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Comparison of trephination techniques in penetrating and lamellar keratoplasty for keratoconus: Results from a large university eye hospital

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Abstract

Penetrating keratoplasty (PK) – using various trephination techniques (mechanical, femtosecond laser with mushroom [FEMTO-MR] or top hat [FEMTO-TH] profile, excimer laser) – and deep anterior lamellar keratoplasty (DALK) are surgical treatments for advanced keratoconus. This retrospective study compares the long-term results of these techniques in a cohort of 605 first-time keratoplasty eyes treated for keratoconus at a tertiary corneal transplant centre in Germany. The 5-year graft survival rate exceeded 90% in all groups. Rejection was significantly more frequent in the FEMTO-MR group (50% at 2.5 years, compared to less than 30% in the other groups). Visual acuity below 0.5 (BSCVA < 6/12 Snellen) was observed in approximately 25% of patients after PK, 2.5 years after complete suture removal, but was not observed after DALK. A refractive cylinder greater than − 5 diopters of astigmatism at 5 years after complete suture removal was found most common after FEMTO-TH (~ 40% vs. ~25% after FEMTO-MR and mechanical trephination, ~ 20% after DALK, ~ 10% after excimer PK). In conclusion, all techniques achieved high graft survival, with less favorable results in femto-groups. Function after DALK and excimer PK tended to be slightly better in comparison to mechanical PK.

Introduction

Keratoconus (KC) is an ocular disease affecting both eyes asymmetrically, characterised by progressive thinning and steepening of the cornea, which leads to irregular astigmatism and reduced visual acuity [1]. Treatment for KC depends on the severity and progression of the disease. It ranges from spectacles for mild cases to contact lenses for moderate cases and corneal surgery, such as corneal cross-linking, intracorneal ring segments or corneal transplantation, for severe cases [2]. The two most common techniques regarding corneal transplantation in advanced cases of KC are penetrating keratoplasty (PK) and deep anterior lamellar keratoplasty (DALK) [3].

PK is a surgical procedure in which the full thickness of the patient's cornea is replaced with donor tissue. PK can be performed using several techniques, such as conventional mechanical (CM) trephination, non-mechanical excimer laser-assisted (EXCIMER) trephination [4], and femtosecond laser-assisted (FEMTO) trephination [5]. Naumann first introduced EXCIMER trephination in 1989 and reported significantly better refractive results than CM trephination, especially lower postoperative astigmatism and better visual acuity [4]. FEMTO trephination, first described by Prince in 2005, is thought to have several advantages over CM trephination due to its precise cuts and ability to create custom-shaped trephinations during PK, such as top hat (FEMTO-TH) and mushroom (FEMTO-MR) [5, 6].

DALK, which selectively transfers the diseased corneal stroma while maintaining the host's healthy endothelium and Descemet's membrane, has become more widely accepted and preferred for treating KC in recent decades [7]. Although DALK and PK result in similar refractive and visual outcomes [8], DALK has some advantages, such as sustaining the eye’s anatomical integrity and eliminating the risk of endothelial rejection [9]. However, the surgical technique is still not fully standardized.

The aim of this retrospective study is to compare the long-term outcomes of different keratoplasty techniques, including PK (CM, FEMTO-TH, FEMTO-MR, EXCIMER), and DALK, in patients undergoing first-time surgery for KC between 2004 and 2024. We assessed long-term graft survival, endothelial cell loss, rejection rates, astigmatism, and visual recovery associated with each surgical technique in a large cohort of patients who received corneal transplantation at a high-volume corneal transplant institution in Germany. The results of this study may help guide ophthalmologists and patients in understanding the potential advantages and limitations of various transplantation techniques for the treatment of advanced KC.

Methods

This retrospective study was approved by the Ethics Committee of Albert-Ludwigs-University Freiburg (Application No. EK-Freiburg: 23-1213-S1-retro). All procedures were carried out in accordance with relevant guidelines and regulations. As this was a retrospective, observational study using anonymized data from existing medical records, informed consent was not required according to the Ethics Committee of Albert-Ludwigs-University Freiburg.

We analysed comprehensive data from medical records at the Eye Centre of the University Hospital of the Albert-Ludwigs-University Freiburg and the Lions Cornea Bank Baden-Württemberg (single-centre study). The study cohort included all first-time keratoplasty cases for KC (PK and DALK) that were successively enrolled at the Eye Centre over a twenty-year period from 2004 to 2024.

All grafts were stored under organ culture conditions, based on German and European guidelines. All surgeries were performed under general anaesthesia and followed systematized protocols that did not change significantly over the study period.

