Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Long-term comparison of full-bed deep anterior lamellar keratoplasty and penetrating keratoplasty in treating keratoconus*

Yong-ming ZHANG[§1], Shuang-qing WU[§1], Yu-feng YAO[†‡1,2]

([1]*Department of Ophthalmology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China*)

([2]*Sir Run Run Shaw Institute of Clinical Medicine of Zhejiang University, Hangzhou 310016, China*)

[†]E-mail: yaoyuf@mail.hz.zj.cn

Received Oct. 11, 2012; Revision accepted Dec. 20, 2012; Crosschecked Apr. 15, 2013

Abstract: Objective: To compare postoperative outcomes of full-bed deep anterior lamellar keratoplasty (DALK) with penetrating keratoplasty (PK) in treating keratoconus. Methods: Seventy-five eyes of 64 patients who received full-bed DALK and 52 eyes of 51 patients who received PK between June 2000 and August 2010 were included in this retrospective study. Full-bed DALK was performed using Yao's hooking-detaching technique. PK was performed using a standard technique. Intraoperative and postoperative complications, visual acuity, rejection, graft survival, endothelial cell density, corneal sensation recovery, and re-innervation were compared between the two groups. Results: A best correct visual acuity of 0.5 or better was achieved in 90.7% of eyes after full-bed DALK and in 92.3% of eyes after PK (P=0.75). By the fifth postoperative year, graft endothelial cell loss reached 34.6% in the PK group vs. 13.9% in the full-bed DALK group (P<0.001). There were no statistical differences in corneal sensitivity recovery or corneal re-innervation between the groups (P>0.05). Intraoperative microperforation occurred in seven out of 75 (9.3%) eyes with a temporally postoperative double anterior chamber in two eyes in the full-bed DALK group. Postoperative complications in the PK vs. the full-bed DALK groups respectively were: rejection (7.7% vs. 0%, P=0.015), high intraocular pressure (IOP) (46.2% vs. 1.3%, P<0.001), secondary glaucoma (9.6% vs. 0%, P=0.006), complicated cataract (19.2% vs. 0%, P<0.001), and wound dehiscence (9.6% vs. 0%, P=0.006). Conclusions: Both full-bed DALK and PK can offer long-term satisfactory visual outcomes for keratoconus. Graft rejection, secondary glaucoma, complicated cataracts, and constant endothelial cell loss were observed in eyes only after PK.

Key words: Full-bed deep anterior lamellar keratoplasty, Penetrating keratoplasty, Keratoconus, Forceps hooking, Viscoelastic detaching

doi:10.1631/jzus.B1200272 **Document code:** A **CLC number:** R779.65

1 Introduction

Keratoconus is a non-inflammatory, progressive thinning, and ectatic disorder of the cornea (Krachmer *et al.*, 1984; Rabinowitz, 1998; Sherwin and Brookes, 2004). Based on the natural course and severity of corneal thinning and ectasia, keratoconus may be divided into four stages: the preclinical stage, early clinical stage, advanced stage, and complication stage. In the advanced stage, keratoconus is characterized by a significantly localized steep conical protrusion associated with prominent stromal thinning in the cone and the adjacent area of the cornea (Rabinowitz, 1998). Highly irregular astigmatism and high myopia in the advanced stage make spectacle correction unsatisfactory or impossible. They also make contact lens correction intolerable, because of the poor fit between the lens and the cornea (Rabinowitz, 1998; Smiddy *et al.*, 1988). Following the advanced stage,

‡ Corresponding author
§ The two authors contributed equally to this work
* Project (No. 2011C13029-2) supported by the Major Program for Science and Technology Research of Zhejiang Province, China
© Zhejiang University and Springer-Verlag Berlin Heidelberg 2013

keratoconus may further develop into the complication stage with spontaneous Descemet's membrane (DM) tears causing highly acute stromal edema (Sharma *et al.*, 2011), and even the occasional occurrence of perforation (Yeh and Smith, 2008). Although most keratoconus with acute hydrops can resolve automatically, a residual scar will persist (Tuft *et al.*, 1994; Rabinowitz, 1998). Therefore, corneal transplantation becomes the only feasible therapeutic approach for keratoconus in the advanced and complication stages. Through transplantation, the corneal stroma can recover its normal thickness, the protruded cone of the cornea will return to normal curvature, and irregular astigmatism and high myopia will be markedly reduced, even to normal refraction, which helps improve vision significantly.

Two types of corneal transplantation can be used for treating keratoconus: penetrating keratoplasty (PK), which transplants full-layers of the donor cornea including the endothelium to the host eye (Sharif and Casey, 1991; Lim *et al.*, 2000; Pramanik *et al.*, 2006; Jensen *et al.*, 2010), and deep anterior lamellar keratoplasty (DALK), which transplants donor corneal tissue to the host eye with the DM retained and intact (Amayem and Anwar, 2000; Coombes *et al.*, 2001; Al-Torbak *et al.*, 2006; Fontana *et al.*, 2007; Feizi *et al.*, 2010). In the advanced stage of keratoconus, as the DM is not involved, either DALK or PK can be used, but for the complication stage, PK may be the only option since tearing of the DM and scarring have occurred in the recipient cornea. However, some investigators have tried DALK in a small series of cases by retaining small amounts of the recipient stroma not reaching the DM for treating keratoconus after acute hydrops (Anwar and Anwar, 2011; Chew *et al.*, 2011; Ramamurthi and Ramaesh, 2011).

Several studies have compared the clinical results of PK with DALK in treating keratoconus (Watson *et al.*, 2004; Funnell *et al.*, 2006; Bahar *et al.*, 2008; Han *et al.*, 2009; Jones *et al.*, 2009). Techniques for performing DALK differed among these studies, creating different intraoperative and postoperative complications (Amayem and Anwar, 2000; Coombes *et al.*, 2001; Watson *et al.*, 2004; Al-Torbak *et al.*, 2006; Funnell *et al.*, 2006; Fontana *et al.*, 2007; Bahar *et al.*, 2008; Han *et al.*, 2009; Jones *et al.*, 2009; Feizi *et al.*, 2010). In addition, many of the studies used fresh donor tissue for DALK grafting, which can cause stromal rejection and even graft failure after surgery (Al-Torbak *et al.*, 2006; Jones *et al.*, 2009; Feizi *et al.*, 2010). We have developed a unique hooking-detaching technique for performing full-bed DALK, which helps to ensure thorough removal of the stroma from the DM in the full bed with few complications (Yao *et al.*, 2006; Yao, 2008; Wu *et al.*, 2012). Moreover, full-bed DALK using the hooking-detaching technique takes advantage of grafting cryopreserved donor corneal buttons without live cells, which avoids the potential risks of graft rejection (Yao *et al.*, 2002; 2006; Yao, 2008; Wu *et al.*, 2012). We have reported a comparison of the long-term results from using full-bed DALK or PK for treating herpetic scars of the cornea (Wu *et al.*, 2012). In this study, the long-term results of full-bed DALK and PK for treating keratoconus are reported.

2 Patients and methods

2.1 Patients

The clinical data of patients who had received full-bed DALK grafts using Yao's hooking-detaching technique (Yao *et al.*, 2006; Yao, 2008; Wu *et al.*, 2012) or PK grafts for treating keratoconus from June 2000 through August 2010 were reviewed. Keratoconus was diagnosed on the basis of clinical slit-lamp findings (stromal thinning, conical protrusion, a Fleisher ring, Vogt striae, and sub-epithelial scarring) and characteristics of topographic pattern. The development of these features had made glass correction unsatisfactory and the use of contact lenses intolerable. Inclusion criteria were that patients with keratoconus had received full-bed DALK or PK with a minimum follow-up of twelve months after surgery. Exclusion criteria included patients who were not grafted by a primary transplant or those who had a history of previous intraocular surgery, coexistence of other ocular disease (such as retinal disorder or glaucoma) not only keratoconus, or postoperative follow-up of less than twelve months.

