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Transcutaneous Electrical Stimulation for the Prevention of Dry Eye Disease after photorefractive keratectomy: Randomized Controlled Trial

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Meeting Presentation: None

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Conflicts of Interest
None of the authors has any conflicts of interest to disclose.

**Running head**: Electrostimulation and dry eye after refractive surgery

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**Key words**: Electrostimulation, dry eye, refractive surgery, corneal nerve regeneration
Abstract

Objective: To evaluate the efficacy and the safety of transcutaneous electrical stimulation (TES) for the prevention of dry eye after photorefractive keratectomy (PRK)

Design: Prospective, single-center, single-blinded, parallel group, placebo-controlled, randomized clinical trial

Patients: Between February 2020 and October 2020 patients at the Samsung Medical Center, scheduled to undergo photorefractive keratectomy (PRK) to correct myopia were screened and enrolled.

Method: The participants in the TES group were instructed to use the electrical stimulation device ((Nu Eyne 01, Nu Eyne Co., Seoul, Korea) at the periocular region after the operation while those in the control group were to use sham device. Dry eye symptoms were evaluated pre and postoperatively at weeks 1, 4, and 12 using the ocular surface disease index (OSDI) questionnaire, the 5-Item Dry Eye questionnaire (DEQ-5), and the standard patient evaluation for eye dryness II (SPEED II) questionnaire. Dry eye signs were assessed using tear break-up time (TBUT), total corneal fluorescein staining (tCFS), and total conjunctival staining score according to the National Eye Institute/Industry (NEI) scale. The pain intensity was evaluated using a visual analog scale (VAS).

Main Outcome Measures: Primary outcomes were OSDI and TBUT.

Results: 24 patients were enrolled and completed follow-up until the end of the study (12 patients in TES group, 12 patients in control group). Refractive outcomes and visual acuity were not different between the groups. No SAEs were reported with regard to device use. No significant difference in OSDI, DEQ-5, and SPEED II questionnaire was observed between the groups in the 12th weeks after surgery. The TBUT scores 12 weeks after the surgery was 9.28±6.90 s in the TES group and 5.98 ± 2.55 s in the control group with significant difference
(p = 0.042). The tCFS and total conjunctival staining score were significantly lower in the TES group than in the control group at postoperative 4 weeks. Pain intensity at 1st week was significantly lower in the TES group compared to the control group by 65% (p = 0.011)

**Conclusion:** The application of TES is safe and effective in improving dry eye disease after PRK.
Introduction

In the second meeting of the Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) in 2017, dry eye disease (DED) was defined as a multifactorial disease of the ocular surface characterized by loss of tear film homeostasis and other ocular symptoms in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play an etiological role. Nerve fibers in the corneal epithelium are responsible for maintaining normal corneal structure and function. Corneal nerves play a significant role in maintaining tear film stability through tear secretion, blinking reaction, and corneal wound healing. However, corneal sensory innervation can be disrupted by various factors, including trigeminal nerve damage, refractive surgery, and chronic contact lens wear. A signaling cascade that leads to an inflammatory reaction and reduced lacrimal secretion can ultimately result in DED-related to corneal nerve damage.

DED-related to corneal nerve damage occurs most commonly post refractive surgery. Refractive surgery is known to induce dry eye owing to decreased corneal sensation, reduced tear production, and impaired wound healing after surgery. A periodic analysis of the corneal nerve physical condition, corneal sensory function, and DED level of patients who underwent laser surgery demonstrated that the corneal nerve density and corneal sensitivity decreased drastically after laser surgery, and the degrees of eye dryness and pain increased simultaneously. Although there are signs of improvement over time, a significant part of the corneal nerve do not recover, leading to persistent eye dryness and pain. Accordingly, it is crucial to develop efficient ways to accelerate and improve the wound healing process, thereby avoiding DED that may arise from damage to the structural integrity of the cornea and the disruption of tear secretion.

