Survival of Mushroom Keratoplasty Performed in Corneas With Postinfectious Vascularized Scars

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• PURPOSE: **To report the visual outcomes and graft survival rates of mushroom keratoplasty for the treatment of postinfectious corneal scars.**

• DESIGN: **Prospective, noncomparative, interventional case series.**

• METHODS: **A microkeratome-assisted mushroom-shaped keratoplasty was performed in 31 eyes of 31 patients with a central vascularized full-thickness leukoma, resulting from infectious keratitis of various origin (herpes simplex virus, n = 16; bacteria, n = 10; *Acanthamoeba*, n = 5), with healthy endothelium. The donor graft consisted of a large anterior stromal lamella (9.0 mm in diameter and ± 250 μm in thickness) and a small posterior button (5 to 6 mm in diameter). Visual acuity, refraction, and endothelial cell density were evaluated before surgery, as well as at 12, 24, and 36 months after surgery, and the postoperative graft survival rate was evaluated.**

• RESULTS: **Three years after surgery, in 26 (83.8%) of 31 patients, best spectacle-corrected visual acuity was 20/40 or better with a refractive astigmatism of 4.5 diopters or less. The endothelial cell count at the last follow-up examination averaged 1584 ± 381 cells/mm^2, with an average cell loss of 40.7% from the preoperative value. The survival rate at 3 years was 90.3%, improving to 96.7% when excluding nonimmunologic causes for graft failure.**

• CONCLUSIONS: **Similarly to penetrating keratoplasty, microkeratome-assisted mushroom keratoplasty restores vision in eyes with postinfectious, full-thickness, central corneal scars. For these vascularized corneas at high risk for immunologic rejection, mushroom keratoplasty combines the visual and refractive advantages of large penetrating keratoplasty grafts with the high survival rate of small penetrating keratoplasty grafts. (Am J Ophthalmol 2012; 153:44–50. © 2012 by Elsevier Inc. All rights reserved.)**

CORNEAL SCARRING RESULTING FROM HERPES SIMplex virus (HSV) or microbial keratitis is one of the main indications for penetrating keratoplasty (PK) in developing countries and is a relatively frequent one also in developed countries.[1,2] Because deep stromal scars of infectious origin usually affect Descemet membrane and endothelium, to date PK is the standard surgical procedure performed to rehabilitate these patients visually. However, long-term survival of full-thickness standard grafts (7.5 to 8.5 mm in diameter) in vascularized corneas is jeopardized by immunologic rejection as well as possible recurrence in herpetic cases.[3–6] In the past, small full-thickness grafts (5 to 6 mm in diameter) performed in vascularized corneas have shown higher survival rates than larger grafts (7 to 8 mm in diameter), but were associated with high-degree and irregular postoperative refractive errors, thus making visual rehabilitation practically impossible.[6,7]

More recently, several deep anterior lamellar keratoplasty (DALK) techniques have been proposed to preserve the healthy endothelium of patients with postinfectious corneal scars.[8–13] However, reduced visibility makes hand dissection particularly difficult in these eyes, whereas the frequent presence of adhesions between deep stroma and Descemet membrane does not allow easy formation of a big bubble when attempting pneumatic dissection, as in keratoconic patients. In addition, femtosecond lasers cannot obtain smooth surfaces when dissecting through nontransparent corneas, and therefore cannot be used successfully for this purpose. Most of all, even if descemetic dissection is obtained, the Descemet membrane and endothelium underlying the central stromal opacity often also are affected centrally, thus preventing full visual recovery if left in place.

We propose a microkeratome-assisted procedure involving transplantation of a relatively small central area of endothelium and deep stroma in conjunction with a large anterior stromal lamella. The resulting mushroom-shaped 2-piece graft shares the refractive advantages of a large anterior lamellar keratoplasty and that of limited removal of the central recipient endothelium (approximately 25% to 35% of the total), while obtaining a central scar-free optical zone of 5 to 6 mm in diameter.[14,15] We present herein the 3-year results of a prospective ongoing study evaluating the outcome of mushroom keratoplasty performed in 31 patients with vascularized postinfectious central scars in corneas with otherwise healthy endothelium.

