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Juliana Lujan Brunetto[1], Aldiéris Alves Pesqueira[1], Daniela Micheline dos Santos[1, 2], Marcelo Coelho Goiato[1, 2] ✉

[1] São Paulo State University (School of Dentistry, Department of Dental Materials and Prosthodontics), Araçatuba, São Paulo, Brazil

[2] São Paulo State University (School of Dentistry, Oral Oncology Center), Araçatuba, São Paulo, Brazil

The Optimization of Platelet Aggregates in Orbital Disorders. An Integrative Review

Conflict of interest: nothing to declare.

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Contacts: m.goiato@unesp.br

Abstract

Due to physical, chemical, and/or pathogenic trauma, the loss of vision, and sometimes of the eyeball, causes to these patients the need of ocular prostheses. For its regeneration and decontamination different approaches have been investigated, but many have a long-term side effect. An interesting alternative is the adhibition of platelet aggregates, obtained from the donor patient's own blood.

Purpose. To compare studies and unite the best treatment techniques that use blood-derived concentrates in the ophthalmic cavity.

Methods. After analyzing the eligibility criteria, of 1.495 initial articles, 23 studies were selected for the integrative review, published in English and between 2011 and 2021. Although the use in eye drops is the most used, the applications of this content have been successfully extended to other necrosis disorders, after LASIK surgery, after ocular burns and even in patients with severe tear dysfunction secondary to Sjogren's Syndrome, Stevens-Johnson Syndrome and pemphigoid. Despite the apparent advantages of re-epithelialization and reduction of inflammation, most techniques are often inadequately regrouped since their inception. Due to the large number of techniques, classifying, comparing and interpreting the preparation and terminology of blood products, has highlighted the difficulty of its systematization. Thus, it is concluded that platelet aggregates present promising results in patients with ophthalmic disorders. Further clinical studies are recommended for a standardization of the technique and investigation in anophthalmic patients.

Keywords: platelet rich plasma, ocular, blood products, platelet concentratesm, platelet aggregates

Брунетто Ж.Л.[1], Пескейра А.А.[1], душ Сантуш Д.М.[1, 2], Гойату М.С.[1, 2] ✉
[1] Государственный университет Сан-Паулу (Стоматологический факультет, кафедра стоматологических материалов и ортопедической стоматологии), Арасатуба, Сан-Паулу, Бразилия
[2] Государственный университет Сан-Паулу (Стоматологический факультет, центр онкологии полости рта), Арасатуба, Сан-Паулу, Бразилия

Оптимизация агрегатов тромбоцитов при поражениях глазницы. Интегрированный обзор

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Контакты: m.goiato@unesp.br

Резюме

Вследствие физических, химических и/или патогенных травм потеря зрения, а иногда и глазного яблока, приводит к необходимости использования глазных протезов. Для их восстановления и обеззараживания разработаны различные подходы, но многие из них имеют долгосрочные побочные эффекты. Интересной альтернативой является применение агрегатов тромбоцитов, полученных из собственной донорской крови пациента.

Цель. Сравнение имеющихся исследований и обобщение наиболее эффективных методик лечения с использованием концентратов, полученных из крови, применяемых в области глазницы.

Методы. После анализа критериев включения из 1495 исходных статей для интегрированного обзора были отобраны 23 исследования, опубликованные на английском языке в период с 2011 по 2021 г. Несмотря на то, что наиболее распространенным является применение вышеописанных концентратов в составе глазных капель, они с успехом используются при других некротических поражениях, после операций по лазерной коррекции зрения, после ожогов глазницы и даже у пациентов с тяжелыми нарушениями слезоотделения вследствие синдрома Сьогрена, синдрома Стивенса – Джонсона и пемфигоида. Несмотря на очевидные преимущества, заключающиеся в реэпителизации и уменьшении воспаления, большинство методик с момента их появления часто в недостаточной степени сгруппированы. В связи с большим количеством методик, классифицирующих, сравнивающих и интерпретирующих приготовление и терминологию препаратов крови, стала очевидной сложность их систематизации. Таким образом, можно сделать вывод, что применение агрегатов тромбоцитов показывает многообещающие результаты у пациентов с офтальмологическими заболеваниями. Дальнейшие клинические исследования необходимы для стандартизации методик и проведения исследований у анофтальмических пациентов.

