Original Article

Comparing the Effect of Mechanical and Tetracaine-Assisted Epithelial Debridement on Central Corneal Endothelial Cells in PRK

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Abstract

Purpose: To compare the effects on central corneal endothelial cells measured by confocal microscopy after photorefractive keratectomy (PRK) with mitomycin C (MMC) using mechanical and tetracaine-assisted epithelial debridement methods.
Patients and Methods: In this prospective, randomized, comparative study, 44 patients (88 eyes) with myopia of up to -6.00 diopters were enrolled. Each patient's right eye was randomly assigned to either the tetracaine-assisted or the mechanical debridement group, with the left eye assigned to the other method. Preoperative and 3-month postoperative assessments were conducted using confocal microscopy. These assessments measured epithelial thickness, the number of keratocytes in the anterior, mid, and posterior stroma, and the characteristics of central corneal endothelial cells. These characteristics included cell density, mean cell area, polymegathism, and hexagonality.
Results: No statistically significant differences were observed between the two groups considering the mean epithelial thickness at the 3-month follow-up. Additionally, there was no statistically significant difference in central corneal endothelial cell characteristics, including mean cell density, mean cell area, mean hexagonality, or mean polymegathism. The density of mid and posterior stromal keratocytes remained unchanged compared to baseline in both groups. However, a significant decrease in anterior stromal keratocyte density was observed in both groups three months after surgery, with no significant difference between the two debridement methods.
Conclusion: The effects of PRK with MMC using mechanical or tetracaine-assisted epithelial debridement methods on central corneal endothelial cells are similar. Additionally, both techniques lead to comparable reductions in stromal keratocyte density in anterior stromal layer.

Keywords: Photorefractive Keratectomy; Cornea; Endothelial Cell Loss; Mitomycin C; Epithelium; Debridement.

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Introduction

Photorefractive keratectomy (PRK) is a laser refractive surgery used to correct refractive errors such as myopia by ablating the corneal stroma [1,2]. A critical step in PRK is epithelial debridement, where the corneal epithelium is removed to make the stroma exposed for laser remodeling [3]. This procedure typically is performed with the use of mitomycin C (MMC) to prevent postoperative haze, a common complication that might impair visual outcomes [4-6].

While the overall efficacy of PRK with MMC in improving vision is well-documented, the differential impacts of various epithelial debridement techniques on corneal health remains in question [1,2]. Two common debridement methods are mechanical and alcohol-assisted techniques, but tetracaine-assisted debridement have also been used in some limited fashion [7-9]. These techniques are different in their approach to epithelial removal, which could potentially influence corneal healing and cellular response post-surgery [7-9].

Central corneal endothelial cells play an important role in maintaining corneal clarity and thickness, which is crucial for optimal vision [10]. Since these cells do not have the ability to reproduce post-surgical damage to them can lead to long-term corneal complications [11]. Similarly, keratocytes are essential in maintaining corneal structure and transparency [12]. Understanding how different debridement techniques influence these cells is vital in optimizing PRK outcomes [13].

Given this background, our study aimed to compare the effects of mechanical and tetracaine-assisted epithelial debridement in PRK with MMC on central corneal endothelial cells and stromal keratocyte density. This comparison is crucial in understanding whether the choice of epithelial debridement technique can influence corneal cellular health and potentially the overall success of the PRK procedure.

Patients and Methods

Patients

This prospective, randomized, comparative study included 44 patients (88 eyes) with myopia from Imam Hossein Medical Center and Vanak Eye Surgery Center, Tehran, Iran. The study adhered to the tenets of the Declaration of Helsinki and received approval from the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Written informed consent was obtained from all participants.

Eligible participants were between 19 and 50 year old, with myopia, spherical equivalent refraction of better than -6.00 diopters (D) and a cylindrical error of less than -3.00 D. Other criteria included a stable refractive error over the past year, no soft contact lens wear for last 2 weeks, and no hard contact lens use for last 6 weeks before the baseline examination. Additionally, participants were required to have an estimated residual corneal thickness of more than 420 microns after surgery. The exclusion criteria were having a history of systemic diseases (such as autoimmune disorders, collagen vascular diseases, or diabetes), being one-eyed, or having conditions like dry eye, corneal ectasia, a history of ocular trauma or intraocular surgery. Other exclusion criteria included an intraocular pressure of greater than 21 mmHg, lens opacity, uveitis, posterior segment diseases, and any other ocular diseases except for refractive errors.

