NEURAL REGENERATION RESEARCH
Volume 7, Issue 20, July 2012
www.nrronline.org

Cite this article as: Neural Regen Res. 2012;7(20):1556-1562.

Corneal subbasal nerve fiber regeneration in myopic patients after laser *in situ* keratomileusis★

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Abstract
A total of 26 myopic patients (52 eyes) underwent laser *in situ* keratomileusis. *In vivo* confocal microscopy revealed that most of the regenerated corneal subbasal nerve fibers in the corneal flap originated from the stump of corneal subbasal nerve fibers outside the ablation zone and extended towards the center of the cornea in all patients. Meanwhile, new fibers were also found to directly regenerate from deep in the stroma in some cases. Approximately 94% of regenerated corneal subbasal nerve fibers (73/78 eyes) regrew vertically into the peripheral central 6-mm circle area 1 month after surgery, 78% (28/36 eyes) grew into the central 3–6 mm area at 2 months, and 23% into the central 3-mm circle area at 3 months. In addition, there was no significant difference in corneal subbasal nerve fiber regenerative capacity between the basic fibroblast growth factor group and the 20% (v/v) deproteinized extract of calf blood group. The majority of corneal subbasal nerve fiber regeneration occurred from the stump of corneal subbasal nerve fibers outside the corneal flap, and the remaining growth occurred deep within the stroma.

Key Words
subbasal nerve fibers; confocal microscopy; cornea; regeneration; keratomileusis; basic fibroblast growth factor; neural regeneration

Research Highlights
This study investigated the regenerative capacity of corneal subbasal nerve fibers in the corneal flap from the margin of the ablation zone to the center of the cornea using a self-made scoring system at different time points after laser *in situ* keratomileusis.

Abbreviations
DECB, deproteinized extract of calf blood; IVCM, *in vivo* confocal microscopy

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Received: 2012-04-29
Accepted: 2012-06-23
(N20110504001/WJ)

Deng SJ, Wang MM, Zhang FJ, Sun XG, Hou WB, Guo N. Corneal subbasal nerve fiber regeneration in myopic patients after laser in situ keratomileusis. Neural Regen Res. 2012;7(20):1556-1562.

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doi:10.3969/j.issn.1673-5374.2012.20.005

INTRODUCTION

Laser *in situ* keratomileusis has become the most common and preferred method to correct ametropia. During surgery, the corneal subbasal nerve fibers in the corneal flap are cut off using a microkeratome, which can cause a series of disorders such as dry eye, hypesthesia and neurotrophic epitheliopathy that can last from months to years after laser *in situ* keratomileusis[1-3]. Recently, many *in vivo* studies have shown a decreased number and density of corneal subbasal nerve fibers in corneal flaps after laser *in situ* keratomileusis, which may initially induce a series of disorders in the cornea that can last for years[4-7]. In this study, we aimed to investigate the pattern, characteristics and regenerative capacity of corneal subbasal nerve fibers at different time points after laser *in situ* keratomileusis. In addition, differences in the speed of corneal subbasal nerve fibers regeneration following two kinds of refractory surgeries currently used, and the response of corneal

subbasal nerve fibers regeneration to drugs were also monitored.

Corneal subbasal nerve fibers reinnervation has been shown to originate from the margin of the ablation zone, and then extend towards the center after laser *in situ* keratomileusis[4]. However, many studies are limited to the central 3 to 4 mm of the cornea and focus on changes in density and length of regenerated corneal subbasal nerve fibers. Observations across the entire laser *in situ* keratomileusis flap are required to describe the complete path of reinnervation.

In vivo confocal microscopy (IVCM), which has been used to study the impairment and reconstruction of corneal nerves after surgery has provided a noninvasive method to observe and estimate the density, length and morphologic changes of nerves in human corneas. In this study, we have for the first time used a scoring system to investigate the regenerative capacity of corneal subbasal nerve fibers in the corneal flap from the margin of the ablation zone to the center of the cornea following (1) laser *in situ* keratomileusis surgery and (2) sub-bowman's keratomileusis surgery, at different time points after laser *in situ* keratomileusis *in vivo* using IVCM. The drug response of corneal subbasal nerve fibers regeneration was also evaluated to identify the most beneficial pharmacotherapy strategy after laser *in situ* keratomileusis.

