ORIGINAL ARTICLE

Confocal Microscopic Examination in Lasek (Laser Subepithelial Keratomileusis)

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ABSTRACT

Purpose: This study aimed to examine the morphological changes in the cornea after laser subepithelial keratomileusis (LASEK) in vivo by confocal microscopy.

Materials and Methods: Patients who underwent LASEK due to myopia and myopic astigmatism were examined with a confocal microscope in the preoperative period, at 1, 3 and 6 months postoperatively. Forty nine eyes of 26 patients were included in the study. The mean age was 35.19±7.3 years (21-50 years). The mean spheric refraction was -5.29±2.4 (-1.50 to -12.0 D) and spherical mean value was -1.52±1.10 (-0.50 to -4.00 D).

Results: No haze was detected clinically in any patient. Confocal microscopically, it was observed that haze peaked in the 1st month, decreased rapidly between the 1st and 3rd months, and continued to decrease towards the 6th month. While there was a statistically significant increase ($p<0.05$) between the preoperative period and the 1st month in terms of haze amount, there was no statistically significant difference between the 3rd and 6th months ($p>0.05$). When the amount of ablation was compared with the haze occurring in the 1st, 3rd and 6th months, no statistically significant difference was found ($p>0.05$). Subepithelial nerve regeneration developed in 27.8% of 36 eyes at 1 month, and 93.2% of 44 eyes at 6 months.

Conclusion: LASEK, with its low haze and early subepithelial nerve regeneration rate even at high myopic values, seems to be a technique that can be safely applied in high myopic and dry eye conditions.

Keywords: Laser Subepithelial Keratomileusis, Confocal Microscope, Keratocyte, Subepithelial Haze, Subepithelial Nerve.

INTRODUCTION

Laser subepithelial keratomileusis (LASEK) is a refractive surgery technique that combines the advantages of photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK). After the corneal epithelium is removed, laser ablation of the stroma is performed and the epithelium is then roll back on the stroma. Since it contains flaps like in LASIK, it has rapid visual rehabilitation, less pain, reduced stromal haze and a faster epithelial healing process than PRK in the postoperative period. Since laser ablation is performed on the anterior surface of the stroma, as in PRK, flap and interphase problems are not observed. Although PRK is successful in low and moderate myopia, the development of haze and myopic regression in high myopia limit the use of PRK.[1]

On the other hand, refractive surgeons have to deal with keratectasia, flap complications and interphase problems of LASIK. The thing that reveals the advantages and disadvantages of these three different techniques is that the cornea exhibits different healing responses in these three different methods. It has been demonstrated by animal experiments and many in vivo confocal microscopic studies that there is an intense keratocyte activation in PRK, and as a result, large amounts of collagen and glycosaminoglycan are synthesized.[2] It has been reported that wound healing is negligible after LASIK.[3] Confocal microscopic studies have also shown that there is a decrease in keratocyte density in the anterior and posterior of the flap in LASIK.[4,5] In vitro studies have shown that keratocyte apoptosis, myofibroblast transformation, and chondroidin sulfate synthesis, which occur in the healing response of the

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cornea, are less in patients who have undergone LASEK compared to PRK.[6,7]

In this study, we aimed to evaluate the corneal morphological changes after LASEK in vivo by confocal microscopy.

MATERIALS AND METHODS

This prospective study was conducted at Gazi University Faculty of Medicine Ophthalmology Refractive Surgery Unit. Patients with myopic and myopic astigmatism were treated with laser subepithelial keratomileusis (LASEK) excimer laser were included in this study. Inclusion criteria were at least 18 years of age, stable refraction of at least 2 year, and normal corneal topography. Patients with unstable refraction, dry eye, blepharitis, corneal disease, who had previous refractive surgery, glaucoma, systemic diseases that may affect corneal wound healing such as collagen vascular disease and diabetes, with a central corneal thickness of 490 µm or less by ultrasonographic pachymetry, and topographical evidence of keratoconus were excluded. Pregnant and lactating mothers were also excluded from the study. Daily-wear soft contact lenses were removed at least 1 month before the surgery. Written informed consent was obtained from all patients. The tenets of Declaration of Helsinki were followed throughout the study.

