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APPLICATION OF PLATELET-RICH PLASMA (PRP) IN CORNEAL LESIONS – A Review

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ABSTRACT

Platelet-rich plasma (PRP) is an autologous blood product rich in proteins and growth factors. Its application has been the subject of many studies in the field of veterinary ophthalmology to stimulate tissue healing and regeneration.

Due to its anatomical features (lack of blood vessels), the cornea is among the most susceptible to damage structures of the eye. Therefore, the study of the impact of various regenerative therapies (autologous blood products, serums, stem cells, amniotic and pericardial membranes) on corneal lesions is important not only scientifically, but also practically for veterinarians.

Numerous reports described the healing effect of PRP on corneal lesions. The present article is a brief overview of the various methods of application of PRP (eye drops, injections, activated clot form) in ophthalmology and the results obtained.

Key words: PRP, ophthalmology, corneal healing, solid eye platelet-rich plasma, regenerative medicine

INTRODUCTION

Regenerative medicine is an area that has attracted increasing scientific interest in recent decades. Its practices have been successfully applied in many areas like orthopedics, spinal surgery, oral and maxillofacial surgery, cardiovascular surgery, cosmetic surgery, implantology, reconstructive surgery, tissue engineering, and especially in hard-to-heal wounds (Lubkowska et al., 2012; Girgin et al., 2016). Ophthalmology is at the forefront of many fields of biomedicine and drug development. Many research groups around the world are focused on treating eye disorders, including corneal repair, and they report on different methods of therapy and techniques.

The cornea is a highly organized group of cells and proteins with three functions: barrier protection, filtration of some of the ultraviolet wavelengths in sunlight, and refraction (the cornea is responsible for 65 to 75 percent of the eye's capacity to focus light on the retina). The cornea must be transparent to refract light properly. Therefore, it has no blood vessels and instead is nourished by tears, environmental oxygen, and the aqueous humor of the anterior chamber (Wilson et al., 2004). The cornea consists of three layers: an outer stratified squamous nonkeratinized epithelium, an inner connective tissue stroma and the innermost layer, a cuboidal endothelium (Fig. 1). Visual acuity can be affected by disorders of any of these layers, including limbal stem cell deficiency, corneal dystrophy, and bullous keratopathy (Oie et al., 2013).

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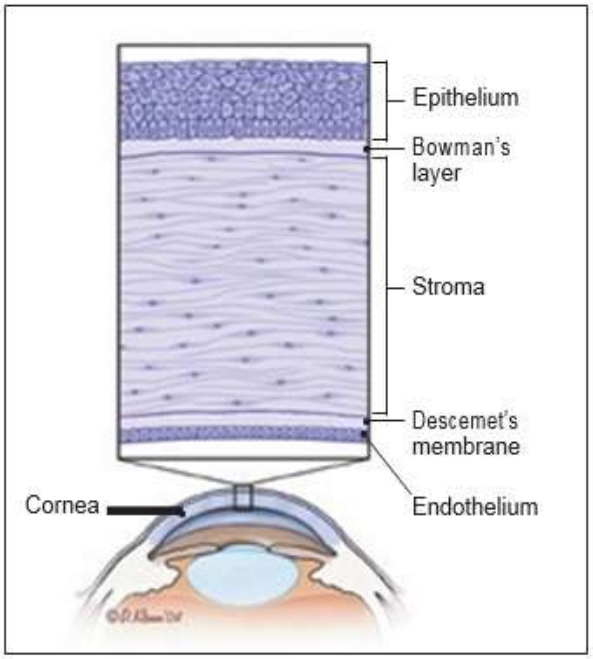

Fig.1. Anatomy of the cornea (Stephen Wilson et al., 2004)

Structural mechanisms of wound healing process require several raw materials, such as growth factors, vitamins, glucose, and so on. The easiest and most efficacious source to obtain these raw materials is the blood (Tanidir et al., 2010). In the literature there are different autologous blood-derived products with variable amounts of platelets and growth factors that are used to promote wound healing and tissue regeneration.

