Loss of endothelial cells in viral DNA-positive grafts after keratoplasty: a 2-year follow-up study

Jing-Hao Qu 1,2, Rong-Mei Peng 1,2, Ge-Ge Xiao 1,2, Hong-Qiang Qu 1,2, Ting Yu 1,2, Shuang Zhang 1,2, Jing Hong 1,2

ABSTRACT

Background To compare endothelial loss between recipients who received viral DNA-positive grafts and controls 2 years after corneal transplantation.

Methods We retrospectively analysed the clinical data and endothelial cell density of recipients of viral DNA-positive grafts and age-, sex-, aetiology- and operation-matched controls from April 2017 to July 2019 at the Peking University Third Hospital, Beijing, China.

Results A total of 23/942 (2.44%) donor corneal buttons tested virus-positive by real-time PCR. A total of 27 recipients (except for 2 recipients) of viral DNA-positive grafts and 48 recipients of viral DNA-negative grafts were included in this study. Recipients of viral DNA-positive grafts had a higher endothelial cell (EC) loss rate post-penetrating keratoplasty and post-descemet stripping automated endothelial keratoplasty (p<0.05), but post-deep lamellar keratoplasty, the EC loss rate was similar to that of the controls. Recipients of herpes simplex virus-1, cytomegalovirus- and varicella-zoster virus-positive grafts all had a higher EC loss rate than the controls during the 12- and 24-month follow-up periods (p<0.05).

Conclusion We inferred that viruses might be hidden in corneal grafts and mainly incubate in the corneal endothelium. Viral DNA-positive grafts do not need to be replaced immediately and can be followed up for a long time.

INTRODUCTION

Donor corneas and preservation fluid are tested for bacterial and fungal infections which are believed to have a direct impact on the quality of grafts and the safety of recipients. Furthermore, cornea donors are also tested for hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and HIV before organ donation. In fact, virus detection is not a routine examination in most eye banks. Surprisingly, data on viral DNA positivity rates in donor corneas and the risk of transmission to recipients are scarce. In 2009, Remeijer et al2 reported that herpes simplex virus (HSV)-1 was detected in 2 of 273 corneoscleral rims, and HSV-2 and varicella-zoster virus (VZV) were not detected in donor corneoscleral rims. That study included the largest number of cases reported so far. Previous studies have mainly focused on HSV and VZV; recently, cytomegalovirus (CMV) was detected in 6 of 30 donor corneas obtained during keratoplasty. 3 There are no data regarding the Epstein–Barr virus (EBV) positivity rate in donor corneas.

HSV-1 can be transmitted from the graft to the recipient with subsequent reactivation of donor-derived HSV-1 in the transplanted cornea. 4 The donor-to-host transmission of infectious agents via corneal transplantation poses a real risk and can lead to graft failure, but there is lack of initial and follow-up data on CMV-, VZV- and EBV DNA-positive grafts after keratoplasty.

In our previous work (unpublished), we tested 942 remaining corneal rims after transplantation for HSV-1, HSV-2, CMV, VZV and EBV, and 23 donor corneal buttons tested positive for viral DNA. We continued to follow-up with recipients who received viral DNA-positive grafts and observed the influence on recipients among different virus types and operation methods. This study might allow a strategy for positive grafts after keratoplasty, for example, immediate graft replacement or long-term follow-up, to be determined.

MATERIALS AND METHODS

Donor corneas and viral DNA detection

Under sterile conditions, an 18-mm trephine was used to strip the sclera from the posterior cornea 3–4 mm within 12 hours after donor death. Then, the corneoscleral buttons obtained and stored in Optisol GS (Bausch & Lomb, Irvine, California, USA) at 4°C. The central endothelial cell density (ECD) of all donor corneas was quantified by a certified technician at our eye bank using the EB-3000 XYZ Eye bank specular microscope (HAI Laboratories, Lexington, Massachusetts, USA). Graft samples were obtained during consecutive cornea transplantation procedures. We extracted DNA from corneal tissues using a QIAamp DNA Mini Kit (catalogue no. 51 304; Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Briefly, corneal rim samples containing endothelium were cut into small pieces, placed in a 1.5-mL microcentrifuge tube and digested with Buffer ATL and proteinase K. The extracted DNA was diluted in water; a total of 50 ng was subjected to PCR.