RESULTS

Quantitative analysis of patients' eyes

Thirty-nine myopic patients (78 eyes) in Beijing Tongren Hospital were included in the study, including 13 patients (26 eyes) in the laser *in situ* keratomileusis group and 26 patients (52 eyes) in the sub-bowman keratomileusis group. All patients in both groups were randomly divided into two subgroups, one group received eye drops containing basic fibroblast growth factor (bFGF group, 54 eyes; 20 eyes in the laser *in situ* keratomileusis + bFGF group and 34 eyes in the sub-bowman keratomileusis + bFGF group); the other group received 20% (v/v) deproteinized extract of calf blood (DECB group, 24 eyes; 6 eyes in laser *in situ* keratomileusis + DECB group and 18 eyes in sub-bowman keratomileusis + DECB group). A total of 78 eyes were included in the final analysis. Data from two patients in the laser *in situ* keratomileusis + DECB group could not be collected at 2 months as patients could not participate in the study at this time.

Baseline analysis of subjects

No significant differences in gender, diopter (spherical equivalent) and soft contact lens wear history were detectable between the LASIK and SBK groups ($P > 0.05$; Table 1).

Table 1 Patient data

Group	Eye (*n*)	Age (mean ± SD, year)	Gender (male/ female, *n*/*n*)	Equivalent sphere diopter (mean ± SD, D)	Time for contact lens wear (mean ± SD, year)
LASIK	26	23±4	5/8	−4.92±−1.17	1.7±2.4
SBK	52	24±5	8/18	−6.98±−1.93	3.9±4.4

LASIK: Laser *in situ* keratomileusis; SBK: sub-bowman keratomileusis. D: Dioptre. Two-sample *t*-test was used for statistical analysis.

Velocity of corneal subbasal nerve fibers regeneration

The regenerative capacity of corneal subbasal nerve fibers was scored as follows. The cornea was divided into four concentric circles (Figure 1). A score of 3 was assigned if regenerated corneal subbasal nerve fibers had grown into the central 3-mm diameter area of the cornea. A score of 2 was assigned when they reached the second circle of 3–6 mm to the corneal apex. A score of 1 was assigned if corneal subbasal nerve fibers had just passed through the margin of the corneal flap and not reached the second circled area. Finally, a score of 0 was assigned when no new corneal subbasal nerve fibers were observed in the corneal flap. Scoring of all regenerated corneal subbasal nerve fibers was performed by an investigator blinded to the treatment group.

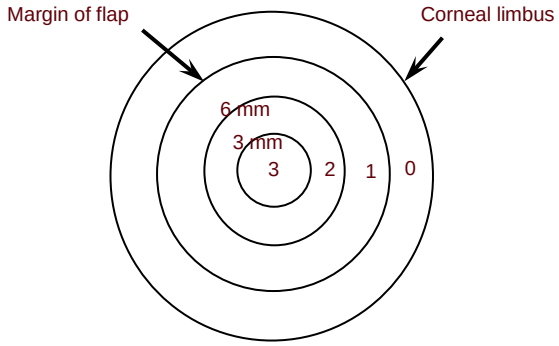

Figure 1 Demarcation and scoring of the regenerated corneal subbasal nerve fibers.

The cornea was divided into four concentric circle areas: the central 3-mm area was scored 3; the circle area of 3–6 mm to the corneal apex was scored 2; the area between the margin of the corneal flap and score 2 was scored 1; the area outside the margin of the flap was scored 0.

