Autologous Platelet-rich Plasma Eye Drops Accelerate Re-epithelialization of Post-keratoplasty Persistent Corneal Epithelial Defects

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Abstract

Purpose: To investigate whether autologous platelet-rich plasma (PRP) eye drops accelerate re-epithelialization of post-keratoplasty persistent corneal epithelial defects (PEDs).

Methods: A total of 34 eyes with PEDs after keratoplasty (24 penetrating keratoplasty and 10 deep anterior lamellar keratoplasty) that were refractory to conventional medical treatments were treated with PRP eye drops every 3 hours. PRP eye drops were prepared with a low- and high-speed centrifugation method and final platelet counts were 700,000-800,000 plt/μl. The mean treatment duration for complete re-epithelialization was compared with the mean treatment duration of conventionally treated corneal defects before the PRP treatment by paired t-test. The mean treatment duration was also statistically analyzed between age groups, gender, indications for keratoplasty, and types of keratoplasty using analysis of variance (ANOVA).

Results: Treatment with autologous PRP eye drops led to rapid re-epithelialization in all eyes. The mean treatment duration for complete re-epithelialization was 2.47 ± 1.21 weeks, which was significantly shorter than the mean treatment duration of conventionally treated corneal defects before PRP treatment (6.82 ± 1.24 weeks) ($P = 0.0001$). There was no significant correlation between re-epithelialization time and patients' age, sex, indications for keratoplasty, and techniques of corneal transplantation.

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Conclusion: Treatment with autologous PRP eye drops is an effective and reliable approach that accelerates re-epithelialization of post-transplantation PEDs.

Keywords: Corneal Epithelial Defects; Eye Drop; Healing; Keratoplasty; PED; Platelet

INTRODUCTION

Persistent epithelial defects (PED) following keratoplasty may occur due to donor, recipient, and surgical technique variables and can reduce the success of corneal transplantation due to corneal opacity and vascularization, stromal melting, and secondary infection.[1] Therefore, management of epithelial defects after corneal transplantation is crucial for surgical outcomes.[2,3] Current standard management of PED after keratoplasty includes frequent lubrication, punctual occlusion, application of serum eye drops, wear of therapeutic contact lenses, and less often tarsorrhaphy.[3-5] In order to promote wound healing in such refractory cases, more potent modalities such as amniotic membrane graft and limbal stem cell transplantation might be required.[6,7]

Corneal wound healing is generally mediated by growth factors such as epidermal growth factors (EGFs), fibroblastic growth factors (FGFs) and platelet-derived growth factors (PDGFs), which stimulate migration, proliferation and differentiation of the corneal epithelial cells.[2,8,9] Role of platelets as a rich source of growth factors has been well established in dermal wound healing.[10,11] They attach to damaged endothelium, release cytokines and growth factors to promote the healing process in the damaged region, and then the healing process occurs due to alterations in the balance between promoting and inhibitory substances.[12]

Platelet-rich plasma (PRP) is supposed to have a high potential for induction of "faster than normal" epithelialization.[13] Alfa granules in platelets release PDGFs, as well as tissue growth factors (TGFs), among which TGFβ, in particular, promotes fibroblastic mitosis and collagen synthesis.[14,15] Several studies revealed the importance of collagen synthesis for a successful corneal graft.[16-18] Moreover, secretion of activated biologic proteins by platelets such as PDGF, TGFβ and EGF augment refractory wound repair.[19-21] In this study, we investigated the efficacy of autologous platelet-rich plasma (PRP) eye drops in the management of refractory cases of post-keratoplasty PEDs.

METHODS

After obtaining full approval from the ethics committee of the Ophthalmic Research Center at the Shahid Beheshti University of Medical Sciences (Tehran, Iran), we enrolled all the patients who developed PEDs following keratoplasty and were unresponsive to standard treatments. PED was defined as the presence of a non-healing epithelial defect that occurred at least 1 week after keratoplasty despite having received standard treatments. Patients with excessively tight suture or donor-host junction misalignment were excluded. After obtaining informed consent, a 30-40 ml blood sample was taken from each patient and autologous PRP eye drops were prepared. Data compiled from the patients' recods included demographic data, indications for keratoplasty, types of corneal transplantation, and duration of corneal epithelial defects.

