Original Article

Outcomes of rebubbling for graft detachment after Descemet's stripping endothelial keratoplasty or Descemet's stripping automated endothelial keratoplasty

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Purpose: To study the outcomes of rebubbling for graft detachment after Descemet's stripping endothelial keratoplasty (DSEK) or Descemet's stripping automated endothelial keratoplasty (DSAEK). **Methods:** From 2260 eyes that underwent DSEK or DSAEK from July 2008 to June 2015, 80 eyes of 80 patients developed graft detachment and were retrospectively reviewed. Host-related, surgery-related and donor-related factors that have a bearing on graft adhesion were looked at retrospectively, and eventual outcomes after rebubbling procedure were studied. **Results:** Successful attachment was observed in 77 (96.25%) eyes and clear grafts were achieved in 55 (68.75%) eyes, while 25 (31.25%) eyes had graft failure. The uncorrected and best-corrected distance visual acuities significantly improved from 1 month to 3 months post-operatively and remained stable till 12 months of follow-up. Three lenticules that failed to attach with the first rebubbling procedure underwent a second rebubbling, two underwent a repeat DSEK with good outcomes and four underwent penetrating keratoplasty. On evaluating possible risk factors for graft failure, lower donor endothelial cell density was found to be a significant factor (P = 0.03). The median graft survival following rebubbling was 30 months. **Conclusion:** Rebubbling procedure in detached grafts after DSEK or DSAEK can reattach the lenticule in 96% of eyes in immediate post-operative period and the majority of the grafts remained clear on long-term follow-up with a median graft survival period of 2.5 years.

Key words: Descemet's stripping automated endothelial keratoplasty, Descemet's stripping endothelial keratoplasty, endothelial cell density, graft detachment, rebubbling, retrospective

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The only method of corneal endothelial replacement for nearly 100 years has been full thickness, penetrating keratoplasty (PK).[1] Outcomes of PK are difficult to predict and patients characteristically experience significant refractive shifts as the full-thickness corneal wound heals and sutures are removed over several months to years.[2-5] Descemet's stripping endothelial keratoplasty (DSEK) or Descemet's stripping automated endothelial keratoplasty (DSAEK) has gained popularity in the surgical treatment of endothelial disorders as an alternative to PK. Selective transplantation of the endothelial layer avoids the potential complications of PK such as wound dehiscence, wound infections and high post-operative astigmatism. Instead, there is structural integrity for the recipient, minimally induced astigmatism and faster visual recovery.[6]

Endothelial keratoplasty (EK) has revolutionized since its inception as posterior lamellar keratoplasty by Melles *et al*.[7] It was later modified and popularized as deep lamellar EK by Terry.[8] Subsequently, Melles *et al*. formulated a newer technique of endothelial transplantation termed Descemet's stripping endothelial keratoplasty (DSEK), which replaced the laborious method of lamellar dissection of the recipient cornea simply by stripping Descemet's membrane (DM) and endothelium.[9] The donor lamellar graft then was inserted and allowed to unfold flush to the bare stromal surface, endothelial side down. Preparation of the donor endothelial graft has been simplified by the use of a microkeratome. This variant in procedure has been termed Descemet's stripping automated endothelial keratoplasty (DSAEK).

The most common complication of DSEK/DSAEK in the early post-operative period is graft dislocation or detachment, with reported incidence varying from 0% to 82% and with an average dislocation rate of 14.5%.[10,11] Usually graft dislocation is detected within the first day or two but occasionally it may be detected after several weeks.[11] Initial graft attachment can be impaired by a full-thickness graft edge from a decentered trephination or by surgical trauma that temporarily or permanently reduces endothelial cell function or inadequate intra-operative air tamponade or leaky wounds or post-operative hypotony or rubbing or squeezing the eye in the early post-operative period.[11-13]

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Detached grafts can be reattached by repositioning of the graft and intra-cameral injection of an air bubble called rebubbling.[13] The common causes of a failed DSEK are persistent detachment after repositioning or re-bubbling, persistent edema despite successful reattachment after repositioning or re-bubbling, persistent edema despite primary apposition of the graft (termed primary graft failure) and edema after graft rejection.[14] Earlier studies reporting the outcomes of re-bubbling after DSEK/DSAEK were of a limited case series.[13,15] The aim of this study was to report the outcomes of rebubbling in graft detachment after DSEK/DSAEK in large number of patients.

