A TWO-PIECE MICROKERATOME-ASSISTED MUSHROOM KERATOPLASTY IMPROVES THE OUTCOMES AND SURVIVAL OF GRAFTS PERFORMED IN EYES WITH DISEASED STROMA AND HEALTHY ENDOTHELIUM (AN AMERICAN OPHTHALMOLOGICAL SOCIETY THESIS)

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ABSTRACT

Purpose: To test the hypothesis that a new microkeratome-assisted penetrating keratoplasty (PK) technique employing transplantation of a two-piece mushroom-shaped graft may result in better visual outcomes and graft survival rates than those of conventional PK.

Methods: Retrospective chart review of 96 eyes at low risk and 76 eyes at high risk for immunologic rejection (all with full-thickness central corneal opacity and otherwise healthy endothelium) undergoing mushroom PK between 2004 and 2012 at our Institution. Outcome measures were best-corrected visual acuity (BCVA), refraction, corneal topography, endothelial cell density, graft rejection, and survival probability.

Results: Five years postoperatively, BCVA of 20/40 and 20/20 was recorded in 100% and over 50% of eyes, respectively. Mean spherical equivalent of refractive error did not vary significantly over a 5-year period; astigmatism averaged always below 4 diopters, with no statistically significant change over time, and was of the regular type in over 90% of eyes. Endothelial cell density decreased to about 40% of the eye bank count 2 years after mushroom PK and did not change significantly thereafter. Five years postoperatively, probabilities of graft immunologic rejection and graft survival were below 5% and above 95%, respectively. There was no statistically significant difference in endothelial cell loss, graft rejection, and survival probability between low-risk and high-risk subgroups.

Conclusions: Refractive and visual outcomes of mushroom PK compare favorably with those of conventional full-thickness keratoplasty. In eyes at high risk for immunologic rejection, mushroom PK provides a considerably higher probability of graft survival than conventional PK.

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INTRODUCTION

In the last decade keratoplasty has undergone a true revolution through the development of new lamellar techniques selectively addressing the diseased part of the cornea, be it the stroma or the endothelium.[1-3] However, to date, no alternative to penetrating keratoplasty (PK) is possible when deep stromal scars of various origin may also affect the central Descemet membrane and endothelium (ie, infections due to viruses, bacteria, fungi, or protozoa; traumata; hydrops in keratoconus).

Since its popularization in the 1960s,[1] the technique of conventional PK has remained basically the same, aiming at suturing a perfectly round, full-thickness donor disc into an equally perfectly round, full-thickness "hole" made in the recipient cornea. All the variables involved in PK surgery have undergone refinements over time with the purpose of improving the outcomes of this procedure and broadening its indications. However, the results of PK still have many limitations, mainly regarding both the residual postoperative refractive error[4-7] and the long-term survival of the graft.[8-10]

POST-PK REFRACTIVE RESULTS

The vertical full-thickness wound of a PK tends to open spontaneously under the effect of the intraocular pressure in the absence of a scar strong enough to counteract it. At least a full year is usually necessary for the PK wound to heal sufficiently, as the surfaces of contact between which the scar tissue is built are relatively limited.[11] In addition, even after suture removal is completed, up to 30% of eyes still have an astigmatic error that cannot be corrected adequately with spectacles and require therefore the use of hard contact lenses or additional surgery.[12] High-degree spherical errors are much less frequent, but can still be seen, especially in eyes with preoperative abnormal curvature,[13] such as keratoconic ones, or if major oversizing and undersizing of donor tissue are employed.

Many approaches have been taken to reduce post-PK refractive error. Modifications of the trephination techniques, including those using excimer or femtosecond lasers,[14,15] were aiming at perfecting the wound edges on both donor and recipient side. However, all of them still necessitated manual cutting with corneal scissors to complete the full-thickness incision and thus were unable to eliminate the variability related to this maneuver. Similarly, the use of different suture materials or suturing patterns has not been found to affect significantly post-PK refraction after suture removal is completed.[16-20] Instead, oversizing the donor disc by 0.25 or 0.50 mm has been proven to reduce the postoperative complication rate,[21] and an inverse relationship between graft size and postoperative astigmatism/surface regularity has been documented previously.[22-24] Nevertheless, grafts larger than 8.0 to 8.25 mm are related to a higher incidence of immunologic rejection, even in eyes at low risk, and are not employed unless strictly necessary (i.e, therapeutic PK for extensive corneal infections unresponsive to conservative treatment).[25]

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POST-PK GRAFT SURVIVAL: THE ROLE OF RECIPIENT ENDOTHELIUM

Long-term graft survival after PK varies according to preoperative indications between more than 80% and less than 50%.[26,27] Failure has been related to many factors, including immunologic rejection and slow decay of endothelial density.[28] Corneas with neovascularization, typically after keratitis of various origins, are at high risk for immunologic rejection because of the loss of immunologic privilege related to avascularity.[27-29] In eyes with herpetic vascularized scars, post-PK recurrence of the disease was considered in the past an important additional threat to graft survival.[30] Systemic antiviral prophylaxis has minimized post-PK herpetic recurrences[31,32] and possibly reduced the risk of immunologic rejection.[32]

Progressive donor endothelial cell loss (ECL) after PK has been documented in several publications and has been explained with various theories. The fact that this phenomenon is more pronounced after PK for bullous keratopathy than after PK for keratoconus suggests the positive effect of a healthy recipient endothelium on the long-term survival of donor tissue.[33-39] Migration from areas with high endothelial cell density to areas with low density is well documented and also takes place postoperatively across the PK wound[40-43] and the posterior corneal surface in general.[44] As a consequence, endothelial density decreases more rapidly in a graft transplanted into a "low density" recipient (ie, bullous keratopathy), thus considerably shortening its survival time, than it does in a "high density" recipient (ie, keratoconus). As shown schematically in Figure 1, transplantation of a full-thickness graft as small as 6 mm in diameter leaves in place approximately 75% of the recipient endothelium in a normally sized cornea. Theoretically, in this situation endothelial migration from a healthy peripheral residual bed across the PK wound could compensate even for a total loss of donor cells. Survival of rather small grafts (6 to 7 mm in diameter) even 30 years after PK for keratoconus is well documented in the ophthalmic literature[45] and can be easily explained by endothelial cell migration.[46] In the first half of the 20th century, very small grafts (5 mm) were transplanted in vascularized corneas.[47] High-degree irregular astigmatism did not allow sufficient improvement of vision, but these grafts survived much longer than larger ones[48,49]: besides the immunologic advantages (less immunogenic load, no antigen-presenting cells in the central cornea), they may have benefitted from migration from the peripheral healthy recipient endothelium to replace donor cells affected by immunologic rejection. With time, a diameter between 8.00 and 8.25 mm has been considered the optimal compromise for sizing full-thickness grafts in order to minimize postoperative refractive error and risk of immunologic rejection while transplanting a sufficient amount of donor endothelium.[48-50]

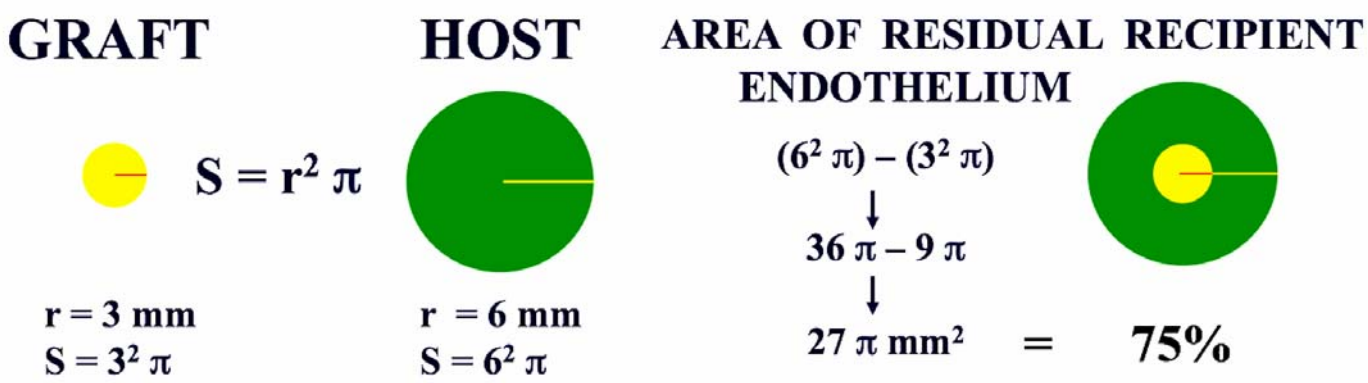

FIGURE 1

Schematic representation of the relationship between the surface of a graft, 6 mm in diameter, and the total surface of the recipient cornea. Transplantation of a 6-mm graft results in preservation of about 75% of recipient endothelium. S, surface; r, radius.

SHAPED WOUND FOR PK

Shaped wounds for PK were introduced in the past by several investigators,[51-57] with the main purpose of improving refractive results by optimizing the "match" between donor disc and recipient bed. However, the use of shaped wounds was soon abandoned because of both the very limited advantages observed and the technical difficulties.

The interest in shaped wounds for PK was revived in 2003 by the publication of a relatively simple method to manually prepare and transplant a "top-hat" graft into a recipient bed dissected to fit with the shape of the donor disc (Figure 2).[58] Among the advantages of this technique were the reduced time required for wound healing and the possibility of transplanting an endothelial layer 9.00 mm in diameter, while limiting the replacement of the recipient surface to the central 7 mm, thus minimizing the exposure to immunologic stimulation (a lower density of antigen-presenting cells and greater distance from limbal vessels).

Since then, the improvement in femtosecond laser technology has led to a more widespread use of shaped wounds for PK.[14,59-61] All new designs (Figure 3) were introduced to improve the fitting between donor disc and recipient bed (top-hat, "mushroom," "zig-zag" or "Christmas tree" pattern) in an attempt at reducing the time necessary for wound healing as well as postoperative refractive error, while increasing the resistance to trauma.[62] The top-hat design was intended to also address the advantage of supplying a larger

amount of donor cells to eyes with decompensated endothelium.[63] In contrast, the purpose of a mushroom PK was to minimize replacement of recipient healthy endothelium (typically keratoconic eyes) while maintaining a large diameter of the superficial, refractive part of the graft, thus optimizing the postoperative refractive result.[64] However, femtosecond laser–assisted preparation of a recipient bed in scarred and often irregular corneas has major limitations. Most of all, the laser does not penetrate sufficiently through nontransparent tissue and does not work if bleeding occurs (i.e, cutting through vascularized corneas). Also, if applanation is required, as with most of the commercially available femtosecond lasers, a regular dissection plane will be obtained only if the corneal thickness is homogeneous, which is usually not the case if ulceration has occurred. Finally, in most eyes the pupil and the corneoscleral limbus are not concentric. Therefore, to center the cap of the mushroom with the corneoscleral limbus and the stem with the pupil would require a precise decentration of the stem portion, in both donor and recipient corneas.

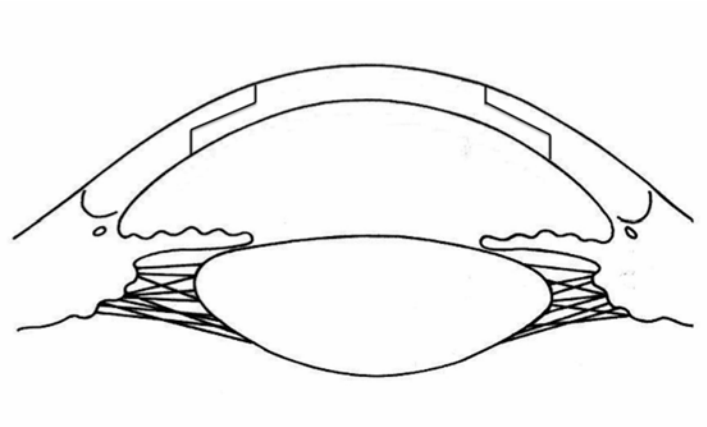

FIGURE 2

Schematic representation of a "top-hat" penetrating keratoplasty.

