

Dye-based identification of the orientation of tissue for Descemet stripping automated endothelial keratoplasty: A laboratory-based study

Pragnya Rao Donthineni, Pravin Krishna Vaddavalli

Purpose: To describe the features enabling the identification of the orientation of Descemet stripping automated endothelial keratoplasty (DSAEK) lenticule with the assistance of vital dyes. **Methods:** This is a blinded experimental lab-based study, including 30 microkeratome prepared pre-cut DSAEK lenticules. The lenticules were divided into control and study arms which included 10 unstained and 20 stained lenticules, respectively. In the study arm, vital dyes like trypan blue (TB), brilliant blue (BB), indocyanine green (ICG) and fluorescein stain (FS) were used to stain 5 lenticules each. They were examined by experienced (group 1) and novice surgeons (group 2) to identify the correct orientation of the lenticule. The results were tabulated and analyzed. **Results:** Of the 30 lenticules examined, the average of total scores obtained by each observer was higher (78%) in group 1 as compared to group 2 (65.3%) which was statistically significant ($P < 0.005$). In group 1, the accuracy of identifying the correct orientation of unstained lenticules was 70% which improved to 82% on staining. The accuracy in group 2 was 58% with unstained lenticules which improved to 69% on staining. Within the study arm, irrespective of surgical experience, the accuracy was highest with BB (86%), followed by TB (82%), ICG (72%) and FS (62%). **Conclusion:** This study found that the accuracy of identifying the orientation of DSAEK lenticules increased with experience and with the assistance of staining using vital dyes. This accuracy improved with blue dyes like brilliant blue and trypan blue, irrespective of the level of experience.

Key words: Descemet stripping endothelial keratoplasty, dye based identification of lenticule orientation, endothelial transplant, loss of orientation of lenticule

Path-breaking advances in corneal transplantation techniques have led to a paradigm shift from full-thickness transplantation towards the selective replacement of the diseased layer.^[1,2] Endothelial transplantation techniques like descemet's stripping automated endothelial Keratoplasty (DSAEK) specifically address the cause, offer accelerated visual recovery, minimize follow-ups and complications compared to full-thickness transplantation.^[3,4] Despite the benefits, complications like loss of orientation of the donor lenticule are unique to DSAEK, leading to primary iatrogenic graft failure (PIGF).^[5] Techniques like double-ring sign aid in identifying lenticule orientation after insertion into anterior chamber (AC).^[6-12] However, loss of orientation can occur even before its insertion into AC, commonly with ultra-thin lenticules and novice-surgeons.^[13-15] Though rare, this could compel the surgeon to replace the tissue, which is undesirable given the preexisting dearth of donor corneas.^[16] Vital dyes like Trypan Blue (TB) have been used to identify stromal fibers and endothelial cells intraoperatively and can be explored to identify lenticule orientation during DSAEK.^[17,18] Thus, in this study, we aimed to identify features enabling identification of lenticule orientation using

vital dyes and correlate the accuracy with type of dye and surgical expertise.

Methods

This is a blinded experimental lab-based study aiming to identify the orientation of DSAEK lenticules with the assistance of 4 vital dyes. A total of 30 donor corneas with normal endothelial morphology but rejected due to medical reasons were included in the study. Informed written consent was obtained from the family members of the donors for use of the tissue for research purpose in case of non-suitability for clinical use. The donor tissues were initially stored in McCarey-Kaufman (MK) corneal preservation medium for the first 4 days and shifted to Eusol C if stored beyond 4 days. All tissues included in the study were used within 7 days of preservation. The study was approved by the institutional ethics committee.