Methods

This was a retrospective interventional case series of patients who underwent DSEK/DSAEK from Jul 2008 to Jun 2015 at the cornea department of a tertiary eye care centre. The study was approved by the Institutional Ethics Committee (date of approval: 19/9/2015) and was conducted according to the tenets of the Declaration of Helsinki.

All surgeries were done under local anesthesia. The donor graft dissection was done either manually using Barron's artificial chamber (Katena, Denville, NJ, USA) and Melles dissectors (DORC, Zuidland, Netherlands) or with the Moria microkeratome (Moria/Microtek Inc., Doylestown, PA, USA) and, in one patient, femtosecond laser (VisuMax, Carl Zeiss Meditec AG, Germany) was used. The recipient's anterior chamber was entered with a 15° sharp blade (Alcon Surgical, Fort Worth, TX, USA) and the anterior chamber was filled with viscoelastic (sodium hyaluronate 1%; Healon, Abbott Medical Optics, Abbott Park, IL, USA). An 8.0 mm trephine was used to mark the corneal surface. Through the paracentesis, the corneal endothelium and DM were scored and stripped along this mark. DM was not stripped in failed grafts. The viscoelastic material was then irrigated out of the eye and balanced salt solution (BSS) was used to repressurize the anterior chamber. The posterior lamellar graft was inserted into the eye using push-in technique on a sheet of intraocular lens (IOL) glide (BD Medical-Ophthalmic Systems, Franklin Lakes, NJ, USA) with the lenticule positioned endothelial side down over a bed of sodium hyaluronate 1%. Following graft insertion, air tamponade was performed with a complete fill of the anterior chamber for 10 minutes followed by air release leaving air bubble of <50% of the anterior chamber. Donor adhesion was assessed the next day on slit lamp microscope.

The grafts were subjected to re-bubbling using air in eyes with partial or complete detachment along with repositioning in eyes with significant decentration. The procedure was performed under local or topical anesthesia. During surgery, gentle irrigation of the anterior chamber was done to remove viscoelastic material in the interface. Re-bubbling with air injection was performed to achieve a complete fill of the anterior chamber for 10 minutes followed by air release leaving air bubble of < 50% of the anterior chamber. Venting incisions were performed in only 15 eyes. The main incision and the side ports were inspected for wound leakage. Extreme care was taken while removing speculum to avoid pressure on the globe.

Unsuccessful re-bubbling was followed by a second attempt at air injection or underwent a repeat EK/PK. Factors related to host, donor, and surgery that have a bearing on graft adhesion were evaluated retrospectively. The eventual outcomes after re-bubbling procedure were also studied. The factors studied were indication for surgery, prior vitreoretinal surgery, or glaucoma surgery, number of surgeries prior to DSEK/DSAEK, additional intervention at the time of DSEK/DSAEK, lens status (phakic/pseudophakic/aphakic), donor preparation method (manual/automated), location of incision, DM stripping, surface venting incisions, side port and tunnel integrity, endothelial cell density (ECD), pachymetry of donor, preservation to utilization time, age of the donor, type of decentration, possible risk factors of graft detachment, and failure. Endothelial cell loss was calculated by measuring the percentage decrease in endothelial cell density of the central cornea as follows:

$$\text{Percentage of endothelial cell loss} = \frac{(\text{Preoperative cell count} - \text{Postoperative cell count})}{\text{Preoperative cell count}} \times 100$$