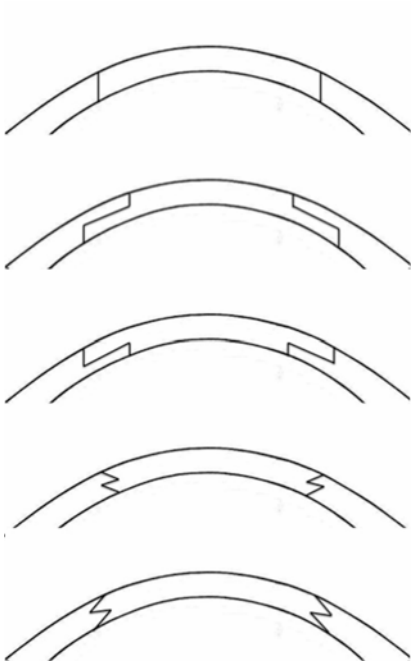

FIGURE 3

Schematic representation of conventional wound and various types of shaped wound for penetrating keratoplasty (from top to bottom): conventional vertical, "top-hat," "mushroom," "zigzag," and "Christmas tree."

TWO-PIECE MUSHROOM PK

A rather simple way to perform mushroom keratoplasty was described in 2005.[65] Manual dissection was used to prepare the recipient bed, and the donor cornea was split into posterior and anterior lamellae by means of microkeratome dissection. These were punched to proper size and reassembled on top of each other to obtain the final mushroom configuration (Figure 4). Dividing the mushroom into anterior and posterior lamellae has the theoretic disadvantage of creating an interface. However, the optical quality of the central part of the interface, since it was obtained with the microkeratome, is compatible with 20/20 vision, and the peripheral part, although rather irregular due to the hand dissection, does not interfere with vision and promotes stronger and faster wound healing.

Moreover, this method overcomes the limitations of the femtosecond-assisted one-piece mushroom PK, as manual dissection can be performed on a nontransparent cornea and at a relatively consistent plane. Therefore, surface irregularities and possible bleeding from vascularized tissue can be managed with ease. A two-piece mushroom PK also allows easy differential centration of the anterior and posterior parts, the former being usually centered on the corneoscleral limbus and the latter on the visual axis. Finally, as no completion with corneal scissors of the 9.0-mm external part of the surgical incision is required, fitting of the anterior lamella into the recipient bed is optimized, thus possibly reducing postoperative refractive astigmatism.

This technique was performed for the first time in an eye with a central perforating corneal injury but an otherwise healthy endothelium. Since then, its indication has been broadened to include eyes with full-thickness scars of any origin, affecting the visual axis, but with healthy peripheral endothelium. Among these were also corneas at high risk for failure after conventional PK, because

of either vascularization or previous herpetic and amoebic infection.[66] Surface irregularity has not been considered a contraindication to the procedure.

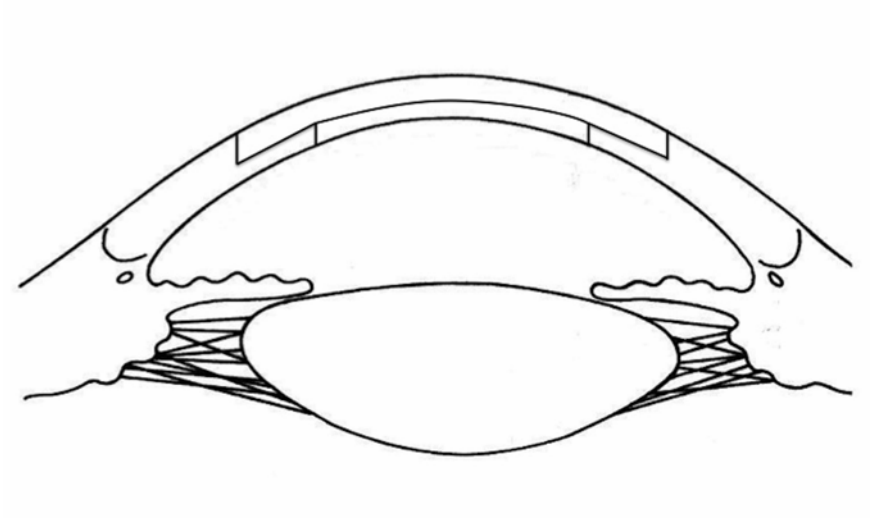

FIGURE 4

Schematic representation of a two-piece mushroom penetrating keratoplasty.

This thesis presents the results obtained in all eyes undergoing a two-piece mushroom PK at our Institution since the introduction of the technique and tests the hypothesis of improved clinical outcomes and graft survival rates in comparison with conventional PK, in eyes at low and high risk for immunologic rejection.

METHODS

The records of all patients undergoing mushroom PK by the same surgeon (M.B.) at "Villa Serena–Villa Igea" private Hospitals (Forlì, Italy) from January 2004 through December 2012 were reviewed retrospectively and included in a noncomparative, nonmasked, interventional case-series. The institutional review board of Villa Serena–Villa Igea Hospitals reviewed and approved the study (approval protocol No. 13/2012). All patients read and signed an informed consent document for the treatment, accumulation of data, and participation in the research. The study was conducted in accord with Good Clinical Practice guidelines, the Declaration of Helsinki, and in conformity with all Italian laws.

The indication for this type of procedure was the loss of visual acuity due to full-thickness corneal scars (with or without vascularization) involving the optical zone, in the presence of otherwise healthy endothelium. In addition, for eyes with post-herpetic scars, surgery was not performed until a period of 6 months or longer without episodes of reactivation of the underlying disease or inflammation had elapsed.

Corneal vascularization was evaluated by slit-lamp examination and rated according to extension (number of clock hours involved) and depth (superficial or deep stroma). Eyes were assigned to the low-risk or high-risk category according to the criteria used in the Collaborative Corneal Transplant Studies.[67] Figure 5, left, portrays an eye at low risk for immunologic rejection (keratoconus with hydrops) and Figure 5, right, an eye at high risk because of the presence of deep stromal vessels (post-herpetic scar) in 2 or more corneal quadrants.

All surgical procedures were performed in a standard fashion as described in detail below. A complete ophthalmological evaluation, including slit-lamp examination, best-corrected visual acuity (BCVA), and refraction, was performed preoperatively as well as at 6 months and 1, 2, 3, 4, and 5 years postoperatively. Snellen BCVA was converted into the logarithm of the minimum angle of resolution (LogMAR) units for statistical analysis. Patients with preexisting comorbidities and failed mushroom PK were considered separately in the visual outcomes analysis.

At each postoperative examination time (6 months, 1 year, and annually thereafter), graft survival probability, cumulative probability of a rejection episode, and endothelial cell density (EM-3000; Tomey Gmbh, Erlangen, Germany) were determined (including subgroup analysis for low-risk and high-risk eyes); corneal topography (EyeSys 2000; EyeSys Technologies, Inc, Houston, Texas) was also performed to differentiate regular patterns (symmetric and asymmetric "bow-tie" patterns as shown in Figure 6, left and middle) from irregular patterns (all others, an example of which is shown in Figure 6, right). Postoperative endothelial cell density was evaluated centrally (i.e, in the area of the posterior donor button); the values recorded were compared with those obtained preoperatively from the eye bank, thus considering the ECL as a percentage of the preoperative in vitro value, as described in a previous report from our group.[68]

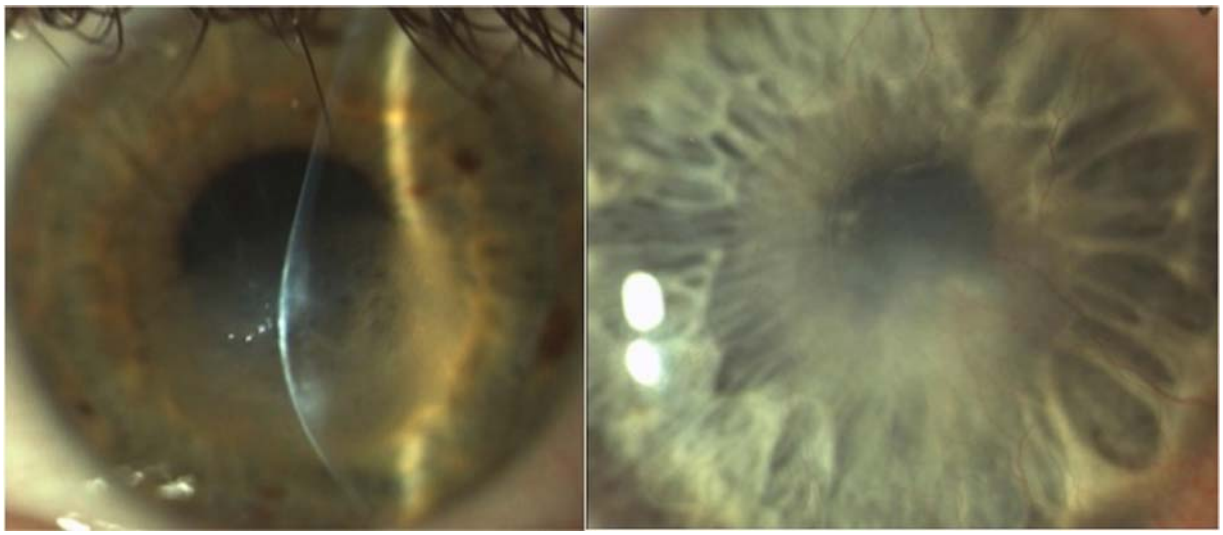

FIGURE 5

Slit-lamp appearance of an eye at low risk for immunologic rejection (keratoconus with hydrops) on the left and an eye at high risk (postherpetic scar with more than two quadrants of deep corneal vascularization) on the right.

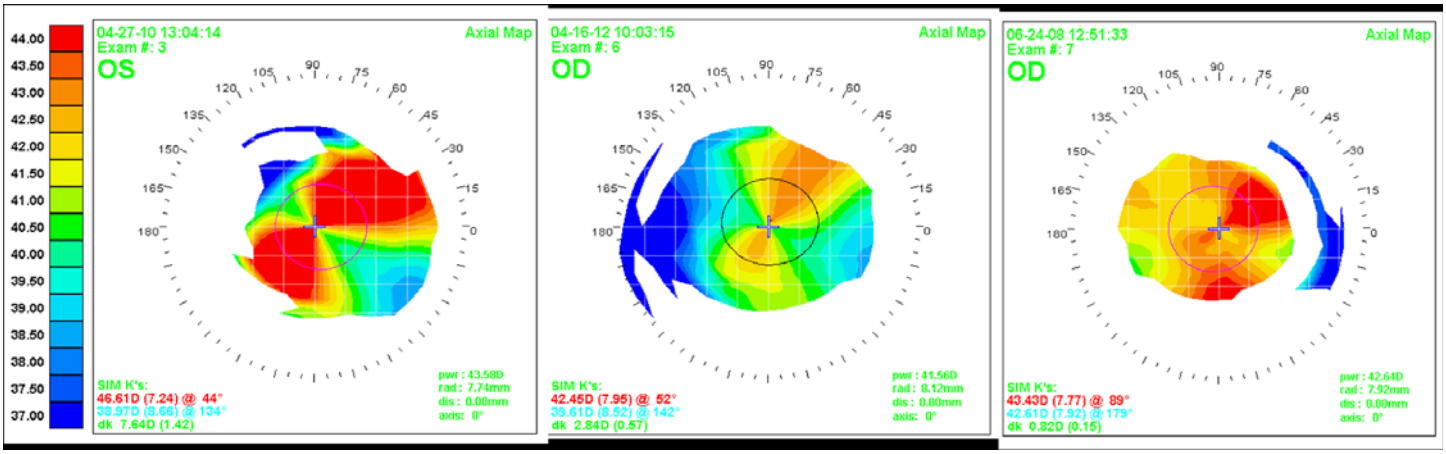

FIGURE 6

Topographic patterns of astigmatism occurring after mushroom penetrating keratoplasty: symmetric "bow-tie" (left), asymmetric "bow-tie" (middle), and irregular (right).

