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Review Article

Autologous serum and plasma rich in growth factors in ophthalmology: preclinical and clinical studies

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ABSTRACT.

The use of blood derivatives represents an alternative therapeutic approach that is gaining interest in regenerative medicine due to its potential to stimulate and accelerate tissue healing. Autologous serum eye drops and platelet-enriched plasma eye drops are being used in the treatment of different ophthalmological disorders. In this review, we summarize the different blood-derived formulations used in the treatment and care of ocular surface disorders. The biological basis and use of autologous serum and plasma rich in growth factors are deeply evaluated as well as the challenges to be addressed in the future in this new generation of blood-derived therapies.

Key words: autologous serum – cornea – dry eye – eye – ocular surface – plasma rich in growth factors – platelet-rich plasma

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Introduction

Ocular surface may suffer from several disorders including dry eye syndrome, persistent epithelial defects (PEDs), neurotropic ulcerations, limbal deficiency and corneal dystrophies among others. Dry eye syndrome is the most common disorder, and its prevalence has tripled in the last decade. These disorders are characterized by impaired tissue repair process. A reduction in epitheliotrophic factors compromises the integrity of the surface epithelia, leading to the formation of epithelial defects that may persist and progress as a result of the compromised wound-healing process (Quinto et al. 2008). Conventional therapeutic options include intensive artificial tear supplements, punctual occlusion, therapeutic contact lenses and appropriate management of adnexal disease. Surgical procedures, as keratoplasty or amniotic membrane transplantation, are used every day to restore the ocular surface but they are no suitable for all patients.

The integrity of the corneal epithelium and the process of re-epithelialization are dependent on many factors. Although underlying mechanisms have not been fully elucidated, it is likely that trophic factors exert a pivotal role in corneal epithelium integrity. For example, nerve growth factor (NGF) is being investigated as a potential treatment in neurotrophic keratopathy (Werner & Grose 2003). Topical NGF shows encouraging results in clinical trials, but its use is limited as still recombinant growth factors are not cost efficient. Heparan sulphate derivates are potential alternatives. ReGeneraTing Agent (RGTA, Cacicol20®, OTR3, Paris, France) is a new type of matrix therapy agent. The latter is based on large polymers, which replace destroyed heparan sulphate molecules, creating a suitable cellular environment that promotes healing. RGTA has been reported to show encouraging results in the treatment of corneal ulcers and dystrophies of various aetiologies, and it is currently being evaluated by means of clinical trials (Aifa et al. 2012).

Several studies have shown that cyclosporin improves corneal epithelium in dry eye patients, including those with graft-versus-host disease (GVHD). Improvements in the ocular surface and tear functions resulted presumably from the decreased inflammation and the increased goblet cell density. However, the use of topical cyclosporin eye drops is correlated with several side-effects such as strong irritation, which limits its use (Wang et al. 2008; Toker & Asfuroglu 2010). Another approach consists of using topical anti-inflammatory agents like

corticosteroids. Although these drugs improve patient′s symptoms, they are also associated with damaging long-term side-effects including cataracts and increased intra-ocular pressure (Dinning 1976; Renfro & Snow 1992). As a consequence, the use of artificial tears, that increase ocular surface humidity and provide additional lubrication, remains as the most widely use treatment. The emergence of presentations without preservatives represents a turning point, as preservatives may induce ocular toxicity, irritation and provokes epithelial damage (Geerling et al. 2001; Noecker 2001). The new types of formulations including hypotonic solutions, tears that contain lipids to prevent evaporation, substances with bioadhesive properties to increase water retention, and formulations that contain protective substances of the cell stress caused by the hypertonicity of the tear represent an advance in the field (Aragona et al. 2013). However, all of them are far from having all the properties of the human tear, as they lack biologically active molecules and growth factors (Klenkler et al. 2007; Dogru & Tsubota 2011).

Blood Derivatives in Ophthalmology

It widely known that natural tears have three main objectives: provide a smooth surface that allows the regular refraction of light, keep the metabolism of the ocular surface and lubricate the eye surface to facilitate blinking. They also have a complex composition, being the main component water (98.3%), followed by salts (1%), proteins and glycoproteins (0.7%), and minor hydrocarbons, lipids and other fractions. Teardrop brings regulatory enzymes and nutrients such as glucose, oxygen, water and electrolytes necessary for the metabolism of the corneal epithelium. It contains numerous active or functional proteins such as growth factors, vitamins, immunoglobulins and neuropeptides that regulate the processes of proliferation, migration and differentiation of the cells of the corneal epithelium and conjunctiva. Furthermore, it also presents antimicrobial properties, not only because of its barrier effect and washing, but because it contains lymphocytes, macrophages and enzymes such as arisulfatase A, peroxidase, lactoferrin and lysozyme with bacteriostatic and bactericide effects (Geerling et al. 2004). Tears thus have lubricating, mechanical and antimicrobial effects, but also epitheliotrophic properties.

Historically, there has been an interest in developing 'natural-tear'-like agents that may be used in the treatment of eye surface diseases. The use of blood and blood derivatives is not new, and their rationale in ophthalmology is based mainly on their potential properties including lubrication, mechanical actions and antimicrobial effects. The Ebers Papyrus, 1534 BC, is the very first reference in history to the implementation of a blood derivate at eye level. It was in 1975 when autologous serum (AS) was initially applied for dry eye (Ralph et al. 1975). The beneficial effect of AS in the treatment of dry eye syndrome was later described by Fox et al. (1984a). But it was at the end of the 1990s of the last century when began its use of a more extended form (Tsubota et al. 1999a,b); since then, AS eye drops have become more popular for treating ocular surface disease.

Through these decades, fetal bovine serum, allogeneic serum and umbilical cord serum have been used for this purpose, but they are heterologous products, with a higher risk of allergic reactions and infectious disease transmission, and their use is possible only in some specialized centres (Yoon et al. 2007a, 2011, 2013; Sharma et al. 2011; Harritshoj et al. 2014). The composition of serum is very similar to that of tears and most concentrations are the same, with the exception of more vitamin A, lysozyme, transforming growth factor-β (TGF-β) and fibronectin, and less IgA, epidermal growth factor (EGF) and vitamin C in serum than in tears (Pan et al. 2013; Table 1). Several studies have shown that AS eye drops contain growth factors such as EGF, vitamin A, TGF-β, fibronectin, substance P (SP), insulin-like growth factor 1 (IGF-1), NGF and other cytokines that are essential for the proliferation, differentiation and maturation of the normal ocular surface epithelium. Some of the main roles of these proteins found in plasma and platelets are summarized below and resumed in Table 1.

Epidermal growth factor

Epidermal growth factor plays an important role in corneal epithelial migration and proliferation that improves and accelerates the wound healing process. EGF stimulates DNA synthesis of epithelial cells and stromal fibroblasts in culture and synthesis of fibronectin by epithelial cells and is chemotactic for human epithelial and stromal cells. It has an anti-apoptotic effect and has been linked with the production of mucin 1 by the Goblet cells of the conjunctiva (López García et al. 2011).

Transforming growth factor-β

Transforming growth factor-β1 has been detected in corneal epithelium, stroma and endothelium. In the epithelium, the TGF-β1 levels are higher during the stromal repair processes. TGF-β is secreted by a large number of cells such as platelets, endothelial cells, lymphocytes and macrophages. In the cornea, TGF-β1 decreases the keratocytes migration, but favours the chemotaxis of fibroblasts as well as the production of extracellular matrix by a dual mechanism: by stimulating the production of collagen, fibronectin and proteoglycans and diminishing its degradation by inhibiting the metalloproteases and other proteolytic enzymes. In a synergistic mechanism with platelet-derived growth factor (PDGF) and integrins, TGF-β promotes the differentiation of myofibroblasts and exerts important anti-inflammatory effects (López García et al. 2011).

