The Role of "Eye Platelet Rich Plasma" (E-Prp) for Wound Healing in Ophthalmology

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Abstract: Blood derived products have demonstrated their capacity to enhance healing and stimulate the regeneration of different tissues and this enhancing effect is attributed to the growth factors and bioactive proteins that are synthesized and present in blood. Eye platelet rich plasma (E-PRP) provides higher concentration of essential growth factors and cell adhesion molecules by concentrating platelets in a small volume of plasma as compared with autologous serum, the latter being used widely in ophthalmology for epithelial wound healing of the cornea for the last two decades. These growth factors and cell adhesion molecules have a major role in wound healing and enhance the physiological process at the site of the injury/surgery *via* eye drops or clot. E-PRP has been used more recently, and has achieved successful outcomes in peer-review articles in the treatment of dormant ulcers (epithelial defects of the cornea that fail to heal), moderate to severe dry eye syndrome, ocular surface syndrome post Laser In Situ Keratomileusis (LASIK), and for surface reconstruction after corneal perforation associated with amniotic membrane transplantation. Preparation of E-PRP in the two available formulations, eyedrops and clot, is inexpensive and easy although it requires following strict sterility conditions using sterile and disposable materials and operating inside a laminar flow hood. No serious adverse effects have been described with the use of these products, and it is generally well tolerated. In summary, Platelet enriched plasma in the form obtained in ophthalmology, E-PRP, is a reliable and effective therapeutic tool to enhance epithelial wound healing in ocular surface disease.

Keywords: Eye platelet rich plasma; corneal dormant ulcer; dry eye; corneal perforations; ocular surface syndrome post LASIK.

INTRODUCTION

The ocular surface comprises the conjunctival mucosa that lines the globe and palpebral surfaces, the corneoscleral limbus, the corneal epithelium and the tear film. This complex structure needs a stable tear film, normal blink, functioning lacrimal system and healthy eyelids to maintain a balanced homeostasis. Several diseases can compromise the stability of the ocular surface, ranging from chemical or physical injuries such as Laser In Situ Keratomileusis (LASIK), to autoimmune diseases such as Sjögren disease or ocular cicatrizial pemphigoid. If corneal wound healing does not occur promptly, it can lead to visual loss, severe scarring, infection and even corneal perforation.

There are several strategies to promote wound healing. Firstly, lubrication can be enhanced with the frequent use of artificial eye drops or ointment. By using bandage contact lenses or patching the eye, closing the eyelids together with a tarsorrhaphy procedure or inducing a ptosis with botulin-toxin we can reduce the exposure of the ocular surface to the outside environment, facilitating cell proliferation and migration.

Finally, the use of biologically active agents to promote wound healing has been known in ophthalmology for several decades, and it has been widely used in the treatment of ocular surface disease such as persistent epithelial defects or neurotrophic keratopathy as well as following corneal grafting [1-4]. Amniotic membrane or hemotherapy derivates can actively stimulate the corneal epithelial turnover and migration. The release of growth factors and other cytokines that promote epithelialisation, suppress inflammation and have microbicidal effects, has been the rationale for using amniotic membrane [4, 5] or autologous serum in patients with wound healing impairment [6].

The goal of this article is to review the use of another blood derived product known as eye platelet-rich plasma (E-PRP) in ophthalmology, and outline its current clinical applications based on the evidence found in the literature.

BACKGROUND

Blood derived products have demonstrated their capacity to enhance healing and stimulate the regeneration of different tissues and this enhancing effect is attributed to the growth factors and bioactive proteins that are synthesized and present in blood [7].

Different blood derived formulations, such as autologous serum, plasma enriched with platelets and preparations rich in growth factors have been used to promote wound healing in multiple tissues.

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The serum is the clear liquid part of full blood after cellular components and clotting proteins have been removed. Since Fox *et al*. [1], first used autologous serum eye drops in the treatment of keratoconjunctivitis sicca, it has been the preferred blood derived topical preparation in the treatment of ocular surface diseases. Autologous serum is commonly used and has been found effective for the treatment of persistent epithelial defects [8], neurotrophic ulcers [2], superior limbic keratoconjunctivitis [9], and other types of dry eye symptoms such as graft versus host disease [10] or after LASIK [11]. Autologous serum has also been used as an adjunctive treatment in ocular surface reconstruction with different results [3, 12]. Unlike artificial tears, serum eye drops have pH, osmolarity and biomechanical properties which resemble natural tears, and they are non-preserved. When used topically they supply essential nutrients to the ocular surface such as growth factors, vitamins and bacteriostatic products such as IgG, lysozyme and complement [13]. Therefore, not only do they provide lubrication, but they also have epitheliotrophic and antimicrobial properties which resemble natural tears, and cannot be found in commercialised artificial tears.

