·Basic Research·

Observation on ultrastructure and histopathology of cornea following femtosecond laser-assisted deep lamellar keratoplasty for acute corneal alkaline burns

Wen-Jing Li [1], Yu-Kun Hu [1], Hui Song [2], Xiao-Wei Gao [1], Xu-Dong Zhao [1], Jing Dong [1], Yun-Lin Guo [1], Yan Cai [1]

[1]Department of Ophthalmology, the 474th Hospital of the Chinese People's Liberation Army, Urumchi 830011, Xinjiang Autonomous Region, China
[2]Department of Ophthalmology, the 309th Hospital of the Chinese People's Liberation Army, Beijing 100000, China
Co-first authors: Wen-Jing Li and Yu-Kun Hu
Correspondence to: Xiao-Wei Gao. Department of Ophthalmology, the 474th Hospital of the Chinese People's Liberation Army, Urumchi 830011, Xinjiang Autonomous Region, China. gxwgaoxw@263.net
Received: 2015-05-21 Accepted: 2015-12-24

Abstract

• AIM: To demonstrate the changes in ultrastructure and histopathology of the cornea in acute corneal alkaline burns after femtosecond laser-assisted deep lamellar keratoplasty.

• METHODS: The New Zealand white rabbits treated with alkaline corneal burn were randomized into two groups, Group A (16 eyes) with femtosecond laser-assisted deep lamellar keratoplasty 24h after burn and Group B (16 eyes) without keratoplasty as controls. All eyes were evaluated with transmission electron microscopy (TEM) at 1, 2, 3, and 4wk follow-up, then all corneas were tested by hematoxylin and eosin staining histology.

• RESULTS: The corneal grafts in Group A were transparent, while those in Group B showed corneal stromal edema and loosely arranged collagen fibers. One week after treatment, TEM revealed the intercellular desmosomes in the epithelial layers and intact non-dissolving nuclei in Group A. At week 4, the center of the corneas in Group A was transparent with regularly arranged collagen fibers and fibroblasts in the stroma. In Group B, squamous cells were observed on the corneal surface and some epithelial cells were detached.

• CONCLUSION: Femtosecond laser-assisted deep lamellar keratoplasty can suppress inflammatory responses, prevent toxic substance-induced injury to the corneal endothelium and inner tissues with quicker recovery and better visual outcomes.

• KEYWORDS: femtosecond laser; deep lamellar keratoplasty; transmission electron microscopy; alkali burn; cornea; rabbit
DOI:10.18240/ijo.2016.04.01

Li WJ, Hu YK, Song H, Gao XW, Zhao XD, Dong J, Guo YL, Cai Y. Observation on ultrastructure and histopathology of cornea following femtosecond laser-assisted deep lamellar keratoplasty for acute corneal alkaline burns. *Int J Ophthalmol* 2016;9(4):481-486

INTRODUCTION

Ocular surface structure and function is essential to maintain the transparency and normal function of cornea. Alkaline injury-induced disruptions in ocular surface structure and function can decrease the visual acuity, even result in blindness. It is necessary to improve allograft survival and decrease the rejection [1]. The current methods for ocular surface reconstruction include amniotic membrane transplantation [2-3], limbal stem cell implantation [4-5], conjunctival flap, lamellar, deep lamellar, penetrating keratoplasty[6-7], and combination of two or three of the above methods [8]. There are few published researches about the occasion of ocular surface reconstruction. Tandon *et al*[9] used amniotic membrane transplantation to reconstruct the ocular surface. This study was to investigative the ultrastructure and histopathological statues following the femtosecond laser-assisted deep lamellar keratoplasty (DLK) for acute alkali burn of cornea.

MATERIALS AND METHODS

Experimental Animal Thirty-two New Zealand white rabbits (32 eyes) and eight homebred rabbits (16 eyes) were provided by the Laboratory Animal Center of Xinjiang Autonomous Region and kept in the animal breeding center of the Key Laboratory in Xinjiang Medical University. All rabbits were males and weighed 2.0-2.5 kg. All the experimental and animal handling procedures were in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and conducted according to the requirements of Animal Research and Ethics Committee of the state scientific and technological committee. Establish the mode of corneal alkaline burn.

