Ultrastructural Alterations of Grafted Corneal Buttons: The Anatomic Basis for Stromal Peeling Along a Natural Plane of Separation

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• PURPOSE: **To examine the ultrastructure of the natural plane of separation in grafted corneas and evaluate the outcomes of stromal peeling.**

• DESIGN: **Interventional case series.**

• METHODS: **In this multicenter study, stromal peeling was attempted in 96 consecutive eyes with unsatisfactory vision following penetrating keratoplasty (PK) for keratoconus (n = 79), herpetic keratitis (n = 11), and granular dystrophy (n = 6). Stromal exchange was performed by (1) 9 mm partial-thickness trephination; (2) creation of a corneal flap across the PK wound; (3) opening of the stromal component of the PK wound until a smooth, translucent natural plane was identified; (4) severing the attachment of the PK scar; (5) stromal peeling along the identified plane; and (6) suturing of donor lamella. Grafted corneas from cases that mandated conversion to PK were processed for transmission electron microscopy.**

• RESULTS: **The natural plane of separation was identified in all cases. Stromal exchange was successfully completed in 84 cases (87.5%). Snellen visual acuity ≥20/40 and ≥20/25 was reached in 93% and 72% of cases at 3 years (n = 49) and 86% and 62% at 4 years (n = 21) postoperatively. Mean endothelial cell loss at 1 year was 6.6% ± 9.5%. Stromal peeling occurred along a plane lined with a continuous layer of keratocytes separating pre-Descemet membrane (DM) stroma, DM, and endothelium from the anterior stroma. Pre-DM stroma was made of poorly organized lamellae containing widely spaced, randomly arranged collagen fibrils.**

• CONCLUSIONS: **Ultrastructural alterations in the stromal microarchitecture of grafted corneas provide evidence of a natural plane of separation identified intraoperatively. Stromal peeling can be successfully performed in post-PK eyes with various stromal pathology.** **(Am J Ophthalmol 2021;231: 144–153.**

According to the 2019 Statistical Report of the Eye Bank Association of America, repeat corneal transplantation is currently the most common indication for penetrating keratoplasty (PK) worldwide.[1] However, not all grafts requiring a repeat PK are associated with impaired endothelial function. Corneal stromal disease, for which PK continues to be the preferred surgical procedure in the United States,[1] can recur years after an uneventful postoperative course and can significantly impair vision, thereby necessitating repeat surgery.[2-4] Considering that both the cumulative probability of graft survival and the median graft survival time decrease after every subsequent PK, performing deep anterior lamellar keratoplasty (DALK) after PK would confer the theoretical benefit of sparing functional corneal endothelium.[5-7] Selective replacement of the affected corneal stroma would obviate the introduction of new endothelium, which is the prime target for alloimmune rejection leading to graft failure.[8]

However, the traditional methods of lamellar dissection employed in a primary DALK are technically challenging in the presence of a PK graft.[9,10] Recently, we have fortuitously identified a natural surgical plane that allows peeling of the stroma of the PK graft and reveals a smooth, homogenous, and translucent recipient bed.[11] In contrast to conventional DALK for keratoplasty-naïve cases, stromal exchange can be performed by simple peeling without the need for air, hydro-, viscoelastic, or layer-by-layer sharp or blunt manual dissection of the PK graft.[11]

We have postulated that the postoperative alterations in the corneal microarchitecture after PK facilitate stromal peeling along the natural plane of separation[11]; however the anatomic basis of the natural cleavage plane identified in eyes that have undergone PK has not been elucidated. In this study, we examined the ultrastructural features of the plane of separation exploited in stromal peeling. Moreover, we evaluated the clinical outcomes of DALK using the stromal peeling technique in eyes that have previously under-

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gone PK for various stromal pathologies, including conditions other than keratoconus.

METHODS

This multicenter interventional case series evaluated the outcomes of 96 consecutive attempted DALK surgeries using the stromal peeling technique for eyes that have previously undergone PK. All surgical procedures were performed by 3 corneal surgeons at Ospedali Privati Forlì "Villa Igea," Forli, Italy (M.B. and C.B.) and University Eye Hospital of "Magna Graecia" University, Catanzaro, Italy (V.S.) between June 2015 and February 2020. The study was compliant to the tenets of the 2013 Declaration of Helsinki and was prospectively approved by the local institutional review board / ethics committee, Comitato Etico Ospedali Privati Forlì (Forlì, Italy). Written informed consent for the surgery and the research was obtained from each patient. Informed consent for tissue donation and medical research was obtained from the donor's next-of-kin.