Vitamin A

Vitamin A is one of the main epithelium-trophic factors in AS, being its concentration 100 times higher than that found in the tear. It seems to prevent processes of squamous metaplasia of the epithelia (Geerling et al. 2004).

Platelet-derived growth factor

Platelet-derived growth factor was one of the first growth factors

Table 1. Main growth factors present in blood and their roles in ocular surface regeneration.

Proteins	Roles	References
EGF	• Induces corneal epithelial migration and proliferation • Stimulates DNA synthesis of epithelial cells and stromal fibroblasts • Stimulates synthesis of fibronectin by epithelial cells • Chemotactic effect for human epithelial and stromal cells • Anti-apoptotic effect • Induces production of mucin 1 by the Goblet cells	López García & del Castillo (2011)
TGF-β	• Decreases keratocyte migration • Favours chemotaxis of fibroblasts • Induces the production of extracellular matrix by stimulating the production of collagen, fibronectin, and proteoglycans and diminishing its degradation by inhibiting the metalloproteases and other proteolytic enzymes • Promotes the differentiation of myofibroblasts • Anti-inflammatory effect	López García & del Castillo (2011)
Vitamin A	• Prevent processes of squamous metaplasia of the epithelia	Geerling et al. (2004)
PDGF	• Chemotactic effect for monocytes, macrophages and fibroblasts • Synergistic effect with TGF-β to promote myofibroblasts differentiation	
Fibronectin	• Promotes wound healing and phagocytosis • Important role on cell migration during the repair process of corneal epithelium	Phan et al. (1987) and Gordon et al. (1995)
Annexin A5	• Stimulates the secretion of the plasminogen activator-type urokinase facilitating cell migration	
Albumin	• Reduces degradation of cytokines and growth factors	Tsubota et al. (1999b), Shimmura et al. (2003) and Unterlauft et al. (2009)
α2 macroglobulin	• Neutralizes the proteolytic enzymes	Tsubota et al. (1999a) and Poon et al. (2001)
bFGF	• Promotes corneal wound healing increasing cell proliferation and motility	Andresen et al. (1997)
IGF-I	• Acts synergistically with substance P to promote corneal epithelial migration	Yamada et al. (2004)
NGF	• Induces neurite sprouting by neural cells • Restores the function of injured neurons • Induces SP and calcitonin gene-related peptide production in the central and peripheral nervous system enhancing epithelial proliferation • Increases epithelial cell proliferation and differentiation • Promotes fibroblast cell growth	Matsumoto et al. (2004)

EGF = epidermal growth factor, TGF-β = transforming growth factor beta, PDGF = platelet-derived growth factor, bFGF = fibroblast growth factor b, IGF-I = insulin-like growth factor 1, NGF = nerve growth factor.

characterized. It is chemotactic for monocytes, macrophages and fibroblasts and stimulates the expression of other factors such as TGF-β.

Fibronectin

It is a soluble protein that promotes wound healing and phagocytosis. On the ocular surface, fibronectin is one of the most important factors in the phase of cell migration during the repair of corneal epithelium (Phan et al. 1987; Gordon et al. 1995). Topical fibronectin has been used in the treatment of persistent corneal epithelial defects and trophic ulcers with different results.

Annexin A5

Annexin A5 is being investigated as an alternative to the fibronectin eye drops, which interacts with the domain kinase of some integrins mimicking its effect. It also stimulates the secretion of the plasminogen activator-type urokinase, whose expression is increased in epithelial defects, facilitating cell migration.

Albumin

Albumin is one of the most important proteins in blood. It reduces the natural degradation of cytokines and growth factors in the areas of tissue injury and shows anti-apoptotic activity. The wound healing effect of albumin eye drops has already been demonstrated *in vitro* and *in vivo* (Tsubota et al. 1999b; Shimmura et al. 2003; Unterlauft et al. 2009).

α2 macroglobulin

Its main function is to neutralize the proteolytic enzymes. It is useful in ocular burns and marginal ulcers (Tsubota et al. 1999a; Poon et al. 2001).

Fibroblast growth factor b

Fibroblast growth factor promotes corneal wound healing, not only by increasing cell proliferation, but also through increased motility (Andresen et al. 1997).

Insulin-like growth factor-I

Insulin-like growth factor-I acts synergistically with SP to promote corneal epithelial migration (Yamada et al. 2004). The use of IGF-I-containing eye drops in patients with neurotrophic keratopathy leads to successful results (Chikama et al. 1998; Yamada et al. 2004). Patients with seasonal allergic conjunctivitis and VKC have showed significant elevation of SP in tears,

which suggests that SP may contribute to the pathogenesis and severity of ocular allergic diseases (Matsumoto et al. 2004).

Nerve growth factor

Nerve growth factor is the best-known neurotrophin. Some reports state the efficacy of the novel use of NGF in resurfacing corneal ulcers resulting from neurotrophic keratopathy. It is well known that NGF induces neurite sprouting by neural cells and restores the function of injured neurons. NGF also has been shown to induce the production of SP and calcitonin gene-related peptide in the central and peripheral nervous system. The biological effects of NGF on the ocular surface are known to be mediated by specific receptors localized on corneal and conjunctival epithelial cells and immune cells (Matsumoto et al. 2004).

Autologous Serum

In general, dry eye treatments attempt to manage symptoms, including burning, irritation and foreign body sensation but fail to repair injured tissues. One potential reason for this is that artificial tears have variable osmolarity, viscosity, electrolyte composition and sometimes the presence of preservative (Lemp 2008). The use of preserved artificial tears may cause toxicity and allergic reactions. Furthermore, artificial tears do not contain the proper mixture of growth factors, neuropeptides and vitamins present in the healthy tear film. As serum composition resembles that of tears (Table 2), it has been proposed as a substitute to treat ocular surface injuries. As early as 1975, Ralph et al. showed the successful use of serum eye drops in treating advanced ocular surface dysfunction (Ralph et al. 1975). After the initial report, Fox et al. and Tsubota et al. elaborated on the use of AS tears in Sjögren dry eye patients, demonstrating that there was not only subjective improvement in symptoms (Fox et al. 1984b), but that there was also a direct effect on the ocular surface epithelium (Tsubota et al. 1999a). Since then, it has been discovered that artificial tears do not maintain intracellular ATP levels and epithelial cell membrane integrity compared to AS eye drops (Poon et al. 2001). Key factors that maintain a healthy ocular surface, such as epithelial growth factor (Pastor & Calonge 1992; van Setten et al. 1992), Vitamin A, SP and insulin-like growth factor albumin, a2-macroglobulin and immunoglobulins, which have a bacteriostatic effect, are present in the AS (Poon et al. 2001; Lopez-Garcia et al. 2007). However, AS eye drops contain also the pro-inflammatory cytokines expressed by the leucocytes and the monocytes. The latter together with the uncontrolled presence of immunoglobulins and complement may be deleterious for many patients, especially those suffering from immunological alterations. In addition, there is not one single approach for preparing the AS eye drops since this product still remains like a 'home-made' approach whose predictability, reproducibility and the molecular mechanisms driving their roles still remains unknown. The preparation and storage of AS is a matter of debate as each clinical centre produces its own AS eye drop and provides different preparation and storage protocols (Geerling et al. 2004; Lopez-Garcia et al. 2014).

Table 2. Comparison of human tears and serum.

