Clinical Outcomes of pre-Loaded Descemet Membrane Endothelial Keratoplasty Grafts with Endothelium Tri-Folded inwards

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Authors: Massimo Busin[1,2,3], Pia Leon[1,2,4], Sergio D'Angelo[5], Alessandro Ruzza[6], Stefano Ferrari[6], Diego Ponzin[6] and Mohit Parekh[6,7]

Affiliations: [1]Ospedale Privato "Villa Igea", Department of Ophthalmology, Forlì, Italy; [2]Istituto Internazionale per Ricerca e Formazione in Oftalmologia (IRFO), Forlì, Italy; [3]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy [4]SS. Giovanni e Paolo" Hospital, Department of Ophthalmology, Venezia, Italy; [5]Department of Biomedical Sciences and Specialized Surgery, University of Ferrara, Ferrara, Italy [6]International Center for Ocular Physiopathology (ICOP), The Veneto Eye Bank Foundation, Zelarino (Venice), Italy [7]Department of Molecular Medicine, School of Biomedicine, University of Padova, Padova, Italy

Correspondence and reprint requests to: Massimo Busin, MD, Ospedale Privato "Villa Igea", Dept. of Ophthalmology, 47122 Forlì, ITALY. Tel.:01139-347-2449343; FAX: 01139 0543 419566;

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ABSTRACT

PURPOSE: To evaluate the initial outcomes and complications of Descemet membrane endothelial keratoplasty (DMEK) utilizing donor tissues tri-folded with the endothelium inwards, pre-loaded at the Eye Bank and delivered with bimanual pull-through technique.

DESIGN: Prospective, non-comparative, interventional case series.

METHODS: Setting: Eye bank and tertiary care Eye Department. Patient. Population: Forty-six consecutive eyes of 41 patients with Fuchs endothelial dystrophy with or without cataract operated between November 2016 and March 2017. Intervention: DMEK tissues prepared with SCUBA technique and punched to a diameter of 8.25 mm were pre-loaded with the endothelium tri-folded inwards in an intra ocular lens (IOL) cartridge with a 2.2 mm opening filled with the same tissue culture medium contained in the vial used for shipment to the surgeon. Standardized DMEK was performed as a single procedure (n=15) or in combination with phacoemulsification and IOL implantation (n=31) within 48 hours from preparation using a bimanual pull-through technique. Main Outcome Measures: Preparation and surgical times, intraoperative and postoperative complications, best spectacle-corrected visual acuity (BSCVA), endothelial cell density (ECD), and graft detachment rate.

RESULTS: Preparation time averaged 26.2±4.1 minutes (range from 17 to 36 minutes), while the surgical time from opening of the stoppers to air fill of the anterior chamber never exceeded 9 minutes (range from 3 to 9 minutes). Surgery was uneventful in all cases. Postoperative complications included graft detachment in 9/46 cases (19.6%), successfully managed in all cases by single re-bubbling within 6 days from surgery, and glaucoma irresponsive to conservative treatment in 1/46 cases (2.1%). In all eyes without co-morbidities (n = 35 of 40) BSCVA was 20/25 (0.097 logMAR) or better as early as 3 months after surgery. Six months postoperatively, ECD was available in 24 of 25 eyes with an endothelial cell loss calculated as a percentage of the preoperative

value determined at the eye bank (range from 2500 to 2800 cells/mm2) of 29.5±14.8% (range from 8.3 to 52.1%).

CONCLUSIONS: Delivering a pre-loaded DMEK tissue, tri-folded with the endothelium inwards, minimizes surgical time and costs without negatively affecting the outcomes of the procedure.

Keywords: DMEK; pre-loaded; clinical investigation; endothelium-inwards; bimanual pull-through technique; eye bank; graft preparation.