Each patient's right eye was randomly assigned to one of two epithelial debridement methods:

mechanical or tetracaine-assisted debridement. The left eye of each patient was treated with the alternate method. Randomization was conducted using a computer-generated random number table to ensure unbiased assignment.

Pre- and postoperative assessment

For the preoperative and three-month postoperative assessments, several procedures were employed. Initially, patients underwent slit lamp examinations, followed by testing of Snellen best-corrected visual acuity (BCVA) and refraction measurements. Dilated fundoscopy was also performed. In addition to these assessments, confocal microscopy (ConfoScan 3.4; NIDEK Technologies, Padova, Italy) was carried out. An ophthalmic pathologist, unaware of the debridement technique used, conducted the confocal microscopy.

The outcome measures, evaluated three months after surgery, included the number of keratocytes and the characteristics of central corneal endothelial cells, specifically in terms of cell density, mean cell area, polymegathism, and hexagonality.

Surgical Procedure

The PRK surgical procedures were uniformly conducted by a singular experienced surgeon, utilizing the Allegretto WAVE Eye Q system (Alcon Laboratories Inc, software version 2.020 default treatment), with a consistent laser nomogram for both patient groups. Prior to surgery, the patients' eyelids and surrounding ocular area were disinfected with a 10 % povidone-iodine solution for one minute, followed by cleansing with 20 ml of balanced salt solution (BSS). The surgical approach in each case involved a 6 mm optical zone and an added 1.0 mm transition zone. The local anesthesia was performed using one drop of 0.5 % tetracaine in each eye.

Epithelial removal in the mechanical method involved centering an 8.0-mm optical zone marker on the cornea over the entrance pupil. The epithelium was excised using a hockey knife, after which photoablation was performed on the stromal layer of the cornea. Conversely, for the tetracaine-assisted debridement approach, an 8.5-mm well containing 0.5 % tetracaine was placed on the epithelium for 20 to 25 seconds before being absorbed by a dry cellulose sponge.

This was followed by a BSS rinse and removal of surplus fluid with another sponge. Mitomycin C was applied in both methods if the ablation depth was more than 70 micron, patient was hyperopic or the astigmatism correction of more than 1 diopter was performed. A blunt spatula facilitated the epithelial rhexis of the loosened epithelium, and immediate stromal photoablation ensued.

Application of 0.02 % mitomycin C (0.2 mg/mL) to the ablated stromal area varied between 30 to 55 seconds based on the depth of ablation. Subsequent to this, a thorough irrigation with 50 ml of BSS was performed, and a protective bandage contact lens (OmniFlex; Coopervision Ltd, Hampshire, United Kingdom) was placed on the eye.

Post surgery patients received betamethasone 0.1 % and ciprofloxacin 0.3 % eye drops, both administered three times daily until the cornea's re-epithelialization was complete. The dosage of betamethasone was gradually decreased over the subsequent month.

Confocal microscopy

The procedure began with the application of 0.5 % tetracaine eye drops for anesthesia.

To facilitate image capture, GenTeal Gel (Novartis Ophthalmics, East Hanover, New Jersey), a viscous gel, was placed on the microscope objective's tip as a contact solution. The microscope, equipped with a 40x objective lens, was precisely aligned and centered to focus on the bright reflex from the posterior surface of the cornea's anterior wall. This setup allowed for the automatic capture of full-thickness corneal images. Following auto-alignment, a digital video camera recorded up to 350 digital photos (25 images per second) as the cornea's whole thickness was scanned from the endothelium to the epithelial surface. An average of 1.5 µm separated each image from the next one. For the assessment of the endothelium, two unambiguous pictures of the layer were picked and endothelial cell density (cells/mm²), hexagonality, polymegathism, and mean cell area were automatically calculated.