Statistical analysis was done using the software Origin v7.0 (Origin Lab Corporation, Northampton, MA, USA). The normality of the continuous data was checked by Shapiro-Wilk test. Visual acuity reported in Snellen measurements were converted to logarithm of the minimum angle of resolution (logMAR) units. Mean and standard deviation summarized the normally distributed data, whereas median, and inter-quartile range (IQR) described the non-parametric ones. Categorical data were described in proportions and compared between graft failures and successes using Chi-square test. Similar comparisons for continuous data were performed using Mann-Whitney test. Pair-wise comparisons between different post-operative visits was done using Wilcoxon signed-rank test. Kaplan-Meier analysis was performed to compute the probability of graft survival following re-bubbling. A P value of <0.05 was considered statistically significant.

Results

During the study period, 2260 eyes underwent DSEK/DSAEK and 96 (4.2%) eyes developed graft detachment that was managed by re-bubbling. A total of 80 (3.5%) eyes had at least 1 year follow-up and were finally included in the retrospective review. There were 45 males and 35 females. Table 1 describes the clinical and surgical details of patients who underwent rebubbling for graft detachment.

Table 2 summarizes the clinical profile of graft detachment and outcomes of rebubbling/repositioning procedure. Fig. 1a-d depict the clinical pictures of a 50-year-old male patient who underwent DSEK for pseudophakic bullous keratopathy in the right eye and Fig. 1e-h those of a 60-year-old male patient who underwent DSAEK for pseudophakic bullous keratopathy in the right eye. The logMAR best corrected distance visual acuity (CDVA) significantly improved from 1 month [median of 0.80 (IQR, 0.60 to 1.09)] to 3 months [median of 0.70 (IQR, 0.47 to 1.00), P = 0.0003] post-operatively and remained stable till 12 months [median of 0.65 (IQR, 0.40 to 1.70)] of follow-up. Two patients with clear grafts had poor visual acuity due to macular pathology and one had glaucomatous disc damage accounting for subnormal vision.

After rebubbling, graft attachment was seen in 77 eyes (96.25%). At the final follow-up, 55 eyes (68.75%) remained

Table 1: Demographics and intraoperative details

Demographics	
Median age (years)	53.5 (IQR, 40-66)
Male:female	1.3:1
Indications for surgery	
Pseudophakic bullous keratopathy	47 eyes (58.75%)
Failed graft	16 eyes (20%)
Aphakic bullous keratopathy	8 eyes (10%)
Fuchs' endothelial dystrophy	4 eyes (5%)
Others (CHED, PPMD, etc.)	5 eyes (6.25%)
Pre-operative lens status	
PCIOL	45 eyes (56.25%)
Phakic	14 eyes (17.5%)
Aphakic	11 eyes (13.75%)
ACIOL	6 eyes (7.5%)
Dislocated IOL	4 eyes (5%)
History of glaucoma surgery	2 patients (2.5%)
	1 trabeculectomy and 1 tube
Number of surgeries before DSEK/DSAEK	
0	8 patients (10.0%)
1	68 patients (85.0%)
2	3 patients (3.75%)
3	1 patient (1.25%)
Surgical intervention	
DSEK/DSAEK alone	50 eyes (62.5%)
ACIOL/SFIOL with AV + DSEK/DSAEK	14 eyes (17.5%)
IOL exchange/implantation/redialing + DSEK/DSAEK	7 eyes (8.75%)
Cataract surgery with PCIOL implantation + DSEK/DSAEK	5 eyes (6.25%)
AV + DSEK/DSAEK	4 eyes (5%)
Surgical details	
Donor dissection	
Moria microkeratome	57 eyes (71.25%)
Manual dissection	22 eyes (27.5%)
Fs laser assisted dissection	1 eye (1.25%)
Incision	
Scleral superior	23 eyes (43.4%)
Scleral temporal	8 eyes (15.1%)
Corneal superior	10 eyes (18.9%)
Corneal temporal	12 eyes (22.6%)
DM stripping	59 eyes (73.75%)
No DM stripping	21 eyes (26.25%)
Surface venting	15 eyes (19.5%)
Suturing of side ports	29 eyes (37.7%)
Suturing of main incision	62 eyes (80.5%)
Median graft size	8 mm (IQR, 8 mm-8 mm)
Donor characteristics	
Donor age, years (mean±SD)	50.0±19.8
Median endothelial cell density (cells/mm^2)	2804.5 (IQR, 2624 to 3003)
Median preservation-to-utilization time (h)	36 (IQR, 24 to 48)
Median donor pachymetry (μm)	509 (IQR, 506 to 514)