HSV-1, HSV-2, CMV, VZV and EBV were detected using qualitative commercial, TaqMan-based methods (HSV-1/HSV-2 Typing Real-Time PCR Kit, Z-SD-0136-02; VZV Real-Time PCR Kit, OD-0024-02; CMV Real-Time PCR Kit, Z-OD-002-02; EBV Real-Time PCR Kit, Z-OD-0023-02; LiVeriver Bio-Tech Corp, China) in accordance with the manufacturer’s instructions. Real-time PCR was performed using reagents from PE Biosystems (PE Applied Biosystems,

To cite: Qu J-H, et al. Br J Ophthalmol 2022;106:26–31. doi:10.1136/bjophthalmol-2020-317629

© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

Check for updates

Clinical science
Study subjects
A total 23/942 (2.44%) donor corneal buttons tested virus-positive by RT-PCR from April 2017 to July 2019. Twenty-five recipients (19 males and 6 females) received viral DNA-positive grafts at 0.25–86 years of age (mean age, 35.7±25.9 years), and 48 age-, sex-, aetiology- and operation-matched controls (31 males and 17 females) received viral DNA-negative grafts from 0.3 to 86 years of age (mean age, 40.3±25.6 years). Viral DNA-positive and viral DNA-negative grafts were used for keratoplasty in the same month. All recipients had no history of ocular viral infection. The study was performed according to the tenets of the Declaration of Helsinki and was approved by the local ethics committee.

Surgical technique
In this study, all procedures were performed by an experienced surgeon (Jing Hong) at the Peking University Third Hospital. Three surgical methods were included in this study.

Penetrating keratoplasty (PK): Graft suturing was performed according to standardised methods in all patients, with 16 interrupted sutures used in most patients. Sutures were removed after at least 12 months. Descemet stripping automated endothelial keratoplasty (DSAEK): The surgical procedures were the same as previously detailed. Deep anterior lamellar keratoplasty (DALK): The anterior and middle stroma was removed using a crescent blade. Air was injected into the posterior stroma. When air injection-induced detachment of the Descemet membrane, sodium hyaluronate was injected between the posterior stroma and the Descemet membrane, and the remaining posterior stroma was completely removed using scissors. Graft suturing was performed according to standardised methods in all patients, with 16 interrupted sutures used in most patients. Sutures were removed after at least 12 months.

Postoperative treatment regimen
The standard postoperative treatment for PK, DSAEK and DALK consisted of topical levofloxacin 0.5% and artificial tears (4 times per day) for 1 month, topical dexamethasone 0.1% eye cream (once every night) for 1 week and topical prednisolone acetate 1.0% (4 times per day), tapered accordingly over 3–6 months; topical cyclosporin 1% (4 times per day) was added 1 week after the surgery and was tapered depending on the status of the graft.

Postoperative follow-up
The clinical outcome of transplantation was assessed by the best-corrected visual acuity (BCVA, LogMAR), intraocular pressure (IOP) (I CARE, TA01, Finland), ECD, graft status and complications at 6, 12 and 24 months postoperatively. The endothelial cell (EC) loss rate was calculated according to the ECD. The average ECD of the central area was measured by in vivo confocal microscopy (HRT III, Heidelberg Engineering, Heidelberg, Germany). Graft attachment and central corneal thickness (CCT) were assessed with anterior segment optical coherence tomography (Carl Zeiss Meditec, Dublin, California, USA). The same certified ophthalmic technician performed all postoperative testing of patients using the same microscope.

RESULTS
Demographics
The total donor cornea viral DNA positivity rate was 2.44% (23/942). The rates of positivity for HSV-1, CMV, VZV and EBV DNA were 30.43% (7/23), 34.78% (8/23), 26.09% (6/23) and 8.7% (2/23), respectively. HSV-2 DNA was not detected in the previous study. Patient nos. 22 and 24 experienced transplant failure caused by viral infection; they underwent a second transplantation and were excluded from this study. A total of 25 recipients who received viral DNA-positive grafts and 48 recipients who received viral DNA-negative grafts were included in this study. There were no significant differences in the mean age (t=−0.717, p=0.476>0.05) or sex (χ²=0.993, p=0.319>0.05) between the recipients with virus-positive grafts and controls. All grafts remained transparent and showed good attachment during the 24-month follow-up period. No keratic precipitate (KP) was found in any recipient.

Recipients with virus-positive grafts
Nine recipients (HSV-1/C MV/VZV/EBV=1/5/3/0) underwent PK, 9 recipients (HSV-1/C MV/VZV/EBV=3/2/2/2) underwent DSAEK and 7 recipients (HSV-1/C MV/VZV/EBV=4/2/1/0) underwent DALK. The diagnoses of the recipients are listed in table 1.