Ключевые слова: обогащенная тромбоцитами плазма, глаз, препараты крови, концентраты тромбоцитов, агрегаты тромбоцитов

■ INTRODUCTION

Corneal ulcers that do not heal, excessive use of steroids, persistent infections, neurotrophic keratopathy, exposure keratopathy, hereditary disorders and chronic conjunctivitis are just some of the injuries that occur due to disorders on the ocular surfaces that involve structures such as the cornea, limbus and conjunctiva, which protect ocular integrity [1]. Whether due to physical, chemical, and/or pathogenic trauma, the loss of vision, and sometimes of the eyeball, makes these patients need ocular prostheses [2]. Ocular prostheses reintegrate the patient into society, protecting the cavity against foreign bodies and preventing the accumulation of secretions in the ophthalmic cavity.

However, they are susceptible to chronic superficial conjunctivitis, constituting an excellent culture medium for bacterial growth [3, 4]. Different approaches aimed at its regeneration and decontamination have been investigated, but many have a long-term side effect. An interesting alternative is that made up of platelet aggregates, obtained from the donor patient's own blood [5]. In addition to being autogenous, its studied benefits include an antimicrobial, anti-inflammatory biophysical effect [6] and, possibly, reduce some undesirable phenomena.

Thus, the objective of this integrative review was to compare studies and unite the best treatment techniques that use blood-derived concentrates in the ophthalmic cavity.

■ MATERIALS AND METHODS

Eligibility Criteria

The inclusion criteria applied in the search strategy for the selection of studies were: 1) in vivo studies; 2) controlled trials; 3) who performed ophthalmic treatments with platelet aggregates; 4) that were published until 11/26/2021.

Excluded articles were: 1) literature reviews or systematic reviews that addressed the topic; 2) duplicate studies; 3) articles focusing on a topic other than the one proposed; 4) presentation of prostheses or association with other treatments; 5) articles that were published after 01/27/2021.

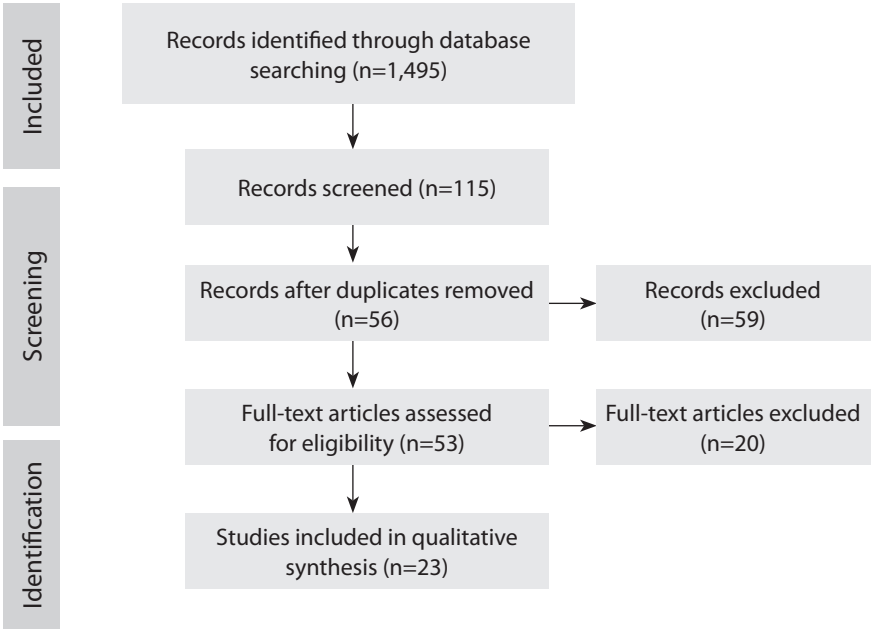
Search strategy

The search and selection were performed by a reviewer (J.L.B.) (Figure) who analyzed the articles. Medline/PubMed, Scopus, VHL, LILACS and Embase databases were used to identify articles related to the topic until 11/27/2021. Keywords were determined by Mesh terms and combined using predetermined Boolean operators: ((("platelet rich plasma" OR "platelet rich fibrin") OR ("prp") OR ("prf")) OR ("leucocyte rich plasma" OR "leucocyte rich platelet" OR "leucocyte rich platelet rich fibrin")) OR ("platelet rich fibrin")) AND ("cornea" OR "ocular" OR "eye").

When it was not possible to collect the article, it was requested via the Bibliographic Commutation Program (COMUT). To complete the search, a manual search of the gray literature was performed in the OpenGrey database.

Data collection and extraction process

The selection of articles was performed by the same reviewer (J.L.B.) with blind and independent analysis, based on eligibility criteria and following the steps: 1) reading the title and abstract; 2) full reading. The data collected from the articles selected by the author (J.L.B.) were: authors, year, type of study, number of patients involved, procedure



Study selection flowchart

performed, adjunct treatments, form of application and tests performed. Afterwards, they were organized using a standardized spreadsheet in the Microsoft Excel® Professional Plus version 2016 (Microsoft Corporation, USA), for later comparison between the studies.