Preparation of the DSAEK lenticules

The donor lenticules were mounted over an artificial anterior chamber (AAC, Moria Inc., Doylestown, PA, USA) and pre-cut

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The Cornea Institute, L. V. Prasad Eye Institute, Hyderabad, Telangana, India

Correspondence to: Dr. Pravin Krishna Vaddavalli, Director, Cornea Institute, L. V. Prasad Eye Institute, Hyderabad, Telangana - 500 034, India. E-mail: pravin@lvpei.org

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lamellar grafts were prepared by a trained eye bank technician using a Moria microkeratome (Moria/Microtek, Doylestown, PA, USA). A 350 μ microkeratome head and a single-pass technique were used during the preparation of the lenticule. The pre-cut grafts were trephined with endothelial side up using an 8 mm trephine which were then examined under an operating microscope. The technician preparing the grafts did not participate in the study at any point.

Distribution of lenticules into study and control arms

The lenticules were divided into study and control arms. The study arm comprised of 20 lenticules with 5 lenticules each stained with Brilliant blue (BB) 0.05% w/v (Ocublue; Aurolab, Madurai, India), Trypan blue (TB) 0.06% (Auroblue; Aurolab, Madurai, India), Indocyanine green (ICG) 5 mg/ml (Aurogreen; Aurolab, Madurai, India) and Sodium fluorescein (FS). 20% w/v (Flures; Aurolab, Madurai, India). The control arm included 10 lenticules that were examined unstained. The procedure of staining included immersion of the lenticules in their respective dyes for 15 seconds followed by immersion in BSS for 2 seconds before washing the excess stain away.

Examination of the DSAEK lenticules

The stained and unstained lenticules were placed on a flat tray with a predetermined orientation (either stromal or endothelial side up) in rows by the investigator and the orientation was masked from the observers. The result key was known to only the investigator and was used to score the responses of the observers with no further manipulation of the lenticules allowed by them. The lenticules were then examined by a total of 10 ophthalmologists who were divided into two groups based on their surgical expertise: (i) group 1 comprising of five-experienced corneal surgeons (who had performed more than 100 DSAEK surgeries each) and (ii) group 2 comprising of five novice surgeons (fellows in the cornea services who had each performed 10 or less DSAEK surgeries). They were asked to examine the orientation of the lenticules under the operating microscope (Opmi 1 FR by Carl Zeiss Meditec, Jena, Germany) using a halogen bulb for illumination. The magnification was set at 10x and test examiners were allowed to change magnification if required. The responses were then tabulated and analyzed using Microsoft Excel (Microsoft Corporation, Redmond, USA) and compared by applying the Wilcoxon rank-sum test using the software Stata (StataCorp. 2015. College Station, TX: StataCorp LP)

Results

Of the 30 lenticules examined, the average of the total scores obtained by each observer in group 1 was 78% (23.4/30) which was higher than the score of 65.3% (19.6/30) obtained in group 2. This difference between both the groups was statistically significant ($P < 0.005$). The overall scores and the scores obtained in control and study arms are depicted in Fig. 1.

Group 1

The average of the score obtained by experienced observers while examining unstained lenticules (control arm) was 70% (7/10). These scores improved to 82% (16.4/20) when the lenticules were stained with the assistance of dyes in the study arm. Among the 4 vital dyes used, the scores obtained were better with brilliant blue (BB) as compared to TB, ICG, and FS. The number of lenticules identified correctly with the assistance

of TB, BB, ICG and FS was 80% (20/25), 96% (24/25), 88% (22/25) and 64% (16/25) respectively.

Group 2

The average score obtained by novice observers while examining unstained lenticules (control arm) was 58% (5.8/10). These scores improved when the lenticules were examined with the assistance of dyes to 82% (13.8/20) in the study arm. The scores obtained were better with the blue dyes (BB and TB) as compared to ICG and FS. The number of lenticules identified correctly with the assistance of TB, BB, ICG and FS was 84% (21/25), 76% (19/25), 56% (14/25) and 60% (15/25), respectively. The scores obtained by both experienced and novice surgeons with respect to the vital dyes used for staining are depicted in Fig. 2.

It was observed that irrespective of the level of experience, the identification of the orientation of the lenticule was better (73.5%) with staining as compared to examining them without staining (64%). Within the study arm where the lenticules were stained, the accuracy was higher with blue dyes with brilliant blue having a score of 86%, followed by trypan blue (82%), ICG (72%), and FS (62%).