METHODS

WE RECORDED THE RESULTS OBTAINED IN ALL PATIENTS undergoing mushroom keratoplasty surgery by the same surgeon (M.B.) at our hospital between January 2004 and

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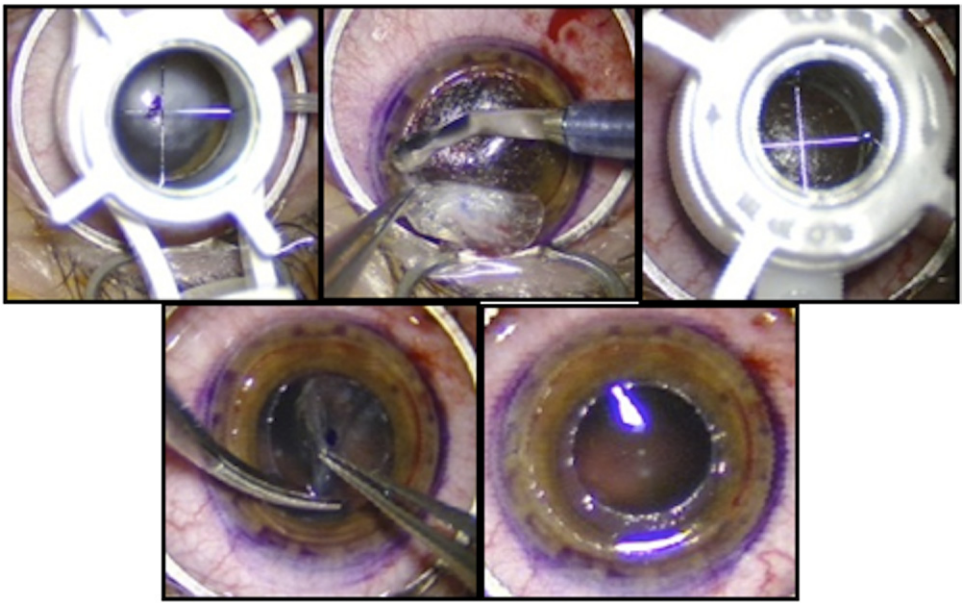

FIGURE 1. Photographs showing the mushroom keratoplasty surgical technique 1. (Top left) A 9.0-mm Barron suction trephine is used to create a circular incision approximately 250 μm in depth. (Top middle) Lamellar dissection is performed by hand and the anterior lamella is removed. (Top right) A 6-mm Barron trephine is used to create a full-thickness incision in the residual recipient bed centered on the pupil. (Bottom left and right) The incision is completed with corneal scissors and the corneal button is excised.

