Mitomycin C–Assisted Photorefractive Keratectomy in High Myopia: A Long-term Safety Study

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Purpose: To evaluate the long-term corneal safety of topical mitomycin C (MMC) used during photorefractive keratectomy to prevent haze formation in highly myopic eyes.

Methods: Twenty-eight patients with bilateral high myopia underwent photorefractive keratectomy. One eye was randomly assigned to intraoperative 0.02% MMC and the fellow eye to conventional treatment. Each eye was checked at baseline and at 5 years after surgery using in vivo corneal confocal microscopy.

Results: At baseline, the endothelial cell density was 2970 ± 295 cells per square millimeter in the MMC-treated eyes and 2839 ± 323 cells per square millimeter in the control eyes. At 5 years, it was 2803 ± 307 and 2780 ± 264 cells per square millimeter, respectively ($P = 0.27$). The number of corneal nerve fibers was 3.9 ± 1.6 in the MMC-treated eyes and 4.4 ± 1.3 in the control eyes. At 5 years, it was 3.0 ± 1.6 and 2.7 ± 1.3, respectively ($P = 0.15$). The density of corneal nerves was 9600 ± 2915 $\mu m/mm^2$ in the MMC-treated eyes and 11,352 ± 3898 $\mu m/mm^2$ in the control eyes. At 5 years, the density was higher in the MMC-treated eyes (6790 ± 2447 $\mu m/mm^2$) than in the control eyes (6024 ± 2977 $\mu m/mm^2$) ($P = 0.003$). The number of nerve beadings at baseline was 12.9 ± 1.7/100 μm in the MMC-treated eyes and 12.3 ± 2.0/100 μm in the control eyes. At 5 years, it was 9.9 ± 2.6/100 and 9.4 ± 2.9/100 μm, respectively ($P = 1.00$). At 5 years, corneal nerve branching and tortuosity were similar in the 2 groups ($P = 0.88$ and 0.54, respectively). Epithelium thickness remained statistically unchanged ($P = 0.69$).

Conclusions: Intraoperative use of topical 0.02% MMC compared with standard treatment does not induce significant long-term corneal changes, as assessed by in vivo corneal confocal microscopy.

Key Words: confocal microscopy, mitomycin C, photorefractive keratectomy

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Photorefractive keratectomy (PRK) has proven to be a predictable and safe method for the treatment of low to moderate refractive errors. Corneal haze, which decreases the best-corrected visual acuity (BCVA) and quality of vision, is a major side effect of PRK. The risk of haze formation is greater in highly myopic eyes, where more corneal stroma needs to be removed.[1,2] To modulate the process of corneal wound healing after PRK, patients are usually treated with topical steroids. Although the prophylactic use of corticosteroid drops may be successful in preventing haze formation, their effectiveness remains controversial.[3,4] Moreover, long-term treatment can lead to several adverse effects: ocular hypertension, glaucoma, and cataracts.[5]

Application of topical mitomycin C (MMC) as a modulator of corneal wound healing after excimer laser surgery was first suggested by Talamo et al.[6] Other studies reported better visual outcome and a lower haze rate in patients treated with MMC after PRK.[3,7,8] MMC is an antibiotic with antimetabolite effects; it forms a covalent linkage with DNA and inhibits DNA synthesis.[3,9] Although effective when used to treat conjunctival–corneal neoplasia, to prevent pterygium recurrence, or to improve the outcome of trabeculectomy, topical application of MMC has been shown to produce different complications.[2,4] These side effects have never been reported to occur after topical MMC application for corneal surface laser ablation, but some concern still exists about possible long-term effects because of a presumed subclinical toxicity of MMC to corneal and intraocular structures.[10,11]

The aim of this study was to determine the in vivo long-term effects, quantified by corneal confocal microscopy, on corneal endothelium, subbasal nerve plexus, and corneal epithelium of eyes that underwent MMC-assisted PRK for the correction of high myopia, compared with fellow eyes that underwent PRK followed by standard treatment with topical steroids. Data about corneal keratocytes have previously been reported.[12]

MATERIALS AND METHODS

Patients and Study Design

This prospective, double-blind, randomized study included 28 subjects (56 eyes) with bilateral high myopia [≥7 diopters (D)] who underwent MMC-assisted PRK in 1 eye and PRK with steroid treatment in the fellow eye. Preoperative evaluation included uncorrected visual acuity (UCVA) and BCVA (Early Treatment Diabetic Study Retinopathy chart, logarithm of the minimum angle of resolution notation) and