INTRODCUTION

Over the last decade, endothelial keratoplasty has become the gold standard for the surgical treatment of endothelial decompensation and therefore eye banks have been confronted with new requirements from corneal surgeons. Lately, several eye banks have started the preparation and shipment of pre-cut grafts with customized diameter and thickness for Descemet stripping automated endothelial keratoplasty (DSAEK) and ultra-thin DSAEK (UT-DSAEK), as well as, more recently, pre-stripped tissues for DMEK[1-3]. Preparation in the eye bank reduces the efforts, time, and costs of surgery, and therefore the popularity of pre-cut/pre-stripped tissues has been increasing rapidly. In addition, it allows validation of the tissue to be grafted and quality control that cannot be obtained by surgeons when they prepare the tissue themselves at the time of surgery. Minimizing the task of surgeons by offering ready to-use grafts is particularly important for DMEK. In fact, the popularity of this procedure is still limited, partly because of the skills required to master the surgical technique, but also because stripping the Descemet membrane (DM)/endothelium complex and handling the graft may cause tissue wastage in a relatively high percentage of cases, especially in the early stages of the learning curve. In an attempt at further facilitating the task of corneal surgeons, grafts pre-loaded in dedicated delivery devices have been introduced and validated for DSAEK and UTDSAEK[4]. We have adapted to DMEK the approach used for DSAEK and pre-loaded in the eye bank the DMEK graft into an intra ocular lens (IOL) cartridge[5], which is shipped to the surgeon and later used for delivery with the pull-through technique described previously[6]. We report herein the initial clinical outcomes of DMEK using grafts pre-loaded this way at the eye bank.

MATERIALS AND METHODS

All eyes undergoing DMEK with pre-loaded tissue at Ospedali Privati Forlì (Forli, Italy) between November 2016 and March 2017 were included in a prospective study aimed at evaluating the outcomes and the possible complications of the procedure. All tissues were collected from Fondazione Banca degli Occhi del Veneto (FBOV) of Venice, Italy, and were pre-loaded in the same Institution after obtaining written consent to be used for transplantation, from the donor's next-of-kin. The study followed the tenets of the 2013 Declaration of Helsinki and approval from the ethics committee of Ospedali Privati Forlì (Forli, Italy). Preoperatively, demographic data was recorded and every patient underwent a complete ophthalmological evaluation including slit-lamp examination, Snellen BSCVA, refraction, tonometry, funduscopy, as well as central (when possible) and peripheral endothelial microscopy (EM-3000, Tomey, Germany). In addition, the power of the intraocular lens (IOL) to be implanted was determined by means of optical biometry (Lenstar LS900, Haag-Streit, Bern, Switzerland). All pre-loaded tissues were prepared according to the technique described earlier5,6 with slight modifications as detailed below and the time required for preparation was recorded. In all cases, a standardized DMEK was performed as specified in the section below; the time elapsing between opening of the tissue vial and the final air filling of the anterior chamber was noted. Patients were scheduled for evaluation of BSCVA at 3 and 6 months after DMEK and assessment of ECD at 6 months after DMEK. Postoperative ECD was compared with that measured preoperatively at the eye bank for the donor corneas using light microscopy after vital staining with trypan blue (0.25%), and cell loss was determined as a percentage of the preoperative in vitro value. Intraoperative and postoperative complications were also recorded. All data collected in the study was entered into an electronic database via Microsoft Excel 2007 (Microsoft Corp., Redmond, WA). Results of descriptive analyses were expressed as means ± standard deviations for quantitative variables, and as counts and percentages for categorical variables.