Preparation of Autologous PRP Eye Drops

Pure PRP eye drops were prepared weekly using a double centrifugation process. Collected whole blood in sodium citrate-containing tubes underwent initial centrifugation in a refrigerator centrifuge (Hettich, Lab Technology, Tuttlingen, Germany) with the speed range of 130-180 g and duration of 13-18 min at 25°C. Second centrifugation with speed of 800 g and duration of 10 min was performed under sterile conditions. A portion of the supernatant plasma was removed and after 20-30 min the platelet pellets were gently suspended, homogenized, and aliquoted into 15 sterile disposable and recappable vials, each containing 350 µl PRP. A platelet count was performed on the final product by using a Sysmex KX-21N Automated Hematology Analyzer (Sysmex America, Inc., Illinois, USA), and counts between 700,000 and 800,000 plt/µl were considered appropriate for the study. A microbiologic culture from the final product was performed to ensure that it was safe.

Patients' Treatment and Follow-up

After obtaining negative microbiologic results, the patients were advised to keep the eye drops at 4°C, use each PRP vial for one day, and apply one drop every 3 h. In this way, approximately 700,000-800,000 plt/µl could be contacted with the wound surface. Patients were instructed not to use topical antibiotics or steroids simultaneously. The patients attended weekly follow-up appointments for slit-lamp biomicroscopic examinations, and the treatment duration required to achieve complete epithelial restoration was recorded.

Statistical Analysis

The frequency, mean, and standard deviation of the duration of corneal epithelial defects before and after topical PRP treatment were calculated and compared

by paired *t*-test. The mean duration of corneal epithelial healing was also compared among different subgroups of age (<40 years, 40-60 years, and >60 years), gender, indication for keratoplasty, and corneal transplantation technique using ANOVA. All statistical analyses were performed using SPSS statistical software (IBM Corp. Released 2014. IBM SPSS Statistics for Windows, version 24.0. Armonk, NY: IBM Corp) and a *P* value less than 0.05 was considered statistically significant.

RESULTS

Thirty-four patients with post-keratoplasty PEDs (24 after penetrating keratoplasty and 10 after deep anterior lamellar keratoplasty) were enrolled. Mean patient age was 45.41 ± 18.28 years (range, 11-80 years), and 65% were male subjects. A history of diabetes mellitus was found only in one patient. All patients received supplemental lubricants, punctual cauterization, serum eye drops and therapeutic contact lenses before the initiation of PRP eye drops. Except for 4 cases in whom tarsorrhaphy was performed prior to the use of PRP eye drops and 3 cases in whom therapeutic contact lenses were worn whilst receiving PRP eye drops, all other cases were balanced in treatments they received before and after treatment with the PRP eye drops.

Indications for keratoplasty, in order of frequency, included corneal degenerations and dystrophies (13 eyes), limbal stem cell deficiency (10 eyes), corneal scar and opacity (4 eyes), failed graft (5 eyes), and aphakic/pseudophakic bullous keratopathy (2 eyes) [Table 1]. All patients with limbal stem cell deficiency had undergone limbal stem cell transplantation before or at the time of keratoplasty.

None of the PRP final products yielded positive cultures and none of the patients reported any adverse effects while receiving autologous PRP eye drops. The mean duration of corneal epithelial defects before PRP treatment was 7.00 ± 1.00 weeks (range, 5-11 weeks). However, the mean duration of corneal epithelial defects until complete re-epithelialization with PRP treatment was 2.00 ± 1.00 weeks (range, 1-7 weeks), which was significantly shorter than that with non-PRP treatment (*P* = 0.0001). Figure 1 demonstrates complete re-epithelialization of a post-keratoplasty persistent epithelial defect after a 2-week treatment with autologous PRP eye drops. The mean duration of corneal epithelial healing with PRP was not statistically different among the 3 age groups (*P* = 0.307), genders (*P* = 0.287), indications for keratoplasty (*P* = 0.153) and transplantation techniques (*P* = 0.694) [Table 2].