	Tears	Serum	References
pH	7.4	7.4	Tsubota et al. (1999b), Geerling et al. (2004), López García & del Castillo (2011) and Pan et al. (2013)
Osmolarity	298	296	
EGF (ng/ml)	0.2–0.3 1.9–9.7	0.5	
TGF-β (ng/ml)	2–10	6–33	
Vit A (mg/ml)	0.02	46	
Fibronectin (μg/ml)	21	205	
Lysozyme (mg/ml)	1.4	6	
SIgA (μg/ml)	1190	2	
IGF-I (ng/ml)	157		
SP (ng/ml)	0.157	0.071	
NGF (pg/ml)	468	54	

EGF = epidermal growth factor, TGF-β = transforming growth factor beta, SIgA = surface immunoglobulin A, IGF-I = insulin-like growth factor 1, SP = substance P, NGF = nerve growth factor.

Plasma Rich in Growth Factors

Platelets: from the knowledge to the technology

Platelets contribute to haemostasis by preventing blood loss at sites of vascular injury and contain a large number of growth factors and cytokines that have a key role in tissue regeneration. In the past two decades, an increased understanding of the physiological roles of platelets in wound healing and after tissue injury has led to the idea of using and concentrating platelets as therapeutic tools. Indeed, after fibrin glue was introduced in the early 1990s as a biomaterial with haemostatic and adhesive properties, the strategic modification of the fibrin to include platelets was reported (Gibble & Ness 1990). The initial rationale of platelet-rich products was to replace the blood clot with a preparation enriched in platelets which could, once activated, secrete a large pool of proteins and factors including PDGF, TGF-b, VEGF, IGF-I, hepatocyte growth factor (HGF), angiopoietins, platelet factor-4 (PF-4) and thrombospondin among others to the local milieu, driving the tissue regeneration mechanism.

The proteins and other substances that are provided by platelets and which can participate in tissue repair and healing are mainly stored in their alpha-granules. The proteins contained in these granules are secreted by exocytosis by the formation of secretory vesicles that fuse with the plasma membrane allowing the release of their contents to the milieu (Reed et al. 2000). Interestingly, by getting rid of erythrocytes and leucocytes, the preparation would take full advantage of the plasma and the concentrated platelets and the stored growth factors, without the presence of pro-inflammatory agents released by the leucocytes.

In the last decade, several technologies have translated the power of the plasma and platelet-derived proteins and growth factors to the clinics by means of a predictable technology that can be easily prepared and used. The

technology of plasma rich in growth factors (PRGF) consists of a limited volume of plasma enriched in platelets, which is obtained from the patient. Once the platelet concentrate is activated, a three-dimensional and biocompatible fibrin scaffold is formed, and a myriad of growth factors and proteins are released, progressively, to the local environment, contributing to the acceleration of wound healing and tissue repair (Anitua et al. 2004). Furthermore, the autologous origin of this preparation eliminates concerns about immunogenic reactions and disease transmission (Ogino et al. 2005). 'PRGF is an autologous 100% platelet-rich plasma with some specific and particular characteristics. Unfortunately, it is almost impossible to reach an agreement about a definition for platelet-rich plasma. There are more than 30 different protocols and platelet-rich plasma products reported in the literature, and many of them are also commercialized. The variability of composition, protein content, platelet enrichment, presence or absence of leucocytes, application protocols, etc. have lead to different scientific results using platelet-rich plasmas in both efficacy and safety. PRGF follows a predictable protocol by which platelets are concentrated twofold in the plasma of the patients. Red cells and white cells including leucocytes are discarded and therefore are not present within PRGF. Furthermore, for ophthalmological purposes, the new PRGF eye drops are not diluted as usually happens with AS. Last but not least, the medical devices needed to prepare PRGF are EU and FDA accepted.'

As it has been previously reported, the use of AS eye drops was the first hemoderivative product used in the ophthalmology field (Tsubota et al. 1999a; Geerling et al. 2004; Matsumoto et al. 2004). Their use in the treatment of ocular surface tissue regeneration was attributed to its content in growth factors, which are mainly provided by blood platelet content (Nurden et al. 2008; Blair & Flaumenhaft 2009). However, the presence of leucocytes during AS preparation procedure increases the level of pro-inflammatory cytokines including IL-6, IL-1β, and TNF-α, involved in the inflammatory process of some ocular diseases (Pflugfelder et al. 1999; Yoon et al. 2007b).

The pioneering use of PRGF in ophthalmology is mainly based on improving and overcoming some of the main limitations that AS products show. As PRGF does not contain leucocytes but doubles the concentration of platelets, it is expected to have more growth factors and neurotrophic factors but without pro-inflammatory cytokines (Fig. 1). The controlled activation process enables the preparation of different therapeutic formulations ranging from an autologous eye drop to a three-dimensional fibrin scaffold (Fig. 2).

Preclinical studies

Plasma rich in growth factors technology has been successfully used in several medical fields like dentistry and oral implantology, orthopaedics, sport medicine and ulcer treatment among others for promoting wound healing and tissue regeneration (Anitua 1999; Sanchez et al. 2007; Anitua et al. 2008, 2010; Torres et al. 2009; Orcajo et al. 2011).

The use of PRGF in ophthalmology begun with a cost-effective study in which it was observed that it was possible to obtain almost the 4.5 mL of PRGF from every 9 mL of blood. The latter significantly reduces the blood volume needed to prepare the month-to-month medication to the patient (Anitua et al. 2011, 2013a). Recently, Freire et al. (2012)

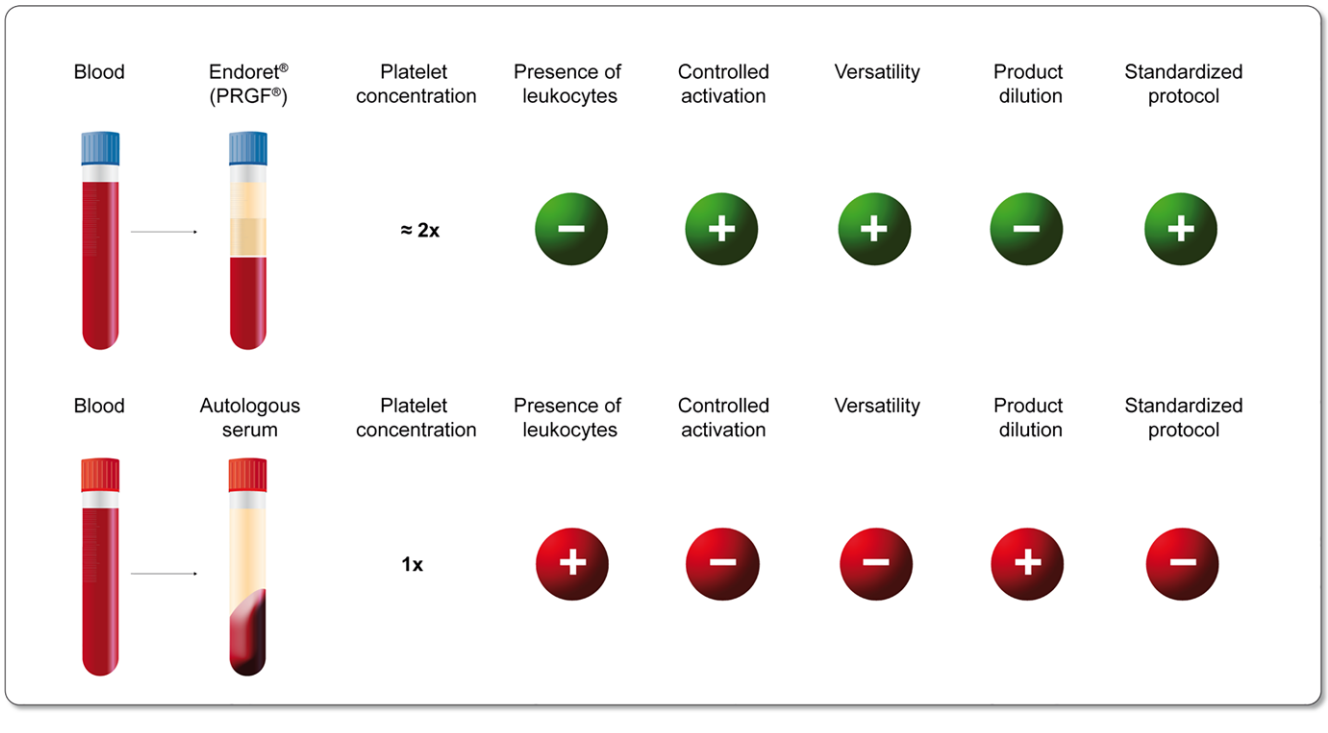

Fig. 1. Differential characteristics of plasma rich in growth factors versus autologous serum. Green ball represents the advantage of one technology against the other.

