Histologic and Ultrastructural Findings in a Case of Traumatic Graft Failure in Deep Lamellar Endothelial Keratoplasty

A Clinicopathologic Case Report

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Purpose: Few complications have been reported for deep lamellar endothelial keratoplasty (DLEK). Endothelial graft failure has rarely been disclosed as a complication. Although the histopathologic and ultrastructural analysis of a failed DLEK graft has been previously described, we are not aware of any reports of these features in a case of traumatic endothelial graft failure.

Methods: We report a case of an 85-year-old man with Fuchs endothelial dystrophy who underwent DLEK for corneal decompensation after cataract extraction and intraocular lens implantation. The graft had dislocated by the fourth postoperative day and was repositioned the following day. Penetrating keratoplasty was performed 3 months later for gradually progressive intractable corneal edema. The excised cornea underwent histochemical, immunohistochemical, and ultrastructural analysis.

Results: Marked endothelial loss resulting in corneal decompensation was diagnosed histopathologically. The graft–host interface line showed no substantial findings for the following histochemical and immunohistochemical stains: colloidal iron, alcian blue (pH 2.5), vimentin, epithelial membrane antigen (EMA), smooth muscle actin (SMA), anti-cytokeratin CAM 5.2, high-molecular-weight keratin, anti-cytokeratin AE1/AE3, and collagen 3. The cornea showed ultrastructural changes similar to, but more pronounced than, those observed in corneas after laser in situ keratomileusis.

Conclusions: This is the second described case of endothelial graft failure after DLEK. Histochemical and ultrastructural analysis revealed that the DLEK-operated cornea contained irregularities that may interfere with optical performance.

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Lamellar keratoplasty is a concept that has long been implemented in the management of disorders of the anterior cornea as an alternative to penetrating keratoplasty (PK). More recently, it has been used in the management of diseases involving the posterior layers of the cornea such as bullous keratopathy and Fuchs endothelial dystrophy (FED).[1] Posterior lamellar keratoplasty (PLK) is a term that was first used to refer to a technique described and used by Tillett[2] in 1956 in the treatment of an advanced case of FED. Melles et al[3] have developed a completely sutureless technique to replace the posterior layers of the cornea, and several advantages of this technique over PK have been proposed. The procedure is now more commonly identified as deep lamellar endothelial keratoplasty (DLEK).[4] We report a case that shows the histopathologic and ultrastructural features of a human cornea that failed 3 months after DLEK.

CASE REPORT

An 85-year-old man with a history of 2 failed PKs in his right eye and a resultant visual acuity (VA) of hand motions presented with FED and a 3+ nuclear sclerotic cataract in his left eye, which had a VA of 20/100 and a central corneal thickness of 624 μm. Keratometry values for the left eye were 42.75 × 78/44.12 × 168, and intraocular pressure (IOP) was 16 mm Hg. The left eye underwent phacoemulsification with posterior-chamber intraocular lens implantation 6 months later. Adjunctive measures such as the soft-shell viscoelastic technique[5] that used a dispersive viscoelastic (Viscoat; Alcon Canada, Mississauga, Ontario, Canada) and balanced salt solution with bicarbonate, dextrose, and glutathione (BSS Plus; Alcon Canada) were used to protect the endothelium. Postoperatively, the patient developed persistent corneal edema with a central corneal thickness of 740 μm, and the VA deteriorated to counting fingers.

Eleven months after the cataract surgery, DLEK was performed by using the small-incision technique as described by Melles et al.[3] This technique involved the meticulous removal of the cohesive viscoelastic, sodium hyaluronate 10 mg/mL (Provisc; Alcon Canada) from the anterior chamber of the host after preparation of the recipient

bed. A donor cornea obtained 10 hours postmortem and preserved in Optisol-GS medium (Bausch and Lomb Canada, Markham, Ontario, Canada) for 6 days postmortem was used. Both the recipient and donor corneas were cut at a diameter of 8.5 mm.