STATISTICAL ANALYSIS

Normally distributed variables were reported as mean ± standard deviation and were compared using a two-tailed Student *t* test. *P* values less than .05 were considered statistically significant. Kaplan-Meier survival analysis was performed to determine graft survival probability and cumulative probability of a rejection episode. The log-rank test was used to compare the curves obtained for the high-risk group with those obtained for the low-risk group.

SURGICAL TECHNIQUE OF MUSHROOM PK

Surgery was performed with all patients under local anesthesia except for those too young to cooperate, who therefore received general anesthesia. Intravenous propofol (3 to 5 mL) was given immediately prior to local anesthetic injection. Local anesthesia was obtained with a peribulbar injection of a mixture of lidocaine hydrochloride 2% and bupivacaine hydrochloride 0.5%, combined with 100 IU of hyaluronidase. Surgery was performed with the surgeon sitting at the 12-o'clock position. Initially, a Barron suction trephine (Katena Products Inc, Denville, New Jersey) was used to create a circular incision, 9.0 mm in diameter and approximately 250 µm in depth, centered in the recipient cornea (Figure 7, top left). Then a manual lamellar dissection was performed circumferentially from the base of the incision about 3 mm toward the center of the cornea (Figure 7, top middle). To facilitate

dissection, cauterization at the limbus was performed, but only when bleeding from the corneal neovessels did not subside spontaneously. The dissected tissue was removed, leaving in place a central island of full-thickness corneal tissue, about 3 mm in diameter (Figure 7, top right). A trephine 6.0 mm in diameter was used to make a full-thickness circular incision in the residual recipient bed, taking particular care to center the incision on the pupil (Figure 7, bottom left). The central button was then excised with corneal scissors (Figure 7, bottom middle and right).

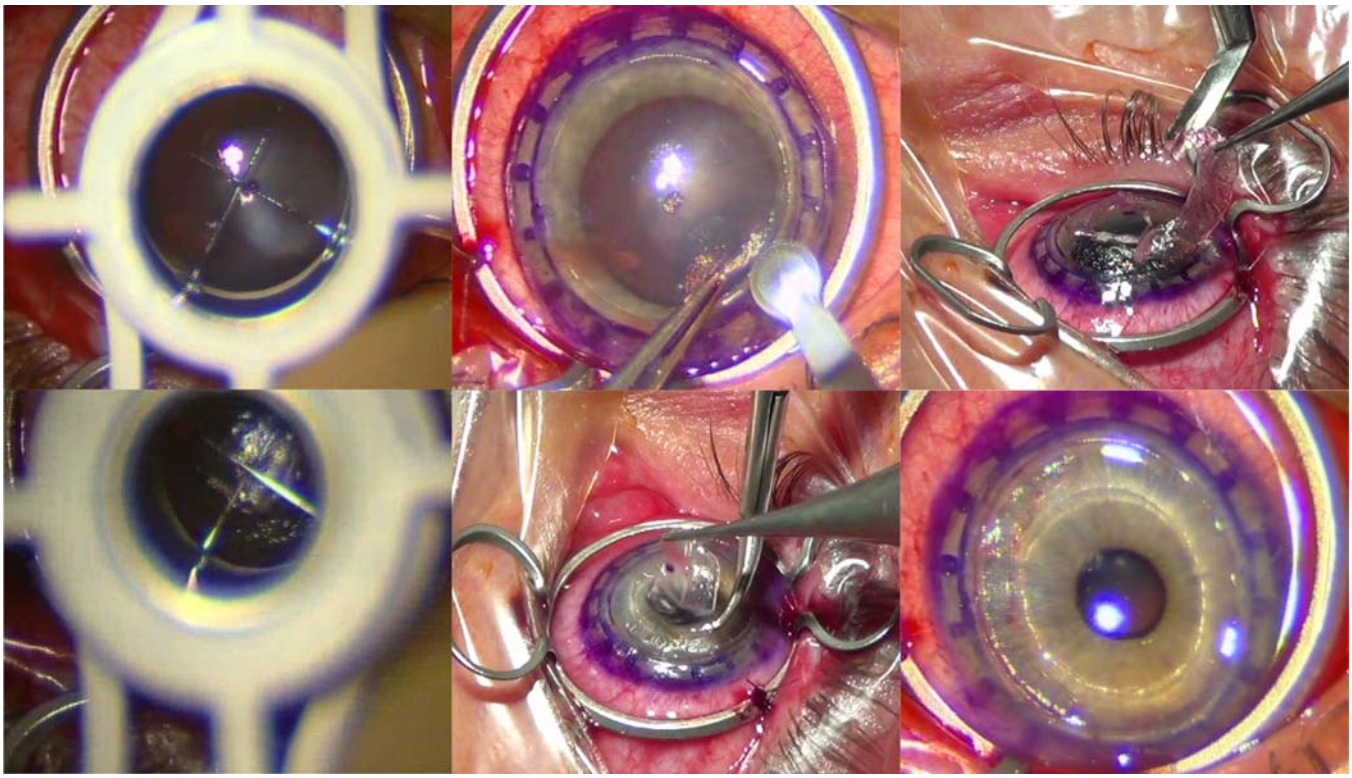

FIGURE 7

Preparation of the recipient bed: Trephination, 9.0 mm in diameter and approximately 250 μm in depth, centered on the recipient corneoscleral limbus (top left); manual lamellar dissection performed circumferentially from the base of the incision toward the center (top middle); removal of the dissected tissue (top right); full-thickness trephination, 6.0 mm in diameter, centered on the pupil (bottom left); excision of the central button with corneal scissors (bottom middle and right).

The donor cornea was mounted on the artificial anterior chamber of the automated lamellar therapeutic keratoplasty system (ALTK; Moria SA, Antony, France). A 200-μm head was used to split the donor cornea into anterior and posterior lamellae (Figure 8, top left). The selection of this head was based on previous reports,[69] showing that it dissects at a level about 20% deeper than the nominal 200-μm value, thus yielding a lamella that approximates the ideal match for the removed tissue. As shown in Figure 8 (top middle and right), both lamellae were then punched to proper size, in order to fit with the recipient bed prepared previously (same size of the recipient bed was used for both the anterior and the posterior lamellae, i.e, 9.0 and 6.0 mm, respectively).

When necessary, before reassembling the mushroom graft, additional intraocular procedures were performed with an "open sky" technique (i.e, extracapsular cataract extraction, intraocular lens [IOL] implantation or exchange, pupilloplasty, anterior vitrectomy).

The donor stem (endothelium and deep stroma) was then fitted into the central hole of the recipient bed without sutures (Figure 8, bottom left). The donor anterior lamella was then placed on top of the posterior one and sutured into position with 4 cardinal 10-0 nylon stitches (Figure 8, bottom middle). Surgery was completed with either a double running 10-0 nylon suture (Figure 8, bottom right) or 16 interrupted 10-0 nylon sutures. In the donor the bites of the sutures were passed only through the anterior lamella, leaving the posterior one free to adapt. Finally, the anterior chamber was filled with balanced saline solution injected with a 30-gauge needle through a limbal puncture. Gentamicin sulfate (40 mg) and methylprednisolone acetate injectable suspension (40 mg) were injected subconjunctivally; ointment with tobramycin sulfate 0.3% and dexamethasone phosphate 0.1% was applied topically.

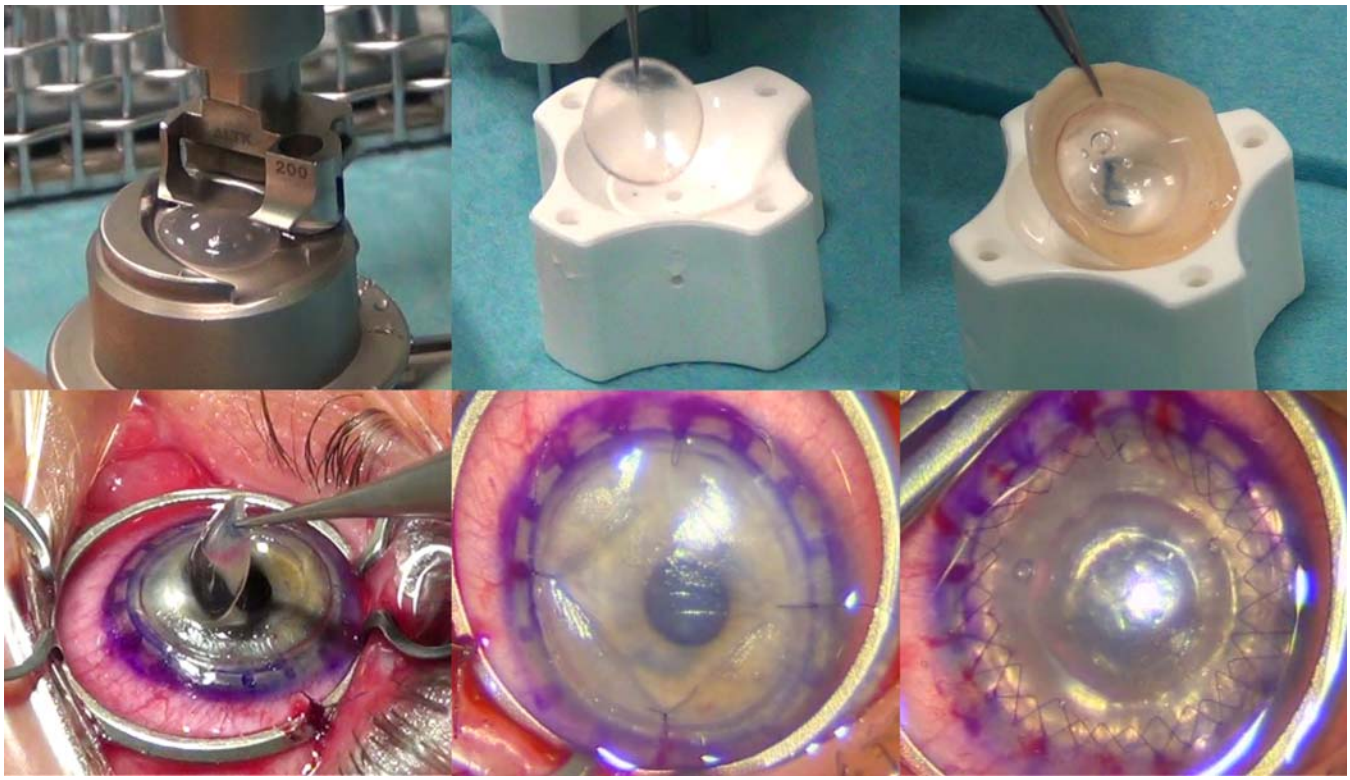

FIGURE 8

Preparation and placement of donor button: Splitting of the donor cornea mounted on the artificial anterior chamber of the automated lamellar therapeutic keratoplasty system into an anterior and a posterior lamella (top left); anterior lamella punched to 9.0 mm (top middle); residual donor tissue before punching of the posterior lamella (top right); placement of the posterior lamella into the recipient bed (bottom left); anterior lamella sutured on top of the posterior lamella with 4 cardinal sutures (bottom middle); final mushroom graft sutured in place with 2 running 10-0 nylon sutures (bottom right).

