A Comparative Study with Confoscan 3 on the Cellular Structure Changes of Cornea after Keratorefractive Surgery

Javad Amoozadeh, MD1 • Soheil Aliakbari, MD2
Amir-Houshang Behesht-Nejad, MD1 • Mohammad-Amin Seyedian, MD2
Bijan Rezvan, DDS2 • Hassan Hashemi, MD2,3

Abstract

Purpose: To assess and to compare stromal and endothelial changes at the cellular level in patients who had photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) using in vivo confocal microscopy

Methods: In this semiexperimental study, 32 eyes of 16 patients (4 males and 12 females) with low to moderate myopia [-1.00 to -4.50 diopters (D)] or low to moderate myopic astigmatism (a total below 4.50 D and a max astigmatism of 1.50 D) were investigated. All participants had the history of PRK or LASIK surgery and were examined with Confoscan 3 confocal microscope (Nidek, Japan) before and 6 months after surgery. The densities of the keratocytes in the anterior and posterior stroma and the endothelial cell count (ECC) were measured. Finally the collected data was analyzed by SPSS16 and T-test.

Results: The mean ablation depth in LASIK and PRK groups were 61.00±17.17 and 62.13±15.4 μm, respectively. Six months after surgery, the mean cell density change in anterior and posterior stroma in LASIK group was 367.12±103.35 cells/mm² (34.7% reduction) and 9.25±28.28 cells/mm² (1.31% reduction) respectively. In the PRK group, these values were respectively 319.71±83.45 cells/mm² (31.13% reduction) and 0.17±38.97 cells/mm² (0.02% reduction). The ECC increased by 0.27% and 1.39% in LASIK and PRK groups, respectively. At retroablation zone in LASIK group, the mean number of keratocytes decreased by 37.2%.

Conclusion: By Confoscan application, after both PRK and LASIK, a decrease in the number of stromal keratocytes which was more significant in the anterior stroma was found. On the other hand, there was a slight increase in the ECC compared to preoperative counts which may be attributed to counting errors. There was not any significant difference in stromal cell densities and the endothelial cell counts between LASIK and PRK groups.

Keywords: Laser in Situ Keratomileusis, Photorefractive Keratectomy, Confocal Microscopy, Confoscan 3, Keratocyte, Endothelium, Endothelial Cell Count


1. Assistant Professor of Ophthalmology, Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences
2. Noor Ophthalmology Research Center, Noor Eye Hospital
3. Associate Professor of Ophthalmology, Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences

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Correspondence to: Hassan Hashemi, MD
Eye Research Center, Farabi Eye Hospital, Tehran, Iran, Tel:+98 21 55414941-6, Email: hhashemi@noorvision.com

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Introduction
In the last decade, the development of improved surgical techniques and instruments especially modern excimer lasers with submicron ablation accuracy have led to an increase in the number of refractive surgeries.1
Excimer laser can be used for the correction of hyperopia, myopia and astigmatism.2 In photorefractive keratectomy (PRK), cornea is ablated with excimer laser so as to remove a layer of the superficial stroma. In laser in situ keratomileusis (LASIK), an anterior corneal flap with 160 μm microns thickness is removed from the cornea and then the excimer laser is applied to the stroma underneath. During LASIK, the anterior stroma and the epithelium are preserved which causes differences in the healing processes observed after PRK and LASIK.3 Surface procedures such as PRK, laser assisted epithelial keratomileusis (LASEK), and epithelial LASIK (Epi-LASIK) have been shown to be safe and predictable for correcting low and moderate refractive errors; moreover, flap related complications and mechanical instability seen with LASIK are minimal with surface approaches.4 However, the major problems related to surface ablation procedures and specially PRK include delayed healing and increased risk of haze formation when high corrections are made.4-6 After PRK, myopia regression and dry eye have also been reported.6 Reports concerning the effect of Mitomycin C on corneal tissue indicate that its use during PRK may help to prevent haze formation.7
In case of LASIK, problems such as epithelial ingrowth, flap complications, keratectasia, mechanical instability, and worsened dry eye have been observed.3,5
Despite improved techniques and technologies, there is limited data regarding the cellular response following different surgical procedures.2 The knowledge about changes in the cellular and structural level of the cornea after each type of surgery, not only may help to understand potential complications, but also may demonstrate the natural processes occurring after each type of surgery. Also, the complex nature of tissue interaction following these surgeries can be learned so that the most appropriate procedure can be chosen for the treatment of different types and degrees of refractive errors.
There are some studies which have used confocal microscopy to investigate the cell morphology and counts in different corneal layers1-6,6-11; however their results are different. In the present study, we used in vivo confocal microscopy, Confoscan 3 (CM, Nidek, Japan), to assess the corneal stroma and endothelium at a cellular level and to compare the changes seen in patients 6 months after LASIK and PRK. It was intended to provide a wiser determination of long-term healing process for future surgeries.

Methods
The study type was semi-experimental.