The donor graft was prepared by using a disposable Barron Artificial Anterior Chamber (Ketena, NJ). After applying viscoelastic to the endothelial side of the corneoscleral button, the graft was placed on the base of the artificial anterior chamber, which contained residual air within the chamber to enhance the visibility of the depth of dissection. The epithelium of the donor graft was scraped off, and a peripheral incision was created with a trifaceted diamond knife set at a depth of 0.4 mm, followed by a typical lamellar dissection. After removal of the donor corneoscleral button from the artificial anterior chamber, the donor cornea was placed epithelial side down onto a punch block, and a donor trephine was used to punch out the donor disc. The resulting donor disc was folded, endothelial side in over a small strip of this cohesive viscoelastic. This graft was inserted into the anterior chamber of the host through a 5-mm self-sealing scleral incision without any additional viscoelastic.

The preoperative endothelial cell density (ECD) by specular microscopy in the donor graft was 2314 cells/mm^2. Keratometry values 9 days postoperatively were 41.87 × 92/43.87 × 2, and the IOP was 14 mm Hg. On postoperative day 2, a faint black space was noted between the posterior host corneal stroma and the donor tissue. On postoperative day 4, the donor disc had dislocated and was repositioned the following day with injection of air into the anterior chamber and manipulation with a reverse Sinskey hook, which was used to gently but firmly drag the peripheral edge of the graft into a central position from the endothelial side. After this, the edge of the graft was tucked along its circumference into the recipient dissection shelf. Air was left in the anterior chamber for 10 minutes, after which an air–fluid exchange was made with balanced salt solution, and the patient was transferred in the supine position to the recovery area. The anterior chamber was not noted to flatten during repositioning or during the original surgery.

During the first postoperative month after the rebubbling,[6] the cornea gradually cleared both centrally and peripherally (Fig. 1), and the patient reported improvement in his vision. No loose stromal tags or strands were observed with the slit lamp. His best spectacle-corrected visual acuity (BSCVA) measured 20/200, and the central corneal thickness reached 561 μm during graft clearing. Mild age-related macular degeneration was noted. Over the following 2 months, however, the cornea decompensated with a central thickness of 850 μm, and endothelial graft failure caused by surgical trauma was diagnosed. Five months after DLEK, a PK was performed uneventfully, and the corneal button was submitted for histopathologic examination. His BSCVA was 20/100 at 5 months after PK, and the patient died shortly thereafter from an unspecified cardiac event.

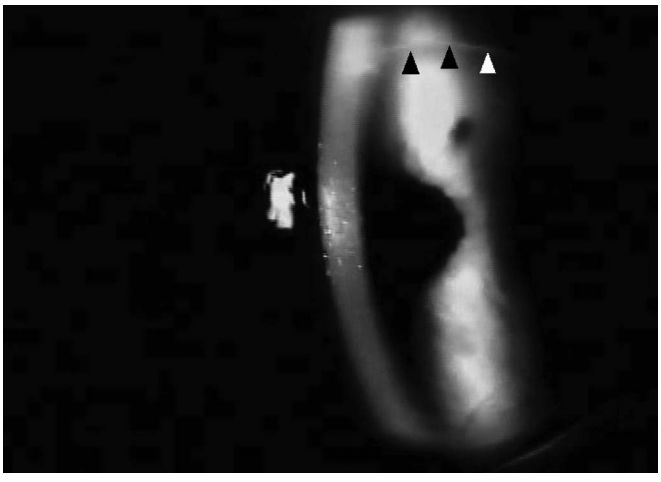

FIGURE 1. Slit-lamp view of the left cornea 1 month after DLEK, with a central corneal thickness of 561 μm. Note the peripheral wound edges, showing no overlap between the graft and peripheral host cornea (arrowheads).