The choice of surgical approach depended on the patient's willingness. The potential advantages and disadvantages of full-bed DALK and PK for treating the keratoconus were explained to the patients, as described by Wu *et al.* (2012). Patients who had a history of acute hydrops were recommended to receive PK rather than full-bed DALK.

2.2 Surgical procedures

All surgery was performed by the same surgeon (YFY). The surgical procedure of full-bed DALK was as described in our previous publications (Yao *et al.*, 2006; Yao, 2008; Wu *et al.*, 2012). In brief, a small area of the DM was exposed using the stromal hooking technique, to create a pocket in the recipient bed at 12 o'clock after a trephination of 7.25 to 8.00 mm in diameter. The full stroma was detached by viscoelastic material injection through the pocket and then removed in a single layer to expose completely the DM in full bed (Fig. 1). In cases where the primary exposure did not exactly reach the layer of the DM, a secondary hooking-detaching procedure was performed (Fig. 2). A cryopreserved donor corneal button was grafted using a continuous 10-0 nylon suture or 16 bits of interrupted 10-0 nylon sutures. The size of the donor graft chosen to match the bed depended upon the axial length of the recipient eye: if the axial length was equal to or shorter than 23.75 mm, the graft was 0.5 mm oversize; if longer than 23.75 mm but shorter than 25.00 mm, the graft was 0.25 mm oversize; if longer than 25.00 mm, the graft was the same size.

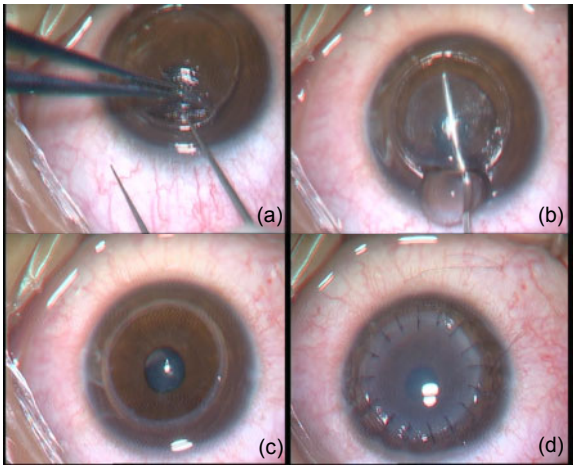

Fig. 1 Demonstration of one-step full-bed deep anterior lamellar keratoplasty (DALK)
The Descemet's membrane (DM) was directly detached in full bed by viscoelastic injection through the initial DM pocket. (a) The DM was exposed as an initial pocket around the trephined margin at 12 o'clock. (b) Viscoelastic injection exactly between the stroma and DM creates a cyst-like elevation of the stroma without edema. (c) The stroma is removed from the DM exposing the full-bed DM. (d) Grafting a cryopreserved donor corneal button

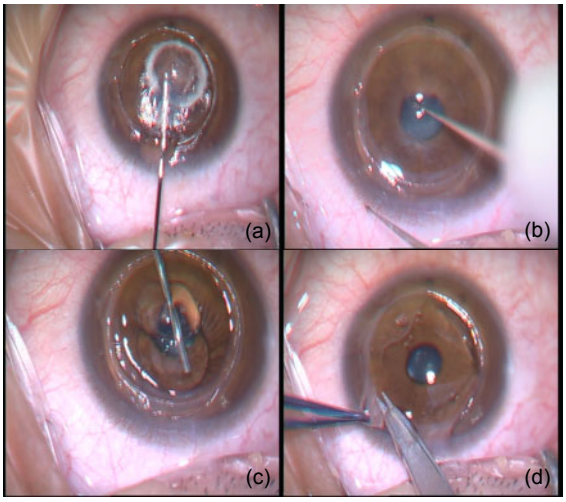

Fig. 2 Demonstration of two-step full-bed deep anterior lamellar keratoplasty (DALK)
Viscoelastic injection through the initial pocket does not go exactly between the stroma and the Descemet's membrane (DM) but runs into the deep stroma layer. Complete exposure of the DM needs a second round of stromal hooking and viscoelastic injection. (a) Viscoelastic injection through the initial pocket creates stromal layer elevation with significant edema. (b) A second pocketing exposure of the DM was performed by hooking on the bed retaining tiny stroma after the first round of viscoelastic injection, viscodelamination, and removal of the stroma. (c) Viscoelastic injection through the second pocket creating thorough detachment of the retained stroma from the DM with a clear detached margin. (d) Removal of the retained stroma sheet around the trephined margin

In PK, a standard surgical procedure was carried out in all cases. The recipient bed was created by trephination using a trephine 7.25 to 8.00 mm in diameter. A fresh donor button, preserved in a moist chamber, was secured by a continuous 10-0 nylon suture or by 16 bites of interrupted 10-0 nylon sutures. The process for selecting a graft size to match the bed was the same as for full-bed DALK grafting. If a planned full-bed DALK for one eye was switched to PK due to large tear of the DM, the eye was included in the PK group for analysis.

2.3 Postoperative medication and follow-up

In the full-bed DALK group, 0.5% (5 g/L) levofloxacin or 0.3% (3 g/L) ofloxacin (Santen pharmaceutical Co., Osaka, Japan) and 0.1% (1 g/L) fluorometholone (Santen pharmaceutical Co., Osaka, Japan) eyedrops were administered four times daily. The PK group was treated with 0.1% (1 g/L) dexamethasone sodium phosphate combined with 0.3%

(3 g/L) tobramycin eyedrops (Tobradex, Alcon, Fort Worth, TX, USA) four times daily. Steroid eyedrops were applied to the eyes after full-bed DALK for at least six months and after PK for at least twelve months, and were then gradually reduced according to the clinical outcome. The application of steroid eyedrops was adjusted when the intraocular pressure (IOP) increased during the follow-up. If this did not work, topical antiglaucoma medication was administered. Rejection episodes were treated with Tobradex eyedrops every 1 to 2 h and then reduced over several weeks, depending on the clinical response.

All patients who received full-bed DALK or PK were scheduled to be followed up after 2 weeks, 1, 3, 6, 12, 18, and 24 months, and every year thenceforth. A detailed clinical examination was performed at every visit, including assessments of IOP, uncorrected visual acuity, best corrected visual acuity (BCVA), refraction, graft clarity, rejection episodes, corneal endothelial density and density of the sub-basal nerve fibers, development of cataracts, and secondary glaucoma. Patients were followed up at shorter intervals or in an emergency if necessary.

In cases with interrupted suture, selective removal of the suture was carried out on the basis of refractive astigmatism and was guided by topography in both groups during the follow-up period. Suture removal was completed in most cases by the end of the follow-up period.

2.4 Major measurements

Sensation in the central cornea was analyzed using a Cochet-Bonnet esthesiometer (Cochet-Bonnet, Lunean, France). Graft corneal endothelial cells and sub-basal nerve fibers were evaluated by slit-scanning confocal microscopy (Confosan 2 or Confosan 3, Nidek Co., Ltd., Gamagori, Aichi, Japan). The cellular density was counted using the manufacturer's software (Navis 3.1.0; Nidek Co., Ltd., Gamagori, Aichi, Japan) and corneal endothelial cell density was determined by counting and averaging the number of cells in an area of 0.05 mm^2 from three confocal microscopic images of the central cornea. The corneal endothelial cell loss was calculated as the decrease in cell density from 1 month to 1 year, and at 2, 3, 4, and 5 years postoperatively, expressed as a percentage of the cell density at 1 month. The number of long (≥200 μm) sub-basal nerve fibers was counted manually from the same confocal microscopic images. The density of the sub-basal nerve fibers was measured in each visit from the best acquired confocal microscopic image and was expressed as $\mu m/mm^2$.