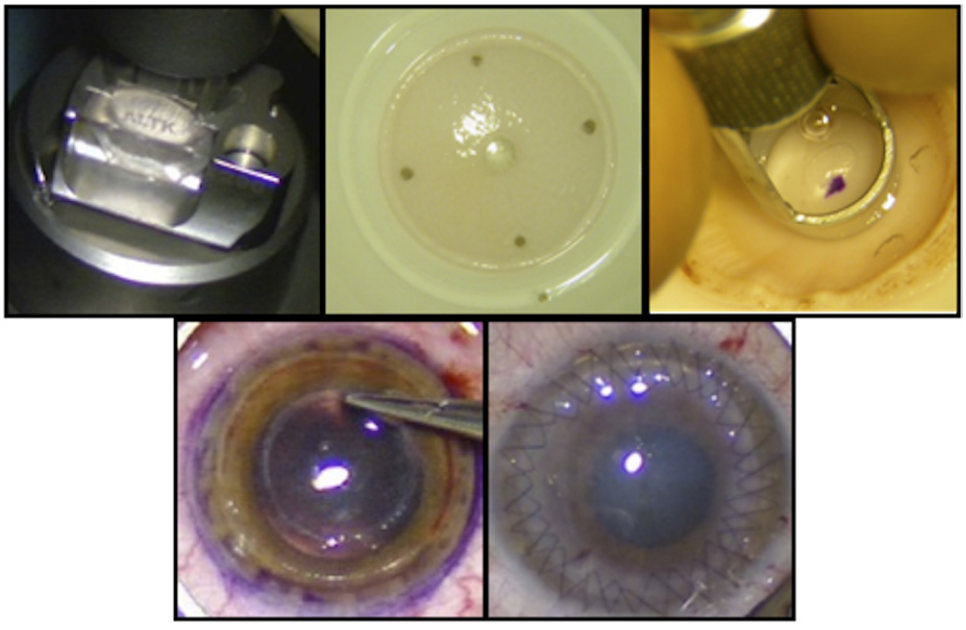

FIGURE 2. Photographs showing the mushroom keratoplasty surgical technique 2. (Top left) The donor cornea is mounted on the artificial anterior chamber, and a 200-μm head is used to split the donor cornea into anterior and posterior lamellae. (Top middle and right) Each of the 2 lamellae then is punched to proper size. (Bottom left) The donor stem is positioned into the central hole of the recipient bed without sutures. (Bottom right) The anterior donor lamella is sutured in place with a double running 10-0 nylon suture.

December 2006 and followed up prospectively thereafter. The main inclusion criterion was the loss of visual acuity resulting from postinfectious deep corneal scars involving the optical zone in the presence of vascularization and otherwise healthy endothelium; in addition, surgery could not be performed unless a period of 1 year or longer without episodes of reactivation of the underlying disease or inflammation had elapsed. All surgical procedures were performed in a standard fashion as described in detail below.

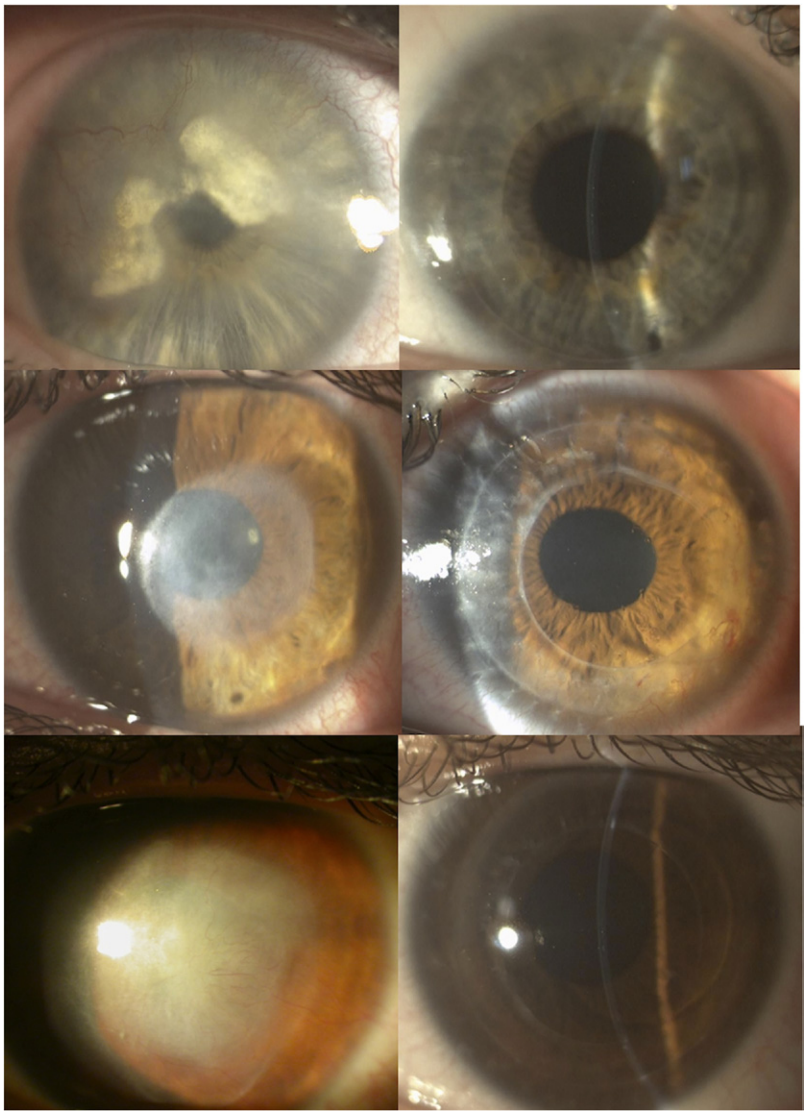

FIGURE 3. Photographs showing mushroom keratoplasty and vascularized scars. Preoperative and postoperative clinical appearance of eyes undergoing microkeratome-assisted mushroom keratoplasty for central, vascularized scars resulting from (Top left and right) herpetic, (Middle left and right) bacterial, and (Bottom left and right) amebic keratitis.