Histopathologic Findings

Formalin-fixed, paraffin-embedded sections of the central cornea showed a variably thickened epithelium with moderate basal cell edema and a mild degenerative pannus above an intact Bowman layer. The stroma was moderately edematous, with the posterior one third showing a compact appearance and a fine demarcation from the anterior two thirds (Fig. 2, top). The demarcation line, corresponding to the graft–donor interface, stained paler than the remaining stroma. The line showed no abnormal staining with the following histochemical and immunohistochemical markers: colloidal iron, alcian blue (pH 2.5), vimentin, epithelial membrane antigen (EMA), smooth muscle actin (SMA), anti-cytokeratin CAM 5.2, high-molecular-weight keratin, anti-cytokeratin AE1/AE3, and collagen 3. There were occasional elliptical shaped thickenings of the interface (Fig. 2, middle). Descemet membrane and the posterior stroma had an undulating appearance. The endothelial cell layer was markedly attenuated with an average of one to two cells per high-powered field (Fig. 2, bottom).

Electron Microscopy

Tissue for electron microscopy was fixed in 2.5% glutaraldehyde, and ultrathin sections were examined with a transmission electron microscope. The interface line was composed of both regularly and irregularly oriented collagen fibrils measuring ~32 μm in diameter (Fig. 3). There was increased spacing between the randomly ordered fibrils, which were interspersed with electron-dense granular material. The keratocytes in most of the host cornea were similar to normal keratocytes (Fig. 4, top). Keratocytes found just adjacent to the interface, both anteriorly and posteriorly, and those throughout the graft showed activated features such as increased rough-surface endoplasmic reticulum (Fig. 4, bottom).

DISCUSSION

Traumatic or iatrogenic endothelial graft failure has not yet been recognized as a substantial complication in DLEK, but there are limited follow-up data thus far. In a similar procedure, Descemet-stripping endothelial keratoplasty (DSEK), traumatic endothelial graft failure has been described by Price and Price,[7] who also found that the incidence of graft repositioning was associated with the incidence of traumatic donor failure. One other case of early postoperative graft dislocation in DLEK needing repositioning reportedly led to endothelial graft failure at 16 months and needed regrafting.[8,9] This case is among 5 early graft dislocations in a recent study of Terry and Ousley[9] of the complications encountered in the first 6 months after 98 consecutive successful cases of DLEK. A significantly lower average ECD in the repositioned corneas (1534 ± 366 cells/mm^2) was shown at 6 months postoperatively compared with those that did not dislocate (2166 ± 411 cells/mm^2). The preoperative ECD in our case was 2314 cells/mm^2, but the graft did not survive to the 6-month postoperative ECD measurement, showing extensive endothelial cell loss in the postoperative PK specimen by the third postoperative month. Early dislocation needing repositioning

