Rotational alignment of corneal endothelial grafts and risk of graft detachment after Descemet membrane endothelial keratoplasty: a double-masked pseudo-randomized study

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ABSTRACT.

Purpose: The posterior cornea is rotationally asymmetric, and Descemet membrane endothelial keratoplasty (DMEK) grafts preferentially scroll vertically. This prospective study assessed whether graft attachment after DMEK differed depending on the rotational alignment of the donor graft in the recipient eye.

Methods: Pseudo-randomization and blinding of the graft orientation in the recipient’s eye were possible by procedural separation: (1) The eye bank recorded the position of an orientation marker in the donor cornea; (2) the surgeon preparing the DMEK graft recorded an upside-down marker relative to the eye bank marker; and (3) the surgeon assessed the position of the upside-down marker in the recipient after DMEK. Surgeons were masked towards the eye bank marker. Using mixed-effects models, we assessed graft attachment relative to the rotational alignment of the donor graft.

Results: Postoperatively, the graft was not fully attached in 59 of 179 eyes (33%). A second air fill (rebubbling) was performed in 11%. The graft axis was in line with the recipient cornea axis in 40%, oblique in 28% and orthogonal in 32%. We did not detect an elevated risk of incomplete attachment (odds ratio [OR], 1.16; 95% CI, 0.61–2.20), risk of rebubbling (OR, 1.25; 95% CI, 0.47–3.31) or larger areas of graft detachment in non-aligned grafts compared to aligned grafts.

Conclusion: Rotational alignment was not strongly associated with the risk of incomplete graft attachment, although modestly elevated risks cannot be ruled out. Efforts are needed to reduce the need for rebubbling after DMEK and to identify modifiable risk factors for graft detachment.

Key words: incomplete graft attachment – descemet membrane endothelial keratoplasty – rebubbling – complication

Introduction

Incomplete graft attachment after Descemet membrane endothelial keratoplasty (DMEK) is the most common postoperative complication and the most common reason for repeat surgery (Heinzelmann et al. 2016; Deng et al. 2018). In areas where the recipient cornea is not covered by an attached graft, corneal oedema can become microcystic and more pronounced than before stripping the diseased Descemet membrane (Reinhard et al. 2002; Moloney et al. 2017). Clinically, visual rehabilitation may be slower (Huang et al. 2018) and may be complicated by pain. To address incomplete graft attachment, repeat surgery with filtered air or gas injection to fully attach the graft can be performed but bears a risk of potentially vision-threatening complications, such as increased intraocular pressure, damage of the graft, epithelial ingrowth and, in rare cases, endophthalmitis (Baydoun et al. 2015; Deng et al. 2018; Le et al. 2020). Given its downstream consequences, understanding of modifiable and non-modifiable risk factors of incomplete graft attachment is essential for devising prevention strategies.

Factors suggested to be associated with postoperative graft attachment are (1) inherent to the donor such as donor age or endothelial cell density (Heinzelmann et al. 2014), (2) associated with
the eye bank’s handling and storage time (Rodriguez-Calvo de Mora et al. 2016), and (3) related to the surgical approach, such as decentralization and mispositioning of the graft, incomplete stripping of the recipient cornea, possibly the use of air or gas tamponade to attach the graft, and postoperative dips in intraocular pressure (Brockmann et al. 2014; Heinzelmann et al. 2018; Rickmann et al. 2018; Siebelmann et al., 2018a, 2018b; Aldave et al. 2019; Yuda et al. 2019). Another potential approach to reducing the risk of incomplete graft attachment may be to take advantage of the cornea’s characteristic shape. The posterior corneal profile has a characteristic ellipsoid shape with a steeper vertical than horizontal meridian, that is the normal posterior cornea has a stable with-the-rule astigmatism (Koch et al. 2012; Ueno et al. 2015; Wacker et al. 2015). In a large prospective study, DMEK grafts preferentially and repeatedly scrolled along the vertical meridian of the donor’s eye (Wacker et al. 2020), indicating that the corneal ultrastructure is not rotationally symmetric (Lewis et al. 2016; Mohammed et al. 2018). Descemet membrane endothelial keratoplasty (DMEK) grafts repeatedly scrolled in the same axis with little deviation, indicating that scrolling axes were stable and likely an inherent property of the individual graft.

In this prospective observational study, we investigated whether rotational alignment of the donor cornea and the recipient cornea results in fewer instances of incomplete graft attachment after corneal transplantation. We leveraged the fact that surgeons were unaware of the rotational axis of the graft in donor eye, allowing for studying the effect of rotational alignment of graft-donor axes in a pseudo-randomized double-masked fashion.