Features aiding in the identification of the lenticule according to the observers: Based on the feedback from the observers, granularity of the stromal surface was the most important feature of the lenticule that aided in identifying its orientation and was reported by 60% of the observers. This was followed by discrepancy at the edge of the lenticule which was reported by 50% of them. The granularity of the stromal surface was accentuated by the use of the dyes. A shiny and smooth appearance of the endothelial surface was reported to be helpful by 20% and drop out areas over the endothelium that were discernible after staining was reported to be useful by 10% of the observers. The features distinguishing between the stromal and endothelial surface of the DSAEK lenticule are depicted in detail in Fig. 3. The appearance of the lenticules after staining with various vital dyes is shown in Fig. 4.

Discussion

With the rapid increase in the number of surgical procedures and surgeons performing DSAEK, there is a pressing need to address the challenges encountered during the learning curve by novice surgeons.^[1,19] One such critical complication unique to DSAEK is the loss of orientation of the lenticule either before or after its insertion into the anterior chamber. Most methods described in literature help in identifying the correct orientation of the lenticule after its insertion into the anterior chamber and are of limited value when orientation loss occurs before insertion. These methods include "double ring" sign, pre-placement of a hitch suture, "acute angled-beveled" sign or pre-marking with gentian violet may.^[6-12] However, loss of orientation can occur prior to insertion that can be due to prior separation of the lenticule from its cap or while tackling incomplete separation, eccentric trephination or flipped graft before insertion.^[15] The double-ring sign can clear the ambiguity to a certain extent at the end of surgery; but it may not always be discernable as its appearance is governed by the thickness and discrepancy between the anterior and posterior curvature of the lenticule.^[20] This is especially true in case of ultrathin lenticules and in planar lenticules that

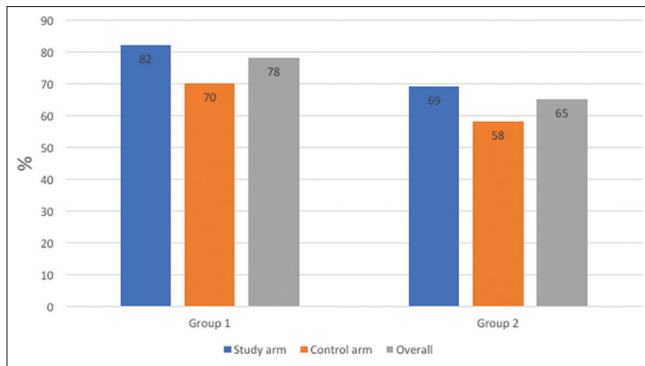


Figure 1: The scores obtained in the overall group and in study and control arms: This bar diagram shows the scores in the study arm, control arm and overall group among experienced (group 1) and novice surgeons (group 2). The scores obtained by observers in group 1 were higher than group two in both study and control groups

lack anterior-posterior curvature discrepancy. Additionally, this sign is applicable only to lenticules inserted into the eye and is of no value if loss of orientation occurs before the graft is inserted into the anterior chamber. Pre-marking with gentian violet is another useful technique to keep a track of the orientation. However it is known to cause toxicity and endothelial cell loss and may not be of help if the loss of orientation occurs prior to marking.^[10] Thus, in this study, we have attempted to identify anatomical and morphological features of the DSAEK lenticule that can aid in identifying the correct orientation in times of need.

