

## Donor cornea quality used for penetrating keratoplasty vs deep anterior lamellar keratoplasty

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### Abstract

Deep anterior lamellar keratoplasty (DALK) has recently been introduced as an alternative procedure to penetrating keratoplasty (PK) for corneal pathologies not affecting the corneal endothelium. DALK does not rely on donor endothelium and requires less rigid criteria for donor corneal tissue quality. Therefore, DALK makes it possible to use donor corneas deemed unsuitable for PK. Furthermore, lamellar keratoplasty allows acellular corneal tissue to be transplanted. As a result, long-term preservation techniques are being revisited to increase the availability of donor corneas and subsequently alleviate constraints of availability, cost, storage, and transportation in many countries. The recent alterations in corneal transplantation techniques and hence the type of donor cornea tissues used for each technique, may require corneal surgeons and eye banks to reevaluate their selection criteria. The purpose of this systematic review is to present an updated analysis on the type and quality of donor corneas used for PK and DALK, assess the influence of donor and eye bank factors on the

quality of donor corneas, and determine whether any of these donor factors affect clinical outcomes, complications, and graft survivals.

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**Key words:** Corneal transplantation; Penetrating keratoplasty; Full-thickness keratoplasty; Deep anterior lamellar keratoplasty; Deep lamellar keratoplasty; Maximum depth anterior lamellar keratoplasty; Donor corneal quality; Graft quality

**Core tip:** Deep anterior lamellar keratoplasty (DALK) is recently used for treating corneal diseases not affecting the corneal endothelium. This makes it possible to use donor tissue with poor endothelium. Furthermore, DALK allows acellular corneal tissue to be used and long-term preservation techniques are being considered as a method for donor storage. The recent alterations may require eye banks and corneal surgeons to reassess their selection criteria to ensure donor grafts which are inappropriate for penetrating keratoplasty because of low endothelial cell count can be safely used for DALK.

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### INTRODUCTION

Penetrating keratoplasty (PK) is a surgical technique in which the full thickness of the recipient cornea is replaced by donor tissue. Deep anterior lamellar keratoplasty (DALK) is intended to selectively replace the abnormal stroma while preserving the recipient's endothelium in place<sup>[1]</sup>. Therefore, DALK can eliminate the risk of

endothelial graft rejection and has minimal detrimental effect on endothelial cell density<sup>[2]</sup>. Some investigators report that visual acuity and refractive error following DALK can be similar to those following PK<sup>[3-6]</sup>.

The recent alterations in corneal transplantation techniques and consequently the type of donor cornea tissues employed for each technique may require corneal surgeons and eye banks to reevaluate their donor selection criteria.

Controversy exists regarding the donor corneal tissue quality used for each transplantation technique. Donor factors such as age, local and systemic diseases, cause of death, and traumatic damages or surgical procedures as well as the storage factors (mainly method of storing, time between death and preservation, and duration of tissue preservation) can influence the final quality of the corneas. When indicated for optical purposes, PK surgeons prefer transplanting donor cornea tissues with quality ranging from good to very good to excellent to provide adequate endothelial cells for a lifelong period. Since the introduction of DALK, many surgeons have been accepting donor corneas with lower quality compared with PK. DALK does not rely on quality of the donor endothelium and requires less strict criteria for donor selection<sup>[7]</sup>. This feature is imperative in increasing the availability of corneal grafts in regions where there is shortage of donor corneas<sup>[7]</sup>.

The purpose of this systematic review is to present an updated analysis on the type and quality of donor corneas used for PK and DALK, to assess the influence of donor and eye bank factors on the quality of donor corneas, and furthermore to determine whether any of these donor factors affect clinical outcomes, complications, and graft survival.

## RESEARCH

### Questions for assessment

The objective of this assessment is to address the following questions: (1) what type and quality of donors are used for PK and DALK? (2) What is the influence of donor and eye bank factors on the appropriateness of corneal grafts for transplantation [endothelial cell density (ECD)] and graft quality? (3) Do donor and eye bank variables affect clinical outcomes, complications, and graft survivals following PK and DALK?

### Description of evidence

A search of the peer reviewed English literature was performed using the PubMed database between January 14, 2014 and April 15, 2014. Article reference lists were also reviewed to identify relevant articles. Keywords used in the search included the MeSH heading “corneal transplantation” combined with text words “deep anterior lamellar keratoplasty” or “DALK” or “deep lamellar keratoplasty” or “maximum depth anterior lamellar keratoplasty” and “penetrating keratoplasty” or “PK” (MeSH and text) or “full-thickness keratoplasty” accompanied with “donor quality” or “graft quality”.

Then, a group of methodologists assigned a level of evidence rating to each article. A level I rating was assigned to well-designed randomized clinical trials; a level II rating was assigned to poor-quality randomized clinical trials or well-designed case-control and cohort studies; and finally a level III rating was assigned to case reports and poor-quality case-control or cohort studies.