Many attempts have been made to accelerate or promote wound healing and nerve
regeneration with electrical stimulation. It has been suggested that the application or enhancement of the electric field can increase the wound healing rate. Electrical stimulation affects cell migration, proliferation, and accelerates nerve regeneration. The promotion of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), through increased calcium influx into the neurons has been suggested to help with nerve and tissue regeneration. In particular, Ghaffariyeh et al. reported that electrical stimulation after surgery improved corneal nerve recovery in an animal study. Moreover, transcutaneous electrical stimulation (TES) around the periorbital regions with DED showed improvement in DED symptoms.

Dry eye after photorefractive keratectomy (PRK) is the most common short- or long-term complication and remains a major concern for practitioners. As described above, this complication results from corneal nerve damage during PRK. Despite, clinical studies showing that periorbital TES improved general dry eye disease and ocular pain, the effects of TES in DED-related to corneal nerve damage has not yet been evaluated. Therefore, we postulated the wound healing and nerve regeneration effects of TES and further investigated the efficacy and safety of TES in treating DED-related to corneal nerve damage after PRK.

Methods

Patients

This prospective, single-center, randomized, single-blind, parallel group, placebo-controlled trial adhered to the tenets of the Declaration of Helsinki. The study protocol was reviewed and approved by the Institutional Review Board and Samsung Medical Center (SMC 2019-
The study was conducted at the Samsung Medical Center. Patients scheduled to undergo PRK to correct myopia were screened and enrolled. The inclusion criteria were as follows: age 19 to 60 years, refractive error of −0.50 to −7.50 diopters (D) of spherical myopia, astigmatism between 0.00 and 3.00 D, and distance visual acuity correctable to ≥ 0.1 logarithm of the minimal angle of resolution. The exclusion criteria were as follows: a history of recurrent corneal erosions, basement membrane disease or keratoconus; an estimated postoperative residual stromal bed thickness of < 350 µm; uncontrolled systemic diseases, including diabetes mellitus and autoimmune disease, retinal disease, and glaucoma; previous ophthalmic surgery within 6 months; systemic medications known to affect the ocular surface (e.g., tetracycline derivative, anti-histamines, and isotretinoin), structural lid abnormalities, active ocular inflammation, and pregnancy. Upon detailed explanation of the study, all patients provided written informed consent. Eligible patients were randomly allocated to the study or control group (Figure 1). The randomization was conducted by computer-generated random number allocation and applied to sequentially enrolled patients. The randomization schedule was pre-determined, prior to commencing participant recruitment, such that the investigator involved in the baseline participant assessment were not involved in the treatment allocation.

Surgical technique

All surgeries were performed by two surgeons (T-YC and DHL) using standardized techniques. Eligible patients were scheduled to undergo bilateral PRK surgery using a WaveLight EX500 excimer laser (Alcon, Fort Worth, Texas, US). The correction target was
based on the manifest refraction adjusted using the Alcon nomogram, with emmetropia being the target in all patients. A few drops of 20% alcohol were instilled into an 8.0-mm well to create a round epithelial defect. After 25 s, the alcohol was removed and a balanced salt solution was poured to irrigate the cornea. The epithelium was then removed smoothly using a spatula, and laser ablation was performed. The eyes were once again irrigated with a balanced salt solution. Sponges soaked in 0.05% Mitomycin-C solution were placed on the eye for 20 sec, followed by vigorous irrigation with a balanced salt solution. Finally, an AcuVue Oasys (Johnson and Johnson, New Brunswick, New Jersey, USA) bandage contact lens was placed on the eye. The same procedure was repeated in the other eye.

**Postoperative medications**

Patients with PRK received the following medications: topical moxifloxacin 0.5% eye drops (Vigamox, Alcon, TX, US) 4 times a day for 2 weeks, and topical loteprednol 0.5% eye drop (Lotepro, Hanlim, Korea) 6 times a day for 2 weeks; this schedule was then tapered for 3 months. All patients were prescribed ketorolac (Acuvail, Allergan, CA, US), which was permitted to be used up to three times a day for unbearable pain on the day of surgery. The artificial tear (hyaluronic acid 0.15%) was also prescribed for their use if needed.