This study found that the accuracy in identifying the correct orientation of the lenticules was better among experienced surgeons as compared to beginners, and this difference was statistically significant. Numerous studies have reported better outcomes following endothelial keratoplasty when performed by experienced surgeons as compared to beginners and similarly, experience could probably aid in better understanding of the morphology of the lenticule as well.^[13,14] The accuracy of identifying the correct orientation of the lenticules improved further when stained with vital dyes among both experienced and novice surgeons. Trypan blue (TB) has been used in the past to identify the stromal fibers during manual dissection for deep anterior lamellar keratoplasty and also to identify endothelial damage during cataract surgery.^[17,18] Thus, the present study attempted to use this property of TB in identifying the orientation of the lenticule and compare it with other vital dyes like BB, ICG and fluorescein. The safety of these dyes for intraocular use has been established, with all of them being safe at lower concentrations.^[21,22] Among the vital stains used, blue stains like BB and TB were more useful in terms of offering better contrast with more distinct identification of the granular appearance and remnant fibers on the stromal surface. BB facilitated better identification of the orientation of the lenticule as compared to TB in our study. The safety and efficacy of BB has been reported to be similar to that of TB in the past.^[23,24] Though ICG is reported to be safe at low concentrations; *in vitro* studies using transmission electron microscopy have shown that it induces organelle swelling, disruption and cell lysis in corneal endothelial cells at higher concentrations.^[21] Thus, it may not be a preferred dye of choice owing to its potential cytotoxicity on endothelial cells. FS on the other hand may not be preferable

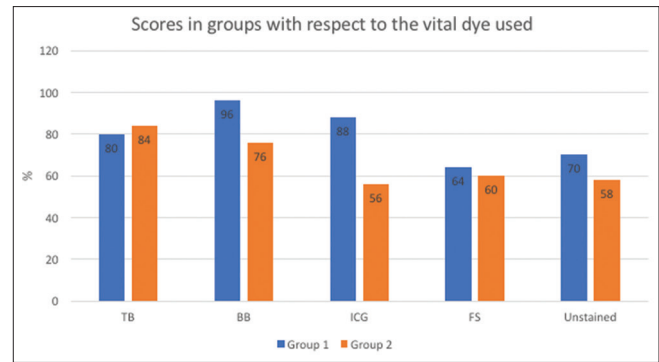


Figure 2: The scores obtained with respect to the vital dyes used for staining: The bar diagram shows the scores of group 1 (experienced surgeons) and 2 (novice surgeons) with respect to the various stains used in the study. The scores in group 2 improved on staining with the blue dyes like brilliant blue (BB) and trypan blue (TB) as compared to Indocyanine green (ICG) and Fluorescein stain (FS)

as it offered the poorest contrast among all 4 dyes in our study and this could explain the poor scores among both experienced and novice surgeons when it was used. We thus recommend the use of TB due to its widespread availability and ease of use.

The observers also reported numerous anatomical and morphological features that helped them in identifying the orientation of the lenticule, which were consistent with the observations of the authors. The stromal side of the lenticule has a more granular appearance with a matted look when compared to the endothelial side and was the most common feature reported by 60% of the observers in the study. This granular or rugged appearance can be attributed to the remnant fibers on the stromal side, which are easily discernable upon staining with vital dyes. The configuration of the edge of the lenticule was another feature that was reported to be useful by 50% of the observers in the study.^[11] When trephination is done with the endothelial side up, the endothelial edge of the lenticule is wider than the stromal side due to the discrepancy between the anterior and posterior curvatures of the cornea.^[14] Other striking features of the endothelial side noted in the study were its smooth and glistening surface with no discernable fibers or irregularities. The Descemet's folds also become more prominent on the endothelial side when the lenticule lies flat on a uniform surface, which could again be attributed to the wider endothelial surface due to discrepancy in anterior and posterior curvature of the lenticule.^[18] In addition, areas of endothelial loss if present get highlighted as patchy areas of drop out upon staining.