Surgical Techniques

CM

In the CM trephination group, a guided trephine system (GTS, Polytech, Germany, diameter: 8 mm) as well as a non-motor conventional trephine were applied to trephine the graft and host cornea. Intraoperatively, after 1% acetylcholine (Miochol®-E, Bausch & Lomb) was applied, a peripheral iridectomy was performed. Non-motor conventional trephination was performed with a 0.25 mm oversized donor graft. GTS was performed without disparity.

FEMTO

Standard FEMTO techniques were carried out and have been described in detail previously [10]. In this study, the top-hat profile (7.5-mm upper and 8.5-mm lower diameters) and mushroom profile (8.5-mm upper and 7.5-mm lower diameters) were included. A 60 kHz Intra-LaseTM FS Laser [AMO (Abbott

Medical Optics), Abbott Park, IL, USA] was used, and an energy of 0.2 µJ, less than the maximum energy (2.0 to 2.7 µJ), was applied. Three cuts were performed (anterior side cut, lamellar cut, and posterior side cut).

EXCIMER

The technique used in this study was the EXCIMER standard technique, which has been fully described earlier [11]. A Schwind Amaris 500E laser system was applied. For donor trephination from the epithelial side, a curved, circular, metal aperture mask (diameter, 8.1 mm; central opening, 3.0 mm for centration; thickness, 0.5 mm; weight, 0.173 g; eight orientation teeth, 0.15 × 0.3 mm) was positioned on a corneoscleral button (16-mm diameter) fixed in an artificial anterior chamber under microscopic control. In all cases, the donor oversize was 0.1 mm. For recipient trephination, a corresponding circular metal mask was used (diameter, 12.9 mm; central opening, 8.0 mm; thickness, 0.5 mm; weight, 0.29 g; eight orientation notches, 0.15 × 0.3 mm). This was the only technique of trephination in this study without mechanical contact.

DALK

In this study, DALK was carried out using big-bubble technique. The recipient's stroma was trephined to a depth of roughly 80% using a guided trephine. After that the anterior stroma was dissected. The depth of dissection was determined according to intraoperative surgical judgment. Lastly, the donor endothelium and the Descemet's membrane were separated by either air injection creating a big bubble. In case of tears in Descemet's membrane or remaining stroma on Descemet's membrane the surgery was converted to PK. So only cases of clearly denuded Descemet's membrane were finalized as DALK.

In all keratoplasty cases, a double running cross stitch suture with nylon 10.0 was applied, according to Hoffmann et al. [12]. Depending on the results and tolerability from patients, the suture was removed between 6 and 18 months postoperatively.

Postoperative Management

Following all procedures, postoperative care included administration of topical Dexamethasone (Dexa EDO 1.3 mg/ml, Dr. Gerhard Mann GmbH, or Monodex 1 mg/ml, Thea Pharma GmbH) five times daily, tapered over six months. In case of epithelial defects, Dexpanthenol (Bepanthen®, Bayer HealthCare) and Ofloxacin (Floxal, Dr. Gerhard Mann GmbH) ointments were administered until reepithelialization.

Clinical Parameters

Postoperatively, graft survival, endothelial cell density (ECD), graft rejection, as well as astigmatism and best spectacle-corrected visual acuity (BSCVA) after complete suture removal were analysed from medical records. Graft failure was defined as a loss of clarity and the ability to function properly,

resulting in blurred vision, or continued corneal cloudiness that did not improve with local treatment. The most common cause of graft failure was corneal oedema due to endothelial decompensation. ECD was calculated using non-contact specular microscopy and a semi-automated cell counting method. A decrease in ECD below 1000 cells/mm² was defined as a clinically significant loss of endothelial cells. Graft rejection was documented if new endothelial precipitates appeared on the graft, accompanied by graft edema with no other explanation. A BSCVA of at least 0.5 (decimal) and astigmatism of more than − 5 dioptres (measured using Scheimpflug corneal topography) were considered to indicate a significant postoperative reduction in visual acuity and high postoperative astigmatism.

Statistics

Kaplan–Meier survival analysis was used to compare long-term outcomes in graft survival, ECD, graft rejection, astigmatism, and BSCVA after complete suture removal between keratoplasty techniques for keratoconus. The log-rank test was performed to assess differences between survival curves. All calculations were conducted using the software R (https://www.r-project.org) with a p-value ≤ 0.05 considered statistically significant.

