Deep Lamellar Keratoplasty by Intracorneal Dissection

A Prospective Clinical and Confocal Microscopic Study

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Objective: To evaluate the clinical findings, visual outcomes, and confocal microscopic corneal features of a surgical technique for manual deep lamellar keratoplasty (DLKP) with intentional sparing of the most posterior stroma.

Design: Noncomparative, prospective, 12-month interventional study.

Participants: Forty-six eyes of 45 patients who had corneal pathologic features without endothelial abnormalities and requiring corneal graft were treated by DLKP by manual stromal delamination. They were examined clinically after surgery and using in vivo confocal microscopy at 2 weeks and 1, 3, 6, and 12 months.

Intervention: The surgical technique consisted of an intracorneal deep manual stromal dissection through a 4-mm limbal incision at 50 μm from Descemet’s membrane (DM). After trephination, an endothelial free graft was sutured.

Main Outcome Measures: Topographic parameters, interface depth and reflectivity, and anterior and postinterface keratocyte density; visual acuity was correlated with these parameters.

Results: Two eyes had rupture of the DM. Two eyes that had delayed epithelial healing because of graft override with stromal inflammation underwent a second surgery (penetrating keratoplasty). Mean uncorrected logarithm of the minimum angle of resolution (logMAR) uncorrected visual acuity and logMAR best-corrected visual acuity (BCVA) improved from preoperative values (1.342±0.239 and 0.923±0.226, respectively) to 0.421±0.122 and 0.104±0.068, respectively, at 12 months. Mean topographic astigmatism was 3.09±1.30 diopters (D) at 3 months after suture adjustment, and 2.87±0.92 D at 12 months after suture removal. Average interface depth was 64.2±6.7 μm at 15 days and showed no significant changes up to 12 months. Mean interface reflectivity was highest at 15 days (95.5±15.7 light reflectance units [LRU]) and showed a progressive decrease over time of 55.3±8.7 LRU at 12 months. A significant negative correlation was observed between BCVA and topographic astigmatism up to 1 month and between BCVA and interface reflectivity starting from 6 months after surgery.

Conclusions: Deep lamellar keratoplasty by intracorneal dissection provides visual and clinical results comparable with that of other DLKP techniques. Visual recovery is slow and progressive, taking up to 1 year. Confocal microscopy enables precise evaluation of corneal features, interface morphologic features, and reflectivity, demonstrating a negative correlation between interface reflectivity and BCVA showing that the progressive recovery over months of the interface transparency is correlated with the increase in visual acuity after 6 months. Ophthalmology 2006;113:1289–1300 © 2006 by the American Academy of Ophthalmology.
the interface between the graft cornea and the recipient stromal bed. Deep lamellar keratoplasty (DLKP) has been proposed as a surgical alternative procedure to avoid interfacing the donor cornea without endothelium over the host bed. Deep lamellar keratoplasty presents the advantage of a greater improvement in postoperative visual acuity with respect to LKP and compares favorably with PKP while maintaining the same advantages of LKP.2,7,9 Deep lamellar keratoplasty is indicated in many pathologic features where PKP was previously regarded as the first surgical choice in patients with healthy endothelium and the absence of stromal edema, such as corneal scarring resulting from keratitis, corneal dystrophies, and keratoconus.1,2,10 The main concern regarding DLKP is the difficulty of the surgical procedure: perforation of DM is the main intraoperative risk, especially when DM is completely exposed free of overlying stroma.1 If perforation occurs, even if DLKP can be completed in some cases, the stromal dissection may be difficult, leading to a low-quality corneal bed. Furthermore, when conversion of the procedure into PKP is needed because of significant breaks in DM, good quality endothelium of donor cornea may not be available.1 It is not clear whether a complete stromal excision baring DM provides better visual results than cases in which a small portion of regraft stroma is left undissected. Sugita and Kondo2 reported that leaving a small amount of stroma does not produce differences in visual acuity compared with complete stromal dissection, as long as the deep stroma is healthy. Thus, intentional sparing of the deepest stroma could be helpful in preventing intraoperative DM rupture.

We developed a new kit of instruments for DLKP based on blunt spatula manual delamination, and we named the surgical technique DLKP by intracorneal dissection; this technique intentionally leaves a minimal thickness of deep stroma on the host bed, without the aid of air or fluid in dissection, before suturing the donor button. The theoretical advantages of such a technique are: (1) the reduced risk of intraoperative complications such as DM rupture and the formation of a double anterior chamber, and (2) the lower complexity of the DLKP procedure, generally demanding greater surgical skills to handle correctly the extremely thin DM–endothelium layer.

The purpose of this prospective study was to evaluate at 12 months of follow-up the complication rate, visual outcome, confocal microscopic interface features, and their correlation with visual acuity in a cohort of patients treated with DLKP by manual intracorneal dissection.

