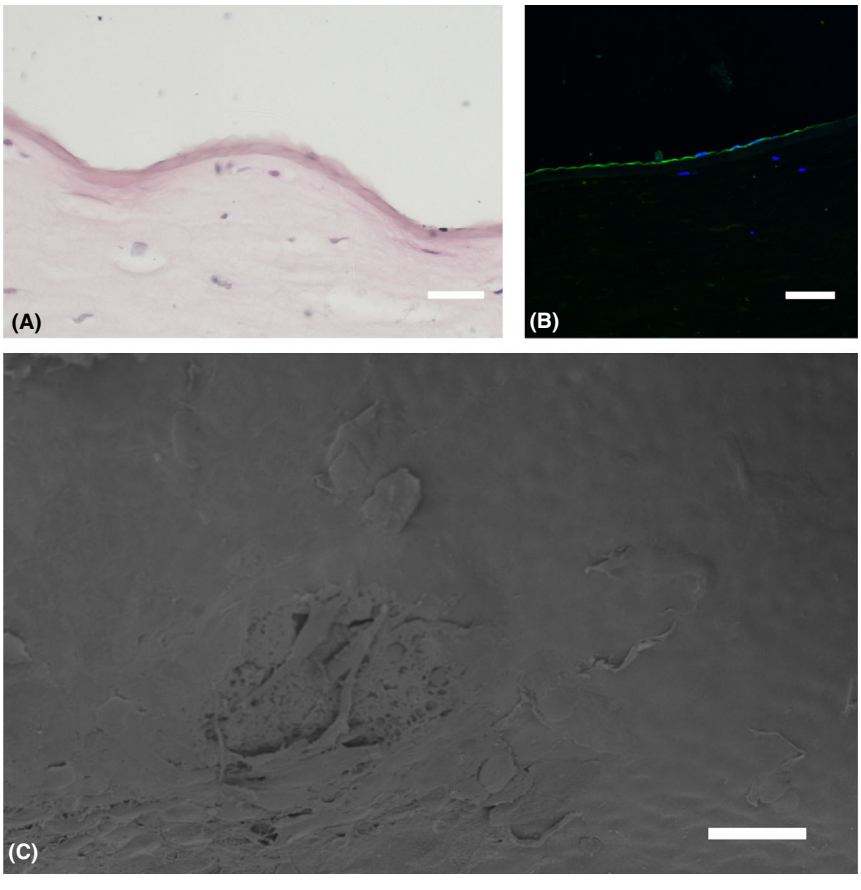

Fig. 6. Immunohistochemistry and electron scanning microscopy after excimer laser ablation and re-endothelialization. (A) Histological image of re-endothelialized previously ablated corneal endothelium (haematoxylin and eosin staining). Scale bar: 50 µm. (B) Immunofluorescence. Staining of endothelial Na^+/K^+-ATPase (green) and cell nuclei (blue). Scale bar: 50 µm. (C) Electron scanning microscopy showing dome-shaped migrated corneal endothelial cells on the wound area. Scale bar: 30 µm.

in this process. Both Okumura and Mehta have also suggested that an intact basement membrane favours cell migration (Okumura et al. 2018; Soh & Mehta 2018). Mehta has even proposed transfer of an acellular Descemet graft to cover the bare stromal defect created in DWEK surgery (Soh & Mehta 2018). Therefore, we chose to analyse whether it is possible to remove guttae while keeping the native basement membrane intact.

Our findings showed that in FECD Descemet membranes *ex vivo*, corneal endothelium and guttae could be ablated by a 308 nm excimer laser clinically used in minimally invasive glaucoma surgery. Furthermore, corneal endothelial cells were able to migrate centripetally after excimer laser ablation from the periphery to the centre of normal human corneas within less than 4 weeks *ex vivo*. By histology and immunostaining against Na^+/K^+-ATPases, we could demonstrate that corneal endothelial cells migrate on the treated area to facilitate wound closure. For the corneal endothelium, proliferation of a minor amount of CEC close to the limbal area as well as a continuous but slow centripetal migration of CECs has been proposed (He et al. 2012). In line, the significant increase of the cell area of immigrated CECs suggests cell expansion and thus the conclusion that migration seems to play a crucial role in regeneration of corneal endothelium following central ablation.