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slit-lamp examination. All eyes underwent corneal confocal microscopy at baseline and at 5 years after PRK. The baseline refractive difference between one eye and the fellow eye of the same subject was <0.75 D, to avoid any relevant influence in the amount of stromal ablation on clinical results. One eye was randomly assigned to intraoperative topical MMC treatment, and the fellow eye was treated with corticosteroids. This approach was chosen to limit the influence of wound-healing individual characteristics. A detailed explanation of the treatment was given to all patients, and free, valid, informed consent was obtained before the procedure. The study protocol was approved by the Institutional Review Board of Padua University Hospital. No patient with lenticular changes, anterior or posterior uveitis, corneal scarring, excessively dry eyes, blepharitis, or systemic disease known to interfere with epithelial healing was included in the study. Patients with keratoconus, corneal dystrophy or degeneration, glaucoma, retinal disease, or a history of severe ocular trauma or previous ocular surgery were also excluded.

Surgical Technique and Postoperative Follow-Up

Operative and postoperative protocols have been previously described in detail.[8] Briefly, after topical anesthesia (oxybuprocaine 0.4% drops, Novesine; Novartis Farma, Milan, Italy) and chemical epithelial removal by 20% ethanol diluted with balanced salt solution (BSS) instilled in a 7-mm optical zone, surgical treatment was performed by the same surgeon using a COMPex 300 laser (InPro, Norderstedt, Germany): a 7-mm broad-beam laser with a pure gaussian profile. Immediately after laser treatment, eyes assigned to be treated with MMC received a single topical application of MMC 0.02% diluted in BSS, administered with a soaked sponge (7 mm in diameter) over the ablated stroma. The sponge was kept in place for 2 minutes. To remove the residual MMC, the corneal surface and the entire conjunctival fornix were irrigated with 20 mL of BSS. The eyes assigned to be treated with corticosteroids received the steroids with a sponge soaked in BSS, using the same posttreatment procedure. Once reepithelialization occurred, all eyes were treated with artificial tears (hyaluronic acid 0.2%, Hyalistil; Sifi, Catania, Italy) 3 times a day for 3 months. Moreover, corticosteroid-treated eyes received fluorometholone sodium 2% (Flumetol S; Farmila, Milan, Italy) 3 times a day for 1 month, whereas MMC-treated eyes received a placebo solution (hyaluronic acid 0.2%) 3 times a day for 1 month. The dosages of corticosteroid and placebo were tapered to twice a day for the second month and once a day for the third month. No additional steroids were used during follow-up. Postoperative clinical evaluation included UCVA, BCVA, and biomicroscopic examination. Corneal haze was graded at the slit lamp according to the haze-grading scale reported by Fantes et al.[13]

Corneal Confocal Microscopy

The morphology of corneal layers was evaluated at baseline and at 5 years after surgery, using the same corneal confocal microscope (ConfoScan 3; Nidek, Gamagori, Japan) with a ×40 surface objective. Corneal confocal microscopy was performed in a standardized manner.[8] Confocal parameters were quantified by a single, blinded, experienced examiner analyzing the study eyes in a random fashion. Endothelial cell density (expressed as cells per square millimeter) was calculated from the best-quality image. Epithelium thickness was defined as the distance between the last focused image of the subbasal nerve plexus and the last image of the epithelium, as described by Li et al.[14] The subbasal nerve plexus was evaluated using the best-quality image for each eye. Density, number of fibers, number of beadings, order of branching, and tortuosity were quantified. Nerve fibers were traced using Neuron J software.[15] We quantified the density of nerve fibers by calculating the total length of all the fibers visible in the selected image: values are expressed as micrometer per square millimeter. To calculate the number of fibers, the number of beadings, and the grade of branching of fibers, the method we previously reported was applied.[16] Briefly, the number of fibers is the sum of the nerve fibers seen in the selected image. Number of beadings is the number of hyperreflective points per unit of length (100 μm) in a single nerve fiber, randomly selected from all nerve fibers. In the subbasal nerve plexus, the mean number of beadings is not significantly different between nerve fibers or their branches (unpublished data). Fiber branching was classified using the following system (with values ranging from 0 to 3): grade 0, no corneal subbasal nerve plexus fiber branching; grade 1, one fiber presenting one or more direct branching from the major nerve trunk; grade 2, one fiber presenting one branching originating from a principal nerve trunk branching; and grade 3, more than grade 2 branching.[16]