The 32 healthy New Zealand white rabbits were randomized into two groups: 16 rabbits in Group A (16 eyes) underwent femtosecond laser-assisted deep lamellar keratoplasty, while 16 rabbits (16 eyes) in Group B were used as controls. All procedures were performed under general anaesthesia by intramuscular injection of ketamine hydrochloride (50 mg/kg) and chlorpromazine hydrochloride (25 mg/kg). One drop of 0.05% proparacaine hydrochloride eye drop for three times was used to perform topic anaesthesia. All right eyes of rabbits were received alkaline burn. Totally, 30 μL of a 1 mol/L NaOH solution was dropped into a diameter of 6 mm dry and sterile filter paper. One minute later, the filter paper was gently placed at the center of the rabbit pupil for 30s. And 50 mL of phosphate buffered saline (pH 7.4) was used to wash the burned area and the conjunctival sac for 1min. Twenty-four hours after the injury, rabbits in Group A were performed the femtosecond laser-assisted deep lamellar keratoplasty while there was no treatment in Group B.

Corneal Graft Preparation The donor homebred rabbits were euthanasia by air embolism under anaesthesia. The eyeballs were enucleated immediately, and washed by 1:1000 gentamicin solution, then placed on the operating table with sterile gauze. Each cornea was placed on the center of cone attached on the VisuMax femtosecond laser (Carl Zeiss AG, Jena, Germany) and started the suction procedure to make the corneal graft.

Femtosecond Laser-assisted Deep Lamellar Keratoplasty The New Zealand white rabbits with alkaline burned corneas were fasted for one day before the keratoplasty. The eyelashes were removed on the day of the surgery. Thirty minutes before the surgery, the chloromycetin eye drop was used to wash the injured eyes. After the anesthesia was administered, the eyelids and surrounding areas were sanitized by iodine and chlorhexidine for three times. An aseptic hole towel and adhesive membrane were used in this procedure. The eyelid was opened using eye speculum. The eyeball was fixed by upper and lower rectus suturing by 1-0 sutures attached to the drapers.

The alkaline burned corneas were received the femtosecond-laser assisted keratoplasty to remove the corneal tissue according to the parameters (energy, 170 nJ; side cut angle, 90°; diameter, 7.5 mm; depth, 300 μm), and the corneal graft was transferred to the acceptor corneal bed using toothless forceps. The corneal graft was fixed at the 12, 6, 3, and 9 o'clock points then suture the cornea with 12 stitches (1-mm-wide each).

Post-operation The subconjunctival injection of 0.2 mL of tobramycin (4 g/L) and antibiotic eye balm were given after the surgery. The chloromycetin eye drop (2.5 g/L) was installed once per day for one week.

Histopathology and Transmission Electron Microscopy The cornea was examined under the slit lamp every two days since 2d after surgery. At 1, 2, 3, and 4wk, 4 rabbits (4 eyes) in Group A and 4 rabbits (4 eyes) in Group B were sacrificed by a 30 mL air embolism. The cornea was removed immediately along the corneal limbus. Two corneas were placed into 10% (v/v) formaldehyde and 4% (v/v) glutaraldehyde for optical microscopy and transmission electron microscopy (TEM) observation respectively at each time point.

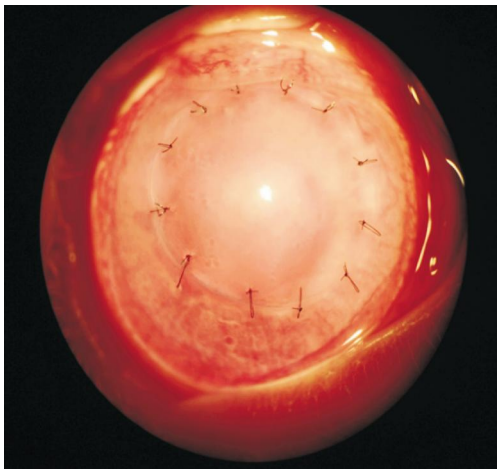

Figure 1 The slit-lamp image at 2wk after the surgery There was pretty light limbal congestion and corneal edema observed.

