Ultrastructural Examination of the Corneal Interface after Predescemetic Deep Anterior Lamellar Keratoplasty (DALK) – A Case Report with Light and Transmission Electron Microscopy

Ultrastrukturelle Untersuchung des Interface-Bereichs nach prädescemetaler tiefer anteriorer lamellärer Keratoplastik (DALK) – ein Fallbericht mit Licht- und Transmissionselektronenmikroskopie

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Key words

deep anterior lamellar keratoplasty, penetrating keratoplasty, light microscopy, transmission electron microscopy, interface, DALK

Schlüsselwörter

tiefe anteriore lamelläre Keratoplastik, perforierende Keratoplastik, Lichtmikroskopie, Transmissionselektronenmikroskopie, Interface, DALK

received	5.11.2021
accepted	18.1.2022
published online	23.3.2022

Bibliography

Klin Monatsbl Augenheilkd 2023; 240: 1010–1016
DOI 10.1055/a-1745-9140
ISSN 0023-2165

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

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ABSTRACT

Purpose To examine corneal buttons with light and transmission electron microscopy (TEM) to visualize the interface area and highlight the ultrastructural corneal changes after deep anterior lamellar keratoplasty (DALK).

Methods Two patients underwent excimer laser-assisted penetrating repeat keratoplasty after predescemetic DALK. The corneal buttons were examined by light microscopy and TEM.

Results The light microscopic examination of the corneal buttons revealed fragments of a second Descemet's membrane in the central and midperipheral areas (Case 1). In both cases, visualization of the interface area was not possible by light microscopy. The donor and host stroma were tightly attached without dehiscence. TEM identified the interface area by irregularities in the collagen distribution between the donor and host stroma. The thickness of the remaining recipient corneal stroma measured approximately 30 µm (Case 1) and 100 µm (Case 2), respectively. In the host stroma, TEM revealed the absence or degeneration of keratocytes, accumulation of amorphous material between the collagen lamellae, and vacuolar inclusions dispersed in the stroma, forming a band-like zone anterior to Descemet's membrane.

Conclusion The interface area after DALK has been mainly investigated by *in vivo* confocal microscopy. Light microscopy and TEM findings indicate remodeling processes after DALK that are associated with increased keratocyte degeneration and structural alterations of the extracellular matrix in the host stroma. The choice of surgical method may influence the postoperative morphological and functional outcome since these findings were primarily apparent in the remaining host stroma. Therefore, complete exposure of Descemet's membrane is an important prognostic factor for the postoperative visual outcome.

ZUSAMMENFASSUNG

Hintergrund Untersuchung von Hornhautexzisaten mit Licht- und Transmissionselektronenmikroskopie (TEM) zur Visualisierung des Interface-Bereichs und Hervorhebung der ultrastrukturellen Hornhautveränderungen nach tiefer anteriorer lamellärer Keratoplastik (DALK).

Methoden Bei 2 Patienten wurde eine Excimerlaser-gestützte perforierende Keratoplastik nach einer prädescemetalen DALK durchgeführt. Die Hornhautexzisate wurden mittels Lichtmikroskopie und TEM untersucht.

Ergebnisse Die lichtmikroskopische Untersuchung der Hornhautexzisate zeigte Fragmente einer 2. Descemet-Membran im zentralen und mittleren peripheren Bereich (Fall 1). In beiden Fällen war die Visualisierung des Interface-Bereichs lichtmikroskopisch nicht möglich. Das Spender- und Wirtsstroma waren ohne Dehiszenz miteinander verbunden. In der TEM wurde der Interface-Bereich durch Unregelmäßigkeiten in der Kollagenverteilung zwischen Spender- und Wirtsstroma identifiziert. Die Dicke des verbliebenen Empfängerhornhautstromas betrug ca. 30 µm (Fall 1) bzw. 100 µm (Fall 2). Im Wirtsstroma zeigte die TEM fehlende oder degenerierte Keratozyten, Ansammlungen von amorphem Material zwischen den Kollagenlamellen und vakuoläre Einschlüsse, die im Stroma verstreut waren und eine bandartige Zone vor der Descemet-Membran bildeten.