BCVA, CCT and ECD
No significant differences in the mean BCVA and CCT were found between the two groups (t=0.062, p=0.951>0.05; t=0.636, p=0.527>0.05). The average ECD of grafts with virus positivity was 3341±508 cells/mm². The average ECD of grafts in the control groups was 3316±450 cells/mm². No significant differences in the mean ECD (t=0.842, p=0.404>0.05) were identified between the two groups. The mean ECDs were 2290, 2052 and 1716 cells per mm² at 6, 12 and 24 months, corresponding to an EC loss of 25.63%, 32.68% and 43.81%, respectively, in the recipients with virus-positive grafts (n=25) (table 2). The mean ECDs were 2547, 2363 and 2156 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 16.85%, 12.89% and 29.13%, respectively, in the controls (n=48) (table 2).

PK
No significant differences in the mean BCVA and CCT were found between the two groups (t=0.209, p=0.836>0.05; t=0.203, p=0.841>0.05). The mean ECDs were 2232, 1942 and 1442 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 33.03%, 41.15% and 56.91%, respectively, in the recipients with virus-positive grafts (n=9) after PK (table 2). The mean ECDs were 2576, 2320 and 2038 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 19.33%, 27.22% and 35.99%, respectively, in the controls (n=16). There was a significant difference in the mean EC loss rate at all follow-up
The mean ECDs were 2375, 2274 and 2198 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 6.85%, 10.91% and 13.67%, respectively, in the controls (n=14). No significant difference in the mean EC loss rate was found at any follow-up point on, the ECD does not include recipients who underwent DALK.

**DALK**

No significant differences in the mean BCVA and CCT were observed between the two groups (t=0.041, p=0.968>0.05; t=0.000, p=1.000>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (table 2). The mean ECDs were 2656, 2471 and 2230 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 22.40%, 27.61% and 35.05%, respectively, in the controls (n=18). There was no significant difference in the mean EC loss rate at 6 months (t=0.197, p=0.846>0.05; t=0.041, p=0.968>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (figure 1C).

**DALK**

No significant differences in the mean BCVA and CCT were observed between the two groups (t=0.041, p=0.968>0.05; t=0.000, p=1.000>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (table 2). The mean ECDs were 2656, 2471 and 2230 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 22.40%, 27.61% and 35.05%, respectively, in the controls (n=18). There was no significant difference in the mean EC loss rate at 6 months (t=0.197, p=0.846>0.05; t=0.041, p=0.968>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (figure 1C).

**DALK**

No significant differences in the mean BCVA and CCT were observed between the two groups (t=0.041, p=0.968>0.05; t=0.000, p=1.000>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (table 2). The mean ECDs were 2656, 2471 and 2230 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 22.40%, 27.61% and 35.05%, respectively, in the controls (n=18). There was no significant difference in the mean EC loss rate at 6 months (t=0.197, p=0.846>0.05; t=0.041, p=0.968>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (figure 1C).

**DALK**

No significant differences in the mean BCVA and CCT were observed between the two groups (t=0.041, p=0.968>0.05; t=0.000, p=1.000>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (table 2). The mean ECDs were 2656, 2471 and 2230 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 22.40%, 27.61% and 35.05%, respectively, in the controls (n=18). There was no significant difference in the mean EC loss rate at 6 months (t=0.197, p=0.846>0.05; t=0.041, p=0.968>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (figure 1C).

**DALK**

No significant differences in the mean BCVA and CCT were observed between the two groups (t=0.041, p=0.968>0.05; t=0.000, p=1.000>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (table 2). The mean ECDs were 2656, 2471 and 2230 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 22.40%, 27.61% and 35.05%, respectively, in the controls (n=18). There was no significant difference in the mean EC loss rate at 6 months (t=0.197, p=0.846>0.05; t=0.041, p=0.968>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (figure 1C).

**DALK**

No significant differences in the mean BCVA and CCT were observed between the two groups (t=0.041, p=0.968>0.05; t=0.000, p=1.000>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (table 2). The mean ECDs were 2656, 2471 and 2230 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 22.40%, 27.61% and 35.05%, respectively, in the controls (n=18). There was no significant difference in the mean EC loss rate at 6 months (t=0.197, p=0.846>0.05; t=0.041, p=0.968>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (figure 1C).