Results

From 2004 to 2024, a total of 6,277 keratoplasties were performed at the Eye Centre of the University Hospital of the Albert-Ludwigs-University Freiburg. Of these, 605 were first-time keratoplasties on eyes treated for KC with at least one follow-up visit. These cases were included in the study. The study cohort was divided into five groups: CM (n = 426), EXCIMER (n = 56), FEMTO-TH (n = 48), FEMTO-MR (n = 36), and DALK (n = 39). All keratoplasties were performed by 6 experienced surgeons. The proportion of females in each group varied from 21% to 28%, and the proportion of right eyes operated on ranged from 44% to 56%. Some patients underwent the so-called "triple procedure," which combines corneal transplantation with phacoemulsification and intraocular lens implantation. The descriptive data of the cohort are shown in Table 1.

Table 1

Descriptive data on study participants. Values are presented as percentage, absolute numbers or mean with standard deviation. Triple procedure means a combined keratoplasty with phacoemulsification and intraocular lens implantation. Abbreviations: CM conventional mechanical trephination. FEMTO-TH femtosecond laser-assisted trephination with top-hat profile. FEMTO-MR femtosecond laser-assisted trephination with mushroom profile. EXCIMER Excimer laser-assisted trephination. DALK deep anterior lamellar keratoplasty.

Techniques	**n**	**CM**	**EXCIMER**	**FEMTO-TH**	**FEMTO-MR**	**DALK**
	∑ = 605	**426**	**56**	**48**	**36**	**39**
Female % (n)	605	24% (101)	27% (15)	21% (10)	28% (10)	26% (10)
Triple procedure % (n)	605	4% (15)	0% (0)	0% (0)	0% (0)	0% (0)
Patient age in years	605	44.5 (32.2–56.7)	39.3 (29.7–45.4)	41.1 (31.3–51.5)	37.8 (26.5–46.7)	43.3 (30.4–58.2)
Follow-up in years	602	3.6 (1.6–9.7)	3.7 (2.0–6.7)	9.2 (4.3–13.0)	7.1 (2.2–13.3)	2.3 (1.6–4.8)
Trephine diameter in mm	344	8.0 (8.0–8.0)	8.0 (8.0–8.0)	8.5 (8.5–9.0)	9.0 (8.6-9.0)	8.0 (8.0–8.0)

Graft survival

All techniques achieved a graft survival rate of at least 90% after five years. Ten years after surgery, the graft survival rates were at least 90% for CM, EXCIMER, and FEMTO-MR, though the rates for FEMTO-TH and DALK were slightly under 90%. The results did not differ significantly from each other (Fig. 1).

Graft rejection

Two and a half years after the operation, the rejection rate of the FEMTO-MR group was significantly higher than that of all other groups (about 50% versus less than 30%, $p < 0.01$). This trend continued for the five- and ten-year periods after surgery, with a slight, though not significant, increase in the rejection rate in the FEMTO-TH group after ten years (Fig. 2). In the DALK group, graft rejection was predominantly stromal, attributable to the fact that the endothelial layer remained intact intraoperatively. Conversely, the graft rejection observed in the other keratoplasty groups was predominantly endothelial.

Endothelial cell density loss

Following all techniques a continued tendency of endothelial cell density loss throughout the five- and ten-year study periods was observed. Five years after surgery, approximately 50% of patients in the FEMTO groups had an ECD below 1,000 cells/mm², whereas the corresponding proportion in the other groups was around 30%. Nevertheless, the difference between groups was not statistically significant.

Ten years after surgery, about 85% of patients in the FEMTO-MR group had an ECD below 1,000 cells/mm², compared to approximately 80% in the other groups.

Visual acuity after suture removal

Visual improvement after surgery was faster in the DALK group after complete suture removal than in other groups. Two and a half years after complete suture removal, none of the DALK patients had a best-corrected Snellen visual acuity (BCSVA) of 6/12 or less. Meanwhile, approximately one-quarter of patients in the other groups still had a BSCVA of 6/12 or less. This difference was significant ($p = 0.04$) (Fig. 3).

Astigmatism development after suture removal

The development of postoperative astigmatism varied significantly among the different techniques, with the FEMTO groups showing the least favourable outcomes (Fig. 4). The cumulative incidence of developing a refractive cylinder greater than − 5 diopters was highest in the FEMTO-TH group, affecting approximately 50% of patients five years after complete suture removal. The FEMTO-MR group performed similarly poorly, with about 40% of patients exceeding this threshold.

In contrast, the other techniques yielded substantially better results. At the same five-year time point, the rate of high astigmatism was approximately 25% for the CM group and 22% for the DALK group. The EXCIMER laser-assisted technique demonstrated the best astigmatic outcomes, with only around 15% of patients developing a cylinder greater than − 5 diopters. The overall difference between the groups was statistically significant ($p = 0.02$).

