Clinical science

Structural and functional changes of binocular corneal innervation and ocular surface function after unilateral SMILE and tPRK

Qianwen Gong,1,2 Kaiyan Huang,1,2 Kexin Li,1,2 Yixuan Tong,1,2 Jian Zhao,1,2 Hui Wang,1,2 Zhiqiang Xu,1,2 Meng Lin,1,2 Fan Lu,1,2 Liang Hu,1,2

ABSTRACTS

Aims To evaluate the bilateral changes in the sub-basal nerve plexus of the cornea and ocular surface function after unilateral small incision lenticule extraction (SMILE) and transepithelial photorefractive keratectomy (tPRK) procedures.

Methods 34 patients were enrolled in the study and underwent unilateral SMILE (21 of 34 patients) or unilateral tPRK (13 of 34 patients). Complete ophthalmic examinations, tear film function tests and Cochet-Bonnet esthesiometry were conducted to assess the effects of the surgeries on the corneal nerves and tear function. Morphological changes were assessed using in vivo confocal microscopy to evaluate the corneal sub-basal nerve plexus and dendritic cells. ELISA was used to measure the tear neuromediators. Clinical and morphological data at each follow-up point were compared with preoperative baseline values.

Results All patients who underwent unilateral SMILE or tPRK procedures exhibited bilateral corneal nerve degenerative changes, decreased corneal sensitivity, worsening of dry eye symptoms and changes in bilateral tear neuromediators. In the SMILE group, bilateral corneal sensitivity was positively correlated with corneal nerve fibre length and negatively correlated with dendritic cell area. The dry eye severity was negatively correlated with corneal sensitivity. Tear levels of substance P and nerve growth factor were positively correlated with mean dendritic cell area and dry eye severity, but negatively correlated with corneal sensitivity. In the tPRK group, bilateral corneal sensitivity was positively correlated with corneal nerve fibre density.

Conclusions Unilateral refractive surgery may bilaterally affect the morphology and function of corneal nerves and ocular surface status postoperatively.

INTRODUCTION

Refractive error is an important reversible cause of vision impairment. Laser vision correction surgery, including small incision lenticule extraction (SMILE) and transepithelial photorefractive keratectomy (tPRK), is widely used in clinical practice to address this issue.

Corneal nerves play a critical role in maintaining ocular surface homeostasis. They are responsible for touch, pain and temperature sensations, blink reflex, wound healing, and tear production and secretion. Laser vision correction surgery can result in reduced corneal sensitivity and tear secretion if the corneal nerve is cut, leading to a dry eye postoperatively. These surgeries also impact the secretion of neuromediators. This influences the immune barrier and nutritional status of the cornea, hence affecting corneal nerve and ocular surface inflammatory responses. Alterations in nerve growth factor (NGF), substance P (SP) and calcitonin gene-related peptide (CGRP) levels are strongly associated with postoperative degenerative changes in corneal nerves and symptoms of dry eye. In addition, it has been established that increased density and altered morphology of dendritic cells (DCs) are associated with dry eye and post-laser-assisted in situ keratomileusis (LASIK) ectasia, since DCs play a crucial role as antigen-presenting cells in inflammatory and immune responses.

Previously, it was considered that the sensory nerves in the cornea were mainly transmitted through the unilateral eye pathway. However, multiple studies have reported that unilateral ocular lesions can also damage the contralateral eye. Paiunik et al reported that a circular corneal incision in one eye can lead to the loss of immune privilege in both ocular surfaces, resulting in a high incidence of corneal allograft rejection. Hamrah et al reported bilateral changes in corneal nerve
density and DCs in patients with unilateral herpes simplex keratitis (HSK). They also demonstrated that the decrease in nerve density was associated with corneal sensitivity loss in patients with HSK. Giannaccara et al.\(^{11}\) observed that unilateral cataract surgery caused bilateral changes in the corneal sub-basal nerve plexus (SNP). Unilateral corneal annular incision induced bilateral changes in the corneal nerve fibre morphology, tear secretory function and ocular surface immune response in animal models.\(^{12}\) However, bilateral changes in the corneal nerves and ocular surface after unilateral refractive surgery in humans have not yet been reported.