**Materials and methods**

**Study population**

This prospective study conformed to the tenets of the Declaration of Helsinki and was approved by the institutional ethics committee at the Faculty of Medicine Freiburg as a non-interventional study, given that standard-of-care treatment consists in graft rotation without knowledge of the rotational axis in donor eye, that is at random. In this cohort study, all patients scheduled for DMEK surgery were screened. Patients with advanced Fuchs’ endothelial corneal dystrophy and pseudophakic bullous keratopathy without previous anterior segment surgery other than cataract surgery and laser capsulotomy, if needed, were invited to participate in this study. All participants gave written consent for this registered study (German Clinical Trials Register, DRKS00020947).

**Preserving the donor cornea’s orientation in the recipient’s eye**

To preserve information about the rotational axis of the graft in the donor eye while maintaining masking of investigators, we used two separate marking steps that were procedurally separated, one being performed by the eye bank and one being performed by the surgeon preparing the graft. In brief, information about the donor rotational axis was recorded by adding a small triangle at the rim of the corneoscleral button (‘eye bank marker’) and recording its clock-hour position (Fig. 1A). Donor tissue was evaluated, organ-cultured and placed in dextran-containing medium before DMEK surgery (Pels & Rijneveld 2009). All DMEK grafts were prepared and stripped from the donor’s stroma as described previously, including adding markers to prevent intraoperative upside-down attachment of the endothelial side of the graft to the stroma (‘upside-down marker’) as part of the routine surgical procedure (Fig. 1B) (Price et al. 2009; Bachmann et al. 2010; Kruse et al. 2011; Heinzelmann et al. 2017). Surgeons stripping the donor grafts recorded the position of these markers relative to the eye bank marker. All marker positions were set at random. Experienced surgeons performed DMEK with air tamponade and, if eyes were phakic, phacoemulsification and posterior chamber intraocular lens placement. Intraoperative events and complications potentially affecting graft attachment were recorded such as bleeding after iridectomy or vitreous prolapse. These eyes were not included in the analyses. At the routine postoperative visit two weeks after DMEK, the position of the upside-down markers was recorded by the surgeon (Fig. 1C).

If markers could not be identified at the visit because of corneal oedema, videos recorded during surgery were assessed and the markers position was recorded at subsequent visits. The presence of graft attachment and the area of incomplete graft attachment were recorded at the slit-lamp examination as well as using anterior segment optical coherence tomography. Eyes with central, non-flat detachment and large areas of more than 1/3 of graft detachment underwent repeat air injection at the surgeon’s discretion. All surgeons remained masked towards the eye bank marker throughout.

**Statistical analysis**

After study completion, the investigators were unmasked to the eye bank markers for data entry and statistical analyses. The rotation of the donor graft’s orientation relative to its position in the participant’s eye was calculated (Fig. 1). The alignment of the donor graft was categorized as in line with the recipient’s axis (0° to <30° and 150° to <180°), oblique to the recipient’s axis (30° to <60° and 120° to <150°), and orthogonal to the recipient’s axis (60° to <120°). Descriptive statistics of donor and recipient characteristics according to alignment group were reported as medians (interquartile range, IQR) or counts and percentages.

The association between rotational alignment status, comparing rotationally aligned versus non-aligned grafts (oblique and orthogonal) and the risk of incomplete graft attachment and of rebubbling because of incomplete attachment was evaluated using mixed-effects logistic regression models that accounted for correlated data between two eyes of each patient, resulting in odds ratios (ORs) and 95% confidence intervals (CI). While alignment axes were considered to be pseudo-randomized, models were additionally adjusted for donor risk factor for incomplete graft attachment, donor age (linear) (Heinzelmann et al. 2018), lenticular status of the donor (binary; phakic versus pseudophakic), donor diabetes mellitus status (binary) (Greiner et al. 2014; Schwarz et al. 2016; Price et al. 2017), as well as potential procedural risk factors, surgeon (four categories) and type of surgery (binary: DMEK versus DMEK combined with...
cataract surgery). To address conflicting data on pseudophakia of the recipient as a risk factor (Leon et al. 2018; Siebelmann et al., 2018a, 2018b), we assessed the association of pseudophakia and incomplete graft attachment in an exploratory analysis in this study.