Visual acuity was recorded using decimal charts and was converted to the logarithm of the minimum angle of resolution (logMAR). Vision levels of counting fingers and hand movements were substituted by logMAR values of 1.7 and 2.0, respectively.

All intraoperative and postoperative complications were reviewed, including microperforation of the DM, large DM tears, double anterior chambers, graft rejection, graft failure, high IOP, and secondary glaucoma. The diagnosis of graft rejection, graft failure, high IOP, secondary glaucoma, or complicated cataract was based on the clinical manifestation as defined by Wu *et al.* (2012).

2.5 Statistical analysis

The Kolmogorov-Smirnov test was used for testing the normality of the datasets. To investigate differences between the groups, the Pearson Chi-square test was used for categorical variables, the independent *t* test for normally distributed variables, and the Mann-Whitney *U* test for non-normally distributed continuous variables. Rejection-free outcomes and graft survival were compared between the two groups by using a Kaplan-Meier survival analysis and a log-rank test. A two-tailed probability of 5% or less was considered statistically significant.

3 Results

3.1 General characteristics

One hundred and twenty-seven eyes of 106 patients met criteria for inclusion in this study. Among them, 75 eyes underwent full-bed DALK grafts and 52 eyes underwent PK grafts. Nine patients received bilateral full-bed DALK, one patient received bilateral PK, and 11 patients received full-bed DALK in one eye and PK in the contralateral eye. There were no significant differences between the two groups regarding age, gender, graft size, and host-graft size discrepancy. The postoperative follow-up period was (46.9±28.0) months for the full-bed DALK group, which was shorter than the (60.2±34.6) months for the PK group (P=0.018). Complete suture removal was

carried out earlier in the full-bed DALK group than in the PK group ((16.9±6.0) months vs. (22.8±9.3) months, respectively, $P<0.001$), and the full-bed DALK group needed less frequent visits than the PK group ((0.29±0.16) times/month vs. (0.35±0.20) times/month, respectively, $P=0.046$). The general characteristics of the both groups are summarized in Table 1.

3.2 Intraoperative and postoperative complications

The intraoperative and postoperative complications of the two groups are listed in Table 2. During full-bed DALK surgery, microperforation of the DM occurred in 7 out of 75 eyes (9.3%). Double chambers appeared temporarily after surgery in two of these eyes, but they resolved automatically at 5 and 20 d, respectively, without any specific intervention. A large DM tear occurred in one eye when scissor tips were being inserted between the detached stroma and the DM to cut the stroma layer around the trephined margin. In the PK group, there were no intraoperative complications.

Postoperatively, in the PK group, four out of 52 eyes (7.7%) suffered endothelial graft rejection. The rejection-free survival rate in the PK group was 98.0% at 1 year, 95.9% at 2 years, and 90.5% from 3 to 10 years (Fig. 3). The median duration of a rejection episode in the PK group was (24.7±7.5) months (range, 17–33 months). Although all rejection episodes in the PK grafts were reversible by frequent topical steroid medication, the complete absence of any such postoperative rejection episode in the 75 eyes with full-bed DALK grafts ($P=0.02$, log-rank test) was a marked difference between the two treatments.

In the full-bed DALK group, only one out of 75 eyes (1.3%) developed temporally raised IOP on the first day of the surgery, possibly caused by air injection in the anterior chamber for tamponading microperforation of the DM during surgery. The raised IOP shortly returned to normal on the second day after surgery by administration of a dehydrolyzing agent and did not need anti-glaucoma medication thereafter, but the raised IOP and air injection in the anterior chamber caused irreversible mydriasis. None of the eyes developed steroid-induced high IOP or glaucoma in the full-bed DALK group, but in the PK group, 24 of the 52 eyes treated developed high IOP. Of those, four returned to normal IOP following a single switching of Tobradex to 0.1% (1 g/L) fluorometholone eyedrops, and 15 returned to normal IOP following adjustment of the topical steroid together with topical anti-glaucoma medication. The remaining five eyes developed secondary glaucoma despite switching topical steroid eyedrops and anti-glaucoma medications, and needed a trabeculectomy to control the IOP.

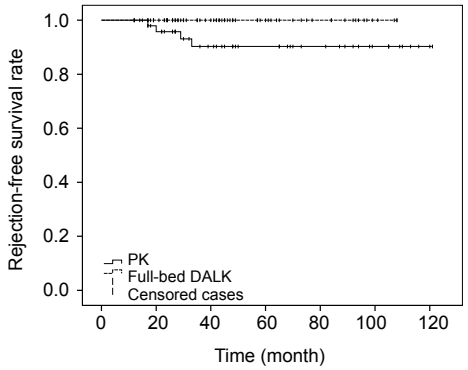

Fig. 3 Overall rejection-free survival rates after full-bed deep anterior lamellar keratoplasty (DALK) and penetrating keratoplasty (PK)

Graph shows overall rejection-free survival rate (Kaplan-Meier method) of 75 consecutive full-bed DALKs and 52 PKs. Rejection-free survival in the full-bed DALK group was significantly better than that in the PK group ($P=0.02$, log-rank test)

Table 1 General characteristics of the full-bed deep anterior lamellar keratoplasty (DALK) and penetrating keratoplasty (PK) groups

Group	Pre-operation		Intra-operation		Post-operation		
	Age (year)	Sex male, female	Graft size (mm)	Graft-bed size discrepancy (mm)	Follow-up (month)	Suture removal (month)	Visit frequency (time/month)
Full-bed DALK (n=75)	20.6±6.8	55, 20	7.53±0.12	0.09±0.13	46.9±28.0	16.9±5.8	0.29±0.16
PK (n=52)	21.9±9.9	45, 7	7.43±1.09	0.10±0.15	60.2±34.6	22.8±9.3	0.35±0.20
P value	0.43	0.07†	0.52*	0.95*	0.018	<0.001	0.046

Values are expressed as mean±standard deviation (SD), except sex. Data with a normal distribution were compared using the independent t-test. * Mann-Whitney U Test; † Pearson Chi-square test

Table 2 Complications in the full-bed deep anterior lamellar keratoplasty (DALK) and penetrating keratoplasty (PK) groups

Complication	Number of patients[#]		*P* value[†]
	Full-bed DALK (*n*=75)	PK (*n*=52)	
Intra-operation			
Microperforation of DM	7 (9.3%)		
Large tear of DM	1 (1.3%)		
Post-operation			
Double anterior chamber	2 (2.7%)		
Rejection	0	4 (7.7%)	0.015
High IOP	1 (1.3%)	24 (46.2%)	<0.001
Secondary glaucoma	0	5 (9.6%)	0.006
Complicated cataract	0	10 (19.2%)	<0.001
Wound dehiscence	0	5 (9.6%)	0.006
Recurrence of keratoconus in the grafts	0	1 (1.9%)	0.23
Mydriasis	1 (1.3%)	0	0.40

[#] Values are expressed as number (percent). [†] Chi-square test. DM: Descemet's membrane; IOP: intraocular pressure

Ten of the 52 eyes (19.2%) in the PK group developed complicated cataracts and eight received cataract surgery during the follow-up period, whereas no complicated cataracts were observed in the full-bed DALK group (P<0.001).