Discussion

This large, single-centre study provides a real-world, long-term comparison of modern and conventional keratoplasty techniques for advanced keratoconus. Our findings indicate that, while all investigated methods achieve excellent long-term graft survival, significant differences emerge in rejection rates and functional outcomes, which have important clinical implications.

Graft integrity: survival and rejection

A key finding is that all techniques achieved graft survival rates exceeding 90% at five years, consistent with previous long-term studies on both PK and DALK for keratoconus [13, 14]. This confirms the general reliability of corneal transplantation for this indication. However, the significantly higher rejection rate in the FEMTO-MR group (50% at 2.5 years) is a critical concern. We hypothesise this is due to the mushroom profile's design, which necessitates a larger anterior graft diameter, thereby increasing the donor tissue surface area and potential immunogenic exposure [10, 15]. The slightly elevated rejection rate in the FEMTO-TH group, which also uses a larger diameter than CM or EXCIMER grafts, further supports this hypothesis. Contrary to theories that the smaller posterior diameter of the mushroom

profile might protect against endothelial rejection [16], our data suggest that the increased overall antigenic load is the dominant factor.

Functional outcomes: visual acuity and astigmatism

Functionally, DALK and EXCIMER-PK demonstrated slightly superior outcomes. Patients in the DALK group achieved significantly faster visual rehabilitation, with no patients remaining below BSCVA < 6/12 up to 2.5 years after suture removal. While long-term studies often find final visual acuity to be comparable between DALK and PK [17, 18], our results highlight a tangible short-term advantage in quality of life for DALK recipients.

Postoperative astigmatism remains a major challenge, and our results clearly show that femtosecond laser–assisted techniques performed the worst in this regard. The FEMTO-TH group had the highest rate of clinically significant astigmatism (< − 5 D). This is likely attributable to the contact applanation required for the laser, which can distort the already irregular keratoconic cornea and lead to an imprecise, pear-shaped host trephination [19, 20]. A previous study described the lower rate of postoperative visual rehabilitation for FEMTO-assisted techniques in comparison with CM trephination. The findings of the present study are consistent with those of the aforementioned study [21]. In contrast, the non-contact EXCIMER laser technique yielded the best astigmatic results, aligning with previous reports showing it creates a more regular corneal topography [19]. DALK and CM-PK also provided favourable long-term astigmatism control, reinforcing their status as reliable techniques for managing this critical outcome.

Endothelial health and practical considerations for DALK

Our study did not find significant differences in ECD reduction among the PK techniques. This contrasts with some literature suggesting DALK's clear superiority in preserving endothelial cells by retaining the host's own endothelium [17, 22]. Nonetheless, although the advantages of preserving endothelial cells postoperatively with DALK techniques have been reported in previous studies, the comparative results with PK were generally presented only within one to three years after surgery [23, 24]. Information regarding the endothelial status ten years after DALK surgery has, until now, remained limited. The comparatively long-term endothelial cell loss observed in DALK may result from trauma to the recipient endothelium during deep stromal dissection as well as postoperative inflammation. Further long-term studies are warranted to elucidate this phenomenon. While the trend in our data did not reach statistical significance, the established biological advantage of DALK in avoiding endothelial surgical trauma and allograft rejection remains questionable as a primary reason for its preference.

However, despite these advantages, the practical challenges of DALK must be acknowledged. The procedure is technically demanding, with a steep learning curve associated with dissecting down to Descemet's membrane. There is a significant risk of intraoperative perforation, which necessitates conversion to a full-thickness PK, thereby negating the primary immunological and structural benefits of the lamellar approach. These difficulties contribute to why DALK is still performed less frequently than PK in many large keratoplasty centres, a trend documented in Germany by Flockerzi et al. [7]. Therefore,

while our study highlights excellent outcomes for successfully completed DALK procedures, these results represent a cohort where the technical challenges were overcome. The potential for conversion and the requisite surgical expertise remain critical considerations in clinical decision-making.

Limitations

Our study's retrospective nature introduces potential for data loss and a "reversed survival bias," where patients with good outcomes may not return for follow-up. Furthermore, the unequal group sizes, reflecting the historical establishment of mechanical PK, may limit the statistical power of some comparisons. We sought to minimize these effects by including all consecutive cases and pre-defining outcomes, but a prospective, randomized trial would be necessary to confirm these findings definitively.

Conclusions

All evaluated keratoplasty techniques provide high long-term graft survival for keratoconus. However, femtosecond laser-assisted keratoplasty should be used with caution for this indication due to a significantly higher risk of graft rejection and unfavorable postoperative astigmatism. DALK and non-contact EXCIMER-PK appear to offer slightly better functional outcomes regarding visual recovery and astigmatism. When selecting a technique, surgeons must weigh these clinical outcomes against practical considerations such as equipment costs, surgical complexity, and the significant learning curve associated with procedures like DALK.