When corneal subbasal nerve fibers in the four quadrants of each cornea were examined, the patient

was asked to fix their gaze to the contralateral side, at which point the cornea was scanned along the regenerated corneal subbasal nerve fibers from the margin towards the center. The longest regenerated corneal subbasal nerve fibers were scored in each quadrant area and the highest score of the four quadrants was regarded as the score for the cornea. At 1 month after surgery, the regenerated fibers derived from the stump of corneal subbasal nerve fibers outside the incision observed in the circle were assigned a score of 1 in 24 eyes (92.3%) from the laser *in situ* keratomileusis group and in 49 eyes (94.2%) from the sub-bowman keratomileusis group. Meanwhile, corneal subbasal nerve fibers from two eyes (3.8%) in the sub-bowman keratomileusis group had reached the 3–6 mm diameter circle area (scores 2) (Figures 2, 3). These regenerated fibers appeared branchless, tortuous and thin, and were directed towards the center (Figure 4C).

At 2 months, new corneal subbasal nerve fibers with more branches, which appeared longer and stronger than that at 1 month in both groups, appeared (Figure 4B). They were mainly detected in the 3–6 mm circle area (score 2), and regenerated fibers in 12.5% eyes from the sub-bowman keratomileusis group had reached the central 3-mm area (score 3).

At 3 months, the new fibers became longer and were mainly in the 3–6 mm circle area, but more fibers were visible in the 3 mm central area (score 3) in both groups (21.4% in the sub-bowman keratomileusis group and 25% in the laser *in situ* keratomileusis group). Although the density of regenerated corneal subbasal nerve fibers remained significantly less when compared to pre-operation, they appeared stronger and had more branches than that at 2 months (Figures 2, 3, 4C).

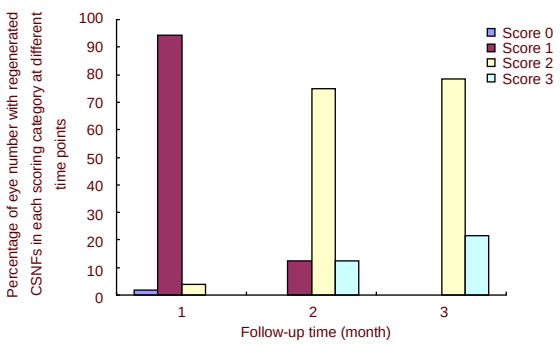

Figure 2 Speed of regenerated corneal subbasal nerve fibers (CSNFs) in the sub-bowman keratomileusis group (n = 52) at various time points postoperation.

Numeration data are expressed as a %. Multinomial logistic regression was used for statistical analysis.

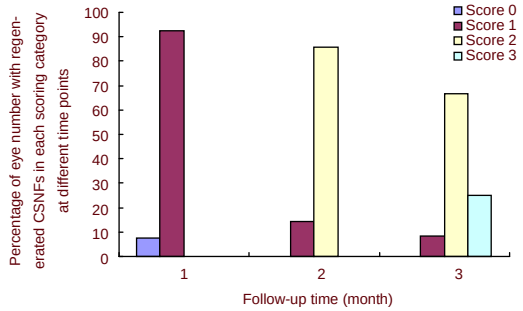

Figure 3 Speed of regenerated corneal subbasal nerve fibers (CSNFs) in the laser *in situ* keratomileusis group (n = 26) at various time points postoperation.

Numeration data are expressed as a %. Multinomial logistic regression was used for statistical analysis.

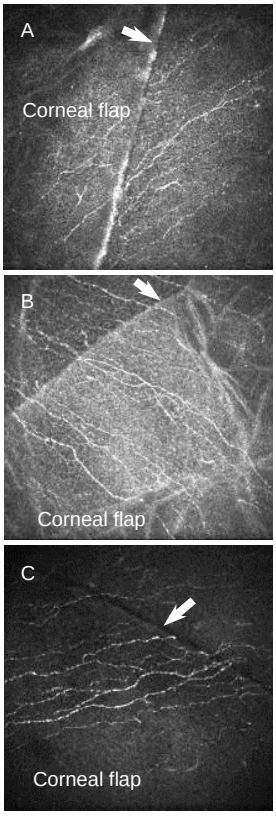

Figure 4 Regenerated corneal subbasal nerve fibers in the corneal flap at 1 month (A), 2 months (B) and 3 months (C) after laser *in situ* keratomileusis (× 800). Arrows: incision.