The front 10 %, middle 33–66 %, and posterior 10 % of the stroma were calculated with Z-scan graphs. Two well-defined, focused photos were chosen in every layer and keratocyte density was calculated by hand counting the brilliant keratocyte nuclei inside a predetermined area. The results of two pictures from each stromal layer were averaged.

Statistical Analysis

All statistical analyses were performed using SPSS software Version 21 (Armonk, NY: IBM Corp). The paired t-test was used to compare the study parameters measured by confocal scanning between the two groups. A P value of less than 0.05 was considered indicative of statistical significance.

Results

A total of 44 patients (88 eyes) were included in the study. The average age of the patients entering the study was 32 ± 9 years, ranging from 20 to 47 years. The study included 21 males and 23 females. All participants successfully completed their follow-up examinations. Baseline characteristics of the patients are summarized in table 1. No significant differences were observed in baseline characteristics like ablation depth,

Table 1: Baseline characteristics of 88 eyes (44 patients) that underwent PRK using tetracaine assisted or mechanical epithelial debridement

	Tetracaine assisted debridement	Mechanical debridement	P*
BCVA, LogMAR	0.05 ± 0.02	0.04 ± 0.03	0.357
Sphere, D	- 2.38 ± 0.74	- 2.61 ± 0.89	0.301
Cylinder, D	- 0.42 ± 0.47	- 0.4 ± 0.41	0.868
Spherical equivalent, D	- 2.71 ± 0.72	- 2.65 ± 0.85	0.605
Ablation depth, µm	50.71 ± 13.45	50.43 ± 13.55	0.814

* Based on Paired t-test
BCVA: Best-Corrected Visual Acuity
LogMAR: Logarithm of the Minimum Angle of Resolution:
D: Diopter

best-corrected visual acuity (BCVA), sphere, cylinder and spherical equivalent, between the two groups.

The preoperative and 3-month postoperative results are compared in table 2. No statistically significant differences were observed in epithelial thickness between the groups three months postoperatively. Additionally, no significant differences were found in variables related to corneal endothelial cells, including endothelial cell density, polymegathism, hexagonality, and mean cell area between the two groups of patients. While there were no significant changes in the density of mid- and posterior stromal keratocytes in either group compared to baseline values, a notable

Table 2: Comparison of the results of confocal microscopy in terms of corneal epithelial. endothelial and stromal cell parameters in PRK using tetracaine assisted or mechanical epithelial debridement

Variables	Mean ± SD		Mean Diff (95 % CI)*	P value*
	Tetracaine-assisted debridement	Mechanical debridement		
Epithelial thickness, µm				
Preop	24.6 ± 5.2	24.6 ± 5.4	0 (- 0.1 to 0.1)	0.88
Postop	24.6 ± 5.1	24.3 ± 5.1	0 (- 0.1 to 0.1)	0.999
Percentage change	0 ± 1	0 ± 1	-2 (- 9 to 5)	0.631
P value	0.999	0.873		
Keratocyte density, cells/mm ^{2}				
Anterior stroma (10 %)				
Preop	868 ± 126	848 ± 121	20 (- 24 to 65)	0.358
Postop	540 ± 91	527 ± 102	13 (- 18 to 45)	0.392
Percentage change	- 36 ± 18	- 35 ± 24	0 (- 1 to 1)	0.874
P value	< 0.001	< 0.001		
Mid-stroma				
Preop	600 ± 75	598 ± 72	2 (- 21 to 25)	0.863
Postop	589 ± 52	606 ± 65	- 17 (- 43 to 9)	0.186
Percentage change	0 ± 13	2 ± 13	- 1 (- 4 to 2)	0.363
P value	0.485	0.620		
Posterior stroma (10 %)				
Preop	596 ± 88	597 ± 66	- 1 (- 33 to 31)	0.935
Postop	626 ± 71	607 ± 91	18 (- 14 to 51)	0.258
Percentage change	22 ± 90	3 ± 18	- 3 (- 11 to 4)	0.384

