Short-Term Results of Acellular Porcine Corneal Stroma keratoplasty for herpes simplex Keratitis

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Abstract

Background: Corneal transplantation is a common surgical intervention for restoring vision loss due to corneal damages. However, due to cultural reasons, there is a huge shortage of donor corneas in China. Acellular porcine corneal stromas (APCS) can be used as corneal substitutes in lamellar keratoplasty for corneal ulcers. This study was conducted to analyze the results of APCS used for herpes simplex keratitis (HSK).

Methods: The study involved HSK patients who underwent keratoplasty with APCS from February 2016 to October 2017 in the second affiliated hospital of Zhejiang University. Patient data was collected at 7 days, 1-month, 3-month, 6 -month and last follow-up (7-25 months) postoperative. The corneal transparency, neovascularization, visual acuity and graft stability were observed.

Results: 13 patients with HSK including 5 patients with corneal perforation were included in this study, 9 patients underwent deep anterior lamellar keratoplasty (DALK) and 5 perforation patients underwent double lamellar keratoplasty. There were 9 men and 4 women with an average age of 62.5±5.6 years old (range from 52 to 70 years old) The mean postoperative follow-up duration was 15.1±5.8 months (range from 7 to 25 months). At the last visit, visual acuity improved in 9 patients (69.2%) compared with preoperative ($p=0.008$).The grafts of 7 individuals (53.8%) were completely transparent or slightly opaque, the score of corneal transparency improved significantly compared with preoperative ($p=0.010$). Various degrees of neovascularization were presented in 11 of the 13 patients (84.6%), most neovascularization gradually stabilized. Graft dissolution occurred in 3 eyes (23.1%)

during the observation period, two underwent regrafting, the other one became stable after treatment. 3 patients underwent second allograft transplantation, 2 of which encountered APCS graft dissolution and 1 of the patients requested a human donor allograft transplantation due to transparency issues despite absence of adverse issues. Conclusion: APCS seems to be effective in the treatment of HSK and can be used in HSK with corneal perforation by using double lamellar keratoplasty in an emergency.

Keywords: acellular porcine cornea, keratoplasty, herpes simplex Keratitis

Introduction

Keratitis caused by herpes simplex virus (HSV) including epithelial, stromal and endothelial keratitis can have detrimental effects on the cornea leading to loss of vision.[1 2] Herpes simplex keratitis (HSK) is characterized by a high risk of relapse that increases over time.[3] After several recurrences, the corneal haze gradually worsens and may even lead to corneal perforation.[4] HSK is a common pathogeny of corneal disease and one of the leading causes of infectious corneal blindness worldwide.[1] Currently，the main treatment of HSK is topical and oral agent antiviral drugs by inhibiting the replication of virus in the cornea.[5] However, in order to preserve the eyeball and recover corneal clarity, surgical intervention may be required for patients with corneal dissolution or long-term complications such as ulcer, necrotizing keratitis, and neurotrophic keratitis.[6]

Currently the lack of donor cornea is a major problem for corneal transplantation especially in Asian countries.[7] Many patients miss the best time for treatment whilst waiting for donor cornea tissue. To solve this problem, many researchers have

attempted to fabricate artificial corneas synthesized from biomaterials, natural polymers materials or synthetic polymers[8-10], amongst various kinds of keratoprosthesis, the porcine cornea has been extensively investigated as a candidate substitute due to its similarity to human cornea in size, thickness and stable refractive status.[11] Previous studies in animals also showed the promise of decellularized porcine cornea as a substitute for human corneal xenotransplantation.[12 13] In 2015, acellular porcine corneal stroma (APCS), the world's first commercialized tissue-engineered cornea, which can be used clinically as a substitute for human cornea in lamellar keratoplasty was approved by the China Food and Drug Administration (CFDA).[14] A study by Zhang, M.C et al showed that APCS grafts appear to be safe and effective in treating corneal ulcers caused by fungal keratitis in human.[15] To a certain extent, APCS grafts can be used as an alternative for~~to~~ donor cornea to alleviate the problem of donor cornea shortage.

