Atomic Force Microscopy for the Evaluation of Corneal Surface Roughness after Femtosecond Laser Flap Creation and Excimer Ablation.

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Abstract

Introduction: It is well known that the femtosecond laser lamellar cut has some degree of roughness. Nevertheless, as in femtosecond laser assisted LASIK (FS-LASIK), an excimer LASIK ablation is performed, the post-ablation stromal bed should show a marked decrease in roughness. We decided to compare, using atomic force microscopy (AFM), the roughness of the corneal stromal bed, after a Femtosecond lasers device (FS) flap was created with or without an excimer myopic ablation.

Methods: Using 6 freshly enucleated porcine eyes, we created in every eye a flap using a femtosecond laser. Additionally in 3 eyes an excimer laser ablation to correct -3 diopters (D) was made. AFM imaging of the remaining corneal stroma was performed. Ten different square areas of 20µm x 20µm at the central area of the stroma of each corneal sample were studied. The roughness parameters used was the root-mean-square (RMS) deviation from a perfectly flap plane.

Results: The RMS deviation were 360 ± 120 nm in femtosecond laser only, and 110 ± 20 nm in those cases where excimer is also involved ($p < 0.0001$).

Conclusions: Our results show that the roughness of the surface treated with excimer is clearly lower than in the group with no excimer ablation, thus the application of laser excimer after a flap creation by femtosecond laser flap creation may soften the nano-irregularities created by this technique.

Introduction

The roughness of the stromal surfaces obtained following femtosecond (FS) laser-assisted keratomileusis (LASIK) has been recognized as a clinically relevant issue, because it is associated with optical aberrations and dysphotopic phenomena ("rainbow glare") that may compromise patients' quality of vision in the postoperative period¹.

Nowadays, it is possible to assess the characteristics of corneal tissue planes by means of three different strategies: at a near-macroscopic scale using anterior segment optical coherence tomography (OCT)², at a microscopic scale using scanning electron microscopy (SEM) and transmission electron microscopy (TEM)³, and in a nano-scale (i.e. with a precision in the range of few nanometers) using atomic force microscopy (AFM)^{1, 4-5}.

Femtosecond laser devices play an increasingly important role in modern refractive surgery and are currently preferred to mechanical microkeratomes for flap creation⁶. Furthermore, FS laser-assisted refractive lenticule extraction (ReLEx) has been proposed as an alternative to laser excimer ablation for the correction of myopia⁷⁻⁸. However, it is well known that the plane of the FS lamellar cut is not absolutely smooth⁵⁻⁹. In fact, the corneal tissue plane has a certain degree of roughness, mostly because the tissue-cutting laser spots are separated from one another (the so called "spot distance")⁵⁻⁹. Recent efforts have focused on the optimal FS laser parameters that would ensure a smoother interface⁹.

Nevertheless, because in FS-LASIK procedures an excimer ablation is performed over the corneal stroma, the post-ablation stromal bed might be expected to show a decrease in roughness.

The aim of the current study was to evaluate the smoothing effect of excimer ablation on corneal tissue planes of freshly enucleated porcine eyes that underwent LASIK flap creation using a FS laser. Using AFM, the stromal bed roughness of eyes that underwent FS LASIK flap creation alone was compared to that of eyes that in addition underwent excimer ablation following the FS LASIK flap creation.

Materials And Methods

Procedure

We designed an experimental comparative study using six freshly enucleated porcine eyes. Within 24 hours from the enucleation, the same experienced surgeon (JGO) created a lamellar corneal flap using the 60KHz IntraLase® FS laser (Abbott Medical Optics, Inc., Santa Ana, CA, USA). The following parameters were used: flap depth 110 microns, flap diameter 9.00 mm, hinge position 90 degrees (i.e., superior), hinge angle: 50 degrees, side-cut energy 0.95 mJ, side-cut angle 135 degrees. Pocket depth and width were set at 230 μm and 0.23 μm , respectively, with 5 μm tangential and radial spot separations. Immediately after the procedure, the flap was lifted and then repositioned using a LASIK spatula. Next, myopic excimer laser ablation to correct -3.0 diopters (D) was performed in three of the six eyes using a WaveLight Allegretto EyeQ 400-Hz laser (Alcon Laboratories, Inc. Fort Worth, TX, USA). The laser parameters of the ablation were: optical zone: 6,5mm, transition zone: 0,3 mm, ablation zone: 7,10 mm, maximum central ablation: 45,5 microns. When the above procedures were completed, a 9 mm trephination of the cornea was performed with a Barron vacuum donor cornea punch (Katena Inc, Denville, NJ, USA) in all eyes. Immediately after the trephination, the flap was lifted and amputated with scissors and the globes were prepared for AFM analysis of the stromal bed roughness. The endothelial side of the corneal button obtained was glued with cyanoacrylate adhesive to a microscope slide and covered with 2.5% glutaraldehyde solution for 24 hours before AFM measurements of the stromal bed roughness were performed.