In the presented article are overviewed the various forms and methods of application of platelet-rich plasma (PRP) on corneal lesions - as one of the main therapies in regenerative medicine.

Autologous blood-derived products. What is PRP?

Different blood-derived formulations, such as an autologous serum, plasma enriched with platelets, and preparations rich in growth factors have been used to promote wound healing in multiple tissues. The serum is the clear liquid part of full blood after cellular components and clotting proteins have been removed. 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 (Fig. 2) that enhance proliferation and wound healing. They are stored in alpha granules, and several endogenous factors are involved in the repair of tissues, including the eye. More than 30 bioactive proteins are described such as EGF, PDGF-AB, VEGF, IGF-1, TGF-β, FGF, as well as cytokines including proteins such as PF4 and CD40 ligand. These factors have been suggested to accelerate epithelial and endothelial regeneration, provoke angiogenesis and cell differentiation, enhance the hemostatic response, increase collagen synthesis, and assist cell migration, thus stimulate soft tissue healing. The plasma also contains concentrated quantities of some important cell-adhesion molecules which promote epithelial migration such as fibrin, fibronectin, and vitronectin. After the complete release of growth factors, platelets can synthesize and secrete additional growth factors for the remaining several days of their lifespan (5 to 9) thus expanding the wound healing effect. (Anitua, 1999; Alio et al., 2007; Márquez de Aracena et al., 2007; Nurden, 2011; Alio et al., 2012; Aminkov et al., 2016; Ibrahim et al., 2019; Marinov, 2020).

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Name	Abbreviation	Function
Platelet derived growth factor	PDGF	Enhances collagen synthesis, proliferation of bone cells, fibroblast chemotaxis and proliferative activity, macrophage activation
Transforming growth factor β	TGF-β	Enhances synthesis of type I collagen, promotes angiogenesis, stimulates chemotaxis of immune cells, inhibits osteoclast formation and bone resorption
Vascular endothelial growth factor	VEGF	Stimulates angiogenesis, migration and mitosis of endothelial cells, increases permeability of the vessels, stimulates chemotaxis of macrophages and neutrophils
Epidermal growth factor	EGF	Stimulates cellular proliferation, differentiation of epithelial cells, promotes cytokine secretion by mesenchymal and epithelial cells
Insulin-like growth factor	IGF	Promotes cell growth, differentiation, recruitment in bone, blood vessel, skin and other tissues, stimulates collagen synthesis together with PDGF
Fibroblast growth factor	FGF	Promotes proliferation of mesenchymal cells, chondrocytes and osteoblasts, stimulates the growth and differentiation of chondrocytes and osteoblasts

Fig. 2. Growth factors in PRP and their biological functions (Voja Pavlovic et al., 2016)

PRP is a nontoxic and nonimmunogenic blood component obtained by centrifuging whole blood to get a clinically useful product of concentrated platelets in a small volume of plasma and is therefore a "platelet-rich plasma". Marx et al. (2001) described in detail what can be accepted as PRP and what makes it different from terminologies like "platelet concentrate", "platelet gel", "plasma rich in platelets". They simply defined PRP as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline.

Preparation of PRP

Usually, normal platelet count ranges between 150,000/µL and 350,000/µL, with an average of 200,000/µL +/- 75,000/µL. Effective PRP is considered to be at least a 4-fold increase of the platelet concentration, up to 1 000 000/µL (Marx, 2004; Liao et al., 2014; Marinov, 2019). Greater platelet concentrations have not been shown to further improve healing (Foster et al., 2009). However, it is important to note that in some cases too high platelet concentrations may have an inhibitory effect on healing processes (Weibrich et al., 2004; Hatakeyama et al., 2014), but no such reports have yet been found in the ophthalmology field.