**Patients and Methods**

**Patient Population and Clinical Examinations**

Between August 2001, and July 2003, 46 eyes of 45 patients (29 males and 17 females; mean age, 35.6 ± 14.1 years; range, 6–70 years) were enrolled consecutively in this prospective noncomparative study. All eyes underwent deep lamellar keratoplasty (DLKP) with intracorneal dissection technique. Criteria for eligibility were: (1) corneal pathologic features requiring graft without evidence of endothelial impairment; (2) endothelial cell density more than 1800 cell/mm²; and (3) corneal thickness at the thinnest point of more than 300 μm; and (4) absence of other ocular pathologic features such as glaucoma or retinal diseases. The original diseases were keratoconus with contact lenses intolerance (34 eyes), stromal dystrophies (5 eyes), corneal leucoma (5 eyes), and stromal opacity occurring after herpes keratitis (2 eyes). All patients enrolled in the study gave informed consent, and the principles of the Helsinki Declaration and good clinical practice were followed. A complete ocular examination, including uncorrected visual acuity (UCVA) and best-corrected visual acuity (BCVA) evaluation, slit-lamp biomicroscopy, application topometry, fundus examination, computerized topography, specular microscopy endothelial cell density, and ultrasound pachymetry were performed before surgery. Each patient was examined in the postoperative period at 15 days and 1, 3, 6, and 12 months after surgery. Each follow-up visit included UCVA and BCVA, slit-lamp biomicroscopy, endothelial specular microscopy (Noncon Robo-P, Konan, Hyogo, Japan), ultrasound pachymetry (Ophthalmic, Teknar, St. Louis, MO), and in vivo confocal microscopy of the cornea (Confoscan 3, Nidek Technologies, Padova, Italy). Visual acuity measurements were performed using Snellen acuities and were analyzed using logarithm of the minimum angle of resolution (logMAR) equivalents for statistical analysis. Corneal topography was performed using an EyeTop instrument (COS Ophthalmic, Frenze, Italy), and topographic astigmatism was measured using a simulated keratometry value given by the power and axis of the steepest meridian and that of 90°. Surface regularity index (SRI) and surface asymmetry index (SAI) were analyzed to study quantitatively the irregularity of topographic astigmatism.7 Intraoperative and postoperative complications also were recorded.

**Surgical Technique**

All the DLKP surgeries were performed under general anesthesia at the Eye Clinic of the University of Verona. To facilitate and speed up the procedure, we designed a special surgery kit composed of a precalibrated diamond knife (Janach, Como, Italy), a 2.25-mm surgical knife with a round head angled 60° with respect to the handle (BD Edge Ahead Circular knife, BD Ophthalmic Systems, Bidford-on-Avon, United Kingdom), a double-ended spatula (short and long) for stromal tissue delamination (Janach, Como, Italy), a Hanna trephine (Moria, Paris, France), forceps for stromal peeling and for donor button endothelium removal (Janach), and a punch for donor button (Moria). Central and periphere corneal thicknesses were measured using an ultrasonic pachymeter (Ophthalmic, Teknar, St. Louis, MO). The surgical procedure started with a 4-mm limbal incision with a precalibrated diamond knife at the 10-o’clock position. The knife calibration was set to the value of the thinnest corneal thickness minus 50 μm to spare the endothelium, DM, and a thin deep stromal layer. Then a cleavage plane was formed by creating a small stromal pocket with the angled bevel up. Subsequently, this pocket was enlarged with the short dissector starting the deep stroma dissection. The intrastromal cleavage delamination continued with the long dissector to dissect 80% of the stromal extension, or at least extending across an area that covered the central 9-mm diameter. The deep stromal pocket was filled up with low cohesive and low molecular weight viscoelastic (IAL-F, Fidia Farmaceutici S.p.A., Abano Terme, Italy). This kind of viscoelastic was chosen to reduce the risk of endothelium perforation during the trephination of recipient button with Hanna trephine. The recipient button was 7.75 mm in 7 cases, 8.00 mm in 31 cases, and 8.25 mm in 8 cases. A sclerocorneal button stored in organ culture at 31°C and provided by the Eye Bank Fondazione Banca degli Occhi del Veneto (Venezia-Mestre, Italy) was used for the preparation of the donor cornea in all cases. The donor cornea was trephined from the endothelial side with Moria punch with a 0.25-mm bigger diameter.
than the recipient bed. Endothelium and DM were removed from the donor button with dedicated forces. This was carried out by placing the donor button on a sterile plastic support with the endothelial face up. While a Pierce forceps blocked the button, a second dedicated forceps peeled out DM and endothelium. Four interrupted 10-0 nylon sutures (Ethilon Black, Johnson & Johnson, St. Stevens Woluwe, Belgium) at 12, 6, 3, and 9 o’clock secured the corneal button in the recipient bed. Then, a single continuous nylon 10-0 running suture (NU-1, Alcon Laboratories, Inc., Fort Worth, TX) with 20 bites was placed. After removal of the interrupted suture, intraoperative adjustment of astigmatism was performed using the Maloney keratometer (Duckworth and Kent, Baldock, UK). An association of 0.05% formocortal and 0.03% sulphate gentamicin was instilled topically at the end of the surgery. An extended-wear therapeutic soft contact lens (Protek T&S, Contact Vision, Marcon, Italy) was placed on the cornea and the eye was patched.

Postoperative therapy included the topical application 6 times daily of a corticosteroid-antibiotic solution composed of 0.1% bisodio sodium phosphated betamethasone, 0.4% chloramphenicol, 0.421% tetracycline, and 18 000 000 IU of sodic colistimethate. Eye drops were tapered within 3 months after surgery with the following schedule: 6 times daily for 20 days, 5 times daily for 10 days, 4 times daily for 10 days, 3 times daily for 10 days, 2 times daily for 10 days, 1 time daily for 10 days, and on alternate days for the final 20 days.