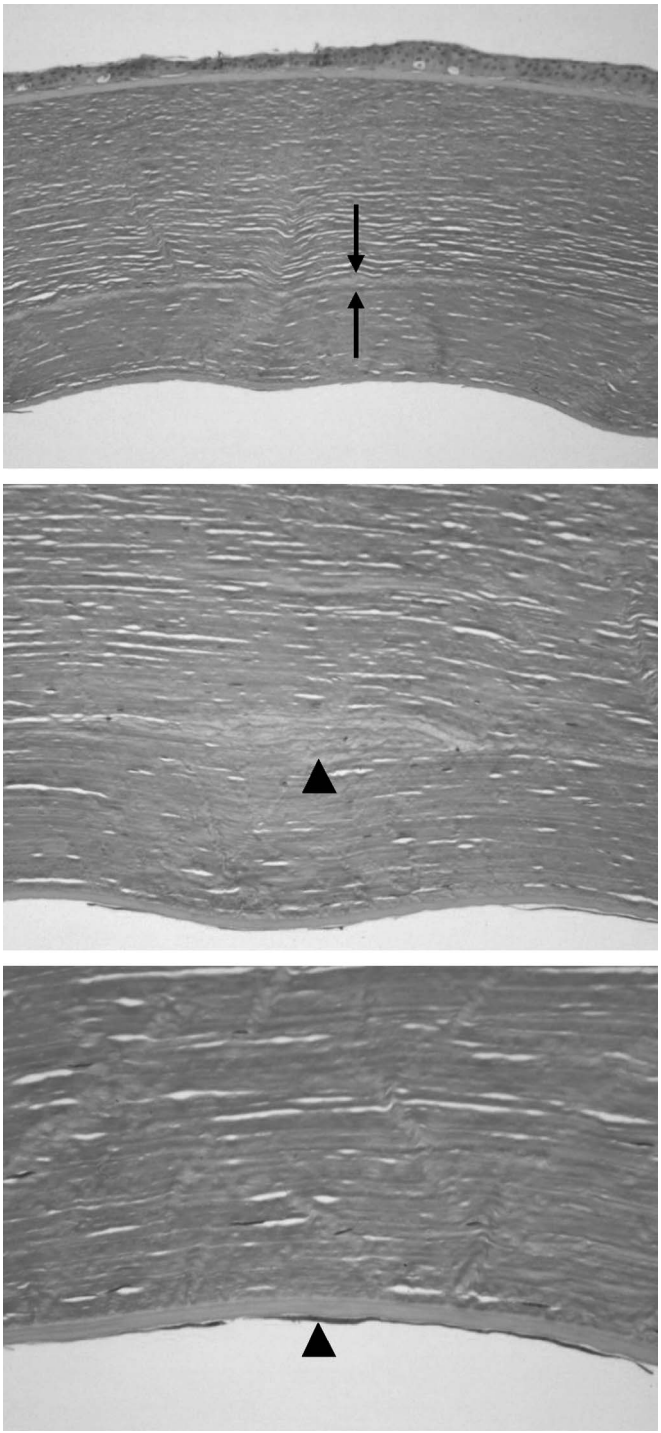

FIGURE 2. Top, The posterior graft is delineated from anterior recipient stroma by a pale line located at 65%–75% of the total stromal depth (between arrows; Masson trichrome; original magnification, ×100). Middle, Elliptical thickening in the interface line on 1 side of the cornea paracentrally (arrowhead), resulting in undulation of the posterior cornea (Masson trichrome; original magnification, ×200). Bottom, The posterior graft shows some remaining flattened cells of the markedly attenuated endothelium (arrowhead; Masson trichrome; original magnification, ×400).

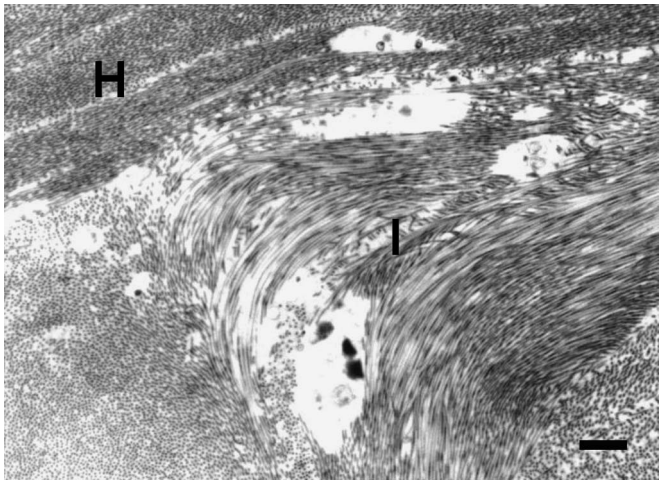

FIGURE 3. Transmission electron photomicrograph of the interface line showing nonuniform orientation of the collagen fibrils (I) with increased spacing between them compared with the more regular lamellar configuration of the host stromal bed (H; original magnification, ×10,000; bar represents 500 nm).

of the donor graft has occurred in 2 other published[10,11] and various unpublished reports, none of which resulted in graft failure.[12] Incomplete evacuation of viscoelastic material from the graft–host interface has been proposed as a risk factor for dislocation.[9] In our case, after a meticulous removal of the cohesive viscoelastic, sodium hyaluronate, from the anterior chamber, a small strip of this viscoelastic was applied only during folding of the donor graft, with no additional viscoelastic being used during the insertion of this graft. Late graft dislodgement has not been reported.

