

Clinical evaluation of deep anterior lamellar keratoplasty using glycerol-cryopreserved corneal tissues for refractory herpetic stromal keratitis

An observational study

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Abstract

The study aimed to evaluate the therapeutic effects of deep anterior lamellar keratoplasty (DALK) using glycerol-cryopreserved corneal tissues (GCCTs) in patients with refractive herpes simplex keratitis (HSK). This article was a retrospective, noncomparative, and interventional case series. Patients with HSK underwent DALK using GCCTs at Shanghai Tongji Hospital from 2012 to 2015. The best spectacle corrected visual acuity, recurrent inflammation, graft status, postoperative central graft thickness, and pre/postoperative complications were detected. The follow-up ranged from 24.4 ± 5.6 months (range: 16–38 months). Overall, the best spectacle corrected visual acuity was increased from HM/10 cm to 0.15 before surgery to 0.41 ± 0.14 (range: 0.1–0.8; $P < 0.05$) at 12 months postoperatively. Intraoperative microperforation occurred in 4 eyes (14.81%), and rejection episodes were encountered in 3 of 27 eyes (11.1%), and all of the eyes reversed. HSK recurred in 2 eyes (7.41%), 1 eye with repeated recurring HSK, and eventually led to perpetual corneal opacity and the patient refused a retransplantation. The mean entire corneal thickness was 0.519 ± 0.018 mm (range: 0.5–0.56 mm) and the mean graft thickness was 0.405 ± 0.033 mm (range: 0.35–0.47 mm) in the final follow-up. The DALK using GCCTs was proven to be an effective and safe therapy in treating refractory HSK.

Abbreviations: DALK = deep anterior lamellar keratoplasty; GCCTs = glycerol-cryopreserved corneal tissues; HSK = herpes simplex keratitis; BSCVA = best spectacle corrected visual acuity; CGT = central graft thickness; DM = Descemet membrane; PK = penetrating keratoplasty; AS-OCT = anterior segment optical coherence tomography; FCTs = fresh corneal tissues.

Keywords: deep anterior lamellar keratoplasty, glycerol-cryopreserved corneal tissues, refractory herpetic stromal keratitis, therapeutic keratoplasty

1. Introduction

Chronic herpetic simplex keratitis (HSK) remains a high-incidence eye disease which is capable of causing potential vision loss. Although a detailed history along with classic clinical manifestations usually suffice in distinguishing the causative agent, sometimes the lack of typical clinical manifestations adds difficulty in making a definite diagnosis, and can lead to late or incorrect treatment.^[1] Antivirals have been used for the treatment of HSK for over 5 decades, and are commonly categorized into idoxuridine, iododexycytidine, vidarabine, trifluridine, acyclovir, and ganciclovir.^[2] However, it is quite difficult to eradicate

the virus from the cornea, and a latency of the viral antigens, which persist in the individuals for life, has been proposed.^[3,4] The viral antigens trigger an immunopathological response causing damage in the cornea.^[5] Moreover, HSK-induced recurrent epithelial disease, stromal keratitis, and iritis can cause scarring and a loss of vision that may require corneal transplantation for visual rehabilitation.^[6]

Deep anterior lamellar keratoplasty (DALK) is a surgical procedure in which the pathological stroma is excised down to Descemet membrane (DM), leaving the original corneal endothelium intact.^[7–9] Compared to penetrating keratoplasty (PK), which replaces the whole layer of the cornea, DALK has the benefits of avoiding the complications which go along with “open sky” surgery, less postoperative management, and a low risk of allograft rejection, relieve the lack of donor corneas in developing countries, and is effective in eliminating infection especially in with infections limited to the anterior or mid stroma.^[10,11] This study aimed to evaluate the outcomes of DALK in cases of refractory HSK.

2. Methods

2.1. Patients

This was a retrospective, noncomparative, and interventional case series including 27 patients (27 eyes) who had a significant history of refractory HSK (3–25 years, mean: 10 years). Refractory HSK was defined as any HSK that recurred perennially for at least 3 years, with a shorter and shorter onset

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interval, exacerbated yearly with a poorly therapeutic effect. Patients with any of the following conditions were excluded: any history of corneal perforation, active keratitis (fungi, bacteria, and virus). Twenty-seven eyes stayed in inactive phase after a systematic antiviral regimen, leaving corneal stromal opacities and low visual acuity. All the 27 eyes were diagnosed via a detailed history, along with classical clinical manifestations under slit-lamp microscopic examination, and therapeutic DALK was conducted between 2012 and 2015. This study was conducted in accordance with the tenets of the Helsinki Declaration, written informed consent was obtained from all of the patients, and ethics approval was obtained from the Institutional Review Board of the hospital.