Fiber tortuosity was classified using the grading system proposed by Oliveira-Soto and Efron,[17] which is based on the frequency and amplitude of changes in the nerve fiber direction. Values range from 0 to 4 (grade 0: nerve fibers appear almost straight; grade 1: nerve fibers appear slightly tortuous; grade 2: nerve fibers appear moderately tortuous, with frequent small amplitude changes in the direction of fibers; grade 3: nerve fibers appear tortuous, with the amplitude of changes in the fiber direction being quite severe; and grade 4: nerve fibers appear very tortuous, showing abrupt and frequent changes in the direction).

Statistical Analysis

Data about corneal endothelium, nerves, and epithelium were analyzed in the MMC-treated eyes and in the corticosteroid-treated eyes using a paired *t* test. The comparison between the 2 treatment groups was performed using analysis of variance for repeated measures (endothelium, epithelium, number and density of nerve fibers, and number of beadings) and the Fisher exact test (branching and tortuosity). Then, to evaluate whether MMC modifies the regeneration of the subbasal nerve plexus, we calculated for each parameter the relative risk of treatment with MMC versus treatment with corticosteroids.

RESULTS

Fifty-six highly myopic eyes of 28 patients (16 women and 12 men) were included in this study. The mean patient age at baseline was 39.9 ± 7 years. The MMC-treated eyes had an attempted spherical equivalent correction of –9.50 ± 1.84 D (range: –7.25 to –14.25 D). The steroid-treated eyes had an attempted spherical equivalent correction of –9.00 ± 1.79 D (range: –7.00 to –14.25 D). BCVA was not significantly different between the 2 groups (*P* = 0.13). The thickness of ablation was 95.4 ± 19.9 μm in the MMC-treated eyes and 93.1 ± 20 μm in the control eyes (*P* = 0.72). No eyes showed signs of delayed reepithelialization or any adverse side effects during follow-up. No eye was retreated during follow-up. Postoperatively, all patients showed improvement in UCVA. At 5 years, the mean UCVA (logarithm of the minimum angle of resolution) was 0.4 ± 0.31 in the MMC-treated eyes and 0.5 ± 0.33 in the control group. The difference was not statistically significant (*P* = 0.8). At 5 years, none of the MMC-treated eyes showed clinical corneal haze, whereas 4 eyes (14.3%) in the control group had grade 1 corneal haze.

Corneal Confocal Microscopy

Mean preoperative endothelial cell density was 2970 ± 295 cells per square millimeter in the MMC-treated eyes and 2839 ± 323 cells per square millimeter in the control group (*P* = 0.12). Five years after PRK, the mean endothelial cell density was 2803 ± 307 cells per square millimeter in the MMC-treated eyes and 2780 ± 264 cells per square millimeter in the control eyes. The difference was not statistically significant (*P* = 0.27).

At baseline, the mean epithelial thickness was 51.4 ± 4.1 μm in the MMC-treated eyes and 51.8 ± 2.8 μm in the control eyes (*P* = 0.68). At 5 years, the corneal epithelium thickness was 53.6 ± 3.8 μm in the MMC-treated eyes and 54.5 ± 2.9 μm in the control eyes. The difference between the 2 groups was not statistically significant (*P* = 0.69).

The preoperative number of corneal nerve fibers was 3.9 ± 1.6 in the MMC-treated eyes and 4.4 ± 1.3 in the control eyes (*P* = 0.27). Five years after surgery, the number of nerve fibers was 3.0 ± 1.6 in the MMC-treated eyes and 2.7 ± 1.3 in the control eyes. The difference between the 2 groups was not statistically significant (*P* = 0.15) (Table 1).

At baseline, the mean corneal nerve density was 9600 ± 2915 μm/mm^2 in the MMC-treated eyes and 11,352 ± 3898 μm/mm^2 in the control eyes (*P* = 0.06). At the 5-year examination, the corneal nerve density was 6790 ± 2447 μm/mm^2 in the MMC-treated eyes and 6024 ± 2977 μm/mm^2 in the control eyes. The difference between the 2 groups was statistically significant (*P* = 0.003) (Table 1).

