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Aspiration of tri-folded, endothelium-in grafts for Descemet Membrane Endothelial Keratoplasty

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Abstract

Purpose: In Descemet Membrane Endothelial Keratoplasty (DMEK), loading and positioning of tri-folded grafts into a cartridge is generally conducted with forceps or a hook, risking graft tear or trauma. We demonstrate the feasibility of loading tri-folded grafts into a cartridge with no touch to the endothelium required beyond the tri-folding process.

Methods: A corneoscleral rim with a pre-stripped DMEK graft is placed into a petri dish. After the graft is tri-folded with forceps and removed from its stromal attachment, the graft is gently wicked into the tip of a saline-filled Alcon B IOL cartridge connected to IV extension tubing and a 3cc syringe, drawn into the cartridge by positioning it adjacent to the graft tip. The remainder of the graft is aspirated with the addition of saline. The cartridge orientation is reversed for graft injection. In this retrospective analysis, we analyzed surgical video for preparation times, and assessed postoperative visual acuity, pachymetry, and endothelial cell density.

Results: Thirteen cases underwent this approach. Median preparation time from stain to cartridge eye contact was 8.5 minutes, and time from graft injection to final centration and bubbling was 2.9 minutes. Corneal thickness decreased from a median of 623 microns preoperatively to 566 microns at one month ($p=0.038$). Visual acuity improved by one month by a median of 0.3 logMAR ($p=0.016$). Endothelial cell density decreased by 32.4% at one month compared to baseline.

Conclusion: Endothelium-in DMEK grafts may be loaded into a plastic cartridge using a skill set similar to aspiration of a scroll.

Keywords

Descemet membrane endothelial keratoplasty; cornea transplantation; corneal endothelium

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INTRODUCTION

Descemet Membrane Endothelial Keratoplasty (DMEK) is a leading method of choice for treatment of Fuchs dystrophy, offering rapid healing time, a smaller incision, and lower risk of rejection than Descemet Stripping Endothelial Keratoplasty.

Most standardized techniques allow injection of a DMEK scroll¹ using a glass device or a plastic injector² attached to a syringe, mobilizing the graft through a fluid flow. However, this approach is limited by two factors. First, the endothelium in a scroll configuration faces the outside, potentially causing trauma to the endothelium as it passes through the tip of the device.³ Second, the graft must be unscrolled inside the eye.

A tri-folding technique allows the tissue to be delivered through an incision or IOL cartridge.⁴ However, this approach generally requires pulling the graft with forceps onto a device, contact lens or platform,^{5–7} a technically challenging approach with risk of trauma to the tissue in the process of transferring it from one surface to another. Even if the graft is coaxed into the posterior aspect of an IOL cartridge with a flow of fluid, it generally still requires a hook or forceps to bring the graft forward to the tip and avoid contact with a plunger.

Plastic is manufactured with microscopic surface irregularities and produces friction with the graft, although cell loss has been shown to be comparable to glass; nevertheless, glass is frequently used for injectors of scrolls due to the smooth inner lumen, thought to reduce trauma to the endothelium.⁸ Therefore, it has been proposed that aspiration of a tri-folded graft into an injector is not feasible as the inverted graft flips inside the Jones tube into a scroll formation.⁵ Here, we describe a technique that leverages the friction of plastic to maintain the tri-folded graft in its inverted position after aspiration. We demonstrate the feasibility of aspirating a tri-folded DMEK graft into a plastic cartridge attached to a syringe, a technique commonly utilized by most DMEK surgeons who aspirate and inject a scroll. We calculate the timing of graft preparation to assess its practicality and demonstrate comparable outcomes.

METHODS

Patients

This retrospective study was approved by the Johns Hopkins University School of Medicine Institutional Research Board. Medical charts of patients who underwent DMEK surgery over a three-month period were reviewed. Eyes were included if the graft was injected as an aspirated tri-fold. Demographic information and clinical data such as visual acuity, corneal thickness, endothelial cell density (ECD), graft position, and relevant clinical information were collected.

Surgical preparation

The eye is prepared in a standardized fashion for DMEK, reducing the pupil size, creating an 8.0mm diameter Descemetorhexis, establishing a patent inferior peripheral iridotomy with a

25G needle, and using a 2.75mm incision that has been expanded slightly to approximately 3mm, to snugly fit an Alcon B cartridge.

