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Effectiveness of autologous serum eye drops in corneal epithelial healing after photorefractive keratectomy

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自体血清滴眼液对屈光性角膜切削术后角膜上皮愈合的影响

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摘要

目的:研究自体血清滴眼液对屈光性角膜切削术(PRK)后角膜上皮愈合的影响。

方法:本研究共纳入 20 例 40 眼近视及近视散光患者,其中男性 9 例,女性 11 例。每例患者随机选取一眼作为研究组应用 20%含自体血清的人工泪液,另一眼为对照组应用常规人工泪液。PRK 术中,40 眼均于角膜表面居中放置直径 8 mm 的酒精储槽, 20%的酒精作用 20s。术后每天观察直到角膜上皮愈合后,分别于 1mo,6mo,12mo 进行随访。上皮愈合时间为主要观察指标,并记录裸眼视力(UCVA),显然验光和混浊度。

结果:两组间术前小瞳检影结果无明显差异。术后 1d, 2d,3d,研究组平均疼痛评分均显著低于对照组($P<0.05$)。术后 1d,3d,研究组的平均水平和垂直上皮缺损低于对照组($P<0.05$)。研究组上皮完全愈合的平均时间比对照组短约 0.7d(3.15±0.366d *vs* 3.85±0.587d, $P=0.00$)。

结论:研究表明,应用自体血清滴眼液,通过加速角膜上皮愈合和减轻疼痛,从而缩短视力恢复时间,降低屈光性角膜切削术术后不适、混浊度和感染风险。

关键词:角膜;自体血清;屈光角膜切除术;上皮愈合

Abstract

• AIM: To study the effect of autologous serum in corneal epithelial healing after photorefractive keratectomy (PRK).

• METHODS: Forty eyes from 20 myopic and myopic-astigmatic patients (9 male and 11 female) were included in this study. One eye of each patient was randomized to receive 20% autologous serum in artificial tears (study group) and one eye received conventional artificial tears (control group). An 8 mm alcohol well was placed centrally in all 40 eyes, and 20% alcohol was applied for 20s during PRK operation. Patients were followed up daily until epithelial closure, and at 1mo, 6mo, and 12mo. Time to epithelial healing was the main outcome measure. Uncorrected visual acuity (UCVA), manifest refraction, and haze were recorded.

• RESULTS: The mean preoperative myopic spherical non-cycloplegic (dry) retinoscopy was not significantly different between two groups. The mean pain score in the study group was significantly lower than the control group on days 1, 2 and 3 ($P<0.05$). The mean horizontal and vertical epithelial defects in the study group was lower than in the control group in all follow up exams on days 1 and 3 ($P<0.05$). The mean total time to epithelial healing in the study group was about 0.7d less than in the control group (3.15±0.366d *vs* 3.85±0.587d, $P=0.00$).

• CONCLUSION: This study demonstrated that using autologous serum eye drops, by accelerating corneal epithelial healing and reducing the pain, which improves recovery time in visual acuity and reduces discomfort, haziness and infection risk after PRK.

• KEYWORDS: cornea; autologous serum; photorefractive keratectomy; epithelial healing
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INTRODUCTION

For many years, excimer laser therapy has become the major technique for the treatment of a wide spectrum of refractive errors[1]. The main procedures for excimer laser therapy are photorefractive keratectomy (PRK) which is also known as surface ablation, and laser-assisted *in situ* keratomileusis (LASIK). Both procedures create flaps before laser treatment and change the shape of the anterior central cornea by ablating (remove by vaporization) a small amount of tissue from the corneal stroma[2]. PRK remodels corneal stroma to compensate refractive errors. Due to its suitability for those eyes in which LASIK surgery is risky and relative safety in terms of complications (specially flap related complications) and technical simplicity compared to LASIK, surface ablation has remained as an alternative technique to LASIK. However, relatively slow visual recovery and early postoperative pain due to delayed epithelial healing remain the main disadvantages of surface ablation[3].