Plasma, unlike serum, does contain clotting proteins of full blood such as fibrin. Although the acellular component of blood contains growth factors, it is well known that platelets are great reservoirs of growth factors that enhance proliferation and wound healing. The alpha-granules in platelets contain more than 30 bioactive proteins such as epidermal growth factor (EGF), platelet derived growth factor AB (PDGF-AB), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-1), transforming growth factor beta (TGF-β), as well as cytokines including proteins such as PF4 and CD40L, which promote tissue repair and influence the reactivity of vascular and other blood cells in angiogenesis and inflammation [7]. Growth factors released from activated platelets initiate and modulate wound healing in both soft and hard tissues [14, 15]. The plasma also contains concentrated quantities of some important cell-adhesion molecules which promote epithelial migration such as fibrin, fibronectin and vitronectin [16]. Laboratory research with different cultured cellular lines such as tendon cells, synovial and skin fibroblasts and corneal epithelial cells, shows the multiple benefits and biological effects of growth factors [13, 17-19].

In the literature there are different autologous blood derived products with variable amounts of platelets and growth factors which are used to promote wound healing and tissue regeneration.

Autologous PRGF has been used for multiple purposes by Anitua *et al*. [20] since 1999. At the beginning PRGF was referred to as "Plasma Rich in Growth Factors" [21], and more recently has changed to "Preparation Rich in Growth Factors" [22] due to the possibility of obtaining different formulations for regenerative purposes in multiple medical conditions. PRGF technology identifies 100% autologous and biocompatible products elaborated using a one-step centrifugation process, sodium citrate and calcium chloride as an anticoagulant and activator, respectively.

Platelet-rich plasma (PRP) is defined by Marx *et al*. [23] as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline. They use a PRP device, concentrate platelets using a double centrifugation technique and activate PRP just when they are ready to use it. The final concentration is at least 1.000.000 platelets/ microlitre. Therefore, it is an autologous concentration of platelets and growth factors. After the complete release of growth factors, platelets are able to synthesize and secrete additional growth factors for the remaining several days of their lifespan thus expanding the wound healing effect [23].

PRP and PRGF have been successfully used for over a decade as a component for tissue regeneration procedures such as oral and maxillofacial surgery, reconstructive orthopaedics, cardiovascular surgery and plastic surgery [21, 24, 25]. Dental implant surgery with guided bone regeneration is one example in which an autologous platelet-rich clot has been shown to accelerate ossification after a tooth extraction and/or around titanium implants, with marked reductions in the time required for implant stabilization and an improved success rate [26-28]. Articular surgery [29], tendon repair [30], reversal of skin ulcers [31], macular hole repair in eye surgery [32], and cosmetic surgery [33] are other situations in which autologous platelets have shown to accelerate healing. Concentrated platelet preparations are also used to induce bone regeneration when prosthetic devices are to be implanted [34] in maxillofacial and implant surgery and also in traumatology, combined with hydroxyapatite, autologous bone, and other biomaterials [35-37].

Other different blood derived preparations such as enriched growth factors solution obtained from activated platelets with human thrombin previously washed with a buffer solution, has been used to study the epitheliotrophic capacity in cell culture models, including human and rabbit corneal epithelial cells [13].

A meticulous review in the literature shows us that blood derived products like plasma enriched with platelets and growth factors has not been as widely used in ophthalmology as it has been in other disciplines that deal with wound healing.

AUTOLOGOUS E-PRP PREPARATION IN OPHTHALMOLOGY: EYEDROP AND CLOT FORMULATIONS

The E-PRP (eye platelet rich plasma) used in ophthalmology is an autologous preparation of plasma rich in platelets, but different from the PRP described by Marx *et al*. [23] and slightly different from PRGF [38]. The E-PRP uses sodium citrate as an anticoagulant and when necessary calcium chloride is used for the activation of the E-PRP clot. The main difference with PRGF is that the E-PRP preparation uses commercial tubes to obtain the blood and a laboratory centrifuge is used for plasma separation. E-PRP does not require specific devices.

The E-PRP obtention is carried out using a one-step centrifugation process and the final rate of the platelet concentration depends on whether it will be used as eyedrops (without activate) or as a clot (activated).

The autologous E-PRP in the form of topical eyedrops is used for surface applications and as a clot for surgical procedures for ocular reconstruction.

E-PRP EYEDROP PREPARATION

80-100 ml of blood is obtained from the patient by venipuncture and collected in 10 ml sterile tubes containing 1ml of 3,2% sodium citrate to avoid coagulation. E-PRP preparation is made following strict sterility conditions using sterile and disposable materials and operating inside a laminar flow hood.

Citrated tubes containing blood are spun at 5ºC, 1400 rpm for 10 minutes and ninety percent of plasma obtained after centrifugation is collected and used as the final product. Nevertheless, the speed and time vary depending on the characteristics of each centrifuge and the size of tubes used. A haemocytometer is needed to quantify the number of platelets in whole blood after the centrifugation in order to obtain the maximum enrichment. We find that PRP made with our conditions yields a 1,6-2,5 fold enrichment of platelets when compared to full blood.

Three to four millilitres aliquots of E-PRP are placed into new, sterilized 10 ml amber glass bottles with eye drop applicators. Patients are instructed to wash their hands before using the product, to keep the area of application of the eye drops clean and to avoid touching the eyedropper. The bottle in use can be kept at +4ºC for one week, after which, the bottle in use needs to be discarded. All these preventions are carried out in order to avoid contamination. Until used, the rest of the PRP tubes should be kept at -20ºC.