Schlussfolgerung Bislang wurde der Interface-Bereich nach DALK hauptsächlich konfokalmikroskopisch untersucht. Die licht- und elektronenmikroskopischen Befunde deuten auf Umbauprozesse nach DALK hin, die mit einer verstärkten Keratozytendegeneration und strukturellen Veränderungen der extrazellulären Matrix im Wirtsstroma einhergehen. Die Wahl der Operationsmethode kann das postoperative morphologische und funktionelle Ergebnis beeinflussen, da diese Befunde vor allem im verbliebenen Wirtsstroma zu beobachten waren. Daher ist eine vollständige Freilegung der Descemet-Membran ein wichtiger prognostischer Faktor für das postoperative Visusergebnis.

Introduction

In recent decades, deep anterior lamellar keratoplasty (DALK) has become increasingly popular for the treatment of corneal pathologies with a healthy endothelial cell layer. One of the major advantages over penetrating keratoplasty (PKP) is the avoidance of an endothelial immune response, which is particularly beneficial for young patients with advanced keratoconus [1,2]. The surgical procedure of DALK includes a variety of different approaches that affect the postoperative visual outcome [3,4]. Most notably, removal of the recipient's corneal stroma down to Descemet's membrane (DM), so-called descemetic DALK (dDALK), has been shown to provide excellent optical quality of the interface, resulting in outcomes comparable to PKP [5].

Nevertheless, little is known about the cellular responses and remodeling processes of the interface area after DALK, which might affect the long-term morphological and functional outcomes. Especially in case of remaining recipient corneal stroma (predescemetic DALK, pDALK), the interaction between both stromal sides may be accompanied with a higher incidence of interface haziness [6].

In this case report, we present two patients who underwent PKP after pDALK. The corneal buttons were further examined with light and transmission electron microscopy (TEM) to visualize the interface area and highlight the ultrastructural corneal changes after DALK.