Although the preparation of PRP in humans is well known, its preparation in veterinarian patients is more delicate due to the reduced volume of blood and the smaller size of the most frequent patients or experimental laboratory animals. Gimeno et al. (2006) used an 8.7 ml blood sample aspirated in 10 ml syringe, preloaded with 1.3 ml of Anticoagulant Citrate Dextrose (ACD) solution to avoid coagulation. Marinov (2019) mentions another option to use 3.2% sodium citrate in a ratio of 10:1. It should be kept in mind that the PRP yield is approximately 10% of the volume of whole blood drawn (Alsousou et al, 2009; Peng et al., 2016). Most of the authors recommended to set apart 1 ml for cell counting.

PRP is prepared by a process known as differential centrifugation (Fig. 3). In differential centrifugation, the acceleration force is adjusted to sediment certain cellular constituents based on different specific gravity (Dhurat et al., 2014). The first spin (called the hard spin) will separate the red blood cells from the plasma, which contains the platelets, the white blood cells, and the clotting

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factors. The second spin (called the soft spin) finely separates the platelets and white blood cells together with a few red blood cells from the plasma. This soft spin produces the PRP and separates it from the platelet-poor plasma (PPP) free from the obstruction provided by a large number of red blood cells. To attempt PRP with a single spin would not produce a true PRP. However, the PRP must be separated from the PPP soon after centrifugation because the concentrated platelets will slowly diffuse into the PPP over time and would reduce the platelet count of the PRP preparation. (Marx, 2001).

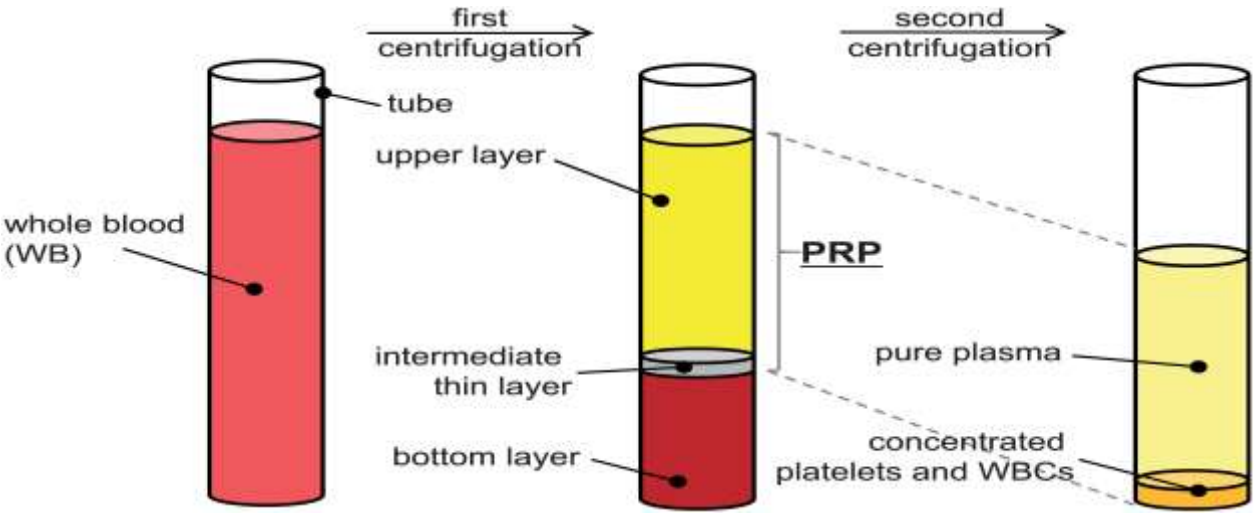

Fig. 3. A two-step process of centrifugal separation of the whole blood in a tube for the preparation of PRP (Linfeng Piao et al., 2017).

Drop form

For eye drop application Alio et al. (2012) suggest different preparation of collected blood by one-step centrifugation. Because this method differs from described by Marx (2001), they even invent the term E-PRP (eye platelet-rich plasma) to describe an autologous platelet-rich preparation specially designed for the treatment of ocular surface diseases that can be used as eye drops or as a solid clot.