(A) Branchless, tortuous and thin regenerated fibers derived from outside the incision extended towards the center of the flap.

(B) New fibers appeared longer and stronger than that at 1 month.

(C) New fibers appeared stronger with more branches than that at 2 months.

Pattern of corneal subbasal nerve fibers regeneration

Most of the regenerated fibers were observed to originate from the stump of corneal subbasal nerve fibers outside the margin of the ablation zone, and extend towards the central cornea (Figure 4). However, some new corneal subbasal nerve fibers were found to directly regenerate into the corneal flaps from deep within the stroma with dilated head ends and many branches (Figure 5). This phenomenon was observed in two cases from the sub-bowman keratomileusis group at 1 month, and five cases (four cases in the sub-bowman keratomileusis group and one case in the laser *in situ* keratomileusis group) at 3 months after surgery. The generated corneal subbasal nerve fibers from the stroma originated from various regions of the corneal flap and even appeared directly in the central cornea (two cases in the sub-bowman keratomileusis group).

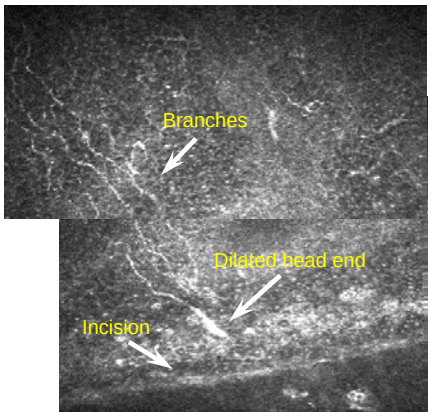

Figure 5 New corneal subbasal nerve fibers were found to directly regenerate from the stroma at 3 months after sub-bowman keratomileusis with dilated head ends and many branches (arrows, × 800).

Drug response to corneal subbasal nerve fibers regeneration

In the sub-bowman keratomileusis group, the new fibers were visible in the 3–6 mm circle area (score 2) in 5.9% of eyes from the bFGF group at 1 month after surgery. However, they did not reach the same circle in the DECB group. New fibers in all eyes from the bFGF group were observed in the 3–6 mm circle area (score 2) at 2 months. Simultaneously, new fibers in 16.7% of eyes in the DECB group were detected in the central 3 mm diameter zone (score 3). At 3 months, new fibers with a score of 3 accounted for 50% of eyes in the DECB group, which was more than that in the bFGF group (33.3%) (Figure 6).

In the laser *in situ* keratomileusis group, new fibers in 95% of eyes in the bFGF group and 83.3% of eyes in the DECB group had passed through the incision (score 1) at 1 month after surgery. At 2 months, statistical analysis was not performed because some follow-up cases were lost. The regenerated corneal subbasal nerve fibers were visible in the central 3-mm diameter zone (score 3) in 50% of eyes in the DECB group, which was faster than that of the bFGF group (30%) at 3 months (Figure 7).

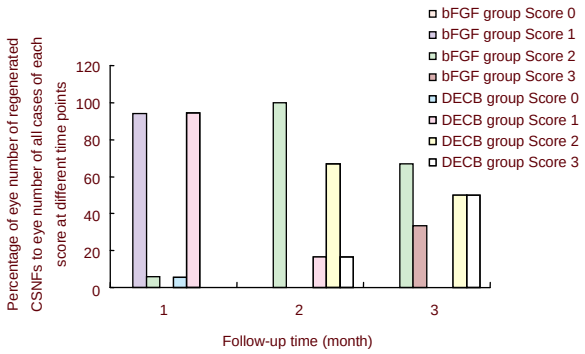

Figure 6 Speed of regenerated corneal subbasal nerve fibers (CSNFs) in the basic fibroblast growth factor (bFGF) and deproteinized extract of calf blood (DECB) groups postoperation (sub-bowman keratomileusis group).