APCS is prepared by a series of strictly-controlled procedures including removal of antigens such as heterogeneous cells, miscellaneous proteins, and polysaccharides from the cornea whilst retaining the natural corneal matrix collagen scaffold.[15] It has shown good biocompatibility, safety, and allows rapid integration with surrounding tissues.[13] Up till now, no studies have shown the efficacy and safety of using APCS for lamellar keratoplasty to treat herpes simplex keratitis. In this study, we observed patients who had HSK-induced corneal scarring and underwent lamellar keratoplasty using a primary graft of acellular porcine cornea. Double lamellar keratoplasty (using both APCS and thin donor posterior lamellar) was conducted in cases where patients

are suffering from corneal perforation but donated allogeneic cornea was not available for immediate use. The clinical efficacy was assessed to evaluate the suitability of APCS as a substitute for donated human corneal in HSK patients.

Methods

This study was approved by the Ethics Committee of the second affiliated hospital of Zhejiang University and was registered in the Chinese Clinical Trial Registry under identifier ChiCTR-ONC-17013979. All eligible patients were enrolled after obtaining their written informed consent.

Patients: From February 2016 through October 2017, patients who had HSK-induced corneal scarring or even perforation received primary graft of acellular porcine cornea by lamellar keratoplasty. The diagnosis of HSK was made on the basis of typical recurrence in history, characteristics of clinical manifestations such as epithelial dendritic or geographic ulcer, typical stromal edema with or without a cluster of keratic precipitates, which had good response to antiviral treatment.[16 17] Patients with any of the following conditions were excluded: corneal scaring caused by other reasons such as fungal keratitis or alkali burns, and have received prior allogeneic corneal transplantation, corneal immune diseases such as Sjogren syndrome and other eye diseases.

Materials: The APCS grafts used in this study were produced by Shenzhen Ainear Cornea Engineering Co., Ltd (Shenzhen, China). They were manufactured through a series of viral inactivation and decellularization processes. Briefly, porcine eyeballs were harvested from a quarantined pig slaughterhouse and the porcine corneas were

removed from the eyeballs with sclera attached. Then, the corneas were placed in sodium hypochlorite solution for viral inactivation, followed by repeated hypertonic-hypotonic treatments by alternating the use of NaCl solution and injectable water for decellularization. After dehydration in glycerol, the APCS were cut into circular discs with different thicknesses (200, 300, 400 and 450um) to serve different clinical requirements. The APCS were then sterilized using Co^{60} radiation. After aseptic vacuum packaging, the APCSs were stored at 2-10℃ until use. This study was conducted using APCS with a thickness of 450um.

Surgery: A brief description of the surgical procedures is as follows: For HSK patients without cornea perforation, the recipient corneal bed is prepared by trephination to a depth of 400-450 um using a trephine 6.25 to 7.50 mm in diameter and debridement of diseased tissue, the APCS is removed from its packaging and soaked in normal saline for 15 seconds and placed on recipient bed. Interrupted 10-0 nylon sutures were made starting at the 12 o'clock position around the trephined margin. Another 1 to 4 stitches were made depending on the size of the graft. For patients with cornea perforation, a procedure named double lamellar keratoplasty (Figure 1) was conducted. In such cases, after debridement of 400-450um diseased tissue, the perforation was first repaired using a very thin layer of remained human posterior lamellar graft which was stored in -80°C, and then APCS graft was transplanted using the same lamellar keratoplasty technique.