SURGICAL TECHNIQUE OF ADDITIONAL PROCEDURES

The details of the procedures performed after mushroom keratoplasty because of postoperative complications are described below.

Detachment of Posterior Lamella

Detachment of the posterior lamella (Figure 9) was managed always on the first postoperative day by repositioning the detached button and injecting air into the anterior chamber through a temporal paracentesis. The air was removed 2 hours after surgery in all cases.

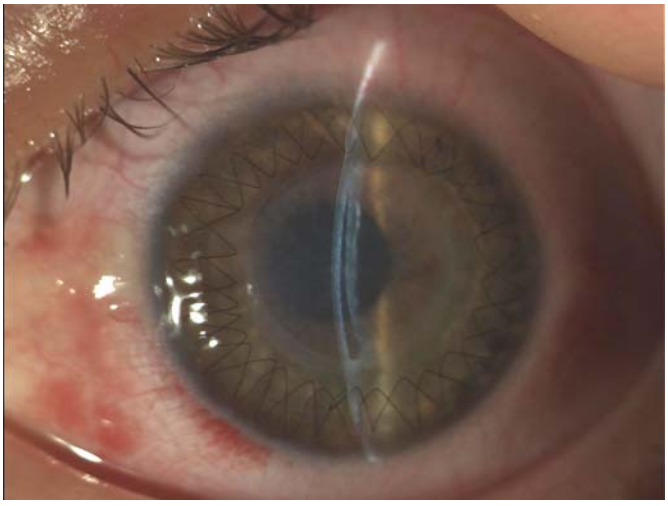

FIGURE 9

Slit-lamp appearance of the detached posterior lamella, on the first day after mushroom PK. A cleft between anterior and posterior lamellae is evident centrally.

Additional Keratoplasty

Keratoplasty surgery was repeated when there were complications involving either one or both lamellae of the two-piece mushroom PK. When the anterior lamella was involved (Figure 10, left), an anterior approach was used. If present, all sutures were removed. The superficial part of the wound of the primary surgery was then easily opened using a Sinskey hook (even months after the primary surgery), and the damaged anterior lamella was stripped off. Then the posterior lamella was inspected and eventually removed as well. Donor tissue was prepared in the same way as for the primary surgery and secured into the recipient bed with a double running or 16 interrupted 10-0 nylon stitches. All sutures were removed by 1 year after the second keratoplasty (Figure 10, right).

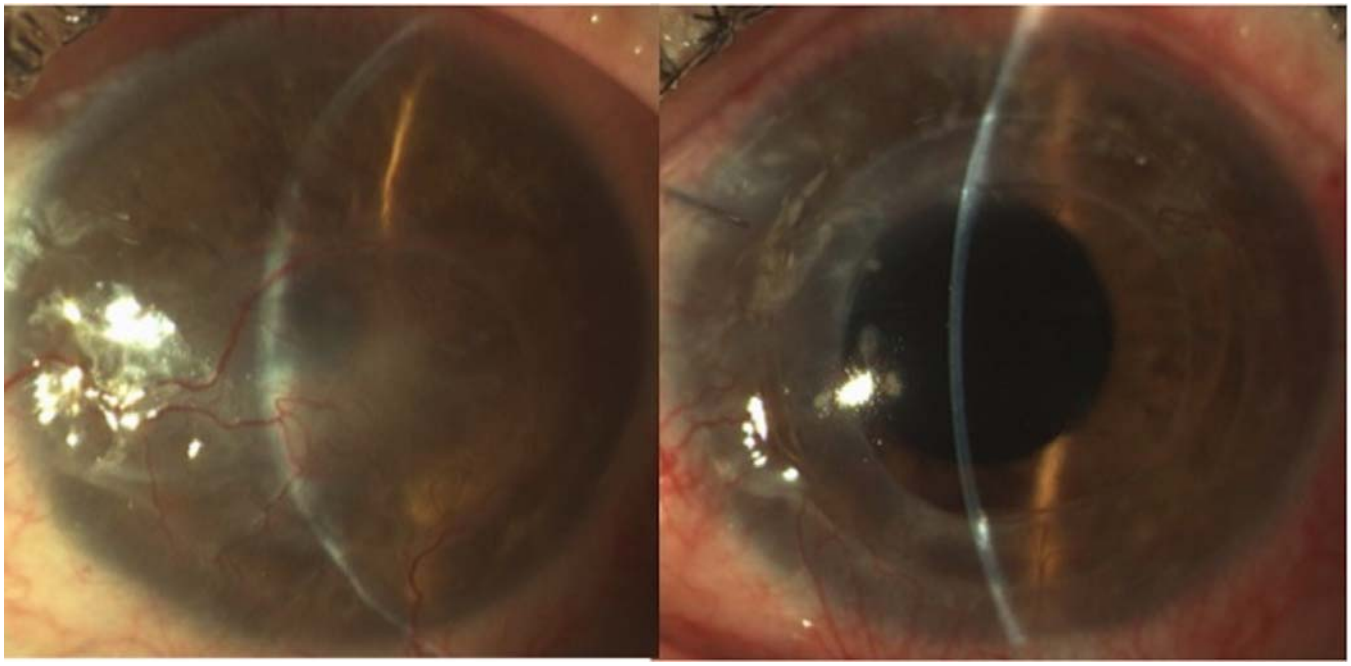

FIGURE 10

Slit-lamp appearance of a mushroom graft failed because of herpetic recurrence (left). Same eye 2 years after a second mushroom penetrating keratoplasty and 1 year after phacoemulsification with posterior chamber intraocular lens implantation (right).

A procedure resembling Descemet stripping endothelial keratoplasty (DSAEK) was performed in those eyes with endothelial decompensation (Figure 11, left). A limbal approach was used to remove the deep button from the overlying anterior lamella. A 25-gauge needle was bent upward and used to open the posterior part of the wound of the primary surgery, detach the posterior disc, and pull it outside through the limbal incision, 3.2 mm in width. A donor lamella of deep stroma and endothelium, 9.0 mm in diameter and about 150 μm in thickness, obtained by microkeratome-assisted dissection was delivered with the same "pull-through" technique used for conventional DSAEK surgery.[68] The donor tissue was attached to the posterior corneal surface by injecting air into the anterior chamber, and all surgical wounds were closed with interrupted 10-0 nylon stitches. The final corneal architecture (Figure 11, middle and right) consisted of 2 layers (old donor anterior lamella and new donor posterior lamella) of corneal tissue in the central 6.0-mm optical zone (as with any DSAEK procedure) and 3 layers (old donor anterior lamella, recipient posterior lamella, and new donor posterior lamella) in the surrounding annular area, about 1.5 mm in width.

Wound Revision

After complete suture removal, wound revision was performed in all eyes with refractive astigmatism higher than 4.5 diopters (D) that could not be adequately corrected with contact lenses (Figure 12, left). The location of the steeper meridian was determined preoperatively based on corneal topography and confirmed in surgery by means of a qualitative keratometer mounted on the surgical microscope (Haag-Streit, Bern, Switzerland). The wound between recipient cornea and anterior lamella was opened using the blunt tips of a tying forceps (Figure 12, middle) for an arc of about 1 clock hour, centered on the superior steeper hemi-meridian. A curved cyclodialysis spatula was inserted between anterior lamella and recipient stroma and then pushed further into the interface between donor anterior and donor posterior lamellae, reaching finally the opposite extremity of the wound at the inferior hemi-meridian of the steeper axis. Using the spatula as a guard, the wound was opened also at this site. The length of the dehiscence created in the surgical wound was based on the refractive effect achieved (as assessed intraoperatively by qualitative keratometry), aiming at a slight undercorrection (Figure 12, right).

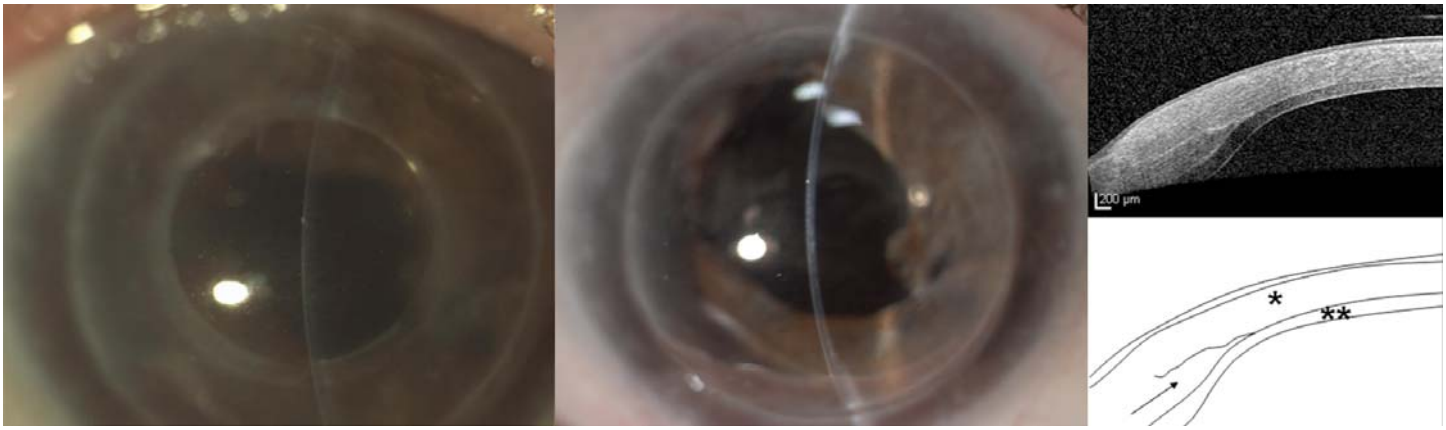

FIGURE 11

Slit-lamp appearance of a mushroom graft that failed after multiple retinal surgery procedures and subsequent phacoemulsification (left). Recovery of normal corneal transparency 1 year after successful Descemet stripping automated endothelial keratoplasty (DSAEK) performed according to the technique described in detail in the "Methods" section of the text (middle). Anterior segment optical coherence tomography (right top) and schematic representation of corneal layers (right bottom): Asterisk indicates anterior donor lamella of mushroom penetrating keratoplasty; double asterisk indicates posterior donor lamella of DSAEK; arrow indicates posterior lamellar bed of recipient cornea.