Patients
From March 1st to May 15th 2007, patients consulted by eye surgeons to have keratorefractive surgery for the correction of low to moderate myopia [-1.00 to -4.5 diopters (D)] or low to moderate myopic astigmatism (a total below 4.50 D and a max astigmatism of 1.50 D) were enrolled after signing consent forms for preoperative and postoperative examinations. Patients with systemic diseases such as diabetes mellitus type 1 or 2, traumatic or infectious complications after keratorefractive surgery, eye diseases, and those who had previous refractive surgery were excluded from the study.
After applying the above exclusion criteria and excluding patients who missed their follow-up visits, data from 32 eyes of 16 patients (4 men and 12 women) was considered for the analysis. It should be noticed that 10 patients were applying contact lenses before surgery (8 in the PRK surgery group and 2 in LASIK group).

Preoperative and postoperative examinations
All patients underwent a series of optometric examinations before and after the surgery including the slit-lamp exam, tonometry, keratometry, topography, pachymetry and cycloplegic refraction.
In addition, the corneal cellular structure, in terms of the number of cells in the superficial and deep stroma, the endothelial cell count (ECC), and hexagonal cells were studied with
the Confoscan 3 confocal microscope (CM, Nidek, Japan). In LASIK group, the number of keratocytes was assessed in the retroablation zone as well. The space located between 5 μm behind the flap and the endothelium was described as retroablation zone and the preoperative measurement was also made in the same described zone.

Patients had their routine follow-up visits and Confoscan 3 examination was repeated 6 months after surgery. Confoscan 3 performed by an ophthalmologist was set on the automatic mode. Topical Tetracaine 0.5% drops were applied to anesthetize the eyes. Methyl cellulose drops (Viscotears Gel; CIBA Vision) were applied on the objective lens of the machine to provide a regular image, and then the lens was aligned with the corneal center. From each eye, 350 frames 5.0 μm apart were acquired. The cells in each layer were marked by an examiner blinded to the type of surgery to avoid double counts.

The number of cells was calculated by counting the number of clear and distinct cells in areas of 379x286 μm². The keratocyte density was studied in two layers; the 25 μm anterior to the Bowman’s membrane as the anterior stroma, and 40 μm posterior to the Descemet’s membrane as the posterior stroma.

Surgeries
The type of surgery was decided by the eye surgeon and the patient without the interference of the researchers. All surgeries were performed by a single surgeon.

Eight eyes of 4 patients had LASIK and 24 eyes of 12 patients had PRK. Ablations were done with the B&L Technolas 217z by applying Planoscan technique. The epithelial removal was performed manually with the hockey knife instrument in the PRK surgeries.

Patients who underwent LASIK were advised to use chloramphenicol drops for 3 days and Betamethasone drops for 1 week after the surgery. Then, artificial tears were recommended for 2 months. Patients who underwent PRK were advised to use Diclofenac Sodium every 8 hours during the first 24 hours, Chloramphenicol drops for 5 days, Betamethasone drops for 2 weeks, and artificial tears for 1 month. Flurometholone drops were recommended for 12 weeks after the cessation of Betametasone drops. At the end of surgery, O2 Optics ciloa vision contact lens was placed to be removed after 3 days.

Method of analysis
Data analysis was performed by the SPSS 16 software. T-test was used for analyzing the mean difference between the superficial stroma cell densities, the mean difference between the deep stroma cell densities, and the mean ECC and hexagonal cells before and after surgery in both groups of patients undergoing PRK and LASIK.

Results
The mean age of the patients was 28.25±5.75 years. The mean ablation depth was 61±17.17 μm in LASIK group and 62.13±15.41 μm in PRK group. The descriptive mean values of the superficial stroma cell density, deep stroma cell density and the ECC both before and after surgery have been mentioned in Table 1. This Table also contains the mean differences between these preoperative and postoperative values. Based on the type of surgery, the changes in these values were not statistically significant for any measured variable.

In LASIK group, there was a mean reduction of 34.70% and 1.31% in the cell density of the superficial and deep stromal layers respectively. On the other hand, a 0.27% increase was observed in ECC.

In PRK group, the mean reduction in the cell density of the anterior and posterior layers was 31.13% and 0.02% respectively; but the ECC increased by 1.39%.

Hexagonal cell density increased by 0.79% in LASIK group, 2.01% in PRK group, and 1.57% in all cases postoperatively. In this case, no statistically significant differences were shown between the two groups and also among all cases.

At retroablation zone in LASIK group, the mean number of keratocytes was 947.13±16.78 which decreased to 610±19.43 postoperatively (37.2%).
Table 1. Mean and standard deviation (SD) of the superficial and deep stromal keratocyte density and endothelial cell count (ECC) (cell/mm²) in LASIK and PRK groups and the total sample before and after surgery along with their differences

<table>
<thead>
<tr>
<th>Keratocyte Density</th>
<th>Superficial stroma</th>
<th>Deep stroma</th>
<th>Endothelial cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preop</td>
<td>Postop</td>
<td>Diff</td>
</tr>
<tr>
<td>LASIK (n=8)</td>
<td>Mean</td>
<td>1058</td>
<td>690</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>95</td>
<td>55</td>
</tr>
<tr>
<td>PRK (n=24)</td>
<td>Mean</td>
<td>1027</td>
<td>707</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>80</td>
<td>63</td>
</tr>
<tr>
<td>Total (n=32)</td>
<td>Mean</td>
<td>1034.7</td>
<td>703.2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>83.7</td>
<td>60.8</td>
</tr>
</tbody>
</table>