**Transcutaneous electrical stimulation (TES)**

TES was administered to the study group, whereas the sham device was assigned to the control group. An electrode patch was designed to provide electrical stimulation at the periocular region, with four electrodes placed on the supra- and infraorbital regions (Figure 2(b)). The electrode patch was connected to a goggle-like device (Nu Eyne 01, Nu Eyne Co.,
Ltd., Seoul, Korea) (Fig. 1(a)) that output an electric current which subsequently generated electric stimulation on the ocular surfaces and around the associated branches of the trigeminal nerve. A 2-Hz biphasic pulse was used with a phase and an interphase interval of 250-μsec and 5-μsec, respectively. For the study group, the amplitude of the pulse was applied between 4-mA and 7-mA and was controlled manually by each patient to the maximum tolerable level. The biphasic pulse was alternatively applied with an inverted waveform (Figure 1(d)) to prevent charge accumulation. The sham device had the same appearance as the real device but only a 0.5 mA biphasic pulse was delivered once every 30 s to confirm proper attachment of device on participant. The participants were instructed to use the device once a day for 15 to 30 min for 2 weeks, and then only once a week for 2 weeks until 3 months after the operation.

Patient evaluation

A complete ophthalmological examination was performed for all patients preoperatively, including uncorrected distant visual acuity (UDVA), best-corrected distant visual acuity (BDVA), manifest refraction, cycloplegic refraction, slit-lamp microscopy, and fundus examination. After the surgery, patients visited the clinic at postoperative days 1 and 3, and weeks 1, 4, and 12 and received the scheduled examination.

Dry eye signs and symptoms were evaluated pre and postoperatively at weeks 1, 4, and 12. Dry eye symptoms were assessed using the ocular surface disease index (OSDI) questionnaire, the 5-Item Dry Eye questionnaire (DEQ-5), and the standard patient evaluation for eye dryness II (SPEED II) questionnaire. Dry eye signs were assessed using tear break-up time (TBUT), Schirmer’s I test without anesthesia, total corneal fluorescein staining (tCFS), and total conjunctival staining score according to the National Eye Institute/Industry (NEI)
The pain intensity was evaluated using a visual analog scale (VAS). The VAS was on an 11-point scale ranging from 0 to 10 points, where 0 corresponded to “no pain” and 10 corresponded to “the worst imaginable pain.” The pain intensity was evaluated before surgery and at post-operative days 1, 3, and 7.

Tear osmolarity tests were performed using a TearLab® Osmolarity System (TearLab Corporation, CA, USA). Lipid layer thickness (LLT) measurement was performed using a LipiView interferometer (TearScience Inc., NC, USA) pre and postoperatively after 12 weeks. The test for matrix metalloproteinase 9 (MMP-9) in the tear film was performed using InflammaDry (Quidel, CA, USA). The test result of InflammaDry was categorized into five levels (negative, trace positive, weak positive, positive, and strong positive) then interpolated to the concentration by comparing with a previous reference.

Corneal sensitivity was assessed using a Cochet-Bonnet esthesiometer (Harada, Tokyo, Japan). “Touch,” “pain,” and “blink” sensations were evaluated and recorded in millimeters.

Statistical analysis

This study was an exploratory clinical trial; therefore, we set 10 patients in each group, which is the minimal sample size for evaluating efficacy and safety. Assuming a 20% dropout rate, the target sample size was calculated as 24 subjects. Statistical analyses were performed using (program). The primary outcomes were the OSDI score and TBUT. Intention-to-treat analysis was conducted for all tests. The Wilcoxon signed-rank test was used for intra-group comparisons and the Wilcoxon rank-sum test was used for inter-group comparisons. The safety index was defined as the ratio of the postoperative CDVA at 3 months to the pre-
operative CDVA. The efficacy index was defined as the ratio of postoperative UDVA at 3 months to the pre-operative CDVA. Data are presented as the mean ± standard deviation (SD) unless otherwise stated. All tests were two-tailed, and p < 0.05 was considered significant.