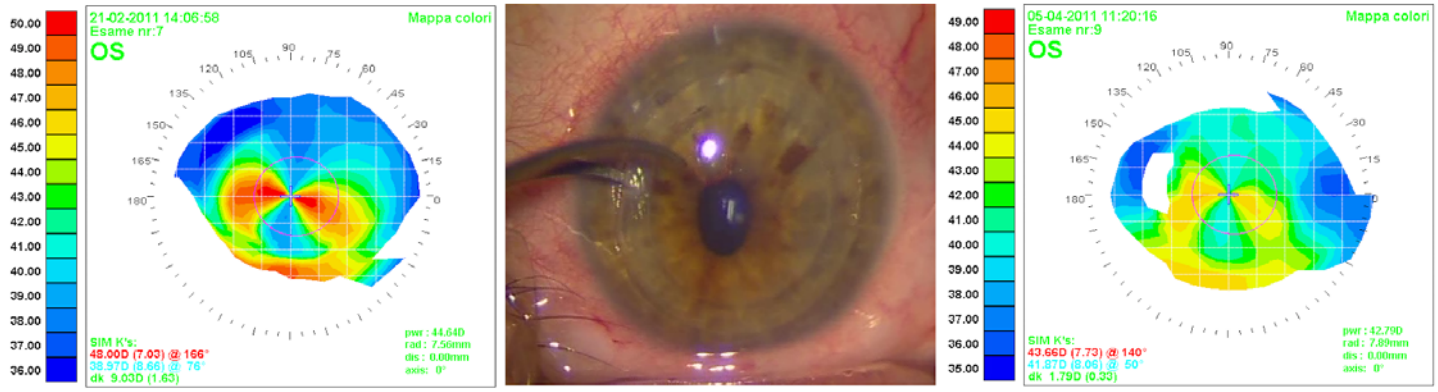

FIGURE 12

Correction of high-degree regular astigmatism occurring after mushroom penetrating keratoplasty (PK): Corneal map used to guide wound revision (left); opening of the anterior part of the mushroom PK with the blunt tip of a tying forceps at the site of steeper meridian (middle); corneal map demonstrating the effect of wound revision as early as one day postoperatively (right).

Phacoemulsification

Phacoemulsification after mushroom PK was always performed after suture removal had been completed. First the conjunctiva was opened, then a sclerocorneal tunnel centered on the steeper meridian was prepared. The width of the tunnel was 3.2 mm, and the anterior chamber was entered through clear cornea not further than 1 mm beyond the limbus, thus well peripheral to the scar between recipient cornea and the posterior donor lamella. In all cases a bimanual technique was used, the crystalline lens was emulsified in the capsular bag under protection of a cohesive viscoelastic substance, and a foldable, hydrophilic IOL was implanted in the bag. The tunnel was not sutured in any case. The conjunctiva was closed with a single 8-0 Vicryl stitch at the limbus.

POSTOPERATIVE THERAPEUTIC REGIMEN

Postoperatively, all patients were given topical tobramycin 0.3% and dexamethasone 0.1% suspension combination therapy (TobraDex; Alcon, Fort Worth, Texas) every 2 hours for 2 weeks, then every 3 hours for an 2 additional weeks. Treatment was then switched to dexamethasone 0.2 % (Luxazone; Allergan SpA, Rome, Italy), four times daily for 1 month, three times daily for 1 month, twice daily for 1 month, then finally once daily and continued indefinitely unless the patient was phakic or a steroid responder.

In eyes at high risk for immunologic rejection, prednisone acetate (Deltacortene; Bruno Farmaceutici SpA, Roma, Italy), 1.5 mg/kg of body weight, was given in a single daily dose after breakfast starting 3 days before surgery and tapered off over a 3-month period.

Systemic acyclovir (400 mg twice daily) was started 3 months before surgery and given for at least 1 year to all patients with herpetic keratitis; after discontinuation of systemic administration, patients were instructed to use topical acyclovir daily at nighttime indefinitely.

Endothelial rejection episodes were treated by steroids given systemically (prednisone acetate, 1.5 mg/kg of body weight in a single daily dose after breakfast, tapered off over a 3-month period), topically (0.2% dexamethasone phosphate eyedrops given hourly during the day and tapered off over a 6-month period), and subconjunctivally (40 mg of methylprednisolone acetate at least twice at 2-week intervals, [Depo-Medrol; Pfizer Italia Srl, Latina, Italy]).

In all cases, all sutures were removed within 12 months after surgery.

RESULTS

DEMOGRAPHICS

One hundred and seventy-two eyes of 171 patients underwent mushroom PK by the same surgeon (M.B.) at Villa Serena–Villa Igea private Hospitals (Forlì, Italy) during the period of time considered in this review. Additional mushroom PK surgery (once n=1; twice n=1), anterior lamella replacement, and DSAEK were performed in two eyes each. Ninety-four patients were female and 77 were male. The average patient age was 41.8±19.0 years (range, 5 to 86). As shown in Table 1, 26 eyes of patients 20 years old or younger were operated on. The youngest patient included in this series was 2 years old.

TABLE 1. PATIENT AGE AT THE TIME OF MUSHROOM KERATOPLASTY

AGE (IN YEARS)	NUMBER OF EYES (% OF TOTAL)
≤10	6 (3.5%)
11-20	20 (11.6%)
≥21	146 (84.9%)
Total	172 (100%)

INDICATIONS

Indications for surgery are detailed in Table 2. The main indication was advanced keratoconus (hydrops was documented in 36 of 68 interventions). There were 76 procedures (44.2%) in eyes at high risk for immunologic rejection because of the presence of 2 or more quadrants of deep vascularization in corneal scars of various origins.

TABLE 2. INDICATIONS FOR MUSHROOM KERATOPLASTY

INDICATION	NUMBER OF EYES (% OF TOTAL)
Keratoconus	68 (39.5%)
Herpetic scar	43 (25.0%)
Nonherpetic infectious scar	20 (11.6%)
Traumatic scar	10 (5.8%)
Interstitial keratitis	6 (3.5%)
Dystrophies	4 (2.3%)
Exposure in paralytic lagophthalmos	4 (2.3%)
Rosacea	3 (1.7%)
Chemical burn	2 (1.2%)
Other	12 (7.0%)
Total	172 (100%)

COMBINED SURGERY

As shown in Table 3, mushroom PK was combined with lens surgery in 15 cases (open-sky extracapsular cataract extraction with IOL implantation, n = 13; secondary posterior chamber IOL implantation with trans-scleral sutures, n = 2), with iris surgery in 2 cases (synechiolysis, n = 1; pupilloplasty, n = 1), and with anterior vitrectomy in 1 case. Lateral tarsorrhaphy was performed in 4 eyes with paralytic lagophthamos.

TABLE 3. SURGICAL PROCEDURES COMBINED WITH MUSHROOM KERATOPLASTY

TYPE OF PROCEDURE	NUMBER OF EYES (% OF TOTAL)
ECCE and PCIOL implantation	13 (7.6%)
Lateral tarsorrhaphy	4 (2.3%)
Secondary PCIOL implantation	2 (1.2%)
Anterior vitrectomy	1 (0.6%)
Pupilloplasty	1 (0.6%)
Synechiolysis	1 (0.6%)
Total	22 (12.8%)

ECCE, extracapsular cataract extraction; PCIOL, posterior chamber intraocular lens.

FOLLOW-UP

In all cases except 3 eyes that underwent early additional surgery (169 of 172), a minimum follow-up of 1 year or longer was available and a full ophthalmologic examination had been performed after complete suture removal. Additional follow-up examinations were performed in 129 (67 low-risk and 62 high-risk) of 148 eyes at 2 years, in 88 (44 low-risk and 44 high-risk) of 99 eyes at 3 years, in 54 (28 low-risk and 26 high-risk) of 65 eyes at 4 years, and in 37 (17 low-risk and 20 high-risk) of 43 eyes at 5 years or longer. The percentage of patients lost to follow-up was 12.8% at 2 years, 11.1% at 3 years, 16.9% at 4 years, and 13.9% at 5 years after surgery. Of the 6 patients lost to follow-up 5 years postoperatively or later, one was deceased and one had moved overseas.

VISUAL OUTCOMES

Twenty eyes (11.3% of all eyes included in the study) were excluded from the analysis of visual outcomes because of preexisting severe comorbidities, including amblyopia, advanced glaucoma, and retinal disease of various types. Table 4 summarizes all the comorbidities recorded preoperatively.

TABLE 4. PREEXISTING COMORBIDITIES LIMITING VISUAL OUTCOME IN EYES UNDERGOING MUSHROOM KERATOPLASTY

TYPE OF COMORBIDITY	NUMBER OF EYES (% OF TOTAL)
Amblyopia	9 (5.2%)
Advanced glaucoma	3 (1.7%)
Diabetic retinopathy	3 (1.7%)
Previous retinal detachment	2 (1.1%)
Age-related macular degeneration	2 (1.1%)
Macular hole	1 (0.6%)
Total	20 (11.6%)

Hard contact lenses were fitted in 9 cases because of high-degree astigmatism persisting after complete suture removal. All other eyes were correctable with spectacles. Table 5 summarizes BCVA results expressed in mean Snellen and logMAR units over the period of time considered. It also shows that the percentage of patients with better vision (20/40, 20/25, or 20/20) increases with time and stabilizes at about 3 years from surgery. Statistical analysis demonstrates that there was no significant change in visual acuity beyond 2 years after surgery (Figure 13).

Figure 14 illustrates the distribution over time of the percentage of patients seeing 20/40, 20/25, and 20/20. BCVA of 20/40 or better was obtained as early as 1 year after surgery in more than three-fourths of patients operated on. With time, this value increases further, reaching 100% 4 years after mushroom PK.

REFRACTIVE OUTCOMES

Refractive results were not compared with preoperative measurements, as these had been taken from highly irregular corneas and therefore considered unreliable. The average spherical equivalent of the postoperative refractive error decreased significantly (P = .0097) from -0.64±3.81 D at 6 months to -1.76±3.69 D at 1 year after mushroom PK. Values recorded at later postoperative examination times did not differ significantly, as shown in Table 6. The absolute value of refractive astigmatism averaged 3.90±1.94 D 6 months after mushroom keratoplasty and did not change significantly at any subsequent examination time (Table 6). However, the percentage of patients with a refractive astigmatism within 4.5 D increased from about 50% to over 70% after suture removal. A further increase to over 80% was found 2 years after mushroom PK, i.e, after astigmatic correction had been obtained with additional

surgery (toric IOL implantation, n=4; wound revision, n=22), and remained stable thereafter. Nine eyes were fitted with hard contact lenses for high-degree irregular astigmatism.

TABLE 5. BEST-CORRECTED VISUAL ACUITY 5-YEAR TREND AFTER MUSHROOM KERATOPLASTY IN ALL EYES WITHOUT COMORBIDITIES

	BEST-CORRECTED VISUAL ACUITY						
	NUMBER OF EYES	LOGMAR	MEAN (Snellen)	≥20/20 (% Eyes)	≥20/25 (% Eyes)	≥20/40 (% Eyes)	*P* VALUE (*t* test)
Preoperative	152	2.05±1.35	20/2224	0.0%	0.0%	0.0%	referent
Month 6	152	0.28±0.16	20/38	0.0%	21.4%	73.8%	<.0001
Year 1	152	0.22±0.20	20/33	11.9%	40.7%	83.1%	.0042
Year 2	113	0.11±0.17	20/25	40.8%	77.6%	93.9%	<.0001
Year 3	79	0.08±0.10	20/24	34.4%	81.3%	96.9%	.16
Year 4	49	0.05±0.07	20/22	51.9%	84.2%	100.0%	.06
Year 5	33	0.04±0.09	20/22	58.8%	82.4%	100.0%	.57

LogMAR, logarithm of the minimum angle of resolution.