Variables	Mean ± SD		Mean Diff (95 % CI)*	P value*
	Tetracaine-assisted debridement	Mechanical debridement		
P value	0.179	0.652		
Endothelial cell parameters				
Density, cells/mm ^{2}				
Preop	3041 ± 302	3057 ± 268	- 16 (- 101 to 68)	0.690
Postop	2943 ± 278	3004 ± 287	- 61 (- 135 to 13)	0.102
Percentage change	- 3 ± 6	- 2 ± 5	1 (- 2 to 5)	0.325
P value	0.014	0.060		
Polymegathism				
Preop	31.5 ± 4.8	31.2 ± 4.9	0.4 (- 1.5 to 2.2)	0.691
Postop	30.3 ± 4	31 ± 5.2	- 0.7 (- 2 to 0.6)	0.270
Percentage change	- 3 ± 10	0 ± 12	- 1 (- 7 to 5)	0.795
P value	0.061	0.825	.	
Hexagonality				
Preop	62.5 ± 7.7	61.9 ± 7.5	0.6 (- 2.5 to 3.6)	0.707
Postop	60.6 ± 7.2	61.1 ± 8	- 0.6 (- 3.7 to 2.5)	0.716
Percentage change	- 2 ± 12	- 1 ± 14	- 3 (- 7 to 2)	0.226
P value	0.238	0.623		
Mean cell area				
Preop	332 ± 37	330 ± 32	3 (- 7 to 12)	0.585
Postop	343 ± 34	336 ± 36	7 (- 2 to 16)	0.133
Percentage change	3 ± 7	2 ± 5	19 (- 16 to 55)	0.277
P value	0.018	0.074		

*CI: Confidence Interval
†Based on Paired t - Test
Preop: Preoperative
Postop: Postoperative

decrease in anterior stromal keratocyte density was seen in both groups three months post-surgery. Across all three layers, no statistically significant differences were detected in postoperative keratocyte numbers between the two patient groups.

Discussion

The present study aimed to compare the outcomes of photorefractive keratectomy (PRK) with intraoperative mitomycin C (MMC) using tetracaine-assisted and mechanical epithelial debridement techniques. The results of our study demonstrated no significant differences in epithelial thickness between the tetracaine-assisted and mechanical debridement groups three months post-operatively. Although to the best of our knowledge no previous comparison of mechanical and tetracaine-assisted debridement has been performed in English literature, studies comparing the mechanical debridement with alcohol-assisted debridement have reported similar results [9,11].

Regarding central corneal endothelial cells, our study found no significant differences in cell density, mean cell area, hexagonality, or polymegathism between the two debridement methods. These findings are in line with previous studies that have reported similar outcomes between different debridement methods [11]. This finding suggests that the use of tetracaine to facilitate epithelial debridement probably does not add to the potential adverse effects of mitomycin C on these cells similar to using alcohol in alcohol-assisted mechanical debridement [11].

In terms of stromal keratocytes, both tetracaine assisted and mechanical debridement groups showed a significant decrease in mean post-operative anterior stromal keratocyte density revealed by confocal microscopy. Previous studies have reported contradictory results when comparing alcohol-assisted and mechanical debridement methods. While Helena et al., [14] and Netto et al., [15] have reported more keratocytes loss after alcohol-assisted corneal debridement in rabbit eyes compared with mechanical technique, Einollahi et al., reported less keratocyte loss in alcohol-assisted and Nassiri et al., reported no statistically significant difference between the two methods [11].

The absence of significant changes in mid and posterior stromal keratocyte density suggests that these regions were less affected by the debridement technique and MMC application. This finding is consistent with previous studies [9,11].

Overall, our study suggests that the tetracaine-assisted and mechanical debridement techniques have comparable outcomes in terms of corneal endothelial cell damage and reduction in anterior stromal keratocyte density. These findings are reassuring for clinicians, as they indicate that both techniques can be utilized with confidence in PRK procedures with MMC, without compromising the health of central corneal endothelial cells.

It is important to acknowledge some limitations of our study. The follow-up period for our study was relatively short at 3 months. Also, the sample size was relatively small, which may have limited the statistical power to detect small differences between the groups. Additionally, we did not compare haze formation and the time needed for re-epithelialization between the two groups.

Conclusion

The effects of PRK with MMC using mechanical or tetracaine-assisted epithelial

debridement methods on central corneal endothelial cells are similar. Additionally, both techniques lead to comparable reductions in stromal keratocyte density in anterior stromal layer.

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.