The strength of the study lies in comparison of effectiveness of 4 different types of vital dyes in identifying the orientation of the lenticule among both experienced and novice surgeons and the inclusion of a control arm. On the other hand, the study is limited by a small sample size and the lack of repetitions of the experiment among the same set of observers. The low sample size was primarily due to the use of donor corneas with viable endothelium within 7 days of retrieval, rejected for use due to medical reasons. It would be worthwhile to conduct studies to know if the accuracy of identifying the orientation of the lenticule improves after the anatomical and morphological features of the lenticule are demonstrated before the tests and if this translates into a shorter learning curve when they begin to operate in

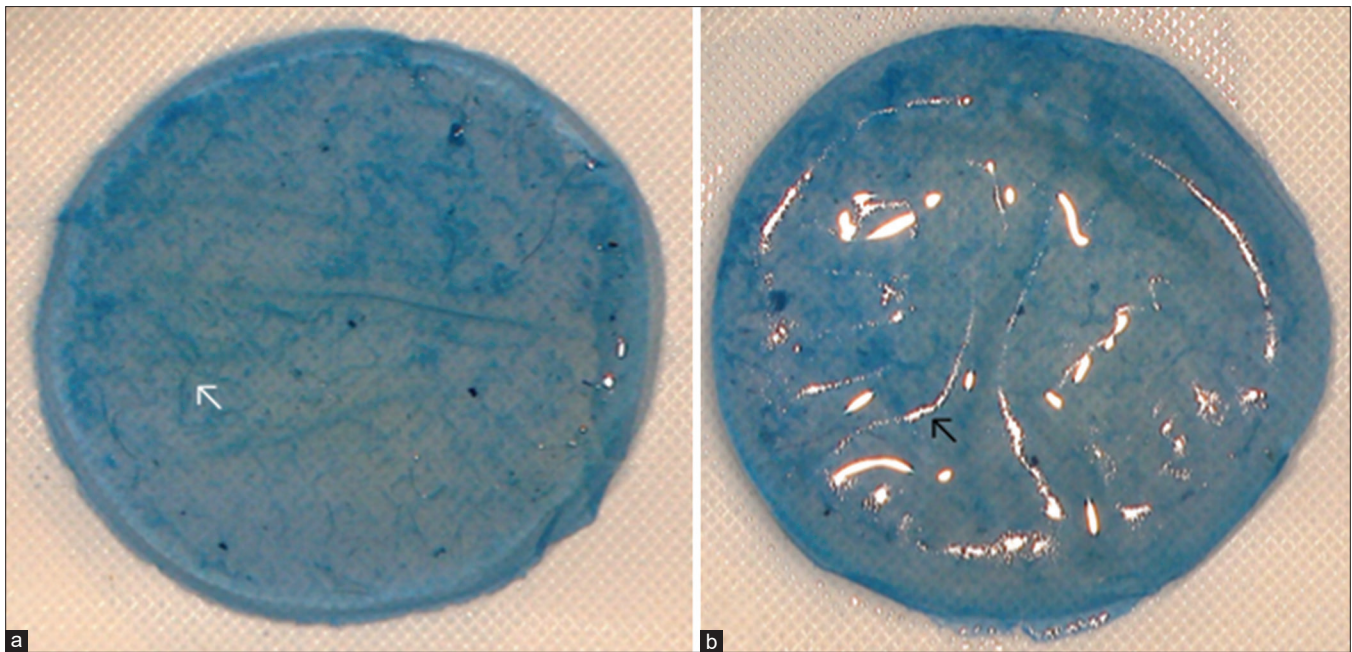


Figure 3: The morphological characteristics of stromal and endothelial surface of the lenticule: The images show stromal and endothelial surfaces of a DSAEK lenticule stained with brilliant blue. The stromal side up (a) shows a characteristic matted look with granular appearance of the surface and stromal fibers (white arrow) being discernible. The endothelial side (b), has a smooth and glistening surface with more prominent Desemet's membrane folds (black arrow) and lack of granularity or discernable fibres