This table describes the demographic features and intraoperative details in the present study on outcomes of rebubbling after DSEK. ACIOL=Anterior chamber intraocular lens; AV=Anterior vitrectomy; CHED=Congenital hereditary endothelial dystrophy; DM=Descemet's membrane; DSAEK=Descemet's stripping automated endothelial keratoplasty; DSEK=Descemet's stripping endothelial keratoplasty; Fs=Femtosecond; IOL=Intraocular lens; IQR=Interquartile range; PCIOL=Posterior chamber intraocular lens; PPMD=Posterior polymorphous corneal dystrophy; SD=Standard deviation; SFIOL=Scleral fixated intraocular lens

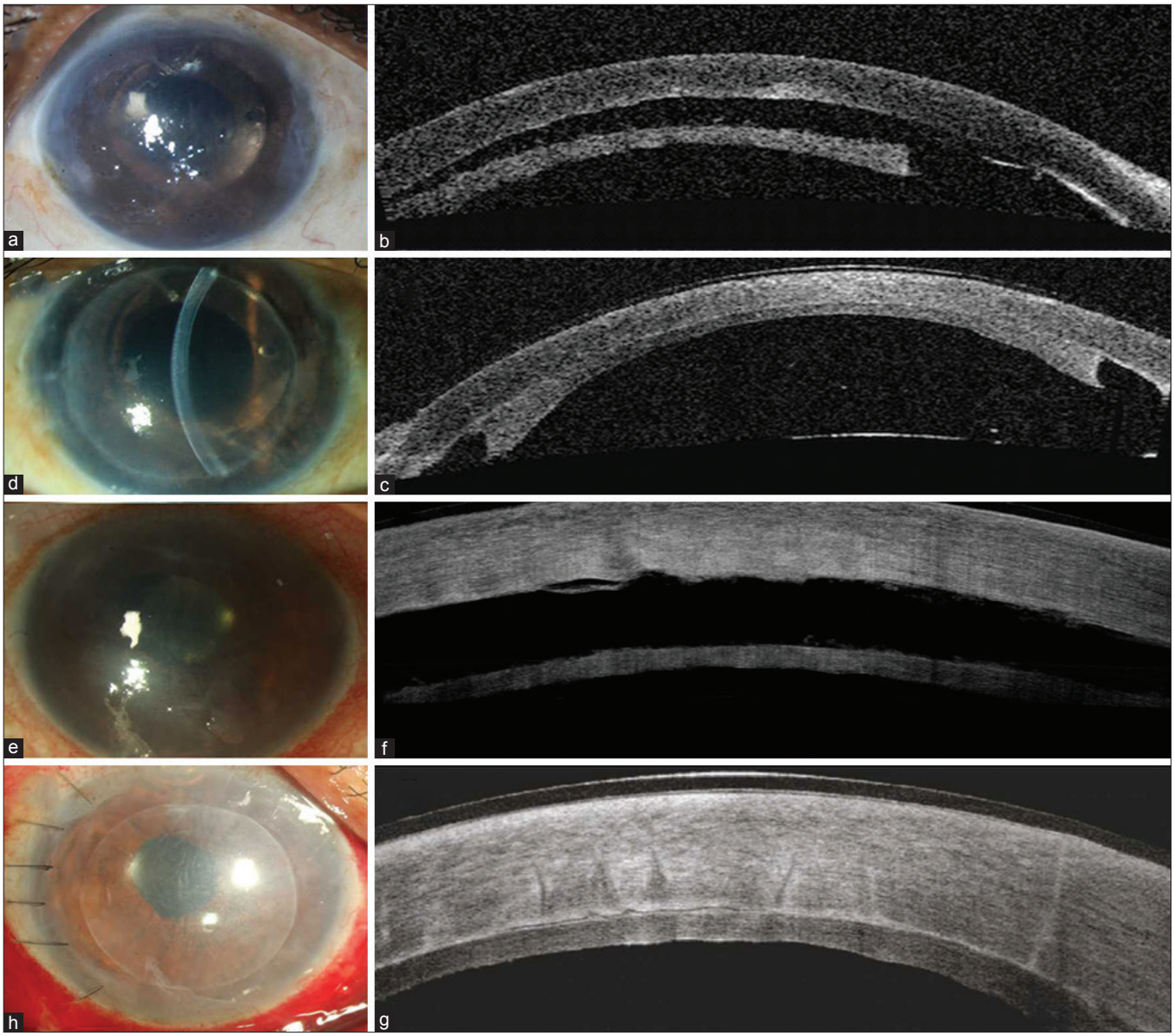

Figure 1: Rebubbling for graft detachment: (a). Diffuse slit-lamp image shows corneal edema with bullae and anterior chamber intraocular lens (IOL), (b). Optical coherence tomography (OCT) shows total graft detachment, (c). OCT shows well attached graft after rebubbling, (d) Diffuse slit-lamp image shows well attached graft 5 years post-rebubbling. (e). Diffuse slit-lamp picture shows corneal edema with bullae and posterior chamber IOL, (f). OCT shows total graft detachment, (g). OCT shows well attached graft after rebubbling, (h). Diffuse slit-lamp picture shows well attached graft 2 years post-rebubbling