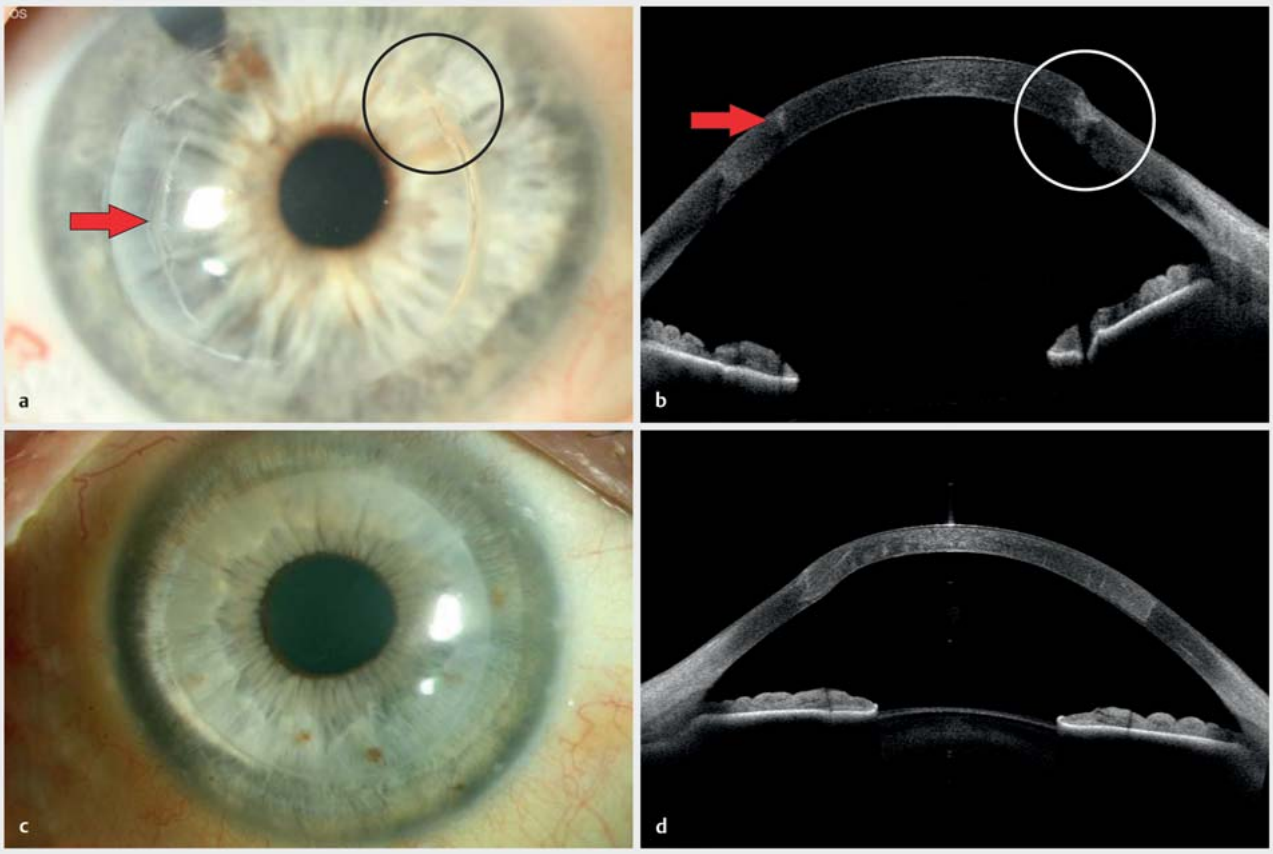
Case Report: Patient History and Clinical Presentation

Case 1

A 58-year-old male patient initially presented to our department due to distinct visual deterioration in the right eye. He underwent DALK previously because of corneal stromal opacification of unknown origin. According to the patient's history, visual acuity was satisfying for years after surgery. One year after, cataract surgery was carried out without any complications. Due to high regular astigmatism, limbal relaxing incisions were performed 4 years after, resulting in rapid visual deterioration with monocular diplopia. At the initial examination at our department, the right eye showed significant interface haziness and a peripheral corneal steepening at 2 o'clock (▶ **Fig. 1**). The fellow eye demonstrated a central crocodile shagreen corneal degeneration. At the time of first presentation, uncorrected near visual acuity in the affected right eye was hand motion. The anterior corneal astigmatism was highly irregular and measured 11.2 diopters. Contact lens fitting to improve visual acuity was not possible due to contact lens intolerance. Therefore, we performed an excimer laser-assisted penetrating repeat keratoplasty (8.5/8.6 mm) because of high irregular astigmatism.

Case 2

A 50-year-old male patient presented to our department due to high irregular astigmatism in the left eye 2 years after manual DALK. The surgical procedure was performed due to deep corneal scarring after foreign body trauma. The corneal graft was clear without interface haziness and did not show any signs of graft rejection (▶ **Fig. 2**). Visual acuity was 6/120 in the affected eye. Tomography demonstrated an anterior corneal astigmatism of 8.9 diopters. There was no tolerance to contact lenses. In case of high irregular astigmatism, we performed an excimer laser-assisted penetrating repeat keratoplasty (8.5/8.6 mm).



▶ **Fig. 1** Anterior segment photograph and anterior segment optical coherence tomography (AS-OCT) of cases 1 (**a, b**) and 2 (**c, d**) after previous DALK. **a** Preoperative examination with interface haziness, limbal relaxing incision (red arrow), and a corneal steepening at 2 o'clock (circle). **b** AS-OCT demonstrates a corneal steepening at 2 o'clock (circle). The red arrow marks the scarred area of the limbal relaxing incision. Moderate haze formation is observed at the interface area. **c** Preoperative findings show a clear corneal graft after previous DALK. **d** AS-OCT displays the margins of the corneal graft. Significant corneal steepening is not present.