Considering that laser vision correction surgery results in varying degrees of corneal nerve transection, our study investigated the effects of unilateral laser vision correction on bilateral corneal nerve morphology and function, ocular surface function and tear neuromediators.

**MATERIAL AND METHODS**

**Subjects and surgical procedures**

Based on clinical experience and previous pre-experimental results, we calculated a sample size of 34 using G power. This prospective study included 34 patients who underwent unilateral tPRK (n=13) or SMILE (n=21) at the Eye Hospital of Wenzhou Medical University between August 2021 and November 2022. In the SMILE procedure, the VisuMax femtosecond laser was used to remove a stromal lenticule, leaving behind a cap with a thickness of 110 µm. The laser energy was 135 nJ at a spot separation of 4.5 µm. The cap diameter was 7.9 mm (with the exception of one patient, in whom it was 7.8 mm) and optical zone was 6.9 mm (with the exception of one patient, in whom it was 6.7 mm). During the tPRK procedure, all surgeries were performed with the Schwind Amaris 750 Hz excimer laser. The optical zones ranged from 6.6 to 7.5 mm, and the ablation depth ranged from 92 to 146 µm. In addition to the initial preoperative visit (S\(_{\text{pre}}\) and T\(_{\text{pre}}\)), patients who underwent SMILE were followed up at 1 week (S\(_{1w}\)), 1 month (S\(_{1m}\)) and 3 months (S\(_{3m}\)) postoperatively, on the other hand, patients who underwent tPRK were followed up at 1 (T\(_{1m}\)) and 3 months (T\(_{3m}\)) postoperatively. At each follow-up time point, the parameters of the operated and unoperated eyes were compared, and the longitudinal changes of the parameters before and after surgery were examined. In terms of postoperative care, patients in the SMILE group were prescribed fluorometholone 0.1% eye drops to be used four times a day. They were gradually decreased every day until they were completely stopped 1 month after surgery. Patients in the tPRK group were initially prescribed tobramycin dexamethasone eye drops for the first week, followed by a gradual reduction of fluorometholone 0.1% eye drops until they were completely stopped at 3-4 months after surgery.

**In vivo laser scanning confocal microscope and image analysis**

Corneal SNP was visualized using an in vivo laser scanning confocal microscope (IVCM, Heidelberg Retina Tomograph with the Rostock Cornea Module, Heidelberg Engineering, Heidelberg, Germany). The IVCM is a non-invasive imaging tool that provides high-resolution images of the cornea, allowing for the visualisation of individual cells and cellular structures. The central and peripheral corneal regions (approximately 3 mm from the apex of the cornea) were scanned for each eye: central, temporal, nasal, superior and inferior regions. At least 10 images of each region were acquired at a focus plane depth of 30-80 µm. Two blinded observers (QG and KH) independently selected three to five images most representative of SNP. The selected images were those located between the basal epithelial layer and the anterior Bowman’s layer, and were in focus, complete and in the same layer, without motion, folds or low contrast.\(^{13}\) The fully automated neural analysis software, ACCMetrics (University of Manchester, Manchester, UK),\(^{14}\) was used to analyse these images to obtain corneal nerve fibre density (CNFD; the number of nerve fibres per mm\(^2\)), corneal nerve branch density (CNBD; the number of branch points on the main nerve fibres per mm\(^2\)), corneal nerve fibre length (CNFL; the total length of nerve per mm\(^2\)), corneal nerve total branch density (CTBD; the total number of branch points per mm\(^2\)), corneal nerve fibre area (CNFA; the total nerve fibre area per mm\(^2\)), corneal nerve fibre width (CNFW; the average nerve fibre width per mm\(^2\)) and corneal nerve fibre fractal dimension (CNFrD), which is used to measure the complexity of corneal nerve fibre structure.\(^{15}\) DCs are characterised by one or more bright dendritic structures with a cell body.\(^{16}\) The density and area of DCs were measured manually in the five regions using Image J software (Heidelberg Engineering, V1.47; NIH, Maryland, USA) as previously described.\(^{16}\) The mean values of the results obtained by two blinded researchers were used as the final results.