Postoperative treatment and follow-up: for postoperative treatment, oral acyclovir tablets 0.4g were prescribed ~~were taken~~, five times daily for 1 month and then tapered

to two times a day for 1 year, antiviral eye ointment was prescribed for four times a day for 1 month then tapered to twice per day until 3 months post-op, topical medications of corticosteroid eye drops were used four times a day for 3 months then tapered to three per day until 6 months post-op，twice per day from 6 months to one year, then once per day after one year. ~~and~~ Artificial eye drops were prescribed for four times per day for 1 year. Patients were asked to return to the clinic ~~follow-up visits~~ at 7 days, and every month after the surgery. The data for 7 day, 1-month, 3-months, 6-months and last visit were collected. A detailed clinical examination was performed at each visit, which included slit-lamp microscopy, anterior segment optical coherence tomography imaging and confocal microscopy. Sutures were generally removed at 6-12 months after surgery. Earlier suture removals were performed for cases with loosened stitches, increased vascularizations, and the presence of refractive astigmatism. Blood routine, urine, liver and kidney function tests were also conducted before surgery and every 3 months after surgery.

Major measurements: A scoring system was used to measure the degree of corneal opacity and neovascularization. Corneal opacity was graded from 0 through 3: 0 (transparent); 1 (mild haze, not obscuring the iris); 2 (moderate haze, partially obscuring iris); 3(opaque area totally obscuring iris). The extent of neovascularization was graded as follows: 0 (no neovascularization); 1 (new vessels appear on the recipient cornea but cannot be seen on the graft); 2 (new vessels appear on graft but not in the center of the graft); 3 (new vessels on the center of the graft). Although it is in general very difficult to confirm diagnosis for recurrence of viral keratitis, dendritic

or geographic ulcers~~are~~ and epithelial defects which respond only to antiviral treatment ~~and~~ can be taken as an indicator of epithelial recurrence of HSK.[18] For herpetic stromal keratitis or herpetic endothelial keratitis recurrences, symptoms such as eye redness, pain and corneal edema similar to the last episode of viral keratitis can be observed. ~~can be observed. Slit-lamp microscopy image.~~ Appearance of endothelial rejection line or subepithelial infiltrates and corneal stroma edema were used as indicators for a rejection episode[19]. To determined virus relapse or graft rejection, the efficacy of anti-rejection therapy and antiviral therapy were considered. Prognosis was defined at the last follow-up as follow: improvement (for HSK with perforation patients, intact cornea; for HSK without perforation patients, improved corneal transparency); Regrafting (those who received a second corneal allograft transplantation from human donor cornea); Unchanged (no improvement in corneal transparency).

Statistical analyses: Categorical variables were evaluated by descriptive statistics using percentages; for continuous variables, mean, standard deviation and range were used. A decimal visual acuity (VA) of 0.0025 for counting fingers (CF), 0.002 for hand motion (HM), and 0.0016 for light perception (LP) were quantified according to a previous study.[20] Wilcoxon signed-ranks test was used to compare the pre/postoperative degrees of corneal transparency and visual acuity. The mean evaluation score before the surgery and each visit after the surgery were compared using Mann-Whitney U test. All statistical analyses were performed in SPSS software, version 20 (IBM, Armonk, NY, USA). A value of $P< 0.05$ was considered statistically significant.

Results

A total of 13 eyes from 9 male and 4 female patients were treated in this study. The average age of patients was 61.9±6.3 years old(range from 51 to 73 years old). Amongst them, 5 patients had a definite perforation. 9 patients had a viral attack within three months before surgery and antivirus as well as~~and~~ anti-inflammatory drugs were ineffective in controlling the attack in 7 of these~~the~~ cases. Preoperative best corrected visual acuity varied from LP to 0.02 and only one patient had visual acuity of 0.02. The mean postoperative follow-up duration was 15.1±5.8 months (range from 7 to 25 months).