2.2. Surgical technique

All of the surgeries were performed by a single surgeon (Dr. Yanlong Bi) under retrobulbar anesthesia (5 mL 2% lidocaine and 0.5% L-bupivacaine mixed solution with 1 drops of 0.1% epinephrine). The diameter and depth of each individual's lamellar dissection were evaluated using a slit-lamp microscope and anterior segment optical coherence tomography (AS-OCT; Carl Zeiss Meditec, Germany) before surgery. A personalized outline of the corneal lesion area was carved first, and then, the corneal stroma was excised from the margin to the center of the cornea. To decrease the risk of DM perforation, in some cases, it was unnecessary to completely expose the DM in the full bed if the residual stroma was uninfected. The donor tissue (-20°C glycerin-cryopreserved corneas) was denuded from its DM, molded to match the size and shape of the recipient bed, and finally, secured with 10/0 nylon interrupted sutures, buried the knots.^[12] The patients wore bandage contact lenses immediately after surgery.

2.3. Perioperative therapeutic protocol

Preoperatively, the patients were administered with antiviral management for oral acyclovir (Neptunus Group, China; 200 mg) 5 times per day, 0.15% ganciclovir ophthalmic gel (Hubei Keyi, China) 4 times per day, tobramycin sulfate eye drops (Alcon, U.S.) 4 times per day and 0.02% fluorometholone eye drops (Santa, Japan) twice a day for at least 3 weeks.

The postoperative treatment consisted of oral acyclovir (Neptunus Group, China) (400 mg) 5 times per day in the first months, and then tapered to 400 mg 2 times per day for a further 12 to 18 months, 0.15% ganciclovir ophthalmic gel 4 times per day for 6 to 12 months (if obvious signs of recurrence were not observed), tobramycin dexamethasone eye drops (TobraDex, Alcon) 4 times daily for the first month, substituted for 0.02% fluorometholone eye drops (Santa, Japan) 3 times daily from then on. All patients orally administered with drugs underwent routine blood and urine examinations as well as liver and kidney function examinations every month to monitor adverse reactions of drugs.

2.4. Perioperative evaluation

The preoperative ocular examination included the best spectacle corrected visual acuity (BSCVA), slit-lamp microscopic examination (including the lesion diameter), AS-OCT, and corneal topography for corneal thickness and the lesion depth.

The postoperative clinical evaluation included the BSCVA, intraocular pressure, slit-lamp microscopic examination (edema

of graft and recipient, healing of graft-recipient interface, recurrent inflammation, uveitis, wound integrity, and rejection episode), postoperative central graft thickness, and postoperative entire central corneal thickness were measured via AS-OCT (Table 1, Figs. 1–3). The follow-up examinations were scheduled weekly during the first month, monthly for 6 months, and every 2 or 3 months thereafter.

2.5. Statistical analysis

SPSS 19.0 was used for the statistical analysis, and the 2-sample *t* test was used to compare the pre/postoperative parameters. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Patient information

Our study included 18 males (66.67%) and 9 females (33%), with a mean age of 40.8 ± 10.6 years old (range 19–56 years). The mean follow-up period was 24.4 ± 5.6 months (range: 16–38 months). All eyes had inactive HSK before DALK. The sutures were selectively removed starting at 6 months in cases of graft neovascularization, severe foreign body sensation, and lacrimation, and completely removed within 12 months postoperatively.

3.2. Visual acuity

The mean preoperative BSCVA was from HM/10 cm to 0.15, while the postoperative visual acuity was significantly improved to 0.41 ± 0.14 (ranging from 0.1 to 0.8; $P < 0.05$) at 12 months. The BSCVA measurement was 0.5 or better in 9 eyes (25.93%), and 0.1 or better in all of the patients at the last follow-up (Table 1).