Graft preparation

Video 1 demonstrates the tri-folding approach for graft preparation, from the surgeon's view. The graft is stained with Trypan blue as desired by the surgeon. After a strip of cohesive viscoelastic is placed onto the corneal surface, each side of the graft is folded onto the center, such that the graft is folded into thirds. The edges may be manipulated from the stromal side to reduce risk of damage. Grafts are delivered from the eye bank with a central attachment, which must be separated in order to allow the graft to be mobilized. The corneoscleral rim is positioned in a petri dish, which prepares it for aspiration. In these cases, grafts were 8.0mm in diameter.

The Alcon B cartridge (Alcon, Forth Worth, TX, USA) is prepared as previously described for scroll injection, with IV extension tubing and a 3 or 5cc syringe,² an off-label use of this cartridge that is utilized for insertion of intraocular lenses. We advise against use of a 10cc syringe, which provides additional room for aspiration but can eject the graft with excessive force. The seal between tubing and the cartridge is primed with balanced salt solution (BSS), an essential step, and tested by aspirating BSS until confirmed to be watertight through an absence of air bubbles. The syringe is filled with approximately 2cc of BSS to allow for room for aspiration.

Once the tissue is tri-folded and separated, it is left on the donor stroma. Often releasing the forceps leaves a sharper edge, which we do not go back to manipulate, and in fact offers easier entry into the cartridge. The cartridge is brought directly to the graft edge and by gently moving the cartridge forward, the graft is drawn into the cartridge upon contact with BSS through capillary action. This is apparent in Video 2.

Once the graft has been maximally drawn into the tip of the cartridge through contact alone, an assistant begins filling the petri dish with BSS. The tip is completely immersed, and the syringe is pulled back to aspirate the remainder of graft into the cartridge. The friction produced by plastic assists to stabilize the graft. We have found advantages to both bevel up and bevel down approaches; importantly however, the orientation must be reversed when inserting the cartridge into the eye.

Graft insertion

In Video 3, we eject the tri-folded graft into the eye. The graft, positioned onto the inner lumen of the plastic of the cartridge, with endothelium oriented inward and protected, is visible in the video. Transparency of the plastic allows the surgeon to properly orient the tissue. Notably, the cartridge is flipped from the orientation of aspiration. We have found that an anterior chamber maintainer can be helpful to facilitate entry of the cartridge, but injecting saline through a syringe is also adequate to deepen the chamber for entry. Neither is necessary once the cartridge is in the eye if saline can be delivered through the device itself.

Upon insertion, the folded edges of the graft are generally posterior rather than anterior to the graft. Therefore, it can be opened with a small air bubble placed directly below the central graft. Cohesive viscoelastic frequently washes off the endothelium during the aspiration process, but placement of an excessive amount of viscoelastic may weigh down one edge of the graft, such that rapid placement of an air bubble may move the graft in the direction of the flap. Therefore, we utilize a 1cc syringe for air to slowly open the graft, followed by a 3cc syringe for SF₆.

Video analysis

Surgeries were recorded using a digital video recorder through the operating microscope. To determine if the graft could be prepared efficiently, the difference was calculated between the time of staining with trypan blue to the time that the cartridge touched the cornea, ready to inject. To determine if the graft orientation resulted in rapid opening and placement onto the posterior corneal surface, the difference was calculated between the time the graft was injected to the final position after opening, centration and bubbling. To provide a conservative estimate, if the graft was recentered or repositioned, the timing was stopped once it reached its final position.

Outcomes

To test whether a high endothelial cell count could be maintained during the learning curve for this technique, specular microscopy was conducted regularly at one week and one month and calculated using the center method. We assessed best-corrected visual acuity by manifest refraction and pachymetry using Scheimpflug imaging (Oculus, Inc., Arlington, Washington), at the thinnest point for thirteen patients who underwent DMEK using this approach. Data were collected at one week, defined as between 5 and 14 days, one month, defined as between 2 weeks to 2 months, and at three months, defined as between 2 months and 4 months. If the patient was refracted at one month but not at three months, the best-corrected visual acuity level achieved at one month was carried forward. Differences between preoperative and postoperative outcomes were assessed by Mann-Whitney U test, due to small sample size and non-normal distribution.

RESULTS

In thirteen patients who underwent this approach, average age was 62.6 years and 61.5% of patients were female. Seven cases were combined with phacoemulsification. Indications included Fuchs dystrophy (n=7), COL8A2-associated corneal dystrophy (n=1), pseudophakic bullous keratopathy (n=4) and corneal graft rejection (n=1). Previous surgeries included trabeculectomy (n=1), DSEK (n=1) and pars plana vitrectomy for retinal detachment repair (n=2), one of which included silicone oil.

Median donor age was 67 (range 52 to 71) and median death-to-preservation time was 8 hours, 39 minutes (range 4 hours, 47 minutes to 14 hours, 44 minutes). Six donors were female.