■ RESULTS

Selection of studies

The search and selection of articles are described below. A total of 1,495 studies were initially looked at (PubMed = 636; Scopus = 230; VHL = 2,728; LILACS = 6; Embase = 323; OpenGrey = 110) for manual searching. After analyzing the titles and abstracts by Mendeley®, 115 studies were selected to separate duplicate articles. Soon after, 56 studies were selected for full reading and application of eligibility criteria. Of these, 03 articles were excluded because they were not performed in humans. Thus, twenty-three studies were included for the qualitative analysis of the integrative review.

Design of studies, materials and tests performed

The characteristics of the included studies are summarized in the paragraph Data collection and extraction process above**.** In the present qualitative analysis of the data (Table), twenty-three articles were included, all were published in English and between the years 2011 and 2021, even without restriction of language and initial date. Among these, several analyzes were performed, such as exfoliative cytology [7], enzyme-linked immunosorbent assay (ELISA) [8], fluorescence, scanning electron microscopy, cell culture and in vitro degradation [9–15] and clinical analysis [16–21].

Quantitative and qualitative data

Author (Year)	Type of study	Number of patients involved	Middle Ages	Cause/ pathology	Procedure performed	Adjunct treatments	Application	Tests	Conclusion
García-Conca (2019)	Double-blind randomized prospective comparison	83	64 years	Dry eye due to hyposecretion	44 patients with PRP and 39 patients with sodium hyaluronate (80 women and 3 men)	–	Eye drops every 3 hours, a total of 30 days	Cytology	Treatment with PRP significantly induced a positive effect on symptomatology, especially in moderate to severe cases
Rodriguez (2020)	Quantify and compare the concentration of different preparations and preservations	6	32,5 years		1) fresh; 2) fresh and centrifuged at 5800g; 3) frozen at –20 °C for 3 months; and 4) frozen at –20 °C for 3 months and centrifuged at 5800 g	–	Laboratorial	Enzyme–linked immunosorbent assay (ELISA)	Freezing E–PRP for 3 months at –20°C increased the concentration of important proteins, such as PDGF–BB and EGF, and maintained the levels of others. The subsequent centrifugation decreased GFR–β1 values
Anitua (2021)	Comparative and quantitative clinical study of different preparations	3	Do not quote	Autoimmune or inflammatory ophthalmic diseases	Collection in tube with 3.8% sodium citrate for 1st centrifugation, 2nd in endoret. This was divided into membrane and liquid activated with calcium chloride, incubated at 37 or 56 °C for 30 min	–	Laboratorial		Heat inactivation of PRGF membranes reducing IgE activity The morphological and physical characteristics of the immunosafe mPRGF were also preserved after heat inactivation

Table continuation

Anitua (2021) 2	Comparative laboratory study	3	Do not quote	Autoimmune or inflammatory ophthalmic diseases	Collection in tube with 3.8% sodium citrate for 1st centrifugation, 2nd in endoret. This was divided into membrane and calcium chloride-activated liquid, incubated at 37 °C for 1h and then at 4 °C for 3 months.	–	Laboratorial	Cell proliferation and migration	Lyophilized growth factor–rich plasma eye drops retain their biological structure even without preservatives for at least 3 months at 4°C
Alio (2017)	Prospective investigative study	80	Adults	Treatment of Post-LASIK Chronic Ocular Surface Syndrome	Collect 10 ml of blood in tubes with sodium citrate to prepare PRP eye drops.	Laser femto-second	6x a day for 6 weeks	Cell count	Significant advantages are the ease preparation, absence of preservatives, its autologous origin, safety, and minimal or no intolerance, without Side effects
Lee (2016)	Prospective investigative study	20 (31 men and 16 women)	44,5 years on average	Traumatic corneal erosions	10 ml of blood was collected in tubes with 1.4 ml of citrate dextrose solution. Afterwards, it was diluted in 20% saline solution	–	Eye drops		In all patients there was recovery of the epithelial defect within the first 5 days
Ronci (2015)		10 (5 men and 5 women)	53,7 years on average	Traumatic corneal erosions	Collected 16 ml of blood, divided into two 8ml RegenKit tubes containing sodium citrate	–	3x per day	Clinical analysis and cell count	High platelet counts do not appear to be necessary to treat corneal injuries
Sanchez-Avila (2020)		20	54,1 years on average	Severe pathologies that have not responded to previous treatments	9 ml of blood was collected in tubes with 3.8% sodium citrate. After the first centrifugation, it was placed in the endoret	–	6 weeks	Symptomatic clinical analysis	Platelet–rich plasma eye drops should help maintain hydration and prevent complications