3.3. Perioperative complications

Four eyes (14.81%) exhibited intraoperative microperforation of DM during the deep stromal dissection, and lamellar keratoplasty were continued since the perforation was tiny enough to neglect the influence to the next procedure, and then with the intracameral injection of a 12% C_3H_8 air bubble. The patients were kept in bed in a supine position for no less than 12 h/d for 3 to 5 days postoperatively. A temporary double anterior chamber occurred in 2 eyes after surgery, fortunately, the fluid interface resolved spontaneously within 3 days after a pressure bandage. For those eyes without microperforation, a sterilized air bubble was injected into the anterior chamber in the last operative step, and the patients were required to keep a supine position in bed for more than 12 hours for 1 day. Two eyes were observed to have a delay of graft epithelization, possibly due to keratitis related limbal stem cells hypofunction. However, the corneal epithelium healed completely within 10 days after surgery.

3.4. Recurrent episodes

HSK recurred in 2 eyes (7.41%) presenting with stromal edema and infection infiltration at 2 and 6 months postoperatively. The 2 patients were given intravenous acyclovir (250 mg) every 8 hours for 3 to 5 days and substituted with oral acyclovir (Neptunus Group, China; 200 mg) 5 times per day, 0.15% ganciclovir ophthalmic gel 6 times per day, tobramycin sulfate eye drops were used 3 times daily. After 4 days, the inflammation

Table 1**Clinical data of HSK before and after DALK.**

| No. | Age | Gender | Infected eye | Preoperative CDVA | Lesion diameter, mm | CNV | Recipient/graft diameter, mm | Complications | Accompanied disease | CGT, mm | BSCVA at last follow-up |
|-----|-----|--------|--------------|-------------------|---------------------|--------|------------------------------|---------------------------------|---------------------|---------|-------------------------|
| 1 | 56 | M | OD | 0.15 | 7.0*6.5 | Mild | 7.5/7.75 | None | Cataract | 0.42 | 0.4 |
| 2 | 26 | M | OS | 0.1 | 6.5*6.0 | Mild | 7.25/7.50 | Rejection | No | 0.44 | 0.6 |
| 3 | 54 | M | OS | HM/10cm | 7.5*7.0 | Severe | 7.75/8.00 | Recurrence and Rejection | Cataract | 0.47 | 0.1 |
| 4 | 25 | F | OS | 0.08 | 7.0*4.0 | Mild | 7.25/7.5 | None | No | 0.35 | 0.5 |
| 5 | 19 | F | OD | 0.1 | 7.0*6.5 | Mild | 7.5/7.75 | None | No | 0.38 | 0.4 |
| 6 | 25 | F | OD | 0.08 | 7.0*7.0 | Mild | 7.5/7.75 | Intraoperative microperforation | No | 0.42 | 0.3 |
| 7 | 40 | F | OS | 0.12 | 7.0*6.0 | Mild | 7.5/7.75 | None | No | 0.35 | 0.3 |
| 8 | 44 | M | OS | 0.02 | 7.0*6.0 | Mild | 7.5/7.5 | None | No | 0.40 | 0.6 |
| 9 | 51 | F | OS | 0.1 | 7.0*6.5 | Mild | 7.5/7.75 | Rejection | No | 0.43 | 0.3 |
| 10 | 25 | M | OD | 0.1 | 5.0*5.5 | None | 6.0/6.25 | None | No | 0.35 | 0.25 |
| 11 | 37 | M | OD | 0.01 | 6.0*5.5 | Severe | 6.5/6.75 | Intraoperative microperforation | No | 0.46 | 0.25 |
| 12 | 42 | F | OS | 0.08 | 6.0*6.0 | Mild | 6.5/6.65 | None | No | 0.40 | 0.4 |
| 13 | 42 | M | OD | 0.1 | 5.0*4.0 | None | 6.0/6.25 | None | No | 0.42 | 0.4 |
| 14 | 47 | F | OD | 0.12 | 7.0*6.5 | Severe | 7.5/7.75 | None | No | 0.39 | 0.5 |
| 15 | 53 | F | OS | 0.1 | 5.0*6.0 | Mild | 6.0/6.25 | None | Cataract | 0.41 | 0.6 |
| 16 | 50 | M | OS | 0.1 | 5.5*6.0 | Mild | 6.0/6.25 | Intraoperative microperforation | No | 0.43 | 0.4 |
| 17 | 20 | M | OS | 0.12 | 6.0*6.0 | Mild | 6.5/6.65 | None | No | 0.40 | 0.4 |
| 18 | 46 | M | OS | 0.01 | 7.0*6.5 | Severe | 8.0/8.25 | None | No | 0.38 | 0.8 |
| 19 | 43 | M | OD | 0.1 | 6.0*6.5 | Mild | 7.0/7.27 | None | No | 0.44 | 0.5 |
| 20 | 48 | F | OS | HM/10cm | 7.0*7.0 | Severe | 7.5/7.75 | Intraoperative microperforation | No | 0.43 | 0.4 |
| 21 | 50 | M | OD | 0.1 | 7.0*6.0 | Mild | 7.5/7.75 | None | No | 0.36 | 0.3 |
| 22 | 45 | M | OD | 0.1 | 6.5*6.0 | Mild | 7.0/7.25 | None | No | 0.38 | 0.4 |
| 23 | 39 | M | OS | 0.12 | 7.0*5.0 | Mild | 7.5/7.75 | None | No | 0.43 | 0.3 |
| 24 | 45 | M | OD | 0.01 | 6.5*6.5 | Mild | 7.0/7.25 | Recurrence | No | 0.39 | 0.3 |
| 25 | 43 | M | OS | 0.1 | 6.5*6.0 | Mild | 7.0/7.25 | None | No | 0.43 | 0.5 |
| 26 | 45 | M | OS | 0.04 | 7.0*7.0 | Mild | 7.5/7.75 | None | No | 0.41 | 0.4 |
| 27 | 41 | M | OD | 0.1 | 5.5*5.0 | None | 6.0/6.25 | None | No | 0.36 | 0.5 |