Twelve of 13 patients presented for one-month followup, and 11 of 13 patients presented at three months.

Preparation time ranged from 4.5 to 12.6 minutes, with a median time of 8.5 minutes. The time from insertion to unscrolling, final centration and bubble placement ranged from 1.0 to 13.5 minutes, with a median time of 2.9 minutes.

Corneal thickness decreased from a median of 623 microns preoperatively to 566 microns at one month ($p=0.038$) and 523 microns at three months ($p=0.001$).

Median visual acuity preoperatively was 0.6 LogMAR (20/80 Snellen equivalent, range 0.1 to 2.3), improving at one month by a median of 0.3 (3 lines Snellen equivalent, $p=0.016$), and at three months by 0.4 LogMAR (4 lines Snellen equivalent, $p=0.036$).

Median post-processing, preoperative endothelial cell count was 2890 cells/mm² as reported by the eye bank (range 2532 to 3135), decreasing to a median of 1873 cells/mm² ($p<0.001$ vs. baseline) at one month (range 1695 to 2481) and 2034 cells/mm² ($p=0.004$ vs baseline) at three months (range 1684 to 2439).

To assess the timing of cell loss, we conducted a post-hoc analysis of 5 eyes for which specular microscopy was able to be conducted at one week due to rapid corneal clearance. In this subset, median ECD decreased from 2890 preoperatively to 2242 cells/mm² at one week ($p=0.016$) and remained relatively stable at 1953 cells/mm² at one month ($p=0.008$ vs. baseline); the difference between one week and one month ($p=0.17$) was not statistically significant.

Six out of 13 cases were rebubbled (46.2%). To assist with both reapproximating grafts and decreasing corneal edema, patients with any degree of visible or suspected detachment at 1 or 2 weeks postoperatively received a 0.1mL bubble of air into the anterior chamber with a 30G needle and 1mL syringe at the slit lamp. If the detachment involved the inferior graft, an additional 0.1mL was placed into the anterior chamber. Aqueous was drained through the inferior cornea with a 30G needle to normalize intraocular pressure after each bubble placement. No cases experienced primary graft failure or rejection.

DISCUSSION

Here, we demonstrate that tri-folded, endothelium-in grafts can be aspirated into a plastic cartridge for DMEK surgery in a manner commonly used for scroll injection.

By removing the need for an instrument to pull the graft into the cartridge or the anterior chamber, it reduces the risk of forceps-related complications, including graft tears which may occur during loading or while pulling the tissue into the eye. Tri-folding is generally safe for grafts,⁹ and this technique shares many characteristics with loading of a scroll, commonly conducted by most DMEK surgeons. Similar to pull-through techniques in which the graft is mechanically introduced into the eye with forceps, the graft opens into position once placed into the eye.

In this technique, we utilize a plastic device that we have also used for injection of a scroll. An advantage of the Alcon B cartridge is its linear, minimally tapered lumen which allows for controlled aspiration and injection, and plastic which provides friction. While a modified

Jones tube or other glass tube may be ideal to minimize graft-glass contact when the endothelium is facing out, such a smooth inner surface may cause the graft to scroll up inside the tube.⁵ Excessive aspiration must be avoided, as the graft may travel too far up the cartridge.

We have used both bevel-up and bevel-down approaches to aspiration and have found advantages to each. We prefer to insert the cartridge into the eye bevel-down for improved anterior chamber stability; this approach requires the graft to be aspirated bevel up. To do so, we find it is essential for the graft to be partially wicked into the lumen of the cartridge before fluid is added to the petri dish, or else it risks unfolding before it can be aspirated. If it begins to unfold, the corneoscleral rim can be lifted out of the dish, weck-cell sponges used to remove saline and the process repeated. If it floats away as a scroll, it can be injected as a scroll.

Generally, unfolding such a graft within the eye follows principles similar to routine DMEK surgery. However, for surgeons transitioning from DSEK to DMEK considering this approach, we found that two aspects of the surgery more closely approximate DSEK. First, in cases where the graft edge was folded in upon entry, we found it could be helpful to deepen the chamber in order to provide room for the flap to open up, an approach that may appear counterintuitive with DMEK scrolls, in which a flatter chamber is desired. Secondly, in cases in which we deepened the chamber and the graft began to open but did not completely do so, we were able to utilize a small air bubble underneath the central graft to push the edges up. Timing for graft opening and positioning in this study is comparable to reported mean times of 5.4 to 6 minutes for endothelium-in and endothelium-out approaches.¹⁰