Ten randomly selected areas from each sample were studied, thus a total of 30 regions from each group were analyzed.

Imaging

AFM imaging was performed using a JPK NanoWizard II® atomic force microscope coupled to a Nikon Eclipse Ti-U inverted optical microscope, in contact mode immersed in liquid, and employing Olympus OMCL-RC800PSA commercial silicon nitride cantilever tips (0.05 N/m, 18 kHz), with typical 15 nm radius at the end. Vertical accuracy of the instrument is in the order of 0.1 nm.

Surface roughness measurements were made in 10 different square areas with dimensions 20µm x 20µm at the central part of each corneal sample, using a 512x512 point resolution. The images were processed and analyzed using the JPK Data Processing software. The roughness parameter used in this study was the root-mean-square (RMS) deviation from a perfectly flat plane within the analyzed area.

Results

The RMS (in microns) from AFM images of every sample analyzed was calculated. The results are depicted in Table 1.

Table 1

Results of the RMS (in microns) from AFM images of every sample analyzed.

	<u>FS-LASIK</u>			FS-LASIK & Excimer		
	<u>Sample 1</u>	<u>Sample 2</u>	<u>Sample 3</u>	<u>Sample 4</u>	<u>Sample 5</u>	<u>Sample 6</u>
Region 1	481	312	396	108	160	113
Region 2	208	312	414	108	132	130
Region 3	292	346	419	90	117	102
Region 4	202	337	672	92	120	122
Region 5	230	338	415	86	119	92
Region 6	288	277	408	69	138	85
Region 7	250	312	504	89	69	89
Region 8	278	376	558	90	82	114
Region 9	237	275	594	106	127	125
Region 10	509	258	358	96	150	148
Mean of each Sample	297.5	314.3	473.8	93.4	121.4	112

For the whole groups, the mean $\pm$ standard deviation of the RMS values were 360 ± 120 nm for the FS-LASIK group, and 110 ± 20 nm for the FS-LASIK and excimer group (P value=0.0001), (Figure 1).

Using atomic force microscopy we obtained three dimensional and vertical deflection images of the stromal surface of the FS-LASIK flap creation without excimer ablation group (Figure 2, Figure 3), and of the FS-LASIK flap creation and excimer laser ablation group (Figure 4, Figure 5).

Discussion

Our results show that excimer laser ablation following flap creation with an FS-LASIK does smoothen the nano-scale irregularities of the corneal stroma surface induced by the FS phototodisruption. This observation is clinically relevant, because the optical effects induced by a rough corneal surface after FS-LASIK surgery may adversely affect the quality of vision in the postoperative period⁵⁻⁹.

Following its validation for ophthalmic purposes, AFM has become a method of choice for studying the roughness of various surfaces¹⁰. In fact, AFM is considered a more attractive option than SEM because it allows the acquisition of three-dimensional images of the surface of samples and offers the possibility of quantitative and objective analysis. An additional advantage of AFM over SEM is that the preparation of specimens for the former is simpler than for the latter. For these reasons, AFM is currently considered the gold standard for the study of stromal surface roughness⁵⁻¹¹.

There are few reports on the use of AFM for the study of the stromal roughness after excimer laser ablation¹²⁻¹³. However, none of them analyzed the stromal roughness before and after the ablation. Instead, these experimental studies only examined the effect of the excimer laser ablation using different parameters for depth and energy following the mechanical removal of the epithelium¹²⁻¹³. Lombardo M et al. were the first to study corneal roughness using AFM after a standard ablation using two different excimer lasers¹⁴, as well as the effect of a smoothening technique with viscoelastic application over the corneal stroma as a masking agent in photorefractive keratectomy⁴. In a later report, the same authors published the results of AFM analysis of the corneal epithelium and the photoablated corneal stroma¹, but did not compare the roughness of the corneal stromal bed before and after the ablation.