clear with a median survival of 30 months [Fig. 2]. Mean ECD (in cells/mm^2) at 3, 12, and 24 months was 1524.6 ± 694, 1355.2 ± 175 and 1429.4 ± 339 respectively. Compared to pre-operative endothelial count, there were 46.6% ECD loss at 3 months and 52.5% at 12 months. On evaluating possible risk factors for graft failure [Table 3], donor ECD was found to be a significant risk factor ($P = 0.03$).

Discussion

Descemet stripping endothelial keratoplasty is increasingly being performed in the recent years with good graft survival rates.[10] Graft dislocation is one of the most common complications of DSEK/DSAEK. This study looked at the long-term success of these grafts after re-bubbling to reattach the dislocated graft. Anatomical attachment was seen in 96% of eyes of which 68% remained clear at the final follow-up.

The majority of detachments occur in the first several days, though they are not uncommon 2 to 3 weeks after surgery and have been reported to occur as long as 6 weeks post-operatively.[16] In our study, all the detachments were noted within the first week of surgery. Many strategies have been described to decrease the rate of graft dislocation such as scraping of the peripheral donor bed, air tamponade, supine positioning postoperatively, venting incisions, and suturing of side port incisions.[15] Graft adhesion in the initial post-operative period depends on maintaining mechanical contact of donor with the host stromal bed. Initial adherence therefore requires pressurizing the anterior chamber with air