*: P<0.05

Discussion

In vivo confocal microscopy is a relatively new technique used for the analysis of morphological and structural changes of the cornea, whether diseased or after surgery. It is a safe method for the study of the dynamic corneal structure. Studies have shown that clinical slit-lamp examination lacks the required sensitivity for studying the biological changes in the cornea after refractive surgeries, whereas the confocal microscopy is free of such limitations. One of the limitations of this technique, however, is the limited area under observation which can be overcome by observing a larger number of samples. Another limitation, in comparison to electron microscopy, is its low resolution. Various studies have used confocal microscopy to study the structure of the cornea and keratocyte density in normal corneas and also after keratorefractive surgery.

Keratocytes are fibroblast-like cells that maintain the health and clarity of the corneal stroma. Compared to corneal endothelial and epithelial cells, keratocytes have medium regenerative capacity. Epithelial cells can be regenerated well and reepithelialization can be completed after injury; however, endothelial cells fail to show such properties. Studying about the changes in keratocyte density after refractive surgeries will help us in the better understanding of the impact of surgery on the cornea.

In a study performed by Ghirlando et al on 50 myopic patients, one month after PRK, the number of activated keratocytes observed in the anterior stroma was more than that in the posterior stroma. McLaren and his colleagues reported a reducing number of cells from the posterior stroma towards the anterior stroma after LASIK. Similarly, we were able to show that after both PRK and LASIK, the number of keratocytes in the anterior stroma showed a greater reduction when compared to the posterior stroma. The mean cell count decrease 6 months after LASIK was 367.12±103.35 cells/mm² (34.7% reduction) for the anterior stroma and 9.25±28.28 cells/mm² (1.31% reduction) for posterior stroma. In case of PRK, these values were 319.71±83.45 cells/mm² (31.13% reduction) and 0.17±38.97 cells/mm² (0.02% reduction) respectively. Of course, one must consider the counting errors as the keratocytes are not all in a single plane.

Versuluma et al believe the anterior stroma cell loss begins 6 months after LASIK. They attribute this observation to the loss of neural input to the keratocytes because the nerves are cut during LASIK, but to a lesser degree in PRK. Although the main cause of keratocyte loss after keratorefractive surgery is unknown, keratocyte necrosis and apoptosis have been suggested as possible causes. In this case, apoptosis and necrosis are the results of cells dying when exposed to large or lethal stresses respectively. Furthermore, it has been reported that the decreases in corneal cellular density are due to the lack of robust cellular proliferative response to cell loss. In a study...
by Erie et al, it was shown that the number of keratocytes in the anterior stroma reduced by 40\% at 6 months following surgery compared to preoperative counts. The authors also stated that the keratocyte density anterior to the retroablation zone reduced by 18\% and 42\% at one and 5 years after LASIK. This reduction was reported to continue in the anterior stroma after PRK and in the stromal flap and retroablation zone after LASIK for at least 5 years. They also observed regions of keratocyte apoptosis at the flap edge which were never replaced by new keratocytes.\textsuperscript{3} Another study reported that the decrease in keratocyte number after LASIK has both an acute and a chronic phase.\textsuperscript{11} Erie et al noted that keratocyte density reduced in the stromal flap and anterior to the retroablation zone during the first 6 months after LASIK and continues to further reduction in the next 2.5 years.\textsuperscript{9}

However, in our study, the reduction of keratocytes in the anterior stroma was observed after both PRK and LASIK. The question is whether the loss of cells following PRK or LASIK can threaten the health and the function of the cornea or not? Although not proven methodologically, clinical studies have shown that this reduction in cell count does not affect the overall health of the cornea.\textsuperscript{13} Even various long-term studies provide evidence of visual improvement after refractive surgeries.\textsuperscript{14,15}

The number of keratocytes in retroablation zone showed a statistically significant decrease (P<0.0001) in LASIK group 6 months after surgery. Compared to the anterior stroma, this higher decrease can be attributed to the direct effect of laser on the retroablation zone.

In the present study, the ECC showed an increase of 0.27\% and 1.39\% after LASIK and PRK respectively. The hexagonal cells also increased insignificantly in both groups after surgery. Since endothelial cells do not divide, and laser does not affect endothelial cells substantially, this slight increase in number can be attributed to errors in counting. It can also be a result of different cross sections applied before and after surgery.

The main result of our study is that there was no significant difference between PRK and LASIK in terms of changes seen in the cell count of different layers of the stroma and endothelium.

The limitations of our study were the unequal number of patients in the PRK and LASIK groups and the lack of a control group. However, these limitations could not be avoided because of the small number of participants and those who completed all their follow-up exams.

**Conclusion**

The keratocyte density in the anterior and posterior stroma reduced after both PRK and LASIK, but no statistically significant differences were found when the two groups were compared.

**References**