Few investigators have used AFM to examine the effect of FS lasers on corneal surface roughness. Serrao et al studied the effect of different parameters on corneal surface roughness using the IntraLase platform for LASIK⁹⁻¹¹, as well as the surface roughness and morphology of the posterior aspect of stromal lenticules created with a femtosecond laser (ReLEx)¹⁵. Ziebarth et al studied the quality of corneal lamellar cuts with the Visumax platform for FS-LASIK⁵. However, none of the aforementioned investigators performed an excimer ablation after the FS laser procedure to study the excimer-induced changes on corneal surface roughness.

To the best of our knowledge, the current study is the first to employ AFM for the assessment of the surface-smoothening effect induced by excimer ablation on a corneal tissue plane created by a FS laser. The fact that surface roughness decreases after the excimer ablation is fortunate, because it indicates that in real-life surgeries at least one of the surfaces created with the FS laser is smoothened. Conceivably, in such cases the improved characteristics of the anterior surface of the stromal bed could translate to better quality of vision in the postoperative period.

Based on our results, one could consider those refractive techniques in which only a FS laser is used (e.g. ReLEx procedures). In these techniques the smoothening effect of excimer ablation is not present. Hence, in such procedures in addition to the roughening effect of the mechanical dissection of the lenticule, irregularities are added on both surfaces created by the FS laser¹⁶. This fact could explain the differences

between LASIK and ReLEx in corrected distance visual acuity at 3 months after surgery in the Multicenter Prospective Cohort Study on Refractive Surgery in 15011 eyes¹⁷. This study clearly showed a lower gain of Snellen lines in eyes treated with ReLEx compared to those treated with LASIK, with a statistically significant difference in the loss of 1 Snellen line and in the proportion of eyes achieving so-called “supervision.” Nonetheless, further studies with sufficiently long follow-up are needed to address these concerns.

A possible limitation of the present study is that the animal eyes that we used were operated within approximately 24 hours after enucleation. Consequently, post-mortem tissue changes may have interfered with the overall quality of the FS cut. In addition, only small areas (20x20 μm^2) of the corneal surface were studied with AFM, thus, morphologic features larger than the single area scanned do not contribute to the analysis.

The results of our study are not necessarily applicable to other commercially available femtosecond laser platforms, or even to the same platform with different settings. Nonetheless, our results clearly indicate the beneficial effect that excimer laser photoablation has on the surface roughness characteristics of tissue planes created with a FS laser.

Conclusion

Our results show that the roughness of the surface treated with excimer is clearly lower than in the group with no excimer ablation, thus the application of laser excimer after a flap creation by femtosecond laser flap creation may soften the nano-irregularities created by this technique.

Declarations

Competing of interest:

None of the authors has any financial or proprietary interest in any aspect of this study.

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Author contribution:

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Data Availability:

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request

Animal research:

All procedures performed involving animals were in accordance with the ethical standards of the institution at which the study were conducted and ethical approval was obtained from COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA REGIONAL DE LA COMUNIDAD DE MADRID (code 216/03, version 2.0 Mayo 2016).

The tables and figures are original and have been produced by the authors for this particular publication

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Figures

Figure 1

Mean $\pm$ standard deviation of the root mean square (RMS) values for the FS-LASIK and the FS-LASIK and excimer group.

Figure 2

Image of 3D atomic force microscopy (AFM) topography of the stromal surface after FS-LASIK flap creation without excimer ablation.

Figure 3

Image of the AFM vertical deflection of the stromal surface after FS-LASIK flap creation without excimer ablation.

Figure 4

Image of 3D atomic force microscopy (AFM) topography of the corneal stromal surface after FS-LASIK flap creation followed by excimer laser (3.0 D myopic ablation).

Figure 5

Image of the AFM vertical deflection of the corneal stromal surface after FS-LASIK flap creation followed by excimer laser (3.0 D myopic ablation).