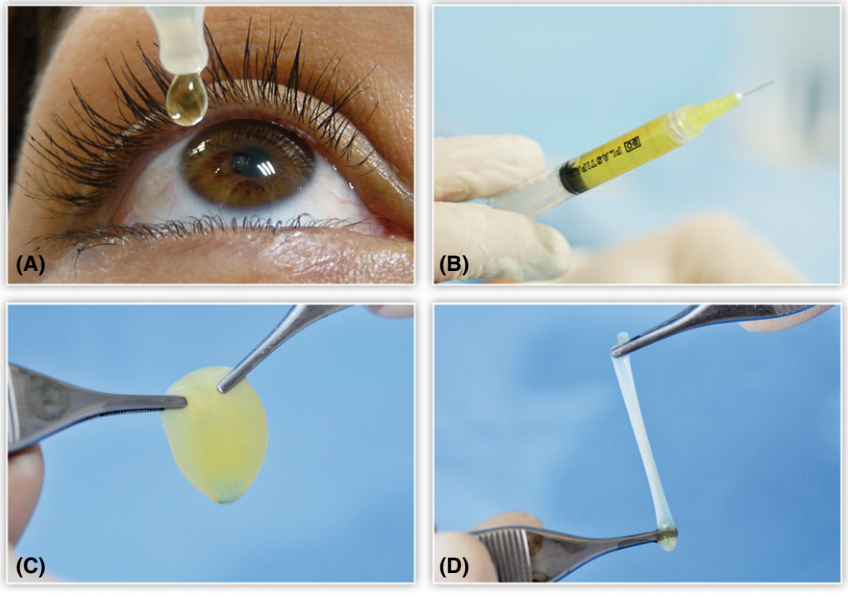

Fig. 2. Different therapeutic fomulations obtained with plasma rich in growth factors (PRGF) technology. (A) PRGF eye drops, (B) injectable PRGF, (C) fibrin scaffold and (D) fibrin membrane.

showed that PRGF eye drops enhance the proliferation of corneal epithelial cells (HCE) compared with other non-activated platelet-based products, suggesting that platelet degranulation is a critical step to enrich the formulation in growth factors and proteins. In this study, it was observed that PRGF upregulated the expression of several genes involved in communication and cell differentiation and significantly improved the biological activity of human corneal epithelial cells compared with AS (Liu et al. 2006; Freire et al. 2012). In another study, it was concluded that PRGF eye drop protects ocular surface tissues against scar formation, reducing the number of SMA-positive cells (myofibroblasts) after TGF-β1 induction (Figs 3 and 4; Anitua et al. 2011). These results were correlated with in '*in vivo*' studies, where mice underwent PRK surgery showing a reduction in scar and haze formation after treatment with PRGF eye drops (Anitua et al. 2013a).

Of particular importance is the dosage and duration of these blood derivatives. Assuming that ocular surface diseases are usually chronic diseases that demand long-term treatments, it is necessary that the biological functionality and stability of the treatments are preserved for weeks or months. PRGF eye drops maintain their protein content and their biological activity potential at least for 3 months after storage at −20 celsius. Moreover, PRGF eye drops can be preserved for their daily use at 4 celsius or room temperature maintaining their composition and biological activity (Anitua et al. 2013b).

Another exciting property of PRGF technology is its versatility. In fact, it is not only an autologous eye drop but also a biocompatible and biodegradable fibrin membrane (Anitua et al. 2007). The fibrin scaffold and membrane can be used in ophthalmology as an autologous sealant or biomaterial to regenerate deep wounds in ocular surface (Anitua et al. 2012).

The promising *in vitro* results obtained with PRGF eye drops have stimulated its use in several animal models of ocular surface diseases. PRGF stimulates ocular surface wound healing, reducing corneal haze formation in mice subjected to PRK surgery. PRGF reduces myofibroblast-transformed cells in the corneal stroma (Anitua et al. 2013a). Tanidir et al. (2010) found similar results in corneal re-epithelization in rabbits. In the same way, successful results were found in a rabbit model of corneal ulcers, showing an acceleration in corneal regeneration, a reduction in ocular inflammation and an improvement in collagen fibre arrangement in the corneal stroma compared to the control group (Khaksar et al. 2013). Last but not least, the fibrin membrane has also been used as a bio-adhesive in preclinical studies, showing successful results in attaching the corneal flap in the lamellar keratoplasty (Luengo Gimeno et al. 2006, 2010).

Clinical application of PRGF eye drops

The positive results obtained with PRGF *in vitro* stimulated the evaluation of the approach in different ocular surface injuries, including dry eye, GVHD and corneal ulcers, among others.

Dry eye

Although dry eye is seen in young adults, its prevalence increases with age. About 15% of adults over the age of 40 have dry eye, and the prevalence of dry eye increases above 19% in people over 80 years old. Women are affected about 1.5 times more frequently than men. Dry eye is a disorder of the tear film caused by an alteration in the amount of tears and/or the composition of it. Artificial tears are the first standard treatment for this disease, but often it is not plenty for the proper management of this pathology. Ideal artificial tears should emulate the physicochemical properties of natural tears, in addition to having the highest possible retention capacity. They should also allow the addition of biological agents, which may be necessary for the metabolism of the cells of the ocular surface.

Over recent years, the biological outcomes of PRGF eye drops on dry eye syndrome treatment have been evaluated in two prospective observational studies (Alio et al. 2007b; Lopez-Plandolit et al. 2011). A total of 34 patients who presented a moderate or severe dry eye syndrome were included in these studies. PRGF technology was applied topically 4–6 times a day per eye for 1–3 months. After PRGF treatment, a total of 82% of the patients showed relevant improvement or full disappearance of symptoms. No cases of poor tolerance or undesirable effects that could be attributed to the PRGF use were observed. On the contrary, it was observed that PRGF eye drops reduced significantly the inflammation in 89% of cases, and fluorescein staining improved significantly in 72% of the cases, indicating an improvement