Corneal grafting and specifically DMEK remains the gold standard therapy in FECD (Dapena et al. 2009). The aim of this therapeutic approach is to remove the damaged corneal endothelium and replace it with normal donor corneal endothelial cells. Migration and regeneration of corneal endothelial cells subsequently lead to an increase of visual acuity (Dirisamer et al. 2011). Excimer laser-aided ablation of corneal endothelium in human corneas *ex vivo* resulted in a recolonization with corneal endothelial cells. This is in keeping with recent literature showing a similar effect of manual descemetorhexis without endothelial keratoplasty followed by regeneration of the corneal endothelium in the treatment of Fuchs' endothelial corneal dystrophy. Still there is a disadvantage of the so-called DWEK procedure: the time until corneal clearance and thus visual improvement is significantly longer than in DMEK (up to 10 weeks postoperatively without ROCK inhibition; Huang et al. 2018). In comparison, the conducted experiments showed a corneal endothelial recolonization within 5–6 weeks in cultured human corneas. This may be due to preservation of the anterior layer of the Descemet membrane serving as a fundament for corneal endothelial cells to migrate, as can be postulated by looking at the electron microscopy. The research group around Soh et al. (2016) showed in several models *in vitro* that endothelial cells migrate significantly more efficient on a basement membrane than on bare corneal stroma. However, biomechanical properties of Descemet's membrane especially in FECD are not yet completely understood (Ali et al. 2016). Furthermore, corneal endothelial cell migration started from the preformed corridors suggesting a better migration from the periphery that cannot be established by manual descemetorhexis.

Light and electron scanning microscopy suggested a viable ablation of guttae and corneal endothelium *ex vivo*. To our knowledge, this is a novel technique and application of excimer lasers used in ophthalmology. Currently, the removal of the Descemet membrane in posterior lamellar keratoplasty is accomplished through manual descemetorhexis (Moloney et al. 2017) including stripping off all layers of the Descemet membrane. As excimer laser ablation of the guttae-like material is done layer by layer, the anterior layer of the Descemet membrane remains and acts as a smooth scaffold for migrating endothelial cells. Furthermore, excimer lasers are already in use in *ab interno* trabeculotomy (Wilmsmeyer et al. 2006) and for smoothening the stromal part of donor grafts prior to posterior lamellar

keratoplasty (Cleary et al. 2012; Trinh et al. 2013). A recent study showed no significant difference of endothelial cell density or corneal thickness before and after femtosecond laser-assisted or excimer laser-aided suture removal after penetrating keratoplasty (Toth et al. 2019) underlining the safety of this technique.

To further examine the ultrastructure of the Descemet membrane and guttae, electron microscopy of the ablated structures was conducted. Cut guttae as well as the laser-affected corneal endothelium showed a fibrillary spongy structure with a subjacent smooth layer. This might fit into the findings described by Xia et al. (2016) suggesting that the Descemet membrane in FECD patients is set up by three layers: the posterior banded layer (PBL), the posterior non-banded layer (PNBL) and the anterior banded layer (ABL). However, we could not specifically distinguish the PNBL and ABL in our analyses as we used scanning electron microscopy, which only illustrates the surface of the inner cornea. Another reason could be the fact that in DMEK procedure, sometimes a splitting in Descemet membrane layers occurs, leading to an adhesion of the ABL on the recipient's corneal stroma and thus the extracted Descemet membrane missed that specific layer (Weller et al. 2016).

Even though the results of our *ex vivo* study are promising, the following issues have to be addressed in prospective studies. (1) *Ab interno* ablation of guttae, corneal endothelium and posterior Descemet membrane: in our setting, *ex vivo* sclerocorneal rings were mounted with the epithelium facing down to approach the corneal endothelium directly with a straight laser tip. *In vivo,* we propose difficulties in ensuring an even ablation of the endothelium when entering the eye via a limbal corneal incision. A possible solution would be a distance-fixed, curved or bent laser tip. This is on one hand important to maintain a flat scaffold for endothelial cells to recolonize the ablated area. On the other hand, an irregular posterior corneal surface can lead to higher order aberrations and thus decreased visual acuity (Yazu et al. 2018). In addition, a control of ablation could be performed by instilling trypan blue intracamerally to stain the ablated area, which seems challenging in severe FECD patients with opaque corneas. (2) Excimer laser specifications: typical excimer lasers in ablative corneal surgery emit ultraviolet light of a wavelength of 193 nm to guarantee precise cuts and a minimum of thermal distortion (Trokel et al. 1983), in comparison with the 308 nm *ab interno* excimer laser used in our experiments. Therefore, further investigations on the tissue response especially of the corneal guttae and Descemet membrane to different light wavelengths must be performed to optimize the ablation effectivity and minimalize potential collateral damage. (3) Corneal endothelial recolonization: our results on corneal recolonization after excimer laser ablation were acquired, using healthy human corneas and under treatment with ripasudil. However, in FECD patients, the endothelial cell density is decreased and thus the recolonization potential may differ. Therefore, experiments on FECD corneas and different sizes of the ablated area are required to investigate the regenerative potential of corneal endothelium following excimer laser treatment of diseased corneas.