**Esthesiometry**

Corneal sensitivity was evaluated using the Cochet-Bonnet esthesiometer (Luneau Ophthalmology, Chartres Cedex, France) by the same physician (KL) who was blinded to the surgical status of the patient during each visit. Bilateral measurements were obtained from the five regions of the cornea: central, temporal, nasal, superior and inferior regions. The specific use of the corneal sensation instrument will be provided in the online supplemental materials.

**Clinical outcome measures**

Each patient underwent the following tests before IVCM imaging according to the dry eye routine clinical protocol\(^{17}\): Ocular Surface Disease Index scores (OSDI) questionnaire, LipiView interferometer ( TearScience, Morrisville, North Carolina, USA), Keratograph 5M (Oculus, Germany), fluorescein tear break-up time test (TBUT) and Schirmer test without anaesthesia. The 12-item OSDI questionnaire was used to assess subjective symptoms of the ocular surface. Lipid layer thickness and partial blink rate were measured using the LipiView interferometer. The non-invasive break-up time, tear meniscus height and bulbar redness were measured using the Keratograph 5M. The ocular surface was stained with fluorescein by introducing a wet Fluoret (Tianjin Jingming New Technology Development Co, Tianjin, China) into the inferior fornix and instructing the participant to blink three times for even corneal distribution. TBUT was recorded as the time from the intact tear film to the ‘rupture point’ observed through a slit lamp with a cobalt blue filter. The Schirmer strip was placed in the lower conjunctival sac at the junction of the lateral and middle thirds, folded back 5 mm and removed after 5 min. The length of the tear-soaked strip was recorded as the Schirmer test value.

**Tear collection and neuromediator analysis**

At each visit, tear samples were collected by Schirmer strips from both eyes for neuromediator analysis. The samples were stored in a ~80°C refrigerator until the day of use for ELISA analysis. The specific methods for collecting tears and detecting tear neuromediator (NGF, SP and CGRP) concentrations using ELISA are provided in the online supplemental materials.
Table 1  Demographic data for SMILE and tPRK participants*

<table>
<thead>
<tr>
<th></th>
<th>SMILE (n=21) (mean±SD)</th>
<th>tPRK (n=13) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Operated eye</td>
<td>Unoperated eye</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.14±3.72</td>
<td>20.08±4.01</td>
</tr>
<tr>
<td>Sphere (D)</td>
<td>−2.69±0.91</td>
<td>−0.07±0.52</td>
</tr>
<tr>
<td>Cylinder (D)</td>
<td>−0.52±0.47</td>
<td>−0.58±0.47</td>
</tr>
<tr>
<td>Spherical equivalent (D)</td>
<td>−2.95±0.97</td>
<td>−0.36±0.55</td>
</tr>
</tbody>
</table>

*Data are presented as mean±SD.
D, diopters; SMILE, small incision lenticule extraction; tPRK, transepithelial photorefractive keratectomy.

Statistical analysis
SPSS V.25.0 (IBM) was used for statistical analysis. Data are presented as mean±SD. A repeated measures analysis of variance test was used to compare preoperative and postoperative data. Multilevel correlation analysis of IVCM parameters, area of DCs, corneal sensitivity and tear neuromediator levels was performed using the correlation package of R studio. The Bonferroni correction for multiple comparisons was applied to confirm statistical significance. Results were considered statistically significant at p<0.05.