Tissue preparation in the eye bank Donors aging between 55 and 70 years were selected for DMEK preparation. After stripping, all tissues were screened using trypan blue staining (0.25%) (VisionBlue, D.O.R.C., Zuidland, The Netherlands) and the endothelial cell density (ECD) and trypan blue positive cells (TBPCs) were recorded by means of a 10x10 reticule mounted in the eyepiece of an inverted microscope (Axiovision, Zeiss, Oberkochen, Germany). The method of preparation was slightly modified from that of previous reports by Busin et al. 6 and Parekh et al.5. The cornea was centered on the base of a suction punch and vacuum was created. A 9.5 mm trephine (Moria, Antony, France) was used to make a superficial incision by gentle tapping. Few drops of trypan blue (0.25%) were applied for about 20 seconds to visualize the incision. The portion of the Descemet Membrane (DM)/endothelium layer outside of the incision was removed using a 120 mm, straight Medium forceps, with pointed tips (Janach, Como, Italy). The endothelium was kept moist during the entire procedure using transport medium [TM], which was prepared in house (FBOV, Mestre, Italy) with full regulatory compliance. Standard stripping was performed using a single quadrant method by peeling the DM/endothelium layer from superiorly up to the inferior periphery except for a thin peripheral hinge (Figure 1, top left). The detached tissue was flipped over to expose the stromal surface and a skin biopsy punch was used to punch the bare stroma (Figure 1, top middle); then the stripped DM/endothelium layer was repositioned back into place (Figure 1, top right). After releasing the vacuum, the tissue was turned upside-down with the corneal convexity facing up. The punched stroma was removed (Figure 1, bottom left) and a letter ‘F’ was marked on the Descemetic side of the graft using a Sinskey hook stained with gentian violet (Figure 1, bottom middle). The stroma was replaced back (Figure 1, bottom right) and the cornea was turned again with the endothelium facing up. The pre-stripped DM/endothelium layer, 9.5 mm in diameter, was punched again with an 8.25 mm punch (Moria, Antony, France) and the excess peripheral crown of the tissue was removed, maintaining the endothelial side facing up. The membrane was then stained again with trypan blue for about 1 minute. It was tri-folded manually with the endothelium inwards from one followed by the opposite side (Figure 2, top left), as

described in our previous article6 and transferred onto a soft contact lens (initial 22 cases) or a sterile aluminium foil (all other cases) that served as scaffold for transportation into the groove of an IOL cartridge (Viscoject, Wolfhalden, Switzerland) with a 2.2 mm opening (Figure 2, top right). The tissue was pulled further inside the funnel of the IOL cartridge filled with TM, using a microincision forceps (Electron Microscopy Sciences, Hatfield, Pennsylvania, USA) inserted from the funnel opening (Figure 2, bottom left). The rear exit of the cartridge was blocked with a white silicon stopper (Figure 2, bottom right), which was hollow to allow TM circulation in the cartridge. The cartridge was then closed by locking its wings and the entire system was immersed and fixated into a dedicated sterile vial that was developed in order to avoid floating and erratic movements of the cartridge during shipment (as opposed to what happens to the other pre-loaded DMEK devices). An illustration of the dedicated shipping container showing a fixed IOL cartridge in the groves with DMEK tissue inside the funnel (Figure 3, top left) and a silicon stopper on its rear end (Figure 3, top right) is shown for appreciating the groves and parts of the device. The actual container with pre-loaded DMEK graft inside the IOL cartridge (Figure 3, bottom left) was filled with TM and shipped to the surgeon for transplantation (Figure 3, bottom right). The time required for preparation of the Surgical technique In all patients, anesthesia and akinesia were obtained by means of peribulbar injection of 10 ml of a 0.75% ropivacaine solution. Surgery was performed in all cases according to the technique described previously with minor modifications.6 In particular, for the main entrance a self-sealing corneoscleral tunnel, 2.8 mm in width, was used instead of a clear-cornea approach. After removing the cartridge with the pre-loaded graft from the vial employed for shipment, a dedicated handle was fixated into the hollow of the rear silicone plug (Figure 4, top left). Then TM was washed out by slowly irrigating the funnel with balanced salt solution. The bimanual pull-through technique was used in all cases for graft delivery (Figure 4, top right). Whenever required, gentle tapping on the cornea was utilized to unfold the graft (Figure 4, bottom right) and air filling of the anterior chamber completed surgery (Figure 4, bottom left). The corneo-scleral tunnel and the side entries were sutured only when an air leak was observed while

filling the anterior chamber. Triamcinolone Acetonide and Gentamicin Sulfate 0.3% were injected subconjunctivally at the end of the procedure. After surgery, a pressure patch was entire procedure was noted. applied and patients were instructed to lie on their back for 2 hours before being checked at the slit-lamp for the possible onset of pupillary block, treated in all cases observed by releasing air through one of the side entries. Beginning the next morning, dexamethasone phosphate 0.1% and tobramycin sulfate 0.3%, both antibiotic eye drops, were administered every 2 hours, then tapered over 3 to 4 months to a single daily steroidal administration, which then was discontinued only in steroid responders. Sutures when present were removed 2-4 weeks after DMEK.