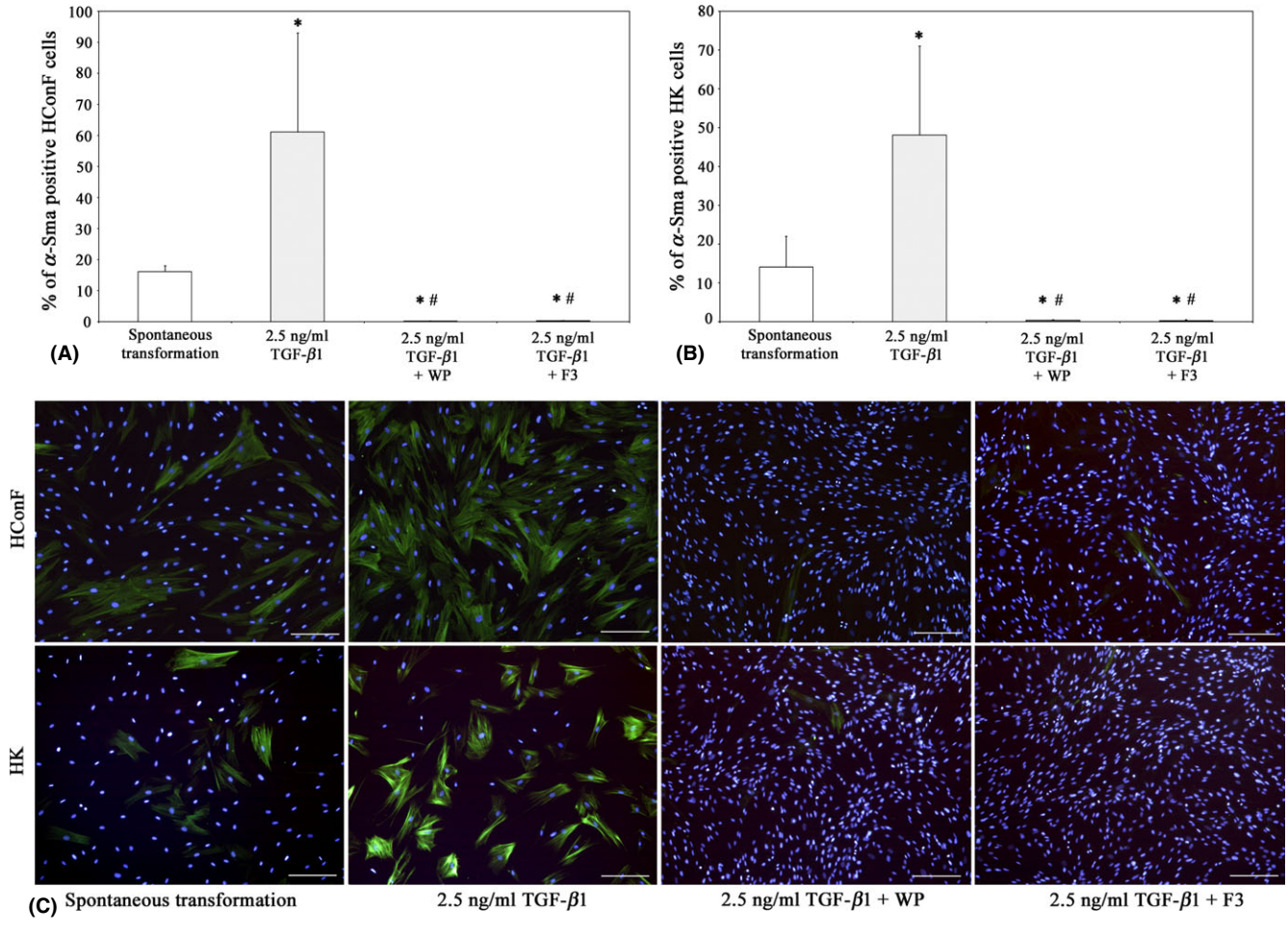

Fig. 3. Protective effect of plasma rich in growth factors (PRGF) against myofibroblast transformation of (A) conjunctival fibroblasts (HConF) and (B) keratocytes (HK). The number of α-SMA-positive cells was significantly lower compared with the TGF-β1 treatment group. (C) Immunofluorescence for the detection of α-SMA protein in HConF- and HK-cultured cells, showing a significant reduction of positive staining in cells treated with PRGF compared to TGF-β1. WP: the whole column of PRGF, which is the final dosage used to prepare the eye drops; F3: the lowest millilitre of PRGF over the buffy coat, which in general contains more platelets. No differences were observed between both PRGF formulations (Anitua et al. 2011). *Statistically significances with regard to spontaneous transformation ($p < 0.05$). # Statistically significances with respect to 2.5 ng/ml TGF-β1 ($p < 0.05$).

in punctate keratitis (Alio et al. 2007b).

Graft-versus-host disease

Ocular GVHD affects approximately 60–80% of patients with cGVHD (Franklin et al. 1983; Arocker-Mettinger et al. 1991). Keratoconjunctivitis sicca (KCS), or dry eye associated with cGVHD, is one of the main complications after allo stem cell transplantation. KCS has a significant impact on patient's life quality and can lead to blindness. Although several therapies including artificial tears, therapeutic contact lenses, punctual plugs, topical or systemic corticosteroids, and other immunosuppressive drugs have been used to minimize the symptoms of dry eyes associated with cGVHD, an effective treatment has not been established (Rocha et al. 2000; Ogawa et al. 2001).

Previous results have shown that PRGF can be applied to the treatment of severe dry eye in patients from different aetiopathologies like Sjögren's syndrome (Lopez-Plandolit et al. 2011). To test its effects in ocular disorders from GVHD patients, Pezzotta et al. (2012) treated a total of 23 patients with refractory GVHD (grade II–IV) unresponsive to conventional treatments. The results showed that 74% of patients (17 of 23) were classified as responders, showing an improvement in the dry eye symptoms. Photophobia was the best symptom improvement (82.6% of patients). Clinical manifestations also improved significantly, showing an improvement in the tear break-up time and fluorescein corneal staining of 86.9% and 69.6%, respectively. These results suggest that this autologous approach may be considered an alternative to the treatment of ocular surface disorders in GVHD patients.

Persistent epithelial defects

Persistent epithelial defects are defined as lesions that measure more than 2 mm in diameter and persist for more than 2 weeks, and are resistant to conventional treatments (Tsubota et al. 1999b). Tear surface and neurogenic dysfunctions are the two main causes of PED, although its etiopathology is very variable (Lopez-Garcia et al. 2007). Other situations that may provoke a PED include burns, immunological factors, infections, dystrophies of the epithelium, metabolic alterations and trauma (Tsubota et al. 1999b; Vajpayee et al. 2003; Lopez-Garcia et al. 2007). Unfortunately, conventional treatments including