Preoperative evaluation included medical history and complete ophthalmologic examination (uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), manifest and

cycloplegic refractions, anterior and posterior segment examination, applanation tonometry ophthalmoscopy, corneal topography, pachymetry, Schirmer testing, and confocal microscopy). Confocal microscopic measurements were done in the preoperative period and at 1, 3 and 6 months postoperatively.

LASEK surgery was performed on all patients by a single surgeon with the same method. All procedures were performed under sterile conditions in an operating room environment. Topical proparacaine 0.5% was used to anesthetize the eyes. A drape and a lid speculum were inserted following the applying of 10% povidone-iodine.

The alcohol chamber was filled with a few drops of 20% ethanol and it was applied for 30 seconds. After then the alcohol was absorbed with a sponge. The epithelium was detached upwards with the help of a special knife, starting from the inferior and lifted. Afterwards, excimer laser (Mel 60, Aesculap-Meditec, Jena, Germany) was applied to the patient in the appropriate amount of ablation. The ablation diameter was 6.5mm with a 0.75mm transition zone in all eyes. Following the ablation, the cornea was irrigated with balanced salt solution and the epithelium was rolled to its original position and dried in place for 2 minutes. A cooled soft contact lens (Focus Night & Day; Ciba Vision, Duluth, Ga) was placed over the cornea with sterile forceps, and a drop of tobramycin 0.3% and dexamethasone 0.1%were instilled. The eyelid speculum and drape were removed. Patients were examined daily until epithelial closure. The contact lens was removed when the corneal epithelium was completely closed (4 or 5 days postoperatively). The patients used 5x1/day topical steroid drops for 1 month, 5x1/day antibiotic drops for 5 days (until epithelial closure in most of them), 1x1/day tear gel for 1 month, 7x1 artificial tears for 1 month.

By selecting the best quality images, corneal morphology, subepithelial haze amount (RI=light intensity) and nerve regeneration rate were evaluated confocal microscopically in the preoperative period, postoperative 1st, 3rd and 6th months.

Statistical analysis was performed by using SPSS 11 software (SPSS, Chicago, IL). Kolmogorov-Smirnov test, Student's T test and Mann-Whitney U test and analysis of variance in repeated measures were used for the statistical evaluation. Statistical significance was considered at $p < 0.05$.

RESULTS

Forty-nine eyes of 26 patients who underwent LASEK surgery were included in the study. Twenty-one of the cases were female and 5 were male. The mean age was 35.19±7.3 years (ranged from 21 to 50 years). The mean preoperative myopic spherical equivalent refraction was -5.29±2.4 D (between -1.50 and -12.0 D). Mean astigmatism was -1.52±1.10 (between -0.50 and -4.00 D). Only 3 patients (6 eyes) were using contact lenses before the surgery. No haze was detected in any patient.

The patients were divided into 2 groups as low-moderate myopia and high myopia. There were 41 eyes with a spherical value between -1.75 and -6.0 D in Group 1, and 8 eyes in Group 2 between -6.5 and -12.0 D. Confocal microscopically, the mean amount of haze (RI) was 56.37±12.24 pixels in Group 1 and 108.0±22.96 in Group 2. The groups were in normal distribution with the Kolmogorow-Smirnov test. There was no statistically significant difference between the two groups in terms of the amount of haze (p>0.05). There was a statistically significant difference between the two groups in terms of ablation amounts (p=0.000). When the amount of ablation was compared with the amount of haze occurring in the 1st, 3rd and 6th months, no statistically significant difference was found (p>0.05).

The mean preoperative RI of all cases was 82.25±13.0 pixels (min 58-max 109 pixels) (Figure 1), 149.6±39.4 pixels (min 76-max 216 pixels) at 1 month (Figure 2), 115.6±32.8 pixels at 3 months (Figure 3) (min 71-max 181 pixels) and at 6 months 104.6±37.8 pixels (min 55-max 214 pixels) (Figure 4) (Table 1).