Light Microscopy

Case 1

Light microscopy (periodic acid-Schiff stain, PAS) of the central corneal button (▶ **Fig. 2 a, b**) revealed several fragments of a second PAS-positive DM of the donor tissue in the peripheral and midperipheral regions of the cornea. The remaining recipient stroma measured about 25 µm in width (distance between both DMs) and showed partial structural alterations with irregularities in collagen lamellae distribution. No keratocytes could be seen in the interface area. Incidental findings included disruption of Bowman's layer with beginning epithelial invasion into the corneal stroma. The corneal endothelium appeared regular.

Case 2

Light microscopic examination (PAS stain) of the corneal tissue (▶ **Fig. 2 c**) demonstrated a mainly intact epithelium that was centrally discontinuous at one location with disruption of Bowman's layer. The anterior corneal stroma revealed an oblique tissue separation surrounded by macrophages and fibroblasts. The remaining corneal stroma was inconspicuous. No clear differentiation of donor and recipient stroma was possible. The DM was intact, and the endothelial cell layer appeared normal.

Transmission Electron Microscopy

Case 1

▶ **Fig. 3 a, b**: The interface area was identified by irregularities in the distribution of the collagen lamellae, although donor and host corneal stromas were tightly attached without any dehiscence. The residual recipient stroma measured approximately 30 µm in width and was characterized by irregular arrangement of collagen lamellae, which were interspersed with amorphous material and vacuolar inclusions, which also formed a band-like zone in the pre-Descemet area. Vital keratocytes were not observed in the recipient stroma, and keratocyte density was also decreased in the posterior donor stroma compared with the anterior regions (not shown). The recipient lamella further showed a regular DM and degenerative appearing endothelial cells. A second DM could not be observed by TEM.

▶ **Fig. 2** Cross-sectional histologic specimen of the corneal explant after previous DALK. **a** Low power view of the corneal explant (Case 1) shows a second Descemet's membrane (black arrow) that is not continuously apparent. More peripherally, it can only be identified dimly (arrowhead). The interface area does not reveal any vital keratocytes. Incidental findings include a defect of Bowman's layer with the start of epithelial invasion (star) (periodic acid-Schiff stain, original magnification 50×). **b** Higher power view of the corneal explant (Case 1) to demonstrate the second Descemet's membrane, which is interrupted abruptly (black arrow). In addition, a disturbed collagen lamellae distribution is seen peripherally (white arrow). The distance between both Descemet's membranes measured 25 µm (double-headed arrow) (periodic acid-Schiff stain, original magnification 100×). **c** Higher power view of the corneal explant (Case 2) reveals an oblique tissue separation of the corneal stroma surrounded by macrophages and fibroblasts. Disruption of Bowman's layer is also visible (circle). No differentiation of donor and recipient stroma is possible (periodic acid-Schiff stain, original magnification 100×).

Case 2

▶ **Fig. 3 c, d**: The interface area was less distinct than in Case 1, but could still be visualized by focal irregularities of the collagen lamellae. Few degenerated keratocytes were seen in the recipient corneal stroma, which measured approximately 100 µm in width. A weak predescemetic vacuolar band-like alteration was also observed. The recipient lamella further showed a regular DM and degenerated endothelial cells. Keratocyte density was again reduced in the posterior donor stroma compared with the anterior stromal regions (not shown).

Discussion

The cellular responses and remodeling processes of the corneal interface after DALK are still largely unknown and are expected to be important for the morphologic and functional outcomes.