Numeration data are expressed as a %. Multinomial logistic regression was used for statistical analysis.

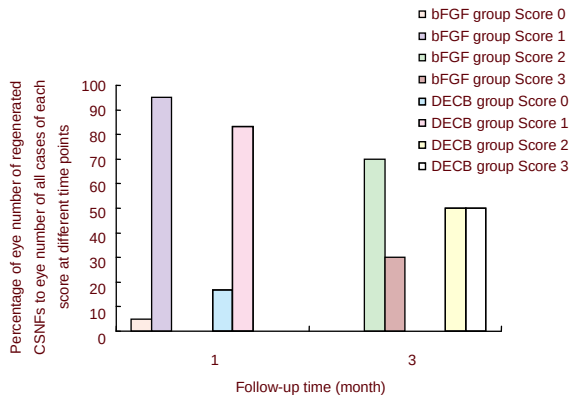

Figure 7 Speed of regenerated corneal subbasal nerve fibers (CSNFs) in the basic fibroblast growth factor (bFGF) and deproteinized extract of calf blood (DECB) groups postoperation (laser *in situ* keratomileusis group).

Numeration data are expressed as the %. Multinomial logistic regression was used for statistical analysis.

The speed of corneal subbasal nerve fibers regeneration between the bFGF and DECB groups was not significantly different ($P > 0.05$) at 1, 2, and 3 months after surgery.

DISCUSSION

Kauffmann *et al* [4] observed corneal subbasal nerve

fibers by confocal video microscopy in 1996 and found that they originated from the margin of the ablation zone and extended towards the center. They also noted that rarefied corneal subbasal nerve fibers were visible at the edges of the corneal flaps at 8 weeks after laser *in situ* keratomileusis, and single branchless nerve fibers were visualized in the center of the ablation zone after 3 months[4]. To date, there has been no other report on the pattern of regeneration of new corneal subbasal nerve fibers, excepting studies that focus on the density and length of nerve fibers in the central cornea. In this study, most of the regenerated fibers in the corneal flap were observed to originate from the stump of corneal subbasal nerve fibers outside the ablation zone and extend towards the center of the cornea in all patients. However, new fibers were also found to directly regenerate from deep within the stroma. These results suggest that the former is the main pattern of corneal subbasal nerve fibers regeneration and that the latter pattern is another important mechanism of fiber regeneration in the corneal flap after laser *in situ* keratomileusis.

The ablation depth of the corneal flap was approximately 130 μm in the laser *in situ* keratomileusis group and 90–100 μm in the sub-bowman keratomileusis group. The new nerve fibers of five patients regenerated from the stromal nerve at 3 months after surgery. We found that four cases (80%) were from the sub-bowman keratomileusis group and 20% were from the laser *in situ* keratomileusis group, suggesting that the deeper the ablation, the deeper the impairment to nerve trunks in the stroma, and the slower the corneal subbasal nerve fibers recovery. It is essential that further research investigates stromal nerve regeneration after different laser *in situ* keratomileusis surgeries. Lee and Patel *et al* compared the relationship between the recovery of corneal sensitivity and corneal subbasal nerve fibers, and confirmed that corneal subbasal nerve fibers did not recover to preoperative conditions even in some patients who had recovered normal corneal sensation[1]. This result is probably not only due to the density of corneal subbasal nerve fibers recovery[1, 8-11], but also due to the different thresholds of different regenerated corneal subbasal nerve fibers patterns.