Suture adjustments were performed when topographic astigmatism was superior to 3 diopters (D) within the third and fourth postoperative week. In case of regression of the effect, postoperative adjustment was repeated for a maximum of 3 times up to 3 months after surgery. Suture removal was performed 6 to 8 months after surgery.

In Vivo Confocal Microscopy

In vivo confocal microscopy (IVCM) was performed using a scanning slit confocal microscope (Confoscan 3). This instrument was equipped with an Achroplan (Zeiss, Oberkochen, Germany) nonapplanating ×40 immersion objective lens designed for full-thickness examination of the cornea, with a working distance of 1.92 mm and a motorized focusing device. The center of the cornea was studied during all examinations. A drop of topical anesthetic 0.4% oxybuprocaine chloride (Novesina, Novartis Farma S.p.A., Origgio, Italy) was instilled before the examination. The patient was seated in front of the microscope, a chin rest and a forehead support were used, and the patient was asked to fix a bright object with the contralateral eye to minimize eye movement during the examination. One drop of 0.2% polyacrylic gel (Viscocitri Gel, CIBA Vision Ophthalmics, Marcon, Venezia, Italy) was applied on the objective tip to serve as an immersion fluid.

Two complete confocal examinations of the entire central cornea were performed for each eye with a total examination time of less than 5 minutes. The Confoscan 3 acquires 350 images per examination at a rate of 25 frames per second, and therefore, the capture time is 14 seconds. The images are stored in memory and directly saved to the hard drive. Each frame is approximately 400×300 μm, with a scanning area of 0.12 mm². The main parameters for the sequence acquisition were set using 3 passes, for each complete corneal examination and a z-axis movement range of 1000 μm, giving a theoretical z-axis distance between images in the scans of 10 μm. The position on the z-axis of the corneal thickness of each image was obtained using the Z-scan function of the instrument. The Z-scan is a graph showing the depth coordinate (expressed in micrometers) on the z-axis and the level of reflectivity (expressed in arbitrary numerical units, called light reflectance units) on the y-axis for each corneal image included in the scan. Because the reliability of the Z-scan graphic is influenced by eye movements during the scan period, only reliable and comparable graphics for each corneal examination were considered for analysis.

Interface was defined as the corneal sublayer located in the posterior stroma with evident discontinuity of the stromal keratocyte and extracellular matrix architecture. The interface depth was calculated as the distance between the proper interface and the endothelial layer. Each value of interface depth was considered as the average of 3 measurements obtained from 3 reliable Z-scan graphics. Interface reflectivity also was calculated as the average of 3 light reflectance unit values obtained by 3 reliable Z-scan graphics.

Mean keratocyte density of anterior and posterior residual stroma was calculated using the analysis software provided with the instrument, which allows manual cell count within a selected region of interest of standardized dimension (0.1 mm²). Keratocyte cell density values of anterior (depth range, 50–100 μm from the basal lamina of epithelium) and residual host stroma (between endothelium and interface layers) were obtained as an average of 5 images per eye.

Confocal microscopy was performed before surgery in all eyes to assess endothelial cell density. Postoperative endothelial cell count was obtained for each eye at every follow-up examination. The endothelial layer was imaged correctly for all patients. The mean of 3 measurements of endothelial cell density was calculated using Confoscan 3 dedicated analysis software and performing a manual cell count processing within a 0.1-mm² standardized region of interest in the central cornea.

Statistical Analysis

Statistical analysis was performed with SPSS 10.1 for Windows (SPSS Inc., Chicago, IL). Data were expressed as mean±standard deviation, and a P value of less than 0.05 was considered statistically significant. Interface depth, interface reflectivity, keratocyte density values, topographic astigmatism, SRI and SAI values, and visual parameters (UCVA, BCVA) at different follow-up examinations were compared using a 1-way analysis of variance. Tukey’s post hoc test was used to detect statistically significant differences between values. The correlation between postoperative visual parameters and interface depth, interface reflectivity, keratocyte density, and topographic parameters was analyzed using Pearson’s correlation.

Results

Graft Clarity and Complications

The surgical procedure was completed in 45 of 46 eyes, and 2 intraoperative complications were observed. In 1 eye, a microperforation of DM occurred during the trephination phase, but this did not affect the subsequent surgical steps, leading to the uneventful completion of the DLKP procedure. This was because the perforation was less than 2 mm and out of the optical zone and because the anterior chamber was maintained by an injection of a high molecular weight viscoelastic. One eye had a bigger perforation during stromal dissection phase requiring conversion of the procedure into PKP. In this case, the DM–endothelium rupture was on the optical zone, and the viscoelastic was unable to maintain the anterior chamber. A full-thickness corneal trephination similar to a standard PKP, followed by the apposition of an entire corneal button sutured with a single continuous running suture, was performed.

Delayed epithelial healing resulting from graft override with stromal inflammation, unresponsive to therapy, occurred within 3
months after surgery in 2 eyes. Both eyes were treated with a subsequent PKP. A mild button haziness and edema were recorded in 1 eye 7 months after DLKP. This complication underwent complete resolution and normal button transparency was achieved within 7 days of medical treatment (dexamethasone 0.2% eye-drops, 6 times daily for the first week and then tapered during the following 8 weeks). No other complications occurred in any eyes over the entire follow-up period.