Results

The study was conducted between February 2020 and October 2020. Thirty-five patients were screened and among these participants, eight did not meet the criteria while three did not agree to the participation terms and conditions. Finally, twenty-four patients were enrolled in this study of whom twelve were included in the TES group with real stimulation and the other twelve were included in the control group with sham stimulation. All registered patients completed follow-up until the end of the study. In the TES group, the mean age of the 12 patients was 28.3 ± 4.7 years (range, 19–37 years), and 6 were female. In the control group, the mean age of the 12 patients was 26.6 ± 5.6 years (range, 20–35 years), and 7 were female. The preoperative refractive error did not differ significantly between the two groups. Demographics and preoperative data of the study population are presented in Table 1.

Three months after the procedure, the UDVA in the TES group was 0.015 ± 0.044 logMAR, whereas that in the control group was 0.010 ± 0.024 logMAR, with no significant difference between the groups (p = 0.775, Wilcoxon rank-sum test). Of the eyes in the TES and control groups, 87.5% and 83.3% had 20/20 or better UDVA, respectively. The spherical and cylindrical error and spherical equivalent also showed no significant differences between the groups. The efficacy index (0.97 in TES group vs 0.98 in the control group) and safety index were similar between groups (1.17 in the TES group vs. 1.13 in the control group, respectively). Detailed information on the refractive outcomes is presented in Table 2.
A total of 73 adverse events were reported during the clinical investigation; 60% of these were associated with visual function, eye dryness, and pain associated with LASEK, whereas 40% were related to the use of the investigation device. While using the device, headaches, skin rashes, drowsiness, mild discomfort, and skin breakout were reported. All events occurred in both the study and control groups and were short-lasting and temporary. A single serious adverse event (SAE) was reported in a patient with pneumothorax, which was concluded to be irrelevant to device use and to this clinical trial. No SAEs were reported with regard to device use, thus demonstrating its safety (Table 3). Additionally, all LASEK procedures were performed successfully, and showed no effect on surgery outcomes upon device usage.

The pre-operative OSDI scores were 36.92 ± 19.48 and 38.17 ± 21.98 the study and control groups, respectively. No significant difference was observed between the groups (p = 0.885, Wilcoxon rank sum test). In both groups, the OSDI scores improved in the 12th week after surgery when compared to the measurements prior to surgery (Figure 3(a)). No significant difference was reported between the groups in the 12th week after surgery (p = 0.885, Wilcoxon rank-sum test). Similar trends were observed in the DEQ-5 and SPEED II surveys between the two groups in terms of the changes before and after surgery. Both groups improved from before to after surgery, but no significant differences were found between the two groups in the 12th week (Figure 3(b), 3(c)).

The TBUT scores prior to the surgery was 5.66 ± 2.88 s in the TES group and 4.40 ± 2.04 s in the control group, and showed no significant difference between the groups (p = 0.081, Wilcoxon rank sum test). The TBUT scores 12 weeks after the surgery was 9.28±6.90 s in the TES group and 5.98 ± 2.55 s in the control group, with significant difference of 63% increase in the TES group than in the control group (p = 0.042, Wilcoxon rank sum test)
(Figure 4(a)). When the study and control groups were compared using the Schirmer’s test measured before and 12 weeks after the surgery, the groups improved by 40% with no significant difference between the two groups (Figure 4(b)). Although the corneal fluorescent staining scores 12 weeks after surgery decreased in both the study and control groups to a similar level, the rate of decrease was more rapid in the TES group. The score at 4 weeks after treatment was 52% lower than that at 1 week, with a significant difference from the control group (p = 0.003, Figure 5(a)). The conjunctival fluorescent staining score decreased more rapidly in the TES group than in the control group. Whereas the TES group showed 81% decrease 4 weeks after surgery and 85% decrease 12 weeks after surgery, the control group decreased by 53% on average 12 weeks after surgery. The differences from weeks 4th to the 12th weeks remained significant (p = 0.0290 at 4th weeks, p = 0.026 at 12th week, Figure 5(b)).