Currently, *in vivo* confocal microscopy is the most common method for examining the corneal interface after DALK. Generally, for quiescent keratocytes, only round or oval-shaped nuclei, but not their cytoplasm, can be visualized. In contrast, activated keratocytes have a higher corneal light backscattering than quiescent keratocytes and their cellular processes are often apparent [7]. After corneal injury, activated keratocytes function as repair cells and repopulate the acellular area by migration and proliferation. Through excessive matrix production, they are able to remodel the extracellular matrix [7].

In vivo confocal microscopy studies have demonstrated an increased keratocyte reflectivity immediately after DALK [4, 6, 8–10]. In addition, differences in the morphology and reflectivity of keratocytes were observed, depending on the depth of the recipient bed preparation. Keratocyte reflectivity was increased in pDALK and dDALK, but less pronounced in the latter. However, reflectivity decreased and keratocyte morphology normalized in the interface area after a few months postoperatively [4, 6, 9].

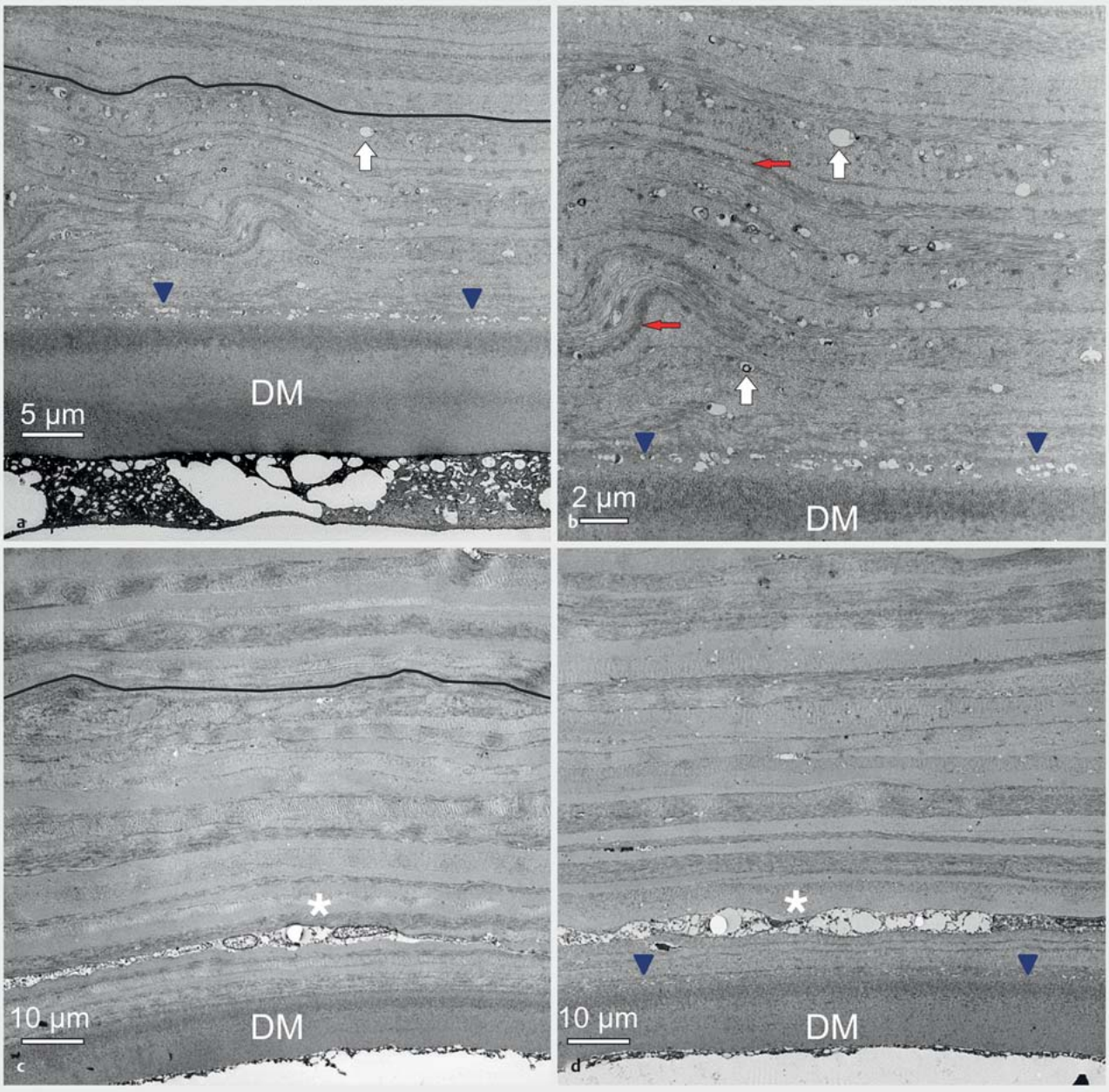
Complete exposure of DM with removal of the predescemetic corneal stroma is an important prognostic factor in terms of postoperative visual outcome. If the residual thickness of the recipient bed is less than 80 µm and is homogenous in its thickness, good visual outcomes may be achieved [5]. Compared to dDALK, postoperative visual recovery in pDALK takes longer, and is reported to have 2–5 years of follow-up [11].