DISCUSSION

Our study demonstrated that administration of autologous PRP eye drops significantly enhanced

Table 1. Patients' characteristics, indications, and types of surgery

|	*n* (%)
Sex	
Female	12 (35.3%)
Male	22 (64.7%)
Age (Years)	
Total	45±18
<40	16 (47.1%)
40-60	10 (29.4%)
>60	8 (23.5%)
Indications for keratoplasty	
Corneal degenerations and dystrophies (Keratoglobus - Keratoconous - Lattice dystrophy)	13 (38.2%)
Limbal stem cell deficiencies (Chemical burn - Aniridia - Mustard gas keratoplasty)	10 (29.4%)
Failed graft	5 (14.7%)
Corneal scar and opacity	4 (11.8%)
Aphakic/pseudophakic bullous keratopathy	2 (5.9%)
Type of surgery	
Penetrating Keratoplasty	24 (70.6%)
Deep Anterior Lamellar Keratoplasty	10 (29.4%)
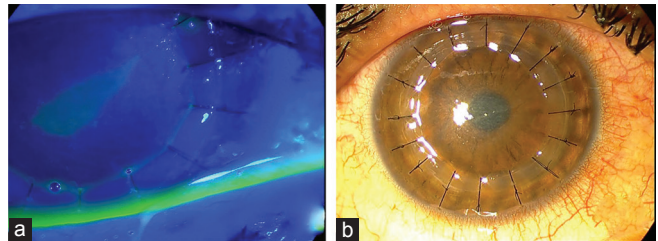
Figure 1. Re-epithelialization of a post-keratoplasty persistent epithelial defect with PRP eye drops. a) Fluorescence staining of corneal epithelial defect in a transplanted cornea. b) Complete re-epithelialization after a 2-week treatment with autologous PRP eye drops.

re-epithelialization in patients with post-keratoplasty PEDs. To the best of our knowledge, this is the first cohort study evaluating the efficacy of autologous PRP eye drops in post-keratoplasty PED. The duration of re-epithelialization with PRP treatment in the current study (2.5 weeks) was comparable with that found by Vajpayee et al,[22,23] who reported the re-epithelialization times for treatment with autologous serum and umbilical cord serum1.8 and 2.4 weeks, respectively. In their study, serum therapy was applied in a heterogeneous group of patients with PED who were refractory to standard therapy, and post-keratoplasty PED accounted for 45% of their cases.

Rapid re-epithelialization in our series was not affected by patients' age or gender, which might be indicative of the appropriate preparation of PRP eye drops in the current study. Our study validates the

Table 2. Duration of corneal epithelial defects and re-epithelialization

	Duration of corneal epithelial defects until complete re-epithelialization (Weeks)	
	Mean±SD (Range)	*P*
Age (Years)		
<40	3.00±2.00 (1.00-7.00)	0.307
40-60	2.00±1.00 (1.00-4.00)	
>60	2.00±0 (2.00-3.00)	
Sex		
Male	2.00±1.00 (1.00-3.00)	0.287
Female	3.00±1.00 (1.00-7.00)	
Indications for keratoplasty		
Keratoglobus - Keratoconus - Lattice dystrophy	2.00±1.00	0.153
Limbal stem cell deficiencies (Chemical burns - Aniridia - Mustard gas keratoplasty)	3.00±1.00	
Failed graft	2.00±0	
Corneal scar and opacity	2.00±1.00	
Aphakic/pseudophakic bullous keratopathy	5.00±4.00	
Type of surgery		
Penetrating Keratoplasty	2.00±1.00 (1.00-7.00)	0.694
Deep Anterior Lamellar Keratoplasty	3.00±1.00 (2.00-4.00)	

SD, standard deviation

fact that the autologous PRP preparation is a safe and effective treatment that can be easily prepared and used in eyes with post-keratoplasty PED.

Since the preservative-free PRP eye drops are prone to contamination, there has always been a concern about the risk of superimposed infection[24] in the grafted corneas. However, none of the cases in our series developed ocular infections during the treatment with autologous PRP eye drops. Microbiologic control of final PRP products, as well as the one-day use of each PRP vial could be key factors that were responsible for the lack of adverse reactions in our series.