Before surgery, the mean number of beadings was 12.9 ± 1.7/100 μm in the MMC-treated eyes and 12.3 ± 2.0/100 μm in the control eyes (*P* = 0.17). At the 5-year examination, the number of beadings was 9.9 ± 2.6/100 μm in the MMC-treated eyes and 9.4 ± 2.9/100 μm in the corticosteroid-treated eyes. The difference between the 2 groups was not statistically significant (*P* = 1.00) (Table 1). Five years after surgery, branching and tortuosity of nerve fibers were not significantly different between the 2 groups (*P* = 0.88 and 0.54, respectively), nor was the difference statistically significant at baseline (Table 1).

DISCUSSION

Corneal refractive surgery involves a variety of techniques, including PRK, laser-assisted in situ keratomileusis (LASIK), laser-assisted subepithelial keratomileusis, and epi-LASIK.[18] Many surgeons perform LASIK to treat moderate to high myopia because of faster visual recovery and lower risk of haze formation, compared with PRK. However, LASIK exposes the eye to flap-related complications that lead to corneal biomechanical instability.[1,2] Intraoperative use of topical MMC after PRK has significantly reduced the incidence of haze formation.[1,7,8] Concerns about MMC use are because of the complications reported when MMC was used to improve the results of pterygium excision or to treat ocular surface neoplasia.[2,4,9] Although these effects have never been reported to occur with the use of topical MMC after

TABLE 1. Descriptive Statistics of Confocal Microscopy Parameters for MMC-Treated Eyes and Steroid-Treated Eyes (5 Years)

	Preoperative		Postoperative (5 Yrs)	
Parameter	**MMC**	**Steroid**	**MMC**	**Steroid**
Fibers (n/image), mean ± SD (range)	3.9 ± 1.6 (2–9)	4.4 ± 1.3 (1–7)	3.0 ± 1.6 (1–7)	2.7 ± 1.3 (1–6)
Density (μm/mm^2), mean ± SD (range)	9600 ± 2916 (3293–15375)	11352 ± 3898 (3714–17087)	6790 ± 2447 (2238–12455)*	6024 ± 2977 (2329–119)21)
Beadings (n/100 mm), mean ± SD (range)	12.9 ± 1.7 (10–16)	12.3 ± 2.0 (9–17)	9.9 ± 2.6 (5–14)	9.4 ± 2.9 (5–15)
Branching, n (%)				
Order 0	1 (3.6)	3 (10.7)	1 (3.6)	3 (10.7)
Order 1	17 (60.7)	14 (50.0)	18 (64.2)	17 (60.7)
Order 2	10 (35.7)	11 (39.3)	9 (32.1)	8 (28.6)
Tortuosity, n (%)				
Grade 1	14 (50.0)	15 (53.6)	12 (42.8)	12 (42.8)
Grade 2	11 (39.3)	11 (39.3)	14 (50.0)	13 (46.4)
Grade 3	3 (10.7)	2 (7.1)	2 (7.2)	3 (10.7)

*Values statistically different between the 2 groups ($P < 0.05$).

surface laser ablation, it is mandatory to investigate the subclinical effects resulting from long-term MMC toxicity to the cornea.[11]

In our study, MMC 0.02% was administered using a soaked sponge kept in place for 2 minutes. This method of administration restricts the diffusion of the drug to the ablated cornea, sparing the surrounding tissues. We studied the cornea by in vivo corneal confocal microscopy at baseline and at 5 years after surgery to evaluate whether MMC causes any long-term alterations of the corneal structure. The lack of significant long-term side effects of MMC on corneal keratocytes, compared with the standard treatment with steroids in the same eyes, has previously been reported.[12]

MMC is an alkylating agent with potential late effects, particularly on cells with elevated proliferative capacity (activated keratocytes and corneal epithelial cells). Moreover, its action is not cell-cycle specific. Therefore, even corneal endothelium considered to be amitotic may be damaged.[19] Amitotic cells require periodic repair of their DNA, and MMC-generated alkylating agents might hinder this activity.[20] In refractive surgery, MMC is applied to the ablated stroma and penetrates into the cornea, and corneal endothelium DNA may be damaged, as experimentally demonstrated by Roh et al.[21]