The duration of complete corneal subbasal nerve fibers recovery after laser *in situ* keratomileusis varies in different studies. Lee *et al* [1] demonstrated that the regenerated corneal subbasal nerve fibers were visible in the central cornea at 6 months after surgery, while Patel *et al*[11] observed that corneal subbasal nerve fibers density did not return to preoperative levels even after 12 months. Calvillo *et al* [7] reported that the number of nerve fiber bundles remained less than 60% of the pre-laser *in situ* keratomileusis number at 3 years. In this study, new fibers were mainly detected in the 3–6 mm circle area (score 2) and approximately 21–25% of eyes had reached the central 3-mm corneal area at 3 months, but the density remained significantly less than that at preoperation. Observations of the complete path of reinnervation and changes in corneal subbasal nerve fibers density in the corneal flap after laser *in situ* keratomileusis require long-term follow-up investigations. Though there was no significant difference between the speed of corneal subbasal nerve fibers regeneration in the laser *in situ* keratomileusis and sub-bowman keratomileusis groups within 3 months, the percentage of regenerated corneal subbasal nerve fibers appearing in the central 3-mm corneal area was different between the two groups (12.5% of eyes in the sub-bowman keratomileusis group at 2 months, 21.4% of eyes in the sub-bowman keratomileusis group, and 25% of eyes in the laser *in situ* keratomileusis group at 3 months), which may be associated with the preoperative conditions of patients, such as contact lens wear history, the preoperative density of corneal subbasal nerve fibers and diopter[10, 12-13]. The effect of different surgeries on corneal subbasal nerve fibers regeneration deserves long-term follow-up.

The drug response of corneal subbasal nerve fibers regeneration after laser *in situ* keratomileusis was also described in the present study. bFGF is a kind of polypeptide growth factor, which exists in each layer of the cornea. Exogenous bFGF promotes the restoration of the corneal epithelium and endothelium[14]. Previous studies have confirmed that exogenous bFGF accelerates the recovery of peripheral nerves after impairment, though it has not been proven for corneal subbasal nerve fibers[14-15], In addition, exogenous bFGF also accelerates the recovery of corneal sensation after laser *in situ* keratomileusis, which suggests that bFGF has the ability to improve corneal subbasal nerve fibers regeneration[14]. As there was a possibility of corneal neovascularization[16], bFGF was only applied for the first 14 days after surgery in this study. The active components of DECB are inositol phosphate oligosaccharide and hyp-molecule polypeptide. Hyp-molecule polypeptide is an essential component for nerve cell protein synthesis and can enhance the regenerative ability of nerve cells under anoxic conditions[17-18]. Nevertheless, it remains unclear whether DECB facilitates the regeneration of corneal nerves. Although there was no significant difference between the bFGF and DECB groups, we detected regenerated corneal subbasal nerve fibers in the central 3-mm zone in 16.7% of eyes from the DECB group at 2 months, while new fibers in all eyes from the bFGF group could not be detected in the same area. Moreover, the percentage of regenerated corneal subbasal nerve

fibers appearing in the central 3-mm area in the DECB group was more than that in the bFGF group at 3 months, suggesting that DECB promotes corneal subbasal nerve fibers regeneration.

MATERIALS AND METHODS

Design

A prospective, nonrandomized, comparative clinical study.

Time and setting

Experiments were performed at the Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, China from May to December 2010.

Materials

Thirty-nine myopic patients (78 eyes) who received laser *in situ* keratomileusis in the Tongren Refractive Center were enrolled as the volunteers of our study from May to December 2010.

Inclusion criteria: Myopic patients with a refractive error between –2 and –20 dioptre (D) for myopia and mean keratometry between 39 and 47 D.

Exclusion criteria: Patients with any history of keratopathy, ocular surgery, autoimmune disorder, diabetes or nervous system disease.

According to the rule of *Administrative Regulations on Medical Institutions*[19], informed consent was signed by each patient after explanation of the goals and the risks of the study.

Methods

Preoperative preparation

Preoperative examinations included uncorrected visual acuity, manifest refraction, cycloplegic refraction, best spectacle-corrected visual acuity, slit-lamp examination, pachymetry, noncontact tonometry, keratometry, computerized corneal topography and fundus examination.

The preoperative information of patients is shown in Table 1. Topical levofloxacin (Santen, Noshu, Japan) was administered into both eyes of all the patients, four times daily, for 3 days before surgery.