Although randomized with each group of 12 patients, the osmolarity scores were significantly higher before surgery in the TES group (p = 0.022, Wilcoxon rank sum test) (Figure 5(a)). When comparing the differences between the two groups with the scores before surgery and 12 weeks after surgery, osmolarity was decreased in the TES group and increased in the control group (p = 0.030, Wilcoxon rank sum test) (Figure 6(b)). Although randomized with each group of 12 patients, Lipid layer thickness (LTT) was significantly higher in the control group before surgery (p = 0.009, Wilcoxon rank sum test). In contrast, the TES group had increased LTT and the control group had decreased LTT 12 weeks after surgery (Figure 7(a)). When comparing the differences between the two groups with the scores before and 12 weeks after the surgery, a significant difference was observed (p = 0.023, Wilcoxon rank sum test) (Figure 7(b)). Moreover, the concentrations of matrix metallopeptidase-9 (MMP-9) were not significantly different between the groups before and 12 weeks after surgery. (Figure 8)
By 12 weeks after surgery, all patients in both groups showed a normal range (55–60 mm) in the esthesiometer test in touch sensation, and no difference was found between the groups. (Figure 9) Pain sensation also showed no difference at 12 weeks. The change in pain sensation before and after surgery was smaller in the TES group, although not significant (7.04 mm decrease in the control group vs. 1.67 mm decrease in the TES group, p = 0.214, Wilcoxon rank sum test). The change in the blink sensation showed a similar trend. When questioned, the eye pain score based on the VAS began to decrease 3 days after surgery. By the first week, the pain had decreased by 65% compared to the control group (p = 0.011, Wilcoxon rank sum test) (Figure 10).

**Discussion**

This prospective, randomized, single-blinded, and placebo-controlled clinical trial revealed that TES was effective in improving DED-related to corneal nerve damage after PRK. TES after PRK showed significant improvement in subjective symptoms, TBUT scores, along with reduction in eye pain and staining scores. In addition, in the TES group, tear film osmolarity and LLT decreased and increased, respectively as opposed to the control group ultimately showing improvements in dry eye status. No serious adverse effects were observed from the application of TES for 12 weeks after PRK.

Several studies have reported the induction of the reinnervation effect through electrical stimulation of the peripheral nerves.\(^\text{24,25,36-39}\) Accordingly, electrical stimulation promotes expression of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) resulting in faster wound healing and reinnervation.\(^\text{24,25}\) In an animal study in rats, BDNF expression was upregulated upon immediate electrical stimulation of damaged facial and sciatic nerves with crush axotomy.\(^\text{36,37}\)
Other studies report peripheral nerves and Schwann cells were induced to express NGF with electrical stimulation.\textsuperscript{24,38,39} Interestingly, NGF is responsible for goblet cell differentiation and mucin production, which is required for better tear film maintenance.\textsuperscript{40} Furthermore, Lambiase et al. demonstrated that NGF was responsible for corneal wound healing.\textsuperscript{41} Additionally, animal study from our group showed both NGF expression and corneal nerve density was higher in the TES group than the control group (unpublished data). Based on these data and those from previous studies, we hypothesized that TES would be efficient in improving DED related to corneal nerve damage.