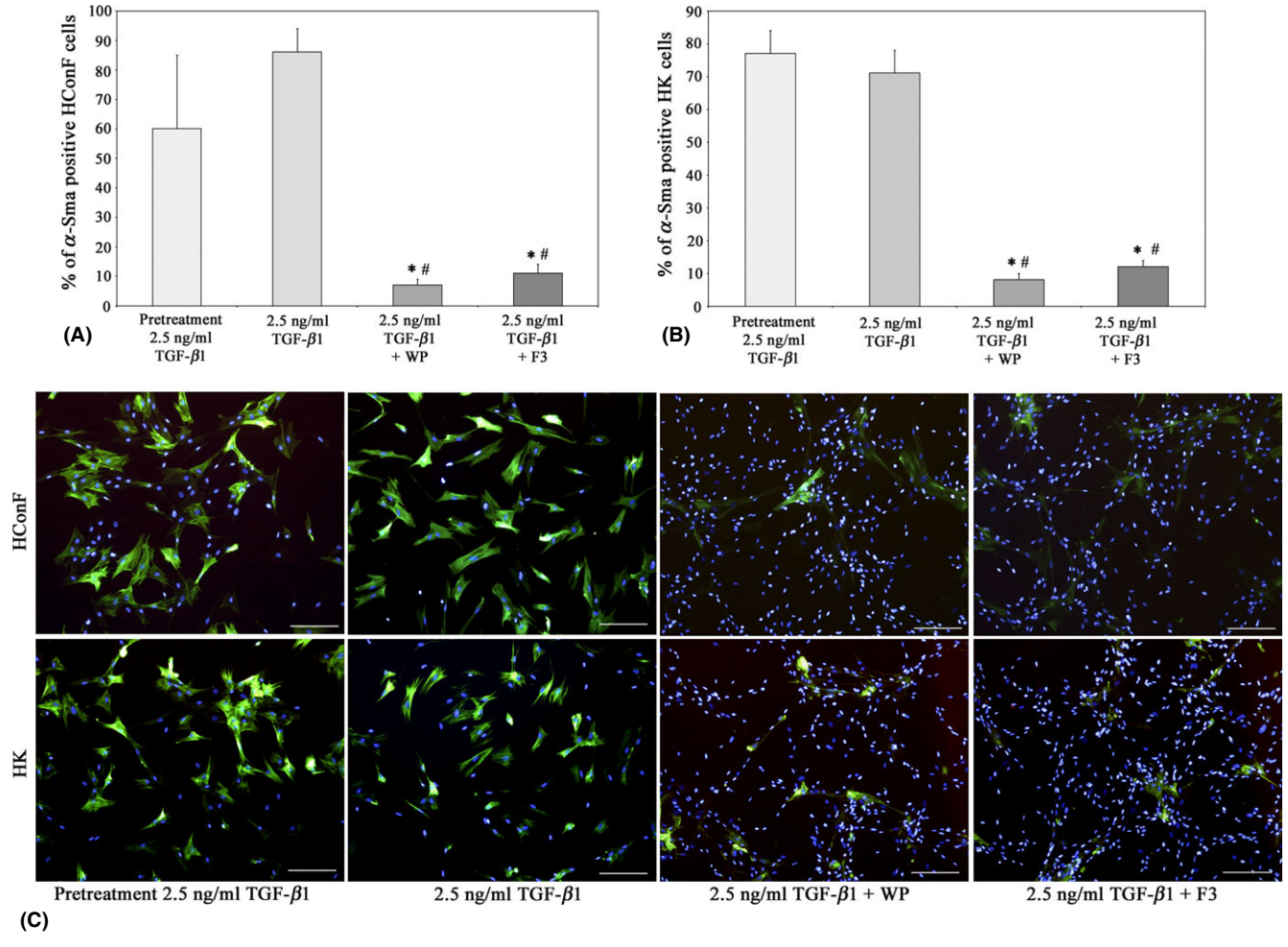

Fig. 4. Plasma rich in growth factors (PRGF) reverts the myofibroblastic phenotype. HConF (A) and HK (B) cells were treated with 2.5 ng/ml TGF-β1 for 3 days to promote differentiation to myofibroblasts. Significant differences were found between the responses induced by PRGF treatment with respect to starting point (2.5 ng/ml). (C) Representative images of α-SMA immunofluorescence showing positive cells before and after treatment with PRGF. No differences were observed between WP and F3 PRGF formulations (Anitua et al. 2011). *Statistically significances with regard to pretreatment with 2.5 ng/ml TGF-β1 ($p < 0.05$). # Statistically significances with respect to 2.5 ng/ml TGF-β1 ($p < 0.05$).

artificial tears, therapeutic contact lenses, tarsorrhaphy, anti-inflammatory agents and oral antibiotics do not improve PED symptomatology and resistant PEDs could degenerate, leading to progressive stromal lysis and subsequent perforation.

The effect of PRGF on PEDs was evaluated by means of a prospective study in 18 eyes (Lopez-Plandolit et al. 2010). Results showed full recovery of the epithelial defect in 85% of cases (17 of 20 eyes). The tolerance to PRGF eye drops treatment was good in 95% of cases (19 of 20). Only one case showed discomfort to PRGF treatment showing redness and itching. No other complications associated with its use were detected. In another retrospective and non-randomized comparative study in patients with PED after infectious keratitis, Kim et al. (2012) reported similar successful results after using PRGF eye drops. In fact, all patients treated with PRGF achieved complete re-epithelialization, while only 77% of patients completed the corneal re-epithelialization after treatment with AS.

Corneal ulcers

A corneal ulcer can be defined as an erosion of the outer layer of the ocular surface, which is often caused by infection, but also by foreign bodies, abrasions, severe dryness and allergic or inflammatory eye conditions. Depending on its aetiology, initial treatment of ocular ulcers relies on accelerating tissue regeneration and reducing the risk of infection and the formation of scar tissue that impairs vision.

The most severe eye ulcers are usually treated with amniotic membranes, which give support to those cells that will colonize the ulcer, and are also treated with growth factors that will promote corneal regeneration. The main disadvantage of the amniotic membrane is that it has heterologous origin and therefore may present biosafety risks. The use of an autologous tissue would be highly desirable for the treatment of corneal ulcers, mainly on corneal perforations. PRGF provides also a fibrin scaffold that can be use as membrane in ocular ulcers (Marquez De Aracena Del Cid & Montero De Espinosa Escoriaza 2009; Geremicca et al. 2010; Panda et al. 2012). In general, the use of PRGF is associated with a reduced healing and epithelization time of the cornea and conjunctiva, with better corneal clarity and best-corrected visual acuity (Marquez De Aracena Del Cid & Montero De Espinosa Escoriaza 2009). A recent study ($n = 38$) showed that 92% of patients with dormant corneal ulcers

improved significantly, reduced inflammation and decreased ocular pain after PRGF treatment (Alio et al. 2007a).

Several studies have evaluated the potential benefits of PRGF-derived fibrin membrane alone (Alio et al. 2013b) or in combination with other membranes like amniotic membrane (Alio et al. 2007a) or Tutopach (Alio et al. 2013a). In all studies, a stable closure of corneal perforation was observed in all patients treated with PRGF fibrin. Furthermore, no evidence of infection, inflammation or pain was observed in any patients treated with PRGF membranes.

Other applications

The use of PRGF in the ophthalmology field has been successfully extended to other ocular surface disorders including the treatment of ocular surface syndrome (Alio et al. 2007c) and flap necrosis (Rocha et al. 2007) after LASIK surgery. A recent study observed that administration of plasma- and platelet-derived proteins adjacent to the lacrimal gland restored the lacrimal function in all patients (Avila 2014). In addition, significant improvement in lacrimal volume, an increase in tear break-up time and a decrease in ocular staining after PRGF treatment were observed.

Future trends and perspectives

Initial studies evaluating blood-based derivatives show efficacy and safety in the treatment of many different ocular surface disorders. However, it is necessary to carry out randomized clinical trials to evaluate and compare properly the potential of these technologies. To succeed, it is mandatory to provide standardized technologies that enable us to fabricate the same type of blood formulations. The development and optimization of PRGF could provide some light on this topic. Furthermore, a more intense basic research focused on the mechanisms of actions of these blood therapies may also help us to better understand how they work and how we can optimize their therapeutic use.

References

Aifa A, Gueudry J, Portmann A, Delcampe A & Muraine M (2012): Topical treatment with a new matrix therapy agent (RGTA) for the treatment of corneal neurotrophic ulcers. Invest Ophthalmol Vis Sci **53**: 8181–8185.

Alio JL, Abad M, Artola A, Rodriguez-Prats JL, Pastor S & Ruiz-Colecha J (2007a): Use of autologous platelet-rich plasma in the treatment of dormant corneal ulcers. Ophthalmology **14**: 1286–1293 e1281.

Alio JL, Colecha JR, Pastor S, Rodriguez A & Artola A (2007b): Symptomatic dry eye treatment with autologous platelet-rich plasma. Ophthalmic Res **39**: 124–129.

Alio JL, Pastor S, Ruiz-Colecha J, Rodriguez A & Artola A (2007c): Treatment of ocular surface syndrome after LASIK with autologous platelet-rich plasma. J Refract Surg **23**: 617–619.

Alio JL, Rodriguez AE & Martinez LM (2013a): Bovine pericardium membrane (tutopatch) combined with solid platelet-rich plasma for the management of perforated corneal ulcers. Cornea **32**: 619–624.

Alio JL, Rodriguez AE, Martinez LM & Rio AL (2013b): Autologous fibrin membrane combined with solid platelet-rich plasma in the management of perforated corneal ulcers: a pilot study. JAMA Ophthalmol **131**: 745–751.

Andresen JL, Ledet T & Ehlers N (1997): Keratocyte migration and peptide growth factors: the effect of PDGF, bFGF, EGF, IGF-I, aFGF and TGF-beta on human keratocyte migration in a collagen gel. Curr Eye Res **16**: 605–613.