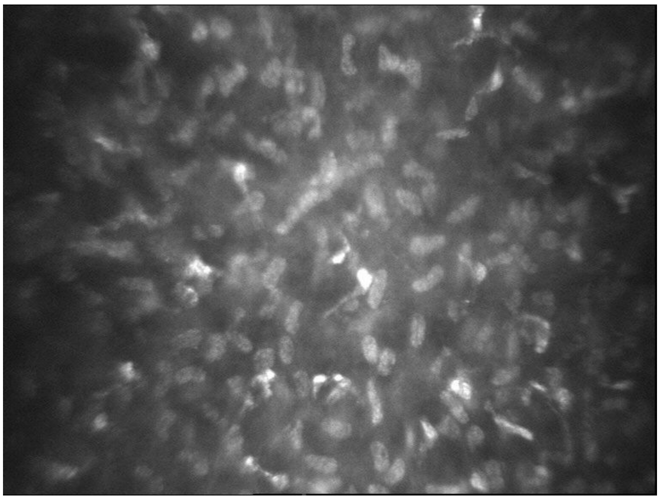

Figure 1: *Confocal microscopic view of the subepithelial area in the preoperative period RI= 99 pixels.*

Table 1: *Comparison of the amount of haze formed in the preoperative period and postoperative 1, 3 and 6 months.*

Time	RI mean (pixel)	p value
Preoperative	82.25±13.0	p=0.000
1st month	149.6±39.4	
3rd month	115.6±32.8	p=0.161
6th month	104.6±37.8	

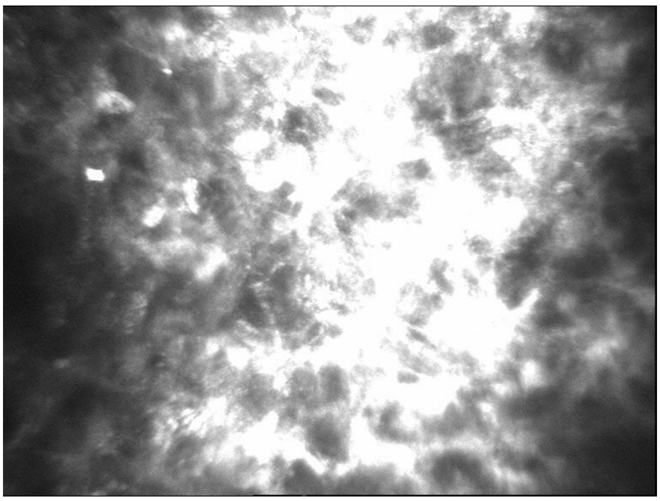

Figure 2: *Confocal microscopic view of the subepithelial haze area at 1 month postoperatively RI= 208 pixels.*

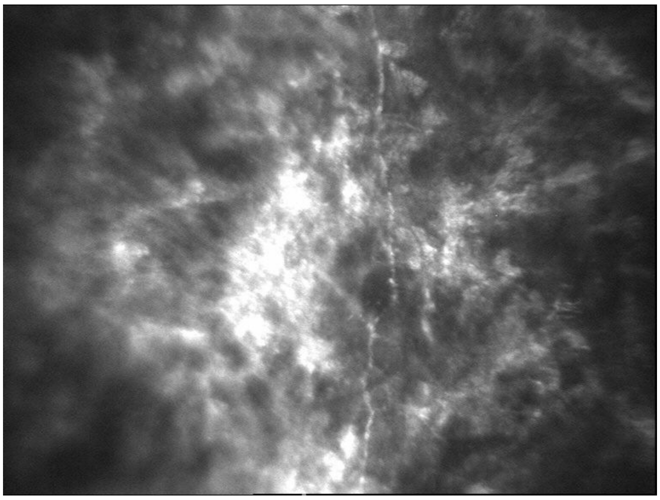

Figure 3: *Confocal microscopic view of the subepithelial haze area at postoperative 3rd month RI= 138 pixels.*

