

Experimental study of trabecular tissue repair for corneal defect in rabbits

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Abstract

• **AIM:** To investigate the mechanism and effect of trabecular tissue repair for corneal defect, and to provide a theoretical basis for its clinical application.

• **METHODS:** Trabeculectomy was performed on 40 (80 eyes) of 70 New Zealand white rabbits. Take trabecular tissue for backup. Thirty (30 eyes) corneal defect models were made, trabecular tissue was filled in the corneal defect, and the oblique cross stitch was used to suture the corneal laceration and debridement. Anterior segment image and optical coherence tomography (OCT) were performed at the time 1d, 1wk, 1 and 3mo after the model was made. After the observation, the cornea was taken and stained with trypanosome blue-alizarin red and the pathological tissue was examined.

• **RESULTS:** Observation 1wk after surgery, the area of corneal defect was edema, but the corneal curvature was basically normal, and the anterior chamber existed under slit lamp. After 3mo of observation, most corneal defects were repaired in the form of corneal leucoma and corneal macula (73.3%), the filled trabecular tissue gradually became transparent, fused tightly with the corneal tissue, and the corneal curvature was relatively smooth. But in one case, the trabecular planter was partially detached, no serious complications such as corneal laceration occurred after the stitches were removed.

• **CONCLUSION:** The trabecular tissue structure is similar to the corneal, and it can be used as a substitute for the corneal tissue defect by providing fiber scaffolds and cell amplification differentiation, and lay a foundation for the second-stage surgical treatment.

• **KEYWORDS:** corneal defect; trabecular tissue; rabbit

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INTRODUCTION

The best surgical method for the treatment of corneal tissue defect is penetrating corneal transplantation, but there are limited sources of fresh corneas and the operation is difficult to carry out.

At present, there are reports that amniotic membrane^[1], sclera, fascial tissue^[2-3], corneal flap transposition^[4-5], lamellar corneal transplantation^[6-7], corneal refractive lenticule^[8-10] can be used to repair corneal tissue defects which caused by trauma, ulcer, degeneration, *etc.* However, different methods have different complications, advantages and disadvantages^[11-13]. The final outcomes of amniotic membrane repair are: new blood vessels grow into the lacerated cornea and form scar tissue, however, the cornea transparency is not ideal, meanwhile the amniotic membrane is weak and soluble, which is also the culture medium for infection. Owing to the opacity and fibrous tissue arrangement of the scleral tissue is different from the cornea, the postoperative effect is not satisfactory. When fascia tissue used to repair the corneal defect, neovascularization grows into the corneal and form scar tissue. Therefore, the transparency of the cornea is not satisfied. The corneal refractive lenticule is relatively thin and poor resistance, so it is difficult to repair the corneal defect with deeper and larger defect range, but it is a good method. Allogeneic cornea is generally taken from the eye bank, which is widely used in clinical, and its advantages are obvious. However, due to the lack of donors, it is important to develop and utilize corneal substitutes.

Li *et al*^[14] used the preserved trabecular tissue to repair the cornea trauma accompanied by defect with trap suture. The seven eyes were healed in the form of corneal leucoma and corneal scar. Postoperative observation for 1-2y, the corneal defect was repaired, the normal appearance of the eye was maintained, the vision of all patients was unchanged or improved, and the curvature was relatively flat, which won the opportunity for the next keratoplasty. It has obtained the good clinical effect.

Trabecular tissue has rich sources benefit from glaucoma surgery, and its structure is similar to corneal tissue structure^[15-16].

If it can be used adequately, it will have important guiding significance for the treatment of clinical ophthalmic diseases. However, because of the volume of the trabecular tissue is small, the range of repairing defect is limited. In order to further observe the possibility of trabecular tissue repair for corneal defect, we made an animal model of rabbit trabecular tissue repair for corneal defect, and discussed its feasibility and role in corneal tissue reconstruct.

MATERIALS AND METHODS

Ethical Approval All animal-based experiments were conducted in compliance with the Experimental Animal Ethics Review Committee of Xinjiang Medical University. All procedures conformed with the guidelines of the Association for Research in Vision and Ophthalmic and Visual Research.

Materials Seventy New Zealand rabbits of clean grade, weighted varied from 2.0 to 2.5 kg, the age ranged from 2 to 3mo of either gender, which were provided by Department of Experimental Animals affiliated of Xinjiang Medical University.