At last visit, visual acuity improved in 9 patients (69.2%) compared to preoperative (p=0.008), while 4 patients (30.8%) had no improvement in visual acuity. The cornea conditions of HSK patients who underwent lamellar keratoplasty were summarized in Table 1. The corneal transparency scores were significantly reduced after the operation compared to preoperative(p=0.010). The grafts gradually became transparent with 7(53.8%) becoming completely transparent or slightly opaque. Although, various degrees of neovascularization were presented in 11 of the 13 patients(84.6%) after the keratoplasty, most neovascularization gradually stabilised from ~~1.85~~ 1.45 to 1.64 (P＞0.05).

~~Figure 2 showedt~~Three HSK patients' slit lamp images preoperative and postoperative were shown in Figure 2. ~~Figure 2 A3,B3,C3 were taken preoperatively,one week after the surger~~Figure 2 A, E, I showed the cornea ulcer (2A), infiltration (2E) and opacity (2I) of the three patients before transplantation. The APCS grafts were slightly opaque

one week after the transplantation as shown in Figure B, F, J. Figure C, G, K exhibited APCS grafts gradually became transparent 3 months post-operation. Figure D, H, L showed the transparencies of the grafts 12 months after surgery which were significantly improved compared with the 3-month follow-up and were similar to the surrounding normal cornea.

At each visit, the corneal neovascularization and graft transparency score as well as visual acuity were evaluated in Figure 3. The corneal neovascularization score of preoperative, 1 week, 1 month, 3 months, 6 months and last visit were 1.85±0.86, 0.92±0.27, 1.17±0.69, 1.00±0.95, 1.45±1.08 and 1.64±0.98. Although the mean neovascularization score was not obvious decreased except for 1 week and 3 months after surgery. Eventually the condition stabilised. The score of graft transparency before the surgery and 1 week, 1 month, 3 months, 6 months and last visit after surgery were 2.77±0.58, 1.62±0.62, 1.17±0.55, 1.27±0.75, 1.36±0.88 and 1.55±0.89. The mean corneal transparency scores were significantly reduced from 1 week after the surgery compared with preoperation ($p<0.05$). Towards the later follow-up visits, two patients experienced APCS graft dissolution, hence causing the slight increase in the average corneal transparency. At the same time, the mean visual acuity was gradually improving from 0.0035 to 0.1681, the mean visual acuity of 1 month,3 months,6 months and last visit had a significant improvement compared with preoperative ($p<0.05$) .

During the observation period, various degrees of graft melting took place in 3 patients (23.1%). Figure 3 showed three patient with dissolved grafts. For patient 7,

the APCS graft started to dissolve 8 months postoperative (C~~Figure 3 A3~~), the graft became stable with neovascularization after topical and oral antiviral drugs were offered (D~~Figure 3 A4~~). As for patient 5, he was attacked by severe inflammation which could not be controlled preoperative, and the posterior elastic layer bulges continuously progressing resulted in corneal perforation (E). After he underwent DALK and received APCS, the corneal epithelium was persistent nonhealing 1 month~~s~~ after operation, the suture was loose 12 days after the surgery, and cornea was oedemic as well as APCS continued to dissolve despite the use of anti-inflammatory, contact bandage lens therapy and amniotic membrane transplantation (F~~Figure 3 B2~~), the patient underwent a second corneal keratoplasty. Graft melting also happened in Patient 12, the corneal stroma started to dissolve 8 month**s** after the transplantation (G~~Figure 3 B4~~), she received allograft transplant after ineffective medicine treatment for 3 weeks. Patient 13 experienced a recurrence episode~~eight~~ 8 months after the operation with corneal stromal edema, the graft restored transparency after timely prescription of antiviral treatment.

Figure 5~~4~~ showed the confocal microscopy image of a patient 6 months after surgery. The graft eventually epithelialized (A~~Figure 4 A1~~), but there was still lack of subepithelial nerve (B~~Figure 4 A2~~). There was a large number of dendritic cells in the subepithelium (B~~Figure 4 A3~~), the nerve regenerated in the stromal layer of the APCS graft (C~~Figure 4 A3~~).