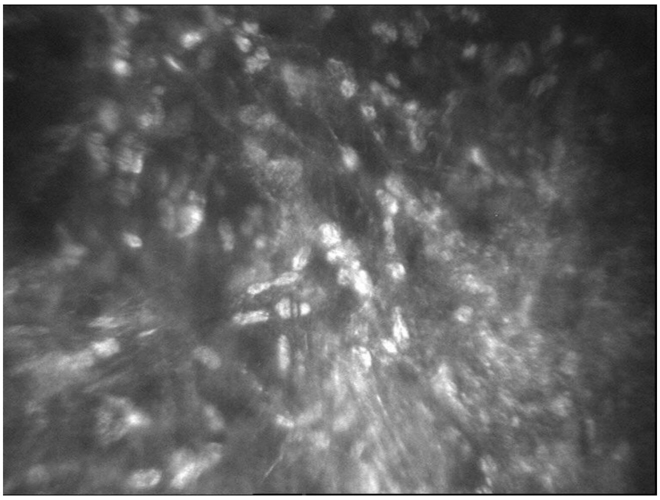

Figure 4: *Confocal microscopic view of the subepithelial haze area at 6 months postoperatively RI= 99 pixels.*

Although there was a statistically significant increase in haze between the preoperative period and the postoperative 1 st month (p=0.000), this increase decreased statistically between 1 and 3 months (p=0.000). There was no statistically significant difference between the third and sixth months (p=0.161) (Table 1).

In figure 5, it is seen that haze peaks in the 1st month, decreases rapidly towards the 3rd month and continues to decrease in the 6th month.

Subepithelial nerve regeneration was observed in 27.8% of 36 eyes at 1 month, 71.1% of 45 eyes at 3 months, and 93.2% of 44 eyes at 6 months on the confocal microscope (Table 2).

These regenerated subepithelial nerves were thin, short and few nerve fiber in the 1st month (Figure 6). Towards the 3rd month, these nerves were observed as longer and

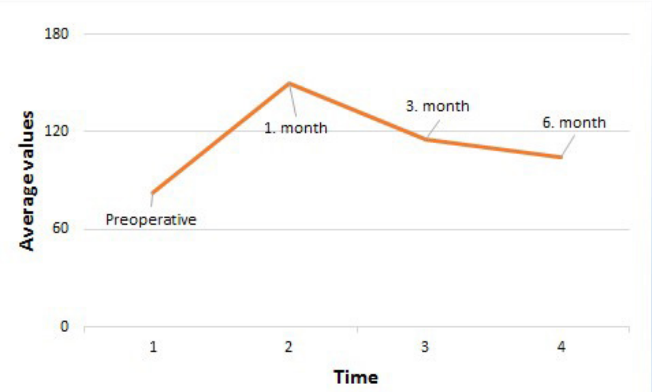

Figure 5: *The change in the amount of haze over time*.

Table 2: *Subepithelial nerve regeneration rate at 1, 3, and 6 months postoperatively.*		
Postoperative time	Subepithelial nerve regeneration rate	Number of eyes
1. month	%27.8	36
3. month	%71.1	45
6. month	%93.2	44