**DALK**

No significant differences in the mean BCVA and CCT were observed between the two groups (t=0.041, p=0.968>0.05; t=0.000, p=1.000>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (table 2). The mean ECDs were 2656, 2471 and 2230 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 22.40%, 27.61% and 35.05%, respectively, in the controls (n=18). There was no significant difference in the mean EC loss rate at 6 months (t=0.197, p=0.846>0.05; t=0.041, p=0.968>0.05). The mean ECDs were 2305, 2230 and 2140 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 7.35%, 10.52% and 13.84%, respectively, in the recipients with virus-positive grafts (n=7) after DALK (figure 1C).
No significant differences in the mean BCVA and CCT were found between the two groups (t=0.189, p=0.854>0.05; t=0.254, p=0.809>0.05). The mean ECDs were 2374, 2059 and 1588 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 32.80%, 39.73% and 58.92%, respectively, in the recipients with HSV-1-positive grafts (n=4) (table 2). The mean ECDs were 2571, 2320 and 2066 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 20.08%, 27.52% and 35.47%, respectively, in the controls (n=7). No significant difference in the mean EC loss rate was noted at 6 months (t=1.897, p=0.090>0.05); a significant difference in the mean EC loss rate was observed at 12 and 24 months (t=2.986, p=0.015<0.05; t=11.418, p=0.000<0.01) (figure 2A).

CMV+

No significant differences in the mean BCVA and CCT were identified between the two groups (t=−0.254, p=0.809>0.05; t=−0.132, p=0.896>0.05). The mean ECDs were 2113, 1797 and 1486 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 36.60%, 46.28% and 56.14%, respectively, in the recipients with CMV-positive grafts (n=7) (table 2). The mean ECDs were 2550, 2343 and 2079 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 20.98%, 27.35% and 35.6%, respectively, in the controls (n=15). There was significant difference in the mean EC loss rate at all follow-up times (t=2.486, p=0.046<0.05; t=3.842, p=0.007<0.01; t=5.027, p=0.001<0.01) (figure 2B).
Qu J-H, et al. Br J Ophthalmol 2022;106:26–31. doi:10.1136/bjophthalmol-2020-317629

**Clinical science**

**Figure 2** The EC loss in different virus-positive recipients and controls after PK and DSAEK. (A) EC loss rate in HSV-1-positive recipients and controls. There was no significant difference in the mean EC loss rate at 6 months and a significant difference in the mean EC loss rate at 12 and 24 months. (B) EC loss rate in CMV-positive recipients and controls. There was no significant difference in the mean EC loss rate at all follow-up times. (C) EC loss rate in VZV-positive recipients and controls. There was no significant difference in the mean EC loss rate at 6 months and a significant difference in the mean EC loss rate at 12 and 24 months. (D) EC loss rate in EBV-positive recipients and controls. There was no significant difference in the mean EC loss rate at any follow-up time (*p<0.05, **p<0.01). CMV, cytomegalovirus; DALK, deep lamellar keratoplasty; DSAEK, descemet stripping automated endothelial keratoplasty; EBV, Epstein–Barr virus; EC, endothelial cell; HSV, herpes simplex virus; PK, penetrating keratoplasty; VZV, varicella-zoster virus.

ECDs were 2651, 1468 and 2203 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 31.85%, 40.34% and 56.64%, respectively, in the recipients with VZV-positive grafts (n=5) (table 2). The mean ECDs were 2651, 1468 and 2203 cells per mm² at 6, 12 and 24 months, corresponding to an EC loss of 21.81%, 27.23% and 35.32%, respectively, in the controls (n=8). No significant difference in the mean EC loss rate was noted at 6 months (t=2.044, p=0.066>0.05); a significant difference in the mean EC loss rate was found at 12 and 24 months (t=2.274, p=0.044<0.05; t=5.56, p=0.000<0.01) (figure 2C).

No significant differences in the mean BCVA and CCT were found between the two groups (t=0.229, p=0.830>0.05; t=0.687, p=0.53>0.05). The mean ECDs were 2750, 2460 and 2212 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 21.34%, 29.38% and 43.23%, respectively, in the recipients with EBV-positive grafts (n=2) (table 2). The mean ECDs were 2893, 2616 and 2366 cells per mm² at 6, 12 and 24 months, corresponding to EC losses of 20.73%, 27.96% and 35.47%, respectively, in the controls (n=4). No significant difference in the mean EC loss rate was noted at any follow-up times (t=0.074, p=0.953>0.05; t=0.135, p=0.931>0.05; t=2.694, p=0.216>0.05) (figure 2D).