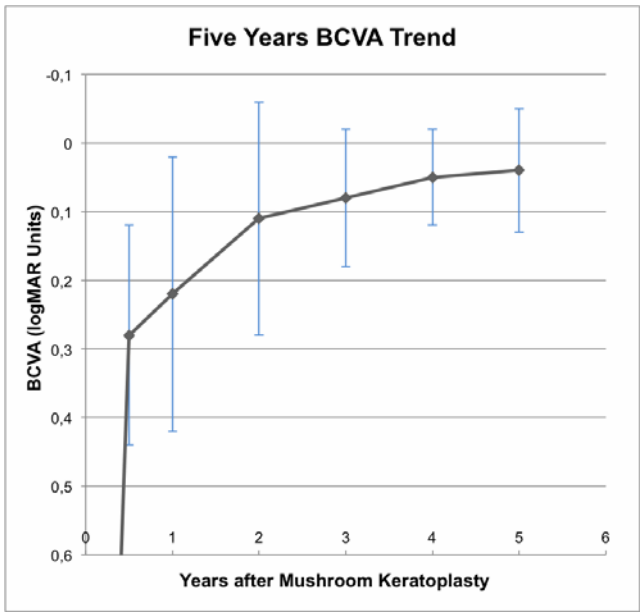

FIGURE 13

Improvement of average best-corrected visual acuity over 5 years following mushroom penetrating keratoplasty (mean values and standard error bars).

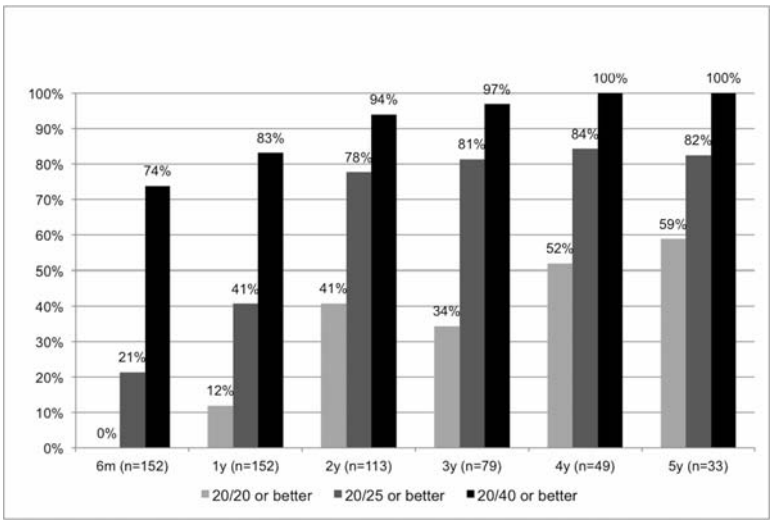

FIGURE 14

Distribution of higher levels of best-corrected visual acuity over 5 years following mushroom penetrating keratoplasty.

CORNEAL TOPOGRAPHY

As to be expected from the refractive results obtained, the mean absolute value of topographic astigmatism also did not change significantly over time (Table 7). The percentage of regular topographic pattern recorded was always extremely high; after complete suture removal, irregular patterns were observed in less than 10% of eyes at all examination times (Table 7).

ENDOTHELIAL CELL COUNT

The mean endothelial cell density in the donor corneas was 2535±263 (range, 2000-3500). Table 8 summarizes the ECL recorded after mushroom PK. The mean percentage loss increased significantly from 26.9±19.8% at 6 months to 35.3±20.6% at 1 year (*P*=.0002) and 41.7±16.9% at 2 years (*P*=.0044) postoperatively. Further increases in ECL recorded at later examination times were not statistically significant. When comparing subgroups at high risk and low risk for immunologic rejection, no statistically significant difference in ECL could be found at any postoperative examination time (Table 9).

Figure 15 confirms the early stabilization of ECL after mushroom PK with flattening of the curve starting at the 2-year examination time for cumulative ECL, as well as ECL of high-risk and low-risk eyes.

TABLE 6. REFRACTIVE PARAMETERS AT DIFFERENT POSTOPERATIVE EXAMINATION TIMES AFTER MUSHROOM KERATOPLASTY

	SPHERICAL EQUIVALENT (IN D)		ASTIGMATISM (IN D)				
	Mean	P Value (*t* test)	Mean	P Value (*t* test)	≤4.5 D (% eyes)	>4.5-8 D (% eyes)	> 8 D (% eyes)
Month 6	-0.64±3.81	referent	3.90±1.94	referent	56.6%	36.8%	6.6%
Year 1	-1.76±3.69	.0097	3.50±1.87	.07	73.0%	23.7%	3.2%
Year 2	-1.51±3.93	.60	3.36±1.09	.47	85.0%	12.4%	2.6%
Year 3	-1.66±3.82	.79	3.34±2.76	.94	87.3%	11.4%	1.3%
Year 4	-1.59±3.76	.91	3.98±2.27	.18	81.6%	16.3%	2.0%
Year 5	-1.48±4.01	.90	3.92±2.26	.91	81.8%	18.2%	0.0%

D, diopters.

TABLE 7. TOPOGRAPHIC ASTIGMATISM AT DIFFERENT POSTOPERATIVE EXAMINATION TIMES AFTER MUSHROOM KERATOPLASTY

	TOPOGRAPHIC ASTIGMATISM (MEAN, IN DIOPTERS)	*P* VALUE (*t* test)	REGULAR TOPOGRAPHIC PATTERN (% OF EYES)
Month 6	4.41±3.47	referent	89.5%
Year 1	4.16±2.45	.44	92.8%
Year 2	3.79±2.40	.22	92.9%
Year 3	3.44±2.80	.35	91.1%
Year 4	4.08±2.48	.19	91.8%
Year 5	3.93±2.68	.80	90.9%

TABLE 8. ENDOTHELIAL CELL LOSS AT DIFFERENT POSTOPERATIVE EXAMINATION TIMES AFTER MUSHROOM KERATOPLASTY

	% ENDOTHELIAL CELL LOSS (MEAN ± STANDARD DEVIATION)	*P* Value (*t* test)
Month 6	26.9±19.8	referent
Year 1	35.3±20.6	.0002
Year 2	41.7±16.9	.0044
Year 3	46.0±18.2	.08
Year 4	48.6±14.4	.37
Year 5	47.8± 8.2	.76

TABLE 9. SUBGROUP COMPARISON OF ENDOTHELIAL CELL LOSS AT DIFFERENT POSTOPERATIVE EXAMINATION TIMES AFTER MUSHROOM KERATOPLASTY

	% ENDOTHELIAL CELL LOSS (MEAN ± STANDARD DEVIATION)		
	High Risk	*Low Risk*	*P* Value (*t* test)
Month 6	28.5±24.5	25.1±13.3	.26
Year 1	40.5±20.0	34.4±19.9	.05
Year 2	41.1±18.4	42.7±14.3	.58
Year 3	47.7±17.2	43.3±20.1	.25
Year 4	49.6±13.4	45.5±18.8	.35
Year 5	49.5± 5.8	45.8±15.5	.33

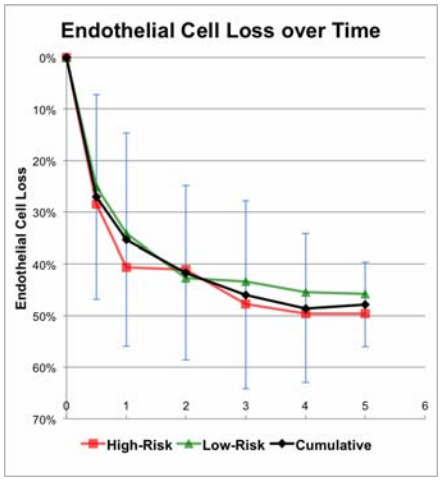

FIGURE 15

Average endothelial cell loss over 5 years following mushroom penetrating keratoplasty. Standard error bars are shown for the cumulative values.

GRAFT SURVIVAL AND IMMUNOLOGIC REJECTION

Six mushroom grafts failed. Three of them failed within 3 months after surgery as a result of persistent epithelial defect with melting of the anterior lamella (n= 2) or both anterior and posterior lamellae (n=1). The cause for this was herpetic infection (n=1) or exposure (n=2). In 2 additional cases, subsequent vitreoretinal surgery (retinal detachment, n=1; epiretinal membrane formation, n=1) and subsequent uneventful phacoemulsification with IOL implantation in the capsular bag resulted in endothelial decompensation 2 and 3 years after mushroom PK, respectively. Finally, recurrence of herpetic infection after discontinuation of antiviral prophylaxis caused graft failure in one eye 3 years after primary surgery.

All failed grafts were regrafted. Substitution of the anterior lamella, mushroom PK, and DSAEK were performed in 2 cases each. In the eye with early failure due to herpetic infection, a second and a third mushroom PK failed again, despite the use of high-dosage systemic and topical antiviral medication (acyclovir 800 mg 5 times daily and acyclovir 3% ointment **every 2 hours** during the day), and the cornea was covered with a conjunctival flap according to the technique described by Gundersen.[70] This procedure was also performed in one of the eyes with exposure due to paralytic lagophthalmos because of subsequent melting. In the remaining 4 cases subsequent surgery was successful and corneal transparency was maintained during the entire follow-up period with no further complications. In particular, the two eyes that underwent a posterior graft exchange had BSCVA of 20/25 and 20/30, respectively, 5 and 3 years after subsequent surgery, and the two eyes that underwent an anterior graft exchange had BSCVA of 20/60 and 20/30, respectively, 2 years and 1 year after subsequent surgery.

Figure 10 shows on the left the late herpetic recurrence with consequent graft failure and on the right the successful result 2 years after subsequent mushroom PK.

Kaplan-Meier analysis of graft survival probability is illustrated in Table 10 and Figure 16. Survival probability was above 90% at 5 years after mushroom PK for both high-risk and low-risk eyes. No statistically significant difference in survival rates was found between the two subgroups (*P*=.33). Figure 17 shows the late postoperative clinical appearance of both eyes portrayed in Figure 5: on the left the eye at low risk for immunologic rejection and on the right the eye at high risk, 4 and 5 years, respectively, after mushroom PK.

The risk for immunologic rejection was high in 76 eyes, as determined according to the criteria of the collaborative corneal transplant studies. Endothelial rejection was documented in 6 eyes (3.2%), of which 4 were at high risk. All but one rejection episode were reversed with steroidal treatment. However, even the cornea unresponsive to the steroidal treatment eventually cleared spontaneously a few months after the onset of immunologic rejection (Figure 18) and discontinuation of therapy. Table 11 and Figure 19 illustrate the cumulative probability of a rejection episode of all eyes undergoing mushroom PK, as well as that of the high-risk and the low-risk groups separately, according to the Kaplan-Meier analysis. Although the 5-year probability of a rejection episode in the high-risk group was more than double that in the low-risk group, this difference was not statistically significant (P=.31).

TABLE 10. KAPLAN-MEIER ANALYSIS OF GRAFT SURVIVAL PROBABILITY AFTERMUSHROOM KERATOPLASTY

		SUBGROUP ANALYSIS	
	Overall	*High Risk*	*Low Risk*
Month 6	98.3%	96.1%	100.0%
Year 1	98.3%	96.1%	100.0%
Year 2	97.5%	96.1%	98.5%
Year 3	95.3%	93.9%	96.3%
Year 4	95.3%	93.9%	96.3%
Year 5	95.3%	93.9%	96.3%

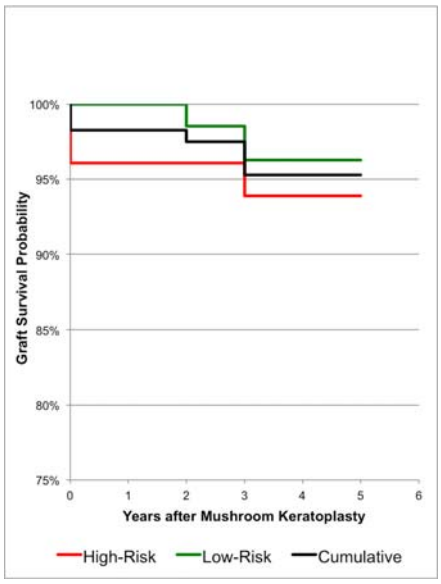

FIGURE 16

Probability of graft survival (Kaplan-Meier analysis) over 5 years following mushroom penetrating keratoplasty.