Graft-host junction dehiscence occurred in five eyes (9.6%) of the PK group, three as a result of trauma and two due to spontaneous wound dehiscence after suture removal at 15 and 23 months after surgery, respectively. No wound dehiscence occurred in the full-bed DALK group (P=0.006).

3.3 Graft survival and endothelial cell changes after surgery

By the last visit, in the full-bed DALK group, all 75 grafts (100%) were clear, whereas in the PK group, 51 grafts (98.1%) were clear and one (1.9%) failed due to recurrence of keratoconus with acute hydrops at 30 months after surgery. Overall, probabilities of graft survival were 100% after 1 to 9 years after full-bed DALK and 97.4% after 3 to 10 years after PK (Fig. 4). There was no significant difference in graft survival between the two groups (P=0.27, log-rank test).

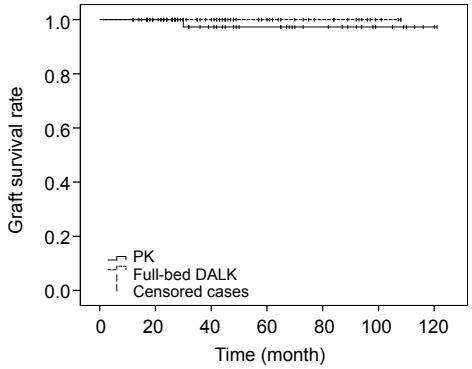

Fig. 4 Overall graft survival rates (Kaplan-Meier method) of 75 consecutive full-bed deep anterior lamellar keratoplasties (DALK) and 52 penetrating keratoplasties (PK)
P=0.27, log-rank test

In the full-bed DALK group, the mean preoperative corneal endothelial cell density was (2634±319) cells/mm^2, significantly higher than (2230±404) cells/mm^2 (n=70) at one month after surgery (P<0.001), with a mean 1-month endothelial cell loss reaching (14.2±11.7)%. Mean 1-month endothelial cell loss was significantly higher in the 6 eyes with perforation of the DM than in the 59 eyes without perforation ((26.0±5.6)% vs. (12.7±11.5)%, P=0.026). Among the six eyes with perforation of the DM, the four in which air was injected into the anterior chamber suffered more endothelial cell loss than the two without air-injection during surgery ((29.1±12.0)% vs. (17.1±25.0)%, respectively, P=0.20). A slight cell loss of graft corneal endothelium was observed in full-bed DALK grafts. The loss was 8.6%, 14.0%, 13.9%, 14.8%, and 13.9% at 1, 2, 3, 4, and 5 years respectively, compared to the 1-month endothelial cell density. By contrast, in PK grafts, a continuous relative decline in corneal endothelial cell density was observed from 14.3% at 1 year to 19.9%, 25.4%, 32.0%, and 34.6% at 2, 3, 4, and 5 years, respectively. The loss in cell density was significantly higher in PK grafts than in the full-bed DALK grafts (Fig. 5).

3.4 Visual and refractive outcomes

Preoperative logMAR BCVA appeared to be worse in the PK group than in the full-bed DALK group (P=0.01, Mann-Whitney U test), possibly due to more eyes in the PK group having experienced acute hydrops or more significant scarring of the stroma.

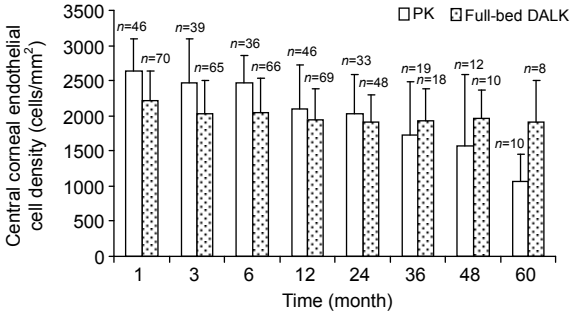

Fig. 5 Periodic changes in corneal endothelial cell density after full-bed deep anterior lamellar keratoplasty (DALK) and penetrating keratoplasty (PK)

Six months after surgery, corneal endothelial cell density was significantly higher in the PK group than in the full-bed DALK group. However, from one to five years after surgery, cell density in the full-bed DALK group remained almost stable, while in the PK group it showed a continuous decline. Values are expressed as mean±standard deviation (SD)

At the last visit, a BCVA equal to or better than 0.5 was achieved in 90.7% of eyes in the full-bed DALK group vs. 92.3% of eyes in the PK group (Table 3). Only in one eye (1.9%) of the PK group was the BCVA lower than 0.1, due to a recurrence of acute hydrops. Postoperative myopia in the full-bed DALK graft group was significantly lower than in the PK group (P<0.001). More eyes in the full-bed DALK group had the absolute spherical equivalent within one diopter (P=0.03), whereas the distribution of astigmatism was similar in the two groups (Table 4). No statistically significant differences in BCVA were found between the two groups at any postoperative follow-up visits (Fig. 6).

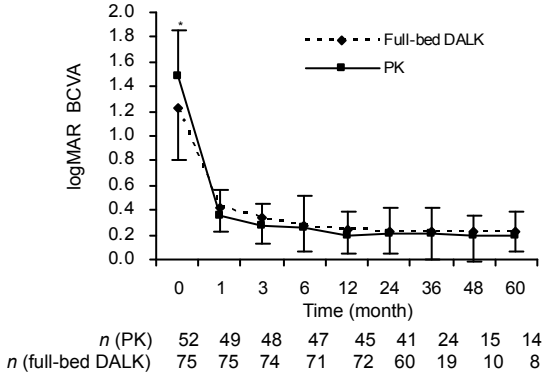

Fig. 6 Best corrected visual acuity (BCVA) in the two groups at all postoperative follow-up visits

Graph shows BCVA expressed as the logarithm of the minimum angle of resolution (logMAR) before and after surgery in 75 eyes with full-bed deep anterior lamellar keratoplasty (DALK) and 52 eyes with penetrating keratoplasty (PK). Values are expressed as mean±standard deviation (SD). * Statistical significance (P<0.01, Mann-Whitney U test)

Table 3 Best corrected visual acuity (BCVA) in the full-bed deep anterior lamellar keratoplasty (DALK) and penetrating keratoplasty (PK) groups at the last visit

BCVA	Number of patients#		P value*
	Full-bed DALK (n=75)	PK (n=52)	
≥0.8	54 (72.0%)	38 (73.1%)	0.89
≥0.5	68 (90.7%)	48 (92.3%)	0.75
≥0.3	74 (98.7%)	49 (94.2%)	0.16
≥0.1	75 (100.0%)	51 (98.1%)	0.23
<0.1	0	1 (1.9%)	0.23

Values are expressed as number (percent). * Pearson Chi-square test. Normally distributed continuous variables were compared using the independent t test

Table 4 Refraction in the full-bed deep anterior lamellar keratoplasty (DALK) and penetrating keratoplasty (PK) groups at the last visit

Refraction	Full-bed DALK (n=75)	PK (n=51)	P value
Spherical equivalent (D)			
Mean±SD (range)	0.09±2.57 (−9.00 to 8.50)	−2.18±3.34 (−9.75 to 7.00)	<0.001
±1 D	18 (24.0%)#	4 (7.8%)	0.03
±3 D	51 (68.0%)	26 (51.0%)	0.06
±5 D	64 (85.3%)	39 (76.5%)	0.24
>5 D and <−5 D	11 (14.7%)	12 (23.5%)	0.24
Astigmatism (D)			
Mean±SD (range)	−3.42±2.26 (−10.00 to 0)	−3.82±2.42 (−9.00 to 0)	0.40
±1 D	9 (12.0%)	8 (15.7%)	0.60
±3 D	41 (54.7%)	24 (47.1%)	0.47
±5 D	54 (72.0%)	38 (74.5%)	0.84
>5 D and <−5 D	21 (28.0%)	13 (25.5%)	0.84