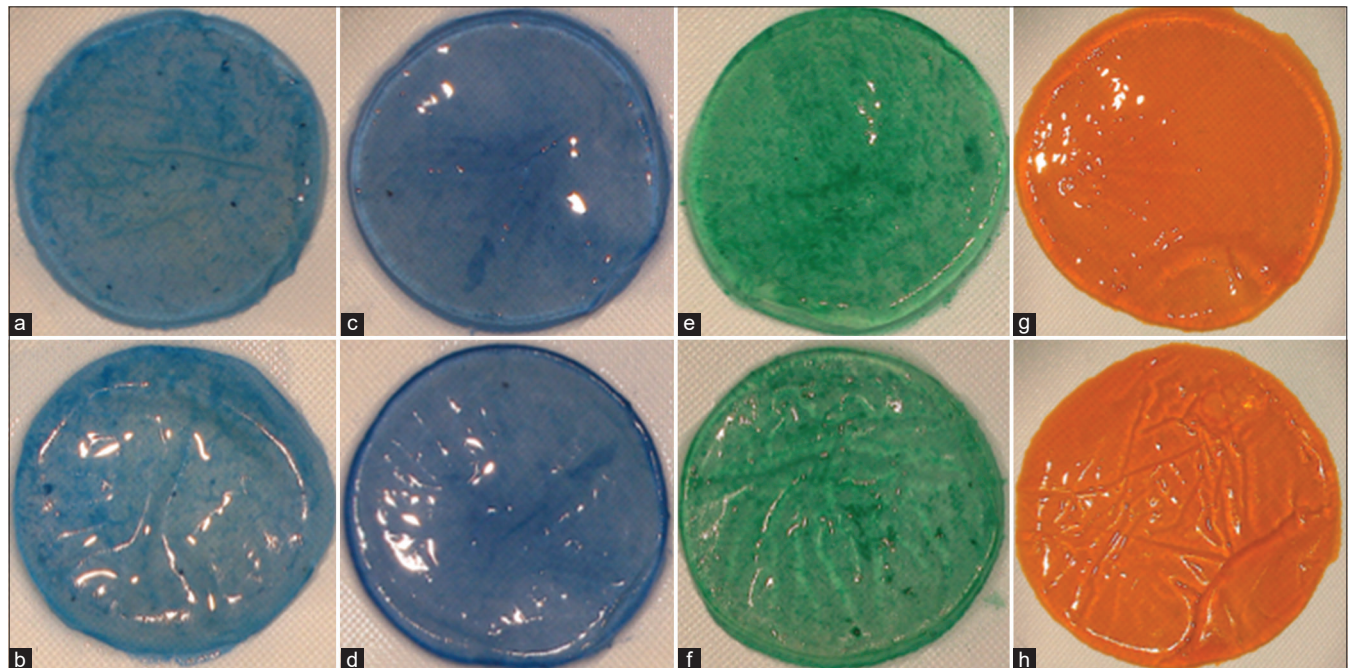


Figure 4: Appearance of DSAEK lenticules when stained with vital dyes: The image is a collage of the lenticules stained with Brilliant blue (a and b), Trypan blue (c and d), Indocyanine (e and f) and Fluorescein stain (g and h). The top row shows the stromal surface and bottom row shows the endothelial surface of the lenticules. The morphological features like stromal fibres, granularity and glossy endothelial surface appear more enhanced on staining with brilliant blue

their clinical practice. Another limitation of the study is that we did not assess the endothelial loss caused by the maneuvers of staining and tissue handling. Though the endothelial loss following eye bank preparation of precut DSAEK lenticules

is well know, we need to understand the extent of endothelial damage caused by this technique. However, we believe that this would potentially be less damaging than inserting the graft in the wrong orientation.

Conclusion

In conclusion, the study found that the accuracy of identifying the orientation of DSAEK lenticules increased with experience and when assisted by staining with vital dyes. Irrespective of the level of experience, this accuracy improved best with brilliant blue followed by trypan blue. Thus, these features could especially help beginners to understand the morphology of the lenticule and in more accurate identification of the orientation of the lenticule in case of an untoward event of loss of orientation intraoperatively. This could also help in an effective training of novice surgeons that can help shorten their learning curve and potentially improve outcomes for the patients.

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Conflicts of interest

There are no conflicts of interest.

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