The removal of epithelium and the ablation of stroma results the disruption of corneal structures and nerves, and release of several peptides and inflammatory cytokines, matrix metalloproteases from epithelium, stroma and nerves into the tear film. On the other hand, a few numbers of growth factors, cytokines, and matrix metalloproteases participate in the process of corneal wound healing. The advantage of autologous serum (AS) is that many of its biochemical characteristics, including PH, nutrient content, vitamins, fibronectin, growth factors such as epithelial growth factor (EGF) or nerve growth factor (NGF), are similar to that of human tears. Several *in vitro* and *in vivo* studies have shown that serum and other blood derivatives enhance corneal epithelial wound healing, probably due to these factors[4-8]. Autologous serum was also found to inhibit the release of inflammatory cytokines and to increase the number of goblet cells and mucin expression in the conjunctiva in a few clinical case series[9-10].

This study was designed to evaluate the effectiveness of autologous serum drops on corneal epithelial healing time and visual and clinical results after photorefractive keratectomy for correction of myopia and myopic astigmatism.

SUBJECTS AND METHODS

Forty eyes from 20 myopic and myopic astigmatic patients (9 male and 11 female) were included in this study. One eye of each patient was simply randomized to receive 20% autologous serum in artificial tears (OFTAGEL Ursapharm Arzneimittel GmbH, Saarbrücken, Germany) (study group) and the other eye received conventional artificial tears (control group). The random allocation software has been used for dividing the eyed into two groups.

The study was designed as double-blind randomized controlled trial style. Considering the fact that, as yet, such a clinical evaluation has not been carried out, in order to examine the plausibility of the research, this study has been designed as a pilot study. Written informed consent was obtained from all patients, the study was approved by the Tabriz Medical University Ethics Committee. The tenets of the Declaration of Helsinki were followed throughout the study.

Inclusion criteria were: age range of 18-40 years, stable refraction of at least 1 year, normal corneal topography, central corneal thickness >500 μm, myopia in the range of -1 to -7 diopters and the astigmatic value under 4 diopters. Daily-wear soft contact lenses were removed at least 1wk before the preoperative examination.

Preoperative evaluation included medical history and complete ophthalmologic examination, including uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA), manifest and cycloplegic refractions, anterior segment examination, ophthalmoscopy, applanation tonometry, ultrasonic pachymetry, corneal topography (Orbscan, Bausch & Lomb, France), and Schirmer testing. Exclusion criteria were: unstable refraction, hyperopic refraction, myopia and astigmatism out of the determined range, dry eye, blepharitis, corneal disease, glaucoma, systemic diseases including infectious and collagen vascular diseases, diabetes, and topographical evidence of keratoconus or other topographical abnormalities.

Whole blood (20 mL) was procured by venipuncture, centrifuged at 3000 revolutions per minute (relative centrifugal force of 25.15×g) for 15min. AS drops were carefully prepared under a laminar airflow cabinet, and diluted with artificial tears (Oftagel) to 20%. AS drops were prepared on the day of surgery and patients were asked to keep AS drops refrigerated at about 4℃.

All twenty patients underwent PRK bilaterally in the same session. Topical Anesthocaine (Tetracaine 0.5%, Sina Darou) was used to local anesthesia. After prep and drape with 10% povidone-iodine lid speculum was inserted and chilled BSS was applied on the cornea. In all eyes, an 8 mm alcohol well was placed centrally, and 20% alcohol was applied for 20s. After the epithelium was detached and discarded, a bare corneal surface with regular epithelial edge was achieved. Spherical ablations were performed according to manifest refraction and after centration and tracking without any reduction using the Technolas 217z Zyoptix excimer laser (BAUSCH & LOMB, Kleinostheim, Germany) excimer laser vision correction was done. The ablation profile was aspheric, but in patients with higher amounts of astigmatism or high aberrations customized ablation profile was accomplished. The optical zone diameter was 6.5 mm in all eyes with varying transition zones determined by Technolas Zyoptix. Total ablation diameter did not exceed 8.0 mm in any of the eyes. Once ablation was finished, Mitomycin C was applied in 30s duration for all patients; by reason of confounding bias of mitomycin the application of the mitomycin was assimilated in all patients. The cornea was irrigated with balanced salt