There are various methods for separating the stroma from the DM of the host cornea, such as viscodissection or pneumodissection, using a "big bubble" [12, 13].

Schlötzer-Schrehardt et al. [14] showed that the distance of stromal keratocytes to DM increases from the central (3.5 to 14 µm) to the peripheral (4.5 to 18 µm) cornea.

Air injection into the corneal stroma of organ-cultured corneas (after DM stripping) resulted in a formation of a big bubble with a bubble wall composed of a stromal sheet varying in thickness from 4.5 to 27.5 µm with keratocytes on the anterior surface. It was demonstrated that the separated stromal layer was thinnest in the central part of the cornea and became thicker in the periphery, suggesting that dissection does not occur along a uniformly thick stromal layer. The authors assume that the variable thickness of the remaining stromal layer may be related to the increasing distance of the keratocytes to DM from center to periphery. This indicates that the intrastromal cleavage after pneumodissection occurs along the posterior keratocytes due to a lower biomechanical resistance [14]. Therefore, pneumodissection should not be considered as a DM-baring technique.

Generally, DALK should only be completed when the DM is fully exposed and not perforated. In case of scarring and uncertain depth during preparation, conversion to PKP should always be chosen. Especially, excimer laser-assisted DALK allows an unproblematic conversion to PKP without disadvantages for the patient, provided the graft is also suitable for PKP [1, 2, 15]. In Case 2, conversion to PKP would have been the method of choice due to the deep stromal scarring, but also due to the possibly limited visibil-



▶ **Fig. 3** Transmission electron microscopy of the corneal explant after previous DALK (Case 1 – **a, b** and Case 2 – **c, d**). **a** The recipient side demonstrates degenerated endothelial cells and a normal Descemet's membrane. The interface area is characterized by irregularities of the collagen lamellae (black line). Vacuolated inclusions are evident in the remaining recipient stroma (white arrow). A predescemetic vacuolated band is also observed (blue arrowhead). **b** The magnified section shows vacuolated inclusions in the recipient cornea (white arrow), amorphous material (red arrow), and a vacuolated predescemetic band (blue arrowhead). **c** The interface area can be identified by irregularities of the collagen lamellae (black line). The recipient side shows a normal Descemet's membrane. In the predescemetic area, the recipient stroma demonstrates degenerated keratocytes (white star). **d** In another sectional area, degenerated keratocytes (white star) are also shown in the recipient stroma. A predescemetic vacuolated band is present (blue arrowhead).

ity of the DM intraoperatively that resulted in a remaining host stromal thickness of 100 µm. The reduced number and degeneration of keratocytes and extracellular changes observed in TEM (▶ **Fig. 3 c, d**) may result in a reduced long-term postoperative outcome. Therefore, the remaining host stroma should be reduced to a minimum in order to obtain PKP-like visual results.