Eyes with stromal disease requiring repeat keratoplasty for unsatisfactory best spectacle-corrected visual acuity (BSCVA) and/or poor contact lens tolerance were included in the study. In all cases, the endothelium was clinically functional with a preoperative endothelial cell density (ECD) of at least 600 cells/mm^2, the threshold that we arbitrarily considered sufficient to maintain corneal deturgescence.[11]

Each patient underwent complete preoperative ophthalmic evaluation including slit-lamp examination, BSCVA testing, manifest refraction, funduscopy, noncontact specular microscopy, and anterior segment optical coherence tomography (Casia; Tomey, Tokyo, Japan). BSCVA was assessed using the Snellen visual acuity chart. ECD was evaluated via noncontact specular microscopy (EM-3000; Tomey GmbH, Erlangen, Germany) using automatic focusing and digital capture. Intraoperative and postoperative complications were recorded. Rates of successful identification of the natural plane of separation and completion of stromal peeling surgery were evaluated. Follow-up visits were scheduled at least once annually.

Graft rejection was defined as the presence of epithelial rejection line, subepithelial infiltrates, and/or keratic precipitates, diffuse or linear; and/or an increase in anterior chamber cells from a previous visit with or without any clinically apparent change in recipient stromal thickness or clarity. Recurrence was considered as the presence of any clinical findings compatible with recurrence of the primary corneal stromal pathology.

Failure of the donor cornea to clear postoperatively could be observed owing to decompensation of the recipient endothelium induced by surgical trauma or by persistent detachment of the recipient bed. The occurrence of both types of complications was also recorded. Primary graft failure in the conventional sense of transplanted endothelium not being able to clear the cornea was not observed, as no endothelium was used for this anterior lamellar procedure. Instead, graft failure was defined as a cornea, initially clear after surgery, becoming subsequently opaque spontaneously or as a consequence of additional interventions (eg, cataract surgery), recurrence of stromal disease, or any external cause.

• **SURGICAL TECHNIQUE:** Stromal peeling in post-PK eyes was performed according to a previously described technique, as shown in the Supplemental Video (Supplemental Material available at AJO.com).[11] Briefly, a vacuum trephine was calibrated within 150 μm from the thinnest anterior segment optical coherence tomography pachymetry value at 9 mm diameter. A partial-thickness trephination, 9 mm in diameter, was carried out outside the old PK graft. From the base of the trephination, lamellar dissection was performed tangentially across the old PK wound to create a partial anterior corneal flap. While lifting the corneal flap, blunt-tipped Vannas scissors were used to open the old PK wound until a natural plane of separation was reached. This plane could be identified by a sudden loss of resistance and by its smooth, glossy, translucent appearance (Figure 1, A). As one of the blades of the same Vannas scissors could be inserted freely along the natural surgical plane without any resistance, the previous PK scar was cut step by step along the 360-degree circumference. The stroma of the PK button was grasped and simply peeled along the natural plane of separation, thereby exposing a recipient bed, which was substantially resistant to surgical manipulation (Figure 1, B). Finally, a 9-mm donor anterior lamella prepared by microkeratome dissection (450 μm depth) was sutured onto the recipient cornea.

When a microperforation occurred, the anterior chamber was filled with air, which was partially released 2 hours postoperatively. On the other hand, in cases with a macroperforation, the procedure was converted to a 2-piece mushroom PK, according to the authors' previously described technique.[12]

At the end of the procedure, triamcinolone acetonide and gentamicin sulfate 0.3% were injected subconjunctivally. A fixed combination of dexamethasone phosphate 0.1% and netilmicin sulfate 0.3% ophthalmic solution was administered every 2 hours and tapered off to 4 times daily over the first postoperative month. Subsequently, antibiotic treatment was discontinued and dexamethasone phosphate 0.1% eyedrops was slowly tapered to once daily over 4 to 5 postoperative months and discontinued after 13 months. For eyes with a history of herpetic keratitis, antiviral prophylaxis treatment protocol was initiated prior to the surgery and extended indefinitely postoperatively according to institutional protocol.[13]

• **DATA COLLECTION AND ANALYSIS:** Stromal peeling was attempted in 96 consecutive eyes of 96 patients that pre-

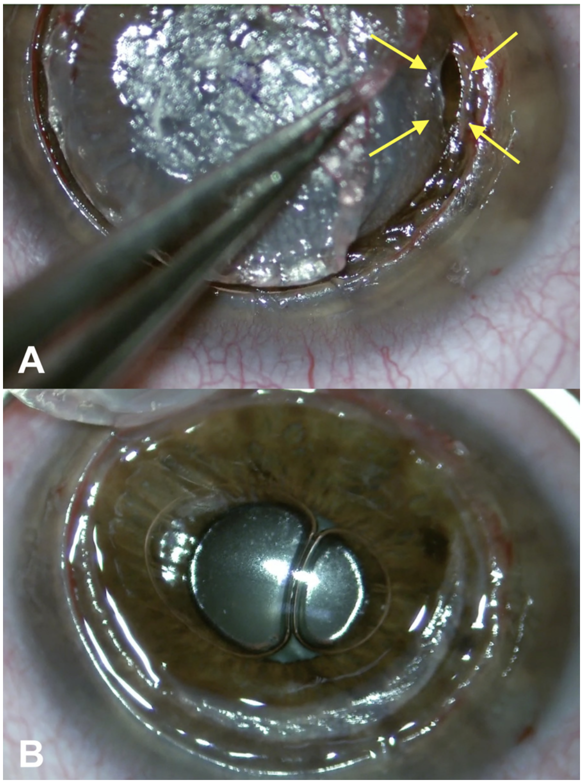

FIGURE 1. Intraoperative photograph of stromal peeling. A. The natural plane of separation upon opening the old penetrating keratoplasty (PK) wound using blunt Vannas scissors. B. Stromal peeling of the PK graft results in a smooth, glossy, and translucent recipient bed.

