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Use of platelets and human platelet-derived products in advanced therapies

Utilização de plaquetas e de produtos derivados de plaquetas humanas em terapias avançadas

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ABSTRACT

Introduction: Platelets have a central role in the tissue response to injury, in hemostasis and clot formation. They also release rapidly and in orchestrated order bioactive molecules, important for angiogenesis and for tissue regeneration. Non-transfusional clinical use of platelet-derived products, such as Platelet Rich Plasma (PRP), is based on their ability to maximize cellular regeneration in lesions that have repair difficulties. **Objectives:** This review addresses the characteristics and the potential clinical use of platelets and their derivatives and discusses the legislative framework of their use in the Brazilian context. **Method:** The databases of PubMed, Brazilian Virtual Health Library, electronic pages of Anvisa and the Brazilian Ministry of Health were consulted between September 2017 and January 2018. **Results:** PRP is a platelet-derived product that cannot be chemically defined, and its proposed clinical uses are not orthologous applications. **Conclusions:** We can thus consider that the applications of PRP are Advanced Cell Therapies. Therefore, it is mandatory to standardize protocols and establish quality control criteria - such as traceability, efficacy and pharmacovigilance - so that their use can be properly controlled by the competent regulatory organs, guaranteeing safety in their use.

KEYWORDS: Blood Platelet; Platelet-Rich Plasma; Regeneration; Regenerative Medicine

RESUMO

Introdução: As plaquetas têm um papel central no processo da resposta tecidual à injúria, atuando na hemostasia primária e na coagulação, e liberando de maneira coordenada as moléculas bioativas importantes para a angiogênese e para a regeneração tecidual. O uso clínico não transfusional de produtos derivados de plaquetas, como o Plasma Rico em Plaquetas (PRP), baseia-se na sua habilidade de maximizar o processo de regeneração celular, em lesões com dificuldades de reparo natural. **Objetivo:** Esta revisão aborda as características e potencialidades do uso clínico de plaquetas e seus derivados e discute seu arcabouço legislativo no contexto brasileiro. **Método:** Foram consultadas as bases de dados PubMed, Biblioteca Virtual em Saúde, páginas eletrônicas da Anvisa e do ministério da Saúde, entre setembro 2017 e janeiro de 2018. **Resultados:** O PRP é produto derivado de plaquetas que não pode ser quimicamente definido, e o seu uso clínico aqui discutido não é ortólogo. **Conclusões:** Dessa maneira, podemos considerar a aplicação do PRP como uma Terapia Celular Avançada, sendo fundamental padronizar protocolos, estabelecer critérios de controle de qualidade (rastreabilidade, eficácia e fármaco-vigilância), tornando seu uso devidamente controlado pelos órgãos competentes, com maior segurança na sua utilização.

PALAVRAS-CHAVE: Plaquetas; Plasma Rico em Plaquetas; Regeneração; Medicina Regenerativa

INTRODUCTION

Platelets are non-nucleated cells circulating in blood, derived from the megakaryocytes of the bone marrow. Approximately 70% of platelets are present in the bloodstream and 30% in the spleen, and their average lifespan is 10 days. Platelets play a key role in the process of tissue response to injury, acting in all its stages: blood coagulation, inflammation, remodeling and healing. Although circulating platelets have simple discoid morphology, they present a complex internal structure of granules, channels and organelles capable of releasing bioactive molecules quickly and in an orchestrated and controlled pattern. Their membrane is highly rich in glycoproteins and molecular transduction receptors, which enable communication and interaction with various molecules and cells[1].

Platelet origin

Platelets (thrombocytes) derive from cytoplasmic fragmentation of megakaryocytes in the bloodstream. In the bone marrow, hematopoietic stem cells (hemocytoblasts) undergo processes of cell proliferation and differentiation that give rise to all precursors of circulating blood cells[2]. Granulocytes, erythrocytes, megakaryocytes, monocytes and other myeloid cells originate from the myeloid progenitor cells derived from the hemocytoblasts.

In response to cytokines, especially thrombopoietin, the myeloid progenitor differentiates in the medullary niche into highly proliferative diploid cells called megakaryoblasts. They initiate a process of endomitotic duplication, in which an increase in the cytoplasmic volume and the number of nuclei and organelles occurs, but without cell division. In this condition, the polyploid cell (promegacarocyte) becomes highly granulated, 5 to 10 times larger than a common cell (<50-100 microns in diameter). These cells contain multiple nuclei, high levels of RNA, prominent ribosomes, rich endoplasmic reticulum, platelet peroxidase, as well as alpha granules, dense granules and primary demarcation membranes. Mature megakaryocyte forms protoplatelet cytoplasmic projections in the venous sinusoid vessels that originate circulating platelets[3] (Figure 1).