Anitua E (1999): Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants **14**: 529–535.

Anitua E, Andia I, Ardanza B, Nurden P & Nurden AT (2004): Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost **91**: 4–15.

Anitua E, Sanchez M, Orive G & Andia I (2007): The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials **28**: 4551–4560.

Anitua E, Aguirre JJ, Algorta J, Ayerdi E, Cabezas AI, Orive G & Andia I (2008): Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. J Biomed Mater Res B Appl Biomater **84**: 415–421.

Anitua E, Sanchez M & Orive G (2010): Potential of endogenous regenerative technology for in situ regenerative medicine. Adv Drug Deliv Rev **62**: 741–752.

Anitua E, Sanchez M, Merayo-Lloves J, De la Fuente M, Muruzabal F & Orive G (2011): Plasma rich in growth factors (PRGF-Endoret) stimulates proliferation and migration of primary keratocytes and conjunctival fibroblasts and inhibits and reverts TGF-beta1-Induced myodifferentiation. Invest Ophthalmol Vis Sci **52**: 6066–6073.

Anitua E, Alkhraisat MH & Orive G (2012): Perspectives and challenges in regenerative medicine using plasma rich in growth factors. J Control Release **157**: 29–38.

Anitua E, Muruzabal F, Alcalde I, Merayo-Lloves J & Orive G (2013a): Plasma rich in growth factors (PRGF-Endoret) stimulates corneal wound healing and reduces haze formation after PRK surgery. Exp Eye Res **115**: 153–161.

Anitua E, Muruzabal F, Pino A, Merayo-Lloves J & Orive G (2013b): Biological stability of plasma rich in growth factors eye drops after storage of 3 months. Cornea **32**: 1380–1386.

Aragona P, Rania L, Roszkowska AM, Spinella R, Postorino E, Puzzolo D & Micali A (2013): Effects of amino acids enriched tears substitutes on the cornea of patients with dysfunctional tear syndrome. Acta Ophthalmol **91**: e437–e444.

Arocker-Mettinger E, Skorpik F, Grabner G, Hinterberger W & Gadner H (1991): Manifestations of graft-versus-host disease following allogenic bone marrow transplantation. Eur J Ophthalmol **1**: 28–32.

Avila MY (2014): Restoration of human lacrimal function following platelet-rich plasma injection. Cornea **33**: 18–21.

Blair P & Flaumenhaft R (2009): Platelet alpha-granules: basic biology and clinical correlates. Blood Rev **23**: 177–189.

Chikama T, Fukuda K, Morishige N & Nishida T (1998): Treatment of neurotrophic keratopathy with substance-P-derived peptide (FGLM) and insulin-like growth factor I. Lancet **351**: 1783–1784.

Dinning WJ (1976): Steroids and the eye–indications and complications. Postgrad Med J **52**: 634–638.

Dogru M & Tsubota K (2011): Pharmacotherapy of dry eye. Expert Opin Pharmacother **12**: 325–334.

Fox RI, Chan R, Michelson JB, Belmont JB & Michelson PE (1984a): Beneficial effect of artificial tears made with autologous serum in patients with keratoconjunctivitis sicca. Arthritis Rheum **27**: 459–461.

Fox RI, Howell FV, Bone RC & Michelson P (1984b): Primary Sjogren syndrome: clinical and immunopathologic features. Semin Arthritis Rheum **14**: 77–105.

Franklin RM, Kenyon KR, Tutschka PJ, Saral R, Green WR & Santos GW (1983): Ocular manifestations of graft-vs-host disease. Ophthalmology **90**: 4–13.

Freire V, Andollo N, Etxebarria J, Duran JA & Morales MC (2012): In vitro effects of three blood derivatives on human corneal epithelial cells. Invest Ophthalmol Vis Sci **53**: 5571–5578.

Geerling G, Daniels JT, Dart JK, Cree IA & Khaw PT (2001): Toxicity of natural tear substitutes in a fully defined culture model of human corneal epithelial cells. Invest Ophthalmol Vis Sci **42**: 948–956.

Geerling G, Maclennan S & Hartwig D (2004): Autologous serum eye drops for ocular surface disorders. Br J Ophthalmol **88**: 1467–1474.

Geremicca W, Fonte C & Vecchio S (2010): Blood components for topical use in tissue regeneration: evaluation of corneal lesions treated with platelet lysate and considerations on repair mechanisms. Blood Transfus **8**: 107–112.

Gibble JW & Ness PM (1990): Fibrin glue: the perfect operative sealant? Transfusion **30**: 741–747.

Gordon JF, Johnson P & Musch DC (1995): Topical fibronectin ophthalmic solution in the treatment of persistent defects of the corneal epithelium. Chiron Vision Fibronectin Study Group. Am J Ophthalmol **119**: 281–287.

Harritshoj LH, Nielsen C, Ullum H, Hansen MB & Julian HO (2014): Ready-made allogeneic ABO-specific serum eye drops: production from regular male blood donors, clinical routine, safety and efficacy. Acta Ophthalmol **92**: 783–786.

Khaksar E, Aldavood SJ, Abedi GR, Sedaghat R, Nekoui O & Zamani-ahmadmahmudi M (2013): The effect of sub-conjunctival platelet-rich plasma in combination with topical acetylcysteine on corneal alkali burn ulcer in rabbits. Comp Clin Pathol **22**: 107–112.

Kim KM, Shin YT & Kim HK (2012): Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis. Jpn J Ophthalmol **56**: 544–550.

Klenkler B, Sheardown H & Jones L (2007): Growth factors in the tear film: role in tissue maintenance,

wound healing, and ocular pathology. Ocul Surf **5**: 228–239.

Lemp MA (2008): Advances in understanding and managing dry eye disease. Am J Ophthalmol **146**: 350–356.

Liu L, Hartwig D, Harloff S, Herminghaus P, Wedel T, Kasper K & Geerling G (2006): Corneal epitheliotrophic capacity of three different blood-derived preparations. Invest Ophthalmol Vis Sci **47**: 2438–2444.

López-García JS, Murube del Castillo J, García-Lozano I & Rivas-Jara L (2011): 'Suero autólogo y derivados hemáticos en oftalmología'. Comunicación solicitada 87 Congreso de la Sociedad Española de Oftalmología. Sociedad Española de Oftalmología. Arch Soc Esp Oftalmol 87.

Lopez-Garcia JS, Garcia-Lozano I, Rivas L & Martinez-Garchitorena J (2007): [Use of autologous serum in ophthalmic practice]. Arch Soc Esp Oftalmol **82**: 9–20.

Lopez-Garcia JS, Garcia-Lozano I, Rivas L, Ramirez N, Raposo R & Mendez MT (2014): Autologous serum eye drops diluted with sodium hyaluronate: clinical and experimental comparative study. Acta Ophthalmol **92**: e22–e29.

Lopez-Plandolit S, Morales MC, Freire V, Etxebarria J & Duran JA (2010): Plasma rich in growth factors as a therapeutic agent for persistent corneal epithelial defects. Cornea **29**: 843–848.

Lopez-Plandolit S, Morales MC, Freire V, Grau AE & Duran JA (2011): Efficacy of plasma rich in growth factors for the treatment of dry eye. Cornea **30**: 1312–1317.

Luengo Gimeno F, Gatto S, Ferro J, Croxatto JO & Gallo JE (2006): Preparation of platelet-rich plasma as a tissue adhesive for experimental transplantation in rabbits. Thromb J **4**: 18.

Luengo Gimeno F, Gatto SC, Croxatto JO, Ferro JI & Gallo JE (2010): In vivo lamellar keratoplasty using platelet-rich plasma as a bioadhesive. Eye (Lond) **24**: 368–375.