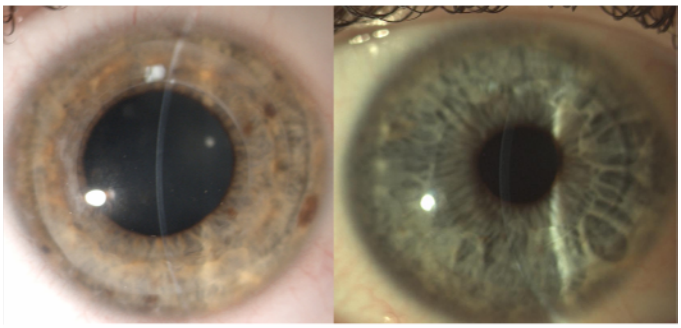

FIGURE 17

Slit-lamp appearance of the same eyes portrayed in Figure 5, 4 and 5 years, respectively, after mushroom penetrating keratoplasty. Both the left eye (keratoconus patient) and the right eye (postherpetic scar) have 20/20 vision. Two separate layers are only barely detectable in the central cornea of both eyes. No corneal neovessels are visible in the postherpetic cornea (right).

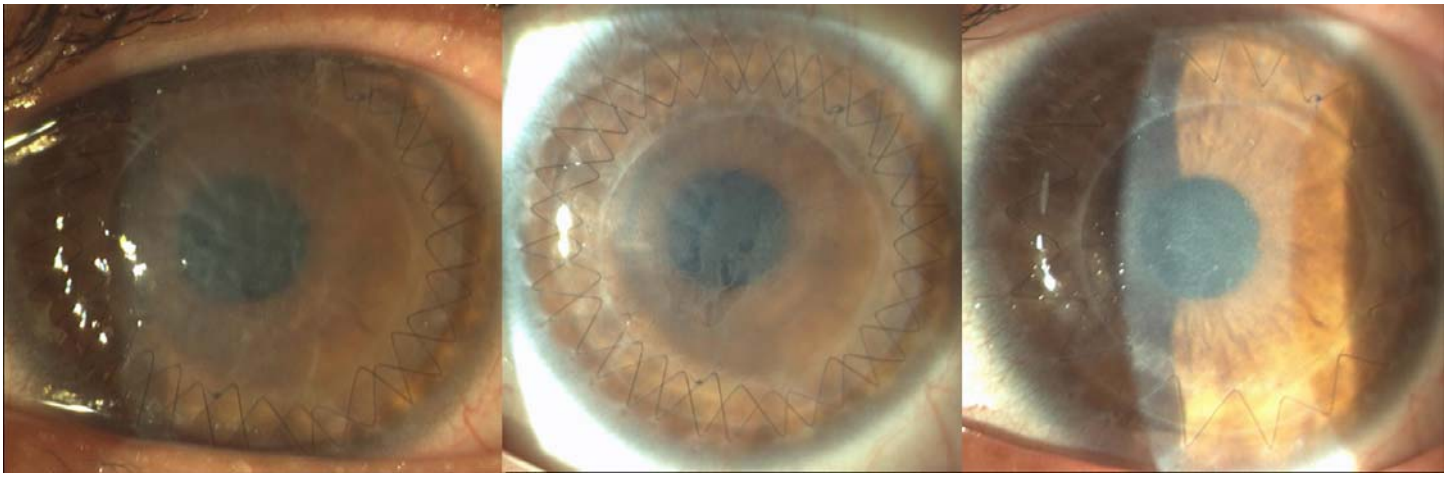

FIGURE 18

Spontaneous clearance of a failed, edematous mushroom graft. The cornea shows marked stromal edema overlying the deep button (left) a few days after discontinuation of steroidal treatment for immunologic rejection of the donor endothelium. Two months later the edema is less pronounced (middle), and 6 months later the cornea has recovered normal transparency with vision improving from 20/100 to 20/30.

COMPLICATIONS (OTHER THAN GRAFT FAILURE)

Complications other than graft failure occurring after mushroom PK are summarized in Table 12. Detachment of the deep button occurred in 2 cases within 24 hours from surgery. It was managed successfully in both cases, and the grafts have survived since then with no further complication. In 19 eyes, progressive lens opacification (high-risk eyes, n=14; low-risk eyes, n=5) developed and a standard phacoemulsification with IOL implantation in the capsular bag was performed. Four of these eyes had a toric IOL

implantation to treat coexisting high-degree regular astigmatism. As mentioned in the previous subsection, postoperative endothelial decompensation occurred in 2 cases at low risk for immunologic rejection that had also undergone retinal surgery.

TABLE 11. KAPLAN-MEIER ANALYSIS OF GRAFT REJECTION PROBABILITY AFTER MUSHROOM KERATOPLASTY

		SUBGROUP ANALYSIS	
	Overall	*High Risk*	*Low Risk*
Month 6	1.2%	1.4%	1.0%
Year 1	1.8%	2.7%	1.0%
Year 2	3.3%	4.4%	2.6%
Year 3	4.5%	6.8%	2.6%
Year 4	4.5%	6.8%	2.6%
Year 5	4.5%	6.8%	2.6%

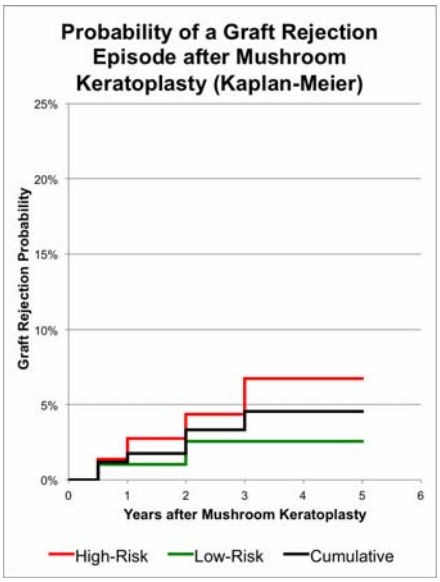

FIGURE 19

Probability of a graft rejection episode (Kaplan-Meier analysis) over 5 years following mushroom penetrating keratoplasty.

TABLE 12. COMPLICATIONS OF MUSHROOM KERATOPLASTY

TYPE OF COMPLICATION	NUMBER OF EYES (%)
High-degree astigmatism	41 (27.0%)
Cataract	19 (11.9%)*
Immunologic graft rejection	6 (3.6%)
Herpetic recurrence	3 (7.3%)†
Persistent epithelial defect	3 (1.7%)
Posterior button detachment	2 (1.2%)

*Percentage of phakic eyes.
†Percentage of herpetic eyes.

Refractive astigmatism higher than 4.5 D was found in 41 of 152 eyes (26.9% as in Table 6) after complete suture removal: 4 eyes were treated with implantation of a toric IOL, 9 eyes were fitted with hard contact lenses, 22 eyes were treated by surgical revision of the keratoplasty wound, and in 6 cases the patient preferred suboptimal vision with spectacles. No wound was opened for more than 2 clock hours. No augmentation sutures were used in any case. In 2 eyes inadvertent opening of the wound between donor posterior lamella and recipient cornea occurred but did not require suturing, as the self-sealing, steplike configuration of the mushroom PK wound blocked any aqueous leakage. All wounds were left to heal spontaneously. The mean reduction of astigmatism was 4.52 D. Wound revision failed to reduce high-degree astigmatism in 2 eyes.

Herpes simplex infection recurred in 3 eyes: one eye underwent successful subsequent mushroom PK, in another eye a conjunctival flap was performed after 2 subsequent mushroom PKs had failed (see previous subsection), and the third eye experienced

multiple recurrences that were managed successfully with antiviral treatment. Persistent epithelial defect and melting due to paralytic lagophthalmos occurred in 2 eyes despite the use of lateral tarsorrhaphy at the time of mushroom PK, and subsequent surgery was performed.

DISCUSSION

The ideal keratoplasty should restore a high level of visual acuity, induce no postoperative refractive errors, and allow long-term graft survival. To achieve these goals, diagnostic tools, surgical technique, and instrumentation have undergone continuous refinement during the last century. More recently, trying to further improve keratoplasty, surgeons have taken two different directions: on one hand, new techniques of anterior and posterior lamellar keratoplasty (LK) have been developed, and on the other hand, the use of shaped, full-thickness grafts has been revived.

Lamellar grafts can be employed to treat selectively a diseased part of the cornea, be it the endothelium or the stroma, but are not indicated when the cornea is affected in its entire thickness, particularly in its central optical zone.

Shaped, full-thickness grafts were introduced in 1921 by Ebeling and Carrel[51] but did not gain popularity, mainly because of the technical difficulty of the procedure. Their initial idea was to create a "joint" in the surgical wound with the purpose of improving the fitting between donor and recipient tissue, thus optimizing both postoperative healing and corneal contour. However, at that time corneal physiology was mostly unknown, and therefore the graft shape represented simply an attempt at optimizing its architecture. The first use of a graft purposely shaped to specifically address a particular corneal disease (i.e, endothelial decompensation) was presented by Busin in 2003.[58] The top-hat graft configuration was aimed at replacing a 9.0-mm area of diseased central endothelium while maintaining the anterior surface of the graft within the central 7.0 mm, therefore far enough from the recipient corneoscleral limbus to reduce the risk of immunologic rejection. At the same time, the increased surface of the surgical wound would allow faster healing and earlier suture removal. The development of femtosecond laser technology has simplified the technique, although the results of cutting through edematous tissue are still far from optimal. In addition, the development and success of posterior (endothelial) LK has made this approach obsolete for the treatment of endothelial decompensation.[2,3]

The mushroom design was conceived to improve the refractive results of PK in eyes with normal endothelium thanks to the large diameter (9.0 mm) of the hat, while maintaining in place most of the healthy recipient endothelium by including into the full-thickness graft only the limited area of the stem (6.0 to 7.0 mm in diameter). This method has been used to treat keratoconic eyes with good results, although in a limited number of cases.[14,64]

Recently, anterior LK techniques, especially the so-called "big-bubble" technique, have become increasingly popular for the treatment of keratoconic patients, mainly because the recipient endothelium remains untouched. However, a mushroom PK offers several advantages over any anterior LK, including the reproducibility, the possibility of standardization, and the feasibility in eyes with ruptured (i.e, previous hydrops or perforating trauma) or centrally scarred (i.e, previous infections) Descemet membrane. We have proposed in the past a microkeratome-assisted procedure, consisting of the combination of a larger anterior LK and a smaller posterior LK resulting in a two-piece mushroom PK.[65] As opposed to one-piece, femtosecond-assisted mushroom keratoplasty, this method allows dissection through densely scarred and vascularized stroma (thus allowing its use in conditions other than keratoconus), as well as proper alignment of hat and stem in relationship, respectively, with the limbus and pupil. Despite the presence of an interface in the optical zone, initial reports have indicated that the visual results are not affected,[65,66] most probably because the surfaces of both anterior and posterior lamellae are cut with a microkeratome, thus being as smooth and regular as those obtained for laser in situ keratomileusis (LASIK).