Bone marrow

Platelet production and release

Deep structural modifications of the megakaryocyte cytoskeleton are required to produce platelets. Extensive internal demarcation of megakaryocytes cytoplasm serves as reservoir for platelets formation in extensions called protoplatelet projections. While some platelet proteins, like von Willebrand Factor and fibrinogen

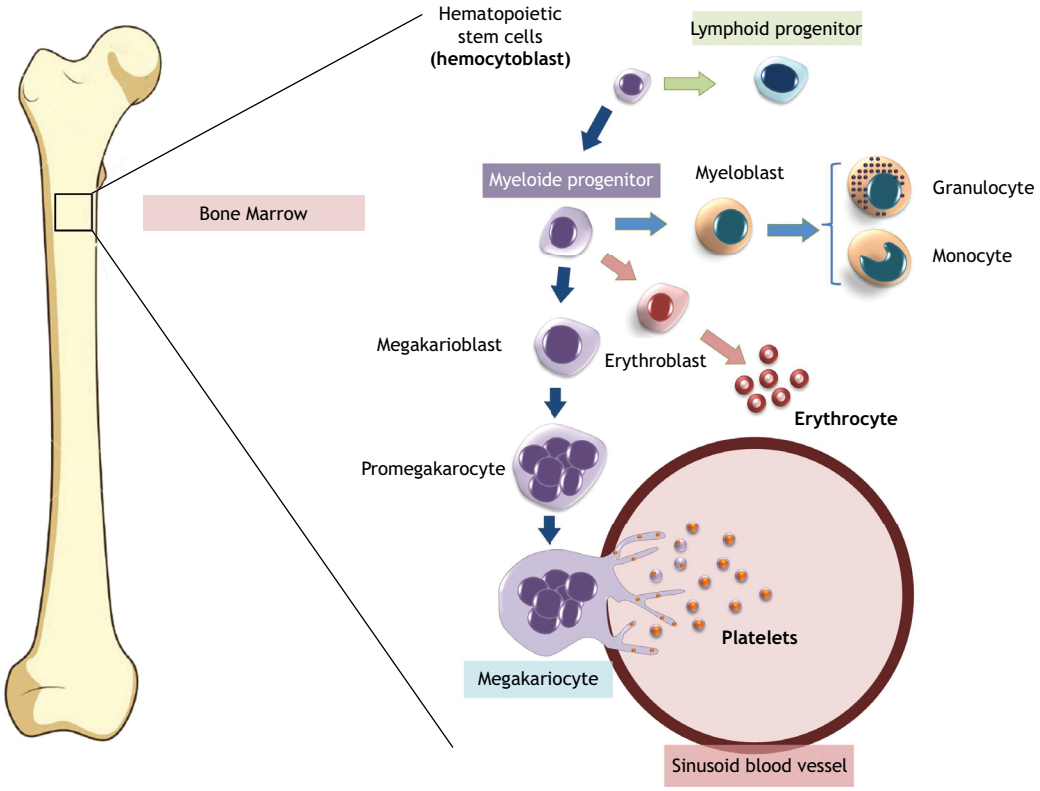

Figure 1. Summary of the genesis of megakaryocytes and platelet release. In the bone marrow, hematopoietic stem cells differentiate into myeloid progenitors. These progenitors differentiate into megakaryoblasts, which later on differentiate into megakaryocytes. Finally, mature megakaryocytes launch protoplantar projections into the sinusoid vessel, into which platelets are released into the bloodstream.

receptors, are produced in the megakaryocyte membrane, part of the organelles and proteins packaged in the granules migrate from the cytoplasm to the protoplatelet extender[2,3].

Two models that explain the formation of platelets are currently accepted. In the first, the release of platelets occurs by the budding of the tips of protoplatelet projections, like in assembly lines. Thus, protoplatelet projections advance through the sinusoidal spaces and through where they separate into individual platelets with the aid of the bloodstream. In the second model, preformed territories with the inner membranes of megakaryocytes demarcate the already packed platelets still within the cytoplasm. Thus they are released by direct cytoplasmic fragmentation in the protoplatelet projections[2].

Primary function of platelets

The primary function of platelets is to trigger a set of responses to a vascular injury, safeguarding the integrity of the blood vessels. They play a central role in the process of urgently restraining the leakage of the blood (hemostasis). Platelets circulate within blood vessels in occasional contact with vascular walls. The endothelium that covers the normal walls is continuous, covered by glycoconjugates that prevent the adhesion of the platelets, keeping them in blood stream. An acute lesion of the endothelium causes exposure of the subendothelial collagen, which is extremely adherent to platelets[4]. This adhesion occurs through the binding of the von Willebrand Factor, associated with subendothelial collagen, to its ligand on the membrane surface of platelets. This immediately promotes platelet adhesion and activation, followed by local mobilization of more circulating platelets and others blood cells. The release of the Tissue Factor by the subendothelial layer also promotes the rapid clot formation by activation of the thrombin cascade, fibrinogen breakdown and the formation of fibrin network (extrinsic coagulation pathway). A blood clot (thrombus) is thus formed with the function of primary blood stasis[5].