RESULTS
Patient characteristics
A total of 34 participants were enrolled in the study (table 1), and divided into the SMILE (21 of 34 participants) and the tPRK (13 of 34 participants) groups. In the SMILE group, the mean age of the patients was 20.1±3.7 years. The mean refractive spherical equivalent was −3.2±1.4 D for the operated eyes and −0.2±0.6 D for the unoperated eyes. All participants completed the 3-month follow-up period, and their data were included in the final analysis.

Corneal nerve analysis
There were no significant differences in baseline values of IVCM parameters between operated and unoperated eyes in all patients preoperatively (all p>0.05). Seven IVCM parameters (CNFD, CNBD, CNFL, CTBD, CNFA, CNFW and CNFrD) in the SMILE group and six IVCM parameters (CNFD, CNBD, CNFL, CTBD, CNFW and CNFrD) in the tPRK groups demonstrated significant differences between the operated and unoperated eyes at each follow-up point postoperatively (as shown in the online supplemental materials).

In both groups, six IVCM parameters (CNFD, CNBD, CNFL, CTBD, CNFA and CNFrD) decreased significantly in the operated eyes, compared with the preoperative values, at each follow-up point (table 2). In the SMILE group, the temporal CNFD was significantly decreased (13.3±2.3 mm/mm² at Spre vs 11.7±3.7 mm/mm² at S1w, p=0.013) and the average CNFW of five corneal regions was significantly increased (0.022±0.001 mm²/mm² at Spre vs 0.023±0.001 mm²/mm² at S1w, p=0.006, figure 1A,B) in unoperated eyes at 1 week postoperatively. Both parameters returned to preoperative levels at 1 month postoperatively.

At 3 months postoperatively, there was a significant increase in central CNFA in the unoperated eyes in the SMILE group (0.005±0.001 mm²/mm² at Spre vs 0.007±0.002 mm²/mm² at S1m, p=0.022, figure 1C). In the tPRK group, the inferior CNFD in the unoperated eyes decreased significantly at 1 month postoperatively (18.7±6.0 n/mm² at Tpre vs 11.9±5.9 n/mm² at T1m, p=0.022) and had not returned to preoperative levels at 3 months postoperatively (9.7±6.7 n/mm² at T3m, p=0.015, figure 1D). Other IVCM parameters did not change significantly in unoperated eyes.

Table 2  Average IVCM parameters, corneal sensitivity and DC parameters in the SMILE and tPRK groups before and after surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SMILE</th>
<th>tPRK</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spre</td>
<td>S1w</td>
<td>S1m</td>
</tr>
<tr>
<td>CNFD (mm²/mm²)</td>
<td>Operated eye</td>
<td>15.46±2.59</td>
<td>15.47±2.69</td>
</tr>
<tr>
<td></td>
<td>Unoperated eye</td>
<td>15.64±2.50</td>
<td>15.47±2.69</td>
</tr>
<tr>
<td>CNBD (n/mm²)</td>
<td>Operated eye</td>
<td>11.45±1.46</td>
<td>11.92±2.29</td>
</tr>
<tr>
<td></td>
<td>Unoperated eye</td>
<td>11.45±1.46</td>
<td>11.92±2.29</td>
</tr>
<tr>
<td>CNFA (mm²/mm²)</td>
<td>Operated eye</td>
<td>29.08±0.09</td>
<td>29.08±0.09</td>
</tr>
<tr>
<td></td>
<td>Unoperated eye</td>
<td>28.88±0.08</td>
<td>28.88±0.08</td>
</tr>
<tr>
<td>CNFA (mm²/mm²)</td>
<td>Operated eye</td>
<td>0.005±0.003</td>
<td>0.005±0.003</td>
</tr>
<tr>
<td></td>
<td>Unoperated eye</td>
<td>0.005±0.003</td>
<td>0.005±0.003</td>
</tr>
<tr>
<td>CNFW (mm²/mm²)</td>
<td>Operated eye</td>
<td>0.022±0.001</td>
<td>0.022±0.001</td>
</tr>
<tr>
<td></td>
<td>Unoperated eye</td>
<td>0.022±0.001</td>
<td>0.022±0.001</td>
</tr>
<tr>
<td>Corneal sensitivity (cm)</td>
<td>Operated eye</td>
<td>5.967±0.058</td>
<td>5.967±0.058</td>
</tr>
<tr>
<td></td>
<td>Unoperated eye</td>
<td>5.967±0.058</td>
<td>5.967±0.058</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001.
The p values for each group are obtained by comparing the postoperative values at each time point with the preoperative values and corrected using Bonferroni correction.
CNBD, corneal nerve branch density; CNFA, corneal nerve fibre area; CNFD, corneal nerve fibre density; CNFL, corneal nerve fibre length; CNFW, corneal nerve fibre fractal dimension; CNFrD, corneal nerve fibre width; CTBD, corneal nerve total branch density; DC, dendritic cell; IVCM, in vivo laser scanning confocal microscope; 1w, 1 month; 1m, 1 month; 3m, 3 months; SMILE, small incision lenticule extraction; Spre, preoperative SMILE group; Tpre, preoperative tPRK group; tPRK, transepithelial photorefractive keratectomy; 1w, 1 week.
Cornea and ocular surface