Before surgery, every patient underwent a complete ophthalmologic evaluation, including slit-lamp examination, both uncorrected and best spectacle-corrected visual acuity, as well as refraction. Corneal vascularization was evaluated by slit-lamp examination and was rated according to extension (number of clock hours involved) and depth (superficial or deep stroma). In addition, at all postoperative examination times (12, 24, and 36 months), corneal topography analysis (EyeSys 2000; EyeSys Technologies, Inc, Houston, Texas, USA) and endothelial cell count (cornea module of HRT-II; Heidelberg Technology, Heidelberg, Germany) were performed. Postoperative endothelial cell density (ECD) was evaluated centrally, that is, in the area of the posterior donor button; the values recorded were compared with those obtained before surgery from the eye bank, thus considering the cell loss as a percentage of the preoperative in vitro value, as described in a previous article from our group.[16]

An analysis of variance was used to determine the significance of changes in ECD values at different postoperative examination times. Analyses were conducted using Stata software version 10.0 (Stata Corp, Inc, College Station, Texas, USA). A *P* value of less than .05 was considered to be statistically significant.

• **SURGICAL PROCEDURE:** All but 2 patients who received general anesthesia because they were younger than 14 years were sedated with 3 mL intravenous droperidol immediately before anesthetic injection. Local anesthesia was administered with a peribulbar injection of a mixture of lidocaine hydrochloride 2% and bupivacaine hydrochloride 0.5%. All steps of the surgical technique are shown in

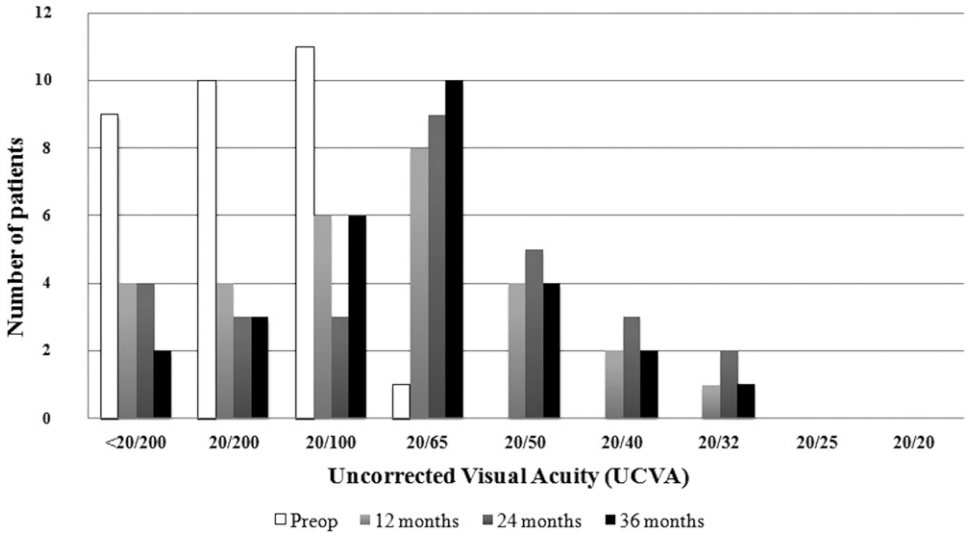

FIGURE 4. Bar graph showing visual outcomes after mushroom keratoplasty: distribution of uncorrected visual acuity (UCVA) at different postoperative examination times.

the Supplemental Video (Supplemental Material available at AJO.com).

Initially, a Barron suction trephine (Katena Products, Inc, Denville, New Jersey, USA) was used to obtain a circular incision 9.0 mm in diameter and approximately 250 μm in depth in the recipient cornea (Figure 1, Top left). Then, a lamellar dissection was carried out by hand from the base of the incision toward the center of the cornea, and the anterior lamella was removed from the recipient cornea (Figure 1, Top middle). A trephine 5 to 6 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 1, Top right). The central button then was excised with corneal scissors (Figure 1, Bottom left and right). At this stage, when necessary, an open-sky extracapsular cataract extraction was performed and an intraocular lens was implanted into the capsular bag.