Materials of Rabbit Trabecular Tissue Forty New Zealand white rabbits (80 eyes) were given intramuscular injection of ketamine (25 mg/kg) and chlorpromazine (12.5 mg/kg) under general anesthesia, and treated with promethvacaine hydrochloride eye drops (5 ml/L) for 3 times under surface anesthesia. Fluorine ketone preoperative disinfection, pull the eyelid with suture and then flushing conjunctival sac with physiological saline containing gentamicin. Completing the preoperative preparation, we made the domine-based spherical conjunctival flap and a size of $3.5 \times 4 \text{ mm}^2$ with thickness of 1/2 of the scleral flap, receted corresponding $2 \times 1 \text{ mm}^2$ trabecularfor backup under the microscope. The distal end of the scleral flap was closed with 10-0 nylon suture. Layers were used to suture the fascia and conjunctiva incisions. Trabecular tissue was taken for backup.

Trabecular Tissue Preservation (40 eyes) The trabecular tissue and medicinal anhydrous glycerol stored in a refrigerator at $-45^\circ\text{C}^{[17]}$. the trabecular tissue was immediately placed in 4°C equilibrium salt and rinsed for three times in order to remove the adhesive glycerol, and then rehydrated in another cup of the same liquid for 5min when we used.

Trabecular Tissue Repair the Model of Corneal Defect The 30 rabbits (30 eyes), anesthesia method with former, first make a $2 \times 2 \times 2 \text{ mm}^3$ triangular filter disinfection, sticking to the right eye cornea and cutting cornea with scalpel, making $2 \times 2 \times 2 \text{ mm}^3$ triangle corneal defect model. Trabecular tissue size is about $1 \times 2 \text{ mm}^2$ size in glaucoma surgery. It's one side is relatively smooth leather and slide down as far as possible. The defect place will filled in 2 or 3 blocks of trabecular tissue with the right direction were filled in the cornea defect. After that, the corneal laceration was sutured with 10/0 suture, and the conjunctival sac was coated with ofloxacin eye cream.

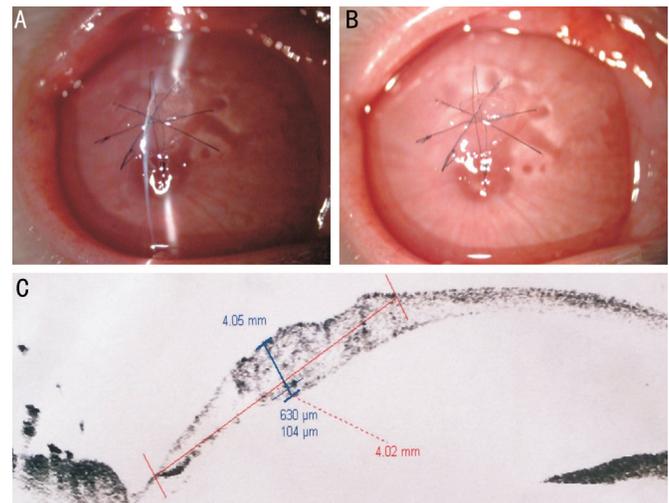


Figure 1 Corneal laceration was packed and sutured with trabecular tissue for 1wk A, B: 1wk after operation, the defect area of cornea was cross sutured, the curvature of cornea was normal, and the anterior chamber existed under slit lamp; C: Anterior segment optical coherence tomography (OCT) showed corneal defect area edema, structure disorder, but closed suture, anterior chamber formation, corneal curvature basically flat.

Postoperative Treatment Dilated the pupil with the compound topiramide and corneal nutrition to promote the healing of corneal laceration. If new blood vessels invade, 25 mg/mL bevacizumab (Avastin) 50 μL can be injected subconjunctivally. After molding, all animals were observed with slit lamp to observe the changes of anterior nodes every day, and the anterior nodes were photographed at each time point 1d, 1wk, 1 and 3mo. After 1mo of observation, the corneal suture was removed if there was any relaxation. After 3mo, all the rabbits were sacrificed. The corneas were examined by trypanosomes blue and alizarin red combined with hematoxylin-eosin (HE) staining.