Previous clinical studies evaluating the effect of TES showed improvement in dry eye\textsuperscript{27,28} and chronic ocular pain;\textsuperscript{40} however, these studies focused primarily on general dry eye and ocular pain populations. DED after PRK is different from general dry eye disease and is known to be associated with damage to the corneal afferent nerve during PRK leading to a disruption in sensory input to the ocular surface lacrimal gland feedback system.\textsuperscript{7} Considering the mechanism of TES, DED related to corneal nerve damage seems to be the best candidate for testing the effects of electrical stimulation. Corneal nerve damage is associated with dry eyes\textsuperscript{42} and dry eye symptoms are common in the setting of corneal nerve injury followed by refractive surgery.\textsuperscript{43} We were able to demonstrate improvement in TBUT and ocular surface staining after TES in this study. Moreover, there was a general decrease in eye dryness measured through subjective questionnaires, such as the OSDI, DEQ-5, and SPEED II (Fig. 2), but no difference was found between the groups despite objective dry eye indicators showing better results in the TES group. This may have originated from the corneal denervation after PRK which relatively requires longer time to recover. Changes in subepithelial plexus and stromal trunks are known to appear from 2-4 months after surgery\textsuperscript{44} while the nerve density continues to improve until 12 months after the surgery. Thus, comparing the results 3 months after the surgery in this study may not be long enough to see
a difference. Also, patients after PRK experience relatively severe pain than the general dry eye disease patients throughout the study period. Such outcome imposes difficulty in detecting improvements in dry eye symptoms by electrical stimulation at the sub-chronic postoperative stage (2-3 months) of PRK. There is a study reporting no difference in symptom scores between the cyclosporine treated study group and control group at 2 months after PRK. Interestingly study showed electrostimulation improved symptom scores in general dry eye wherein corneal nerve received milder damage. This suggests, DED from relatively mild corneal nerve damage such as from cataract surgery would be more feasible to show improvement of symptoms by electrostimulation.

The effects of electric stimulation and neuromodulation on pain relief have been well-established. In our previous clinical study, oral medication for neuropathic pain was also effective for early stage pain relief after PRK. However, the TES group showed lower pain scores 1 week after surgery than did the control group and did not differ at days 1 and 3 between the two groups in the present study. In general, patients’ complaints associated with pain after PRK were clinical manifestations and developed during the first 3 days. This implies that the lower pain score in the TES group at 1 week might have been due to an improvement in DED related to corneal nerve damage in the study group.

Additionally, the present study did not demonstrate a significant difference in corneal sensitivity between the study and control groups (Fig. 9). For a typical study, it is suggested that central corneal sensitivity recovers within 6 months after surgery, and corneal nerve density to take 12 months or more. Thus, it would take more than 12 weeks to monitor and measure the impact of electrical stimulation on corneal sensitivity. Moreover, the results of esthesiometer were almost all in normal range for both groups at 12 weeks. As mentioned above, the changes in subepithelial plexus and stromal trunks after PRK starts 2 to 4 months
post-operatively\textsuperscript{44} however, it is hard to assume that corneal nerve had been fully regenerated at 12 weeks in both groups. Previous report noted that while corneal nerve density and length had been decreased after 12 month post-PRK in \textit{in vivo} confocal microscopy, corneal sensitivity measured by Cochet-Bonnet esthesiometer recovered to preoperative levels within 1 month after surgery.\textsuperscript{50} We assume the lack of difference in corneal sensitivity did not directly correlate with the disuse of electrostimulation since the use of esthesiometer had no effect on corneal nerve status in PRK patients. Further study using \textit{in vivo} confocal microscopy may be required in order to come up with a deductive conclusion.

An interesting result in this study is that TES increased LLT and decreased tear osmolarity. The lipid layer of the tear film prevents evaporation of the aqueous layer and maintains tear osmolarity thus, playing an important role in tear film stability.\textsuperscript{51} Inflammation of the cornea and conjunctiva as well as high osmolarity of the tear film causes inflammation in the meibomian glands, which secrete lipid components.\textsuperscript{6,52} The improvement of TBUT and both corneal and conjunctival staining upon TES, along with increase in LLT and decrease in tear osmolarity, suggests TES improves dry eye via various mechanism. The faster improvement in the corneal and conjunctival staining scores in the TES group seems to be associated with increased mucin and lipid production and reduced tear osmolarity through TES. Additionally, this result may demonstrate that electrical stimulation induces wound healing in the corneal and conjunctival tissue aligning with the findings of Song et al.\textsuperscript{22} and Ghaffarieh et al.\textsuperscript{23}