RESULTS

Corneal Grafts At 2d after surgery, there was obvious limbal hyperemia in Group A. The corneal graft was opaque and edema. No exudation was observed in the anterior chamber. All sutures have matched well. At 1wk after surgery, the limbal congestion, corneal edema and opaque were lighter than before. At 2wk after the surgery, there was very light limbal congestion and corneal edema observed (Figure 1). At 3wk after the surgery, no limbal congestion was observed. The cornea was transparent. The sutures were removed. At 4wk after the surgery, no limbal congestion was observed. The cornea was transparent.

Histopathology At 1wk after the surgery, in Group A there was mild corneal graft edema while the orderly arrangement of collagen fibers was found in the graft, and the epithelium was well (Figure 2A); there was edematous and thickened stroma with a loose arrangement of the collagen fibers, and corneal epithelium was detached in Group B (Figure 2B). At 2wk after the surgery, the corneal edema in Group A was decreased and the neat arrangement of collagen fibers in graft was observed (Figure 3A); in Group B, corneal edema was observed, part of the corneal epithelium was lost and untidy arrangement of the collagen fibers and neovascularization were found in cornea (Figure 3B). At 3wk after the surgery, the corneas in Group A regained their normal thicknesses, no obvious corneal edema and untidy arrangement of the collagen fibers was observed, inflammatory cell infiltration was observed only around the sutures (Figure 4A); in Group B, the corneal epithelium in

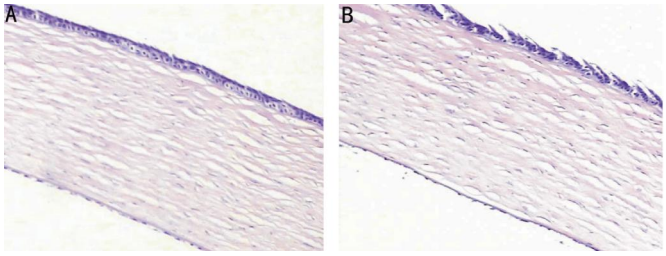

Figure 2 Represented the histopathological pictures at 1wk after the surgery A: Mild corneal graft edema and the orderly arrangement of collagen fibers in Group A (HE ×100); B: Edematous stroma and loose arrangement of the collagen fibers in Group B (HE×100).

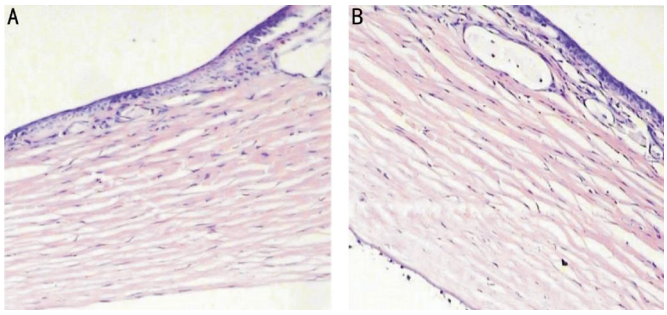

Figure 3 The histopathological pictures at 2wk after the surgery A: Neat arrangement of collagen fiber in Group A (HE× 100); B: Untidy arrangement of the collagen fibers and much neovascularization in Group B (HE×100).