In a rabbit model, Chang[22] found that MMC causes corneal edema and endothelial apoptosis, related to MMC concentration and exposure time. But the effect of MMC on human cornea may be different, considering that rabbit corneal endothelium is capable of mitosis.[21] In humans, there are no reports of early corneal decompensation after the use of intraoperative MMC 0.02% in refractive surgery.[23] Data related to the endothelial effects of MMC after refractive surgery are sometimes inconclusive. Morales et al[24] and Nassiri et al[25] found statistically significant corneal endothelial changes in eyes treated with MMC. However, the other authors concluded that the use of MMC to prevent haze after refractive surgery does not reduce the endothelial cell density compared with the control eyes.[2,7,26–29] Moreover, a concentration of MMC substantially greater than that used in refractive surgery, applied in a cyclic manner for the treatment of ocular surface squamous tumors, does not significantly affect the corneal endothelium.[30] In the current study, the loss of endothelial cells 5 years after surgery was not statistically significant and is consistent with the physiologic decrease in corneal endothelial cells.[31]

The subbasal nerve plexus is ablated during PRK, and the recovery of nerve fibers is a slow process. Erie et al[32] reported that subbasal nerves can recover to preoperative densities by 2 years after PRK. In our patients, 5 years after PRK, the number and density of nerve fibers were still reduced compared with baseline values. Nevertheless, we found that the density of nerve fibers is significantly higher in the MMC-treated eyes compared with the corticosteroid-treated eyes. It is unknown whether the lower density of subbasal nerve plexus in the corticosteroid-treated eyes relates to a toxic effect of topical corticosteroids; this observation has never been reported.

The subbasal nerve plexus recovers after PRK, but its fibers may be abnormal for many years after photoablation.[33] In our patients, at 5 years, the number of corneal nerve beadings was lower, the order of fiber branching was reduced, and the grade of fiber tortuosity was increased compared with baseline (Fig. 1).

The mean number of corneal subbasal nerve plexus beadings, which are accumulations of mitochondria along the nerve, significantly decreases after surgery and does not fully return to preoperative values. However, there were no differences between the 2 treatment groups, suggesting that MMC does not by itself have long-term effects on mitochondrial DNA during the regeneration of nerve fibers. Whereas higher corneal nerve tortuosity may represent a morphologic marker of nerve regeneration, a reduction in branching is suggestive of limited regeneration.[34] Five years after surgery, no significant difference in these parameters (branching and tortuosity) between the MMC-treated eyes and the corticosteroid-treated eyes was documented. We also analyzed whether intraoperative MMC affects the corneal

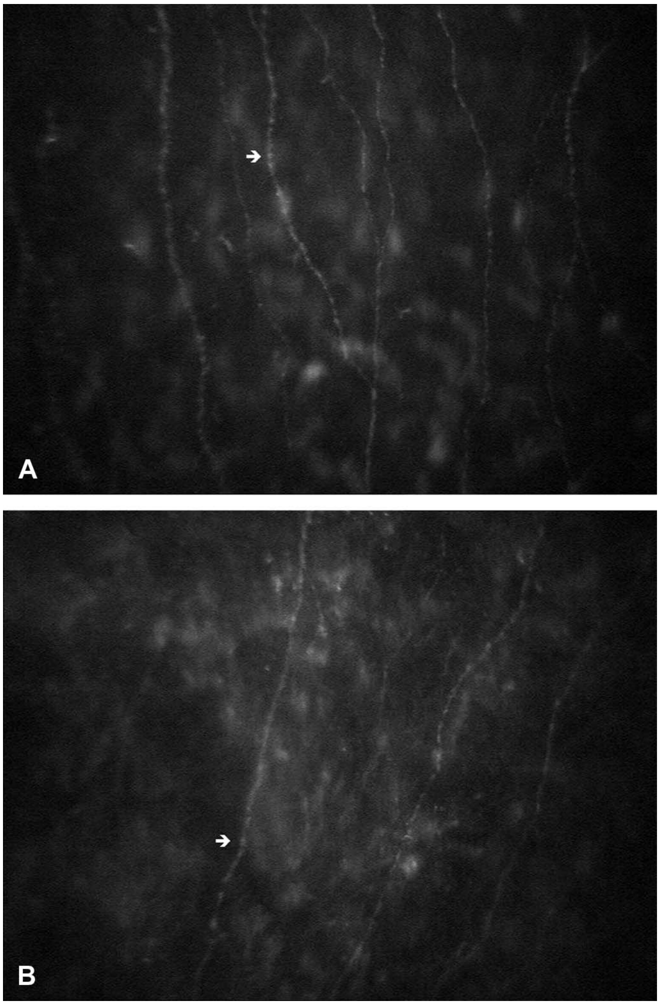

FIGURE 1. Corneal subbasal nerve plexus (confocal microscopy) before (A) and 5 years after (B) PRK; the number and density of nerve fibers and the mean number of beadings (arrow) are below normal values. The eye in this case underwent MMC-assisted PRK.