In addition to the preparation of the recipient bed with exposure of the DM, the method of donor preparation is also relevant. Using a full-thickness corneal graft (Descemet-on) was associated with significantly higher haze formation in the interface area [10]. The histological section (▶ **Fig. 2 a, b**) of the corneal button demonstrated a second DM, which was not continuously present and

was only weakly pronounced peripherally. One explanation would be a failed preparation of the donor tissue with a tear during the attempt to remove the DM and leaving it partially attached to the donor stroma.

On the other hand, increased keratocyte activation after DALK, which was confirmed by confocal studies, could come along with remodeling and degradation of DM and could be caused by increased expression of matrix metalloproteinases [16]. Although enzymatic digestion is only hypothetic, it may provide another potential explanation for the faded DM.

Localization of the interface area was possible by *in vivo* confocal microscopy due to hyporeflective striae in the posterior stroma, representing microfolds, hyperreflective amorphous material, and high contrast microdots [8, 10].

In our cases, TEM (▶ **Fig. 3**) was clearly able to differentiate the recipient and donor stromas and thus identify the interface area based on irregularities in the distribution of the collagen lamellae. However, no clear interface area could be detected by light microscopy, which was also reported by Favuzza et al. in a recent case series [17]. Differentiation between recipient and donor stromas based on light microscopy alone would not have been possible without indications like the presence of the second DM. It is interesting that the group of Favuzza et al. could not identify any interface area by TEM. In contrast to our results, the keratocytes had a normal morphology and the posterior stroma was arranged with regularly organized collagen lamellae without evidence of stromal irregularities [17]. Considering the fact that the group was able to identify the exact location of the interface area by AS-OCT 3 weeks postoperatively, the absence of any evidence in the TEM even 2 years after DALK is surprising.

Abdelkader et al. investigated the corneal wound healing after DALK in rabbits. It was demonstrated that the keratocytes disappeared in the area adjacent to the wound edge and began to repopulate 7 days postoperatively. Furthermore, highly reflective interface particles were detected, which could represent foreign cell debris or inflammatory cells [8].

In our cases, TEM revealed structural alterations in the interface region and recipient stroma, particularly irregularities in the collagen fiber arrangement, inclusion of amorphous and vacuolar material, and formation of a vacuolar band-like zone anterior to DM. Vacuolar inclusions and amorphous material interspersed between collagen fibers were also described in confocal studies [8, 10]. We hypothesize that these changes may indicate remnants of degenerated keratocytes due to increased apoptosis after DALK, since increased keratocyte density is found directly predescemetally in healthy corneas [18]. TEM showed absence or degenerated keratocytes in the interface area and in the recipient stroma as well as in the adjacent donor stroma. A confocal microscopy study by Feizi et al. revealed more distinct cellular changes in the donor tissue after DALK compared to PKP. Keratocyte density after PKP was comparable to those of a normal cornea in the anterior, mid, and posterior stromas, whereas in DALK, keratocyte density was significantly reduced in all three stromal layers. Also, the decrease in keratocyte density from anterior to posterior stroma was not seen in the DALK group [19].

The disturbed alignment of the collagen lamellae in TEM confirms the assumption of extracellular matrix remodeling processes after DALK.

In summary, up to now, the examination of the corneal interface has been performed mostly by confocal microscopy studies, while results from light and electron microscopy studies have been described only sporadically [17]. Differentiation of the interface area could only be achieved with TEM and the findings seem to concur with results of previous confocal microscopy studies. For a successful long-term postoperative result, the surgical method is of major importance, and it must be assumed that a stroma-stroma interaction is accompanied with higher interface remodeling and haziness. Therefore, complete exposure of DM is an important prognostic factor regarding the postoperative visual outcome.

Conflict of Interest

The authors declare that they have no conflict of interest.

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