BSCVA=best spectacle corrected visual acuity; CGT=central graft thickness; CNV=corneal neovascularization; F=female; HM=hand movement; M=male; OD=oculus dexter; OS=oculus sinister.

subsided in one of the recurring eyes, while the other eye recurred more than once, and complicated with graft rejection, eventually leaving a permanent corneal opacity, and he refused to receive a retransplantation.

3.5. Graft rejection and evaluation

Rejection episodes were encountered in 3 of the 27 eyes (11.11%), clinically manifested with ciliary congestion, neovascularization, graft edema, or opacity (Figs. 1 and 3). The rejection episodes reversed in all of the eyes using a regimen of intravenous hydrocortisone (100mg) daily, tobramycin-dexamethasone eye drops (TobraDex, Alcon) every 4 hours, oral acyclovir (Neptunus Group, China) (200mg) 5 times per day, 0.15% ganciclovir ophthalmic gel 6 times per day. Meanwhile, 1 eye complicated with repeated HSK recurrence, eventually leading to a perpetual corneal opacity.

The mean entire central corneal thickness was 0.519 ± 0.018 mm (range: 0.5–0.56mm), and mean central graft thickness was 0.405 ± 0.033 mm (range: 0.35–0.47) as measured by the AS-OCT at the final follow-up (Table 1). Overall, the graft and the recipient matched well, and the graft-recipient interfaces were hardly distinguishable under slit-lamp microscopic examination (Figs. 1–3).

4. Discussion

Corneal disease had been reported as a major cause of blindness, ranking second to cataracts worldwide.^[13] The first China

National Sample Survey on Disability suggested that corneal blindness ranked second (11.44%), causing 1/4 of the blindness in China, and the prevalence was 12/10,000 individuals.^[14] In developed countries, HSK has been the leading infectious corneal disease leading to blindness, with an incidence of HSK from 2.07/10,000 to 3.15/10,000 individuals annually.^[15–17] A national, multicenter, epidemiological survey organized by the Shangdong Eye Institute in 2010 showed that the prevalence of infectious keratitis was 19.2/10,000 individuals, and the prevalence of HSK was 11.0/10,000 individuals, ranking first in infectious keratitis in China.^[18] In summary, HSK is a major cause of corneal blindness worldwide, and more studies concerning the prevention and rehabilitation of infectious corneal blindness should be conducted, in this study we applied DALK using GCCTs for management of HSK.