viously underwent PK for keratoconus (n = 79), herpetic keratitis (n = 11), and granular corneal dystrophy type 1 (n = 6). Mean age at the time of stromal peeling was 55 ± 11 years (range: 29-81 years). Fifty-seven (59.3%) were male and 39 (40.6%) were female. Mean interval between primary keratoplasty and stromal peeling was 11 ± 9 years.

Clinical data from cases successfully completed by stromal peeling (n = 84) were recorded and collected prospectively. BSCVA was converted to logarithm of the minimum angle of resolution (logMAR) units. Endothelial cell loss (ECL) was calculated by subtracting postoperative ECD from baseline donor ECD, dividing by the baseline donor ECD, and multiplying by 100. Mean follow-up was 38 ± 13 months. Follow-up data and the number of potentially available eyes at each examination time, as well as the reasons for exclusion, are summarized in the Table 1.

Statistical analyses were performed using IBM SPSS (version 27.0; IBM, Armonk, New York, USA). Values were expressed as mean ± standard deviation for continuous variables and individual counts and percentages for categorical variables. Analysis of repeated measures using linear mixed models was used to assess the changes in BSCVA and ECD over the follow-up period. Adjustment with Bonferroni method was applied to multiple pairwise comparisons. The significance threshold was set at .05. Cumulative probabilities of graft rejection and survival were generated by Kaplan-Meier analysis.

• **TRANSMISSION ELECTRON MICROSCOPY:** Grafted corneas from 8 cases in which the plane of separation was identified but mandated conversion to mushroom PK were processed for transmission electron microscopy. Five corneal specimens obtained from normal human donor eyes without any known ocular diseases but unsuitable for transplantation owing to low ECD were used as controls.

Samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 3 hours and post-fixed in 2% buffered osmium tetroxide for 1 hour. The specimens were then dehydrated with graded concentrations of acetone and embedded in Araldite epoxy resin (Durcupan ACM, Fluka; Sigma-Aldrich Co, St. Louis, Missouri, USA) according to standard protocols. Semithin sections (1.5 μm) were cut using an ultramicrotome (Reichert Ultracut S; Leica Microsystems, Vienna, Austria) and stained with a 1% aqueous solution of toluidine blue. Ultrathin sections (90 nm) were prepared using the same ultramicrotome, counterstained with uranyl acetate in saturated solution and lead citrate, and examined with a transmission electron microscope (Hitachi H-800 100 kV; Hitachi Ltd, Tokyo, Japan).

RESULTS

The natural plane of separation was identified intraoperatively in all cases (100%). Stromal peeling was successfully completed in 84 eyes (87.5%). Twelve eyes (12.5%), including 10 that underwent primary PK for keratoconus and 2 for herpetic keratitis, required conversion to mushroom PK owing to significant Descemet membrane (DM) perforation. All perforations occurred at the site of the PK surgical scar during attempts of identification of the surgical plane (n = 2) and during severing of the PK surgical wound (n = 10). During peeling of the stroma from the underlying tissue, no inadvertent tears or rupture of DM were observed. The rate of successful completion of stromal peeling surgery did not differ significantly among the 3 surgeons ($P = .96$).

Following complete suture removal, baseline logMAR BSCVA significantly improved, from 0.90 ± 0.37 to 0.20 ± 0.14 ($P < .001$) and 0.12 ± 0.10 ($P = .038$) at 1 and 2 years after surgery, respectively. Subsequently, BSCVA remained stable throughout the follow-up period. Sensitivity analysis adjusting for stromal pathology and primary surgeon did not significantly change the analysis. No patient lost any Snellen lines of vision. Three years after surgery, 93% of eyes reached ≥20/40 and 72% reached ≥20/25 (Table 1).

One year postoperatively, ECD averaged 1004 ± 263 cells/mm^2 with an ECL of 6.6% ± 9.5%. Average annual ECL after the first year was 2.8% ± 2.7% (Table 1). Double