In summary, we could demonstrate a promising experimental approach to excise guttae and parts of the Descemet membrane by excimer laser ablation, which in turn enables corneal endothelial cells to migrate easily onto the wounded area. However, several methodological problems have to be solved in advance and *in vivo* studies need to be performed. Advantages of this novel technique for potential treatment of FECD might be the avoidance of a corneal graft in combination with the creation of a wound that does not present bare stroma, cannot initiate an unfavourable stromal healing response and may encourage faster cell migration over an intact basement membrane.

References

Abbott RL, Fine BS, Webster RG Jr, Paglen PG & Spencer WH (1981): Specular microscopic and histologic observations in nonguttate corneal endothelial degeneration. Ophthalmology **88**: 788–800.

Adamis AP, Filatov V, Tripathi BJ & Tripathi RC (1993): Fuchs' endothelial dystrophy of the cornea. Surv Ophthalmol **38**: 149–168.

Ali M, Raghunathan V, Li JY, Murphy CJ & Thomasy SM (2016): Biomechanical relationships between the corneal endothelium and Descemet's membrane. Exp Eye Res **152**: 57–70.

Arba Mosquera S & Verma S (2015): Analysis of the change in peak corneal temperature during excimer laser ablation in porcine eyes. J Biomed Opt **20**: 78001.

Bleyen I, Saelens IE, van Dooren BT & van Rij G (2013): Spontaneous corneal clearing after Descemet's stripping. Ophthalmology **120**: 215.

Borboli S & Colby K (2002): Mechanisms of disease: Fuchs' endothelial dystrophy. Ophthalmol Clin North Am **15**: 17–25.

Cleary C, Liu Y, Tang M, Li Y, Stoeger C & Huang D (2012): Excimer laser smoothing of endothelial keratoplasty grafts. Cornea **31**: 431–436.

Dapena I, Ham L & Melles GR (2009): Endothelial keratoplasty: DSEK/DSAEK or DMEK–the thinner the better? Curr Opin Ophthalmol **20**: 299–307.

Dapena I, Yeh RY, Baydoun L, Cabrerizo J, van Dijk K, Ham L & Melles GR (2013): Potential causes of incomplete visual rehabilitation at 6 months postoperative after descemet membrane endothelial keratoplasty. Am J Ophthalmol **156**: 780–788.

Dirisamer M, Dapena I, Ham L, van Dijk K, Oganes O, Frank LE, van der Wees J & Melles GR (2011): Patterns of corneal endothelialization and corneal clearance after descemet membrane endothelial keratoplasty for fuchs endothelial dystrophy. Am J Ophthalmol **152**: 543–555.e1.

Elhalis H, Azizi B & Jurkunas UV (2010): Fuchs endothelial corneal dystrophy. Ocul Surf **8**: 173–184.

Gain P, Jullienne R, He Z, Aldossary M, Acquart S, Cognasse F & Thuret G (2016): Global survey of corneal transplantation and eye banking. JAMA Ophthalmol **134**: 167–173.

Garcerant D, Hirnschall N, Toalster N, Zhu M, Wen L & Moloney G (2019): Descemet's stripping without endothelial keratoplasty. Curr Opin Ophthalmol **30**: 275–285.

He Z, Campolmi N, Gain P et al. (2012): Revisited microanatomy of the corneal endothelial periphery: new evidence for continuous centripetal migration of endothelial cells in humans. Stem Cells **30**: 2523–2534.

Huang MJ, Kane S & Dhaliwal DK (2018): Descemetorhexis without endothelial keratoplasty versus DMEK for treatment of Fuchs endothelial corneal dystrophy. Cornea **37**: 1479–1483.

Iwamoto T & DeVoe AG (1971): Electron microscopic studies on Fuchs'combined dystrophy. I. Posterior portion of the cornea. Invest Ophthalmol **10**: 9–28.