Declarations

Data Availability

The data from this manuscript are not publicly available due to privacy concerns expressed by the healthcare providers. Despite anonymisation, individual access to the data can be arranged if appropriate precautionary measures are taken, ensuring compliance with the German data protection laws. Access will be granted only under strict conditions to safeguard patient privacy and data security. Please send requests via email to daniel.boehringer@uniklinik-freiburg.de.

Conflict of Interest

The authors declare that they have no conflict of interest.

Generative AI and AI-assisted technologies in the writing process

DeepL Write, Google Gemini and Perplexity AI were used for linguistic improvements. The authors carefully checked the text suggestions for accuracy and made corrections where necessary. They take full responsibility for the publication's content.

Additional information

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Author contributions

B.D.T. wrote the main manuscript text. B.D.T. and D.B. prepared the figures and tables. D.B. and P.M. provided corrections and revisions to the manuscript. T.R. conceived the idea and supervised the entire project. All authors reviewed the manuscript.

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Figures

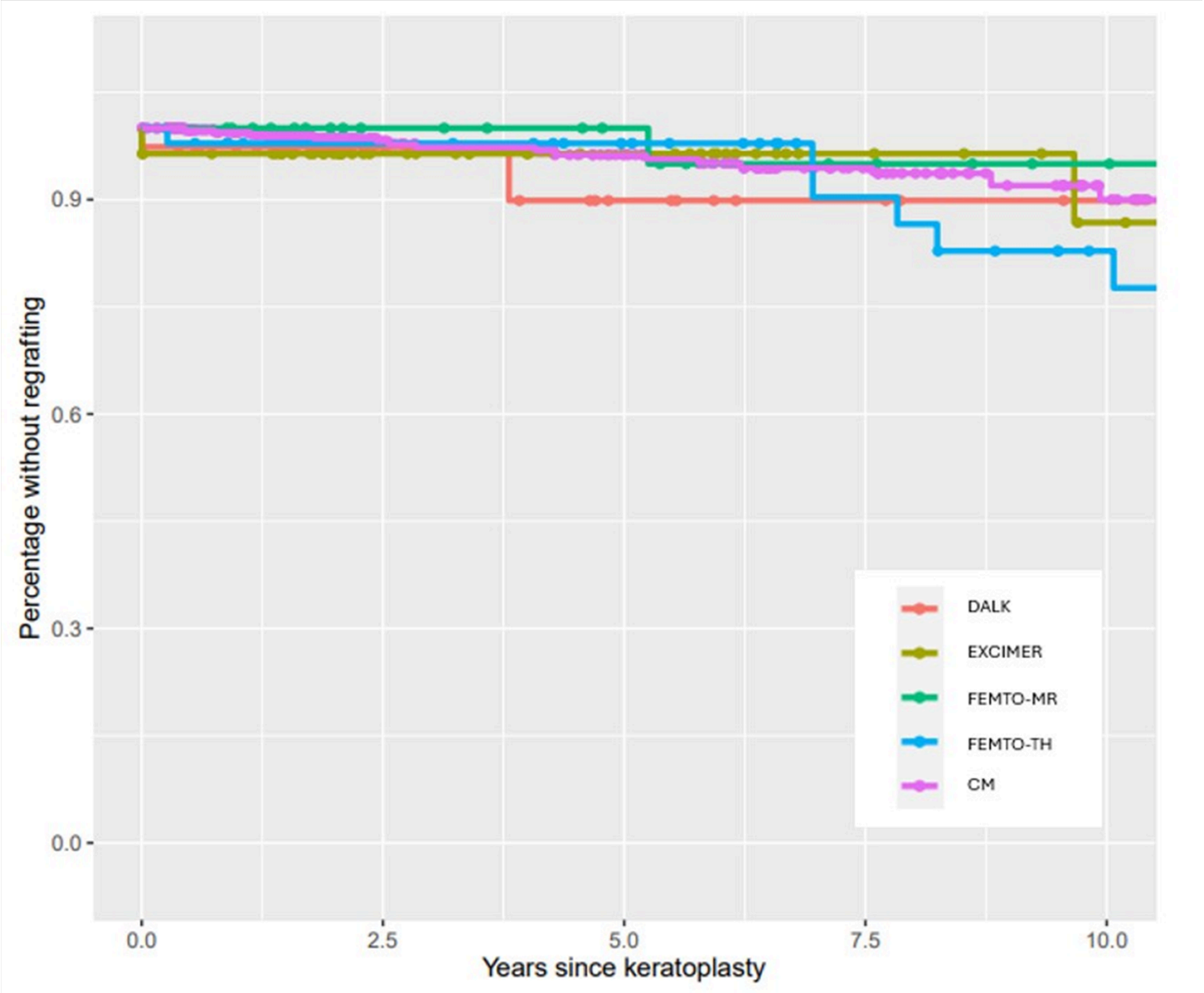


Figure 1

Kaplan-Meier curve of graft survival within ten years after surgery. DALK Deep anterior lamellar keratoplasty, EXCIMER Excimer laser-assisted keratoplasty, FEMTO-MR Femtosecond laser-assisted