Table 1 Uncorrected visual acuity based on Snellen chart over times

UCVA (LogMar)	Study group	Control group	*P*
Preoperative	0.66±0.13 (0.5-0.9)	0.64±0.11 (0.5-0.9)	0.401
1mo	1.03±0.12 (0.9-1.2)	1.11±0.15 (0.9-1.2)	0.233
6mo	0.97±0.13 (0.8-1.2)	0.98±0.13 (0.8-1.2)	0.345
12mo	0.97±0.12 (0.8-1.2)	0.97±0.12 (0.8-1.2)	0.357

UCVA: Uncorrected visual acuity.

solution, and a drop of Chloramphenicol was instilled. A cooled soft contact lens was placed over the cornea with sterile forceps. A drop of AS or artificial tears was instilled. The eyelid speculum and drape were removed.

At the executive stage, the assistant was aware of the type of intervention and its assignment to relevant groups, but the follow-up examiner (the surgeon) and the patient were not aware of the type of intervention assigned to which eye.

Patients were visited daily by the same examiner in the morning until epithelial closure. All patients were examined in 1wk, 1mo, 6mo and 12mo. After PRK, all patients received chloramphenicol 4 times daily until complete epithelial healing. Betamethasone started every 4h, followed by tapering every week until 1mo, followed by fluorometholone 0.1% (Sina Darou) four times daily for another two to four weeks depending on refraction and haze level.

AS drop in the study group and artificial tears (Oftagel) in the control group every 2h was administrated. AS eye-drops were changed to artificial tears after epithelial healing. All medications were discontinued after 3mo.

In daily visits, patients were asked to grade their level of ocular discomfort until epithelial healing as follows: Level 0: no pain; Level 1: mild pain; Level 2: moderate pain; Level 3: severe pain. Postoperative haze was graded as follows: 0: completely clear cornea; +0.5: barely visible corneal opacity; +1: reticular subepithelial opacities not interfering with visibility of fine iris details; +2: punctate or coalesced subepithelial opacities with mild obscuration of iris details; +3: confluent subepithelial opacities with moderate obscuration of the iris and lens; +4: dense opacities with complete opacification of the stroma[2].

Data were analyzed by SPSS software version 21 as qualitative data (percentage and frequency) and quantitative data (mean± SD). The normality test was performed by the Kolmogorov-Smirnov test. To compare the quantitative data, *t*-test was used. Statistical significance was considered as $P<0.05$.

RESULTS

Considering that one eye of each patient received the autologous serum eye drop and the other eye of the same patient received conventional artificial tear, the effect of the age and gender on the results was eliminated. The mean age of the patients was 31.5±10.5 (range: 21-35) years old. 45% of patient was male and 55% was female. There was no statistically significant difference between male and female patients considering age ($P=0.391$).

Table 2 Mean pain score during 1d to 4d after surgery

Postoperative	Study group	Control group	*P*
1d	1.58±0.77	1.95±0.22	0.034
2d	0.80±0.77	1.45±0.51	0.005
3d	0.16±0.69	0.95±0.22	0.001
4d	0.05±0.22	0.15±0.37	0.073

The mean preoperative non-cycloplegic (dry) refraction was -3.55±1.68 (range: 0 to -7.25) D in eyes receiving AS drops (study group) and -2.97±1.37 (range: 0 to -6.5) D in eyes receiving conventional artificial tear (control group), ($P=0.154$) and mean cycloplegic refrarction was -3.30±1.70 D in study group and -2.65±1.63 D in control group ($P=0.211$).

Mean astigmatism was 0.77±0.53 (range: 0 to 4.5) D in the study group and 0.72±0.51 (range: 0 to 4.5) D in the control group ($P=0.311$). The mean preoperative central corneal thickness based on pentacam findings was 558 in the study group and 561 in the control group ($P=0.321$). 90 % of patients in both groups achieved complete vision (10/10 or better) in the first month. Changes in visual acuity over time are presented in Table 1.

The mean pain score in the study group was significantly lower than the control group in day 1, 2 and 3 postoperative ($P=0.034$, $P=0.005$ and $P=0.001$), such a difference was not found in fourth day (Table 2).