TABLE 1. Clinical Outcomes of Completed Stromal Peeling Surgeries

	Baseline[a]	Month 12	Month 24	Month 36	Month 48	Month 60
Total number of eyes potentially available	84	84	67	49	21	6
Number of eyes evaluated	84 (100%)	84 (100%)	66 (99%)	46 (94%)	19 (91%)	6 (100%)
Number of eyes excluded						
Regraft	0 (0%)	0 (0%)	0 (0%)	1 (2%)	2 (9%)	0 (0%)
Lost to follow-up	0 (0%)	0 (0%)	1 (1%)	2 (4%)	0 (0%)	0 (0%)
BSCVA						
Mean ± SD (logMAR)	0.89 ± 0.37	0.20 ± 0.14	0.12 ± 0.09	0.13 ± 0.11	0.11 ± 0.09	0.13 ± 0.10
Corneal ectasia	0.90 ± 0.37 n = 69	0.19 ± 0.14 n = 69	0.13 ± 0.10 n = 53	0.13 ± 0.11 n = 40	0.11 ± 0.10 n = 19	0.13 ± 0.10 n = 6
Herpetic keratitis	0.93 ± 0.38 n = 9	0.19 ± 0.19 n = 9	0.10 ± 0.09 n = 8	0.08 ± 0.04 n = 2	-	-
Granular dystrophy	0.75 ± 0.35 n = 6	0.23 ± 0.12 n = 6	0.09 ± 0.02 n = 5	0.09 ± 0.03 n = 4	0.10 ± 0.00 n = 2	-
No. of eyes ≥20/50		84	66	46	19	6
No. of eyes ≥20/40		69	64	43	18	4
No. of eyes ≥20/25		39	48	33	13	4
Endothelial cell density	1073 ± 253	1004 ± 263	983 ± 260	975 ± 255	970 ± 293	894 ± 188

BSCVA = best spectacle-corrected visual acuity; logMAR = logarithm of the minimum angle of resolution.

[a] Stromal peeling surgery was completed in 84 of 96 attempted cases that previously underwent penetrating keratoplasty for keratoconus (69 of 79 cases), herpetic keratitis (9 of 11 cases), and granular corneal dystrophy type 1 (6 of 6 cases).

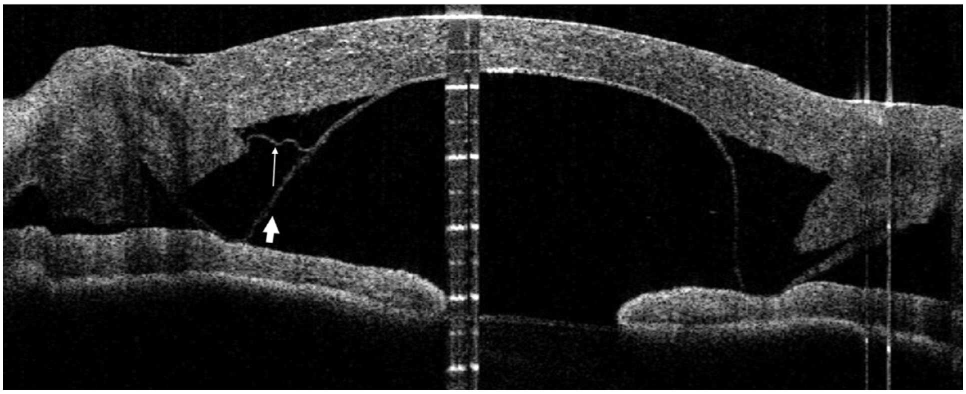

FIGURE 2. Anterior segment optical coherence tomography image of a partial recipient bed detachment shows 2 hyperreflective bands representing pre-Descemet membrane stroma (long thin arrow) and Descemet membrane (short thick arrow).

anterior chamber formation occurred in 22 eyes (26.2%) (Figure 2), 7 of which were associated with a microperforation intraoperatively. All cases were managed successfully with a single injection of air into the anterior chamber. Sequential cataract surgery via phacoemulsification with posterior chamber intraocular lens implantation was performed in 14 (16.7%) eyes, 4 of which had an immature cataract prior to stromal peeling surgery. No clinical signs of recurrence were observed in any case. The 4-year cumulative risk for immunologic rejection and graft failure was 4.1% and 4.0%, respectively. Kaplan-Meier estimates are presented in Figure 3, A and B. All episodes of rejection were successfully reversed with topical steroids. Both cases of graft failure were due to progressive ECL observed 2 and 6 months following sequential cataract surgery. Descemet stripping automated endothelial keratoplasty was performed in both cases. Failure of the donor cornea to clear postoperatively from decompensation of the recipient endothelium induced by surgical trauma or by persistent detachment of the recipient bed was not observed in any case.

In grafted corneas from cases in which the plane of separation was identified but mandated conversion to mushroom PK, transmission electron microscopy demonstrated that the natural plane identified intraoperatively occurred along a single row of keratocytes separating a thin layer of pre-DM stroma from the overlying anterior stroma