With respect to graft clarity, an interface mild opacity, visible at slit-lamp examination, was noted in all cases during the first period after surgery. This slight interface haziness presented biomicroscopic clinical characteristics of edema that resolved within 2 to 4 months after DLKP. However, the donor corneal button was found to be biomicroscopically clear in all eyes during the entire follow-up, except in those 2 patients who experienced the above-mentioned postoperative complications. Epithelial pathologic features, such as epithelial edema or microerosions, were not observed in any eye.

**Visual Outcome and Topographic Astigmatism**

In the 43 eyes observed for 12 months, the average preoperative logMAR BCVA was 0.923±0.226, and the mean postoperative logMAR BCVA improved to 0.104±0.068 at the end of follow-up (P<0.001). Average logMAR UCVA improved from a preoperative value of 1.342±0.239 to 0.421±0.122 at 12 months after surgery (P<0.001). In 41 of 43 eyes (95.3%), logMAR BCVA was 0.2 or better, whereas in 30 of 43 eyes (69.7%), it was 0.1 or better, at 12 months. Figure 1 shows the changes in postoperative BCVA and UCVA during the follow-up. A statistically significant improvement of both BCVA and UCVA over time, at each follow-up examination, was observed. Notable relationships between speed of recovery of visual acuity and either age or original disease were not observed.

Mean postoperative corneal topographic astigmatism was 6.86±3.51 D 15 days after surgery, 3.09±1.30 D at 3 months after eventual suture adjustment, and 2.87±0.92 D at 12 months after suture removal. The mean time of suture removal after surgery was 209±62 days. Variations of mean corneal topographic astigmatism, SRI, and SAI over the 12-month follow-up are reported in Table 1. Ninety-one percent of eyes (39 of 43) underwent 1 or more (up to 3) suture adjustments between 15 days and 3 months after surgery. Topographic data at 15 days indicated postoperative astigmatism before suture adjustment and at 1, 3, and 6 months after eventual suture adjustments, and at 12 months after suture removal. A significant overall decrease (P<0.001, analysis of variance) of topographic astigmatism was related to the differences between 0.5 to 1 month and 1 to 3 months after surgery. Surface regularity index and SAI postoperative values showed a significant tendency toward decrease (P = 0.013 and P = 0.004, respectively, analysis of variance). However, the comparison at different time points indicated a significant difference only for SRI between 0.5 and 12 months and for SAI between 0.5 and 6 to 12 months, as showed in Table 1.

**In Vivo Confocal Microscopy**

A deep lamellar interface was identified in all examined eyes at all follow-up examinations. This appeared at confocal examination as a corneal sublayer with unique morphologic characteristics in the
cornea represented by evident discontinuity of stromal cellular and extracellular architecture of the overlying and underlying rear stroma, absence of distinguishable keratocytes, presence of homogeneous reflectivity, variable transparency, and possible presence of bright microdots. In vivo confocal microscopy images of interface morphologic features at different postoperative times are presented in Figure 2; a typical Z-scan graphic showing the interface depth determination is presented in Figure 3.

Mean interface depth (defined as distance from endothelium) at 15 days after surgery was 62.2±6.7 μm (range, 45–74 μm) and remained stable without significant variations during the entire follow-up (Table 2). Mean interface reflectivity values at each examination are reported in Table 2. Interface reflectivity was found to be highest at 15 days and at 1 month (95.5±15.7 and 84.5±8.4 light reflectance units, respectively) and presented a subsequent and progressive decrease, indicating that tissue transparency was regained. Statistically significant changes were observed between 15 days and 1 month and between 3 and 6 months. Morphologically, the cause of the increased reflectivity of the interface, evidenced in the first period after surgery, was likely to be interface edema presenting an accumulation of reflective interface fluid, rather than cellular activation or interface scarring (Fig 2).

<table>
<thead>
<tr>
<th>Time (mos)</th>
<th>Astigmatism (D)</th>
<th>Surface Regularity Index</th>
<th>Surface Asymmetry Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6.86±3.51</td>
<td>2.15±0.94</td>
<td>1.55±0.81</td>
</tr>
<tr>
<td>1</td>
<td>4.91±2.68*</td>
<td>1.83±0.77</td>
<td>1.33±0.60</td>
</tr>
<tr>
<td>3</td>
<td>3.09±1.30†</td>
<td>1.77±0.73</td>
<td>1.24±0.50</td>
</tr>
<tr>
<td>6</td>
<td>2.52±1.03</td>
<td>1.73±0.68</td>
<td>1.18±0.46§</td>
</tr>
<tr>
<td>12</td>
<td>2.87±0.92</td>
<td>1.62±0.64†</td>
<td>1.11±0.42§</td>
</tr>
</tbody>
</table>

D = diopters.
*P<0.001 between 0.5 and 1 month.
†P<0.001 between 1 and 3 months.
‡P = 0.007 between 0.5 and 12 months.
§P = 0.020 between 0.5 and 6 months.
‖P = 0.003 between 0.5 and 12 months (analysis of variance–Tukey post hoc test).

Figure 2. Confocal images of the interface of the same patient at (A) 15 days, (B) 3 months, (C) 6 months, and (D) 12 months after deep lamellar keratoplasty. Note the progressive reduction of haziness and brightness, associated with the recovery of transparency: keratocytes became visible from adjacent layers, as well as some bright microinclusions similar to those visible in LASIK interfaces.
of images of the posterior corneal layers, including interface, after DLKP for keratoconus.