Overall, during the minimum follow-up of 6 months, 10 of the 13 APCS grafts became stable over the prolonged follow-up period and no rejection episodes were

identified eventually. 9 out of 13 patients (69.2%) had an improvement compared with preoperation. One remained unchanged with the same degrees of graft opacity and neovascularization compared with preoperation. 3 of 13 patients (23.1%) received regrafting, of which 2 were due to graft melting, the other patient was dissatisfied with corneal transparency two months after surgery and strongly urged a second transplantation with human donor cornea even without recurrence or rejection episode，although the score of corneal transparency was only 1. Besides, no significant changes in postoperative systemic safety indicators were observed.

Discussion

With the shortage of donor corneas for cornea transplantation, there is a critical need to find an ideal substitute for donor corneas.[15] Compared with other materials, the APCS has its own unique advantages and is more widely used clinically. For treatment of corneal fungal ulcers in 47 human eye, most porcine grafts (87%) gradually became transparent and an improvement of best corrected visual acuity was achieved in 34 eyes, four patients encountered graft melting, all grafts eventually epithelialized despite graft dissolution, and there were no perforations, rejection or infection relapses.[15]To the best of our knowledge, there were no studies about these engineering corneal tissues applied for HSK patients except three studies which reported the long-term outcomes of the Boston type I Kpro in eyes with a history of HSV keratitis, the postoperative complications such as persistent epithelial defect, corneal infiltrate were approximately twice of those without HSV infection.[21]

The conventional surgical procedure for treating HSK-induced corneal ulcer is

penetrating keratoplasty (PKP), but recent studies show an increase anterior lamellar transplants (DALK).[2] [22]A study by Wu, SQ et al compared full-bed deep lamellar keratoplasty with penetrating keratoplasty in treating corneal scar caused by Herpes Simplex Keratitis,121 eyes were involved in this study with 58 eyes received full-bed DALK and 63 eyes received PKP. 6 eyes (10.3%) in the full-bed DALK group and 13 eyes (20.6%) in the PKP group encountered recurrence episodes. There were no rejection episodes in full-bed DALK group while 26 ~~(41.3%)~~ of 63 eyes (41.3%) encountered graft rejection in eyes that received PKP.[18] In our study, no eyes encountered graft rejection and 3 eyes (23.1%) encountered recurrence episodes. Table 2 showed a comparison with Wu et al's study. We used double lamellar keratoplasty to treat HSK with perforation by repairing the perforation with human donor-derived corneal graft, which significantly reduced rejection. Our results were not as good as using allogeneic grafts for lamellar keratoplasty (LKP) but far better than for PKP. As lamellar keratoplasty retains the endothelial layer of the recipient, the incidence rate of postoperative rejection was reduced.

For the first time, we have observed the clinical efficacy of lamellar keratoplasty by using acellular porcine cornea for severe herpes simplex keratitis. Graft melting occurred in 3 of the 13 patients. The pathogenesis of corneal graft melting is complex and infection and immune response play a key role. Previous studies had shown HSK patients undergoing LKP are at higher risk for graft failure when compared to individuals receiving keratoplasty for conditions such as keratoconus and Fuchs' corneal dystrophy, which often requiring repeat grafting.[21] [23]Timely recognition and

management to prevent recurrence of HSV-related inflammation and graft rejection. A study in animals showed both innate and adaptive immune mechanisms associated with anti-HSV CD4 T-cell response plays a significant role.[24] Most of the dissolved grafts were related to recurrence, showing the importance of taking appropriate postoperative measures to prevent recurrence. It is recommended to continue taking oral antiviral drugs after surgery, but our patients failed to adhere to the treatment programme.