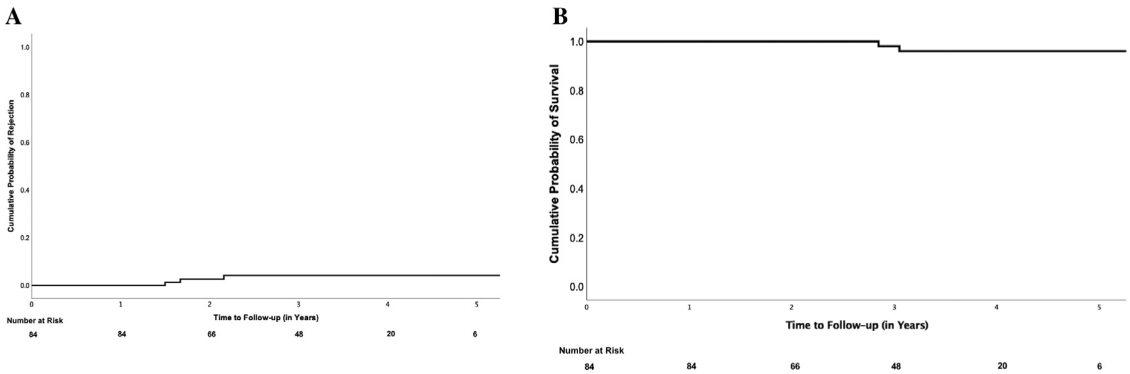

FIGURE 3. Kaplan-Meier curves of stromal peeling surgery. A. Cumulative probability of graft rejection. B. Graft survival.

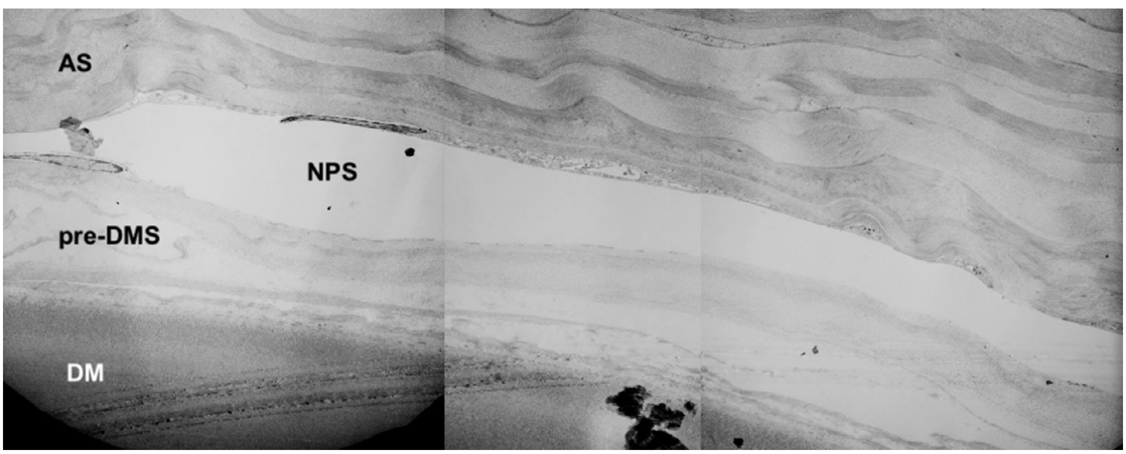

FIGURE 4. Low-magnification montage of transmission electron micrographs (× 2000) showing the intraoperatively identified natural plane line separating anterior stroma and thin pre-Descemet membrane stroma overlying Descemet membrane and endothelium. AS = anterior stroma; DM = Descemet membrane; E = endothelium; NPS = natural plane of separation; pre-DMS = pre-Descemet membrane stroma.

(Figure 4 and Figure 5, A). The keratocytes were oriented parallel to the corneal surface and appeared as flattened cells with large distinct nuclei and thin, long cytoplasmic processes between stromal lamellae (Figure 5, B). Figure 5, C demonstrates the single layer of keratocytes, which were arranged between parallel stromal lamellae and continued uninterrupted along the surgical plane of separation. Although there was increased cellularity both anterior and posterior to the plane of separation, keratocytes were not found continuously arranged between any other stromal lamellae.

Directly anterior to the plane of separation, the corneal stroma was made of tightly packed collagen fibrils with relatively regular spacing and orderly arrangement (Figure 6, A). On the other hand, the thin corneal stroma posterior to the plane of separation was made of poorly organized collagen lamellae containing widely spaced, randomly arranged collagen fibrils, interspersed with long-spacing collagen fibers anterior to DM (Figure 6, B, and Figure 7, A and B). Mean central thickness of the pre-DM stroma was 11.01 ± 3.48 μm (range: 6.25-15.98 μm). The individual collagen fibrils in the posterior stroma had a considerably uniform diameter measuring 22.01 ± 2.49 nm.

In control eyes, the deep posterior stroma anterior to DM contained scarce keratocytes randomly arranged between tightly packed collagen lamellae made of uniform-sized collagen fibrils, which measured 24.69 ± 2.56 nm, were typically arranged in the longitudinal, transverse, and oblique directions and were interspersed with long-spacing collagen fibers anterior to DM (Figure 6, C, and Figure 7, C).

