

## **Clinical observation of long-term deep-cryopreserved cornea in penetrating keratoplasty.**

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### **Abstract**

The aim of this study was to investigate the effects of using LC-DCP versus fresh corneas on graft transparency after penetrating keratoplasty. The observation group (25 eyes of 25 patients) was performed penetrating keratoplasty with LT-DCP cornea, while the control group (23 eyes of 23 patients) was performed penetrating keratoplasty with fresh cornea from human corpses. The follow-up was 3 to 32 months. 18 corneal grafts in the observation group were transparent (accounting for 72.0%), 5 cases were semi-transparent, 1 case was un-transparent, and 1 case was performed eyeball removal. The control group had 17 cases of transparent corneal grafts (73.5%), 5 cases were semi-transparent and 1 case was un-transparent. The results of transparent grafts between the 2 groups had no statistical significance ( $\chi^2=0.003$ ,  $p>0.05$ ). LT-DCP cornea had important clinical and promotional values towards penetrating keratoplasty.

**Keywords:** Keratoplasty, Penetrating, Cornea, Deep cryopreserved.

*Accepted on July 11, 2016*

### **Introduction**

Corneal disease is one of the world's leading causes of blindness [1], and penetrating keratoplasty is the primary means in treating blinding corneal disease [2], with the indications including keratoconus, corneal scarring, degeneration, malnutritional and purulent corneal ulcers, especially in keratoconus, the success rate of penetrating keratoplasty could be up to 90% [3], so it is a widely used measure of corneal grafting.

The preservation of cornea and the establishment of eye bank were the basis for corneal transplantation [4], which could take full advantages of the limited corneal resources. In China, because the corneal sources were lacked, the corneal transplantation was thus limited. Because fresh cornea would not be easily or timely obtained, partial acute corneal disease might be difficult to control, which resulted in blindness or loss of eye [5]. Therefore, adequate and timely access to the corneal materials was the key towards penetrating keratoplasty in treating the acute blinding corneal diseases. The success of corneal transplantation depended on maintaining the corneal transparent state, while the corneal endothelial cells were the basic conditions to maintain the corneal transparent state (only the survival rate of corneal endothelial cells was up to 70%). The preservation technology shift to preserve the activities of endothelial cells been used for corneal transplantation [6].

The key of corneal preservation was to protect the activities of corneal endothelial cells [7], long-term deep-cryopreservation was a method suitable for the corneal preservation, under the protection of refrigerants, the cornea was cooled with

controlled rate to  $-80^{\circ}\text{C}$ , then stored in liquid nitrogen ( $-196^{\circ}\text{C}$ ) [8,9], this process could inhibit the cellular metabolism, so that the "dormant status" could avoid accumulation of metabolites and microbial growth, and the cornea could preserved for more than a year [10]. Studies had shown that [11] the clinical manifestations and effects of LT-DCP cornea grafts were similar to those of the fresh cornea, they were both applicable for penetrating keratoplasty. The effects of using LC-DCP versus fresh corneas on graft transparency after penetrating keratoplasty was investigated.

### **Materials and Methods**

#### **General information**

Twenty-five cases (25 eyes) of penetrating keratoplasty with LT-DCP cornea since 1997 were performed in our hospital, and all achieved good results, which were reported as follows. The observation group: 25 patients (25 eyes), were performed penetrating keratoplasty with LT-DCP cornea, including 18 males and 7 females; 15 cases were right eyes and 10 cases were left eyes; aged 23 to 68 years old, and the mean age was 46.7 years old; The mean visual acuity of the log arithm of the minimum angle of resolution (log 10 MAR) was  $0.6 \pm 0.4$ ; among the patients, 11 cases were of fungal corneal ulcer, 7 cases were of other keratitis, 4 cases were of leukoma, 2 cases were of corneal trauma, and 1 case was performed the surgery twice. Shen and his colleagues performed the penetrating keratoplasty in the study.

**Preoperative visual acuity:** light perception ~ determined by fingers in front of the eye. The control group: 23 patients (23 eyes) were performed penetrating keratoplasty with fresh cornea from human cadaver, including 15 males and 8 females; 16 cases were right eyes, and 7 cases were left eyes; aged 28 to 60 years old, the average age was 38-year-old. 11 cases were of leukoma, 6 cases were of anterior corneal dystrophy or degeneration, 4 cases were of viral keratitis, and 2 cases were of bullous keratopathy.

**Preoperative visual acuity:** light perception ~ determined by fingers in front of the eye, among the 14 cases, 6 cases were of 0.01 ~ 0.05, and 3 cases were of 0.05 ~ 0.1.

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of The People's Hospital of Rizhao. Written informed consent was obtained from all participants.