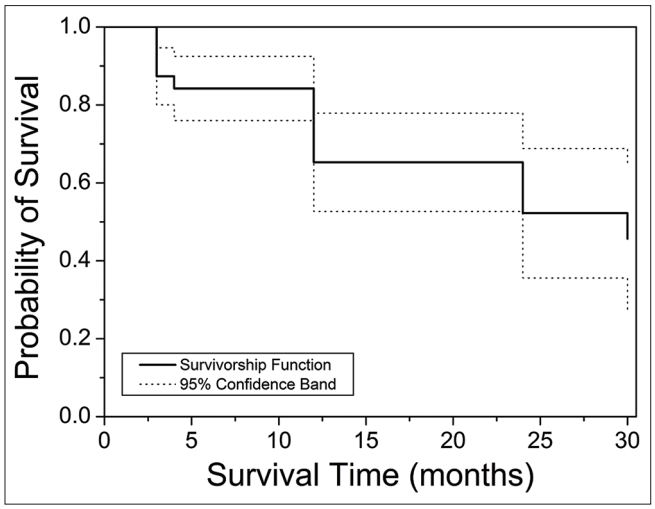

Figure 2: Kaplan Maier Survival analysis: This figure shows the survival probability of the grafts following rebubbling

and maintaining the tamponade. The factors affecting graft adhesion are largely unknown; however, donor adherence is likely most dependent on a healthy donor endothelium that begins pumping fluid from the overlying cornea as soon as possible.[17] Excessive surgical manipulation and poor quality donor grafts are likely to affect graft attachment by interfering with the endothelial pump physiology. In our study, lower donor endothelial cell density was a significant risk factor for graft failure, although the count was still a good indication for keratoplasty procedures.

Graft detachment can be managed by a re-bubbling procedure; however, the increased risk of endothelial cell loss and graft failure is of concern with additional intervention.[18] In our study, graft failure was found in 31% of cases and endothelial cell loss was also seen after rebubbling procedure. Price and Price described endothelial cell loss in a longitudinal analysis of a subset of 34 patients of DSEK that showed 34% cell loss at 6 months, 36% at 12 months, and 41% at 24 months.[18] Mean ECD (in cells/mm^2) was 2000 ± 540 at 6 months, 1900 ± 480 at 1 year and 1800 ± 490 at 2 years.[18] In our study, mean ECD was lower both at 12 (1355.2 ± 175 cells/mm^2) and 24 months (1429.4 ± 339 cells/mm^2) after re-bubbling. Compared to preoperative endothelial cell counts, there were 46.6% ECD loss at 3 months and 52.5% at 12 months after re-bubbling. The mean ECD was lower and the percentage ECD loss was higher at 12 months as compared to previous report.[18]

During the re-bubbling procedure, despite all possible measures to facilitate graft reattachment (irrigation of the anterior chamber to remove any retained viscoelastic material in the interface, adequate air tamponade, and secure wounds), persistent detachment was observed in 4% of eyes. In a series by Suh *et al.*, out of 23% detached cases that underwent re-bubbling or repositioning, 68% of the grafts reattached and 76% of these cleared subsequently.[12] In our study, 96% of detached grafts attached after the first re-bubbling procedure, only 4% required a second re-bubbling procedure; a clear graft was achieved in 68% of eyes with successful attachment and approximately one-third of eyes had primary graft failure. Chaurasia *et al.* showed that 17/27 (62.9%) detached grafts attached after the first re-bubbling procedure and 3/27 (11%) required a second re-bubbling procedure;[15] however, a clear graft was achieved

Table 2: Post-operative graft detachment and rebubbling details

Median duration from surgery to diagnosis of detachment (days)	2 (IQR, 1 to 5)
Median duration between detachment and rebubbling (days)	3 (IQR, 1 to 7)
Air bubble in anterior chamber on first post-operative day	
Absent	20 eyes (36.4%)
One-third	25 eyes (45.5%)
Half	8 eyes (14.6%)
Full	2 eyes (3.6%)
Graft centration	
Centered	20 eyes (35.7%)
Decentered	36 eyes (64.3%)
Type of decentration	
Superior	3 eyes (8.1%)
Inferior	30 eyes (81.1%)
Temporal	2 eyes (5.4%)
Nasal	1 eye (2.7%)
Rebubbling procedure	
Once	76 eyes (95%)
Twice	3 eyes (3.75%)
Thrice	1 eye (1.25%)
Interventions after graft dislocation	
Rebubbling	74 eyes (92.5%)
PK	4 eyes (5%)
Repeat DSEK/DSAEK	2 eyes (2.5%)