DISCUSSION

The development of modern lamellar keratoplasty has generated a renewed interest in the microarchitecture of the human cornea.[14-19] With the introduction of big-bubble

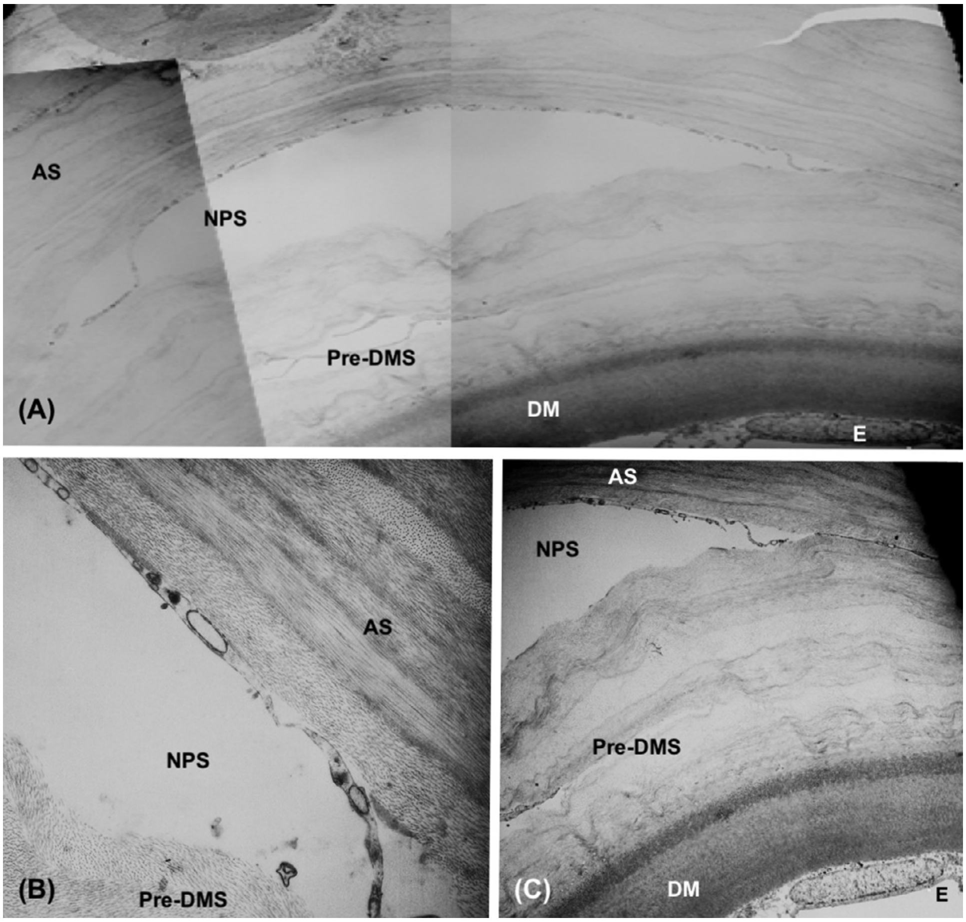

FIGURE 5. A,B. Transmission electron micrographs demonstrate (A) keratocytes continuously arranged along the plane of separation (× 2,000) (B) appearing as elongated cells with thin, long cytoplasmic processes (× 5,000). C. The single row of keratocytes continued uninterrupted along the surgical plane of separation (× 5,000). AS = anterior stroma; DM = Descemet membrane; E = endothelium; NPS = natural plane of separation; pre-DMS = pre-Descemet membrane stroma.

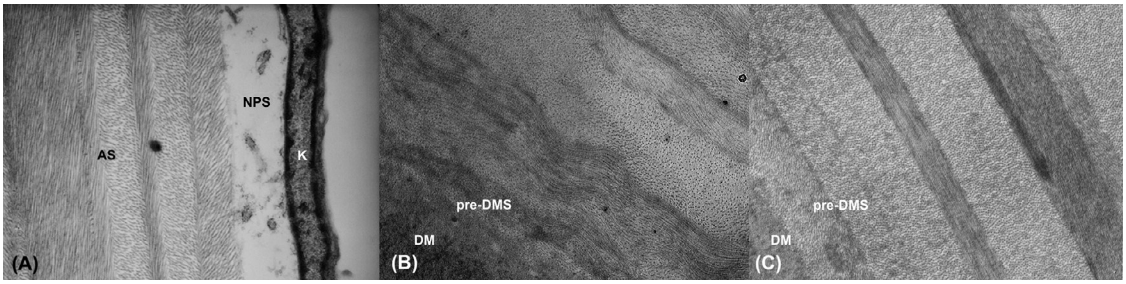

FIGURE 6. A-C. Transmission electron micrographs (× 8,000) showing the arrangement of collagen fibrils (A) anterior and (B) posterior to the plane of separation in a grafted cornea and (C) in a control cornea. AS = anterior stroma; DM = Descemet membrane; K = keratocyte; NPS = natural plane of separation; pre-DMS = pre-Descemet membrane stroma.