epithelium. Corneal epithelial cells have a rapid turnover rate, and there are reports of limbal stem cell deficiency after treatment of corneal–conjunctival neoplasia with MMC.[35] However, delayed epithelialization after MMC-assisted PRK has been reported in only 0.2% of eyes in noncomparative series.[2] In our patients, 5 years after surgery, epithelium thickness was slightly increased compared with the baseline values, but there are no significant differences between the 2 treatment groups. This observation confirms that the previous studies reported no delay in epithelial healing after MMC-assisted refractive surgery.[8,26,28]

Our findings show that 0.02% topical MMC, applied for 2 minutes after PRK in highly myopic eyes, does not induce significant clinical or morphologic alterations in the corneal endothelium and epithelium.[2,7,8,26–28] Moreover, at 5 years, morphologic parameters of nerve fibers (number of beadings, branching, and tortuosity) were similar in the 2 treatment groups, suggesting that MMC does not modify nerve regeneration. Subbasal nerve plexus density in our patients was higher in the MMC-treated eyes than in the corticosteroid-treated eyes.

Intraoperative use of topical 0.02% MMC does not induce long-term side effects on corneal layers, as assessed by in vivo corneal confocal microscopy. According to our data, 0.02% topical intraoperative MMC seems to be a safe treatment for preventing haze formation in patients with high myopia undergoing PRK, although a longer follow-up may be useful to confirm these observations.