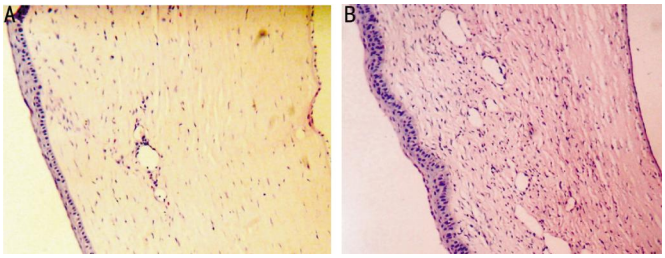

Figure 4 The histopathological pictures at 3wk after the surgery A: The corneas regained their normal thicknesses, no obvious corneal edema and untidy arrangement of the collagen fibers was observed in Group A (HE ×100); B: The corneal epithelium in the burned areas was gone in Group B (HE×100).

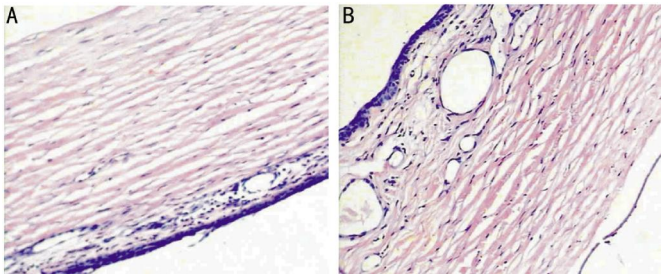

Figure 5 The histopathological pictures at 4wk after the surgery A: Neat arrangement of collagen fibers and normal thickness in Group A (HE ×100); B: Thickened and edematous stroma on the boundary with epithelium, loose arrangement of collagen fibers with large numbers of vacuoles in Group B (HE×100).

the burned areas was gone and there was an ulcer in the cornea. Edematous and thickened stroma were observed in the burned area, especially on the boundary with epithelium. Collagen fibers were loose arrangement with large numbers of vacuoles, a small amount of neovascularization, and the infiltration of numerous inflammatory cells (Figure 4B).

At 4wk after the surgery, the corneas graft with neat arrangement of collagen fibers in Group A remained of normal thickness without edema and there was infiltration of inflammatory cells observed around the sutures (Figure 5A). In Group B, the corneal epithelium in the burned areas has disappeared and the ulcers has aggravated, and there was thickened and edematous stroma observed especially on the boundary with epithelium, loose arrangement of collagen fibers was observed with large numbers of vacuoles, a small amount of neovascularization, and the infiltration of numerous inflammatory cells (Figure 5B).

Transmission Electron Microscopy In Group A corneal epithelial growth was observed at 1, 2, 3, and 4wk after the surgery. Many desmosomes were observed at 1wk after the surgery, the desmosomes were clearly seen in the epithelium and organelles were scattered throughout the cytoplasm, the dark cytosol and mild edema were also observed (Figure 6A). At 2wk after the surgery, large numbers of fibroblasts were observed in the corneal stroma with extension of the endoplasmic reticulum, there were intact nuclei in the endothelium; some vacuoles were seen between the endothelial and descemet layers (Figure 6B). At 3wk after the surgery, microvilli were found on the top of the corneal epithelium and the collagen fibers were orderly arranged; fibroblasts were seen in the stroma with microfilaments inside the cytoplasm (Figure 6C). At 4wk after the surgery, thriving microvilli were observed on the top of the corneal epithelium and there were neat arrangement of the collagen fibers and fibroblasts in the stroma (Figure 6D). The basement membranes were tightly connected, there were hemidesmosome-like substance in the basement membranes; and large amounts of microfilaments in the cytoplasm.

In Group B, at the 1wk after the surgery, there was obvious stromal edema with a wavy arrangement of collagen fibers and incomplete fibroblasts (Figure 7A). At 2wk after the surgery, stromal edema and large amount of vacuoles were observed without nuclei (Figure 7B). At 3wk after the surgery, the corneal epithelial cells were irregularly arranged with broken collagen fibers (Figure 7C). At 4wk after the surgery, thousands of infiltration of inflammatory cells in the corneal stroma was observed, there were uneven morphological and irregularly arranged corneal epithelial cells and broken, uneven and deranged collagen fibers in the stroma, squamous cells were observed on the top of corneal epithelium and part of the epithelial cells was detached (Figure 7D).