Wróbel-Dudzińska et al. (2018) describe the use of the final product collected in sterile bottles with eye drop applicator and the patients were given autologous platelet-rich plasma drops five times a day. Thee bottle in use should be kept at +4°C for a maximum of 7 days and the rest at –20°C for a maximum of 3 months (Alio et al., 2017). The main risk with the eye drops is bacterial contamination, so Wu et al. (2015) recommended preparing a new bottle every week to keep autologous PRP fresh and avoid patient infection. 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. The 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 (Alio et al., 2012).

Injectable form

The major limitation of PRP treatment in animals with low circulation volumes is to obtain enough blood to prepare the end product. On the contrary to the drop form, injectable applications can be used as a one-shot and do not need any treatment combinations (Tanidir et al., 2010).

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Subconjunctival injection of platelet-rich plasma (sPRP) revealing an acceleration and improvement in corneal epithelial wound healing. The injection should be made as soon as possible after the injury in the superior bulbar conjunctiva. Ibrahim et al. (2019) studies alkali burns in rats and concluded that earlier single-shot sPRP injection in 2 hours after burns had good efficiency in restoring the corneal healthy surface. Injection after 48 hours is not as promising as the earlier application. Elbassiouny (2010) reported an experimental study of a single injection of 0,5 ml sPRP into the superior conjunctival fornix in a group of rabbits with stromal injuries and concludes better remodeling of stroma with a decreased population of cells and uniform arrangement of collagen bundle, along with rapid regular epithelial healing. It should be mentioned that some patients may manifest some side effects like red-orange discoloration of sPRP injection and formation of granulation tissue at the infiltration point. Similar complications were noted by Tanidir et al. (2010) and Marquez-de-Aracena et al. (2007), but they also notice none of the complications observed after sPRP application was reported to persist in the long-term follow-up.

Charalambidou et al. (2018) reported the first experimental study that uses PRP via intrastromal injection to the cornea in a group of rabbits with alkali burns (Fig. 4). They preferred this application, used routinely to administer stem cells, as they can increase the concentration of growth factors in the deep layers of the cornea in a minimally invasive way.

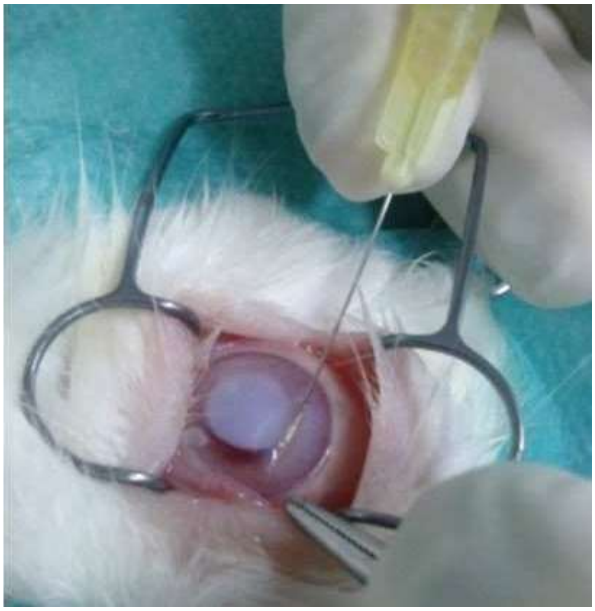

Fig. 4. Intrastromal injection (Glykeria Charalambidou et al., 2018)

Clot form (activated platelet-rich plasma)

The clot form of PRP was also reported to be used in corneal epithelial defects. The inevitable addition of a secondary occlusive therapy to a clot form is its major disadvantage. However, some studies still report the efficiency of the clot form (Alio et al., 2007). When PRP is combined with thrombin and calcium, the clotting cascade is activated, converting fibrinogen to fibrin (platelets then become trapped in a fibrin matrix/mesh that produces a stable clot) with subsequent degranulation and releasing growth factors (Englert et al., 2005). More than 95% of the presynthesized growth factors are secreted within 1 hour. Therefore, PRP must be developed in an anticoagulated state and

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should be used within 10 minutes of clot initiation - clinicians should not activate PRP in advance. Studies that have not used anticoagulated whole blood, which is then clotted to activate the PRP, are not studies of PRP and therefore are misleading (Marx, 2004).