The donor cornea was mounted on the artificial anterior chamber of the automated lamellar therapeutic keratoplasty system (Moria SA, Antony, France); a 200-μm head was used to split the donor cornea into anterior and posterior lamellae (Figure 2, Top left), which then were both punched to proper size (Figure 2, Top middle and right) to fit with the recipient bed prepared previously (the same size of the recipient bed was used both for the anterior and the posterior lamellae). To prepare a donor lamella with a thickness similar to that of the excised one, a 200-μm head was used, basing this selection on previous published data showing that microkeratome-assisted dissection creates a donor lamella, the thickness of which in general is thicker (10% to 20%) than the intended value, based on the width of the head slit.[17]

The donor stem (endothelium and deep stroma) was fitted into the central hole of the recipient bed without sutures (Figure 2, Bottom left). The 9-mm donor anterior lamella then was placed on top of the posterior one and was sutured into position with 4 cardinal 10-0 nylon stitches. Surgery was completed with a double-running 10−0 nylon suture (Figure 2, Bottom right). 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 salt solution injected with a 30-gauge needle through a limbal puncture.

Based on treatment regimens previously used for patients at high risk for immunologic rejection, steroids were given systemically (1 mg/kg body weight prednisolone tapered off over a 3-month period) and topically (dexamethasone 0.1% eye drops every 2 hours and tapered off to daily over a 5-month period).[18–21] Systemic acyclovir (400 mg twice daily) was given for at least 1 year to all patients with herpetic keratitis. In all cases, all sutures were removed within 12 months after surgery.

RESULTS

THIRTY-ONE PHAKIC OR PSEUDOPHAKIC EYES OF 31 PATIENTS were included in this series. The average age at surgery in the study was 38.3 ± 18.4 years (mean ± standard deviation), ranging from 7 to 76 years. Eighteen patients were males (58.0%) and 13 patients were females (42.0%).

Sixteen scars (51.6%) were of herpetic origin (Figure 3, Top left and right), 10 scars (32.2%) of bacterial origin (Figure 3, Middle left and right), and 5 scars (16.1%) resulted from acanthamebic infection (Figure 3, Bottom left and right); in all eyes, corneal deep vascularization was present, with 3 clock hours or more involvement. In 2 (6.4%) eyes with cataract, a mushroom keratoplasty was

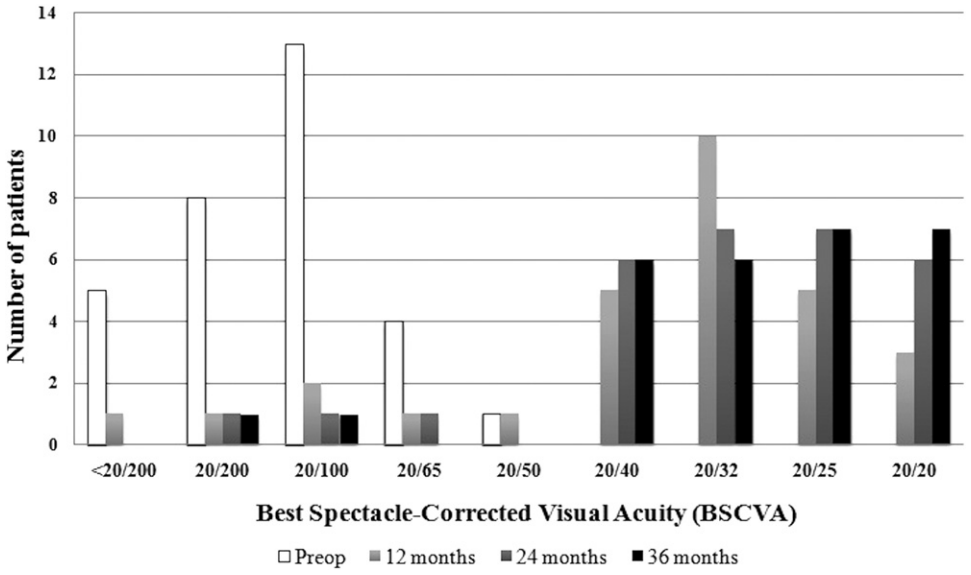

FIGURE 5. Bar graph showing visual outcomes after mushroom keratoplasty: distribution of best spectacle-corrected visual acuity (BSCVA) at different postoperative examination times.