As eye drops need to be used in the liquid form, there is no activation of the coagulation until the drops are instilled and aggregation takes place. Endogenous release of activators of the coagulation in the site of application results in a slower release of growth factors and chemical mediators, providing a longer effect.

E-PRP CLOT PREPARATION

For the preparation of E-PRP clot, 40-60 ml of blood is obtained from the patient just before the surgery and centrifuged by the one-step process as previously described for the E-PRP eye drop formulation. However in this case, only the plasma nearest to the red cells is harvested, avoiding the white blood cell layer due to the unknown effects of leukocytes [39]. 1 ml of E-PRP is placed into each 4 well tissue culture plates (Nunc®) and 50 µl of 10% calcium chloride (Braun®) are added to each well for activation. After mixing carefully with a sterile pipette, the plates are incubated at 37ºC for 30 minutes. After that time the E-PRP clot is formed and it is ready to be applied onto the ocular defect immediately afterwards. In the E-PRP clot the rate of the platelets enrichment is about 2-3 times over the full blood values. The manipulation of the tubes to obtain the E-PRP must be done under strict sterile conditions using a laminar flow hood.

SAFETY

The concern about transmissible diseases such as HIV, hepatitis, or Creutzfeldt-Jakob disease, which arises when using allogenic blood derivates do not apply to either serum drops or E-PRP as they are entirely autologous. Bovine thrombin is not used anymore as the clotting initiator so the stimulation of antibody production is not an issue. No serious adverse events have been described using PRP for ocular surface diseases. Only one patient from our series developed tolerability problems and discomfort with the topical administration of E-PRP [40]. There is not a single case reported of infection secondary to microbial contamination of the eye drops, as it is prepared under strict sterile conditions.

CLINICAL APLICATIONS

Dormant Ulcers

Dormant ulcers are epithelial defects of the cornea that fail to heal in spite of at least 2 weeks of conventional treatment, and are most commonly caused by neurotrophic keratopathy (including metaherpetic disease), dry eye, or immunological disorders such as rheumatoid arthritis or ocular cicatricial pemphigoid.

In a prospective study Alió *et al*. [41] included 26 eyes with dormant corneal ulcers, 12 had neurotrophic keratopathy, 9 had herpetic keratopathy and 5 had ulcers of immunological origin Fig. (**1**). They were treated with eye drops made from autologous platelet rich plasma at a dose of 6 times a day in addition to routine medication. Primary outcome measures were the reduction in size or depth of the corneal ulcer and improvement in best-corrected vision. Secondary outcome measures were the reduction of pain or discomfort, decrease in conjunctival or ciliary hyperemia, or conjunctival edema if present.

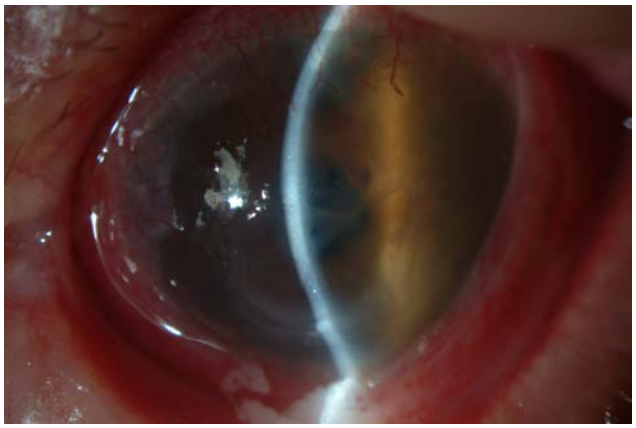

Fig. (1). Patient with neurotrophic keratopathy, showing intense inflammation.

Significant clinical improvement was found in 92 % of the eyes (24/26), with a complete resolution of the ulcer in 50% of the cases (13/26) Fig. (**2**). Only two eyes did not show significant changes after the treatment. Reduction in inflammation and decrease of ocular pain were the other parameters that clearly improved in the majority of the cases. Two eyes with a relapsing epithelial defect, defined as observation of a new epithelial defect located at the level of the previous ulcer with positive fluorescein staining under slit-lamp examination, occurred after 6 months to 1 year and were treated successfully by keratoplasty.

Visual acuity also improved in more than half of the patients, with 31% of the eyes gaining 1-3 lines of visual acuity, 15% with an improvement of 4-5 lines of visual acuity, and 12% with more than 6 lines of improvement. A reduc-

tion in inflammation occurred in 1 to 2 weeks, with a decrease in ocular pain.