Endothelial cell density values recorded over the follow-up are shown in Table 2. Mean preoperative endothelial cell density was 2565±512 cell/mm², whereas it was 2210±489 cell/mm² at 15 days after surgery (cell loss with respect to preoperative values, 13.8%; \( P < 0.001 \)) and 2135±501 cell/mm² at 1 month (cell loss with respect to 15-day values, 3.4%; \( P = 0.048 \)). Ensuing changes that were detected were not significant.

**Correlations**

Uncorrected visual acuity showed a significant negative correlation with topographic astigmatism during the entire follow-up. A significant negative correlation between UCVA, SRI, and SAI were detected only up to 1 month and 3 months, respectively (Table 3). A significant negative correlation was observed between BCVA and topographic astigmatism, SRI, and SAI up to 1 month after surgery. Best-corrected visual acuity and interface reflectivity values showed a significant negative correlation starting from 6 months (Table 3). Other tested parameters did not show a significant correlation with visual acuity at the follow-up time points.

**Discussion**

Several authors have reported a favorable visual outcome after DLKP.\(^2,7–9,11–17\) Recent results of comparison studies suggest that DKLP can be considered as a safe alternative to PKP in the treatment of different anterior corneal pathologic characteristics with stromal opacity and in keratoconus.\(^7,17,18\) In addition to comparable results in terms of visual recovery, DLKP presents several advantages with
respect to PKP, mainly linked to the preservation of corneal host endothelium, leading to a lower risk of endothelial rejection and late endothelial failure, as well as a greater availability of donor corneas that do not need perfectly healthy endothelium and high endothelial cell density to be suitable for corneal grafting. Other favorable aspects concerning DLKP are represented by a faster stabilization of the wound, leading to earlier suture removal, and a lower risk of open sky surgery-related complications such as endophthalmitis. However, different theoretical disadvantages are thought to influence DLKP procedures. Deep lamellar keratoplasty is technically difficult, demanding a surgeon well-trained in corneal grafts. It is still an evolving technique, lacks a standardization of procedures in the different indicating pathologic characteristics, lacks long-term (years) results, and presents potential interface haze or irregularity, with the ensuing related negative influences on visual performance. Interface scarring leading to poor visual acuity represents one of the greatest reservations that has limited the applications of LKP in the past. The deeper interface obtained with new DLKP techniques has not been associated with a significant incidence of opacification and irregularity occurring at the level of donor–host corneal lamellar interface. This aspect may affect the better visual

Figure 4. Sequential confocal scan images of the central cornea 12 months after deep lamellar keratoplasty for keratoconus. The images represent different corneal layers on the z-axis starting from the endothelial plane toward the posterior stroma (from top left to bottom right). The endothelial layer was evident in the sequence (first line) on top (>1 endothelial image was captured). The posterior residual host stromal bed was clearly imaged in the following frames, and typical stromal keratoconus hyporeflective striae were visible within keratocytes and extracellular architecture (arrowhead). Preinterface and postinterface stromal layers were imaged in the following frames of third and fourth lines. The proper interface (arrow) presented absence of distinguishable keratocytes and presence of bright microinclusions that extend toward the layer adjacent to the interface. Note that the interface was optically clear with low reflectivity. The last 2 frames of the fourth line indicated that the initial posterior stromal layers of donor button evidenced normal architecture of keratocytes and extracellular tissue transparency (star).
Acuity results of DLKP, which presents values comparable with those after PKP.\(^7\)\(^,\)\(^7\)\(^,\)\(^17\)\(^,\)\(^18\)

A possible explanation for the better interface transparency of DLKP over traditional LKP may be the smooth host surface obtained by separating DM from the deepest stroma and the very slight optical disturbance of the donor stromal lamellae.\(^2\)\(^,\)\(^14\) However, it has not been established clearly in literature which reasons are responsible for the different behavior affecting interface transparency of corneal tissues occurring either after LKP or DLKP. A recent report documented that separation may occur between the anterior banded and the posterior nonbanded layer of DM in some cases during DLKP.\(^19\) Several techniques of stromal dissection down to DM have been proposed over the past years to facilitate a safe and efficient deep stromal delamination.\(^20\)

The original article by Archila,\(^11\) followed by other studies,\(^12\)\(^,\)\(^13\) described air injection into the Stromal bed. Other proposed delamination methods include hydrodelamination, in which a saline solution is used as a dissecting fluid,\(^2\)\(^,\)\(^14\) viscoelastic dissection alone or after air injection into the anterior chamber,\(^3\)\(^,\)\(^15\) and the recent big bubble technique based on air injection into the paracentral corneal stroma after partial-thickness trephination.\(^16\) Although providing smoother surfaces, barering the DM as a surgical goal requires that the surgeon deals with a very thin layer of residual host cornea (less than 40 μm), resulting in a variable incidence (between 0% and 39%) of DM perforation.\(^2\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^18\)\(^,\)\(^20\)

Sugita and Kondo, when comparing patients in whom the DM was exposed with patients who retained some portion of non-pathologic deep stroma left during surgery, reported that no differences in visual acuity and endothelial cell density were found in a prospective study during a 12-month follow-up.\(^2\)

It is possible, although not clearly proven in literature, that intentional sparing from dissection of a thin layer of healthy rear stroma during deep lamellar grafts may provide the advantage of reducing the risk of DM puncture, allowing surgeons to perform the delicate phase of deep delamination in a simpler manner.