Values are expressed as number (percent). Discrete variables were compared using the Pearson Chi-square test. Normally distributed continuous variables were compared using the independent t test. D: diopter; SD: standard deviation

3.5 Recovery of corneal sensation and sub-basal nerve regeneration

Recovery of central corneal sensation (Fig. 7) along with sub-basal nerve regeneration (Tables 5 and 6, and Fig. 8) occurred sooner in the PK group than in the full-bed DALK group, but was not statistically significant between the two groups throughout the whole follow-up period. The earliest appearance of the sub-basal nerve was found at about 6 months after

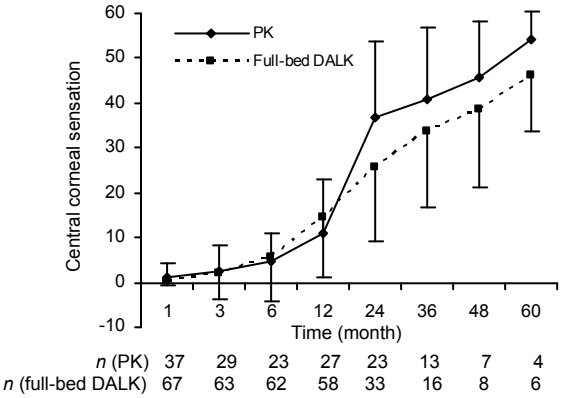

Fig. 7 Recovery of central corneal sensitivity after full-bed deep anterior lamellar keratoplasty (DALK) and penetrating keratoplasty (PK)

Values are expressed as mean±standard deviation (SD). No significant difference was found between the two groups at any time point

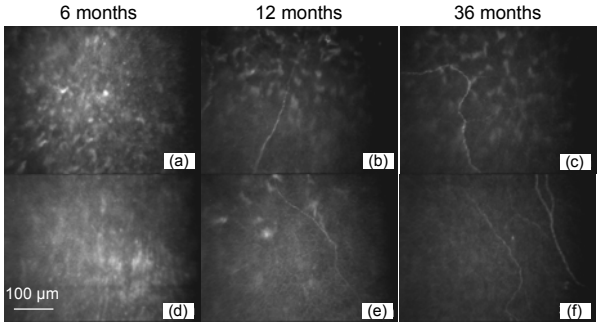

Fig. 8 Sub-basal nerve regeneration in representative cases after full-bed deep anterior lamellar keratoplasty (DALK) or penetrating keratoplasty (PK)

Graph shows regeneration of sub-basal nerves after PK (a, b, c) or full-bed DALK (d, e, f). Similar corneal re-innervation was observed in the two groups

Table 5 Number of the sub-basal nerve fibers in grafts after full-bed deep anterior lamellar keratoplasty (DALK) or penetrating keratoplasty (PK)

Time (month)	Full-bed DALK			PK			*P* value
	Number of eyes	Number of nerve fibers		Number of eyes	Number of nerve fibers		
		Mean±SD	Range		Mean±SD	Range	
1	62	0		46	0		
3	55	0		36	0		
6	59	0.07±0.52	0–4	33	0.04±0.20	0–1	0.53
12	69	0.29±0.93	0–5	43	0.47±1.24	0–4	0.54
24	45	0.86±1.58	0–5	28	0.65±1.23	0–4	0.83
36	19	0.86±1.51	0–5	15	0.95±1.05	0–3	0.41
48	9	1.14±2.04	0–5	7	1.30±1.25	0–5	0.52
60	6	1.33±2.16	0–5	5	1.67±1.37	0–4	0.60

Continuous variables are expressed as mean±standard deviation (SD) and compared using the Mann-Whitney *U* test

Table 6 Density of the sub-basal nerve fibers in grafts after full-bed deep anterior lamellar keratoplasty (DALK) or penetrating keratoplasty (PK)

Time (month)	Full-bed DALK			PK			*P* value
	Number of eyes	Nerve fiber density (μm/mm^2)		Number of eyes	Nerve fiber density (μm/mm^2)		
		Mean±SD	Range		Mean±SD	Range	
1	62	0		46	0		
3	55	0		36	0		
6	59	103.4±800.9	0–6204.0	33	97.8±489.0	0–2445.1	0.53
12	69	559.1±1688.5	0–7761.2	43	855.4±2110.1	0–6614.8	0.55
24	45	1743.0±3127.9	0–11088.5	28	1224.3±2277.8	0–7830.0	0.72
36	19	1666.9±2606.5	0–7392.4	15	2101.4±2286.4	0–7992.2	0.44
48	9	1743.3±3069.0	0–7283.9	7	2856.2±2813.6	0–7321.1	0.40
60	6	2228.3±3815.1	0–9253.1	5	4015.3±3454.7	0–8663.3	0.39

Continuous variables are expressed as mean±standard deviation (SD) and compared using the Mann-Whitney *U* test

surgery in both groups. The percentage of eyes with sub-basal nerves was 2.9% (1/34), 14.3% (6/42), 25.8% (8/31), 58.8% (10/17), 60.0% (6/10), and 66.7% (4/6) in the PK group, and 1.7% (1/60), 12.3% (8/65), 25.6% (11/43), 35.7% (5/14), 42.8% (3/7), and 50.0% (3/6) in the full-bed DALK group at 0.5, 1, 2, 3, 4, and 5 years after surgery, respectively.

4 Discussion

The purpose of corneal transplantation for advanced keratoconus includes reconstruction of normal corneal thickness of the thinning cornea, restoration of normal curvature of the protruded cornea, and improvement of the refractive state of the eye for achieving significantly better vision. Consistent with many previous studies (Watson *et al.*, 2004; Funnell *et al.*, 2006; Bahar *et al.*, 2008; Han *et al.*, 2009), our data show that full-bed DALK can achieve at least the same effect as PK in long-term clinical observation. However, our results and those of many others (Watson *et al.*, 2004; Funnell *et al.*, 2006; Bahar *et al.*, 2008; Han *et al.*, 2009) contradict to some extent those of a recent multiple center study (Jones *et al.*, 2009), which suggested that the improvement in vision created by DALK may not be as favorable as that created by PK. However, DALK surgery is very dependent on the technique and skill of individual surgeons. Only when surgical techniques among groups of surgeons are matched consistently, can meaningful multi-center comparisons be made. Therefore, further studies are needed to compare conclusively the improvement in vision achievable with DALK and PK. Our current study also showed that refraction and astigmatism created by full-bed DALK and by PK were not significantly different, with mean astigmatism of three to four diopters in both groups, consistent with the results of other studies (Watson *et al.*, 2004; Han *et al.*, 2009; Jones *et al.*, 2009).

Regardless of the need for further multi-center studies, our current study suggests that full-bed DALK has many obvious advantages over PK. The most important advantage is the absence of allograft rejection in full-bed DALK grafts. This is attributable to the retention in situ of the recipient DM together with endothelium, for grafting. Moreover, the use of cryopreserved donor tissue for grafting in the full-bed DALK clearly contributed to the absence of postoperative rejection, since no live cells were retained in the cryopreserved donor tissue (Yao *et al.*, 2002; 2006; Yao, 2008; Wu *et al.*, 2012). Epithelial and stromal types of rejection may occur when fresh donor tissue is used in DALK for grafting (Al-Torbak *et al.*, 2006; Jones *et al.*, 2009; Feizi *et al.*, 2010). The absence of allograft rejection in the full-bed DALK group was consistent with our previous studies (Yao *et al.*, 2006; Yao, 2008; Wu *et al.*, 2012) in which we treated other corneal diseases using the same technique of full-bed DALK grafting. Another advantage of using cryopreserved donor tissue for DALK grafting is that we can expand the donor sources, because there is no need to consider the health of the endothelium for long-term cryopreservation of donor tissue. Thus, an elderly donor or a donor with unhealthy endothelium but healthy stroma can still be used effectively for full-bed DALK grafting. This is particularly important for saving corneal tissue in a country like China in which there is an extreme shortage of donors for transplantation.