There is an ongoing debate about potential exogenous platelet activation before application to the damaged tissue. In PRP, with inactivated platelets, contact with fibrillary collagen, thrombin, or basement membrane of the cells leads to thrombocytes activation, with releasing huge amounts of bioactive molecules from platelet alpha granules. However, by using certain protocols platelets may be activated before PRP application and making that great amount of growth factors may be immediately available to the target cell in the damaged tissue (Cavallo et al., 2016).

Arnalich et al. (2016) reported the use of E-PRP (previously described by Alio et al., 2012) activated with 10% calcium chloride (Fig. 5A). Different materials were used to maintain the solid clot attached to the site where treatment is necessary. For these purposes, amniotic membrane (Fig. 5B) or other biomaterials such as bovine pericardium or autologous fibrin membrane can be used with different grades of interdonor variations or biological hazards, providing surgical alternatives to be used depending on the availability of them for the emergency management of corneal perforations secondary to different severe ulcerative diseases.

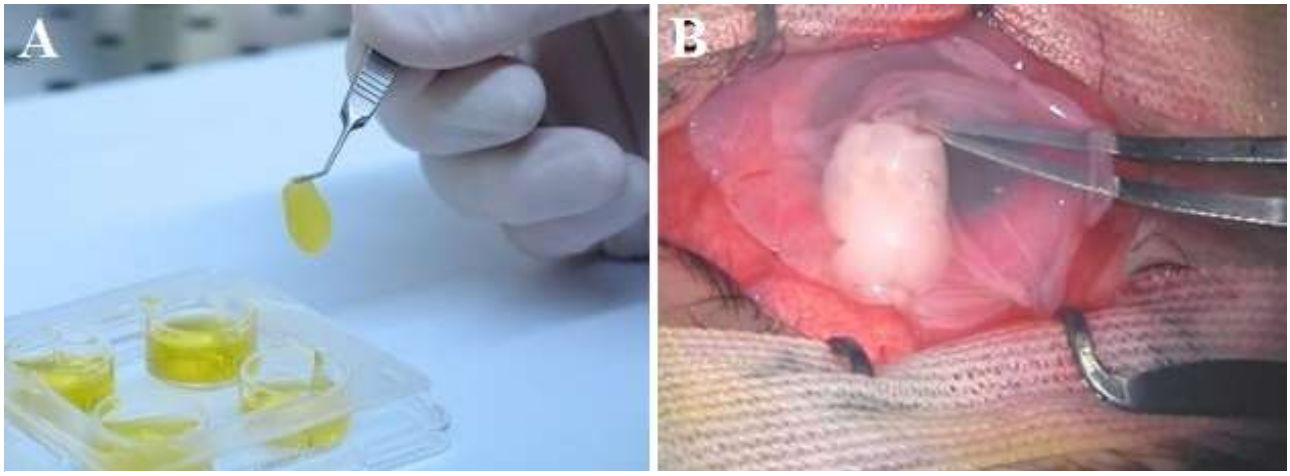

Fig. 5. A - Aspect of a clot of eye platelet-rich plasma (E-PRP) immediately after its preparation; **B** - Placing eye platelet-rich plasma (E-PRP) clot below the amniotic membrane (Francisco Arnalich et al., 2016)

Minutes after clot formation, rapid degeneration of platelet alpha granules begin and release of growth factors in the application area. While some authors recommend that activation be performed with a mixture of calcium dichloride and thrombin (Man et al., 2001), others advise that thrombin be eliminated because of the high risk of immune responses and transmission of infectious diseases (Marinov, 2019). Du et al. (2018) performed a novel method of PRP preparation-activation, by which the coagulation was inhibited previously in the hypothermic environment (4°C) and then the activation of platelets through autologous thrombin activity restoration followed only by rewarming (37°C). They named it temperature controlled PRP (t-PRP) and the method requires no exogenous additives, neither anticoagulants nor bovine thrombin or citrate.