In the current study, autologous topical PRP treatment was initiated in post-keratoplasty eyes with PED after about a 7-week failure of standard therapy. In routine keratoplasty cases, corneal epithelial defects occur in the immediate postoperative phase and are mostly managed with conventional medical therapies.[3] However, the use of growth factors containing topical preparations for the successful treatment of post-keratoplasty epithelial defects, as evidenced by the current study and the study by Kamble et al,[3] lead to faster re-epithelialization as compared with standard therapy. Whether autologous PRP had any extra advantage over other growth factors containing sera needs further investigations.

The re-epithelialization time, in the current study, after topical administration of autologous PRP was not correlated with indications for keratoplasty or the technique of corneal transplantation. With the PRP treatment as a growth factor-rich preparation, the mean healing time in the group of patients with chemical burns

was not even significantly different from patients that had other indications for keratoplasty.

Diabetes mellitus is one of the risk factors for the occurrence of post-keratoplasty PEDs and delayed epithelialization.[25] In our series, the only diabetic patient showed complete re-epithelialization after a very short-term PRP therapy. One limitation of the current study is that donor variables such as graft size, death to preservation time, donor graft quality, and corneal storage time that might have had the potential risk of delayed epithelial healing after keratoplasty[25-27] were not evaluated. However, we strongly suggest topical PRP therapy for all patients that have a potential risk for developing post-keratoplasty PED.

The main outcome measure of our study was duration of complete epithelial repair. The sizes of epithelial defects, in the current study, were not measured and analyzed. Additionally, this study was a clinical assessment of the short-term safety and efficacy of autologous PRP eye drops for the management of PEDs after corneal transplantation. Long-term effects of PRP eye drops at the cellular and molecular levels remain to be determined.

In conclusion, our study showed that autologous PRP eye drops are safe and effective in rapid re-epithelialization of PEDs following corneal transplantation. Whether PRP eye drops have any superiority over autologous serum eye drops warrants further investigations.

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Conflicts of Interest

There are no conflicts of interest.

REFERENCES

1. Raj A, Dhasmana R, Bahadur H. Factors associated with surface epithelial keratopathy after optical penetrating keratoplasty. *J Curr Ophthalmol* 2017;29:108-115.

2. Szaflik J, Fryczkowski AW, Liberek I, Czubak M, Brix M, Broniek G, et al. [Corneal wound healing after penetrating keratoplasty with EGF application. Experimentalstudies]. *Klin Oczna* 1999;101:409-416.

3. Kamble N, Sharma N, Maharana PK, Bandivadekar P, Nagpal R, Agarwal T, et al. Evaluation of the role of umbilical cord serum and autologous serum therapy in reepithelialization after keratoplasty: A randomized controlled clinical trial. *Eye Contact Lens* 2016. [Epub ahead of print]

4. Ricci F, Missiroli F, Ciotti M, Perno CF, Cerulli L. Persistent epithelial defect after penetrating keratoplasty caused by adenoviral infectious keratitis. *New Microbiol* 2010;33:171-174.

5. Chen YM, Hu FR, Huang JY, Shen EP, Tsai TY, Chen WL. The effect of topical autologous serum on graft re-epithelialization after penetrating keratoplasty. *Am J Ophthalmol* 2010;150:352-359.

6. Seitz B, Das S, Sauer R, Mena D, Hofmann-Rummelt C. Amniotic membrane transplantation for persistent corneal epithelial defects in eyes after penetrating keratoplasty. *Eye* (*Lond*) 2009;23:840-848.

7. Capozzi P, Petroni S, Buzzonetti L. Combined HLA matched limbal stem cells allograft with amniotic membrane transplantation as a prophylactic surgical procedure to prevent corneal graft rejection after penetrating keratoplasty: Case report. *Ann Ist Super Sanita* 2014;50:298-300.