In order to evaluate the minimal endothelial cell count limits, the upper and lower limits of donor age, maximal time intervals from death, enucleation or excision to preservation, and maximal storage time deemed suitable for corneal transplantation, studies on the characteristics of donor corneas used for transplantation were reviewed.

The author assessed the 61 citations and selected 48 articles that potentially or definitely met the inclusion criteria. The full text of these 48 articles was obtained for further evaluation.

Nine studies were prospective, double-masked clinical trials (level I) reported by the Cornea Donor Study<sup>[8-16]</sup>. Four studies were prospective non-randomized trials (level II)<sup>[17-20]</sup>. The remaining 35 articles were comparative or non-comparative case series and were rated as level III evidence<sup>[21-47]</sup>.

### Type and quality of donors used for PK and DALK

Assigned corneas for PK with quality ranging from good to excellent had the following characteristics: donor age varied from 1 to 96 years<sup>[9,10,19-23,46]</sup>; endothelial cell density varied from 2000 to  $\geq 3000$  cells/mm<sup>2</sup><sup>[29,10,18,24]</sup>; death-to-preservation time was between 45 min and 22.3 h<sup>[19,22,23,25]</sup>; maximum storage time was 14 d in cool-storage media and 4 wk in organ culture<sup>[19-21]</sup>.

In contrast to penetrating keratoplasty, donors with quality ranging from fair to excellent were employed for DALK<sup>[26,27]</sup>. Furthermore, long-term preserved donor tissues completely devoid of cells were also transplanted<sup>[28-31]</sup>. One DALK study used donor cornea tissues with age between 12 and 72 years, graft rating from fair to excellent, ECD between 1128 and 4255 cells/mm<sup>2</sup>, death-to-preservation time up to 56 h, and storage time up to 13 d in Optisol medium ( $-4^{\circ}\text{C}$ )<sup>[26]</sup>. Another DALK study used donors with the following features: age between 28 and 88 years, ECD between 100 and 3300 cells/mm<sup>2</sup>, and storage time up to 35 d in organ culture medium ( $31^{\circ}\text{C}$ )<sup>[27]</sup>.

Long-term preserved corneas with mean storage time between 2.7 and 9.6 mo were also used for DALK by some surgeons<sup>[28-31]</sup>. The majority of studies used lyophilization or chemical agents to dehydrate corneas before cryopreservation<sup>[28-30]</sup>. One study, however, employed cryopreservation without dehydration before freezing<sup>[31]</sup>.

### Effect of donor and eye bank variables on endothelial cell density and graft quality

Most studies that evaluated effects of donor characteristics and eye bank variables on graft quality and ECD concluded that the age of donor and time interval in organ culture were the main variables influencing the quality of endothelium. Gavrilov *et al*<sup>[32]</sup> reported that the rate

of organ-cultured corneas which were inappropriate for PK as a result of inadequate endothelium increased from 13% in donors < 40 years to 32% in donors > 80 years. The Cornea Donor Study revealed a negative correlation between donor age and ECD<sup>[16]</sup>.

Armitage *et al*<sup>[24]</sup> revealed that the age of donor and preservation time in organ culture were the main variables which could affect endothelial suitability for PK. The odd of ECD less than 2500 cells/mm<sup>2</sup> was increased with longer preservation time and increasing donor age. Increasing time interval from enucleation to corneoscleral disc excision also increased the likelihood of ECD less than 2500 cells/mm<sup>2</sup> but the overall impact was small and significant only for a time interval greater than 18 h<sup>[24]</sup>.

Grabska-Liberek *et al*<sup>[33]</sup> found that the rating of the morphological state of corneas suitable for PK depended mostly on the time between death and preservation, donor's age, cause of death and duration of preservation. The overall rating of tissues obtained in a very short time after death (to 5 h) was higher (excellent and very good) compared with corneas removed 8-12 h after the donor's death. An increasing percentage of endothelial cell loss was observed after 7 d of preservation independent of other factors<sup>[33]</sup>.

One study found that initial ECD was lower and elimination for low ECD was more frequent in donors aged 85 years and over, compared to younger donors<sup>[17]</sup>. However, after storage in organ culture, very old corneas lost fewer endothelial cells than younger ones resulting in ECD which did not differ at the end of storage<sup>[17]</sup>.

One study measured endothelial cell loss during preservation in organ culture<sup>[25]</sup>. The donor's gender, age, cause of death, and postmortem interval had no significant correlation with the percentage of endothelial cell loss. However, the preservation time demonstrated a significant correlation with a loss of 0.07% for each day of preservation<sup>[25]</sup>.