In this study, we utilized viscoelastic on the endothelial surface to prevent crush injury to the central flap. However, since performing these cases, it has been demonstrated that tri-folding without viscoelastic does not result in increased cell loss relative to a scroll,⁹ and we have recently transitioned to performing pre-loaded, tri-folded DMEK without viscoelastic. One advantage that we have observed is that viscoelastic slows the opening of the flap such that for post-vitrectomy eyes with deep anterior chambers, the grafts to which viscoelastic have been applied open in a more slow and controlled fashion. Nevertheless, the total amount applied should be minimal, such as a single ribbon, in order to avoid contamination of Descemet membrane, which may interfere with graft attachment. Moreover, if the central air bubble technique is used to open the peripheral flap, we have found that the additional mass on the flap from viscoelastic can push the graft laterally if the bubble is vigorously injected.

Overall, the rates of endothelial cell loss in this study are comparable to alternative techniques throughout the DMEK learning curve, with reported rates of 30% cell loss at three months¹¹ and 43% loss at six months¹² in earlier cases, and approximately 30% cell loss at 6 months in a comparison of endothelium-in and endothelium-out surgery by an experienced DMEK surgeon.¹⁰

We have found that this approach is versatile and may be utilized in a variety of complex DMEK cases, while remaining relatively atraumatic to the corneal endothelium. DMEK

surgeons currently aspirating grafts as a scroll may find this to be a straightforward transition to an endothelium-in approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Terry MA, Straiko MD, Veldman PB, et al. Standardized DMEK Technique: Reducing Complications Using Prestripped Tissue, Novel Glass Injector, and Sulfur Hexafluoride (SF₆) Gas. *Cornea*. 2015;34:845–52. [PubMed: 26075461]
2. Kim EC, Bonfadini G, Todd L, et al. Simple, inexpensive, and effective injector for descemet membrane endothelial keratoplasty. *Cornea*. 2014;33:649–52. [PubMed: 24763123]
3. Downes K, Tran KD, Stoeger CG, et al. Cumulative Endothelial Cell Loss in Descemet Membrane Endothelial Keratoplasty Grafts From Preparation Through Insertion With Glass Injectors. *Cornea*. 2018;37:698–704. [PubMed: 29561351]
4. Busin M, Leon P, Scordia V, et al. Contact Lens-Assisted Pull-Through Technique for Delivery of Tri-Folded (Endothelium in) DMEK Grafts Minimizes Surgical Time and Cell Loss. *Ophthalmology*. 2016;123:476–83. [PubMed: 26686969]
5. Parekh M, Ruzza A, Ferrari S, et al. Endothelium-in versus endothelium-out for Descemet membrane endothelial keratoplasty graft preparation and implantation. *Acta Ophthalmol* 2017;95:194–198. [PubMed: 27422483]
6. Busin M, Leon P, D'Angelo S, et al. Clinical Outcomes of pre-Loaded Descemet Membrane Endothelial Keratoplasty Grafts with Endothelium Tri-Folded inwards. *Am J Ophthalmol* 2018;193:106–113. [PubMed: 29940169]
7. Muraine M, Gueudry J, He Z, et al. Novel Technique for the Preparation of Corneal Grafts for Descemet Membrane Endothelial Keratoplasty. *American Journal of Ophthalmology* 2013;156:851–859. [PubMed: 23932263]
8. Schallhorn JM, Holiman JD, Stoeger CG, et al. Quantification and Patterns of Endothelial Cell Loss Due to Eye Bank Preparation and Injector Method in Descemet Membrane Endothelial Keratoplasty Tissues. *Cornea*. 2016;35:377–82. [PubMed: 26583280]
9. Lohmeier J, Christy J, Chiang E, et al. Viability of Descemet Membrane Endothelial Keratoplasty Grafts Folded in the Eye Bank. *Cornea*. 2018;37:1474–1477 [PubMed: 30095494]
10. Price MO, Lisek M, Kelley M, et al. Endothelium-in Versus Endothelium-out Insertion With Descemet Membrane Endothelial Keratoplasty. *Cornea*. 2018;37:1098–1101. [PubMed: 29863544]
11. Price MO, Giebel AW, Fairchild KM, et al. Descemet's membrane endothelial keratoplasty: prospective multicenter study of visual and refractive outcomes and endothelial survival. *Ophthalmology*. 2009;116:2361–8. [PubMed: 19875170]
12. Rose-Nussbaumer J, Alloju S, Chamberlain W. Clinical Outcomes of Descemet Membrane Endothelial Keratoplasty During the Surgeon Learning Curve Versus Descemet Stripping Endothelial Keratoplasty Performed at the Same Time. *J Clin Exp Ophthalmol* 2016;7:599. [PubMed: 28018708]