Since its initial use for the treatment of a corneal perforating injury,[65] microkeratome-assisted mushroom PK has been used routinely at our Institution to treat all eyes with full-thickness central opacities but otherwise healthy endothelium, including keratoconus, especially after hydrops, post-infectious scars of various origin, posttraumatic scars, and dystrophies.

As with any type of new surgical procedure, the issues of technical complexity, safety, efficacy, stability of outcomes and, in case of corneal transplantation, graft survival must be addressed in order to validate this technique.

TECHNICAL COMPLEXITY AND SAFETY

Mushroom PK involves substantially the same surgical skills required for conventional PK, and dedicated instrumentation is limited to a microkeratome. Dissection of the recipient bed is certainly the most demanding step of the procedure, especially in corneas with previous ulcers (i.e, postherpetic scars), where it is also essential in creating a recipient bed of uniform thickness. However, it is not necessary to achieve a perfectly smooth surface, as it is performed only in the peripheral, nonoptical part of the cornea; on the contrary, some roughness in the annular area of contact between anterior donor lamella and recipient bed promotes healing and therefore creation of a stronger wound. Mushroom PK shares the open-sky approach of a conventional PK but minimizes to 6.0 mm the size of the full-thickness opening. Combined surgery, including extracapsular cataract extraction with IOL implantation, was easily performed through an opening of this size, but at the same time, vitreous pressure was managed more easily than through a conventional opening, 7.5 to 8.0 mm in diameter. The two-piece architecture did not affect the final mushroom configuration of the graft as a whole. Adhesion of the posterior lamella to the overlying anterior one was achieved primarily in all eyes except 2 (1.2% of the total), which required air injection into the anterior chamber for successful reattachment. As we previously reported,[71] it must be noticed that mushroom PK was performed successfully in all patients 10 years of age or younger, for whom traumata are often the

cause of postoperative failure.[72] Figure 20 shows the preoperative (left) and late (3 years after mushroom PK) postoperative (right) slit-lamp appearance of the right eye of a boy 4 years old at the time of central perforating corneal injury.

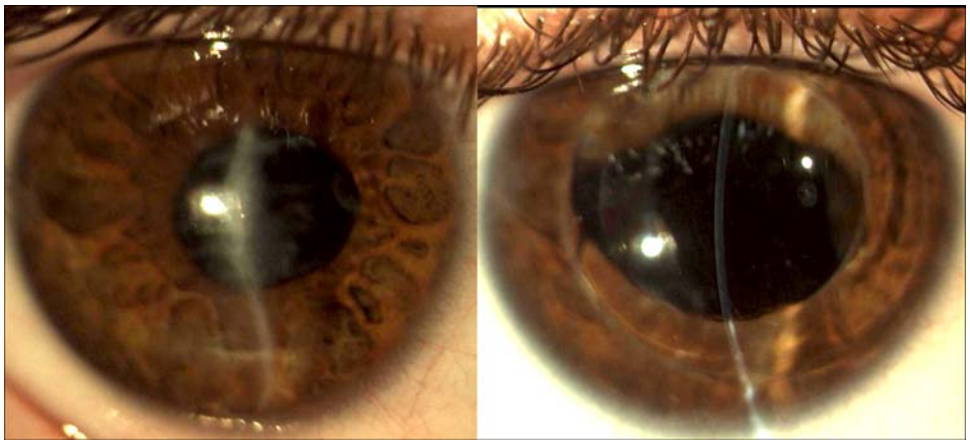

FIGURE 20

Slit-lamp appearance of the cornea of a 4-year-old boy before (left) and 3 years after mushroom penetrating keratoplasty combined with anterior synechiolysis and secondary posterior chamber intraocular lens implantation over the remnants of the posterior capsule (right). The graft is clear (endothelial cell density of about 1600 cells/mm^2 was measured) in the presence of a pupillary capture of the intraocular lens. Best-corrected visual acuity was 20/25.

In mushroom PK the steplike configuration of the surgical wound creates a much larger area of contact between donor and recipient tissue, theoretically resulting in faster healing and increased resistance to trauma. We have not tested in our patients whether a period of time much shorter than 1 year could be sufficient to create a safe scar and allow earlier suture removal. However, we did not observe any case of wound dehiscence after suture removal was completed (within 1 year from surgery in all cases), thus contrasting with previous literature reporting an incidence up to 7.2% of post–suture removal wound dehiscence for conventional PK.[73,74] Finally, secondary procedures, in particular opening of the surgical wound for up to 2 clock hours, were performed in 22 eyes without compromising the stability of the mushroom PK wound.

EFFICACY AND STABILITY OF OUTCOMES

In the vast majority of eyes without comorbidities, mushroom PK restored excellent levels of visual acuity in a relatively short period of time and maintained them over the 5-year period considered in this thesis. BCVA of 20/40 or better was achieved by over 80% of eyes at 1 year postoperatively, after all sutures had been removed, and by a percentage of eyes close or equal to 100% at subsequent examination times. Instead, BCVA of 20/20 was limited to only slightly more than 10% at year 1 after surgery, but increased thereafter, reaching over 50% at years 4 and 5. Several reasons may explain these outcomes. First, the indications for mushroom PK apply in general to a rather young population, thus excluding subclinical macular disease or other retinal conditions that often limit the visual outcome in older patients. Most important, the architecture of mushroom PK was instrumental in minimizing corneal distortion even with sutures in place, as indicated by the very low percentage (≤10.5%) of irregular topographic patterns recorded. As a consequence, although the entity of average postoperative astigmatism did not differ substantially from values reported for conventional PK,[1,4-7] starting from the 2-year examination time, when most secondary procedures for the correction of high-degree astigmatism had been performed, only less than 3% of all eyes had a refractive astigmatism of 8 D or more. Finally, some of the patients missing at the follow-up examinations (drop-off rate always below 17%) may have not shown up because of unsatisfactory results, thus masking part of the negative outcome.

A relative discrepancy was found in our series between the fast recovery of 20/40 vision and the relatively longer time required by most eyes in order to achieve 20/20 vision. Mushroom PK shares with other types of LK this finding, i.e, the slow recovery of optimal optical quality of corneal tissue in the presence of an interface.[75] However, the extreme smoothness of the surfaces obtained by means of microkeratome-assisted dissection as with LASIK would explain the high percentage of 20/20 BCVA recorded at later postoperative examination times.

The stability of visual and refractive outcomes of mushroom PK is demonstrated by the absence of statistically significant changes after the 2-year examination time. With conventional PK for keratoconus, late changes in refraction and astigmatism have been reported even many years after surgery, supposedly as a consequence of the progression of the ectatic disease in the host rim.[76,77]

Again, at least theoretically, the different structure of the surgical wound of a mushroom PK, with a larger area of diseased tissue being replaced, should protect against this possibility.

GRAFT SURVIVAL

Graft survival is related to both progressive postoperative ECL and immunologic rejection.

Endothelial cell density is known to decrease progressively after PK,[33-39] but surgical trauma can be held responsible only for early changes. Migration of endothelial cells over the posterior corneal surface from areas with higher cell density to areas with lower cell density has been documented in both experimental models[40] and humans[41-44] and may account for some of the continuing cell loss after keratoplasty performed in eyes with diseased endothelium (i.e, bullous keratopathy). It also points out the possible role of high-density healthy endothelium with high cell density in the residual recipient bed helping reduce ECL in the donor endothelium.[46,78]

The presence of a healthy endothelium was one of the two main inclusion criteria for the eyes undergoing mushroom PK. Changes in endothelial cell density recorded later than 2 years postoperatively were not statistically significant, showing that stabilization of ECL in the graft could be obtained much earlier than after conventional PK.[33-37,39,78] The mushroom PK minimizes transplantation to about only 25% of the healthy recipient endothelium: early stabilization of ECL could therefore be explained by the large reservoir of healthy endothelial cells in the recipient cornea, which, if necessary, could migrate across the surgical wound onto the posterior surface of the graft. This mechanism has been suggested in the past,[42] and an indirect demonstration is given by the case illustrated in Figure 18: after endothelial decompensation in the central donor tissue following an immunologic rejection episode unresponsive to steroidal treatment, the cornea of this keratoconic patient cleared spontaneously over a 9-month period, possibly because of the reservoir of healthy endothelium in the peripheral recipient bed.

In our series the average decrease of ECL stopped below 50% of the preoperative value, therefore far above the minimum required for corneal transparency. The maintenance of a stable ECL at this level would be compatible with a much longer survival time than the 5-year period considered in this study.

Interestingly, there was no statistically significant difference in ECL between the groups at low and high risk for immunologic rejection. As opposed to what was proposed by other investigators,[38] this finding suggests that immunologic mechanisms may not be involved in progressive ECL following PK.

Immunologic rejection episodes were extremely rare after mushroom PK, and the cumulative probability of their occurrence, calculated according to Kaplan-Maier analysis, remained below 5% 5 years postoperatively. Quite surprisingly, there was again no statistically significant difference between the groups at low and high risk for immunologic rejection, although the value for the former subgroup (2.6%) was less than half of that for the latter subgroup (6.8%). Minimizing the amount of donor endothelial tissue to an area 6 mm in diameter may be responsible for reduced immunologic stimulation and therefore a lower incidence of rejection.

Both stabilization of ECL below 50% and graft immunologic rejection probability below 5% contributed to the recorded cumulative graft survival probability of more than 95% 5 years postoperatively. As a corollary of the previous results, survival probability rates were not significantly lower in the group at high risk for immunologic rejection. These findings contrast sharply with those obtained after conventional PK. In eyes at high risk for immunologic rejection, as defined by the Collaborative Corneal Transplant Studies,[67] post-PK probability of occurrence of an immunologic rejection is significantly higher and graft survival probability is significantly lower than in eyes at low risk.[26,28,29] In this respect it must be noted that over the past few years the implementation of antiviral prophylaxis has dramatically improved the prognosis of PK for postherpetic scars, almost eliminating post-PK herpetic recurrences and reducing significantly the incidence of immunologic rejection episodes.[31,32] This would only partially explain our results, as at least half of the eyes at high risk for immunologic rejection included in our thesis did not suffer from herpetic disease. The outcomes of mushroom PK suggest that minimal endothelial transplantation may be the reason for the rare occurrence of immunologic rejection after this type of transplant. In addition, at least theoretically, if rejection would occur, endothelial cell migration from the large healthy recipient bed into the rather small graft could easily replace the damaged donor cells, thus explaining the extremely high 5-year graft survival probability, regardless of the type of indication.

In our study, follow-up was available for 87.2%, 83.1%, and 86.1% of all patients, respectively, 2, 4, and 5 years after surgery, whereas in the Collaborative Corneal Transplantation Studies, the percentages of patients with follow-up of 2, 4, and 5 years were 91%, 40%, and 14%.[67]

Finally, complications following mushroom PK, other than immunologic rejection, were relatively rare and could be managed successfully in the vast majority of cases, thus minimally affecting the prognosis of this procedure.

CONCLUSIONS

Two-piece, microkeratome-assisted mushroom PK is a relatively simple procedure designed to share the refractive advantages of a large anterior LK and those related to minimal endothelial transplantation via a small posterior LK. The outcomes recorded 5 years postoperatively support the use of this technique for eyes with full-thickness, central opacities in corneas with otherwise healthy endothelium. Visual and refractive results compare favorably with those obtained after conventional PK, and even in eyes at high risk for immunologic rejection, the probability of a rejection episode and/or graft failure is extremely low. Further studies involving more surgeons operating on a larger number of eyes with long-term follow-up are required to confirm our initial results and further validate the use of mushroom PK.

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