Analysis of DC parameters

In the SMILE group, the area of DCs was significantly increased in both eyes at 1 week postoperatively (30.4±14.8 µm² at Spre vs 54.9±32.9 µm² at S1w, p=0.007 in operated eyes, table 2). In operated eyes, it returned to preoperative levels at 3 months postoperatively. However, in unoperated eyes, the area had not returned to preoperative levels at 3 months postoperatively (43.4±13.8 µm² at S3m, p=0.031). At 3 months postoperatively, the average density of DCs in the five corneal regions, and more specifically, in the superior corneal region in the unoperated eyes, demonstrated a significant increase (average DC density, 14.5±13.6 cells/mm² at Spre vs 39.5±36.3 cells/mm² at S1w, p=0.031). There were no significant changes in DC parameters in the tPRK group.

Esthesiometry

The corneal sensitivity of the operated eyes in the SMILE and tPRK groups exhibited a significant decrease at 1 week and 1 month after surgery, respectively (average corneal sensitivity, 6.0±0.1 cm at Spre vs 5.1±0.1 cm at Tpre vs 5.8±0.1 cm at T1m, p<0.001; inferior corneal sensitivity, 6.0 cm at Tpre vs 5.7±0.3 cm at T3m, p=0.037, figure 2D).

Clinical dry eye assessments

In the SMILE and tPRK groups, the OSDI of the operated eyes increased significantly at 1 week and 1 month after surgery, respectively (16.9±8.8 at Spre vs 25.8±6.2 at Tpre, p<0.01; 10.8±8.1 at Tpre vs 22.5±10.3 at T3m, p<0.01, figure 3A), while Schirmer test and TBUT values were significantly decreased (Schirmer test, 13.6±8.3 mm at Spre vs 6.7±4.7 mm at S1w, p<0.001; 14.6±7.2 mm at Tpre vs 6.6±5.4 mm at T1m, p=0.004; TBUT, 10.3±4.3 s at Spre vs 4.2±2.1 s at S1w, p<0.001; 9.2±3.8 s at Tpre vs 4.7±2.5 s at T1m, p<0.001, figure 3B,C). These values returned to the preoperative level at 3 months postoperatively.
In the SMILE group, both Schirmer test and TBUT values in the unoperated eyes had significantly decreased at 1 week postoperatively (Schirmer test, 14.1±8.2 mm at Spre vs 9.7±6.4 mm at S1w, p<0.001; TBUT, 9.6±4.7 s at Spre vs 6.0±3.7 s at S1w, p=0.005, figure 3B,C) and returned to the preoperative level at 1 month postoperatively. In the tPRK group, the Schirmer test values in unoperated eyes had also significantly decreased at 1 month postoperatively (14.4±7.6 mm at T pre vs 10.0±6.0 mm at T1m, p=0.001, figure 3B) and recovered at 3 months postoperatively.