RESULTS

The corneal curvature of all models was basically normal, and the anterior chamber existed. One week after the operation, at the meanwhile, the corneal defect area was swollen and structurally disordered, but the anterior chamber was formed (Figure 1). At the end of observation (3mo), the corneal edema subsided, corneal leukoplakia and scar healing, there was a case of partial release of trabecular graft (Figure 2). The surgical corneal endothelium (CE) was stained with trypanosoma blue-alizarine red and HE. The results show that the CE in the defect area was edema, the cells were of different sizes, and very few nuclei were slightly stained (Figure 3). The cornea showed infiltration of monocytes, neutrophils and lymphocytes by HE staining. The corneal epithelium was normal, and the stromal layer showed the neovascularization lumen (Figure 4).

Long-Term Results Three months after the operation, The results of corneal healing were corneal leucoma in 10 eyes

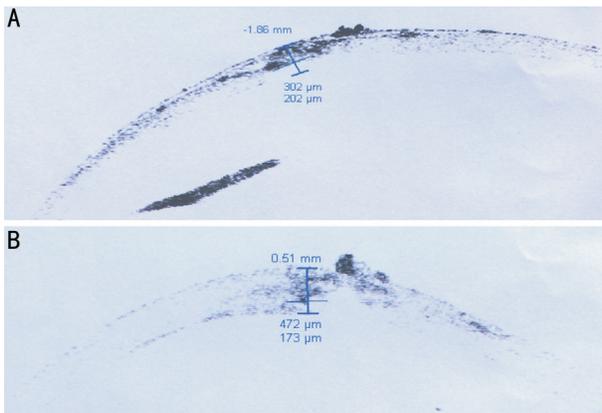


Figure 2 The anterior section OCT (3mo) A: 3mo after operation, anterior segment OCT showed that the curvature of the cornea was basically normal, the corneal edema subsided, and the structure was slightly disturbed. However, in one case, the trabecular planter was partially detached, the inner orifice was open, but the anterior chamber was still present (B).

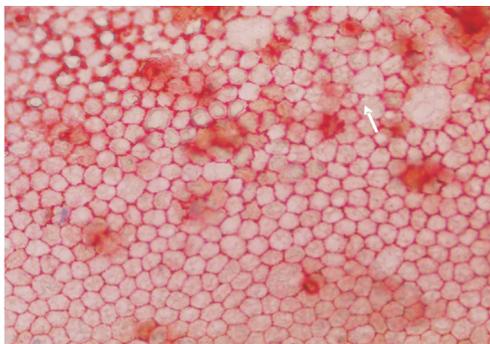


Figure 3 Corneal endothelial cells in the defect area of cornea were edema and varied in size with few light-stained nuclei (white arrow area, ×100).

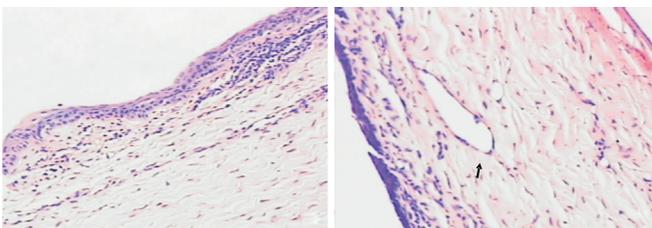


Figure 4 Corneal tissue staining (HE, 3mo) The cornea is infiltrated by a large number of monocytes, neutrophils and lymphocytes, the corneal epithelium was normal, neovascularization lumen was visible in the stromal layer (black arrow), there were a lot of plasma cells and a few fibroblasts (×100).

(33.3%), corneal macula in 12 eyes (40.0%), nebula in 4 eyes (13.3%), near transparent in 3 eyes (10.0%), trabecular tissue graft out in 1 eye (3.3%). All animals with trabecular tissue to repair corneal defects had no rejection reaction. In 3 eyes (10.0%), corneal neovascularization was obvious, and 50 μL of bevacizumab (25 mg/mL) was injected subconjunctival at 4wk. Except for 1 case of partial trabeculae prolapse, all eyes maintained normal ocular appearance without shallow anterior



Figure 5 Trabecular tissue excision in glaucoma.

chamber, anterior chamber hemorrhage, corneal laceration, and severe corneal distortion.