The study has also shown that the recurrence of HSK was more likely to occur 8 months after surgery as all three recurrence episodes in our study happened at that time. This may be due to the timing of nerve regeneration into the bioengineered cornea, the virus that remain latent in the trigeminal ganglion is able to travel via axonal transport. This hypothesis is consistent with Liya et al's finding that subepithelial nerve fibers appeared 6 months after LKP using APCS.[25] Our data in Figure 4 was consistent with the above conclusions. Therefore, prophylactic antiviral therapy 6 months after transplantation is of vital importance to reduce the recurrence of the virus and improve the survival rate of the APCS graft. Furthermore, those who were resistant to antiviral therapy preoperatively were more likely to encounter graft melting. This high-risk of graft dissolution is attributed to extensive corneal neovascularization and Inflammatory cell infiltration preoperatively associated with HSK. Host immune responses to residual viral antigens or virally-altered cell proteins as carriers of inflammation exist even after successful removal of virus in the surgery.[16] The following three patients were in active phrase postoperatively, and all the grafts were dissolved after the operation. For

patient 7 and patient 12, it was likely that the corneal melting incidents were caused by viral recurrence. For the patient 5, we considered neither rejection nor recurrence of the virus induced graft melting. Recurrence and rejection often did not occur in the early post-transplantation. He had a recurrence of the virus one week before surgery that the antiviral and anti-inflammatory drugs could not control. We believe that APCS used on the cornea with obvious preoperative inflammation are more likely to result in corneal epithelial persistent nonhealing, loose sutures and APCS melting.

One problem encountered during the course of our study was that most patients were not compliant with taking antiviral drugs as prescribed. The prescribed duration of antiviral therapy may be not enough. Numerous studies had proven oral antiviral can reduce the risk of recurrence of herpetic keratitis, it prolongs the recurrence interval and reduces the duration of herpetic disease.[26] Besides, in our study, it seemed that the longer the time from the last onset of herpes simplex virus, the better the prognosis was. It is best to have inflammation controlled for more than 3 months before surgery. However, there were 5 perforation cases and 5 uncontrolled inflammation cases in our study. We performed corneal transplantation because inflammation could not be controlled, drug treatment was ineffective. Transplantation in eyes with active inflammation has an even more dismal prognosis with less than a quarter of eyes surviving compared to 85% of those with quiescent scars.[27] Rational and effective antiviral therapy before surgery ensuring that the virus is in the quiescent phase is crucial for the prognosis of surgery. Another limitation of our study was that the number of cases was small and the follow-up time was not long enough. However,

as a preliminary study, this study provides a proof-of-concept of the feasibility of using APCS for keratoplasty for the treatment of HSK. Future studies will expand the number of cases in order to provide more robust and reliable clinical evidence to support the widespread use of APCS for corneal transplantation for HSK.

Conclusion

APCS may solve the problem of shortage in corneas and can be used for corneal transplantation for HSK. In addition, APCS seems to be effective in the treatment of HSK and can be used in HSK with corneal perforation by conducting double lamellar keratoplasty in an emergency. The timing for conducting the surgery, postoperative monitoring and timely management of complications also determines the survival rate of graft and hence the success rate of the transplantation.

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Table 1. The Corneal summary of conditions of HSK patients who underwent lamellar keratoplasty