On the basis of the above-mentioned observations, we performed a standardized surgical technique for lamellar dissection with the aim of obtaining a reproducible DLKP, using spatula-mediated manual deep delamination by which an intended host stromal residual layer of approximately 50 μm is left undissected above the DM–endothelial layer. When performing a dissection in a nontrephined cornea, it is easier to extend the cleavage plane on the entire corneal area creating a wide pocket, which allows a better apposition of the donor button on the recipient bed even in presence of more advanced ectasic pathologic features.

In the present study, we prospectively evaluated visual results, complications rate and interface morphology, over a 12-month follow-up period in eyes that underwent DLKP by intracorneal dissection for keratoconus (34 eyes) or anterior stromal opacity (12 eyes). The main outcome measures chosen for this study were specifically related to the parameters for evaluating donor–host interface characteristics of the graft. The potential for interface haze, although minimal, is considered to be a possible occurrence interfering negatively with visual performance in any kind of corneal lamellar grafts; therefore, an objective method for interface evaluation seemed to us as to be an important issue for at least 2 reasons. First, although a recent study of DLKP reported interface opacification as a relatively infrequent complication (incidence of 8% in the reported 25-patient series),\(^18\) to our knowledge, quantitative methods have not been used to measure interface transparency, and this has been evaluated by using subjective slit-lamp biomicroscopic examination. In fact, interface haze should not be considered as a discrete parameter (i.e., present or not present), but as a continuous variable inducing optical disturbance, which may interfere differently with visual performance with a dependence on opacity location, quantity, and homogeneity. Moreover, the visual acuity obtained in previous studies of DLKP was correlated with interface transparency, which may be a factor influencing visual acuity over time. Second, DLKP using deep delamination but without baring

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### Table 3. Pearson's Correlation between Logarithm of the Minimum Angle of Resolution Uncorrected and Best-Corrected Visual Acuity with Parameters That Showed Significant Correlation at a Minimum of 1 Follow-up Visit

<table>
<thead>
<tr>
<th>Time (mos)</th>
<th>0.5</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>12</th>
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<tbody>
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<td>UCVA</td>
<td></td>
<td></td>
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<tr>
<td>Astigmatism</td>
<td>(r = -0.700)</td>
<td>(r = -0.780)</td>
<td>(r = -0.647)</td>
<td>(r = -0.513)</td>
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<td></td>
<td>(P&lt;0.001)</td>
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<td>(P&lt;0.001)</td>
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<td>SRI</td>
<td>(r = -0.491)</td>
<td>(r = -0.370)</td>
<td>(r = -0.211)</td>
<td>(r = -0.253)</td>
<td>(r = -0.218)</td>
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<tr>
<td></td>
<td>(P&lt;0.001)</td>
<td>(P = 0.011)</td>
<td>NS</td>
<td>NS</td>
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<td>SAI</td>
<td>(r = -0.308)</td>
<td>(r = -0.491)</td>
<td>(r = -0.345)</td>
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<td>(P = 0.037)</td>
<td>(P = 0.001)</td>
<td>(P = 0.019)</td>
<td>NS</td>
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<td>BCVA</td>
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<td>Astigmatism</td>
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<td>(r = -0.352)</td>
<td>(r = 0.270)</td>
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<td></td>
<td>(P&lt;0.001)</td>
<td>(P&lt;0.016)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SRI</td>
<td>(r = -0.561)</td>
<td>(r = -0.540)</td>
<td>(r = -0.235)</td>
<td>(r = -0.198)</td>
<td>(r = -0.203)</td>
</tr>
<tr>
<td></td>
<td>(P&lt;0.001)</td>
<td>(P&lt;0.001)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SAI</td>
<td>(r = -0.602)</td>
<td>(r = -0.405)</td>
<td>(r = -0.222)</td>
<td>(r = -0.232)</td>
<td>(r = -0.212)</td>
</tr>
<tr>
<td></td>
<td>(P&lt;0.001)</td>
<td>(P = 0.009)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Interface</td>
<td>(r = -0.104)</td>
<td>(r = -0.073)</td>
<td>(r = -0.273)</td>
<td>(r = -0.734)</td>
<td>(r = -0.945)</td>
</tr>
<tr>
<td>Reflectivity</td>
<td>NS</td>
<td>NS</td>
<td>P = 0.047</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
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</table>

BCVA = best corrected visual acuity; NS = not significant; SAI = surface asymmetry index; SRI = surface regularity index; UCVA = uncorrected visual acuity.
DM theoretically may present greater problems of interface optical quality (being somewhat similar in principle of interface haze generation to LKP) than DLKP with DM exposure.

In vivo confocal microscopy has been used widely in recent years as a valid diagnostic technique for the in vivo microscopic imaging of corneal structure, both in healthy and pathologic corneas. \(^2\) One of the fields of application in which IVCM was shown to be a potentially useful tool is that of corneal refractive surgery. \(^2\) In vivo confocal microscopy has been used to evaluate corneal structure and morphologic features in LASIK-treated corneas. \(^2\)–\(^8\)–\(^2\)–\(^4\) Specifically, IVCM has been shown to be effective in the microscopic imaging of the LASIK flap–stroma interface, allowing identification of the interface layer, which is characterized by the presence of microscopic interface bright particles, \(^2\)–\(^4\)–\(^5\)–\(^7\)–\(^8\)–\(^1\) variable reflectivity (low to high), \(^2\)–\(^4\)–\(^7\)–\(^8\)–\(^1\) and disappearance of keratocytes around the wound interface. \(^2\)–\(^4\)–\(^7\)–\(^8\) In addition, IVCM allowed flap thickness and residual stromal thickness measurements by using confocal microscopy sequences providing light intensity profiles or Z-scan graphics, through the z-axis (anteroposterior) distance calculation between the identified interface layer and epithelium or endothelium. \(^2\)–\(^4\)–\(^7\)–\(^8\)

In our study, IVCM was used to evaluate interface parameters (depth and reflectivity), as well as keratocyte and endothelial cell density over a 12-month follow-up. We also evaluated the clinical outcome (complication rate and visual acuity) and topographic parameters that could affect visual results. Correlations between visual outcome and detected topographic and confocal microscopy interface data also were investigated.