DALK, evidence gathered from empiric observations and ultrastructural analysis of the cornea has clarified previous knowledge on the general principles of keratoplasty.[15-19] Although the proposal of a separate stromal layer directly anterior to DM has spurred wide debate,[20] the recent characterization of pre-Descemet layer (PDL) has highlighted the complexity of the human cornea and has underscored the need to establish the anatomic basis of corneal lamellar surgery.[17-19]

Most of the reports in the literature, however, examine the corneal morphology in the setting of normal anatomic relationships.'[14–19] Although it is known that surgery can

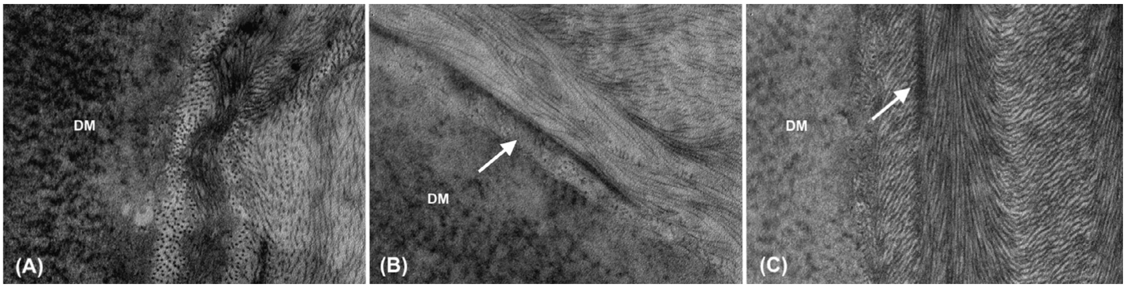

FIGURE 7. A-C. High-magnification transmission electron micrographs (× 25,000) showing the (A) arrangement of collagen fibrils in the pre-Descemet stroma of a post–penetrating keratoplasty (PK) eye and (B) presence of long-spacing collagen fibrils (arrows) in a post-PK eye and (C) in a control eye. DM = Descemet membrane.

induce alterations in the corneal anatomy, an examination of the specific structural features would provide a better understanding of their impact on the surgical strategy and allow optimization of the outcomes of repeat keratoplasty in post-PK eyes.

In the current study, regardless of the primary stromal pathology, a natural plane of separation was consistently visualized and identified intraoperatively by its smooth, glossy, and translucent surface (Figure 1, A). Akin to the PDL–DM–endothelium complex observed in type 1 bubble DALK, the residual bed after stromal peeling was substantially resistant to manipulation with surgical instruments. From a surgical standpoint, both the consistently identifiable plane of separation and the relative strength of the residual bed against manipulation have equally contributed to the high completion rate of stromal peeling surgery (88%). Considering the inherent surgical challenges in postsurgical eyes, this rate is comparable with those reported for DALK in keratoplasty-naïve eyes (75%-85%).[21-24]

However, unlike the rather "rough" appearance of the cleavage plane in a type 1 bubble,[25] the surface of the residual bed after stromal peeling was smooth, homogenous, and featureless, with no visible stromal fibers or broken strands of collagen (Figure 1, B). These gross features explain how the blades of the scissors can slide freely within the natural plane of separation without encountering any resistance. After the peripheral attachment of the PK surgical scar was completely severed, the stroma could be easily peeled from the underlying bed even without applying mechanical force.

For nongrafted eyes with keratoconus, Malbran has described a "peeling off" technique for lamellar keratoplasty using up to 2 forceps to pull the anterior stroma while the globe is fixed at the limbus.[26] However, just as in any form of manual dissection in nongrafted corneas, considerable mechanical force would be required to separate the anterior corneal stroma from the recipient bed, which may result in a layer of variable thickness.[24] In contrast to conventional DALK performed in virgin corneas, pneumatic, hydro-, viscoelastic-assisted, or blunt dissection maneuvers were not required for stromal peeling in post-PK eyes.[11]

Also, unlike the cleavage plane exploited in Descemet stripping procedures,[27] no separation resistance within the deep stroma was encountered along the natural plane of separation. In fact, pulling the stroma of the PK graft straight across the underlying bed did not result in inadvertent tears or rupture of DM.

Several lines of evidence support the surgical observation of a weakened interlamellar attachment in the posterior stroma of grafted corneas. Firstly, spontaneous DM detachment can occur several years after an uneventful PK.[28,29] Secondly, inadvertent retention of the host DM during a repeat PK is a common observation of many surgeons and has been described in various reports.[30,31] Finally, we have recently observed a spontaneous intracorneal hemorrhage 26 months after PK wherein the stromal infiltration of blood was found to be delimited posteriorly by the same natural plane of separation.

For the first time, the current study demonstrates the ultrastructural basis allowing the performance of stromal peeling in post-PK eyes. Separation occurred along a plane that was lined with a continuous layer of keratocytes. These keratocytes may have preferentially migrated along a natural plane of separation, which presumably represents the path of least resistance and is located approximately at the same level where pneumatic dissection succeeds to create a type I bubble in virgin corneas. In terms of average thickness, the measurement of the residual pre-DM stroma after stromal peeling ($11.01 \pm 3.48\ \mu m$) was similar to the reported values of the PDL in a type 1 DALK ($10.15 \pm 3.6\ \mu m$).[17]

To date, there are very few studies that have investigated the movement of stromal keratocytes following PK.[32,33] Analysis of sex-mismatched corneal transplants through in situ hybridization has demonstrated that donor keratocytes are eventually replaced with recipient cells.[32,33] Thus, it is conceivable that the keratocytes found within the natural surgical plane in grafted corneas may have also been derived from the host, which would require in situ hybridization to be confirmed. In a future study, it would be desirable to investigate the cellular dynamics of these keratocytes, including the mechanisms that regulate cell migration in grafted corneas.