A second and also very important advantage of full-bed DALK is that it needs only very mild anti-inflammatory medications, such as 0.1% fluorometholone eyedrops for short-term use postoperatively, which certainly helps reduce the incidence of postoperative complications. Moreover, simple, short-term, and very mild anti-inflammatory medications for the full-bed DALK grafts are very helpful for patients who live in remote areas or those who comply poorly with regular follow-ups and medication instructions. In such situations the behavior of the patients will not affect the quality of graft survival. In the PK group, not only was there a 7.7% incidence of allograft rejection, but also complications presumably induced by topical steroids. These results were strikingly different from those of the full-bed DALK group. To prevent postoperative allograft rejection of the PK grafts, we routinely apply Tobradex eyedrops (0.1% dexamethasone sodium phosphate with 0.3% tobramycin) four times daily for at least 12 months to PK-grafted eyes. The difference in postoperative medications between the two groups is clearly associated with the different incidences of high IOP (46.2% in PK grafts vs. 1.3% in full-bed DALK grafts, $P<0.001$), secondary glaucoma (9.6%

in PK grafts vs. 0% in full-bed DALK grafts, P=0.006), and complicated cataracts (19.2% in PK grafts vs. 0% in full-bed DALK grafts, P<0.001).

Potential graft failure and postoperative endothelial cell loss were other concerns. Our current study showed that, in the PK group, the overall probability of graft survival was 97.3% after 3 to 10 years. Although there was 7.7% allograft rejection, it did not cause graft failure by endothelial decompensation. Our results regarding PK for keratoconus are very consistent with many previous reports (Lim *et al.*, 2000; Watson *et al.*, 2004; Funnell *et al.*, 2006; Pramanik *et al.*, 2006; Bahar *et al.*, 2008). Only one graft failure in the PK group was observed, which was due to a recurrence of keratoconus in the graft with acute hydrops. This result suggests that long-term graft survival, even in the PK group, is quite satisfactory. However, when endothelial cell loss is considered, the difference between the PK and full-bed DALK groups is remarkable. Our data show a decline in corneal endothelial cell density in PK grafts of 14.3%, 19.9%, 25.4%, 32.0%, and 34.6% at 1, 2, 3, 4, and 5 years, respectively, significantly higher than that of 8.6%, 14.0%, 13.9%, 14.8%, and 13.9% at 1, 2, 3, 4, and 5 years respectively, in the full-bed DALK group (Fig. 5). In the PK group, a steady and continuous decline in endothelial density was observed, even in the absence of allograft rejection. This suggests that chronic cell loss in the graft endothelium induced by non-immunologic factors may lead to eventual graft failure in extended long-term observation (Ing *et al.*, 1998; Patel *et al.*, 2005). Although endothelial cell loss was significantly lower than in the PK grafts, it did occur in the full-bed DALK grafts. The most critical period for cell loss was within one month after surgery when cell loss reached 14.2% compared with the preoperative endothelial cell density. This result suggests that thorough removal of the recipient stroma and complete exposure of the DM, even using our viscoelastic detaching technique, may lead to a certain degree of endothelial cell loss due to the indirect impact of surgical manipulation of the endothelium. Endothelial cell loss within one month after surgery was also observed in other studies using different DALK techniques (Sugita and Kondo, 1997; van Dooren *et al.*, 2004; Marchini *et al.*, 2006; Fontana *et al.*, 2007; Cheng *et al.*, 2011). We observed a slight decline in endothelial cell density in the full-bed DALK grafts one year after surgery, although the decline was significantly less than that in the PK grafts. The occurrence of endothelial cell loss one year after surgery was in contrast to the results of other studies (Shimazaki *et al.*, 2002; van Dooren *et al.*, 2004), and also contradicted our own previous study using full-bed DALK to treat herpetic corneal scars (Wu *et al.*, 2012). A further study is needed to explain why a slight endothelial cell loss occurred following full-bed DALK over the 5-year period after surgery.

Previous studies have demonstrated that corneal nerve bundles are derived mainly from the ophthalmic branch of the trigeminal nerve and partly from the maxillary branch of the trigeminal nerve. The thick stromal nerve trunks enter the cornea at the periphery below the anterior third of the stroma, moving in a radial fashion parallel to the corneal surface (Muller *et al.*, 1996; 2003; Patel and McGhee, 2009). Therefore, both sub-basal nerve fibers and stromal nerve trunks will be severed by PK and by full-bed DALK, causing loss of corneal sensation in the grafts. An important issue is whether corneal sensation recovery and corneal re-innervation may differ between the cryopreserved donor grafts of the full-bed DALK group and the fresh donor grafts of the PK group. In the present study, based on Cochet-Bonnet esthesiometer measurements, corneal sensation recovery in the full-bed DALK group was slightly, but not significantly, slower than in the PK group (Fig. 7). In both groups, the corneal sensation recovery began slowly at 6 months after surgery, gradually increased from 12 through 24 months after surgery, then noticeably accelerated from 36 to 48 months after surgery, reaching close to normal levels at 60 months after surgery (Fig. 7). The appearance and recovery of corneal sensation in the grafts were confirmed by confocal microscopy, showing the earliest appearance of the sub-basal nerve at about 6 months after surgery in both groups. Quantitative analysis of nerve fiber numbers and density through 60 months postoperative observation by confocal microscopy (Fig. 8) showed no statistical difference between the full-bed DALK group using cryopreserved donor tissue for grafting, and the PK group using fresh donor tissue (Tables 5 and 6). To our knowledge, this is the first report of corneal sensation recovery and corneal re-innervation in grafts of cryopreserved donor tissue.

Further observations of stromal cell reconstruction in the cryopreserved donor grafts after full-bed DALK are currently underway, and the results will be reported elsewhere.

Of particular concern were the two eyes in which spontaneous junction dehiscence occurred during suture removal at 15 and 23 months after surgery, respectively, in the PK group, suggesting poor stromal wound healing at the host-graft junction. Wound dehiscence was not noticed in the full-bed DALK grafted eyes, even with earlier suture removal. We suspect that the intact DM retained in the recipient eye provided tectonic support to prevent occurrence of spontaneous dehiscence in full-bed DALK grafted eyes, or possibly that stromal wound healing is better in full-bed DALK than in PK.