keratoplasty with mushroom-shaped trephination, FEMTO-TH Femtosecond laser-assisted keratoplasty with top-hat-shaped trephination, CM Conventional mechanical keratoplasty.

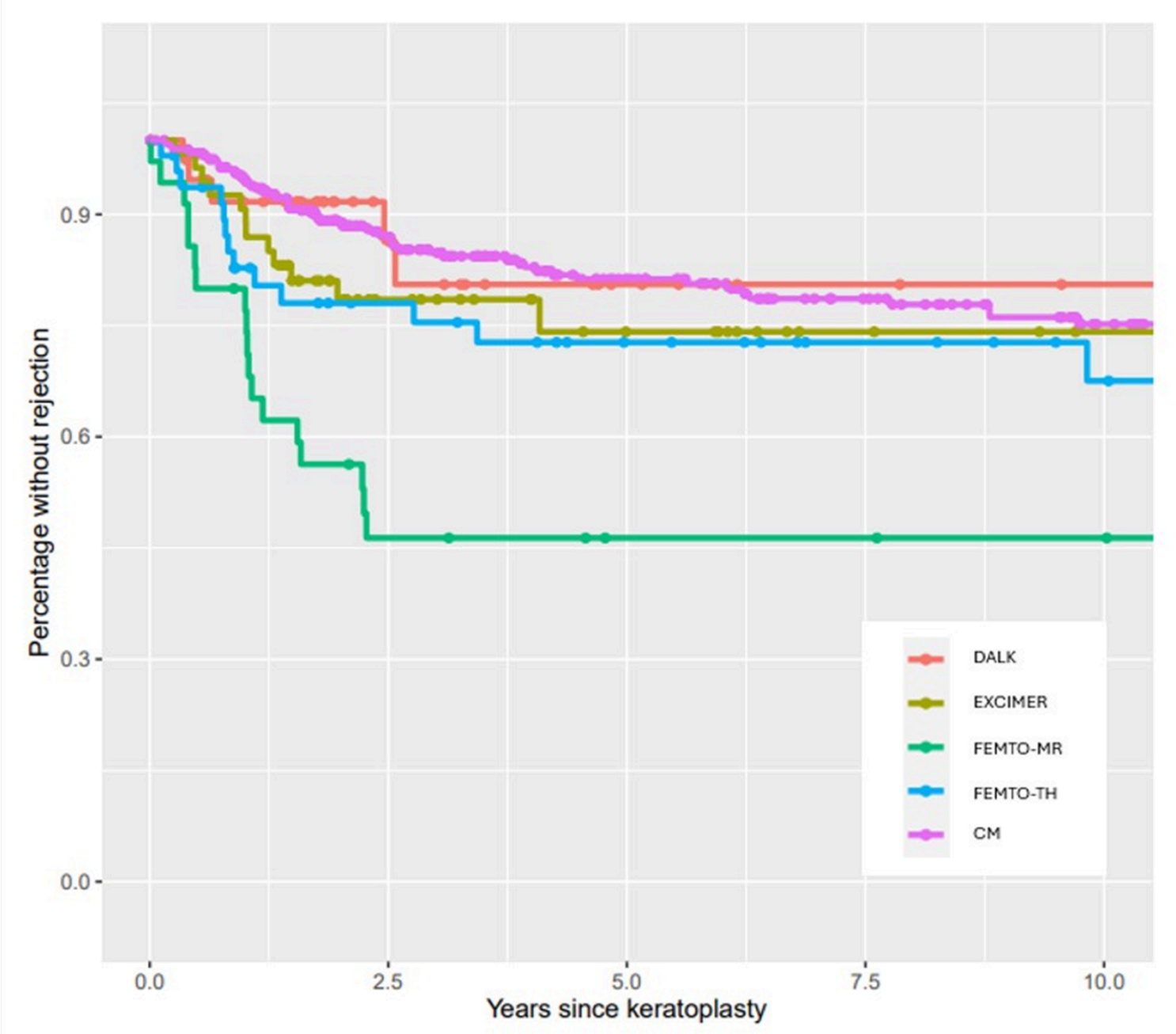


Figure 2

Kaplan-Meier curve of rejection rate within ten years after surgery. DALK Deep anterior lamellar keratoplasty, EXCIMER Excimer laser-assisted keratoplasty, FEMTO-MR Femtosecond laser-assisted keratoplasty with mushroom-shaped trephination, FEMTO-TH Femtosecond laser-assisted keratoplasty with top-hat-shaped trephination, CM Conventional mechanical keratoplasty.

