CORRESPONDENCE

Pull-through technique for delivery of a larger diameter DMEK graft using endothelium-in method

Busin et al. recently reported the results of an innovative surgical technique, describing the delivery of trifolded (endothelium-in) Descemet membrane (DM) endothelial keratoplasty (DMEK) graft using a pull-through technique.[1] We performed a similar (n = 9), but in vitro, study with larger DMEK grafts (9.5 mm) to evaluate surgical endothelial cell loss (ECL) and the learning curve. We intentionally used larger DMEK grafts because it has been demonstrated that they increase graft survival after ultrathin Descemet stripping automated endothelial keratoplasty (UT-DSAEK).[2]

A written consent from the donor's next of kin was obtained before the use of tissues for research purposes.

The tissue was stripped and trifolded by using acute forceps (E. Janach, Como, Italy) to manipulate the DM with the endothelial side facing inward to avoid potential endothelial damage. Using the same forceps, the graft was gently pulled inside a 2.2 intraocular lens (IOL) cartridge (Viscoject, Wolfhalden, Switzerland), maintaining the graft in the same orientation (DM side touching the cartridge bottom and the endo-in on the top). Using 25G end-grasping forceps (Grieshaber forceps; 25G Alcon, Tex.), the graft was pulled inside the funnel of the IOL containing sterile phosphate-buffered saline (PBS) ready for delivery.[3] After the DM-endothelium was removed, the cornea was mounted on an artificial anterior chamber (AAC; Moria, Antony, France). The pressure inside the AAC was controlled by adjusting the height of an infusion bottle at 20 cm (15.3 mm Hg).[4] At the 12 o'clock surgical position, a 3-mm limbal incision was made with a slit knife. Three side ports were created at the 10:30, 1:30, and 7:30 clock positions. The cartridge was inverted so that the exposed DM side was on the top of the funnel and the flaps on the bottom to facilitate opening the graft inside the eye. The IOL introducer cartridge was then inserted through an incision, and the graft was pulled from the opposite side by using end-grasping forceps. An air bubble was then used to attach the unfolded graft to the donor corneal stroma. The cornea was dismounted from the AAC and the graft gently removed using PBS and stained with trypan blue for 20 seconds followed by a wash with PBS. Placed in a hypotonic (sucrose - 1.8%) solution in a petri plate, the endothelium was examined for damage, ECL, and uncovered areas by using an inverted microscope (Primovert; Zeiss, Milan, Italy). The cells were counted by using a 10 × 10 eye piece reticule. Time required for stripping, loading, injecting, unfolding and the total surgery time were recorded.

All the tissues were successfully peeled in one attempt (100% success rate). On average, 18.78 ± 5.65 minutes were required to prepare a prestripped DMEK graft in the Eye Bank. Poststripping, postloading and postdelivery mean endothelial cell density, mortality, uncovered area, time intervals, and total time of the procedure are listed in Table 1. We did not observe any disorientation of the graft in any of the 9 cases. The ECL at the end of the procedure was 22.28%, which is less than that reported for DMEK surgery (endothelium-out) of 35% to 37% at 6 months.[5]

We had similar results in a previous in vitro study, in which we compared the ECL and total time of the surgery after injecting a 9.5-mm DMEK graft with endothelium flapped in or rolled out.[6] In addition, a 9.5-mm DMEK graft will transplant increased numbers of cells initially and may help in maintaining an adequate number of functional endothelial cells in the long term. It is worth considering that an increase from an 8.25-mm to a 9.5-mm diameter graft would result in transplanting approximately 20% more cells.[2,7] Even compared with the very low ECL of 9.9% reported by Busin et al.,[1] a larger (9.5-mm) graft with an ECL of 22.28% would still provide a greater number of cells compared with a 8.25-mm graft with a 9.9% ECL. Using the method proposed by Unterlauft et al.[8] for estimating the area of the endothelial corneal surface, 8.25 mm and 9.5 mm grafts will have areas of approximately 124.9 mm^2 and 156.9 mm^2, respectively.[7] If the preoperative endothelial cell density of the donor corneoscleral disc was, for example, 2700 cells/mm^2, an increment from a 8.25-mm diameter to a 9.5-mm diameter graft would result in transplanting of

Table 1—Endothelial cell loss and surgical timing

Endothelial cell evaluation	
Poststripping endothelial cell density (ECD) (cells/mm^2)	2044.44 ± 427.53
Poststripping mortality (%)	0.52 ± 0.99
Poststripping uncovered areas (%)	0.17 ± 0.35
Postloading ECD (cells/mm^2)	2000.00 ± 409.27
Postloading mortality (%)	0.59 ± 0.96
Postloading uncovered areas (%)	0.29 ± 0.43
Postdelivery ECD (cells/mm^2)	1588.89 ± 321.89
Postdelivery mortality (%)	0.79 ± 1.00
Postdelivery uncovered areas (%)	17.84 ± 24.19
Timing (min)	
Time to prepare	1.07 ± 0.29
Time to inject	3.51 ± 1.00
Time to unfold	5.84 ± 3.95
Total time	10.42 ± 3.68

approximately 424,000 cells (9.5 mm) rather than 339,705 cells (8.25 mm). Even if the ECL is 9.9% with an 8.25-mm graft and 22.28% with a 9.5-mm graft, the remaining number of cells that will be transplanted with a 9.5-mm graft (330,720 cells) will still be substantially greater than that with an 8.5-mm graft (306,000 cells). Although these calculations are speculative, such a procedure may potentially translate to higher graft survival, as also suggested by Anshu et al.[9]

We observed that similar to 9.5-mm UT-DSAEK grafts, larger DMEK grafts were easier to handle in terms of folding and loading compared with smaller grafts. This is also highlighted by no significant ECL during loading time. The time required for the entire procedure was 10.42 ± 3.68 minutes, and this may give an additional advantage to the surgeons. Despite the limitations of this in vitro study, the results suggest that the described technique using large (9.5-mm) grafts for DMEK has an acceptable ECL and learning curve and therefore may be useful for the long-term survival of grafts compared with that of smaller grafts.

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