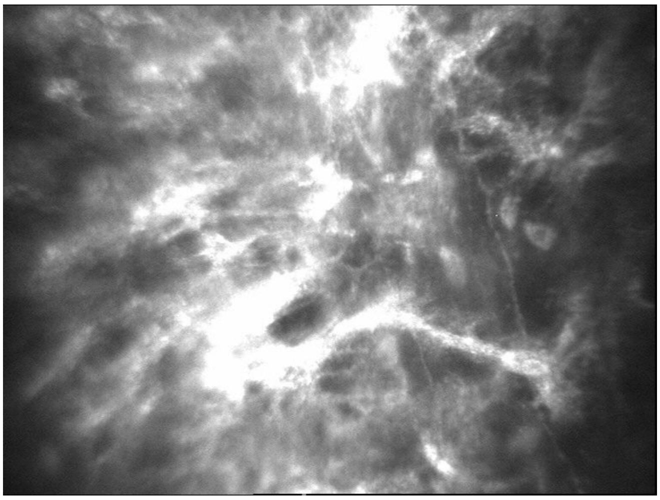

Figure 6: *Subepithelial nerve appearance at 1 month postoperatively.*

more reflective fibers (Figure 7) and at the 6th month control, these nerves were similar to the preoperative nerves in morphological appearance and number in most of the cases (Figure 8). In Graphic 2, it is observed that subepithelial nerve sprouts, which were observed in 1/3 of the cases in the 1st month, appeared in almost all cases in the 6th month. There was no statistically significant difference between the nerve regeneration rate at the 3rd and 6th months postoperatively (p=0.691). Also it was found that the rate of nerve regeneration was not affected by the amount of ablation in the 1st month postoperatively (p=0.885).

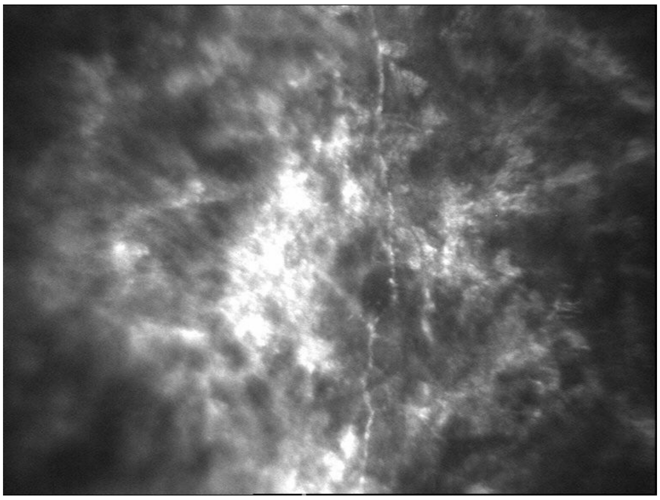

Figure 7: *Subepithelial nerve appearance at postoperative 3rd month.*

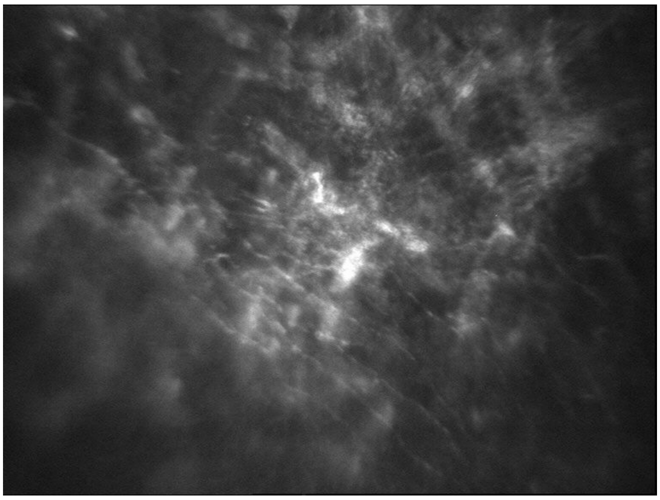

Figure 8: *Subepithelial nerve appearance at postoperative 6th month.*

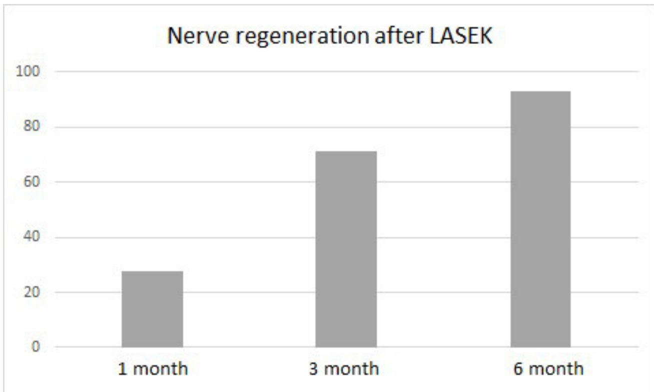

Figure 9: *Nerve regeneration rate by time in the postoperative period.*

DISCUSSION

Corneal wound healing remains a popular factor that influence the efficacy and safety of refractive surgical procedures. Clinical outcomes and many complications are directly related to this healing process and corneal cell response. The introduction of confocal microscopy into clinical use has enabled the in vivo examination of the wound healing response in the cornea after excimer laser in the human eye. The haze appearance, known as keratocyte activation during wound healing, prominence of nuclei in keratocytes and increase in extracellular matrix, is a prominent parameter in confocal microscopic evaluations. Refractive surgeries trigger this healing by activating keratocytes and ablating subepithelial nerves.