McCartney MD, Robertson DP, Wood TO & McLaughlin BJ (1987): ATPase pump site density in human dysfunctional corneal endothelium. Invest Ophthalmol Vis Sci **28**: 1955–1962.

McCartney MD, Wood TO & McLaughlin BJ (1989): Moderate Fuchs' endothelial

dystrophy ATPase pump site density. Invest Ophthalmol Vis Sci **30**: 1560–1564.
Melles GR, Ong TS, Ververs B & van der Wees J (2006): Descemet membrane endothelial keratoplasty (DMEK). Cornea **25**: 987–990.
Moloney G, Chan UT, Hamilton A, Zahidin AM, Grigg JR & Devasahayam RN (2015): Descemetorhexis for Fuchs' dystrophy. Can J Ophthalmol **50**: 68–72.
Moloney G, Petsoglou C, Ball M et al. (2017): Descemetorhexis without grafting for Fuchs endothelial dystrophy-supplementation with topical ripasudil. Cornea **36**: 642–648.
Okumura N, Inoue R, Okazaki Y, Nakano S, Nakagawa H, Kinoshita S & Koizumi N (2015): Effect of the Rho kinase inhibitor Y-27632 on corneal endothelial wound healing. Invest Ophthalmol Vis Sci **56**: 6067–6074.
Okumura N, Matsumoto D, Fukui Y et al. (2018): Feasibility of cell-based therapy combined with descemetorhexis for treating Fuchs endothelial corneal dystrophy in rabbit model. PLoS ONE **13**: e0191306.
Price MO, Giebel AW, Fairchild KM & Price FW Jr (2009): Descemet's membrane endothelial keratoplasty: prospective multicenter study of visual and refractive outcomes and endothelial survival. Ophthalmology **116**: 2361–2368.
Shah RD, Randleman JB & Grossniklaus HE (2012): Spontaneous corneal clearing after Descemet's stripping without endothelial replacement. Ophthalmology **119**: 256–260.
Soh YQ & Mehta JS (2018): Regenerative therapy for Fuchs endothelial corneal dystrophy. Cornea **37**: 523–527.
Soh YQ, Peh G, George BL, Seah XY, Primalani NK, Adnan K & Mehta JS (2016): Predicative factors for corneal endothelial cell migration. Invest Ophthalmol Vis Sci **57**: 338–348.
Toth G, Butskhrikidze T, Seitz B, Langenbucher A, Hager T, Akhmedova E, El-Husseiny M & Szentmary N (2019): Endothelial cell density and corneal graft thickness following excimer laser vs. femtosecond laser-assisted penetrating keratoplasty-a prospective randomized study. Graefes Arch Clin Exp Ophthalmol **257**: 975–981.
Trinh L, Saubamea B, Auclin F et al. (2013): A new technique of endothelial graft: the femtosecond and excimer lasers-assisted endothelial keratoplasty (FELEK). Acta Ophthalmol **91**: e497–e499.
Trokel SL, Srinivasan R & Braren B (1983): Excimer laser surgery of the cornea. Am J Ophthalmol **96**: 710–715.
Verma S, Hesser J & Arba-Mosquera S (2017): Optimum laser beam characteristics for achieving smoother ablations in laser vision correction. Invest Ophthalmol Vis Sci **58**: 2021–2037.
Wacker K, Baratz KH, Maguire LJ, McLaren JW & Patel SV (2016): Descemet stripping endothelial keratoplasty for Fuchs' endothelial corneal dystrophy: five-year results of a prospective study. Ophthalmology **123**: 154–160.
Weller JM, Schlotzer-Schrehardt U, Kruse FE & Tourtas T (2016): Splitting of the recipient's descemet membrane in descemet membrane endothelial keratoplasty-ultrastructure and clinical relevance. Am J Ophthalmol **172**: 1–6.
Wilmsmeyer S, Philippin H & Funk J (2006): Excimer laser trabeculotomy: a new, minimally invasive procedure for patients with glaucoma. Graefes Arch Clin Exp Ophthalmol **244**: 670–676.
Xia D, Zhang S, Nielsen E et al. (2016): The ultrastructures and mechanical properties of the descement's membrane in fuchs endothelial corneal dystrophy. Sci Rep **6**: 23096.
Yazu H, Yamaguchi T, Dogru M, Satake Y, Tsubota K & Shimazaki J (2018): Decreased visual acuity by an irregular corneal posterior surface after repeat descemet stripping automated endothelial keratoplasty. Eye Contact Lens **44**(Suppl 1): S249–S254.

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