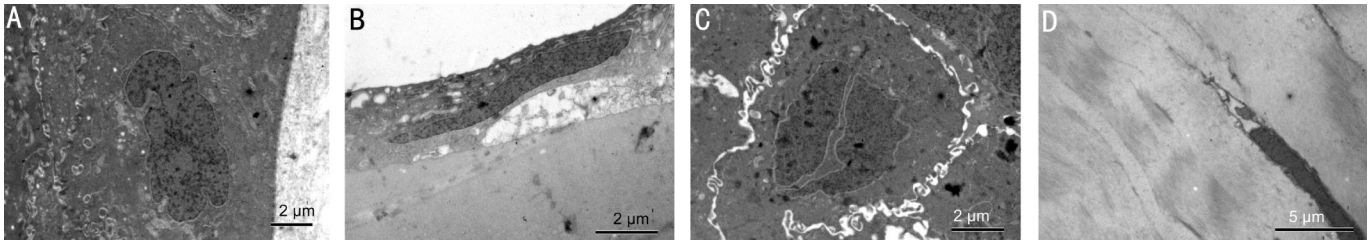

Figure 6 The pictures through transmission electron microscopy in Group A A: At the 1wk after the surgery, many desmosomes were observed, desmosomes were clearly seen in the epithelium (TEM×8K); B: At 2wk after the surgery, large numbers of fibroblasts were observed in the corneal stroma (TEM×12K); C: At 3wk after the surgery, microvilli appeared on the surfaces of the corneal epithelia (TEM×10K); D: At 4wk after the surgery, thriving microvilli were observed on the surface of the corneal epithelia with neat arrangement of the collagen fibers and fibroblasts (TEM×6K).

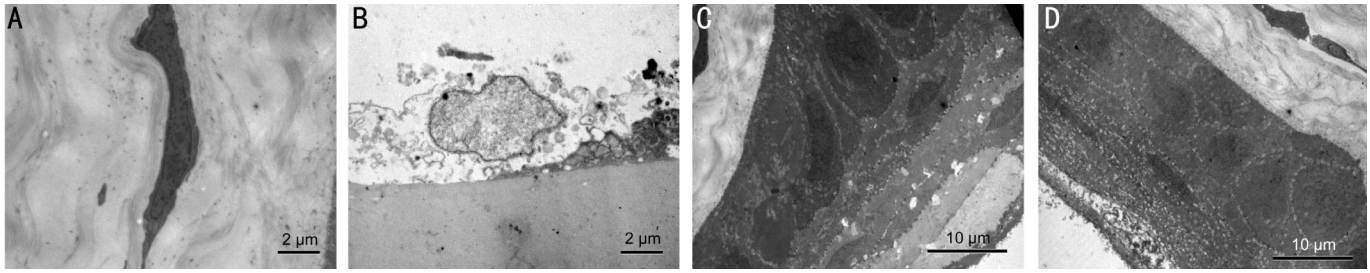

Figure 7 The pictures through transmission electron microscopy in Group B A: At the 1wk after the surgery, the corneal stromas showed significant edema with incomplete fibroblasts (TEM×8K); B: At 2wk after the surgery, large amount of vacuoles were observed without nuclei (TEM×10K); C: At 3wk after the surgery, the corneal epithelial cells were irregularly arranged with broken collagen fibers (TEM×3K); D: At 4wk after the surgery, the corneal epithelial cells were irregularly arranged with broken, uneven, and deranged collagen fibers (TEM×3K).

DISCUSSION

Corneal transparency is the fundamental basis of normal visual function. The alkaline burned eye can result in rapid protein denaturation and cell necrosis. The dysregulation of cellular fluid homeostasis induced by dehydration accelerates necrosis. Saponification between the alkaline and lipid-like substances can disrupt the cellular membrane structure and create a softened or liquefied environment that enables the alkaline to spread over and impair adjacent or inner tissues. The thrombus that results from the alkaline burns can lead to ischemia and lack of nutrition in the corneal tissues, which deteriorates the impairment and prevents the tissues from recovery [10-11]. Therefore, alkaline burns to the eye can cause corneal hypoxia, severe under-nutrition, poor regeneration of the corneal epithelium, and long-term ulcers. They can also lead to severe complications such as corneal neovascularization, disruption of the immunosuppressive microenvironment in the anterior chamber, severe adhesion of the eyelids and conjunctiva, corneal ulcers, perforation, and secondary bacterial infections.