### **Corneal materials**

The LT-DCP corneas for the observation group were provided by the cryogenic research department of Qingdao Medical College, the shelf life was 1 to 60 months, obtained from the cornea of healthy young human corpse, aged 18 to 40 years old, the preservation process was completed with 10 h of death. The control group used the fresh corneas donated by human corpse within 6 h of death, aged 21 to 35 years old. All groups were performed penetrating keratoplasty.

### **Surgical methods**

The two groups were performed dual-approach retrobulbar anaesthesia with 7 ml mixture of 2% lidocaine hydrochloride and 0.75% bupivacaine hydrochloride (mixed with equal amount). Then the eye ball was intermittently pressed for softening, the surgery was performed by the same surgeon and under the same surgical microscope (OPMI Lumera SN: 6215101546, Carl Zeiss Surgical GmbH, Carl Zeiss-Strabe 73446 Oberkochen). The diseased eye was marked with the lesion as the center and the diameter of planting hole was 6.75 ~ 7.75 mm, the graft was 0.25 ~ 0.5 mm greater than the planting bed and the planting hole.

**Observation group:** Before surgery, the LT-DCP cornea was removed from the liquid nitrogen and rewarmed in 40°C water bath. During the period, the cornea were slightly wobbled to melt the cryoprotectant, when only thin ice residued around the corneal graft, removed the corneal graft with one sterile tweezers, and balanced in 2% albumin at 4°C for 10 min, then placed on a pad of silica, with inner surface upwards, and perpendicular drilled and cut from the inner surface by the hammer method.

**Control group:** The fresh cornea, with scleral edge, was firstly cut from human cadaver, and then placed on a pad of silica, with inner surface upwards, and perpendicular drilled and cut from the inner surface by the hammer method. When the drilled on planting bed and planting hole (diameter 2.0-10 mm, XHZ10B, Suzhou Medical Instrument) succeeded, the graft

and the planting bed was continuously or interruptedly sutured with 10/0 nylon suture, and the cut wound was then sealed for water and gas prevention. The patients with inflammation was performed the intraoperative iris root resection, the patients with fungal hypopyon was fully rinsed the anterior chamber with 0.02% fluconazole. After surgery, 20,000 U of gentamicin and 2.5 mg of dexamethasone were subconjunctivally injected, and the eye was bandaged under pressure.

Secondary glaucoma in patients improved following peripheral iridectomy, some peripheral iridectomies were carried out after transplantation.

### **Postoperative treatment**

The patients were routinely intravenously injected 8,000,000 U of penicillin (Amresco, Solon, Ohio, USA) and 10 mg of dexamethasone (Sigma-Aldrich, the USA) for 3 ~ 5 d, 24 h after surgery, opened the dressing, and added F-Dexamethasone (Sigma-Aldrich, the USA) as the eye drops, the patients with fungal infections were applied 0.02% fluconazole eye drops (Changchun di Rui pharmaceutical co., LTD, China) simultaneously, and orally administrated itraconazole (Sporanox<sup>®</sup>, Janssen Pharmaceuticals) for 5 ~ 7 d, the patients with virus were administrated acyclovir eye drops at the same time, and orally administrated prednisone after discharge, while the dosages should be gradually reduced, and stopped six months after treatment. The follow-up was 3 to 32 months.

### **Statistical analysis**

All data were expressed as means ± standard error. Data between groups were analysed by a Student's t-test, among groups were analysed by one-way analysis of variance (ANOVA) using SPSS 13.0 software. A value P<0.05 was considered statistically significant.

## **Results**

### **Transparent situations of grafts**

Among the 25 patients (25 eyes) of the observation group, 18 corneal grafts were transparent (accounting for 72.0%), 5 cases were semi-transparent (20%), 1 case was un-transparent (4%), and 1 case was performed eyeball removal (4%). Among the 23 patients (23 eyes) of the control group, 17 cases were transparent (73.5%), 5 cases were semi-transparent (21.7%) and 1 case was un-transparent (4.3%). The results of transparent grafts between the 2 groups had no statistical significance ( $\chi^2=0.022$ ,  $p>0.05$ ) as shown in Table 1.

### **Postoperative visual acuity**

In the observation group, 9 cases exhibited the visual acuity  $\geq 0.5$ , while 10 cases were within 0.2 to 0.4, 5 cases were within 0.02 and 0.1, 1 case had the light perception, and 1 case had no light perception. The control group: 8 cases exhibited the visual acuity  $\geq 0.5$ , 9 cases were within 0.2 to 0.4, 5 cases were within 0.02 and 0.1, while 1 case had light perception. There

was no significant difference between the two groups ( $\chi^2=0.003$ ,  $p>0.05$ ).

**Intraoperative and postoperative complications**

In the observed group, the graft exhibited heavier oedema 2 ~ 18 d after grafting, which gradually subsided, and restored transparent two months later.