Surgery technique of sub-bowman keratomileusis and laser in situ keratomileusis

After topical anesthesia (0.4% (v/v) oxybuprocaine hydrochloride eye drops, Santen, Osaka, Japan), the corneal flap with a superior hinge in the laser *in situ* keratomileusis group was created by mechanical microkeratome (Moria, Antony, France); the corneal flap with a nasal hinge in the sub-bowman keratomileusis group was created by One Use Plus microkeratome (Moria, Antony, France). Stroma was ablated by the same excimer laser (Visx S4IR, Irvine, CA, USA) and the stroma bed was thoroughly washed with balanced salt solution. The corneal flap was repositioned to smooth the surface as much as possible, and superfluous moisture was absorbed. All surgical procedures were performed by the same surgeon.

Management of patients after surgery

Postoperatively, patients received topical levofloxacin four times daily for 10 days. Fluorometholone (Santen, Osaka, Japan) was instilled four times daily and reduced once every 3 days, for a total period of 10 days. All patients in each group were randomly divided into two sub-groups. One sub-group received eye drops containing bFGF (21 000 IU/5 mL; Zhuhai, China) (20 eyes in the laser *in situ* keratomileusis group and 32 eyes in the sub-bowman keratomileusis group) once daily before sleep in the first 14 days after surgery, and then artificial tears four times daily for 3 months (the bFGF group). Another sub-group received eye drops of 20% (v/v) DECB (Shenyang, China; six eyes in the laser *in situ* keratomileusis group and 20 eyes in the sub-bowman keratomileusis group) once daily before sleep in the first 14 days, and then three times daily for 3 months (the DECB group).

Technique of confocal microscopy examination

IVCM examination was subsequently performed in all subjects at 1, 2 and 3 months separately after surgery (The Heidelberg Retina Tomograph III Rostock Corneal Module, Heidelberg, Germany). IVCM uses a diode laser with a wavelength of 670 nm. The dimension of each image was 400 μm × 400 μm with a lateral digital resolution of 1 μm/pixel and digital depth resolution of 2 μm/pixel. The enlargement ratio of each image was 800. Each eye was anesthetized with a drop of 0.4% (v/v) oxybuprocaine hydrochloride twice before examination. The cornea was scanned from the margin of the ablation zone to the center at each four quadrants (superior, inferior, nasal and bitemporal) using a section mode to obtain high-quality images of the subbasal nerve plexus.

Score criteria of regenerated corneal subbasal nerve fibers

When corneal subbasal nerve fibers in the four quadrants of each cornea were examined, the patient was asked to fix their gaze to the contralateral side, and then the cornea was scanned along the regenerated corneal subbasal nerve fibers from the margin towards the center. The longest regenerated corneal subbasal nerve fibers were scored at each quadrant area and the highest score of the four quadrants was regarded as the score of the cornea.

Statistical analysis

Measurement data were expressed as mean ± SD, and numeration data were presented as the rate. The data were analyzed using SPSS 11.5 (SPSS, Chicago, IL, USA). Intergroup differences were compared utilizing the two-sample *t*-test and multiple regression analysis. A *P* value of < 0.05 was accepted as statistically significant.

Author contributions: Shijing Deng was responsible for experimental design, data analysis and manuscript preparation. Mengmeng Wang was in charge of experimental performance and manuscript preparation. Fengju Zhang guided the research. Xuguang Sun, Wenbo Hou and Ning Guo participated in experimental performance and data supply.

Conflicts of interest: None declared.

Ethical approval: Because confocal microscopy examination is routine and a noninvasive technique, ethical approval was not required from the ethical committee.

REFERENCES

[1] Lee SJ, Kim JK, Seo KY, et al. Comparison of corneal nerve regeneration and sensitivity between LASIK and laser epithelial keratomileusis (LASEK). Am J Ophthalmol. 2006;141(6):1009-1015.