Tear neuromediator profiles
In the SMILE group, tear NGF concentration in the operated eyes was significantly increased at 1 week postoperatively (33.0±1.7 pg/mL at Spre vs 63.8±3.9 pg/mL at S1w, p<0.001, figure 3D). It gradually decreased afterwards, but remained higher than the preoperative level at 3 months postoperatively (44.7±7.6 pg/mL at S3m, p<0.001, figure 3D). Tear SP concentration in the operated eyes also significantly increased at 1 week postoperatively (1732.5±82.4 pg/mL at Spre vs 1947.2±43.9 pg/mL at S1w, p<0.001, figure 3E) and returned to the preoperative level at 3 months postoperatively. Tear NGF and SP concentrations in unoperated eyes also significantly increased at 1 week postoperatively (NGF, 33.1±1.8 pg/mL at Spre vs 56.1±3.9 pg/mL at S1w, p<0.001; SP, 1738.0±75.8 pg/mL at Spre vs 1916.5±44.0 pg/mL at S1w, p<0.001, figure 3D,E) and returned to the preoperative levels at 3 months postoperatively.

In the tPRK group, tear NGF and SP concentrations in both operated and unoperated eyes had significantly increased at 1 month postoperatively (NGF, 33.1±1.5 pg/mL at Tpre vs 55.3±9.6 pg/mL at T1m in operated eyes, p<0.001; 33.4±1.7 pg/mL at Tpre vs 43.7±6.8 pg/mL at T1m in unoperated eyes, p=0.001; SP, 1749.6±88.0 pg/mL at Tpre vs 1900.6±51.1 pg/mL at T1m in operated eyes, p=0.003; 1737.3±81.7 pg/mL at Tpre vs 1856.1±58.9 pg/mL at T1m in unoperated eyes, p=0.012, figure 3D,E) and returned to the preoperative level at 3 months postoperatively.

Correlation analysis
In the SMILE group, the temporal corneal sensitivity and CNFL were positively correlated in both eyes (r=0.66 in operated eyes, r=0.63 in unoperated eyes; both p<0.001). However, the average corneal sensitivity and average DC area were negatively correlated (r=-0.38 in operated eyes, r=-0.42 in unoperated eyes; both p<0.01). Both Schirmer test and TBUT were correlated positively with average corneal sensitivity in both eyes (Schirmer test, r=0.45 in operated eyes, p<0.001, r=0.34 in unoperated eyes, p<0.05; TBUT, r=0.48 in operated eyes, r=0.50 in unoperated eyes, both p<0.001). Tear SP and NGF

**Figure 2** Changes in corneal sensitivity measures from baseline (pre) to last follow-up (3m) in operated eyes and unoperated eyes of SMILE and tPRK groups: (A) corneal sensitivity in operated eyes of the SMILE group; (B) corneal sensitivity in unoperated eyes of the SMILE group; (C) corneal sensitivity in operated eyes of the tPRK group; (D) corneal sensitivity in unoperated eyes of the tPRK group. *P<0.05, **p<0.01, ***p<0.001. pre, preoperation; 1w, 1 week; 1m, 1 month; 3m, 3 months; SMILE, small incision lenticule extraction; tPRK, transepithelial photorefractive keratectomy.
Cornea and ocular surface

levels were both positively correlated with the average DC area and OSDI in both eyes, but negatively correlated with Schirmer test, TBUT and average corneal sensitivity. In the tPRK group, there was a positive correlation between inferior corneal sensitivity and inferior CNFD in both eyes (r=0.85 in operated eyes, r=0.71 in unoperated eyes, both p<0.001).