Marquez De Aracena Del Cid R & Montero De Espinosa Escoriaza I (2009): Subconjunctival application of regenerative factor-rich plasma for the treatment of ocular alkali burns. Eur J Ophthalmol **19**: 909–915.

Matsumoto Y, Dogru M, Goto E, Ohashi Y, Kojima T, Ishida R & Tsubota K (2004): Autologous serum application in the treatment of neurotrophic keratopathy. Ophthalmology **111**: 1115–1120.

Noecker R (2001): Effects of common ophthalmic preservatives on ocular health. Adv Ther **18**: 205–215.

Nurden AT, Nurden P, Sanchez M, Andia I & Anitua E (2008): Platelets and wound healing. Front Biosci **13**: 3532–3548.

Ogawa Y, Okamoto S, Kuwana M et al. (2001): Successful treatment of dry eye in two patients with chronic graft-versus-host disease with systemic administration of FK506 and corticosteroids. Cornea **20**: 430–434.

Ogino Y, Ayukawa Y, Tsukiyama Y & Koyano K (2005): The effect of platelet-rich plasma on the cellular response of rat bone marrow cells in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod **100**: 302–307.

Orcajo B, Muruzabal F, Isasmendi MC, Gutierrez N, Sanchez M, Orive G & Anitua E (2011): The use of plasma rich in growth factors (PRGF-Endoret) in the treatment of a severe mal perforant ulcer in the foot of a person with diabetes. Diabetes Res Clin Pract **93**: e65–e67.

Pan Q, Angelina A, Zambrano A, Marrone M, Stark WJ, Heflin T, Tang L & Akpek EK (2013): Autologous serum eye drops for dry eye. Cochrane Database Syst Rev **8**: Cd009327.

Panda A, Jain M, Vanathi M, Velpandian T, Khokhar S & Dada T (2012): Topical autologous platelet-rich plasma eyedrops for acute corneal chemical injury. Cornea **31**: 989–993.

Pastor JC & Calonge M (1992): Epidermal growth factor and corneal wound healing. A multicenter study. Cornea **11**: 311–314.

Pezzotta S, Del Fante C, Scudeller L, Cervio M, Antoniazzi ER & Perotti C (2012): Autologous platelet lysate for treatment of refractory ocular GVHD. Bone Marrow Transplant **47**: 1558–1563.

Pflugfelder SC, Jones D, Ji Z, Afonso A & Monroy D (1999): Altered cytokine balance in the tear fluid and conjunctiva of patients with Sjogren's syndrome keratoconjunctivitis sicca. Curr Eye Res **19**: 201–211.

Phan TM, Foster CS, Boruchoff SA, Zagachin LM & Colvin RB (1987): Topical fibronectin in the treatment of persistent corneal epithelial defects and trophic ulcers. Am J Ophthalmol **104**: 494–501.

Poon AC, Geerling G, Dart JK, Fraenkel GE & Daniels JT (2001): Autologous serum eyedrops for dry eyes and epithelial defects: clinical and in vitro toxicity studies. Br J Ophthalmol **85**: 1188–1197.

Quinto GG, Campos M & Behrens A (2008): Autologous serum for ocular surface diseases. Arq Bras Oftalmol **71**: 47–54.

Ralph RA, Doane MG & Dohlman CH (1975): Clinical experience with a mobile ocular perfusion pump. Arch Ophthalmol **93**: 1039–1043.

Reed GL, Fitzgerald ML & Polgar J (2000): Molecular mechanisms of platelet exocytosis: insights into the 'secrete' life of thrombocytes. Blood **96**: 3334–3342.

Renfro L & Snow JS (1992): Ocular effects of topical and systemic steroids. Dermatol Clin **10**: 505–512.

Rocha EM, Pelegrino FS, de Paiva CS, Vigorito AC & de Souza CA (2000): GVHD dry eyes treated with autologous serum tears. Bone Marrow Transplant **25**: 1101–1103.

Rocha GA, Acera A & Duran JA (2007): Laser in situ keratomileusis flap necrosis after trigeminal nerve palsy. Arch Ophthalmol **125**: 1423–1425.

Sanchez M, Anitua E, Azofra J, Andia I, Padilla S & Mujika I (2007): Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. Am J Sports Med **35**: 245–251.

van Setten GB, Tervo T, Tervo K & Tarkkanen A (1992): Epidermal growth factor (EGF) in ocular fluids: presence, origin and therapeutical considerations. Acta Ophthalmol Suppl **202**: 54–59.

Sharma N, Goel M, Velpandian T, Titiyal JS, Tandon R & Vajpayee RB (2011): Evaluation of umbilical cord serum therapy in acute ocular chemical burns. Invest Ophthalmol Vis Sci **52**: 1087–1092.

Shimmura S, Ueno R, Matsumoto Y, Goto E, Higuchi A, Shimazaki J & Tsubota K (2003): Albumin as a tear supplement in the treatment of severe dry eye. Br J Ophthalmol **87**: 1279–1283.

Tanidir ST, Yuksel N, Altintas O, Yildiz DK, Sener E & Caglar Y (2010): The effect of subconjunctival platelet-rich plasma on corneal epithelial wound healing. Cornea **29**: 664–669.

Toker E & Asfuroglu E (2010): Corneal and conjunctival sensitivity in patients with dry eye: the effect of topical cyclosporine therapy. Cornea **29**: 133–140.

Torres J, Tamimi F, Martinez PP, Alkhraisat MH, Linares R, Hernandez G, Torres-Macho J & Lopez-Cabarcos E (2009): Effect of platelet-rich plasma on sinus lifting: a randomized-controlled clinical trial. J Clin Periodontol **36**: 677–687.

Tsubota K, Goto E, Fujita H, Ono M, Inoue H, Saito I & Shimmura S (1999a): Treatment of dry eye by autologous serum application in Sjogren's syndrome. Br J Ophthalmol **83**: 390–395.

Tsubota K, Goto E, Shimmura S & Shimazaki J (1999b): Treatment of persistent corneal epithelial defect by autologous serum application. Ophthalmology **106**: 1984–1989.

Unterlauft JD, Kohlhaas M, Hofbauer I, Kasper K & Geerling G (2009): [Albumin eye drops for treatment of ocular surface diseases]. Ophthalmologe **106**: 932–937.

Vajpayee RB, Mukerji N, Tandon R, Sharma N, Pandey RM, Biswas NR, Malhotra N & Melki SA (2003): Evaluation of umbilical cord serum therapy for persistent corneal epithelial defects. Br J Ophthalmol **87**: 1312–1316.

Wang Y, Ogawa Y, Dogru M et al. (2008): Ocular surface and tear functions after topical cyclosporine treatment in dry eye patients with chronic graft-versus-host disease. Bone Marrow Transplant **41**: 293–302.

Werner S & Grose R (2003): Regulation of wound healing by growth factors and cytokines. Physiol Rev **83**: 835–870.

Yamada N, Yanai R, Nakamura M, Inui M & Nishida T (2004): Role of the C domain of IGFs in synergistic promotion, with a substance P-derived peptide, of rabbit corneal epithelial wound healing. Invest Ophthalmol Vis Sci **45**: 1125–1131.

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

Yoon KC, Jeong IY, Park YG & Yang SY (2007b): Interleukin-6 and tumor necrosis factor-alpha levels in tears of patients with dry eye syndrome. Cornea **26**: 431–437.

Yoon KC, Choi W, You IC & Choi J (2011): Application of umbilical cord serum eyedrops for recurrent corneal erosions. Cornea **30**: 744–748.

Yoon KC, Oh HJ, Park JW & Choi J (2013): Application of umbilical cord serum eyedrops after laser epithelial keratomileusis. Acta Ophthalmol **91**: e22–e28.

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