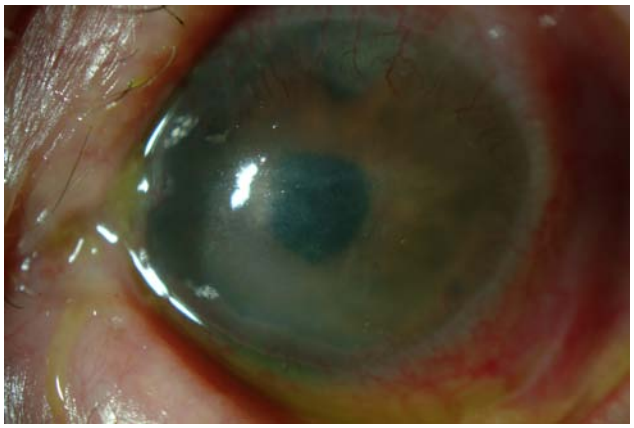

Fig. (2). Same patient as in fig. (**1**), two weeks after treatment with E-PRP. Inflammation has been reduced and there is no epithelial defect.

The same study [41] included 14 eyes treated surgically with clot of autologous E-PRP and amniotic membrane in perforated corneas or with impending perforation due to corneal melting following chronic corneal ulcer. We will review this subgroup of patients in detail in the paragraph dedicated to surgical use of E-PRP.

This report shows that E-PRP improved photophobia, pain and inflammation; facilitated reepithelialisation, promoting corneal wound healing and improving the clinical condition, which in the end resulted in improved vision in the majority of the patients included in the study Fig. (**1a** and **1b**). Although based on a single study and therefore further studies are necessary, this article shows that autologous E-PRP is a reliable and efficient procedure to restore the corneal epithelial surface in patients with dormant corneal ulcers. As it has been described for autologous serum, E-PRP facilitates reepithelialisation and promotes corneal wound healing in intractable corneal ulcers. The advantage of E-PRP over autologous serum is that E-PRP has a large quantity of growth factors that are released from the platelets once these are activated in the ocular surface [41]. Whether this new therapeutic approach is better than previous approaches is a question that should be answered with a larger series of studies with a contemporaneous comparison group.

Dry Eye Syndrome

Dry eye is a disorder of the tear film caused by a disturbance in the composition and quantity of tears, and can be due to: a) deficiency of the aqueous phase of the tear, as in Sjögren´s syndrome or lacrimal gland disease b) deficiency of the mucin layer caused by a vitamin A deficiency, trachoma, diphtheric keratoconjunctivitis, mucocutaneous disorders and certain topical medications or c) abnormalities of the lipid tear layer caused by blepharitis and rosacea.

Dry eye is very common in ophthalmology and causes disturbing subjective symptoms such as dryness, burning, and a sandy-gritty eye irritation and is accompanied by superficial punctate keratopathy Fig. (**3**), tiny abrasions on the surface of the eyes and conjunctival hyperemia. Dry eye can also compromise visual acuity if it is severe enough. Artificial tears are the main standard treatment for this disease, but very often not sufficient enough to stop all the symptoms or signs of the disease.

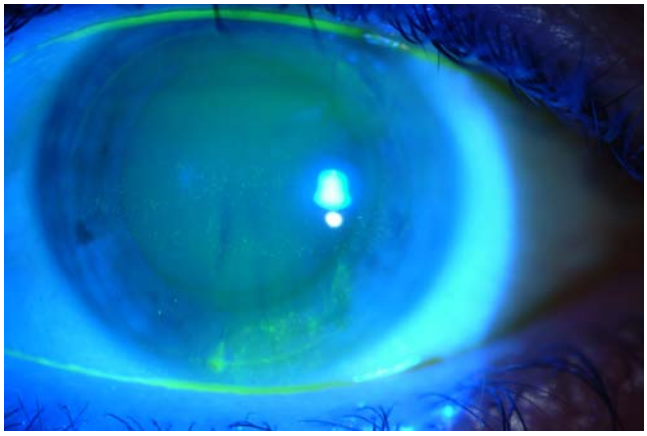

Fig. (3). Patient with severe dry eye. Fluorescein stains in green and shows punctate keratopathy with blue cobalt light.

Some studies have shown that dry eye is often associated to a low-grade inflammatory reaction on the ocular surface with production of pro-inflammatory cytokines, and a status of activation of T cells in the lacrimal glands and conjunctiva either with or without Sjögren's syndrome [42, 43]. Dry eye symptoms usually correlate with the severity of the disease but, according to recent reports, when accompanied by inflammation, they may cause a down regulation of sensory receptors leading to a decrease in symptoms while milder conditions may cause the opposite effect.

Alio *et al*. [44] conducted a prospective, nonrandomized, observational consecutive pilot study that included 36 eyes of 18 patients with moderate to severe dry eye syndrome according to the triple classification of Madrid [45]. Twelve patients were suffering from moderate dry eye and 6 patients had severe dry eye symptoms related to Sjögren's syndrome or Stevens-Johnson disease. All the patients had punctate keratitis, with fluorescein staining on at least 50% of the corneal surface, were highly symptomatic and showed severe ocular surface disturbances Fig. (**4**). Main outcome measurements included the disappearance of subjective symptoms after treatment; the increase in visual acuity, tear meniscus height and tear breakup time (BUT); the decrease in inflammation and fluorescein staining and improvement of impression cytology. E-PRP was given topically as eye drops (4-6 times a day per eye) to patients suffering from moderate to severe dry eye symptoms. Patients were monitored for improvement every week. Data was analyzed after 1 month of treatment.