REFERENCES

1. Esquenazi S, He J, Bazan NG, et al. Comparison of corneal wound healing response in photorefractive keratectomy and laser-assisted subepithelial keratectomy. *J Cataract Refract Surg*. 2005;31:1632–1639.
2. Lee DH, Chung HS, Jeon YC, et al. Photorefractive keratectomy with intraoperative mitomycin-C application. *J Cataract Refract Surg*. 2005; 31:2293–2298.
3. Shalaby A, Kaye GB, Gimbel HV. Mitomycin in photorefractive keratectomy. *J Refract Surg*. 2009;25:S93–S97.
4. Kim TI, Lee SY, Pak HJ, et al. Mitomycin C, ceramide, and 5-fluorouracil inhibit corneal haze and apoptosis after PRK. *Cornea*. 2006;25:55–60.
5. Gartry DS, Kerr Muir MG, Lohmann CP, et al. The effect of topical corticosteroids on refractive outcome and corneal haze after photorefractive keratectomy. *Arch Ophthalmol*. 1999;110:944–952.
6. Talamo JH, Gollamudi S, Green WR, et al. Modulation of corneal wound healing after excimer laser keratomileusis using topical mitomycin C and steroids. *Arch Ophthalmol*. 1991;109:1141–1146.
7. Carones F, Vigo L, Scandola E, et al. Evaluation of the prophylactic use of mitomycin-C to inhibit haze formation after photorefractive keratectomy. *J Cataract Refract Surg*. 2002;28:2088–2095.
8. Gambato C, Ghirlando A, Midena E, et al. Mitomycin C modulation of corneal wound healing after photorefractive keratectomy in highly myopic eyes. *Ophthalmology*. 2005;112:208–219.
9. Netto MV, Mohan RR, Sinha S, et al. Effect of prophylactic and therapeutic mitomycin C on corneal apoptosis, cellular proliferation, haze, and long-term keratocyte density in rabbits. *J Refract Surg*. 2006;22: 562–574.
10. Wilson SE. Long term outcomes after photorefractive keratectomy. *Ophthalmology*. 2006;113:1693–1694.
11. Thornton I, Puri A, Xu M, et al. Low-dose mitomycin C as a prophylaxis for corneal haze in myopic surface ablation. *Am J Ophthalmol*. 2007;144: 673–681.
12. Midena E, Gambato C, Miotto S, et al. Long-term effects on corneal keratocytes of mitomycin C during photorefractive keratectomy: a randomized, contralateral eye confocal microscopy study. *J Refract Surg*. 2007;23:S1011–S1014.
13. Fantes FE, Hanna KD, Waring GO III, et al. Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys. *Arch Ophthalmol*. 1990;108:665–675.
14. Li HF, Petroll WM, Møller-Pedersen T, et al. Epithelial and corneal thickness measurements by in vivo confocal microscopy through focusing (CMTF). *Curr Eye Res*. 1997;16:214–221.
15. Meijering E, Jacob M, Sarria JC, et al. Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. *Cytometry A*. 2004;58:167–176.
16. Midena E, Brugin E, Ghirlando A, et al. Corneal diabetic neuropathy: a confocal microscopy study. *J Refract Surg*. 2006;22:S1047–S1052.
17. Oliveira-Soto L, Efron N. Morphology of corneal nerves using confocal microscopy. *Cornea*. 2001;20:374–384.
18. Randleman JB, Loft ES, Banning CS, et al. Outcomes of wavefront-optimized surface ablation. *Ophthalmology*. 2007;114:983–988.
19. Rajan MS, O'Brart DPS, Patmore A, et al. Cellular effects of mitomycin-C on human corneas after photorefractive keratectomy. *J Cataract Refract Surg*. 2006;32:1741–1747.
20. Torres RM, Merao-Lloves J, Daya SM, et al. Presence of mitomycin-C in the anterior chamber after photorefractive keratectomy. *J Cataract Refract Surg*. 2006;32:67–71.
21. Roh DS, Cook AL, Rhee SS, et al. DNA cross-linking, double-strand breaks, and apoptosis in corneal endothelial cells after a single exposure to mitomycin C. *Invest Ophthalmol Vis Sci*. 2008;49:4837–4843.
22. Chang SW. Early corneal edema following topical application of mitomycin-C. *J Cataract Refract Surg*. 2004;30:1742–1750.
23. de Benito-Llopis L, Teus MA, Ortega M. Effect of mitomycin-C on the corneal endothelium during excimer laser surface ablation. *J Cataract Refract Surg*. 2007;33:1009–1013.
24. Morales AJ, Zadok D, Mora-Retana R, et al. Intraoperative mitomycin and corneal endothelium after photorefractive keratectomy. *Am J Ophthalmol*. 2006;142:400–404.
25. Nassiri N, Farahangiz S, Rahnavardi M, et al. Corneal endothelial cell injury induced by mitomycin-C in photorefractive keratectomy: non-randomized controlled trial. *J Cataract Refract Surg*. 2008;34:902–908.
26. Wallau AD, Campos M. Photorefractive keratectomy with mitomycin C versus LASIK in custom surgeries for myopia: a bilateral prospective randomized clinical trial. *J Refract Surg*. 2008;24:326–336.
27. Diakonis VF, Pallikaris A, Kymionis GD, et al. Alterations in endothelial cell density after photorefractive keratectomy with adjuvant mitomycin. *Am J Ophthalmol*. 2007;144:99–103.
28. Leccisotti A. Mitomycin C in photorefractive keratectomy. Effect on epithelialization and predictability. *Cornea*. 2008;27:288–291.
29. Goldsberry DH, Epstein RJ, Majmudar PA, et al. Effect of mitomycin C on the corneal endothelium when used for corneal subepithelial haze prophylaxis following photorefractive keratectomy. *J Refract Surg*. 2007; 23:724–727.
30. Panda A, Pe'er J, Aggarwal A, et al. Effect of topical mitomycin C on corneal endothelium. *Am J Ophthalmol*. 2008;145:635–638.
31. Bourne WM, Nelson LR, Hodge DO. Central corneal endothelial cell changes over a ten year period. *Invest Ophthalmol Vis Sci*. 1997;38: 779–782.
32. Erie JC, McLaren JW, Hodge DO, et al. Recovery of corneal subbasal nerve density after PRK and LASIK. *Am J Ophthalmol*. 2005;140: 1059–1064.
33. Linna T, Tervo T. Real-time confocal microscopic observations on human corneal nerves and wound healing after excimer laser photorefractive keratectomy. *Curr Eye Res*. 1997;16:640–649.
34. Kallinikos P, Berhanu M, O'Donnell C, et al. Corneal nerve tortuosity in diabetic patients with diabetic neuropathy. *Invest Ophthalmol Vis Sci*. 2004;45:418–422.
35. Dudney BW, Malecha MA. Limbal stem cell deficiency following topical mitomycin C treatment of conjunctival-corneal intraepithelial neoplasia. *Am J Ophthalmol*. 2004;137:950–951.