There are limitations to this study as this was a pilot study involving small number of participants. The subjective symptoms did not significantly differ between the TES and control groups thus, suggests the small number of participants might not have been sufficient to detect these differences in the present study. Increasing the number of participants is
necessary for future studies. In addition, the overall study period was relatively short to further investigate the outcome of nerve regeneration. To fully evaluate the effect of TES on DED-related to corneal damage after PRK, a larger study with a longer follow-up period is required.

In conclusion, the application of TES is safe and effective in improving DED after PRK. Further studies are needed to evaluate patients with a more general type of DED without exposure to external events such as laser surgery.
Acknowledgement

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Supervision: Dong Hui Lim, Tae-Young Chung

Project administration: Young Sik Yoo, Pyungkyu Kim, Dong Hui Lim, Tae-Young Chung
References


34. Brujic M KD. Making matrix metalloproteinase-9 levels more meaningful. presented at: Global Specialty Lens Symposium; January 21-24 2016; Las Vegas


Figure Legends

Figure 1. Flow diagram of the study

Figure 2. Electrical stimulator and electrode (NuEyne 01, Nu Eyne inc., Seoul, Korea) (a), examples of electrode attachment (b), device application (c), and waveform of electrical stimulation (d).

Figure 3. Subjective dry eye symptom scores from Study and Control groups. (Pre: screening prior to the surgery, 12Wk: 12 weeks after the surgery, * : p<0.05, ** : p<0.01, Wilcoxon Signed-Rank Test) (a) Ocular Surface Disease Index (OSDI), (b) 5-Item Dry Eye Questionnaire (DEQ-5), (c) Standard Patient Evaluation for Eye Dryness II (SPEED II)

Figure 4. Tear break-up time (A) and Schirmer test (B) results before and 12 weeks after the surgery. (Pre: Screening prior to the surgery, 12Wk: 12 weeks after the surgery, * : p<0.05, Wilcoxon Rank Sum Test)

Figure 5. Corneal (a) and conjunctival (b) fluorescent staining scores 1 week, 4 weeks, and 12 weeks after the surgery compared between the study and control groups. (1Wk: 1 weeks after the surgery, 4Wk: 4 weeks after the surgery, 12Wk: 12 weeks after the surgery, p<0.05, Wilcoxon rank sum test)

Figure 6. Osmolarity test scores before and 12 weeks after the surgery compared between the test and control groups (a), Differences of osmolarity test scores between before and 12weeks after the surgery of the study and control groups (b). (Pre: Screening before the surgery, 12Wk: 12 weeks after the surgery, * : p<0.05, Wilcoxon rank sum test).

Figure 7. Lipid layer thickness (LTT) before and 12 weeks after the surgery compared between the study and control groups (a). Differences of LLT between before and 12 weeks after the surgery of the study and control groups (b). (Pre: Screening before the surgery,
12Wk: 12 weeks after the surgery, * : p<0.05, ** : p<0.01, Wilcoxon rank sum test)

**Figure 8.** Concentrations of MMP-9 (matrix metalloproteinase-9) before and 12 weeks after the surgery compared between the study and control groups. (Pre: Screening before the surgery, 12Wk: 12 weeks after the surgery, * : p<0.05, ** : p<0.01, Wilcoxon rank sum test)

**Figure 9.** Corneal sensitivity measured with corneal aesthesiometer before and 12 weeks after the surgery to be compared between the study and control groups. (Pre: Screening before the surgery, 12Wk: 12 weeks after the surgery, * : p<0.05, ** : p<0.01, Wilcoxon rank sum test) Touch: sensing level (a), Pain: pain level (b), Eye_Closed: While the eyes were closed (c)