Table continuation

Avila (2018)	Comparative in vivo clinical study	30		Dry eye due to Sjogren's syndrome	10 ml of blood was collected in tubes with 0.5 ml of sodium citrate	–	Injected 1ml of prp at 0, 30, 60 and 90 days into the lacrimal gland	Analysis of tear volume and clinical question-naire	PRP injections are considered safe and more effective than hyaluronic acid for eye lubrication
Sanchez-Avila (2018)	Clinical study with multiple corneal pathological variables	15	27 to 82 years	Various corne-al pathologies	9 ml of blood was collected in tubes with 3.8% sodium citrate. After the first centrifugation, it was placed in the endoret	Surgical technique for suturing the corneal membrane	Eye drops 4x a day for 6 weeks + surgery for membrane placement	Ex vivo culture	Effective and safe as an adjunct treatment in surgeries related to ocular surface disorders, accelerates tissue regeneration by minimizing inflammation and fibrosis
Alio (2017)	Clinical study	80	Do not quote	chronic ocular surface syndrome (OSS)	Collected 10 ml of blood with 1 ml of sodium citrate	LASIK Laser	Eye drops 6x a day for 6 weeks	Fluores-cence and clinical analysis	Well–tolerated, safe and effective treatment for the management of post–LASIK ocular surface syndrome
Sanchez-Avila (2017)	Clinical study	26	Do not quote	Sjogren Syn-drome	Collected 9 ml of blood in tubes with 3.8% sodium citrate. After the first centrifugation it was placed in the endoret	–	Eye drops 4x a day for 6 weeks	Clinical and tear analysis	Signs and symptoms were reduced after treatment
Panda (2012)	Clinical study	20	Do not quote	chemical injury	100 ml of blood was collected from each	–	Eye drops applied 10 times a day	Fluores-cence and clinical analysis	Topical therapy with autologous platelet–rich plasma is safe and effective, and promotes rapid re–epithelialization of the surface and can be administered along with standard medical therapy

Table continuation

Yang (2021)	Clinical study	108	Do not quote	pterygium	Collect 5 ml of blood in an anticoagulant-free tube	Surgery, membrane suture	–	Clinical analysis of membrane curability	Can be widely used in pterygium management
Anitua (2015) 2	Laboratorial study	6	Do not quote		Do not quote	–	Store at −20°C for up to 6 months	Cell culture	There was no reduction of growth factors in up to 6 months
Freire (2012)	In vitro laboratory test	16	30 to 60 years	–	Collected in tubes with sodium citrate	–	–	Cell culture and pro-liferation, microscopy and RT–PCR	Promotes corneal re–epithelialization
Anitua (2015) 3	Laboratorial	3	Do not quote	Do not quote	9 ml of blood was collected in tubes with 3.8% sodium citrate. After the first centrifugation, it was placed in the endoret	–	Do not quote	Fibroblastic growth and cell prolif-eration	The results obtained here suggest that PRGF protects and reverses the myofibroblast phenotype while promoting cell proliferation and migration
Anitua (2011)	In vitro	1	Do not quote	–	9 ml of blood was collected in tubes with 3.8% sodium citrate. After the first centrifugation, it was placed in the endoret	–		Cell prolif-eration and migration and immu-nofluores-cence	Further studies are needed to determine the exact mechanism
Wu (2014)	Clinical case	3	11 to 66 years		9 ml of blood was collected in tubes with 3.8% sodium citrate. After the first centrifugation, it was placed in the endoret	Surgery for membrane application		cynical as-sessment	Compared with diffuse superficial corneal ulcer, PRP has effect on the deep corneal scar

End of table

Avila (2021)	Clinical study	74	56,8 years average		9 ml of blood was collected and after centrifugation, 10% calcium chloride was added		Eye drops 4x a day for 6 weeks	Intraocular pressure (IOP), visual analogue scale (VAS) and the Ocular Surface Disease Index (OSDI)	could be a therapeutic alternative for this type of corneal disease
Alio (2017)	prospective study	68	–	dry eyes	Collected 10 ml of blood in a tube with 1 ml of sodium citrate	–	Eye drops applied for 6 weeks	Immunofluorescence and cell count	PRP eye drops proved to be a good option
Anitua (2021)	in vitro study	3	–	–	9 ml of blood was collected in tubes with 3.8% sodium citrate. After the first centrifugation, it was placed in the endoret	–	In vitro	Cell count, scanning electron microscopy and fluorescence	Heat inactivation of PRGF membranes (is–mPRGF) dramatically reduces IgE
Anitua (2021) 2	In vitro study	3	–	–	9 ml of blood was collected in tubes with 3.8% sodium citrate. After the first centrifugation, it was placed in the endoret	–	In vitro	Cell culture	PRP maintained at 4°C for up to 3 months has maintained characteristics