combined with an open-sky extracapsular cataract extraction and intraocular lens implantation. All procedures were uneventful, no recurrence of infection occurred in patients with microbial keratitis, and all grafts but 3 were clear at the final examination. In 2 eyes, progressive lens opacification developed and a standard phacoemulsification with intraocular lens implantation in the capsular bag was performed between 6 months and 1 year after mushroom keratoplasty; in one of them, the surgical trauma caused severe endothelial cell loss requiring regrafting. One patient experienced an episode of allograft rejection revealed by central corneal edema and endothelial precipitates; treatment with topical and systemic steroids failed to control the rejection and the graft decompensated. One eye with glaucoma unresponsive to maximal medical treatment underwent repeat cyclocryocoagulation, but phthisis bulbi eventually occurred.

Visual results are summarized in Figures 4 and 5. Thirty-six months after surgery, best spectacle-corrected visual acuity was 20/40 or better in 26 (83.8%) eyes, ranging between 20/200 and 20/20. The mean refractive astigmatic error was within 4.50 diopters in 26 of 31 cases, averaging 3.10 ± 1.59 diopters.

The donor ECD, calculated from eye bank data, averaged 2671 ± 236 cells/mm^2. Central ECD was 2044 ± 360 cells/mm^2, 1751 ± 398 cells/mm^2, and 1584 ± 381 cells/mm^2 12, 24, and 36 months after surgery, respectively. The endothelial cell loss in the posterior donor button calculated from the preoperative value was 23.5% at 12 months, 34.5% at 24 months, and 40.7% at 36 months. The statistical analysis showed that the reduction of ECD induced by surgery was statistically significant only up to 2 years after surgery ($P < .05$). Instead, the difference from preoperative values obtained 3 years after mushroom keratoplasty did not differ significantly from that recorded 2 years after surgery ($P > .1$).

Graft survival rate at 3 years showed a value of 90.3%, improving to 96.7% when excluding nonimmunologic causes for graft failure.

DISCUSSION

ALTHOUGH PENETRATING KERATOPLASTY HAS ESTABlished itself over the past years as the standard surgical treatment for all pathologic changes impairing corneal function, long-term survival of donor grafts is still problematic in patients at high risk for immunologic rejection. Clinical studies have reported corneal vascularization to be one of the main risk factors for the development of endothelial rejection and consequent graft failure after PK, especially when deep stromal vascularization exceeds 3 clock hours in extension, as is almost always the case in eyes with postinfectious scars of various origin.[5,21–23] In these eyes, full-thickness grafts of small diameter, usually less than 6 mm, have shown higher long-term survival rates than those of conventional size (7.0 to 8.0 mm), but their initial use has been discontinued because of the resulting irregular and high-degree astigmatism.[6,7]

Small full-thickness grafts are known to survive longer than larger grafts in vascularized corneas. This could relate to a lower antigenic load and consequent lower frequency of rejection episodes, or, most probably, to endothelial spreading from the recipient bed into the graft compensating the loss of donor cells (host–donor endothelial rearrangement). Transplantation of a 6-mm graft results in preservation of approximately 70% of recipient endothelium, which could be sufficient to repopulate the graft in case of irreversible endothelial rejection, as easily could be the case in corneas with postinfectious vascularized

scars.[24–26] In our series, although a trend toward progressive endothelial cell loss was still present, the values recorded between 2 and 3 years after surgery did not differ significantly. These values are not lower than those reported after conventional PK, but demonstrate stabilization of endothelial cell loss at a much earlier postoperative time, thus supporting this theoretical possibility. Further eventual loss of donor cells could be compensated fully by rearrangement between donor and recipient endothelium, unless pre-existing damage of the recipient endothelium would make this impossible, as may have been the case in the only immunologic graft failure recorded in our series. The minimal endothelial transplantation model of mushroom keratoplasty therefore offers an alternative approach to improve graft survival in high-risk vascularized corneas.