In the present study, we aimed to evaluate the wound healing response after LASEK. While clinically haze was not detected in any of the cases, it was observed that the confocal microscopically observed haze decreased rapidly in the early postoperative period in our study. Subepithelial nerve regeneration started as thin and short fibers in the early postoperative period, and it was observed that they regained their normal appearance in size and number in the 6th month. Azar et al. defined a low incidence of corneal haze after LASEK.[8] While Bilgihan K. et al. detected grade 1 haze in 3 eyes after LASEK performed on 61 eyes of 36 patients with high myopic values (-6.0 to -10.0 D) with refraction levels similar to those in our study, no haze was detected in any of our high myopic patients.[9] There are many studies in the literature evaluating the wound healing response in LASEK compared to PRK because of the similar characteristics of these two techniques. Similar to our results, these previous studies have also reported lower grades of haze after LASEK, particularly in the early postoperative months documented with confocal microscopy.[10-13] As a result of clinical studies in which LASEK was performed in one eye and PRK in the other eye of the same patient, it was also found that the haze level and postoperative pain were significantly lower after LASEK than in eyes with PRK.[10,11,14,15] In our study, it was observed that the haze detected by confocal microscopy peaked in the postoperative 1st month, decreased rapidly towards the 3rd month, and approached normal values in the 6th month in the patients that had spherical values between -1.75 and -12.0 diopters. On the other hand, it was reported that the haze peak appeared in the 3rd month and decreased in the 1st year of the haze after PRK.[2,16]

When the amount of ablation applied in our study was compared with the preoperative period and the amount of haze occurring in the 1st, 3rd and 6th months, no statistically significant difference was found. In an another study it was stated that there was no correlation between these two parameters similar to our results.[17]

In our confocal microscopy study, the morphological features of the corneal wound healing process especially the subepithelial nerves were determined after LASEK. In the present study, subepithelial nerve fibers, which were observed in 1/3 of the cases in the 1st month, were observed in almost all cases in the 6th month. Moreover, it was observed that these nerve fibers were similar to their preoperative appearance and values in morphology and number in most cases at 6 months. Similar to the studies, no statistically significant difference was observed between the amount of ablation and the rate of nerve regeneration in the postoperative 1st month. This suggests that the rate of subepithelial nerve regeneration is not affected by ablation depth, just like haze. Supporting these results, there are studies reporting that tear secretion is affected very little after LASEK. Herrmann WA et al. reported that tear secretion and stability reached preoperative values in the postoperative 1st month after LASEK.[18] The early regeneration of the subepithelial nerves, which play an important role in the tear secretion cycle, after LASEK has also been demonstrated in our study. Unlike LASEK, nerve regeneration occurs in the late period after PRK. In a study by Erie JC et al., it was reported that the subepithelial nerves reached preoperative values in the 2nd year after PRK and remained at the same level in the 3rd and 5th years. It was stated that it was low at the rate of 24%, but the difference was not statistically significant. It was also stated that there was no correlation between ablation depth and nerve density in both groups similar to our results.[19] In an another study, it was emphasized that subepithelial nerve regeneration reached normal levels at a rate of 70% in the 5th year after PRK.[17]

In conclusion, the important advantages of LASEK such as low haze rate and early subepithelial nerve regeneration which have been demonstrated by clinical studies have been proven morphologically by this confocal microscopic study. LASEK can be used safely in high myopic and dry eye patients due to these characteristic properties.

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Conflict of Interests

The authors declare that they do not have any kind of financial, commercial, or proprietary interests in any method or material mentioned, directly or indirectly. No public or private support has been received.

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