After 1 month of treatment with E-PRP eye drops Fig. (**5**), 89% of the patients (16/18 patients) experienced relevant improvement or full disappearance of subjective symptoms, and none of the patients' symptoms deteriorated whilst receiving treatment. Twenty-eight percent of patients (5/18) had at least 1 line or more of visual acuity, with no cases of visual loss documented. Improvement of the quality of the tear film was also reported in more than half of the patients with an increase of the tear meniscus height and tear break

up time (BUT). As in the report on the use of E-PRP in dormant ulcers, a significant reduction of the inflammation was found in 89% of those patients suffering from conjunctival inflammation (6 out of 7 patients). Moreover, improvement of fluorescein staining of the cornea due to superficial punctate keratitis was found in 72% of the cases (13 out of 18). Lastly, with the available data of impression cytology 12 out of the 18 patients presented an increase in the density of goblet cells (mucin producing cells) that was statistically significant on the superior bulbar conjunctiva, and that almost reached significance (p value 0.053) in the inferior bulbar conjunctiva.

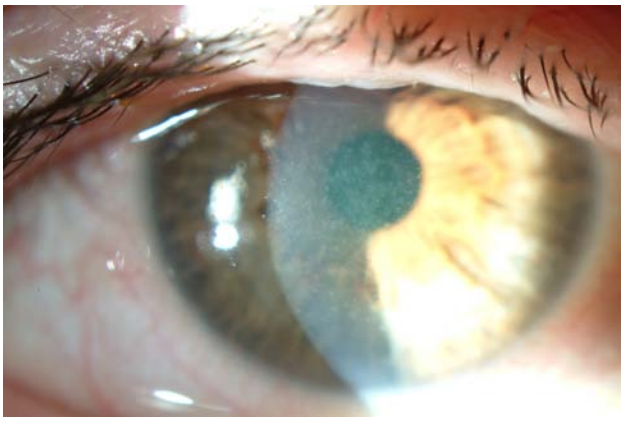

Fig. (4). Patient with severe dry eye prior to E-PRP treatment showing intense keratitis.

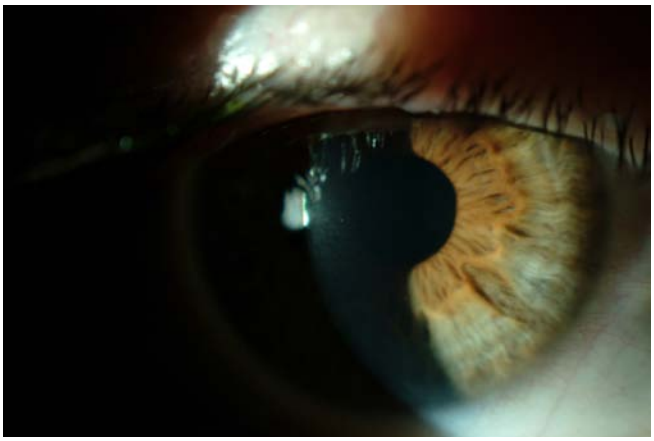

Fig. (5). Same patient as in fig. (**4**), two weeks after treatment with E-PRP.

In this clinical study, E-PRP eye drop application improves regeneration of the ocular surface and relieves symptoms in patients with symptomatic dry eye, with no adverse events with a follow-up of up to 6 months. Autologous E-PRP is a preservative-free biological product obtained directly from the patient's own blood. Its main components are platelets and growth factors known for their properties of increasing and accelerating the normal healing process by stimulation of cell growth and function, and the symptoms are relieved rapidly. Topical steroids have also been proved to quickly relieve symptoms, but their long-term usage may result in side effects such as an increased risk of infection, intraocular pressure elevation and cataract formation. The preservative component present in these steroid solutions can also affect the bonds between epithelial cells in the cornea inducing punctate keratopathy that may develop into a corneal ulcer [46].

A comparative study with other therapeutic options such as autologous serum, which has also shown to be effective [1], is needed. No formal pilot study has been designed yet but we have observed from data obtained in our institute, that E-PRP has a slight benefit compared to 100% autologous serum, probably due to the fact that the presence of platelets produces a prolonged release of growth factors. E- PRP should be considered the product of choice in cases of severe dry eye where the presence of growth factors acting for longer periods of time is more beneficial.

Ocular Surface Syndrome Post Laser In Situ Keratomileusis (LASIK)

Ocular surface syndrome is a well-known side effect following refractive procedures using LASIK technology. Clinically it is characterized by dry eye, micropunctate keratitis, decreased and unstable tear film, and decreased best spectacle-corrected visual acuity (BSCVA). Although the exact mechanism for this disease is not completely understood, it is thought to be secondary to corneal nerve damage which occurs following the cutting of the corneal stroma in refractive procedures which involve creating a corneal flap. One of the main functions of the ocular nerves is the regulation of secretion activities of the lacrimal and meibomian glands. Their damage causes neurotrophic epitheliopathy and affects tear composition [47]. All lipidic, aqueous and mucus components of tears are involved in the quality of the tear film and any misbalance can compromise the ocular surface. When a deficiency on the aqueous phase is present in the tear film, the risk of infection by pathogens increases. A decrease in the quantity or quality of the mucus associated with goblet cells of the conjunctiva decreases tear stability. The same is observed when the alteration of the lipid composition of the tears affects the tears' evaporation control [48]. Treatment of ocular surface syndrome post LASIK surgery with artificial tears is often disappointing [49].