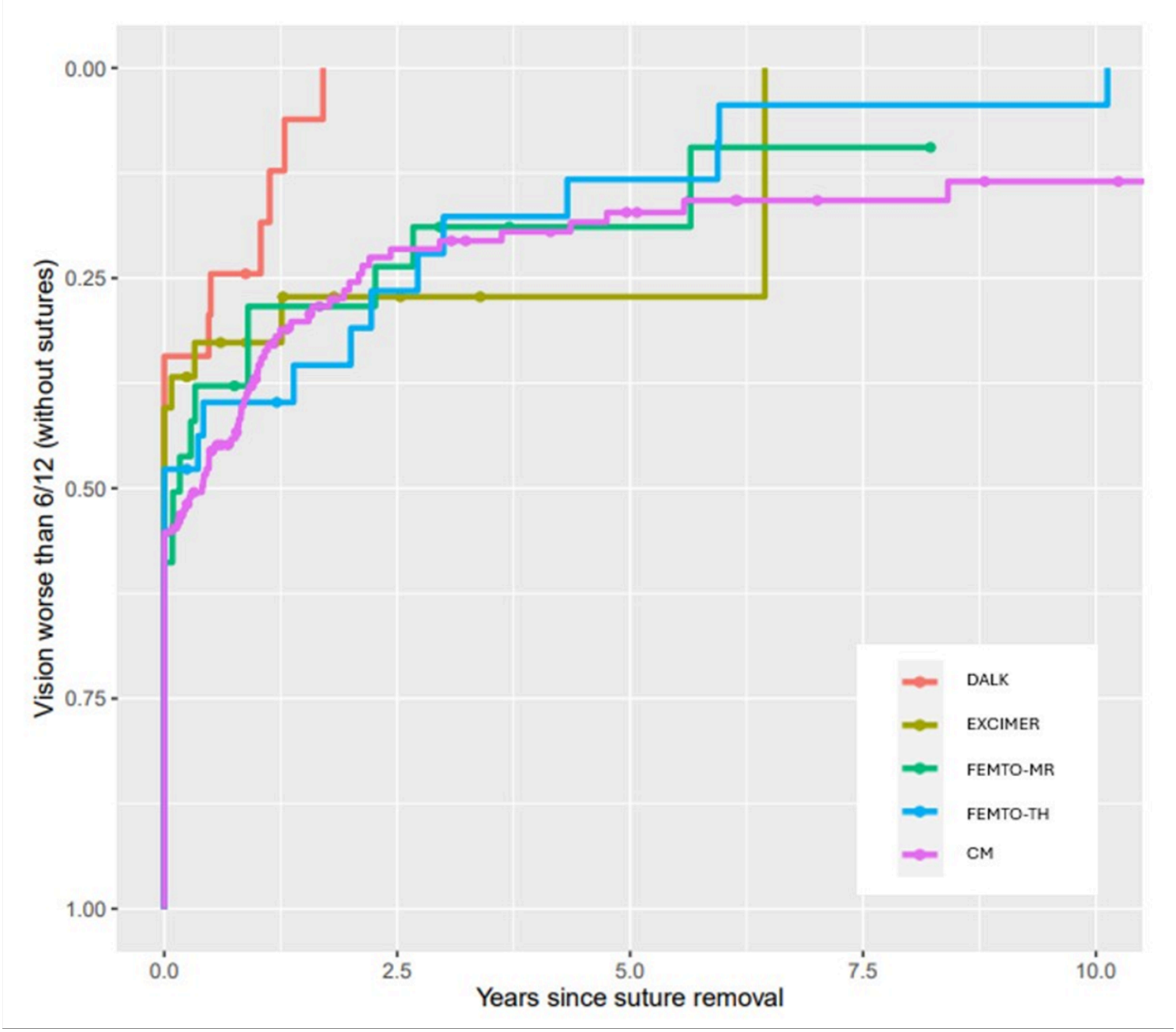


Figure 3

Kaplan-Meier curve of visual rehabilitation within ten years after suture removal following surgery. DALK Deep anterior lamellar keratoplasty, EXCIMER Excimer laser-assisted keratoplasty, FEMTO-MR Femtosecond laser-assisted keratoplasty with mushroom-shaped trephination, FEMTO-TH Femtosecond laser-assisted keratoplasty with top-hat-shaped trephination, CM Conventional mechanical keratoplasty.