Results

Participants’ and donor characteristics

A total of 223 eyes of 209 individual participants were included in the study between August 2018 and January 2020. 13 eyes were not included in the analysis because of missing eye bank markers; 9 eyes were not included because of difficulties with graft preparation; and 9 eyes were not included in the analysis because of intraoperative complications potentially affecting graft attachment. Graft orientation could not be recorded at the postoperative follow-up visits or on videotapes in 13 eyes. Video records of surgery were used in 37 eyes to assess the position of upside-down markers.

Among 169 participants (179 eyes) included in the analysis, median age was 70 years (interquartile range, 63–78). 65% of participants were women. 177 recipient eyes had advanced Fuchs' dystrophy and two eyes required DMEK because of pseudophakic bullous keratopathy. At the time of study inclusion of the first eye, 30% of participants had already had a transplant in the fellow eye for Fuchs’ dystrophy (n = 50). 61% of eyes received a combined DMEK with cataract surgery, and 39% were already pseudophakic before surgery. 7% of eyes received an epithelial removal for better visualization during surgery in oedematous corneas. At the first postoperative evaluation within hours after DMEK, median eye pressure was 21 mmHg (IQR, 16–27). In 7 eyes, it was necessary to release air the day of surgery because of air bubble-induced pupillary block (median eye pressure, 57 mmHg; range, 45–63) (Table 1).

Postoperative graft attachment and rotational alignment

Postoperatively, participants were assessed for graft attachment after a median of 16 days (IQR, 10–40; n = 59). 8% of eyes were evaluated at an additional follow-up visit (n = 14) and additional air injection in the anterior chamber (rebubbling) because of incomplete graft attachment was performed in 11% of eyes (n = 20).

Axes of donor grafts were in line with recipient corneas in 40% of eyes

Fig. 1. Study design. Pseudo-randomization and blinding of the orientation of the donor graft in the recipient’s eye were possible by procedural separation of the following steps. (A) The local eye bank recorded the position of an orientation marker at the scleral rim of the donor cornea (triangle). Importantly, all surgeons were masked towards the eye bank marker. (B) Surgeon’s view at the back of the cornea: The surgeon preparing the DMEK graft recorded the position of the upside-down marker at periphery of the graft relative to the eye bank marker. (C) Two weeks after DMEK, the DMEK surgeon assessed graft attachment and the position of the upside-down marker in the patient’s eye. After unmasking the investigators to the eye bank markers, the rotational alignment of the donor graft in the participant’s eye was calculated and categorized as (C1) in line with the recipient’s axis, (C2) oblique to the recipient’s axis or (C3) orthogonal to the recipient’s axis. Figure illustrated by the authors.
Donor grafts were oblique to the recipient axis in 28% (n = 50) and orthogonal in 32% (n = 58). Non-aligned grafts were not at a statistically significantly higher risk of incomplete graft attachment than aligned grafts (OR, 1.16; 95% CI, 0.61–2.20), although lower risk in aligned grafts could not be ruled out. Likewise, the risk of rebubbling for incomplete graft attachment did not clearly differ (OR, 1.25; 95% CI, 0.47–3.31), nor did non-aligned grafts have a statistically significantly higher proportion of graft detachment (mean, 9.8% of the graft area, 95% CI, 6.8–12.8) than aligned grafts (mean, 7.1%, 95% CI, 3.4–10.8; difference in means, 2.7%; 95% CI, −2.1 to 7.5) (Fig. 2).

In a sensitivity analysis, additionally controlling for donor age, donor diabetes mellitus status, lenticular status of the donor, surgeon, and the type of surgery, results on the association between graft alignment and risk of incomplete graft attachment remained null (OR, 0.80; 95% CI 0.37–1.72). The risk of incomplete attachment was not different in DMEK compared to DMEK combined with cataract surgery (unadjusted OR, 0.99, 95% CI, 0.52–1.88).

**Discussion**

In this prospective, pseudo-randomized study, we investigated whether orienting donor grafts in rotational alignment within the recipient’s eye was associated with lower risk of incomplete graft attachment after DMEK. Pseudo-randomization was possible through Table 1.