Eye platelet-rich plasma has a lubricating effect and has been effective in regenerating the ocular surface in cases of micropunctate keratitis, decreasing inflammation in patients suffering from dry eye [44] and stimulating wound-healing processes in dormant corneal ulcers [41]. This motivated a prospective study [40] by Alió *et al*. which included 26 eyes of 13 patients who had had LASIK surgery to correct myopia. All surgeries were performed using a mechanical microkeratome (Moria M2, disposable head; Moria, Paris, France). A 9.5-mm flap was created with a superior hinge of 3.5 to 4 mm. Flaps were tentatively programmed to be 130-µm thick based on an intended flap thickness of 160 µm.7 Laser in situ keratomileusis was performed using the Esiris excimer laser (Schwind, Kleinostheim, Germany). They were all affected by severe to moderate dry eye syndrome (according to the Triple Classification of Madrid REF) for at least 6 months after the surgery. Six of the 13 patients suffered from moderate ocular surface dysfunction symptoms prior to treatment Fig. (**6**) showed severe symptoms. Best spectacle-corrected visual acuity decreased from 1 line to 4.

All patients were positive for fluorescein staining and showed a tear break-up time between 4 and 9 seconds. Main outcome measures included subjective symptom disappearance, increased visual acuity, increased tear meniscus and tear break-up time, decreased inflammation and fluorescein staining and improvement in impression cytology.

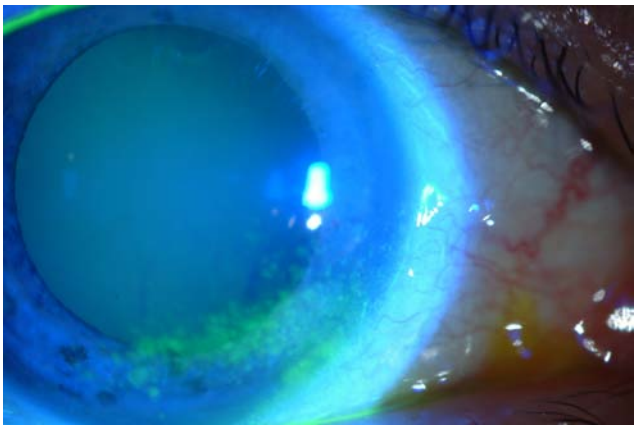

Fig. (6). Fluorescein staining of a patient with punctate keratitis suffering ocular surface syndrome post LASIK before treatment.

E-PRP eye drops applied 6 times daily improved subjective symptoms in the vast majority of patients and the improvement was considered good or excellent in 84% of the enrolled patients (11/13 patients). Fluorescein staining analysis showed complete resolution of punctate keratitis in 69% of the cases (18/26 eyes) Fig. (**7**) and almost complete in another 23% of the cases (6/26 eyes). E-PRP also improved visual acuity, providing a gain in vision in 69% of the cases (18/26 eyes) ranging from 1 to 4 lines of improvement in visual acuity. Tear break-up time increased to 2 seconds in 46% (12/26 eyes). Fourteen (54%) eyes showed a 0 to 2 second tear break-up time increase. One patient (8%) developed intolerance after 4 weeks and has been the only case reported in the literature.

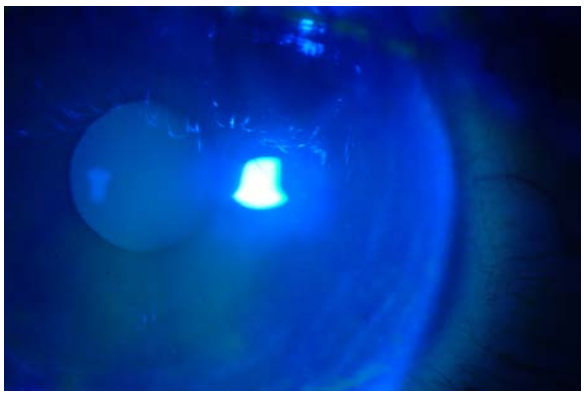

Fig. (7). Same patient as in Fig. (**6**), one month after treatment with E-PRP.

Autologous E-PRP adds a new tool for ocular surface syndrome treatment after LASIK as it provides subjective and objective improvement. E-PRP is rich in vitamins and growth factors. It also contains platelets, which upon activation are capable of providing a prolonged release of growth factors, thus increasing the endurance of their effects with fewer applications if compared to autologous serum. In a previous study [11], no differences were noted in the subjective scores for dryness between the autologous serum eye drops and artificial tears groups. However, further studies comparing conventional treatment such as autologous serum and E-PRP are necessary.