The patients in the study group mostly reported a moderate (Level 2) pain on day 1, which decreased to mild level (Level 1) afterwards and completely resolved on day 2 or 3 when their contact lenses were removed. Meanwhile, the epithelium healed on day 4 (100% of the eyes, $n=20$) with conventional treatment (Table 2). Patients in the control group also had moderate pain (Level 2) on day 1, which decreased to mild (Level 1) before epithelial closure. However, pain lasted about 1 day longer in the control group because of delayed complete epithelial healing.

Mean epithelial defect size during 1-5d after surgery horizontally and vertically are presented in Table 3. The mean horizontal and vertical defect in the study group was lower than the control group in all follow up exams. The decrease in horizontal and vertical epithelial defect in study group was statistically significant in day 1 and day 3, such a statistical significance was not achieved in day 2 and day 4.

On third day total epithelial healing was occurred in 70% (14 eyes) of eyes received serum autologous and 35% in the control group (7 eyes) ($P=0.027$). On forth day, 100% of

Table 3 Horizontal and vertical epithelial defect (mm) during 1-5d after surgery

Parameters	Study group	Control group	*P*
1d horizontal defect	4.62±1.10	5.13±1.08	0.016
2d horizontal defect	2.56±1.18	2.85±1.10	0.063
3d horizontal defect	0.44±0.69	0.79 ±0.74	0.002
4d horizontal defect	0.00	0.10±0.21	0.066
5d horizontal defect	0.00	0.00	1.00
1d vertical defect	4.76±1.04	5.24±0.90	0.005
2d vertical defect	2.67±1.15	2.97±0.96	0.066
3d vertical defect	0.44±0.68	0.85±0.79	0.001
4d vertical defect	0.00	0.11±0.26	0.066
5d vertical defect	0.00	0.00	1.00

Table 4 Total epithelial healing after surgery *n*(%)

Parameters	Study group	Control group	*P*
3d postoperative	17 (85%)	5 (25%)	
4d postoperative	20 (100%)	18 (90%)	
5d postoperative	20 (100%)	20 (100%)	
Total healing duration	3.15±0.37	3.85±0.587	0.000

No eye in either group showed more than +1 haze during the follow-up period and all laser ablations were completed successfully without any complication.

eyes received serum autologous *vs* 85% (17 eyes) of control group were totally healed ($P = 0.072$). The mean total epithelial healing time in the study group was about 0.7d less than in the control group (Table 4).

DISCUSSION

Currently, surface ablation procedures are more commonly employed compared to LASIK in order to avoid the complications of LASIK, especially the risk of ectasia[11]. However, the relatively slow visual recovery and early postoperative pain, mostly due to prolonged total duration of epithelial healing, remain the main disadvantages[12]. Although with introducing the femtosecond laser many drawbacks of conventional LASIK has been dissipated but surface ablation techniques still have preserved their popularity. Shortening the epithelial healing duration with novel methods may shorten the duration of pain, and decrease other negative aspects of delayed healing, such as risk for haze and infectious keratitis due to a bare epithelial surface. In addition to this, patients may return to their daily regular activities earlier and the number of postoperative follow-up visits decreases.

Autologous serum eye-drops are effective in improving epithelial healing in various ocular surface disorders because they positively impact proliferation, migration and differentiation of the ocular surface epithelial cells[13-15]. There are few clinical reports regarding the effect of autologous serum drops on the rate of epithelial healing in refractive surgery especially surface ablation techniques. Conversely, autologous serum drops have been utilized in the management of dry eye after refractive surgery. After LASIK, autologous serum drops have been reported to provide a prolongation of the tear breakup time and a reduction in rose bengal staining score[16].

Hondur *et al*[17] demonstrated that AS drops can accelerate epithelial healing and shorten postoperative discomfort after LASEK by about one day. In other study by Akcam *et al*[2] the duration of epithelial healing was about one day shorter after PRK in more than half of the eyes in the study group (study group: 2.2±0.25d and control group: 3±0.0d).

Our study results were in accordance with aforementioned studies (while in this study, we measured the vertical and horizontal epithelial defect precisely) and duration of both horizontal and vertical epithelial healing was about 0.7d shorter in the AS group, although in this study the healing time in both study and control groups was about one day longer (3.15 ± 0.366 and 3.85 ± 0.587 respectively). This elongation of healing time can be attributed to higher range of sphere and astigmatic correction in our study and prescription of routine topical corticosteroid in the early postoperative period, although the type of excimer laser and ablation profile cannot be overlooked.