DISCUSSION

This is the first study to identify significant bilateral changes in corneal nerve morphology and corneal sensitivity after unilateral SMILE or tPRK surgeries, with associated changes observed in ocular surface function. In addition to the expected changes in IVCM parameters of the operated eyes after both procedures, temporal CNFL decreased significantly and average CNFW increased significantly in the unoperated eye 1 week after SMILE surgery. This change was transient and a return to preoperative levels was observed at 1 month after SMILE surgery. The central CNFA in the unoperated eye increased significantly 3 months after SMILE surgery. However, only inferior CNFD in the unoperated eyes of the tPRK group was observed to be significantly decreased at 1 month after surgery.

Cruzat et al.18 reported that bilateral corneal nerve density was reduced and DC density was increased in patients with unilateral bacterial keratitis. They also demonstrated a strong correlation between reduced corneal nerve density and increased bilateral DC density, suggesting that the connection between the immune and nervous systems contributes to the development of reactive lesions in the contralateral eye. Two main hypotheses could explain this phenomenon. One suggests10 that unilateral nerve damage induces central nervous system sensitisation, which subsequently affects contralateral nerves through central regulation. The other proposes that the trigeminal pathway crosses the midline to reach the contralateral main nucleus.19 This bilateral connection may allow the direct spread of the virus from the primary affected eye to the contralateral eye.20 This may explain the findings of our research that unilateral corneal refractive surgery can affect bilateral corneal nerves and the ocular surface.

In the present study, a significant increase in bilateral DC area was also observed 1 week after the SMILE surgery, indicating that DCs in both corneas were activated. This change was synchronised with the changes in temporal CNFL and average CNFW. We could therefore speculate that transection of the corneal nerve in the operated eye may lead to Wallerian degeneration of distal axons and recruitment of inflammatory cells, including DCs.21 These activated immune cells may migrate to the contralateral eye surface through the draining lymph nodes and aggravate the degenerative changes in the bilateral corneal nerves, leading to the SNP changes in the contralateral eye.12 The bilateral alteration may also be attributed to the retrograde signals transmitted through the centripetal neural interconnection. The neurogenic inflammation resulting from the local release of proinflammatory neuropeptides may contribute to changes in the fellow eye.11 The DC area of the operated eyes returned to the preoperative level earlier than that of the unoperated eyes. This could be due to the use of fluorometholone eye drops within 1 month after the surgery. These drops have an anti-inflammatory effect.22 However, the increase in CNFA in unoperated eyes was not observed until 3 months after the surgery, suggesting that the inflammatory response in the unoperated eyes could be a chronic process. Insignificant changes in DC parameters in the tPRK group could be attributed to the use of corticosteroid eye drops postoperatively, which would have suppressed inflammation in the operated eye. The usage of topical corticosteroids in the operated eye is a possible

Figure 3  Changes in dry eye symptoms and tear neuromediator measures from baseline (pre) to last follow-up (3m) in operated eyes and unoperated eyes of SMILE and tPRK groups: (A) OSDI scores; (B) Schirmer test; (C) TBUT; (D) tear NGF concentration; (E) tear SP concentration. *P<0.05, **p<0.01, ***p<0.001. pre, preoperation; 1w, 1 week; 1m, 1 month; 3m, 3 months; OSDI, Ocular Surface Disease Index; TBUT, tear break-up time; NGF, nerve growth factor; SP, substance P; SMILE, small incision lenticule extraction; tPRK, transepithelial photorefractive keratectomy.
Corneal innervation is associated with neurotrophic factors, neuropeptides, neurotransmitters and other biologically active chemicals, collectively referred to as neuromediators. The three neuromediators most commonly affected by refractive surgery in the cornea are NGF, SP and CGRP. These have demonstrated significant potential in promoting corneal healing and nerve regeneration. We also observed that the corneal sensitivity in the operated eyes decreased postoperatively in both groups. The corneal sensitivity in the unoperated eyes also decreased to a certain extent but returned to the preoperative levels 3 months postoperatively. The corneal sensitivity was positively correlated with CNF and negatively correlated with DC area in the unoperated eyes in the SMILE group. It was positively correlated with CNFD in the tPRK group. This confirms our findings regarding the functional level of corneal nerves.