**Figure 10.** Pain rating score with visual analogue scale (VAS) change before and after the surgery. (1Day: 1 day after, 3Day: 3 day after, 1Wk: 1 week after the surgery, * : p<0.05, Wilcoxon rank sum test)
Table 1. Demographics and preoperative data of study participants

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<td>Male</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Spherical error(D)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.88 ± 1.23</td>
<td>3.80 ± 1.39</td>
<td>3.95 ± 1.20</td>
<td>0.694</td>
</tr>
<tr>
<td>Cylindrical error(D)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.67</td>
<td>1.00 ± 0.80</td>
<td>0.80 ± 0.49</td>
<td>0.311</td>
</tr>
<tr>
<td>Central corneal thickness (μm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>551.8 ± 24.9</td>
<td>545.9 ± 22.3</td>
<td>557.8 ± 26.4</td>
<td>0.101</td>
</tr>
</tbody>
</table>

<sup>a</sup>Value was expressed as mean ± standard deviation, <sup>b</sup>Student t-test, <sup>c</sup>chi-squared test

Table 2. Refractive outcomes of the participants at 12 weeks after the surgery

<table>
<thead>
<tr>
<th></th>
<th>Total Subjects</th>
<th>Study Subjects</th>
<th>Control Subject</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=24)</td>
<td>(N=12)</td>
<td>(N=12)</td>
<td></td>
</tr>
<tr>
<td>UDVA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.012 ± 0.035</td>
<td>0.015 ± 0.044</td>
<td>0.010 ± 0.024</td>
<td>0.775</td>
</tr>
<tr>
<td>BDVA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.054 ± 0.073</td>
<td>-0.061 ± 0.080</td>
<td>-0.046 ± 0.068</td>
<td>0.579</td>
</tr>
<tr>
<td>Spherical error(D)</td>
<td>0.19 ± 0.30</td>
<td>0.14 ± 0.26</td>
<td>0.24 ± 0.34</td>
<td>0.142</td>
</tr>
<tr>
<td>Cylindrical error(D)</td>
<td>-0.08 ± 0.21</td>
<td>-0.06 ± 0.15</td>
<td>-0.10 ± 0.26</td>
<td>0.207</td>
</tr>
</tbody>
</table>
Spherical equivalent(D) | 0.15 ± 0.33 | 0.11 ± 0.26 | 0.19 ± 0.40 | 0.310  
Efficacy index | 0.97 | 0.97 | 0.98 | 0.775  
Safety index | 1.15 | 1.17 | 1.13 | 0.579  

UDVA=uncorrected distant visual acuity, BDVA=best-corrected distant visual acuity

^aVisual acuity is presented as logMAR scale.

^bWilcoxon rank sum test

Table 3. Summary of Overall Adverse Events (Safety Population)

<table>
<thead>
<tr>
<th></th>
<th>Study Subjects (N=12)</th>
<th>Control Subject (N=12)</th>
<th>Total (N=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of AEs</td>
<td>27</td>
<td>46</td>
<td>73</td>
</tr>
<tr>
<td>Total number of SAEs</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total number of device-related AEs</td>
<td>8 (3)</td>
<td>21 (4)</td>
<td>29 (7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AEs</th>
<th>Study Subjects (N=12)</th>
<th>Control Subject (N=12)</th>
<th>Total (N=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>4 (2)</td>
<td>9 (2)</td>
<td>13 (4)</td>
</tr>
<tr>
<td>Skin rash</td>
<td>3 (2)</td>
<td>4 (2)</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>1 (1)</td>
<td>5 (1)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Skin discomfort</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin breakout</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (1)</td>
<td>1 (1)</td>
<td></td>
</tr>
</tbody>
</table>

AE=Adverse Event, SAE=Serious Adverse Event
Précis
Transcutaneous electrical stimulation was safe and effective in improving DED after photorefractive keratectomy.