Although the pain is a subjective item but can be quantitated by some kinds of questionnaire. This study is similar to previous studies the duration of pain and discomfort in the AS group was about one day shorter than the control group.

A beneficial effect of autologous serum drops on corneal haze after LASEK and PRK has been reported, and this effect was attributed to the serum components including epidermal and fibroblast growth factors, and fibronectin[18]. Similar to recently mentioned studies we did not note any beneficial effect of autologous serum drops on corneal haze in the present study, no eye showed haze intensity greater than + 1. Moreover, autologous serum drops were discontinued upon epithelial healing, which probably is a very short duration for an effect on corneal haze.

The composition of the autologous serum obtained depends on a number of production parameters, including clotting or centrifugation time, and this is likely to have an impact on the epitheliotrophic effects[19]. To optimize the product and to permit comparison between studies, standardized protocols have been developed based on *in vitro* work, but these have not yet been confirmed in clinical comparative studies[20-21]. In addition to production issues, further issues exist around product storage, as the concentration of growth factors in autologous serum can reduce over time when stored at 4 ℃. At -20 ℃ the composition of autologous serum was found to be stable for up to 9mo[22-23].

In this study the concentration of AS was diluted to 20%. While evidence from cell cultures suggests that proliferation of epithelial cells is enhanced by diluting the serum to 20% or less, epithelial migration and extracellular matrix deposition from fibroblasts is better stimulated by 50% or 100% serum[24-26]. In a rabbit model, undiluted serum was more

effective than diluted serum in healing a corneal epithelial wound[27]. So further studies are needed to evaluate the effectiveness of 100% AS eye drops after refractive surgery.

An interesting topic for future research to accelerate epithelial healing after surface ablation may be the investigation of the use of biological tear substitutes.

There are some other alternatives for autologous serum such as adult allogeneic serum, umbilical cord serum and platelet preparations. Allogeneic serum can be prepared from previously stored blood, is quicker to produce and thus potentially more convenient. However, concerns remain in using allogeneic sources for proteins due to the theoretical risk of an immune response to foreign antigens.

Umbilical cord serum has similar advantages to allogeneic serum, in that it can be prepared in large quantities (up to 250 mL) from a single donor and be used for many patients. Moreover, it is useful in patients with systemic inflammation, anemia or chronic diseases, who may not be ideal candidates for autologous serum drops. Umbilical cord serum has a higher concentration of tear components such as EGF, NGF and transforming growth factor (TGF)-b compared to peripheral blood serum[28].

There are many different platelet preparations used in clinical studies, including platelet-rich plasma, plasma rich in growth factors and platelet lysate. These preparations differ widely in their method of processing[7-8,29-33].

Liu *et al*[29] compared the growth factor content of fresh frozen plasma and platelet releasate to that of serum and evaluated their effects on proliferation, migration and differentiation of corneal epithelial cells in an *in vitro* system. They found that the growth factor content was higher in platelet releasate than plasma or serum, with better cell proliferation, but that serum had better cellular migration and differentiation owing to its higher content of fibronectin and vitamins.

Although its preparation procedure is more sophisticated, positive results with platelet rich plasma after refractive surgery have been reported[34-36] and it may provide more positive effects compared to AS drops.

The majority of clinical trials and case series studying autologous serum suggest that it can be effective in the management of OSD secondary to DED, probably due to its anti-inflammatory, epithelio- and neuro-trophic functions, significantly improving signs and symptoms within a few weeks[35]. In the opinion of authors this study is unique due to ascertaining the study group and control group in the same patient and thus preventing bias in pain sensation or epithelial healing time. Furthermore this is the only study measuring the horizontal and vertical epithelial defects separately.

In conclusion this study demonstrated that using autologous serum eye drops by accelerating the corneal epithelial healing and reducing the pain, helps faster recovery of visual acuity and reduces discomfort and haziness or infection risk after PRK. Further studies with greater patient numbers and longer follow-up and different types of excimer lasers and with concerning molecular mechanisms would be valuable.

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