E-PRP as Adjuvant for Ocular Surface Reconstruction: Role of E-PRP Clot as a Potential Surgical Tool

Ocular surface reconstruction includes limbal autograft or allograft keratoplasty, amniotic membrane transplantation (AMT), sectorial epitheliectomy, etc. Theoretically, the adjuvant use of PRP would enhance the regenerative effect of these interventions, by release of growth factors that promote wound healing and decrease inflammation. Other biologically active products such as autologous serum have been used successfully for this purpose [3].

Alió *et al.* [41] presented a series of cases with perforated eyes or high risk of perforation due to deep chronic corneal ulcers treated with amniotic membrane transplantation combined with a clot of autologous E-PRP Figs. (**8** and **9**). Sur-

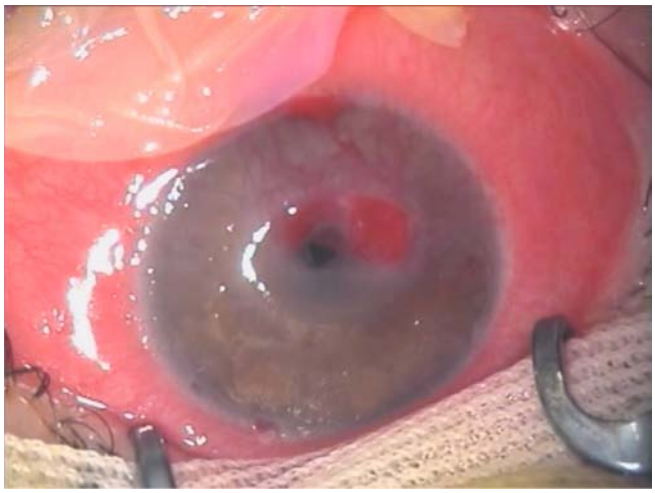

Fig. (8). Perforated eye before the treatment with amniotic membrane transplantation combined with a clot of autologous E-PRP.

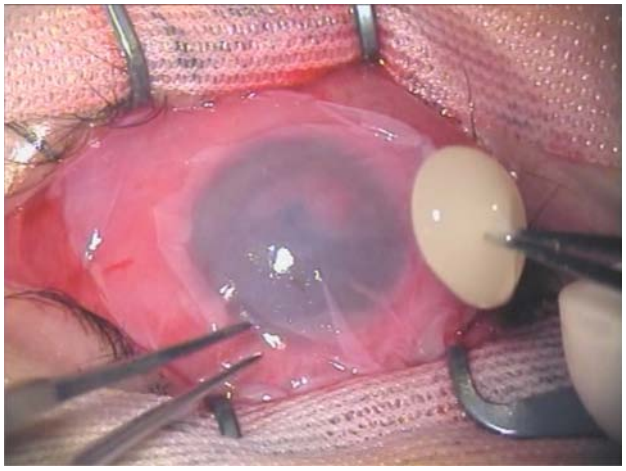

Fig. (9). Amniotic membrane transplantation with a clot of E-PRP before placing it underneath.

gery consisted of wound debridement, excision and removal of devitalized tissue and an application of amniotic membrane to the wound site with the epithelial side up. A clot of autologous platelet-rich plasma was placed under the amniotic membrane to seal the impending or actual corneal perforation and to increase the therapeutic effect of the amniotic membrane Figs. (**10** and **11**). The membrane was sutured into place with a continuous 10-0 nylon suture to the conjunctiva and a running purse-string suture was applied so that the membrane tightly adhered to the entire corneal surface. The eye was closed with a temporary tarsorrhaphy. Primary outcome measures were the reduction in size or depth of the corneal ulcer and improvement in best-corrected vision.

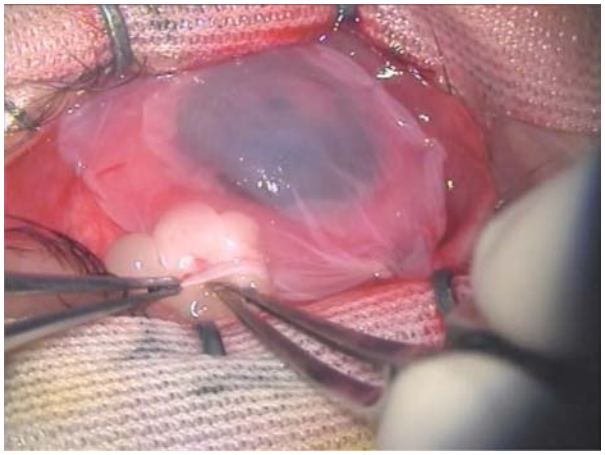

Fig. (10). Introducing the clot of E-PRP under the amniotic membrane.

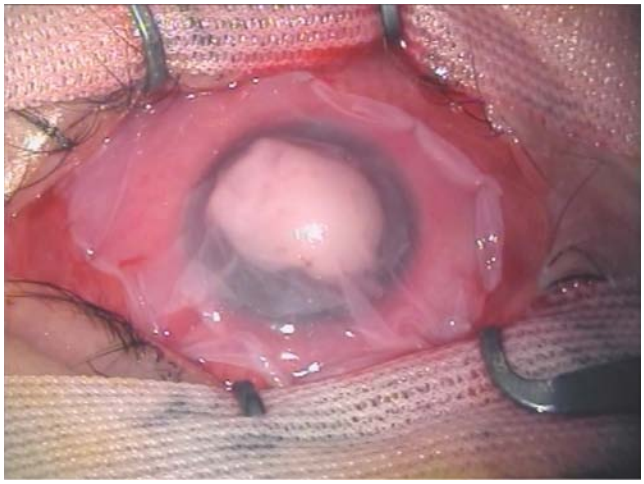

Fig. (11). Final result after amniotic membrane transplantation with a clot of E-PRP in a perforated eye.