In our series of 46 eyes, 2 DM intraoperative ruptures occurred during the delamination phase. The larger rupture required conversion into PKP. Two other DLKP procedures failed after surgery because of delayed epithelial healing resulting from graft override with severe stromal inflammation and needed subsequent PKP within 5 months of the first surgical procedure. The eyes that underwent PKP were excluded from data analysis. The incidence of DM split in our group was 4%, and this frequency is consistent with the most favorable outcome reported in previous studies. \(^2\)–\(^7\)–\(^8\)–\(^1\)–\(^4\)–\(^7\) Recent reports from Watson et al \(^1\) and from Shimazaki et al \(^7\) documented a DM rupture in 15% of the eyes treated with DLKP with DM exposure. The relatively lower incidence of DM breaks that occurred in our study may be related to the presence of the deepest stromal layers left undissected above the endothelium, which may protect DM from surgical damage during the dissection phase.

The logMAR UCVA and BCVA after surgery improved to average values of 0.421 and 0.104, respectively, at 12 months. The logMAR BCVA was 0.2 or better in 95% of eyes, and 0.1 or better in 70% of eyes at the end of follow-up. These findings are similar to those of other studies investigating visual outcome after DLKP for keratoconus and other anterior corneal pathologic features. \(^2\)–\(^7\)–\(^9\)–\(^1\)–\(^7\)–\(^8\) As already mentioned, Sugita and Kondo, \(^2\) in their clinical report on DLKP that included 120 eyes with various corneal diseases other than keratoconus, found that there were no differences in BCVA at 12 months between the 2 subgroups of patients (DM exposed or some deep stromal layers left undissected above the endothelium). Moreover, Shimazaki et al \(^7\) in their DLKP series for corneal pathologic features, except from keratoconus, with 24-month follow-up observed that visual acuity stabilized within 6 months after surgery and that no significant differences were observed during follow-up between these values and PKP group visual acuity score. However, in our patients, visual recovery was slow and progressive, with continuous increments over time, as shown in Figure 1.

The results of this study thus indicate that visual acuity outcomes, obtained with intentional sparing of the most posterior stromal layers, are comparable with other DLKP techniques baring the DM, confirming the observation by Sugita and Kondo, \(^2\) but that visual recovery requires at least 6 to 12 months to reach satisfactory levels. Over the 12-month follow-up, we evaluated topographical parameters and interface microscopic morphologic parameters that could affect visual performance over time.

Although starting from mean postoperative values of nearly 7 D, before suture adjustment, the average topographic cylinder at the end of follow-up was less than 3 D after suture removal. Final astigmatism in our series was comparable with or slightly lower than other reported data obtained using DLKP. \(^7\)–\(^9\)–\(^1\)–\(^7\)–\(^8\)–\(^1\) and these findings confirm the efficacy of suture adjustment to control astigmatism occurring after DLKP. \(^1\)–\(^3\) as was observed for PKP. \(^3\)–\(^1\) Topographic index SRI, a measure of local fluctuations in central corneal power, and SAI, a measure of the differences in corneal power at every ring over the entire cornea, evaluated at all follow-up examinations, tended to decrease over time. Surface regularity index and SAI values observed at 12 months corresponded to results reported in other studies of DLKP \(^1\) and PKP. \(^3\)