The current treatment for early stage of alkaline burned cornea includes washing out the chemicals and removing the necrotic tissues as soon as we can. In addition, we have performed the symptomatic treatment such as washing the conjunctival sac, a subconjunctival injection of vitamin C, and paracentesis in the anterior chamber. Amniotic membrane transplantation, limbal stem cell implantation, and lamellar or therapeutic penetrating keratoplasty can be applied when appropriate. However, most corneal grafts have shown vascularization or continuous dissolution. The pathological characteristics of early corneal alkaline burns are tissue ischemia and necrosis as well as constant infiltration of inflammatory cells, which results in excessive immune responses against cornea-specific proteins. Therefore, corneal impairments are aggravated over time with severe complications [12]. If the injured corneal tissues were removed by surgery at the early stage of chemical burn and the ocular surface was reconstructed to prevent invasion of the toxic substances and inflammatory cells, the excessive immune responses against cornea-specific proteins and complications including neovascularization and corneal dissolution would be away from cornea. Therefore, it is very important to surgically remove the necrotic tissues and toxic substances at the early stage of corneal alkaline burns to protect the cornea, which plays a critical role in preventing or reducing the long-term complications caused by corneal alkaline burns and retains the visual function of the injured eyes.

The current methods for ocular surface reconstruction include amniotic membrane transplantation [2-3], limbal stem cell implantation [4-5], conjunctival flap, lamellar, deep lamellar, penetrating keratoplasty[6-7], and combination of two or three of the above methods[8]. According to Yoeruek *et al*[13], the surgery doesn't work if the neovascularization was still

observed in corneal grafts after surgery for corneal chemical burns. The corneal transparency could only be maintained by long-term taking the neovascularization inhibitors. Doganay *et al* [14] research shows that the subconjunctival administration of bevacizumab could inhibit corneal neovascularization effectively in the rabbit corneal alkali burn model. Yao *et al* [15] discovered that deep lamellar keratoplasty for the treatment of corneal chemical burns could suppress neovascularization to maintain the corneal transparency for long term. Yao *et al* [16] results suggest that subconjunctival injection of MSCs significantly accelerates corneal wound healing, decreases inflammation and reduces CNV in alkaline-burned corneas. Kheirkhah *et al* [17] used a sutureless application of an amniotic membrane patch allows the early delivery of its biologic actions, which may help preserve limbal stem cells for rapid extension and prevent late cicatricial complications in eyes with mild and moderate acute alkaline burns. Peng *et al* [18] have demonstrated that in mice locally administrated AMD3100 can reduce the number of alkali burn induced CNV, the number of inflammatory cells and inflammatory responses in corneal tissue.

There are few published researches about the occasion of ocular surface reconstruction. Tandon *et al* [9] used amniotic membrane transplantation to reconstruct the ocular surface. Hackett *et al* [8] replaced the injured cornea with an artificial biological corneal graft to treat early severe chemical burns. However, none of the above studies successfully achieved the purpose of the long-term prevention of corneal complications.

Dastjerdi *et al* [19] demonstrates that bevacizumab can penetrate the neovascularized cornea through topical application, in addition subconjunctivally injected bevacizumab can penetrate into the corneal stroma with an intact cornea. Dastjerdi *et al* [20] have reported that subconjunctival injection of bevacizumab may offer therapies in preventing graft rejection in high-risk corneal transplantation. We have studied the inhibition of neovascularization after corneal alkaline burns [12,21] and achieved the good results. In this study, we applied VisuMax femtosecond laser-assisted deep lamellar keratoplasty to treat the early stage of corneal chemicals burns. The injured cornea was removed to prevent further impairment to the surrounding and inner tissues and reduce even prevent complications such as corneal dissolution. Meanwhile, the surgery can block the pathways of excessive immune responses against the corneal-specific proteins and block neovascularization invasion. It can enhance the corneal epithelialization and maintain the normal corneal structure and function. Our results suggested that the corneal grafts in Group A matched well without obvious edema.