The use of lamellar keratoplasty to treat full-thickness vascularized stromal scars has not become popular, despite its advantage of leaving the recipient endothelium in place, thus eliminating the risk of its immunologic rejection. In the past, the main reason for this was the technical difficulty to remove the entire scarred stroma while creating a hand-dissected interface of optical quality compatible with final visual acuity as good as that obtained after PK.

More recently, the development of DALK has revived the interest in lamellar keratoplasty to treat full-thickness corneal scars. However, also with DALK, postoperative visual acuity can compare favorably with that reported after PK only when Descemet membrane is exposed and the recipient stroma is removed in its entirety.[27] Baring Descemet membrane is a challenging step with a steep learning curve even in transparent corneas, and the presence of a dense scar located deeply in the stroma is an additional hindrance that very few experienced surgeons can overcome.[28,29] Moreover, when the infectious process involves the whole cornea, adhesions may result between Descemet membrane and overlying stroma, thus increasing the difficulty of a dissection at this level.

Finally, several studies have shown that DALK also is associated with endothelial damage, causing an endothelial cell loss of up to 25% of the preoperative value even in uncomplicated cases.[12,27,30] Keratoplasty surgery using full-thickness grafts shaped like a mushroom combines the minimal effect on corneal curvature of large donor buttons with the preservation of most of recipient endothelium, which is possible only if small donor buttons are transplanted. In 1921, Ebeling and Carrel introduced the concept of a mushroom-shaped graft and performed the first mushroom keratoplasty.[31] However at that time, ophthalmic surgery was still in its infancy, and even the operating microscope was not yet available. Because of the technical difficulties and the outcome, the procedure did not gain popularity and was abandoned.

We have revisited mushroom keratoplasty by modifying the technique as described in our previous report.[14] The key to success for this new technique is to separate the stem from the hat by performing a microkeratome-assisted dissection in the donor cornea and then punching to proper size both the anterior and the posterior lamella. Possible minor discrepancies between the depth of manual trephination and the depth of microkeratome-assisted dissection did not seem to affect negatively the outcome of the procedure, as the results reported herein have shown. The resulting 2 sections are then mounted in place in the recipient bed and maintain their positions without the need of any sutures, similar to deep lamellar endothelial keratoplasty. The potential adverse effect on visual acuity caused by creating a stromal interface is minimized by the use of the microkeratome. As shown also by other types of anterior and posterior microkeratome-assisted lamellar surgery, good vision is the rule, rather than the exception, in patients undergoing these procedures.[32–34] In addition, the large diameter of the hat (9.0 mm) minimizes, although does not eliminate, postkeratoplasty astigmatism and therefore allows spectacle correction in a very high percentage of patients (83.8%).

Indeed, splitting the mushroom in a hat and a stem offers several advantages over a 1-piece mushroom graft. Most importantly, the hat can be centered on the limbus, while the stem is centered on the pupil, thus achieving optimal fitting even in eyes with rather eccentric pupils. In these cases, because the optical zone coincides with the diameter of the stem (usually approximately 6.0 mm) and is rather small, proper alignment is essential in determining the final visual outcome, as well as in avoiding glare and halos at night. Although glare was not tested in our study, none of the patients in this series reported subjective glare. The fact that the incision is buried under the anterior lamella and the contour of the corneal surface remains unaffected may be responsible for minimizing the possible adverse effects of using rather small optical zones.

In conclusion, visual and refractive results of mushroom keratoplasty compare favorably with those of conventional PK; in high-risk patients with vascularized corneal scars occurring after various types of infections, mushroom keratoplasty is associated with an extremely high survival rate in the medium term. Possible explanations for this are either a slow replacement of rejected donor endothelium with cells from the host reservoir, spreading across the host–donor wound, or a reduced stimulation of the immune system by minimal endothelial antigenic load. Additional studies in a larger number of patients followed up for a longer period are required to confirm our initial very encouraging results obtained with mushroom keratoplasty in patients at high risk for immunologic rejection of transplanted corneal grafts.

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the conduct of the study, including collection, analysis, and interpretation of data, with overall supervision and approval by V.S. The study was approved by the local institutional review board (protocol no. 299/CE) and adhered to the tenets of the Declaration of Helsinki. Informed, written consent was obtained from all patients.

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Biosketch

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