The hooking-detaching technique for performing full-bed DALK was described in detail previously (Yao *et al.*, 2006; Yao, 2008; Wu *et al.*, 2012), and the results of treating herpetic corneal scars and corneal stromal dystrophy using the technique have been reported by Wu *et al.* (2012) and Huo *et al.* (2011). The many techniques that have been developed for performing DALK share the disadvantages of being time-consuming and technically difficult. DM perforation has a rate of incidence of between 4.0% and 39.2% (Sugita and Kondo, 1997; Coombes *et al.*, 2001; Fontana *et al.*, 2007; Marchini *et al.*, 2006; Sarnicola *et al.*, 2010), and large DM tears needing conversion to PK have a rate of incidence of from 2.3% to 3.5% (Feizi *et al.*, 2010; Kubaloglu *et al.*, 2011; Sarnicola and Toro, 2011). Using the stromal-hooking and viscoelastic-detaching technique in handling keratoconus for 76 eyes, microperforation of the DM occurred in 9.3% (7 out of 75) of eyes. Since microperforation was created by a very tiny forceps tip, like the tip of a needle, only two out of seven eyes developed temporary double chambers. The others did not show postoperative double chambers even without air injection into the anterior chamber to tamponade the detached membrane. The double chambers in the two eyes resolved automatically at 5 and 20 d, respectively, without any specific intervention. One eye had a large DM tear which occurred during the insertion of scissors to cut the stromal layer around the trephined margin, suggesting that before removing the stromal layer, it may be necessary to ensure that the stroma has been fully detached from the DM. Aside from these intraoperative complications, our technique enables thorough removal of the stroma with a completely exposed DM in full bed. This was confirmed using scanning electron microscopy of the removed tissue (Yao *et al.*, 2006). The average operating time for each eye was 1 h and 10 min. We believe that the stromal-hooking and viscoelastic-detaching technique for performing DALK is worthy of further promotion among ophthalmologic surgeons.

In summary, many advantages of full-bed DALK over PK in treating keratoconus suggest that the technique deserves further promotion among ophthalmologic surgeons to master the surgical skills required, so that patients can receive surgery with fewer complications and much longer graft survival.

Compliance with ethics guidelines

Yong-ming ZHANG, Shuang-qing WU, and Yu-feng YAO declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the Ethics Committee of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, China and with the Helsinki Declaration of 1975, as revised in 2000(5). Informed consent was obtained from all patients for being included in the study.

References

Al-Torbak, A.A., Al-Motowa, S., Al-Assiri, A., Al-Kharashi, S., Al-Shahwan, S., Al-Mezaine, H., Teichmann, K., 2006. Deep anterior lamellar keratoplasty for keratoconus. *Cornea*, **25**(4):408-412.

Amayem, A.F., Anwar, M., 2000. Fluid lamellar keratoplasty in keratoconus. *Ophthalmology*, **107**(1):76-79, discussion 80.

Anwar, H.M., Anwar, M., 2011. Predescemetic dissection for healed hydrops—judicious use of air and fluid. *Cornea*, **30**(12):1502-1509. [doi:10.1097/ICO.0b013e31822018b9]

Bahar, I., Kaiserman, I., Srinivasan, S., Ya-Ping, J., Slomovic, A.R., Rootman, D.S., 2008. Comparison of three different techniques of corneal transplantation for keratoconus. *Am. J. Ophthalmol.*, **146**(6):905-912. [doi:10.1016/j.ajo.2008.06.034]

Cheng, Y.Y., Visser, N., Schouten, J.S., Wijdh, R.J., Pels, E., van Cleynenbreugel, H., Eggink, C.A., Zaal, M.J., Rijneveld, W.J., Nuijts, R.M., 2011. Endothelial cell loss and visual outcome of deep anterior lamellar keratoplasty

versus penetrating keratoplasty: a randomized multicenter clinical trial. *Ophthalmology*, **118**(2):302-309. [doi:10.1016/j.ophtha.2010.06.005]

Chew, A.C., Mehta, J.S., Tan, D.T., 2011. Deep lamellar keratoplasty after resolution of hydrops in keratoconus. *Cornea*, **30**(4):454-459. [doi:10.1097/ICO.0b013e3181f0b1f3]

Coombes, A.G., Kirwan, J.F., Rostron, C.K., 2001. Deep lamellar keratoplasty with lyophilised tissue in the management of keratoconus. *Br. J. Ophthalmol.*, **85**(7):788-791. [doi:10.1136/bjo.85.7.788]

Feizi, S., Javadi, M.A., Jamali, H., Mirbabaee, F., 2010. Deep anterior lamellar keratoplasty in patients with keratoconus: big-bubble technique. *Cornea*, **29**(2):177-182. [doi:10.1097/ICO.0b013e3181af25b7]

Fontana, L., Parente, G., Tassinari, G., 2007. Clinical outcomes after deep anterior lamellar keratoplasty using the big-bubble technique in patients with keratoconus. *Am. J. Ophthalmol.*, **143**(1):117-124. [doi:10.1016/j.ajo.2006.09.025]

Funnell, C.L., Ball, J., Noble, B.A., 2006. Comparative cohort study of the outcomes of deep lamellar keratoplasty and penetrating keratoplasty for keratoconus. *Eye*, **20**(5):527-532. [doi:10.1038/sj.eye.6701903]

Han, D.C., Mehta, J.S., Por, Y.M., Htoon, H.M., Tan, D.T., 2009. Comparison of outcomes of lamellar keratoplasty and penetrating keratoplasty in keratoconus. *Am. J. Ophthalmol.*, **148**(5):744-751. [doi:10.1016/j.ajo.2009.05.028]

Huo, Y.N., Yao, Y.F., Yu, P., 2011. Pathogenic mutations of *TGFBI* and *CHST6* genes in Chinese patients with Avellino, lattice, and macular corneal dystrophies. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **12**(9):687-693. [doi:10.1631/jzus.B1100011]

Ing, J.J., Ing, H.H., Nelson, L.R., Hodge, D.O., Bourne, W.M., 1998. Ten-year postoperative results of penetrating keratoplasty. *Ophthalmology*, **105**(10):1855-1865. [doi:10.1016/S0161-6420(98)91030-2]

Jensen, L.B., Hjortdal, J., Ehlers, N., 2010. Longterm follow-up of penetrating keratoplasty for keratoconus. *Acta Ophthalmol.*, **88**(3):347-351.

Jones, M.N., Armitage, W.J., Ayliffe, W., Larkin, D.F., Kaye, S.B., 2009. Penetrating and deep anterior lamellar keratoplasty for keratoconus: a comparison of graft outcomes in the United Kingdom. *Invest. Ophthalmol. Vis. Sci.*, **50**(12):5625-5629. [doi:10.1167/iovs.09-3994]

Krachmer, J.H., Feder, R.S., Belin, M.W., 1984. Keratoconus and related noninflammatory corneal thinning disorders. *Surv. Ophthalmol.*, **28**(4):293-322. [doi:10.1016/0039-6257(84)90094-8]

Kubaloglu, A., Sari, E.S., Unal, M., Koytak, A., Kurnaz, E., Cinar, Y., Ozerturk, Y., 2011. Long-term results of deep anterior lamellar keratoplasty for the treatment of keratoconus. *Am. J. Ophthalmol.*, **151**(5):760-767. [doi:10.1016/j.ajo.2010.11.020]

Lim, L., Pesudovs, K., Coster, D.J., 2000. Penetrating keratoplasty for keratoconus: visual outcome and success. *Ophthalmology*, **107**(6):1125-1131. [doi:10.1016/S0161-6420(00)00112-3]

Marchini, G., Mastropasqua, L., Pedrotti, E., Nubile, M., Ciancaglini, M., Sbabo, A., 2006. Deep lamellar keratoplasty by intracorneal dissection: a prospective clinical and confocal microscopic study. *Ophthalmology*, **113**(8):1289-1300. [doi:10.1016/j.ophtha.2006.01.071]

Muller, L.J., Pels, L., Vrensen, G.F., 1996. Ultrastructural organization of human corneal nerves. *Invest. Ophthalmol. Vis. Sci.*, **37**(4):476-488.