To our knowledge, this study is the first investigation reporting corneal confocal microscopic morphologic and interface features in DLKP. As previously explained for LASIK, IVCM allowed easy identification of the interface layer in DLKP corneas. Deep stromal interface appeared as an atypical stromal layer characterized by discontinuity of tissue and cellular stromal architecture, absence or reduced density of keratocytes, and variable background extracellular reflectivity. Data obtained from averaged Z-scan interface depth measurements revealed that the mean depth (distance of the interface from the endothelium) was 60 \(\mu m\) (range, 45–74 \(\mu m\)) 2 weeks after surgery and remained unchanged over the entire follow-up period. These results indicate a reliable depth standardization achievable with the technique used. This presumably suggests that significant processes of stromal remodeling or deep stromal rethickening behind the interface do not take place in DLKP. Moreover, in corneas that underwent surgery for keratoconus, the images of residual stromal bed showed a characteristic presence of hyporeflective linear striae (Fig 4) that were present before surgery, which have been shown to be typical confocal microscopic features of panstromal involvement in keratoconus. \(^3\) This aspect further confirms that the deep stroma under the interface persists without significant morphologic changes, to the extent observable by IVCM. However, it was reported previously that recurrent corneal ecta-
sia after DLKP for keratoconus is a rare but potential late-onset complication that may be related to donor or host factors, and IVCM assessment of preinterface and postinterface stromal morphologic features and thickness could improve the understanding of stromal changes in case of recurrent keratoconus. Interface morphologic features and reflectivity showed notable variations during the year of follow-up as opposed to host residual stromal layers. Mean interface reflectivity values (Table 2) were highest at 2 weeks and 1 month and showed a continuous decrease over time, reaching reflectivity levels comparable with normal rear stromal values at 12 months. In all cases, no (or rare) distinguishable keratocytes or myofibroblasts were detected within the interface layers. Interface brightness apparently was the result of extracellular diffuse and discretely homogeneous reflectivity, compatible with edematous fluid accumulation. This interface extracellular opacity tended to clarify over months (Fig 2), demonstrating a progressive gain of transparency of the stroma at the interface layer. The reabsorption of interface fluid is the hypothesis that best describes the reduction of interface reflectivity observed in our study, and apparently no visible scarring features mediated by activated keratocytes or myofibroblast were found at the interface level during the follow-up period. However, further in vivo micromorphologic studies, with a longer follow-up, could clarify if such scarring phenomena may affect deep stromal interfaces at late postoperative periods. In the present study, interface morphologic features at 6 and, particularly, at 12 months after surgery showed a stromal layer with low reflectivity and lack of keratocyte cells. These features were similar to normal LASIK interface microscopic morphologic features. Anterior and posterior stroma (behind the interface) did not show significant opacification, loss of transparency of the extracellular lamellae, or relevant keratocyte activation or inflammation. Anterior and posterior keratocyte density were within normal limits without significant changes over the 12-month follow-up. Thus, the only corneal sublayer that evidenced variations in transparency was the proper donor–host interface. Epithelial morphologic features, nerve fiber regeneration, and button stromal cellular architecture were not investigated in this study, but could be interesting parameters to evaluate in future studies to understand more thoroughly the microscopic behavior of tissues in DLKP-transplanted corneas. By using the surgical technique presented in our study, a certain degree of interface opacity occurring within the first months after surgery was a constant behavior of DLKP wound healing and should not be considered a complication. We did not observe severe or persistent interface opacity limiting the recovery of visual acuity. Also, slit-
lamb examination allowed the visualization of a mild inter-
face haziness mainly up to 3 months after surgery. How-
ever, biomicroscopic evaluation was not effective in
detecting a fine degree of opacity (at 6 months after surgery, 
almost all interfaces appeared clear at slit lamp) and did not 
permit quantification or grading of this interface parameter 
as objectively as IVCM.

Correlation analysis of visual with other measured pa-
rameters showed that interface reflectivity presents a sig-
nificant negative correlation with BCVA starting from 3 
months, when the effect of astigmatism and topographic 
parameters was reduced. In fact, a significant negative cor-
relation was detected between UCVA and topographic 
astigmatism during the entire follow-up as expected, and 
between UCVA and SRI and SAI up to 1 and 3 months, 
respectively. As opposed to BCVA, which showed only 
slight but significant negative correlations with topographic 
astigmatism, SRI and SAI during the first month after sur-
gery showed significant correlations, suggesting that the 
reduction of astigmatism and the amelioration of topo-
graphic indexes after suture adjustment decrease the influ-
ence of these parameters on corrected visual acuity. How-
ever, we detected significant correlation between BCVA 
and interface reflectivity only between 3 and 12 months. 
This may indicate that interface transparency plays a role in 
visual recovery, which becomes more evident when the ef-
fects of other influencing parameters, such as irregular astig-
matism, have been controlled. As summarized in Figure 5, 
average BCVA continued to increase after the stabilization of 
astigmatism at 3 months, with a parallel decrease of interface 
reflectivity.

In our series, endothelial cell density showed a signifi-
cant reduction between preoperative values and those ob-
tained 15 days after surgery (13.8% cell loss) and a further 
decrease between 15 days and 1 month (3.4% cell loss), but 
farther significant decreases were not observed. These re-

cuits are consistent with other studies that reported that 
DLKP reduces endothelial cell density by approximately 
10% at 1 year, by half as much as PKP, and that 
endothelial cell loss occurs principally during the first post-
operative period, followed by a physiological cell decre-

Deep lamellar keratoplasty induces a lower 
postoperative endothelial cell loss, does not lead to an 
accelerated endothelial decrement, and theoretically is free 
from endothelial rejection, thus presenting the advantage of lower risk of graft failure resulting from endothelial 
causes.

In conclusion, our study documented that morphologic 
characteristics of the donor–host interface after DLKP, as-
sessed by means of IVCM, present significant functional 
correlation with visual performance after surgery.

Deep lamellar keratoplasty by intracorneal dissection, in 
our series, resulted in a good visual improvement, compa-
rable with PKP or other DLKP techniques, and presented a 
limited incidence of intraoperative complications (DM rup-
ture). Visual recovery takes several months, and astigmatism 
and interface transparency are the main factors involved. 
Our study documented that morphologic characteristics of the 
 donor–host interface after DLKP, assessed by IVCM, 
present significant functional correlation with visual perfor-
ance after surgery.

After the 3-month follow-up, no eyes had substantial 
irregular astigmatism (severely interfering with the visual 
acuity), but progressive reduction of interface reflectivity 
was correlated with subsequent BCVA increases up to 1 
year after surgery. In vivo confocal microscopy is a nonin-
vasive method for quantitative evaluation of interface trans-
parency in lamellar grafts that could be applied in the other 
DLKP techniques to find possible differences.

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