Over 95% of the cases of HSK are caused by HSV-1, excluding neonatal HSK, which is caused mostly by HSV-2.^[19] HSK threatens visual acuity and is liable to recur, causing repeated torment and eventually permanent diminution of vision.^[14] In order to alleviate patient pain, rehabilitate visual acuity, and recover corneal clarity, therapeutic corneal transplantation is needed to eliminate most of the virus existing in the stroma, and thus, reduce the viral load.^[20]

Compared to PK, DALK has several advantages, including the removal of the pathological cornea without breaking into the anterior chamber, a reduction in the quantity and duration of the postoperative steroid dosage, avoiding the happening of endothelial rejection and chronic endothelial failure, better visual acuity with less astigmatism, and a lower graft failure rate, and

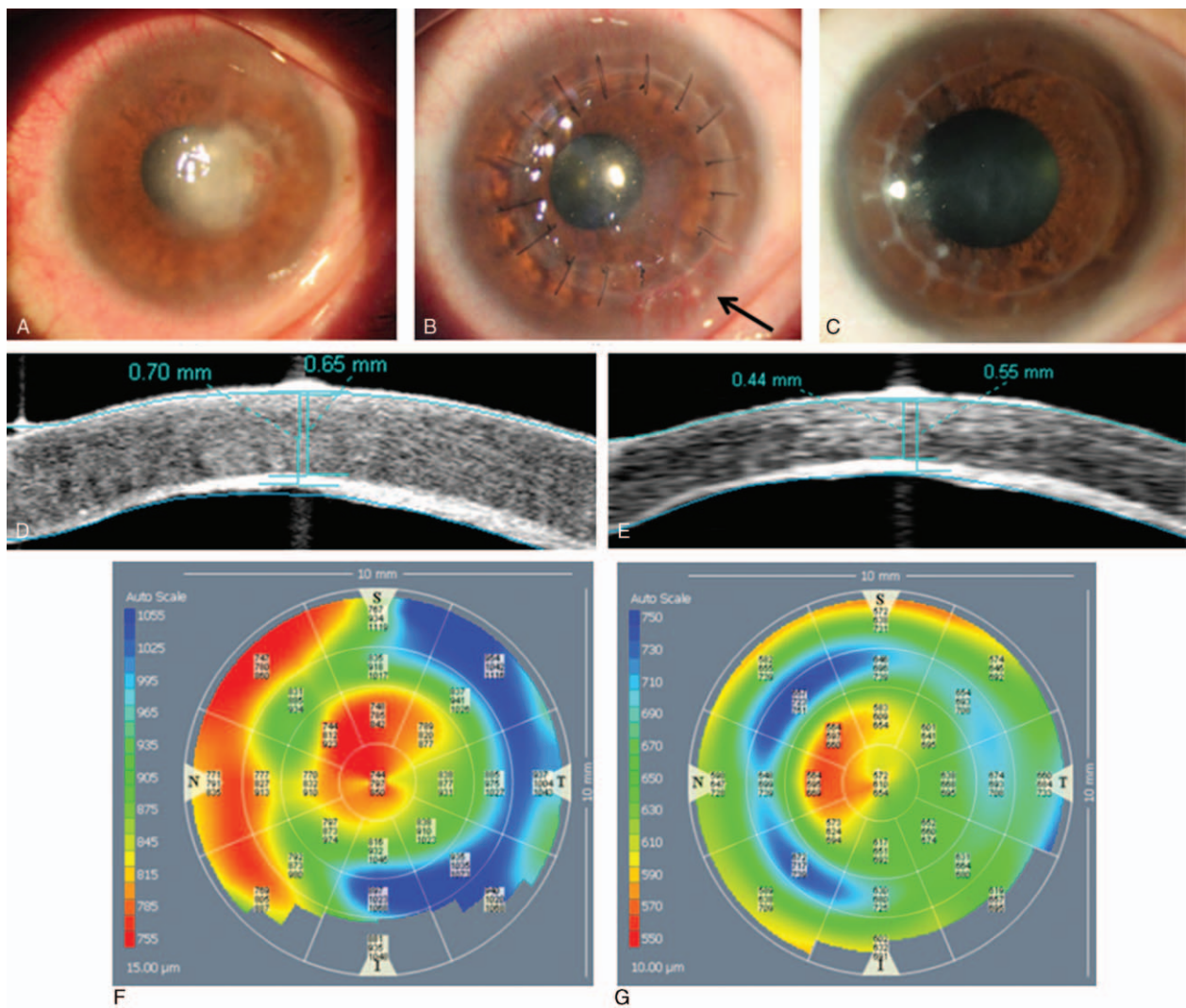


Figure 1. Preoperative and postoperative examinations of a typical case undergoing DALK. (A) The image showed corneal leukoma and neovascularization into the stroma (BSCVA: 0.08). (B) Postoperative image showing mild graft edema with corneal inferotemporal neovascularization (black arrow) 2 months after DALK, and treated with the regimen of stromal rejection. (C) The graft remained transparent 12 months after DALK, and the new vessels faded away after proper medication (BSCVA: 0.5). (D, E) AS-OCT images showed that the DM of the recipient was bared without residual stroma. The CCT decreased between 3 months and 6 months. (F, G) The whole corneal thickness decreased gradually with the subsiding of edema. AS-OCT=anterior segment optical coherence tomography, BSCVA=best spectacle corrected visual acuity, CCT=cryopreserved corneal tissues, DALK=deep anterior lamellar keratoplasty, DM=Descemet membrane.