8. Ljubimov AV, Saghizadeh M. Progress in corneal wound healing. *Prog Retin Eye Res* 2015;49:17-45.

9. Lu L, Reinach PS, Kao WW. Corneal epithelial wound healing. *ExpBiol Med* (*Maywood*) 2001;226:653-664.

10. Lacci KM, Dardik A. Platelet-rich plasma: Support for its use in wound healing. *Yale J Biol Med* 2010;83:1-9.

11. Fernandez-Moure JS, Van Eps JL, Cabrera FJ, Barbosa Z, Med1el Rosal G, Weiner BK, et al. Platelet-rich plasma: A biomimetic approach to enhancement of surgical wound healing. *J Surg Res* 2017;207:33-44.

12. Abegão KG, Bracale BN, Delfim IG, Santos ES, Laposy CB, Nai GA, et al. Effects of heterologous platelet-rich plasma gel on standardized dermal wound healing in rabbits. *Acta Cir Bras* 2015;30:209-215.

13. Panda A, Jain M, Vanathi M, Velpandian T, Khokhar S, Dada T.

14. Topical autologous platelet-rich plasma eyedrops for acute corneal chemical injury. *Cornea* 2012;31:989-993.

14. Alio JL, Abad M, Artola A, Rodriguez-Prats JL, Pastor S, Ruiz-Colecha J. Use of autologous platelet-rich plasma in the treatment of dormant corneal ulcers. *Ophthalmology* 2007;114:1286-1293.e1.

15. Geremicca W, Fonte C, Vecchio S. Blood components for topical use in tissue regeneration: Evaluation of corneal lesions treated with platelet lysate and considerations on repair mechanisms. *Blood Transfus* 2010;8:107-112.

16. Kato T, Nakayasu K, Kanai A. Corneal wound healing: Immunohistological features of extracellular matrix following penetrating keratoplasty in rabbits. *Jpn J Ophthalmol* 2000;44:334-341.

17. Saika S, Ohnishi Y, Ooshima A, Liu CY, Kao WW. Epithelial repair: Roles of extracellular matrix. *Cornea* 2002;21 (2 Suppl 1):S23-S29.

18. Liu CY, Kao WW. Lumican promotes corneal epithelial wound healing. *Methods Mol Biol* 2012;836:285-290.

19. Yuan T, Zhang CQ, Tang MJ, Guo SC, Zeng BF. Autologous platelet-rich plasma enhances healing of chronic wounds. *Wounds* 2009;21:280-285.

20. Carducci M, Bozzetti M, Spezia M, Ripamonti G, Saglietti G. Treatment of a refractory skin ulcer using punch graft and autologous platelet-rich plasma. *Case Rep Dermatol Med* 2016;2016:7685939.

21. Asadi M, Alamdari DH, Rahimi HR, Aliakbarian M, Jangjoo A, Abdollahi A, et al. Treatment of life-threatening wounds with a combination of allogenic platelet-rich plasma, fibrin glue and collagen matrix, and a literature review. *ExpTher Med* 2014;8:423-429.

22. Vajpayee RB, Mukerji N, Tandon R, Sharma N, Pandey RM, Biswas NR, et al. Evaluation of umbilical cord serum therapy for persistent corneal epithelial defects. *Br J Ophthalmol* 2003;87:1312-1316.

23. Yoon KC, Heo H, Im SK, You IC, Kim YH, Park YG. Comparison of autologous serum and umbilical cord serum eye drops for dry eye syndrome. *Am J Ophthalmol* 2007;144:86-92.

24. Hessen M, Akpek EK. Ocular graft-versus-host disease. *Curr Opin Allergy Clin Immunol* 2012;12:540-547.

25. Chou L, Cohen EJ, Laibson PR, Rapuano CJ. Factors associated with epithelial defects after penetrating keratoplasty. *Ophthalmic Surg* 1994;25:700-703.

26. Feizi S, Javadi MA, Ghasemi H, Javadi F. Effect of donor graft quality on clinical outcomes after penetrating keratoplasty for keratoconus. *J Ophthalmic Vis Res* 2015;10:364-369.

27. Cheour M, Nasri H, Kamoun H, Lamloum H, Kasri A, Hamdi S, et al. Factors associated with graft reepithelialization after penetrating keratoplasty. *J Fr Ophtalmol* 2008;31:786-789.