This table summarizes the post-operative graft detachment and rebubbling details in the present study on outcomes of rebubbling after DSEK. DSAEK=Descemet's stripping automated endothelial keratoplasty; DSEK=Descemet's stripping endothelial keratoplasty; IQR=Inter-quartile range; PK=Penetrating keratoplasty

Table 3: Evaluation of risk factors for graft failure after rebubbling

Parameter	*P*
Combined surgery (DSEK/DSAEK + AV/DSEK/DSAEK + IOL exchange or redialing)	0.80
DSEK/DSAEK in failed graft/dislocated IOL	0.69
DSEK/DSAEK in failed graft only	1.00
Donor age	0.10
Superior (corneal or scleral) incision	0.64
Post-operative air bubble in anterior chamber - absent to one-third	0.08
Decentered graft	0.21
Interval between DSEK/DSAEK and rebubbling	0.45
Endothelial cell density of donor	0.03
Absent venting incision during DSEK/DSAEK	0.11

This table summarizes the risk factors that were evaluated for graft failure following rebubbling in the present study on outcomes of rebubbling after DSEK/DSAEK. AV=Anterior vitrectomy; DSAEK=Descemet's stripping automated endothelial keratoplasty; DSEK=Descemet's stripping endothelial keratoplasty; IOL=Intraocular lens

in 13/20 (65%) eyes with successful attachment and 7/20 (35%) eyes had primary graft failure, which is comparable to the present study.

Lee *et al*. reported average Snellen visual acuities after DSEK with follow-ups from 3 to 21 months, vision ranged from 20/34 (logMAR 0.23) to 20/66 (logMAR 0.51) and the percentage of subjects whose visual acuity was 20/40 or better after DSEK yielded a range of 38% to 100% from 3 to 20 months.[10] In our study, the logMAR CDVA significantly improved from 1 month (median of 0.80) to 3 months (median of 0.70, $P = 0.0003$) post-operatively and remained stable till 12 months (median of 0.65) of follow-up after re-bubbling.

The recipient endothelium and DM is usually stripped from within the area that will be covered by the donor graft. In particular, guttae cause significant light-scattering and must be removed for optimal post-operative vision.[11] In certain cases, it can be prudent to leave the recipient endothelium and DM intact. For example, in a failed PK without guttae or central DM scarring, leaving DM intact helps preserve the strength of the healed PK incision, especially if relaxing incisions had been made in the incision to treat astigmatism.[19,20] In our study, DM stripping was performed in 77% of eyes and avoided in failed PK patients undergoing DSEK.

Patients with medically managed glaucoma can achieve equivalent visual acuity outcomes as those without glaucoma after EK. Patients who underwent previous glaucoma surgery also realize significant visual improvement with EK although mean CDVA maybe one to two lines worse than that achieved in eyes without glaucoma.[21] Two patients in our study had history of glaucoma surgery of which one achieved CDVA comparable to that achieved in eyes without glaucoma and one had graft failure at 12 months follow-up. Graft survival after DSEK ranges from 55% to 100%, with an average of 94% graft survival at 1 year.[10] The median graft survival period after re-bubbling procedure was 2.5 years in our study. The limitations of the study include all aspects of a retrospective study. In this study, we did not analyze the outcomes based on technique (DSEK vs DSAEK) but would be worth to look at in subsequent work that we do on endothelial keratoplasty.

Conclusion

In summary, graft detachment is one of the most important complications seen in the immediate post-operative period after DSEK/DSAEK. Re-bubbling using air helps in improving both anatomical and functional outcomes. The median graft survival period after re-bubbling procedure was 2.5 years. Lower donor endothelial cell density was found to be a significant risk factor for graft failure but would need further validation.

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Conflicts of interest

There are no conflicts of interest.

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