All patients showed an improvement in the size of the ulcer, and 71% (10/14 eyes) had a complete resolution of the ulcer. Vision also improved in 57% of the cases (8/14 eyes) along with a decrease in inflammation that occurred 1 to 2 weeks after the surgery.

It cannot be ascertained in this study whether the healing effect of the E-PRP in combination with AMT was higher than using AMT alone. However, theoretically, E-PRP combined with AMT should further promote corneal wound-healing processes and further decrease inflammation due to the intrinsic characteristics of E-PRP mentioned throughout the review.

We also have experience (unpublished data) with the use of E-PRP clot in other surgical interventions to restore the ocular surface such as limbal keratoplasty Figs. (**12** and **13**) with good results, as seen with autologous serum by other authors [3, 12]. We believe that the prolonged synthesis and release of growth factors by the E-PRP clot provides additional long acting that would increase the benefit of E-PRP over autologous serum.

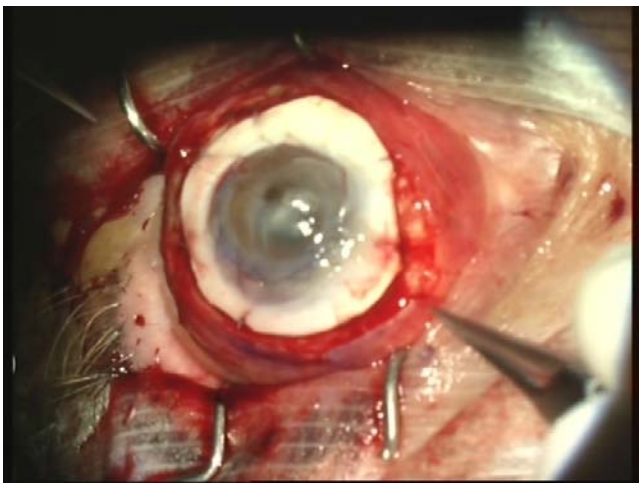

Fig. (12). 360º Limbal keratoplasty previous to autologous clot of E-PRP and Tutopatch membrane application, in a patient with limbal deficiency.

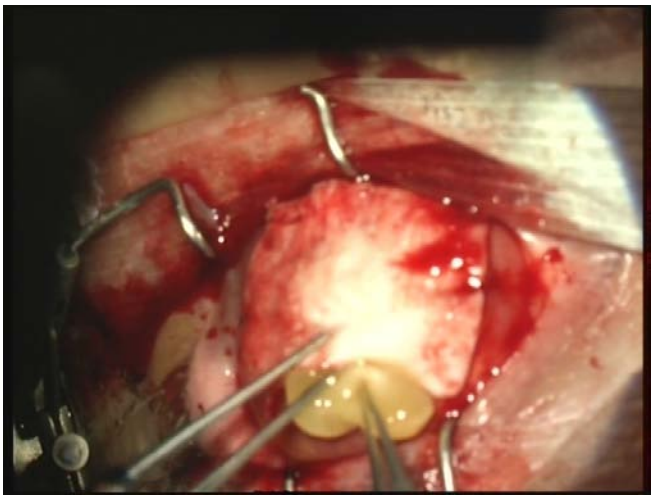

Fig. (13). Same patient as in fig. (**8**) after limbal keratoplasty followed by autologous clot of E-PRP and Tutopatch membrane (bovine pericardium).

Other Uses in Ophthalmology

The use of autologous platelets concentrates has also been described to enhance the healing of the idiopathic macular hole. A significant improvement in the anatomic success rate of surgery for idiopathic macular holes of less than 3 years' duration was found, but postoperative visual acuity of the group treated with autologous platelets concen-

trates was not statistically different from the control group [50].

CONCLUSIONS

Platelet enriched plasma in the form obtained in ophthalmology, E-PRP, is a reliable and effective therapeutic tool to enhance epithelial wound healing in ocular surface disease. PRP provides a high concentration of essential growth factors and cell adhesion molecules by concentrating platelets in a small volume of plasma. These growth factors and cell adhesion molecules have a major role in wound healing and enhance the physiological process at the site of the injury/surgery *via* eye drops or clot.

There are only a few controlled clinical studies that provide evidence that the use of autologous E-PRP promotes wound healing. Larger series and comparative studies using other treatment options such as autologous serum should be conducted to determine clinical differences between therapeutic options. In theory, E-PRP should provide a higher amount of growth factors in an initial burst as well as late synthesis and secretion of growth factors for the remaining 7 days of the life span of a platelet.

CONFLICT OF INTEREST

None declared.

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