van Dooren et al[13] created an endothelial cell decay model with their 3-year follow-up data, comparing the 5.0-mm graft folding delivery technique used in our case with the former 9.0-mm incision technique. Although the 5.0-mm incision technique resulted in a lower initial postoperative ECD, endothelial cell loss was more rapid in the 9.0-mm incision technique. Extrapolation of the model predicts a critical ECD of 500 cells/mm^2 after 5.5 years with the latter technique compared with 7.8 years for the 5.0-mm folding technique, as used in our case. The report of Terry and Ousley[14] of their prospective case series performed with the 5.0-mm incision disclosed 6-month postoperative endothelial counts comparable to both large-incision DLEK and PK. In addition to possible trauma induced by repositioning the graft, our patient's history of 2 failed PKs in the fellow eye indicates that host factors may have been operative in the endothelial demise of the graft at 3 months.

Interface optical clarity is the most unpredictable challenge with DLEK. Terry and Ousley[15] estimated that the manually dissected interface causes a loss of ~1 line of VA, noting no reliable correlation between BSCVA and clinical perceptibility of interface haze. On light microscopy, manually dissected irregularities were present at the donor–host interface, around which fluid may have pooled, taking time to reabsorb, and through which refractive aberrations may

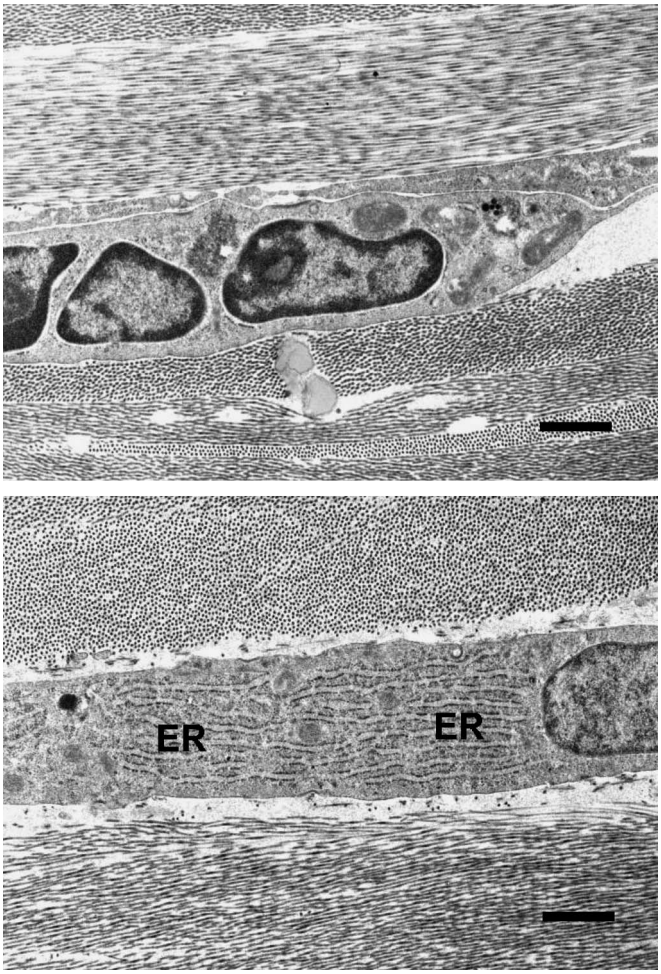

FIGURE 4. Transmission electron photomicrograph of keratocytes found in the operated cornea. Top, Relatively quiescent keratocyte located anterior to the interface in the host stromal bed, showing no prominent rough-surface endoplasmic reticulum. Bottom, Activated keratocyte located posterior to the interface in the graft, showing increased rough-surface endoplasmic reticulum (ER; both top and bottom frames, original magnification, ×12,000; scale bar = 500 nm).

have been induced (Fig. 2, middle). However, no stromal strands, irregularities, or haze were noted with the slit lamp postoperatively.