**IOP**

There was no correlation between the IOP and EC loss in the graft-positive or control group (p=0.918, p=0.891).

**DISCUSSION**

During our observation, except for two recipients who received HSV-1-positive grafts, acute viral infection occurred within 1 week after endothelial keratoplasty (aqueous humour tested HSV-1 DNA-positive, and endothelial grafts showed viruses by electron microscopy on replacement). Interestingly, two recipients who underwent DALK showed no evidence of viral infection. This study was recently published by our research group. Other grafts remained completely transparent over the 24-month follow-up period. However, the EC loss rate in the viral DNA-positive graft group was higher at 1 and 2 years after PK or DSAEK than that in the control group, but there was no significant difference between the viral DNA-positive graft group and the control group after DALK. Therefore, we inferred that viruses might be hidden in the grafts and mainly incubate in the corneal endothelium. In PK and DSAEK, viruses can be transmitted from donors to recipients. The presence of the virus in the endothelium continued to affect the morphology and function of the corneal endothelium, resulting in a higher EC loss rate than that observed in the controls.

The EC loss rate also varied by type of viral DNA. The EC loss rates in HSV-1 and VZV DNA-positive recipients were higher than those in the control groups from 1 year after the operation. HSV-1 has been proven to be transmitted from donors to recipients; we also observed this phenomenon. Similar results pertaining to latent viral infections in donor corneas and infections in recipients have been confirmed in animal experiments. The cornea might serve as a reservoir of latent HSV-1 and a source of virus reactivation. Polcicova et al found that in mice infected with a strain of HSV that could not move from the sensory ganglion back to the cornea, herpes simplex keratitis developed because the virus was still in the cornea. Our study showed that HSV-1 might exist in the corneal endothelium. VZV has been found hidden in multiple ganglia throughout the body. The two most frequently involved are the thoracic ganglion (87%) and the trigeminal ganglion (53%). However, the means by which VZV enters the sensory ganglia remains uncertain. Our findings might provide a possible explanation for why VZV was not found in some ganglia but still established a latent infection. VZV could be hidden in the corneal endothelium.
However, CMV DNA-positive recipients had a higher EC loss rate at 6 months after surgery. A previous study inferred that graft-to-host transmission scarcely occurred in cases of CMV DNA-positive grafts. However, our study found that CMV might exist in corneal ECs and continue to affect corneal ECs. According to anterior chamber-associated immune deviation (ACAID), we can infer that if the viral load is large or immune system is abnormal, the virus can enter the anterior chamber of recipients from the infected corneal endothelium and replicate. If the viral load is low and immune function is normal, the virus might exist in corneal ECs and not replicate or continue to affect corneal ECs. The EC loss rate in EBV DNA-positive recipients showed no significant differences from that in the controls during the 24-month follow-up. However, the number of cases was small, and further study is needed.

In our study, the EC loss rates in the control group were 19.33%, 27.22% and 35.99% at 6 months, 1 year and 2 years after PK, respectively. The EC loss rates in the control group were 22.90%, 27.61% and 35.05% at 6 months, 1 year and 2 years after DSAEK, respectively. In the literature, the reported EC loss rates after PK were 11–33%, 16–42% and 29–49% at 6 months, 1 year and 2 years, respectively, while the EC loss rates after DSAEK were 16–40%, 16–44%, and 23–44% at 6 months and 1 and 2 years, respectively. Our EC loss rates are similar to those reported by others.

Ideally, corneal tissue and other materials intended for transplantation should be free of pathogens. Donor corneas and preservation fluid are tested for bacterial and fungal infections, but viral DNA detection in grafts is difficult to complete before transplantation. Recipients who of viral DNA-positive grafts in keratoplasty. Recipients who of viral DNA-positive grafts in keratoplasty. Recipients who of viral DNA-positive grafts in keratoplasty.

LIMITATIONS
The follow-up period in this study was 24 months, and further research is needed. Therefore, we plan to continue observing patients with positive DNA results to further confirm our hypothesis.

Contributors JH-Q, GG-X-J-H: design of the study(); JH-Q, T-Y: conduct of the study; S-Z, HQ-Q: collection and management of the data; RM-P: analysis and interpretation of the data; JH-Q: writing of the manuscript; J-H: review or approval of the manuscript.