Patient	Sex/Eye Affected	Age	Last episode (Drag control)	Perfo-ration	Pre/post[1] Visual acuity	Pre/post[1] Corneal opacity	Post[1] Neovascu-larization	Follow-up time (months)	Compli-cation	Prognosis
1	F/R	67	1 month(no)	Yes	HM/FC	3/2	2	24	No	Improvement
2	F/L	65	2months(yes)	Yes	LP/0.02	3/2	1	25	No	Improvement
3	M/L	67	3 months(yes)	Yes	LP/HM	3/3	2	12	No	Improvement
4	M/R	52	2 months(yes)	Yes	FC/0.2	3/1	0	14	No	Improvement
5	M/R	62	1 week(no)	Yes	HM/HM	3/3	2	12	Graft melting (Inflammation)	Regrafting
6	M/L	52	2 years(yes)	No	0.02/0.5	3/1	0	23	No	Improvement
7	M/L	63	2 weeks(no)	No	HM/HM	3/1	2	18	Graft melting (Recurrence)	Improvement
8	M/L	59	Unknown(yes)	No	FC/0.4	3/1	2	13	No	Improvement
9	M/L	69	1 month(yes)	No	FC/0.02	3/3	3	9	No	Unchanged
10	M/R	61	6 months(no)	No	FC/0.2	3/1	2	11	No	Improvement
11	F/R	70	unknown(no)	No	HM/HM	1/1	2	7	No	Regrafting
12	F/R	62	1 month(no)	No	HM/HM	2/2	2	13	Graft melting (Recurrence)	Regrafting
13	M/R	63	3 weeks(no)	No	FC/0.5	3/0	2	15	Recurrence	Improvement

LP= light perception;HM=hand motion; FC=counting fingers;[1]the datas were documented at the last visit.

Table 2.A comparison with Wu et al's study		
	Wu et al (allogeneic)	This study (APCS)
Follow-up time	24-60months	7-25months
Full-bed DALK (eyes)	58	8
Double LKP (eyes)	0	5
PKP (eyes)	63	0
Recurrence (DALK)	6/58 (10.3%)	3/13 (23.1%)
Recurrence (PKP)	13/63 (20.6%)	N/A
Graft rejection (DALK)	0	0
Graft rejection (PKP)	26/63 (41.3%)	N/A

Figure 1. Double lamellar lamellarkeratoplasty surgical procedures.A1.Corneal scar with perforation(A).A2.Corneal scar was removed(B).A3.Perforation was repaired using a very thin layer of self or donor posterior lamellar graft(C).A4.Then APCS graft was transplanted(D).

Figure 2:Slit-lamp biomicroscopic photographs of pre- and post-operative corneas.A1.Vision loss caused by repeated virus attacks in the past two years(A).B1.Severe vision loss caused by the virus for 3 weeks, the patient screamed for corneal transplantation(E).C1.The herpes simplex virus lead to corneal opacity for two years(I);A2,B2,C2. one week after the surgery, the APCS grafts were slightly opaque(B,F,J);A3,B3,C3. The APCS grafts gradually became transparent3 months postoperation(C,G,K); A4,B4、C4.12 months after surgery, the transparency of the grafts significantly improved compared with the 3-month follow-up and was similar to the surrounding normal cornea(D,H,L).

Figure 3.The corneal neovascularization and transparency score and changes in visual acuity at every time-serial.*The score has significant statistical difference compare with preoperative (*P＜0.05,**P＜0.01，***P＜0.001).

Figure 4:The dissolved graft observed with slit-lamp microscopyimage.A1.Preoperatively,the patient presented with corneal ulcer and neovasculization(A).A2.One month after the operation, the graft was mildly opaque(B). A3. At 8 months postoperatively, the graft started to dissolve, topical and oral antiviral drugs were offered(C). A4.At 10 months after surgery, the graft became stable with the

growth of neovascularization(D).~~B1.~~ Preoperatively,the cornea was perforated(E);~~B2.~~The the corneal epithelium was persistent nonhealing, the suture was loose and corneal was edemaas well as stroma continued to dissolve even with anti-inflammatory, contact bandage lense therapy and amniotic membrane transplantation(F).~~B3.~~Preoperatively,the patient presented with corneal ulcer(g);~~B4.~~ The corneal stroma started to dissolve 8 months after the transplantation, she recieved allograft transplantafter invalid medicine treatment(H).

Figure 5. Confocal microscopy of a patient 6 months after surgery.~~A1.~~the graft eventually epithelialized(A).~~A2.~~there was no subepithelial nerve but a large number of dendritic cells(B).~~A3.~~The black arrow showed the nerve in the stromal layer of the APCS graft(C).