Corneal nerves are essential for the sensory function of the cornea. They maintain the integrity of ocular surface function by releasing nutrients that promote corneal epithelial homeostasis, and stimulate the secretion of reflex tears and the blink reflex. Corneal nerve injury can lead to a dry eye after SMILE and tPRK. The reduced corneal sensitivity caused by corneal nerve transection leads to decreased blink frequency, longer ocular surface exposure, increased tear evaporation and osmolarity, and unsteadied tear film. Damage to afferent nerve pathways of the ocular surface can also lead to reduced reflex tear secretion. The occurrence of dry eye symptoms in operated eyes could also be due to the postoperative increase in the partial blink rate, and DCs have been demonstrated to be crucial for the dry eye pathogenesis. OSDI scores reflect the subjective dry eye symptoms of patients. In our study, OSDI scores were higher in the operated eyes compared with the unoperated eyes, which might be related to the patients’ stronger feeling of discomfort in the operated eye after surgery. Schirmer test and TBUT results reflect tear secretion ability and tear film stability, respectively. The decrease in bilateral TBUT and Schirmer test results in the SMILE group postoperatively and the decrease in Schirmer test in the tPRK group postoperatively suggest that surgery may have caused damage to the quality and quantity of the tear film in both eyes. The deterioration of these dry eye parameters was negatively correlated with corneal sensitivity. This indicates that the ocular surface function of both eyes is affected after unilateral laser vision correction surgery.

SP is an 11-amino acid long neuropeptide, which is secreted by neuronal and non-neuronal cells, including the immune cells in the cornea. It binds to neurokinin receptors (neurokinin 1 receptor), affecting corneal healing and ocular surface inflammation. In our study, the tear SP concentration increased bilaterally after unilateral SMILE and tPRK surgeries. It was positively correlated with DC area and dry eye severity in both eyes in the SMILE group. Previous studies have reported that tear SP concentration increased after LASIK. It was positively correlated with dry eye symptoms and negatively correlated with CNFD and corneal sensitivity. Our results are consistent with these findings. This suggests that after unilateral refractive surgery, inflammatory reactions also occur in the unoperated eyes, leading to dry eye symptoms. The changes in tear NGF and SP in unoperated eyes might be due to the transection of corneal nerve in the operated eyes.

The study had some limitations. The follow-up period was only 3 months. However, we will conduct more long-term follow-up investigations. Our patient population was limited to those aged between 18 and 20 years and neural recovery may be closely related to age. Therefore, future research should consider expanding the age range of participants to better improve the generalisability of the findings. Finally, our study only included SMILE and tPRK procedures, and the optical zone and cap diameter of all SMILE patients were the same in all patients except for one. It will be interesting to explore the influence of different optical zones and cap diameters on sub-basal nerve changes after SMILE in future studies; additionally, we plan to add patients who underwent LASIK to expand our research.

Unilateral refractive surgery is commonly performed for patients with visual impairment. In conclusion, our study demonstrates that even in patients who undergo unilateral refractive surgery, changes in corneal nerves and ocular surface function occur bilaterally after surgery. This highlights the importance of monitoring the unoperated eye after unilateral refractive surgery, such as recommending the bilateral use of artificial tears after surgery. Additionally, as the unoperated eye may be impacted by changes in the operated eye, it would not be an appropriate control in certain clinical studies.
Cornea and ocular surface

includes any translated material. BMI does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID IDs
Fan Lu http://orcid.org/0000-0002-8794-6944
Liang Hu http://orcid.org/0000-0003-1422-4008

REFERENCES