Funding This work was supported by the National Natural Science Foundation of China under Grant No. 81970768, No. 81800801 and No. 31271045.

Competing interests The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution-Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs Jing-Hao Qu http://orcid.org/0000-0003-2368-3708 Rong-Mei Peng http://orcid.org/0000-0001-9613-932X Shuang Zhang http://orcid.org/0000-0002-0047-8456 Jing Hong http://orcid.org/0000-0002-8079-2073

REFERENCES
1 Broniek G, Langwinski-Wesoło E, Sybińska M, et al. Occurrence of viral DNA in paired samples of corneal rim and cornea preservation fluid. J Med Virol 2017;89:732–6.
2 Remeijer L, Duan R, van Dun JM, et al. Prevalence and clinical consequences of herpes simplex virus type 1 DNA in human cornea tissues. J Infect Dis 2009;200:11–19.
3 Hisao CH, Hwang YS, Chuang WY, et al. Prevalence and clinical consequences of cytomegalovirus DNA in the aqueous humour and corneal transplants. Br J Ophthalmol 2018.
4 Remeijer L, Maertzdorf J, Doornenbal P, et al. Herpes simplex virus 1 transmission through corneal transplantation. Lancet 2001;357:442.
5 Busin M, Arfia RC, Sebastiani A. Endokeratoplasty as an alternative to penetrating keratoplasty for the surgical treatment of diseased endothelium: initial results. Ophthalmology 2000;107:2077–82.
6 Hong Y, Hong J, Xu YG, et al. Comment on phakic descemet stripping automated endothelial keratoplasty: prevalence and prognostic impact of postoperative cataracts. Cornea 2013;32:217.
7 Zhang S, Xiao G, Peng RM, et al. Clinical consequences of herpes simplex virus DNA in donor corneas: different prognosis and management of endothelial keratoplasty and deep anterior lamellar keratoplasty. J Clin Virol 2020;129:104508.
8 Lobo AM, Alegidis AM, Shukla D. Pathogenesis of herpes simplex keratitis: the host cell response and ocular surface sequelae to infection and inflammation. Ocul Surf 2019;17:40–9.
9 Zheng X. Reactivation and donor-host transmission of herpes simplex virus after corneal transplantation. Cornea 2002;21:590–3.
10 Polcicova K, Biswas PS, Banerjee K, et al. Herpes keratitis in the absence of anterograde transport of virus from sensory ganglia to the cornea. Proc Natl Acad Sci USA 2005;102:11462–7.
11 Starr CE, Pavan-Langston D. Varicella-zoster virus: mechanisms of pathogenicity and corneal disease. Ophthalm Clin North Am 2002;15:7–15.
12 Mahalingam R, Wellish M, Wolf W, et al. Latent varicella-zoster viral DNA in human trigeminal and thoracic ganglia. N Engl J Med 1990;323:627–31.
13 Borderie VM, Sandali O, Bullet J, et al. Long-term results of deep anterior lamellar versus penetrating keratoplasty. Ophthalmology 2012;119:249–55.
14 Price MO, Gorovoy M, Price FJ, et al. Descemet’s stripping automated endothelial keratoplasty: three-year graft and endothelial cell survival compared with penetrating keratoplasty. Ophthalmology 2013;120:246–51.
15 Lass JH, Beck RW, Benetza BA, et al. Baseline factors related to endothelial cell loss following penetrating keratoplasty. Arch Ophthalmol 2011;129:1149–54.
16 Lass JH, Gal RL, Donchev M, et al. Donor age and corneal endothelial cell loss 5 years after successful corneal transplantation. Specular microscopy ancillary study results. Ophthalmology 2008;115:627–32.
17 Berrin Mann E, Pleyer U, Reise P. Risk factors for endothelial cell loss post-keratoplasty. Acta Ophthalmol Scand 2006;84:766–70.
18 Anshu A, Price MO, Price FJ, et al. Descemet stripping automated endothelial keratoplasty for Fuchs endothelial dystrophy-influence of graft diameter on endothelial cell loss. Cornea 2013;32:5–8.
19 Price MO, Fairchild KM, Price DA, et al. Descemet’s stripping endothelial keratoplasty five-year graft survival and endothelial cell loss. Ophthalmology 2011;118:725–9.
20 Price MO, Bidros M, Gorovoy M, et al. Effect of incision width on graft survival and endothelial cell loss after Descemet stripping automated endothelial keratoplasty. Cornea 2010;29:523–7.