**Fig. 2.** Rotational alignment of corneal endothelial grafts and risk of graft detachment and rebubbling. All eyes were evaluated for graft attachment and the need of an intervention using slit-lamp biomicroscopy and anterior-segment optical coherence tomography at a median of 16 days after Descemet membrane endothelial keratoplasty (DMEK). (A) The risk of graft detachment of any amount was not statistically different in eyes with grafts not-aligned with the recipient’s eye (oblique and orthogonal) compared to grafts aligned with the recipient’s eye (OR, 1.16; 95% CI, 0.61–2.20), nor did non-aligned grafts have a statistically significantly higher area of graft detachment (difference in means, 2.7% of graft area; 95% CI, −2.1 to 7.5). The number of eyes (circles) with incomplete attachment per group is listed below each box. The upper and lower edges of the boxes represent the interquartile ranges, and the bold line is the median of each group. (B) The bars display the percentage of eyes that required rebubbling (bottom, dark grey), a subsequent visit to re-evaluate the need for rebubbling (middle, grey), and of those eyes that did not require further follow-up (top, light grey). The risk of a subsequent intervention for incomplete graft attachment with rebubbling did not clearly differ between non-aligned grafts compared to rotationally aligned grafts (OR, 1.25; 95% CI, 0.47–3.31).
procedural separation of donor harvesting, graft preparation and surgery with markers chosen at random. One third of eyes in our study had some degree of incomplete graft attachment, although the extent of detachment was generally relatively low, covering one fifth of the graft’s surface only. Our data did not suggest a strong association between rotational alignment and risk of incomplete graft attachment. However, our data are entirely compatible with aligned grafts having modestly decreased (or increased) risks of incomplete graft attachment, and thus, further study is required.

After DMEK, between 3% to 76% of eyes require repeat injection of air or diluted sulphur hexafluoride (SF6) for eyes requiring repeat injection of air or diluted sulphur hexafluoride (SF6) for incomplete graft attachment (rebubbling), depending on the intervention threshold as well as on length of follow-up (Deng et al. 2018). In this study, incomplete attachment of any degree was present in 33% of eyes. Only one third of these eyes underwent repeat surgery with rebubbling, corresponding to a risk of 11% of rebubbling among all eyes, which is lower than the risk of rebubbling in our previous study of Fuchs’ dystrophy and bullous keratopathy (Heinzelmann et al. 2018). One potential explanation is the non-inclusion of patients with additional anterior segment diseases in the current study and the special attention to pressurize the eye at the end of the surgery in order to specifically attach the graft (Heinzelmann et al. 2018).

Modifying risk factors for incomplete graft attachment is challenging. After applying basic quality criteria for donor selection, adhering to local eye banking regulations (Laaser et al. 2011; Heinzelmann et al. 2014) and performing state-of-the-art surgery (Brockmann et al. 2014; Heinzelmann et al. 2018; Rickmann et al. 2018; Siebelmann et al., 2018a, 2018b; Aldave et al. 2019; Yuda et al. 2019), the remaining risk factors are currently still unclear. One such potentially modifiable risk factor could be the asymmetry of the posterior cornea and the preferential vertical scarring pattern of DMEK grafts, yet this present study suggested no strong impact of rotational alignment on graft attachment. Among potential procedural risk factors (Leon et al. 2018; Siebelmann et al., 2018a, 2018b) that were pseudo-randomized in this prospective study, the risk of incomplete graft attachment was not different in eyes requiring DMEK combined with cataract surgery compared to DMEK alone. Nevertheless, other procedural factors, such as postoperative positioning, that were not recorded as part of this study may have affected graft attachment, causing every tenth eye to develop graft detachment that needed to be addressed interventionally. While the collagen lamellae rotational orientation was not relevant for postoperative visual acuity in a case series of DSEAK eyes (Nahum et al. 2016), the impact of DMEK attachment on visual function and long-term graft health is still a major concern (Siebelmann et al. 2020).

The precision of our study to determine the exact donor graft alignment may have been affected by potential postural cyclorotation (Hummel et al. 2017) in donors and in those participant’s eyes with video recording only. Additionally, estimating the position markers at three steps – at the eye bank, at the graft preparation and in the participant’s eye – might have limited our precision in quantifying rotational axes. Donor or participant characteristics or procedural factors such as differences in air tamponade or the institutional decision to use air instead of gas were unlikely to affect the estimated risks because of pseudo-randomization and near-equal distribution across groups (Table 1). In summary, the present study did not show a significant impact of donor alignment on graft attachment after DMEK. Based on this study, however, a modest effect size on the risk of graft attachment in eyes with rotational misalignment cannot be ruled out. Future studies may assess the impact on purposefully rotating the graft’s scroll to a desired axis to fit the donor’s orientation. Technically, we were able to repeatedly rotate the graft with irrigation only before unfolding and attaching the graft in a specific orientation (observations from our surgical training laboratory). Such studies will also require assessing long-term effects on the corneal endothelium and on duration of surgery.

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5

Acta Ophthalmologica 2021
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