2 cases of secondary glaucoma were cured after iris root resection; 4 cases had the postoperative adhesions around iris; 4 cases exhibited the concurrent cataract; 1 case exhibited the sphincterolysis, 2 cases exhibited the epithelial defects; and 6 cases exhibited the rejection, while cured by the cyclosporine and corticosteroid treatment, among who 3 cases recovered transparent, 2 cases recovered translucent, while 1 case became cloudy because of not adhering the medication.

**Control group:** The grafts exhibited mild oedema, the irises of two cases were intraoperatively damaged, thus causing the pupil deformed; 2 cases of secondary glaucoma were performed the iris root resection; 1 case of intraocular lens dislocation was cured with suture fixation method; 5 cases exhibited the rejection, who were also treated with the cyclosporine eye drops and the corticosteroid treatment, among who 4 cases restored transparency, while 1 case still maintained turbid.

The average endothelial cell density for all transplants in the research was 2150+/-938 cells/mm<sup>2</sup>.

**Table 1.** The results of transparent grafts in the two groups.

	Transparent	Semi-transparent	Un-transparent	P value
The observation group (25 eyes) (23 eyes)	18	5	1	P > 0.05
Control group	17	5	1	

P<0.05 means the significant difference.

**Discussion**

The effects of using LC-DCP versus fresh corneas on graft transparency after penetrating keratoplasty was investigated. The transparent grafts between the LC-DCP and fresh corneas groups had no statistical significance.

Corneal transplantation is an important sight restoring surgery clinically [12], it is not only the only means for the patients of corneal blindness to regain their sight [13], but also the most effective method to treat fungal corneal ulcer [14]. Due to lacking qualified sources of corneal materials, partial corneal grafting that drew the materials from the donor with diabetes or hyperlipidaemia exhibited higher failure rate [15], thus limiting the extensive spread of this work, and many patients with blinding corneal disease lost their chances of treatment and sight regaining [16].

In this paper, besides electively collected the fresh corneas from fresh corpse, the emergency surgery all used LT-DCP cornea. There were many corneal preservation ways [17], including short-term preservation, also known as wet-room preservation, which was invented earliest and still most widely used, while it required the transplantation to be completed within 24 hours [18]; mid-term preservation, referred to the preservation in 4°C active solution, it could be maintained for 4-14 days [19]; the mid and long-term preservation, referred to the preservation in the simulated physiological environment, similar to the preservation of tissues and organs, and this method was also widely used in many eye banks currently [20]; the long-term preservation, referred to the cryopreservation, furthermore, the "vitrification preservation" referred to the rapid freezing method, so that the cornea could be directly amorphously fixed, but this method was still in the laboratory study, and not used clinically yet, but viewing from the preservation duration, this method was undoubtedly the future direction of corneal preservation [21].

As for the corneal preservation methods currently used clinically, due to longer preservation time, cryopreservation could better alleviate the supply and demand of corneal transplantation, therefore it was the preferred method of clinical corneal preservation currently [11]. The results of this study showed no significant difference in the clinical outcomes of penetrating keratoplasty with these 2 corneal materials. Except for the heavier oedema 2 ~ 18 d after grafting, the LT-DCP corneal materials showed no other serious complications, and achieved the goals of treatment and sight restoring. The success of LT-DCP cornea in penetrating keratoplasty could not only ease the contradictions between supply and demand, but also provide favourable conditions for planning surgical arrangements and assuring the surgical qualities. But the early post-grafting oedema was heavier, indicating the graft endothelium and cells were in a certain degree of decompensation, which needed to be gradually recovered from freezing-rewarming under hydatoid nutrition, the recovery of endothelial cells' functions required a gradual process [22]. The key to the success of cryogenics penetrating cornea transplantation depends on endothelial cell survival rate. The precoolant enhanced the endothelial cell survival rate. Furthermore, washing the cornea with balanced salt solution can reduce endothelium injury during the surgery. The corneal endothelial cell survival rate cannot be lower than fresh corneal standards (over 70%). Therefore, the oedema regression of LT-DCP corneal graft and the time for cornea to recover the normal thickness might be relatively longer than the fresh one.

Although the LT-DCP corneal materials were more used for inflammatory diseases, the postoperative reactions were relatively heavier, no serious complications such as graft endothelial dysfunction occurred, which also showed the mature and progress of LT-DCP technology. The preservation technology can increase the survival rate of endothelial cells. Only the survival rate of corneal endothelial cells was up to 70%, could it be used for corneal transplantation. This study demonstrated that the LT-DCP corneal materials had important

and promotional values in clinical practice, as well as the important supplementary source of corneal materials.

### Conflicts of Interest

All of the authors declare that they have no conflicts of interest regarding this paper.

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