The histology of a failed DLEK graft has been described by Faia et al.[16] Similar to the patient in this report, their patient was pseudophakic with a history of Fuchs dystrophy, and the DLEK graft was also complicated by early dislocation, needing repositioning twice with air injection. However, in contrast to our case, a Sinskey hook was not used during graft repositioning. Faia et al observed clinically apparent vertical folds at the graft–host interface, which they felt contributed to the poor visual outcome, ultimately needing a repeat DLEK. In our case, irregularities or haze were not detectable on the slit lamp, although there were elliptical-shaped thickenings at the interface observed on light microscopy. The use of a Sinskey hook during repositioning in our case may have resulted in sufficient surgical trauma to produce critical endothelial cell loss, resulting in graft failure. In the case of Faia et al, endothelial cells were adequately preserved, but a suboptimal visual outcome occurred, presumably because of the persisting folds in the donor–host interface.

In another case of a failed DLEK graft reported by Kapur et al,[17] histologic analysis showed the presence of red blood cells and a pigmented line at the graft–host interface. Special stains revealed the presence of hemosiderin and melanin pigment. The authors suggested that the erythrocytes and blood breakdown products came from bleeding limbal vessels during lamellar dissection.[17] Melanin pigment may have been introduced from the iris during manipulation of the donor tissue. The patient had a poor visual outcome, presumably because of the diffuse deposits of pigment at the interface line.

Terry et al[18] has described ultrastructural features in cadaver eyes on which DLEK and DSEK were performed. They found the recipient corneal surface after DLEK stromal dissection displayed a rough surface with stromal fibers cut at a relatively uniform level, giving a "short-cut carpet" appearance. The corresponding surface in the DSEK eyes had a "glassy smooth" surface that did not show the presence of cut fibrils. The authors hypothesized that, in DSEK, the lack of cut stromal fibrils on the host surface results in a higher rate of graft dislocation than with DLEK procedures.[18] Using a "Terry Scraper" to roughen the peripheral donor surface in DSEK was associated with a reduction in graft dislocation from 50% to 4%. In another study, electron microscopy was performed on a failed DLEK graft, but the graft–host interface could not be identified in the examined grids.[17] The electron microscopic findings in our case showed a nonuniform orientation of the interface collagen fibrils with increased spacing between them (Fig. 3), in an electron-dense granular background material. These observations are similar to those seen in postmortem corneas of patients who previously had laser in situ keratomileusis (LASIK)[19] but were more pronounced in our case, perhaps as a result of nonuniformity at the manually dissected interface.

The alteration of fibril configuration as light passes through the interface affects optical performance, even in the context of a clinically imperceptible interface.[15] Current efforts to perfect a protocol to create smoother stromal surfaces to minimize interface haze include use of the femtosecond laser, although its microscopic superiority over manual dissection has yet to be shown.[20,21] Other avenues being pursued are improved techniques such as DSEK, which seems to produce a donor–host interface that is smoother on clinical examination and on electron microscopy compared with manually dissected stromal interfaces in DLEK.[7,18] Implementing a microkeratome to prepare the donor lamellar graft would theoretically furnish a smoother donor surface. In a series of DSEK procedures, microkeratome-prepared grafts were found to be associated with a lower incidence of tissue perforation and traumatic donor failure than with grafts prepared by hand dissection.[7]

Although still early in our patient's postoperative course (3 months), collagen synthesis and remodeling were already well under way. The keratocytes in most of the host cornea were similar in size and morphology to those found in a normal

cornea, suggesting quiescence (Fig. 4, top). Other keratocytes found just adjacent to the interface line and throughout the graft showed activated features such as increased rough-surface endoplasmic reticulum (Fig. 4, bottom). This finding suggests that the wound healing mechanism was still active after 3 months throughout the graft.

Our case shows some of the potential complications of DLEK, most notably early endothelial graft failure. Analysis of the decompensated cornea showed the histopathologic and ultrastructural irregularities of the manually dissected interface line, potentially affecting optical performance.

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