more donor corneas can be utilized since DALK does not require a healthy donor endothelium.^[6,11,21,22] Moreover, in a long-term comparative study, the recurrence episodes were more severe and complicated in the PK group when compared with the DALK group.^[23] Therefore, PK is commonly recommended in severe and progressive infections which are nonresponsive to medications, risking corneal perforation or scleral extension.^[11,24]

Until now, many surgeons have preferred fresh corneal tissues (FCTs) for DALK, since it was believed to be essential for the graft survival; however, rejections still occur as reported.^[25–28] Previous research has indicated that the spherical equivalent, astigmatism, BSCVA, central corneal thickness, and endothelial cell density were not significantly different between the acellular glycerol-cryopreserved corneal tissues (GCCTs) and FCTs.^[29–31] In contrast to the FCTs, GCCTs can decrease allograft rejection rate and certainly improve the graft survival rate due to the lack of antigen-presenting cells, keratocytes, and other resident bone-marrow derived cells.^[26,31,32] Li et al^[29] found that the rejection-

free graft survival rate at 2 years was 100.0% in the GCCT group, and 78.8% in the FCT group ($P=0.006$). In our previous study, we performed lamellar keratoplasty combined with keratopigmentation in 22 corneal leukoma eyes using GCCTs, and no graft-rejection occurred during the 3 years of follow-up.^[12] Moreover, the outcome of a low graft rejection rate in GCCTs was also confirmed by our preceding study in treating Terrien marginal degeneration.^[33] In this study, three eyes (11.1%) suffered allograft stromal rejection, all eyes reversed after prompt medication. Meanwhile, 1 eye complicated with repeated HSK recurrence, eventually leading to a perpetual corneal opacity.

Previous reports have clarified that up to 33% of patients have suffered HSK recurrence using fresh grafts. The recurrence rate in FCTs may be partially related to the long-term usage of topical steroid eye drops^[22,23]; however, it may be much more closely correlated with fewer keratocytes in the cryopreserved donor tissue to reactivate immune-inflammatory responses. In our study