■ DISCUSSION

Despite reaching a relevant final total sample, this integrative review has some biases such as the variation of the protocol established by the articles for the analysis of content and purpose. Therefore, it is not possible to carry out a systematic review with comparison in meta-analysis.

Most articles reported the need for a second centrifugation, after blood collection in tubes with sodium citrate. Apparently, a higher concentration of growth factors (PRGF), caused by the use of Endoret, may bring potential benefits to the ocular surface. With these factors, the saline solution obtained facilitates the proliferation, migration and differentiation of epithelial cells. To obtain it, all the authors who used the Endoret (mentioned in the table above) performed a light centrifugation in the first step, collected the platelet-rich plasma (PRP) and centrifuged again, but this time at high speed, to obtain of the PRGF. Despite the benefits presented by being a material with low cost and autologous, there is still a concern about the storage and durability of the product.

Anitua (2021) analyzed the biological content of PRGF (Plasma Rich in Growth Factors) in eye drops in a refrigerated storage at 4ºC and at room temperature, for a period of 3 months. And despite an initial reduction (within 24h) [22] in the amount of growth factors, in the long term, there was no statistically significant difference detected in the pure content or with the addition of 2.5% trehalose, a saccharide used to reduce oxidation of the molecules. Thus, the benefits attributed to tissue cell regeneration caused by the factors EGF, TGF-β1, VEGF and PRGF, presented levels similar to those observed in natural tears [9].

Although the use in eye drops is the most used, the applications of this content have been successfully extended to other disorders flap necrosis, after LASIK surgery, after eye burns and even in patients with severe tear dysfunction, secondary to Sjogren's Syndrome, Stevens-Johnson syndrome and pemphigoid [10]. Under normal circumstances, cellular oxidative stress occurs in a balanced way between levels of antioxidants and natural oxidants. When one of the aforementioned dysfunctions occurs, the retina is the main one affected, since it is the area most susceptible to oxidation.

Anitua (2020) [22] indicates, from his report, that this stress could be neutralized or reduced through the application of PRGF. Since this preserves the activity of mitochondrial cells and their cell viability, emerging as a treatment option for disorders related to oxidative damage.

This same explanation is given by Lee (2016) [23] and Panda (2012) [11] years earlier, but analyzing erosions and ophthalmic chemical injuries. The second Panda [11], despite not having a large sample, proved that topical therapy with PRP is a safe and effective adjuvant for the treatment of chemical lesions of the ocular surface from grade III to grade V, due to its effectiveness.

Other authors, such as Sanchez-Avila (2018) [24], carried out their human clinical study using PRGF membranes immediately after several ophthalmic surgeries, together with topical therapy (applied four times a day for 6 weeks). Although the initial diagnosis was not standardized, it was reported that none of the 15 patients had a side effect and that the processes of postoperative inflammation and fibrosis were reduced.

Whether in the investigation by Yang (2021) [19], which presented the largest sample number (108 patients), or in the investigation by Anitua (2021) [9], one of the smallest samples (3 patients), both presented positive results using PRGF membranes. There

are several ways to prepare the products generated by blood products and each one with its own parameters. Most techniques are often inappropriately regrouped since their inception. Therefore, classifying the technique, comparing and interpreting its preparation and terminology, has highlighted the difficulty of its systematization. And despite the need to maintain the aseptic chain, as well as that already performed in any surgery, blood products are products that do not present a risk of disease transmission, when not transplanted.

It is known that PRF contains a large amount of immune cells, which can inhibit an infection [25], through antimicrobial peptides [26], reducing the growth of S. aureus, E. coli, Pseudomonas aeruginosa and Klebsiella pneumonia [27]. However, knowing the differentiation of the microbiota, this information was not proven in the ophthalmic cavity. Despite the promising results of the studies cited, specific protocols in randomized studies, with follow-up, employing larger and sufficient sample sizes to produce a consistent conclusion.

■ CONCLUSION

Platelet aggregates show promising results in patients with ophthalmic disorders. However, further clinical studies are recommended for a standardization of the technique and investigation in anophthalmic patients.

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