[2] Moilanen JA, Holopainen JM, Vesaluoma MH, et al. Corneal recovery after lasik for high myopia: a 2-year prospective confocal microscopic study. Br J Ophthalmol. 2008;92(10):1397-1402.

[3] Benitez del Castillo JM, Wasfy MAS, Fernandez C, et al. An in vivo confocal masked study on corneal epithelium and subbasal nerves in patients with dry eye. Invest Ophthalmol Vis Sci. 2004;45(9):3030-3035.

[4] Kauffmann T, Bodanowitz S, Hesse L, et al. Corneal reinnervation after photorefractive keratectomy and laser in situ keratomileusis: an in vivo study with a confocal videomicroscope. Ger J Ophthalmol. 1996;5(6):508-512.

[5] Slowik C, Somodi S, Richter A, et al. Assessment of corneal alterations following laser in situ keratomileusis by confocal slit scanning microscopy. Ger J Ophthalmol. 1997;5(6):526-531.

[6] Lee BH, McLaren JW, Erie JC, et al. Reinnervation in the cornea after LASIK. Invest Ophthalmol Vis Sci. 2002; 43(12):3660-3664.

[7] Calvillo MP, McLaren JW, Hodge DO, et al. Corneal reinnervation after LASIK: prospective 3-year longitudinal study. Invest Ophthalmol Vis Sci. 2004;45(11):3991-3996.

[8] Zhao LJ, Zhang FJ, Zhang ZH, et al. The use of confocal microscopy to study the characteristics of corneal nerve regeneration after different excimer laser operations. Yanshiguangxue Zazhi. 2008;10(4):314-317.

[9] Gallar J, Acosta MC, Moilanen JA, et al. Recovery of corneal sensitivity to mechanical and chemical stimulation after laser in situ keratomileusis. J Refract Surg. 2004; 20(3):229-235.

[10] Bragheeth MA, Dua HS. Corneal sensation after myopic and hyperopic LASIK: clinical and confocal microscopic study. Br J Ophthalmol. 2005;89(5):580-585.

[11] Patel SV, McLaren JW, Kittleson KM, et al. Subbasal nerve density and corneal sensitivity after laser in situ keratomileusis: femtosecond laser vs mechanical microkeratome. Arch Ophthalmol. 2010;128(11): 1413-1419.

[12] Oliveira-Soto L, Efron N. Morphology of corneal nerves in soft contact lens wear. A comparative study using confocal microscopy. Ophthalmic Physiol Opt. 2003;23(2):163-174.

[13] Yan D, Zhang FJ. Evaluation of corneal changes from long-term contact lens wear using a confocal microscope for analysis. Yanzhiguangxue Zazhi. 2008;10(4):310-313.

[14] Li Y, Zhong LXY, Wang Z, et al. Clinical study of repairing effect of basic fibroblast growth factor on epithelium, endothelium and nerves after laser assisted in situ keratomileusis. Yanke Xinjinzhan. 2008;28(2):125-130.

[15] Grothe C, Nikkhah G. The role of basic fibroblast growth factor in peripheral nerve regeneration. Anat Embryol. 2001;204(3):171-177.

[16] Tong S, Yuan F. Dose response of angiogenesis to basic fibroblast growth factor in rat corneal pocket assay: II. Numerical simulations. Microvasc Res. 2008;75(1):16-24.

[17] Ren SP, Zhang J. Recent progress of efficacy of deproteinized hemoderivative of calf blood injection on cerebrovascular disease. Zhongguo Yiyao Daobao. 2006; 8(3):200-201.

[18] Luo BY, Rong R, Zhao M, et al. Efficacy of deproteinized hemoderivative of calf blood injection on diabetic peripheral neuropathy: multicenter, positive parallel controlled clinical trial. Zhongguo Linchuang Yixue. 2006; 6(6):963-964.

[19] State Council of the People's Republic of China. Administrative Regulations on Medical Institution. 1994-09-01.

(Edited by Jiang CH, Li R/Qiu Y/Song LP)