It has been reported that lamellar, deep lamellar, and endothelial keratoplasty are the major methods in chemical burn treatment and ocular surface reconstruction. However, the prognosis of each case is different, as it is related with the degree of injury, opportunity, and surgical techniques, especially the preparation of lamellar grafts and the grafting beds. The injured cornea was removed using blades in traditional deep lamellar keratoplasty, and the effect was dependent on the skills of operators. The smoothness of the grafting bed and the match between the graft and the grafting bed were limited. Therefore, it was difficult to obtain an optimal optical result. Our study used femtosecond laser-assisted deep lamellar keratoplasty [22-23] to treat early corneal alkaline burns. The femtosecond laser is capable of removing the injured area and preparing the grafts and grafting beds with highly precise smoothness, size, and depth. Our results suggest that the use of this technique is feasible for the treatment of early corneal alkaline burns and separating the affected areas. This would greatly shorten the disease course, improve outcomes, and retain visual function in combination with amniotic membrane transplantation, conjunctival grafting, or stem cell keratosplasty depending on the degree of injury.

In summary, the use of femtosecond laser-assisted deep lamellar keratoplasty to treat early corneal alkaline burns can suppress the inflammatory responses, promote epithelial growth, and effectively stimulate recovery of the corneal epithelium, which provides great insight into clinical application.

ACKNOWLEDGEMENTS

Foundations: Supported by the Military Medical Science and Technology General Project During the 12th Five-Year Plan Period (No. CWS11J239); Autonomous Region of Xinjiang the Mandatory Project of Science and Technology (No. 201491171).

Conflicts of Interest: Li WJ, None; **Hu YK,** None; **Song H,** None; **Gao XW,** None; **Zhao XD,** None; **Dong J,** None; **Guo YL,** None; **Cai Y,** None.

REFERENCES

1 Klebe S, Coster DJ, Williams KA. Rejection and acceptance of corneal allografts. *Curr Opin Organ Transplant* 2009;14(1):4-9.

2 Takahashi H, Igarashi T, Fujimoto C, Ozaki N, Ishizaki M. Immunohistochemical observation of amniotic membrane patching on a corneal alkali burn in vivo. *Jpn J Ophthalmol* 2007;51(1):3-9.

3 Tandon R, Gupta N, Kalaivani M, Sharma N, Titiyal JS, Vajpayee RB. Amniotic membrane transplantation as an adjunct to medical therapy in acute ocular burns. *Br J Ophthalmol* 2011;95(2):199-204.

4 Carifi G. Clinical outcomes of penetrating keratoplasty after autologous cultivated limbal epithelial transplantation for ocular surface burns. *Am J Ophthalmol* 2012;153(4):782-783.

5 Marchini G, Pedrotti E, Pedrotti M, Barbaro V, Di Iorio E, Ferrari S, Bertolin M, Ferrari B, Passilongo M, Fasolo A, Ponzin D. Long-term effectiveness of autologous cultured limbal stem cell grafts in patients with limbal stem cell deficiency due to chemical burns. *Clin Experiment Ophthalmol* 2012;40(3):255-267.

6 Singh G, Singh Bhinder H. Evaluation of therapeutic deep anterior lamellar keratoplasty in acute ocular chemical burns. *Eur J Ophthalmol* 2008;18(4):517-528.

7 Toor GS, Basu S, MacNeil S, Sangwan VS. Successful deep anterior lamellar keratoplasty following multiple failed limbal transplantations for chronic ocular burns. *BMJ Case Reports* 2012;2012.