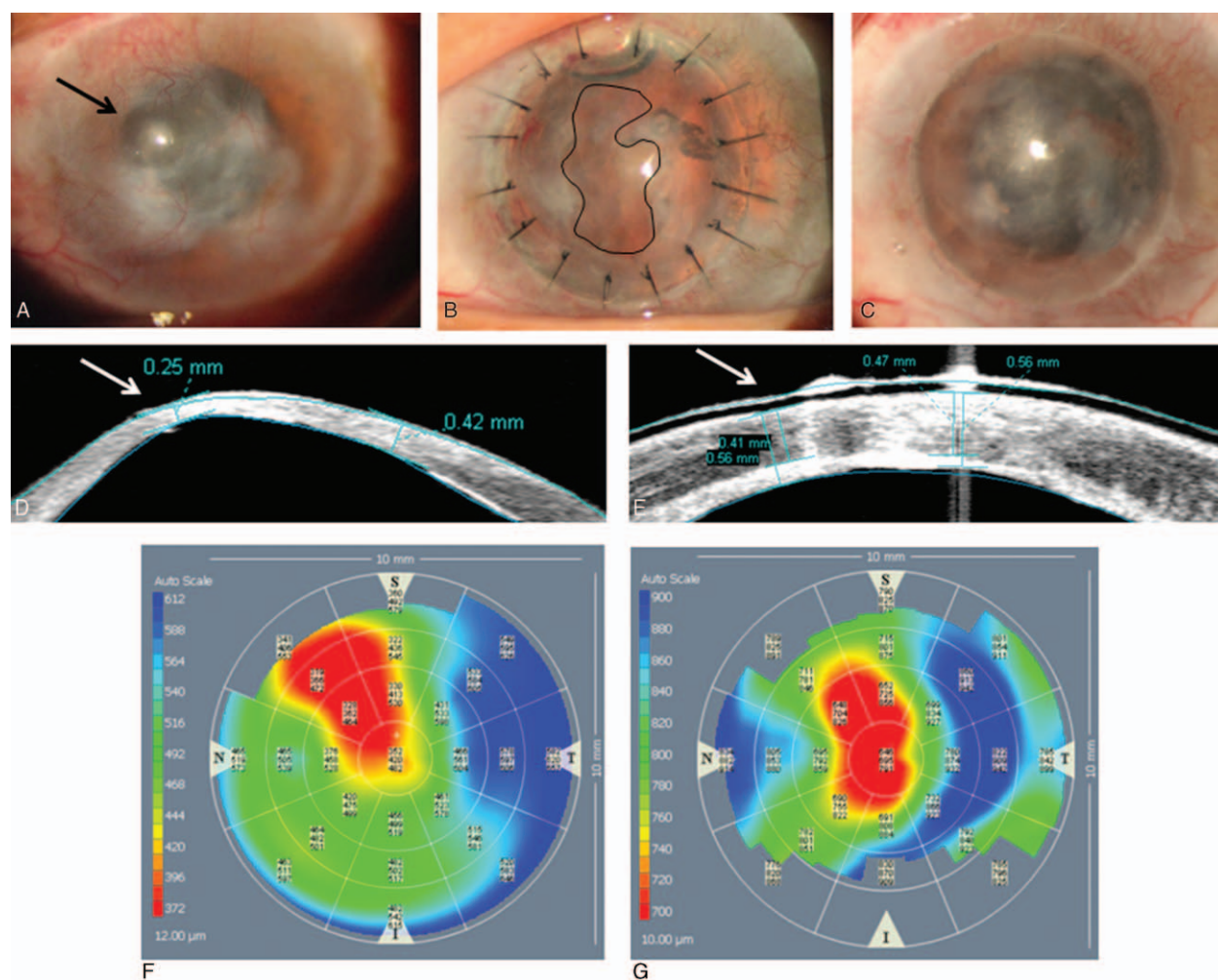


Figure 2. Preoperative and postoperative examinations of another typical case undergoing DALK. (A) Preoperative image showing severe corneal opacity, neovascularization spread over the cornea, and descemetocoele (black arrow) (BSCVA: HM/10 cm). (B) The graft was transparent with residual DM opacity 10 days after surgery. In this case, only the DM in the optical area (black circle) was bared. (C) The graft in the optical axis remained transparent, and the BSCVA achieved 0.1 although the peripheral recipient bed stayed somewhat opaque 12 month after surgery. (D) AS-OCT images showed corneal stroma relatively attenuated in the whole cornea, especially near the site of the descemetocoele before the surgery. (E) The CGT restored to a normal value 8 months after surgery, with contact lens (white arrow). (F) Corneal topography before surgery showed obvious corneal thinning at the site of descemetocoele (G) CGT is thicker than normal, and indicated graft thickness, DALK=deep anterior lamellar keratoplasty, DM=Descemet membrane.

using GCCTs, only 2 eyes (7.41%) exhibited HSK recurrence and the main site was located at the margin of the graft and the recipient bed. This result is consistent with the theory that grafts survive better in inactive HSK when compared with active cases.^[34,35] Wang et al^[20] demonstrated that HSV antigens were identified in 84.2% of the active cases, but 15.4% of the inactive cases via immunohistochemistry staining. Based on the above information, GCCTs can be effectively and biosafely used with a low rejection and recurrence rate in DALK.