Additionally, the combined effects of cause of death and donor age on ECD were evaluated. It was identified that chronic and long-lasting, severe diseases like cancer reduced ECD to a greater extent as compared to diseases causing a more rapid death. This negative impact of chronic diseases was aggravated by the general reduction in ECD observed with increasing age<sup>[34]</sup>.

### **Effect of donor and eye bank variables on clinical outcomes, complications, and graft survivals following PK**

Five studies investigated the effect of donor and eye-bank variables on epithelium-related problems following PK<sup>[19,23,35-37]</sup>. Death-to-preservation time and total storage time were significantly associated with an increased prevalence of epithelial defects on day 1 or hurricane and filamentary keratopathy<sup>[19,23]</sup>. Kim *et al*<sup>[35]</sup> outlined that the degree of epithelial defect had a statistically significant association with the time interval from preservation to surgery. Borderie *et al*<sup>[36]</sup> reported that death-to-storage time, storage time, and deswelling time significantly influenced the graft reepithelialization time in univariate analysis. In

multiple regression however, none of the donor variables significantly influenced the graft reepithelialization time. As for the surface keratopathy 1 wk following PK, Mananis *et al*<sup>[37]</sup> observed no correlation between this complication and donor age, death-to-preservation time, preservation-to-surgery time, and the donor epithelial status.

Another widely investigated correlation was the effect of donor and eye-bank variables on postoperative ECD which yielded contradicting results. Langenbucher *et al*<sup>[21]</sup> reported no significant association between the annual endothelial cell loss and the donor age as well as post-mortem interval. However, the storage time had a statistically significant correlation with the annual endothelial cell loss. Parekh *et al*<sup>[25]</sup> reported postmortem interval  $\geq 10$  h tends to have a higher percentage of endothelial cell loss than < 10 h of interval at both 1 year and 3 years postoperatively.

Postoperative higher ECD values were significantly associated with higher baseline ECD and younger donor age in one study<sup>[9]</sup>. When the follow-up period was extended to 10 years, the study group observed that the donor age influenced ECD, although this finding was primarily influenced by a small group of the youngest donors (12 to < 34 years of age) that had the least cell loss and the best graft survival<sup>[9]</sup>. Lass *et al*<sup>[11]</sup> observed the younger age and female gender of the donor had a significant correlation with higher ECD over time. However, cause of death and time interval from death to preservation or to surgery failed to demonstrate any significant association with changes in ECD during follow-up<sup>[11]</sup>. One study found a statistically significant negative influence of postmortem time and donor age on chronic loss of endothelial cell density after PK for keratoconus<sup>[38]</sup>.

Two studies reported the effect of donor age on visual outcomes. Gain *et al*<sup>[17]</sup> found no significant difference between the two groups (donors younger than 85 years and donors aged 85 years and older), in terms of visual acuity and astigmatism. Halliday *et al*<sup>[39]</sup> found no significant correlation between the time taken to reach a postoperative acuity of 6/12 and the age of donor.

One study reported that donor age, ABO compatibility, and other donor factors were not associated with graft rejection<sup>[8]</sup>. Younger donor age, however, was found a risk factor for graft rejection (but not for graft failure) by three other studies<sup>[17,40,47]</sup>.

Despite contradictory results of studies evaluating the effect of donor and eye-bank variables on ECD and morphology, the majority of studies showed that donor preservation method and time, donor age, cause of death, and preoperative donor ECD and/or morphometric measures (coefficient of variation and hexagonality) had no influence on overall graft failure<sup>[12,13,17,20,22,40,41]</sup>. However, one study reported that preoperative risk factors for developing late endothelial failure included low ECD and older donor age<sup>[46]</sup>. Authors from the Cornea Donor Study observed that grafts from donors aged between 66 and 75 years old that met the eligibility criteria of their study had a 5-year graft survival rate, comparable to grafts from younger donors<sup>[10]</sup>. However, higher donor

age was significantly associated with lower graft success during a longer follow up period<sup>[15]</sup>.

### **Effect of donor and eye bank variables on clinical outcomes, complications, and graft survivals following DALK**

Borderie *et al*<sup>[27]</sup> evaluated the effect of donor variables on the result of different anterior lamellar keratoplasty techniques in a heterogeneous group of corneal disorders with normal endothelium. The age of donor was the only factor which influenced visual rehabilitation postoperatively; visual acuity was significantly lower in recipients who received corneas from donors > 80 years<sup>[27]</sup>. Heindl *et al*<sup>[42]</sup> did not observe any significant association between donor storage time intervals and visual results one year following DALK. Feizi *et al*<sup>[26]</sup> observed that graft rating and preservation-to-surgery time had a significant correlation with the presence of graft stromal edema and epithelial defects on postoperative day 1 following DALK. Suture-related complications, graft rejection episodes, graft clarity, visual acuity and refractive outcomes at the final follow-up examination were found to have no correlations with any donor or eye-bank factors<sup>[26]</sup>.