However, clotting of the PRP should be done only at the time of use. Clotting activates platelets, which begin secreting their growth factors immediately. Within 10 minutes they secrete 70% of their stored growth factors and close to 100% within the first hour (Marx, 2001)

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DISCUSSION

PRP was used for the first time by Ferrari et al. (1987) to reduce the transfusion of homologous blood products following open-heart surgeries. Since then, many authors demonstrated the variety of platelet-rich plasma applications not only in several medical specialties but especially in several applications in ophthalmology. The role of PRP in eye diseases has demonstrated in moderate-to-severe dry eye, persistent epithelial corneal defect, recurrent corneal erosion, alkali burn, dormant ulcers, neurotrophic keratopathy, superior limbic keratoconjunctivitis, graft-versus-host disease, post-LASIK ocular surface syndrome, corneal surface reconstructions in corneal perforations, etc. (Foster et al, 2009; Alsousou et al, 2013).

Corneal wound healing is a unique process because the cornea is an avascular tissue. Stimulation and regulation of healing rely on growth factors that can reach the cornea through the tears, aqueous humor, and the limbic vessels (Swank et al., 1996). In the organism, the normal healing response begins the moment the tissue is injured. As the blood components spill into the site of injury, the platelets come into contact with exposed collagen and other elements of the extracellular matrix. This contact triggers the platelets to release clotting factors as well as essential growth factors and cytokines (Diegelmann and Evans, 2004).

PRP provides and delivers a high concentration of essential growth factors and cell adhesion molecules by concentrating platelets in a small volume of plasma. Monotherapy with any form of PRP revealed good results. But as the cornea becomes more vulnerable to infections during the process of epithelial wound healing, it is advisable to use antibiotics prophylactically to achieve scatheless ocular healing and to prevent potential infections (Tanidir et al., 2010). PRP cannot promote the infection on its own. However, it is no different in substrate than the blood clot that forms in every wound and therefore could not support bacterial growth any more than any other blood clot. PRP has a pH of 6.5 to 6.7 compared with a mature blood clot of 7.0 to 7.2. It has thus been counter suggested that PRP actually inhibits bacterial growth. (Marx, 2004). There is not a single case reported of infection secondary to microbial contamination of the e-PRP drops, as it is prepared, used, and stored under strict sterile conditions. (Alio et al, 2012)

There are many protocols for the preparation of PRP, each having its own standardized parameters and claimed. Most techniques are often regrouped inappropriately under the historical term of Platelet-Rich Plasma (PRP). Since 25 years, their use dramatically increased in human medicine, veterinary medicine, and experimental medicine fields. It is very difficult to sort and interpret the available data, due to a large number of preparation techniques, terminologies and forms of these materials, and the endless list of potential applications (Ehrenfest et al., 2013).

Prolonged episodes of corneal de-epithelialization increase the production of collagenase in the corneal stroma, which may reach corneal perforation (Marquez-de-Aracena et al., 2007). A single subconjunctival injection gave good results in this case. Single subconjunctival injections have proved their good results. If the objective is a rapid corneal epithelization, eye drops are also a good way of administration PRP. Significant advantages are ease of preparation, absence of preservatives, autologous origin, safety, and the possibility of storage. They are also an option for the treatment of moderate to severe chronic dry eye diseases. As a disadvantage of eye drops can be noted the need for frequent application. In lesions with large stromal loss, the association of solid PRP and amniotic membrane or other biomaterial is recommended, as well as its use in isolation (Ribeiro et al., 2017)

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The clinician needs to be well aware of the corneal lesion to select the most reliable method for the particular patient. By the use of 100% autologous blood technique, and strictly aseptic conditions, PRP products are free from the risk of disease transmission.

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