During the surgery, there was still a residual whitish semiopacity in 4 of the eyes until excising nearly pre-DM or even baring the DM, this may related to repeated corneal edema, inflammation, and scarring secondary to HSK. In these cases, we continued applying LK instead of changing to PK since the occurrence or high risk of “open sky” surgery, although it may impact the visual acuity to some extent. No incidence of recurrence occurred, and the results proved to be encouraging in these cases (Figs. 2 and 3). With regard to the aforementioned

advantages of DALK, this further illustrates it is an excellent alternative to PK for treating HSK.

In summary, for patients with refractory HSK but healthy endothelium, therapeutic DALK using GCCTs in treating HSK can achieve satisfactory visual acuity, less drug-induced complications, less recurrence episodes, less allograft rejections, early drug withdrawal of steroids and antivirals, a long-term graft survival rate, and efficient use of donor corneas. It is therefore recommended first for treating refractory HSK-induced corneal opacities in patients with relatively healthy endothelium, and no perforation history especially in developing countries where good donor corneas are difficult to get.

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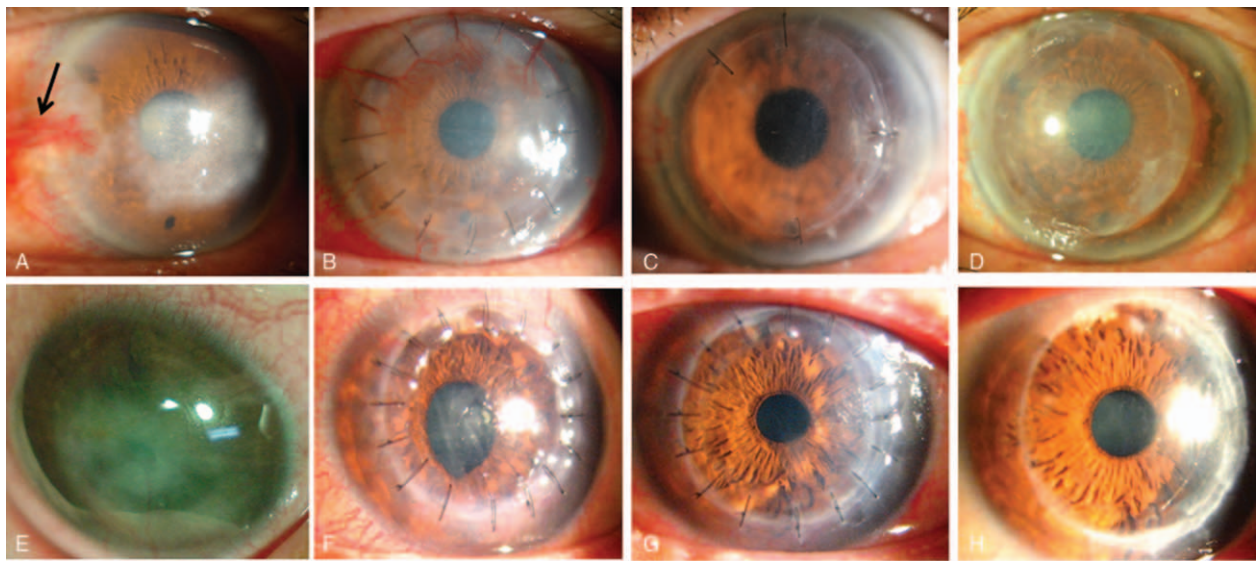


Figure 3. Slit-lamp observation of 2 patients that underwent DALK. (A) In patient 1, the preoperative image showed pseudopterygium (black arrow), HSK opacity invaded the central corneal stroma but left the pre-DM intact (BSCVA: 0.02). (B) We excised the anterior stroma and left the pre-DM stroma during the surgery, so the interface was still residual whitish semiopaque. At the 2-month follow-up, stromal rejection occurred, and the stroma was severely vascularized and treated with a rejection regimen. (C) The graft was clear and new vessels subsided at the 6-month follow-up. (D) The graft kept clear 1 year after surgery (BSCVA: 0.6). (E) In patient 2, the HSK invaded the whole stroma before surgery (BSCVA: 0.01). (F) The graft was clear with mild graft-recipient interface edema 1 week postoperatively. (G) The edema subsided and no recurrence occurred 1 month after surgery. (H) The graft remained clear at the 2-year follow-up (BSCVA: 0.8). BSCVA=best spectacle corrected visual acuity, DALK=deep anterior lamellar keratoplasty, HSK=herpes simplex keratitis, DM=Descemet membrane.

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