Five studies concluded that long-term cryopreserved donors can provide similar visual results comparable to fresh corneal tissues following DALK<sup>[28,29,31,43,44]</sup>. Another advantage of long-term preservation of the cornea by lyophilization and chemical glycerin-dehydration is to eliminate cells such as epithelium, keratocytes, and antigen-presenting cells and create the acellular biological materials<sup>[28,45]</sup>. As such, acellular corneal tissues may significantly reduce or even eliminate the incidence of graft rejection after lamellar keratoplasty<sup>[28,29,31]</sup>. Complications such as persistent epithelial defects, filamentary keratitis, and suture-related complications were more likely to occur when such a low quality graft was transplanted<sup>[31]</sup>.

There is currently a paucity of evidence for setting an acceptable minimum donor conditions for corneal transplantation, especially for lamellar keratoplasty. According to Eye Bank Association of America standards for human corneal transplantation, minimal endothelial cell count limits, the upper and lower limit of donor age, time intervals from death, enucleation or excision to preservation are left to the discretion of the eye banks<sup>[48]</sup>. An understanding of the effect of donor variables including age, time interval from death to enucleation and to preservation, storage time, and endothelial cell density both on the quality of donor corneas and on post-transplantation outcomes help to set eye banking standards. To establish the criteria, it is vital to find out the correlation between these donor parameters and the appropriateness of corneas for transplantation as well as between donor parameters and post-transplantation outcomes. The aim of this review, therefore, was to assess the influence of donor and eye bank factors on the graft quality as well as clinical outcomes.

Based on the results of this review, the minimum ECD for PK is 2000 cells/mm<sup>2</sup> and there is no age limit for donors. Death-to-preservation time up to 24 h is ac-

cepted and maximal storage time is 14 d in cool-storage media and 4 wk in organ culture.

Because endothelial cell graft attrition takes place at an accelerated rate<sup>[49]</sup>, a higher initial endothelial cell density of the donor tissue can improve long-term graft survival<sup>[9]</sup>. Older donor age and longer storage time are more likely to be associated with lower ECD but, as long as the ECD is greater than a given minimum at the time of corneal transplantation, these parameters will have insignificant influence on long-term graft survival. The Cornea Donor Study results indicate that donor age is not an important factor in most penetrating keratoplasties performed for endothelial disease<sup>[15]</sup>. Therefore, functional and cellular results of PKs are not dramatically influenced by very old donor age and the very elderly should not be deemed off limits for corneal procurement.

The results of this review showed that epithelium-related complications such as filamentary keratitis and persistent epithelial defects correlate with longer death-to-harvest time and longer storage time<sup>[19,23,35]</sup>. In addition to donor endothelial status, graft corneal surface is a determinant for the success of corneal transplantation in the postoperative period. Although the donor cornea is ultimately resurfaced by the recipient's epithelium, an intact donor epithelium on postoperative day 1 implies a smoother course after corneal transplantation. An unstable graft surface can lead to poor visual acuity due to an irregular tear film interface, discomfort, permanent damage to Bowman's layer, sub-epithelial scarring, and even infectious keratitis<sup>[19]</sup>.

DALK has recently been introduced to replace abnormal corneal stroma while preserving the host's healthy endothelium. DALK does not rely on donor endothelial cells and less strict criteria can be used for donor graft quality. Therefore, it increases the availability of donor tissues that do not require high-quality endothelium to be appropriate for PK. However, the use of low quality donors for DALK could cause epithelium-related complications more frequently, besides more edematous alterations of the graft necessitating a closer follow-up immediately after surgery. Apart from that, low quality donors can provide good visual acuity and refractive outcomes with complication rates comparable to those achieved after the use of good quality donors following DALK.

The two main techniques for storing corneas are organ culture and hypothermia<sup>[50,51]</sup>. Since lamellar corneal transplantation makes it possible to use acellular corneal tissue, long-term preservation methods have emerged as a means to provide a greater availability of corneal tissue to alleviate constraints of availability, cost, storage, and transportation in many countries. The results of several studies indicate that cryopreserved corneal tissues can successfully substitute for fresh grafts in DALK using the big-bubble technique. One advantage of long-term preservation of the cornea is to significantly reduce or even eliminate the incidence of graft rejection. However, complications such as persistent epithelial defects, filamentary keratitis, and suture-related complications are more likely to develop with such low quality grafts making closer



follow-up visits essential immediately after DALK.

## CONCLUSION

Although both donor and eye bank variables have effects on the quality of donor corneas, and post-PK outcomes and complications, these effects would be little provided that the minimum selection criteria set by eye banks are respected. DALK makes it possible to transplant corneas with low quality and allows using the long-term methods of storage. Because, epithelial defects and stromal edema are more frequently encountered, closer follow-up visits are required when a low-quality graft is transplanted.

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