DISCUSSION

Severe open anterior segment injury caused by eye explosion injury is often accompanied by corneal tissue deficient and loss of eye contents. If corneal laceration is forcibly sutured, it will lead to wound leakage, pre-iris adhesion and corneal tissue deformation, which will seriously affect vision, stage II and III surgical treatment and prognosis of the disease^[18]. Penetrating keratoplasty (PKP) is feasible in clinical treatment, but it is difficult to operate because of the limited fresh cornea and the lack of glycerol preserved corneal tissue.

The trabecular tissue in glaucoma is abundant (Figure 5). If we can make use of it, it will be of great clinical significance.

Normal trabecular tissue consists of four components. The outermost layer is endothelial cells, the inner layer is elastic fibers and basilar membrane-like substance, and the central axis is collagen fibers. The corneal tissue is made up of 90% collagen fibers, so the structure of trabecular tissue is similar to that of the cornea.

Since the clinical application of argon laser trabeculoplasty (ALT) for the treatment of glaucoma, Schwalbe's line cells in trabecular tissue have received much attention^[19], and it has been found that ALT stimulates trabecular cell division to reduce intraocular pressure, and laser stimulates the expansion of peripheral CE. The rates of cell division in trabecular tissue was four times that in the untreated group^[20]. In addition, CE and trabecular meshwork (TM), both are derived from embryonic neural crest cells. In bovine eye experiment, ready-made fibroblast growth factors and cytokines can stimulate the differentiation and amplification of CE and TM cells. In conclusion, there are stem/progenitor cells in Schwalbe's line of anterior chamber horn structure^[21-23]. It is assumed that progenitor cells (PET cells) with potential differentiation ability exist between trabecular reticulum and CE, and PET

cells have the potential to transform into CE and TM. TM that is removed contains TM and CE during glaucoma, and it also contains PET cells. Numerous scholars^[24-25] reported that there is a potential resource in the trabecular tissue-stem cells for the treatment of CE diseases and glaucoma. Stem cells were cultured and differentiated into CE and trabecular, and personalized stem cells for the treatment of CE diseases and glaucoma. Therefore, in the perspective of tissue culture engineering, TM tissue can completely replace corneal tissue.

Glycerin is the most frequently used cryoprotectant^[17], which is widely used for long-term preservation of cells, bacteria, human tissues and organs, etc. Glycerin is a good solvent, which is easy to clean, and it has bacteriostatic effects. The results of electron microscopy and clinical application showed that the cornea preserved with glycerol for a long time had certain activity, the corneal structure was intact, and the transparency rate was higher after corneal transplantation.

In this study, trabecular tissue was used to repair 2 mm corneal defects. After 3mo of observation, all the experimental rabbits had good anterior chamber formation, smooth corneal curvature, fiber scar healing. Except for one case of trabecular tissue detachment, no corneal laceration was found. CE staining revealed the presence of CE in the defective area, but the sizes were different. This may be caused by compensatory repair of peripheral CE expansion and displacement filling. After the corneal defect is filled with trabecular tissue, a fiber scaffold is provided, which is conducive to the repair and healing of corneal scar. Meanwhile, the presence of suture also induces the growth of new blood vessels. If neovascularization invades the cornea obviously, anti-vascular endothelial growth factor (VEGF) drugs can be injected under the conjunctiva according to the actual situation. The author chose bevacizumab (3 eyes, 10.0%). In this study, most corneal defects were repaired in the form of corneal leucoma and corneal macula (73.3%), and even 3 corneas were basically transparent (10.0%). However, at 3mo after HE staining, a large number of monocytes, neutrophils and lymphocytes were still visible in the cornea, and neovascularization lumen was visible in the stromal layer. so it is still needed to further extend the experiment time, observe the final results.

This study has certain limitations, because the small trabecular tissue makes it difficult to repair large defects, and only 2 mm defects were observed. During the observation, immunohistochemical examination or other methods should be carried out to identify whether the trabecular tissue plays the role of stem cells. If corneal defects larger than 2 mm, it is difficult to repair corneal defects with TM, so multiple TM or lamellar corneal transplantation can be applied.

In conclusion, the results show that the trabecular tissue structure preserved with glycerine can be used to repair

corneal tissue defects smaller than 2 mm. However, clinically, trabecular tissues of glaucoma patients are infiltrated by mitomycin C or fluorouracil, so the long-term effects need to be further observed.

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