Muller, L.J., Marfurt, C.F., Kruse, F., Tervo, T.M., 2003. Corneal nerves: structure, contents and function. *Exp. Eye Res.*, **76**(5):521-542. [doi:10.1016/S0014-4835(03)00050-2]

Patel, D.V., McGhee, C.N., 2009. In vivo confocal microscopy of human corneal nerves in health, in ocular and systemic disease, and following corneal surgery: a review. *Br. J. Ophthalmol.*, **93**(7):853-860. [doi:10.1136/bjo.2008.150615]

Patel, S.V., Hodge, D.O., Bourne, W.M., 2005. Corneal endothelium and postoperative outcomes 15 years after penetrating keratoplasty. *Am. J. Ophthalmol.*, **139**(2):311-319. [doi:10.1016/j.ajo.2004.09.045]

Pramanik, S., Musch, D.C., Sutphin, J.E., Farjo, A.A., 2006. Extended long-term outcomes of penetrating keratoplasty for keratoconus. *Ophthalmology*, **113**(9):1633-1638. [doi:10.1016/j.ophtha.2006.02.058]

Rabinowitz, Y.S., 1998. Keratoconus. *Surv. Ophthalmol.*, **42**(4):297-319. [doi:10.1016/S0039-6257(97)00119-7]

Ramamurthi, S., Ramaesh, K., 2011. Surgical management of healed hydrops: a novel modification of deep anterior lamellar keratoplasty. *Cornea*, **30**(2):180-183. [doi:10.1097/ICO.0b013e3181ca2b8c]

Sarnicola, V., Toro, P., 2011. Blunt cannula for descemetic deep anterior lamellar keratoplasty. *Cornea*, **30**(8):895-898. [doi:10.1097/ICO.0b013e3181e848c3]

Sarnicola, V., Toro, P., Gentile, D., Hannush, S.B., 2010. Descemetic DALK and predescemetic DALK: outcomes in 236 cases of keratoconus. *Cornea*, **29**(1):53-59. [doi:10.1097/ICO.0b013e3181a31aea]

Sharif, K.W., Casey, T.A., 1991. Penetrating keratoplasty for keratoconus: complications and long-term success. *Br. J. Ophthalmol.*, **75**(3):142-146. [doi:10.1136/bjo.75.3.142]

Sharma, N., Mannan, R., Jhanji, V., Agarwal, T., Pruthi, A., Titiyal, J.S., Vajpayee, R.B., 2011. Ultrasound biomicroscopy-guided assessment of acute corneal hydrops. *Ophthalmology*, **118**(11):2166-2171. [doi:10.1016/j.ophtha.2011.03.040]

Sherwin, T., Brookes, N.H., 2004. Morphological changes in keratoconus: pathology or pathogenesis. *Clin. Exp. Ophthalmol.*, **32**(2):211-217. [doi:10.1111/j.1442-9071.2004.00805.x]

Shimazaki, J., Shimmura, S., Ishioka, M., Tsubota, K., 2002. Randomized clinical trial of deep lamellar keratoplasty vs. penetrating keratoplasty. *Am. J. Ophthalmol.*, **134**(2):159-165. [doi:10.1016/S0002-9394(02)01523-4]

Smiddy, W.E., Hamburg, T.R., Kracher, G.P., Stark, W.J., 1988. Keratoconus. Contact lens or keratoplasty?

Ophthalmology, **95**(4):487-492.

Sugita, J., Kondo, J., 1997. Deep lamellar keratoplasty with complete removal of pathological stroma for vision improvement. *Br. J. Ophthalmol.*, **81**(3):184-188. [doi:10.1136/bjo.81.3.184]

Tuft, S.J., Gregory, W.M., Buckley, R.J., 1994. Acute corneal hydrops in keratoconus. *Ophthalmology*, **101**(10): 1738-1744.

van Dooren, B.T., Mulder, P.G., Nieuwendaal, C.P., Beekhuis, W.H., Melles, G.R., 2004. Endothelial cell density after deep anterior lamellar keratoplasty (Melles technique). *Am. J. Ophthalmol.*, **137**(3):397-400. [doi:10.1016/j.ajo.2003.09.053]

Watson, S.L., Ramsay, A., Dart, J.K., Bunce, C., Craig, E., 2004. Comparison of deep lamellar keratoplasty and penetrating keratoplasty in patients with keratoconus. *Ophthalmology*, **111**(9):1676-1682. [doi:10.1016/j.ophtha.2004.02.010]

Wu, S.Q., Zhou, P., Zhang, B., Qiu, W.Y., Yao, Y.F., 2012. Long-term comparison of full-bed deep lamellar keratoplasty with penetrating keratoplasty in treating corneal leucoma caused by herpes simplex keratitis. *Am. J. Ophthalmol.*, **153**(2):291-299. [doi:10.1016/j.ajo.2011.07.020]

Yao, Y.F., 2008. A novel technique for performing full-bed deep lamellar keratoplasty. *Cornea*, **27**(s1):S19-S24. [doi:10.1097/ICO.0b013e31817f445f]

Yao, Y.F., Zhang, B., Zhou, P., Jiang, J.K., 2002. Autologous limbal grafting combined with deep lamellar keratoplasty in unilateral eye with severe chemical or thermal burn at late stage. *Ophthalmology*, **109**(11):2011-2017. [doi:10.1016/S0161-6420(02)01258-7]

Yao, Y.F., Jin, Y.Q., Zhang, B., Zhou, P., Zhang, Y.M., Qiu, W.Y., Mou, S.L., Wu, L.Q., 2006. Recurrence of lattice corneal dystrophy caused by incomplete removal of stroma after deep lamellar keratoplasty. *Cornea*, **25**(10 Suppl. 1):S41-S46. [doi:10.1097/01.ico.0000247212.86014.35]

Yeh, S., Smith, J.A., 2008. Management of acute hydrops with perforation in a patient with keratoconus and cone dystrophy: case report and literature review. *Cornea*, **27**(9):1062-1065. [doi:10.1097/ICO.0b013e31817618c2]

Recommended paper related to this topic

Therapeutic efficiency of tissue-engineered human corneal endothelium transplants on rabbit primary corneal endotheliopathy

Authors: Ting-jun FAN, Jun ZHAO, Xiu-zhong HU, Xi-ya MA, Wen-bo ZHANG, Chao-zhong YANG

doi:10.1631/jzus.B1000199

J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.), 2011 Vol.12 No.6 P.492-498

Abstract: To evaluate the therapeutic efficiency of tissue-engineered human corneal endothelia (TE-HCEs) on rabbit primary corneal endotheliopathy (PCEP), TE-HCEs reconstructed with monoclonal human corneal endothelial cells (mcHCECs) and modified denuded amniotic membranes (mdAMs) were transplanted into PCEP models of New Zealand white rabbits using penetrating keratoplasty. The TE-HCEs were examined using diverse techniques including slit-lamp biomicroscopy observation and pachymeter and tonometer measurements in vivo, and fluorescent microscopy, alizarin red staining, paraffin sectioning, scanning and transmission electron microscopy observations in vitro. The corneas of transplanted eyes maintained transparency for as long as 200 d without obvious edema or immune rejection. The corneal thickness of transplanted eyes decreased gradually after transplanting, reaching almost the thickness of normal eyes after 156 d, while the TE-HCE non-transplanted eyes were turbid and showed obvious corneal edema. The polygonal corneal endothelial cells in the transplanted area originated from the TE-HCE transplant. An intact monolayer corneal endothelium had been reconstructed with the morphology, cell density and structure similar to those of normal rabbit corneal endothelium. In conclusion, the transplanted TE-HCE can reconstruct the integrality of corneal endothelium and restore corneal transparency and thickness in PCEP rabbits. The TE-HCE functions normally as an endothelial barrier and pump and promises to be an equivalent of HCE for clinical therapy of human PCEP.