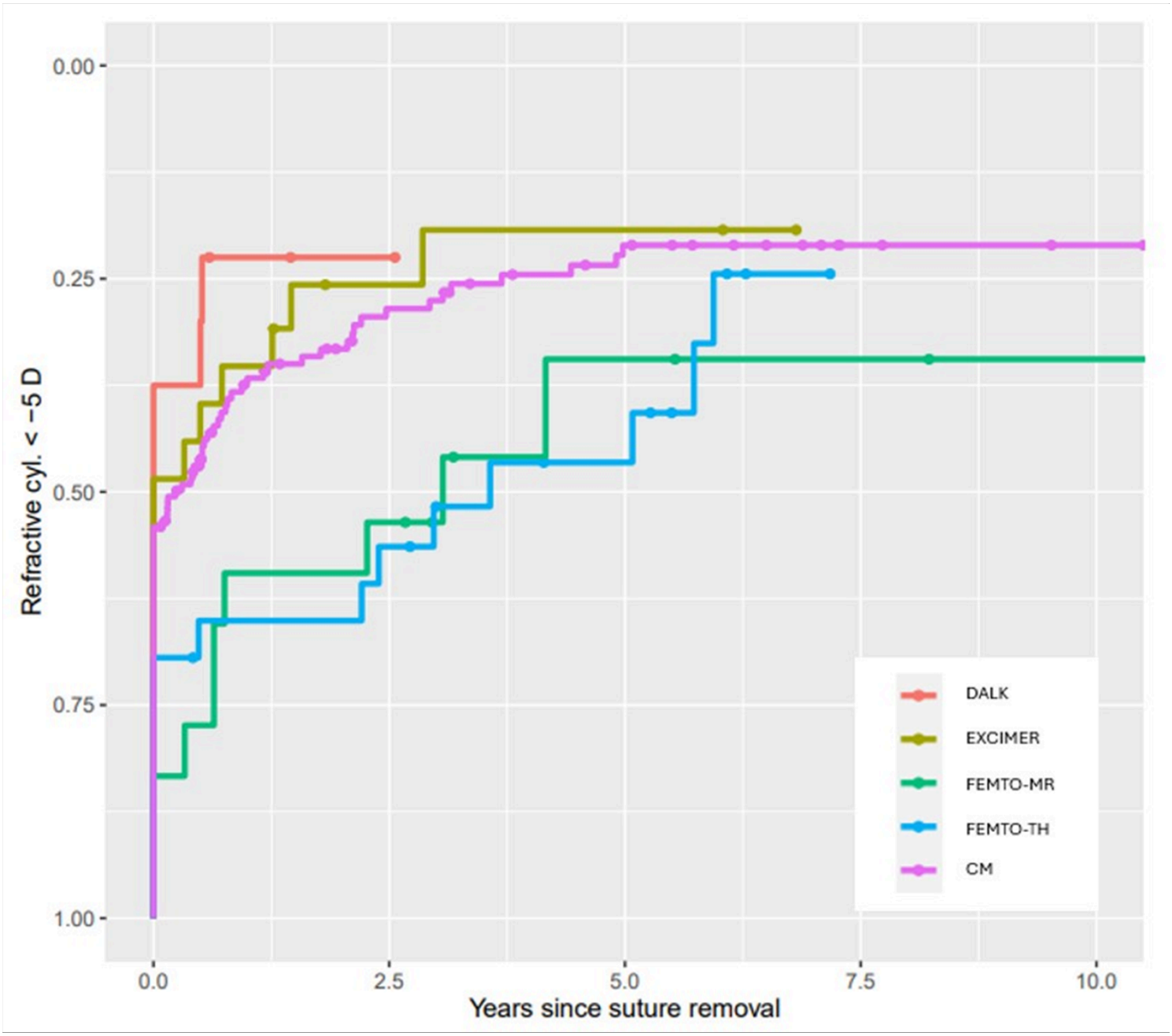


Figure 4

Kaplan-Meier curve of astigmatism development within ten years after suture removal following surgery. DALK Deep anterior lamellar keratoplasty, EXCIMER Excimer laser-assisted keratoplasty, FEMTO-MR Femtosecond laser-assisted keratoplasty with mushroom-shaped trephination, FEMTO-TH Femtosecond laser-assisted keratoplasty with top-hat-shaped trephination, CM Conventional mechanical keratoplasty, Refractive cyl. – Refractive cylinder.