RESULTS

At the time of this review, 46 eyes of 41 patients had entered this study; 40 eyes of 36 patients had a minimum follow-up of 3 months and 25 eyes of 23 patients had a minimum follow-up of 6 months. There were 30 females and 11males; the average age was 68.2 ± 8.4 years (range from 51 to 86). Stripping and pre-loading of DMEK grafts at the eye bank was uneventful in 45 of 46 (98%) cases. One case was complicated by extensive tissue rupture during the loading phase. The time required for preparation averaged 26.2 ± 4.1 minutes (range from 17 to 36 minutes), Surgery was uneventful in all cases. No change in tissue orientation occurred between loading at the eye bank and delivery in surgery. The time required to perform DMEK, from opening of the stoppers to filling of the anterior chamber with air, never exceeded 9 minutes (range from 3 to 9 minutes). After surgery, no primary failure was observed. Graft detachment was seen in 9 of 46 eyes (19.6%), in all cases but one after DMEK combined with phacoemulsification. Single re-bubbling within 4 days from surgery succeeded in reattaching all grafts. At the time of the review all corneas were clear with perfectly attached grafts. In some patients, the “F” mark visible at day 1 (Figure 5, left) could still be seen at month 3 (Figure 5, middle), but faded away at month 6 (Figure 5, right) after DMEK. As early as 3 months after DMEK, BSCVA had improved from 20/60 or worse in all cases to 20/25 or

better in overall 35 of 40 eyes, i.e. in all eyes when 5 eyes with comorbidities were excluded. BSCVA did not change substantially at the 6-month examination time. In the 5 cases with BSCVA below 20/25, moderate to severe glaucomatous optic atrophy was observed. The average cell loss determined 6 months after DMEK as a percentage of the preoperative value determined at the eye bank (range from 2500 to 2800 cells/mm2) in 24 of 25 eyes was 29.5±14.8% (range from 8.3% to 52.1%).

DISCUSSION

The need for high surgical skills and lack of standardization, as well as the high rate of intraoperative and postoperative complications, have all contributed to slowing down the rate of adoption of DMEK among corneal surgeons. However, in recent years, substantial progress has been made in the preparation of DMEK grafts, and pre-stripped tissue is available from many eye banks today. The use of pre-stripped tissue not only eliminates intraoperative waste and allows quality control of the tissue eduring graft preparation but also considerably reduces the time required for the procedure, and yields results comparable with those obtained with donor tissue stripped at the time of surgery2[,3].At the winter meeting of the European Society of Cataract and Refractive Surgery held in Maastricht (The Netherlands) in February 2017, we presented a video illustrating a further step towards standardization and simplification of DMEK, i.e. those of grafts loaded at the eye bank into a delivery device and, therefore, shipped pre-loaded to the surgeon. This approach offers several advantages over the use of tissue that is only prestripped. Firstly, the delicate phase of tissue loading is delegated to the eye bank and complications related to this maneuver, which might lead to the surgeon aborting the DMEK procedure intraoperatively, can instead be easily dealt with at the eye bank. In addition, no punches or other instruments required for graft preparation are necessary in surgery, thus reducing costs considerably in countries like the USA, where the insurance reimbursement for tissue is separate from the forfeited payment of hospital and doctors' fees. Finally, the surgical time is further minimized6, as shown by the results