A recent study by Dua and associates[29] has also demonstrated such increased cellularity in the deep posterior stroma of eyes that developed DM detachment, some of which occurred following a PK. Although they did not perform an ultrastructural analysis, Dua and associates[29] have postulated that the infiltration of cells observed in the posterior stroma was made of myofibroblasts or fibroblasts, which invaded from the adjacent stroma and induced cicatrization within the PDL. In the grafted corneas examined in the present study, however, the stromal cells were morphologically and functionally consistent with mature keratocytes, rather than fibroblasts or myofibroblasts, as corneal stromal transparency was not affected. Further studies using immunohistochemistry staining may confirm our hypothesis.

As described by Dua and associates,[17] the PDL of naïve corneas consists of 5 to 8 thin lamellae of tightly packed collagen bundles that are oriented in longitudinal, transverse, and oblique directions interspersed with long-spacing collagen fibers but absent or sparse keratocytes. However, Schlötzer-Schrehardt and associates[15] and Meek and associates[34] did not observe distinct differences, nor a specific demarcation, in the ultrastructure of the PDL in normal corneas. On the other hand, in grafted corneal buttons, the alterations in the ultrastructural arrangement and alignment of the collagen fibrils of pre-DM stroma were sufficiently distinct from the rest of the stroma. Likely induced by corneal stromal remodeling following transplantation, these changes represent the anatomic basis for the consistent plane of separation identified during stromal peeling.

Since the initial pilot study, we have broadened the surgical indications of stromal peeling, including primary pathologies other than keratoconus. Our findings confirm that the residual bed after stromal peeling is compatible with satisfactory visual outcomes regardless of the indication. One year postoperatively, average approximate Snellen BSCVA was 20/32 and the outcomes were maintained throughout the follow-up period. As observed after a primary DALK, an initial, limited ECD decline was followed by a slower rate of ECL.[22,35] The ECL over time appears to be only marginally increased over the rate normally seen in post-PK eyes,[36] which in turn accounts for the excellent survival rate (96%) 4 years after stromal peeling. Thus, avoiding unnecessary replacement of functional endothelium through selective keratoplasty is particularly beneficial to patients with corneal stromal disease who tend to be younger and may possibly require multiple grafts throughout their lifetime.[11]

Of note, the 2 cases of graft failure in this series occurred after cataract surgery. Thus, it may be prudent to defer cataract surgery after stromal peeling for as long as possible. However, should it ever be deemed necessary, these patients should be adequately counseled about the postoperative complications as well as the possible need for sequential endothelial keratoplasty.

Although granular dystrophy can recur as early as 1 year following keratoplasty, late recurrence has also been described 15 years postoperatively.[37,38] While the mechanism remains poorly understood, this wide range may be related to the interindividual variability in phenotypic expression.[39] In a series of 33 cases, Lyons and associates[38] did not find a significant correlation between graft size and time to recurrence. However, most of the published data included cases performed before the year 2000 based on conventional keratoplasty techniques using 8 mm grafts or anterior lamellar keratoplasty employing traditional manual dissection.[37,38] In the present study, recurrence of primary stromal pathology was not observed over a mean follow-up of 3 years. These findings appear to corroborate previous observations on 9 mm big-bubble DALK for recurrent granular dystrophy ($n = 8$) following previous anterior lamellar keratoplasty with no clinical recurrence noted over a mean follow-up of 4 years.[40] Although our experience is based on a small sample size, deep lamellar dissection along the natural plane of separation in post-PK eyes and excision of an area of diseased stroma as large as 9 mm in diameter could contribute to delayed recurrence. Extended longitudinal follow-up of a larger sample of cases would be needed to determine the actual recurrence rates and evaluate the true magnitude of treatment effect.

Three experienced surgeons, who have each performed more than 100 primary big-bubble DALK surgeries, contributed almost equally to this study. While the results may not be generalizable to all corneal surgeons, in view of the fact that the natural plane of separation was consistently identified, we believe that this technique is fairly reproducible, especially among those who have achieved sufficient proficiency in conventional DALK. Furthermore, the study includes an unequal number of patients with primary stromal pathology other than keratoconus, for which a head-to-head statistical analysis could not be performed. Nevertheless, the current study demonstrates the technical feasibility of stromal peeling for the breadth of indications that may require selective stromal exchange after PK. Another study limitation would be the noncomparative design. As we do not routinely perform a repeat conventional PK for post-PK eyes with functional endothelium, a randomized comparative study design for the sole purpose of obtaining higher levels of evidence could not be justified.

In conclusion, the ultrastructural alterations in the stromal microarchitecture of grafted corneas provide evidence of the natural plane of separation identified intraoperatively. Stromal peeling can be successfully performed in post-PK eyes with various stromal pathology, yielding excellent visual outcomes, minimal ECL, and satisfactory graft survival.

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