8 Hackett JM, Lagali N, Merrett K, Edelhauser H, Sun Y, Gan L,Griffith M, Fagerholm P. Biosynthetic corneal implants for replacement of pathologic corneal tissue: performance in a controlled rabbit alkali burn model. *Invest Ophthalmol Vis Sci* 2011;52(2):651-657.

9 Tandon R, Gupta N, Kalaivani M, Sharma N, Titiyal JS, Vajpayee RB. Amniotic membrane transplantation as an adjunct to medical therapy in acute ocular burns. *Br J Ophthalmol* 2011;95(2):199-204.

10 Dohlman CH, Cade F, Pfister R. Chemical burns to the eye: paradigm shifts in treatment. *Cornea* 2011;30(6):613-614.

11 Gicquel JJ. Management of ocular surface chemical burns. *Br J Ophthalmol* 2011;95(2):159-161.

12 Gao XW, Zhao XD, Li WJ, Zhou X, Liu Y. Experimental study on the treatment of rabbit corneal melting after alkali burn with collagen cross-linking. *Int J Ophthalmol* 2012;5(2):147-150.

13 Yoeruek E, Ziemssen F, Henke-Fahle S, Tatar O, Tura A, Grisanti S, Bartz-Schmidt KU, Szurman P; Tübingen Bevacizumab Study Group. Safety, penetration and efficacy of topically applied bevacizumab:evaluation of eyedrops in corneal neovascularization after chemical burn. *Acta Ophthalmol* 2008;86(3):322-328.

14 Doganay S, Firat PG, Cankaya C, Kirimlioglu H. Evaluation of the effects of resveratrol and bevacizumab on experimental corneal alkali burn. *Burns* 2013;39(2):326-330.

15 Yao YF, Zhang B, Zhou P, Jiang JK. Autologous limbal grafting combined with deep lamellar keratoplasty in unilateral eye with severe chemical or thermal burn at late stage. *Ophthalmology* 2002;109 (11): 2011-2017.

16 Yao L, Li ZR, Su WR, Li YP, Lin ML, Zhang WX, Liu Y, Wan Q, Liang D. Role of mesenchymal stem cells on cornea wound healing induced by acute alkali burn. *PLoS One* 2012;7(2):e30842.

17 Kheirkhah A, Johnson DA, Paranjpe DR, Raju VK, Casas V, Tseng SC. Temporary sutureless amniotic membrane patch for acute alkaline burns. *Arch Ophthalmol* 2008;126(8):1059-1066.

18 Peng LH, Shen W, Yong W, Lu L, Liu L. Effects of AMD3100 subconjunctival injection on alkali burn induced corneal neovascularization in mice. *Int J Ophthalmol* 2011;4(1):44-48.

19 Dastjerdi MH, Sadrai Z, Saban DR, Zhang Q, Dana R. Corneal penetration of topical and subconjunctival bevacizumab. *Invest Ophthalmol Vis Sci* 2011;52(12):8718-8723.

20 Dastjerdi MH, Saban DR, Okanobo A, Nallasamy N, Sadrai Z, Chauhan SK, Hajrasouliha AR, Dana R. Effects of topical and subconjunctival bevacizumab in high-risk corneal transplant survival. *Invest Ophthalmol Vis Sci* 2010;51(5):2411-2417.

21 Gao XW, Fu Y, Li WJ, Du AJ, Li X, Zhao XD. Mechanism of immune tolerance induced by donor derived immature dendritic cells in rat high-risk corneal transplantation. *Int J Ophthalmol* 2013;6(3):269-275.

22 Baradaran-Rafii A, Eslani M. Femtosecond laser-assisted corneal transplantation. *Br J Ophthalmol* 2013;97(6):675-676.

23 Chamberlain WD, Rush SW, Mathers WD, Cabezas M, Fraunfelder FW. Comparison of femtosecond laser-assisted keratoplasty versus conventional penetrating keratoplasty. *Ophthalmology* 2011;118(3):486-491.