obtained in our study. To reduce the bias related to maneuvers performed by a technician under a sterile hood rather than by a surgeon in the theatre, a pre-clinical laboratory validation of the preparation of pre-loaded DMEK grafts was undertaken by FBOV5.Tri-folding the pre-stripped tissue with the endothelium inwards was relatively easy while the detached graft was still lying on the donor cornea. Maintaining the tri-folded architecture during transportation into the cartridge, however, can be challenging as lifting the tissue with forceps leads to loss of its configuration. For this reason, instead of the soft contact lens used by Busin et al. in their series of experiments6,we resorted to using a customized sterile aluminum foil. The aluminum foil is a flexible support that can be easily molded to perfectly adapt to both, the hollow of the donor cornea and the groove of the cartridge while, unlike the soft contact lens, maintaining its shape and therefore allowing the surgeon to easily maneuver the device during preparation. In addition, the smoothness of the aluminum foil allowed us to drag the graft in its tri-folded shape uneventfully onto its surface in all cases. Also, the aluminum foil offers other advantages including easy availability, low costs and possibility of multiple sterilizations. As shown by Busin et al.6, grasping the donor tissue at its periphery damages about75 endothelial cells each time, which means that more than 15 touches will destroy 1% of a donor endothelium with 2,500 cells/mm2. At the eye bank we grasped the periphery of the tissue immediately below the “F” mark, which served as a reference also for the surgeon in theater, in order to limit contact with the forceps in the same area and minimize cell damage. The ‘F’ marking was also instrumental for the surgeon in avoiding upside down attachment of the tissue, thus eliminating primary graft failure in our series.

In the series published to date, ECL following DMEK has ranged between 31 and40% at 3 months, between 36 and 40% at 6 months[8-14] between 19 and 36% at 1year and up to 39% at 5 years[7,11,15,16] showing an early flattening of the curve similar to that described after DSAEK, but possibly at lower levels of ECL. In our series, early results of DMEK utilizing pre-loaded tissue showed values of ECL similar to those reported for conventional DMEK employing grafts prepared in theater or tissues that are pre-stripped but not pre-loaded, all delivered with conventional methods. Instead,

ECL appears higher than that we have recorded using the pull through technique with pre-stripped tissue loaded in surgery[6]. As the surgical technique was not modified,[6] damage at the eye bank during the loading phase correlated to delayed surgery are suggested as possible explanations for this discrepancy. In particular, no substantial difference in cell loss was seen between tissue loaded with the use of a contact lens and tissue loaded employing the aluminum foil, thus making us exclude this as a possible factor influencing ECL. Specular microscopy performed at later postoperative times is mandatory anyway to confirm our encouraging early outcomes. A disadvantage of using pre-loaded DMEK grafts is that the surgeon must choose the graft size preoperatively, rather than deciding in theater. However, surgeons should be guided in this choice more by the preoperative condition of the eye than by intraoperative variables. Grafts with larger diameter, usually between 8.5 and 9 mm, allow replacement of a larger amount of diseased cells, as required by eyes with totally decompensated endothelium, but may be more difficult to handle. On the other hand, guttae may concentrate in the central part of the endothelium and leave the periphery with a rather high number of good cells, which can be saved by transplanting DMEK grafts of a diameter between 7.5 and 8 mm. A careful preoperative evaluation of the recipient endothelium and especially of its peripheral portion is instrumental in deciding whether a smaller diameter can be employed, thus facilitating surgery. In this series, to optimize standardization of the procedure, for all cases we chose a diameter of 8.25 mm, which represents a good compromise for treating both totally and partly decompensated corneas. Finally, with the current technique it is impossible to evaluate the endothelium after loading it into the IOL cartridge. Improving the transparency of the device, which is at the moment is somewhat glossy, may allow the use of specular microscopy to detect possible damage occurred during the loading phase, thus optimizing quality control of pre-loaded tissue. In conclusion, the use of pre-loaded DMEK grafts minimizes intraoperative tissue wastage, costs and surgical time, while yielding outcomes comparable to those obtained with tissue loaded in theater. These results were obtained in our series with grafts loaded with the endothelium inwards and delivered employing the pull-through technique, a technique used

to date by a minority of surgeons[6,17]. Specific validation is still required if DMEK grafts are pre-loaded in the conventional configuration with the endothelium outwards and delivered by injection into the anterior chamber, as possible prolonged contact of the endothelium with the cartridge wall may produce increased endothelial damage and affect graft survival.

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