During their activation, platelets secrete several molecules that are important for the maintenance of the thrombus and trigger the tissue inflammation process. The major soluble mediators released from the platelet alpha-granules are: platelet factor IV, coagulation factors, plasminogen activator inhibitor and von Willebrand factor. They are accompanied by adhesive proteins, thrombospondin and vitronectin, which capture and activate new circulating platelets into the forming clot. From the dense granules are released: ATP, ADP and calcium. Platelet aggregation also induces intracellular signaling involved in cellular responses, such as the production of serotonin, ADP and TXA2, which amplify platelet responses. Several cytokines are released by the alpha granules, which act on the pro-inflammatory signaling, guiding the activation and differentiation of monocytes and neutrophil adhesion.

Secondary functions of platelets

In the late and chronic context of blood vessel injuries, platelets are involved in the recruitment of immune cells, new blood vessels formation (angiogenesis), and in tissue remodeling and regeneration processes. Activated platelets release a plethora of factors that stimulate the metabolism and proliferation of both resident and mobilized cells from bloodstream. These factors are known as “growth factors”, and are intended to promote the repair and regeneration of injured tissues.

While the activation of coagulation and clot formation is rapid and limited to the initial phase of response to injury, the release of growth factors is extended, sustained by inflammatory factors and by the cascade activation of mobilized platelets from the blood stream. The released growth factors associate with the fibrin network, collagen and glycoconjugates of the extracellular matrix, maintaining a long term regeneration. While proinflammatory factors initiate the process of local inflammation, neoangiogenic platelet-derived factors stimulate endothelial proliferation and formation of new capillary vessels. In addition to tissue feeding and oxygenation, vascular networks ensure the supply of circulating progenitor cells present in biological fluids. Vascular stabilizing factors mobilize the pericytes (resident progenitor cells that migrate on the abluminal side of the vascular walls) and induce the proliferation of the mural cells of larger vessels. Finally, the growth factors involved in the proliferation of various cell types ensure broad tissue regeneration.

Providing blood stasis (primary function) and promoting tissue repair and regeneration (secondary function) are the major platelet activities that occur in different contexts and times. The primary function acts essentially in an acute and immediate context, while the second function acts in a chronic and late context. Both cases may involve similar components, but the operational logic and sequence are distinct, albeit complementary. It is not surprising that the production and coordinated release of this set of platelet factors of tissue regeneration attract attention, and can be used in many areas of regenerative medicine. Blood platelets, their source, can be used in various contexts as donors of growth factors. For this reason, their potential use in medical practice and in health care follows the same dichotomy. With that in mind, the present review intends to address the characteristics and potentialities of the clinical use of platelets and their derivatives.

METHOD

This study was performed as an integrative review, considering the collection of scientific papers with the objective of understanding the use of Platelet Rich Plasma (PRP) in advanced therapies and to discuss the regulatory framework of this therapy in the Brazilian context. The research of scientific literature was carried out by consultation based on PubMed and the Virtual Health Library (VHL), using keywords like: Plasma Rich in Platelets, Platelet-rich Plasma, Platelets, Platelets, Regeneration, Regenerative medicine, Therapy. The websites of the Brazilian National Sanitary Surveillance Agency (Anvisa - portal.anvisa.gov.br) and the Brazilian Ministry of Health (portalsaude.saude.gov.br) were also consulted. This research was carried out from September 7 to 14, 2017, and from January 15 to 23, 2018.

RESULTS AND DISCUSSION

Transfusional use of platelets

The clinical use of platelets is a well-established procedure in the prophylaxis and treatment of problems arising from thrombocytopenia or abnormalities of platelet functions[6]. In the context of blood stasis and coagulation control, the therapeutic intervention involves the platelet concentrate. It is estimated that in the United States, about 2.2 million units of platelet concentrate are transfused annually. Platelet Concentrate (PC) is a blood component obtained from fractioning of whole blood, or directly through apheresis. In Brazil, the guidelines of the Ministry of Health[7] recommend that each (random) PC unit contains approximately 5.5 x 10^{10} platelets in about 50 to 60 mL of plasma. In these cases, the platelets are obtained from a total blood unit[8]. In the case of plateletpheresis, PC are obtained by apheresis and contain about 3.0 x 10^{11} platelets in 200 to 300 mL plasma. Their therapeutic use is transfusional in cases of thrombocytopenia or abnormalities of platelet function, as well as in surgical patients with active bleeding, in massive transfusions, extracorporeal circulation, disseminated intravascular coagulation. Prophylactic use applies to patients with spinal aplasia who undergo chemo or radiation therapy, and those who undergo critical surgical procedures, as well as in ophthalmologic and neurological surgeries[7].

The use of PC by vascular infusion functions as a supplement to the quantity and/or quality of existing platelets, and is characterized as an orthologous and conventional therapy. It is evident that the use of platelet transfusion in these procedures is due solely to the primary function of platelets: blood stasis. This product is under the responsibility of the hemotherapy centers, and these therapies are submitted to the quality controls of these products, in a context of allogeneic therapies. Anvisa's RDC n. 57, of December 16, 2010[9] determines the sanitary regulation of services that carry out activities related to the productive cycle of human blood and transfusion components and procedures. Ministerial Act 1353/2011[10] approves the Technical Regulation of Hemotherapeutic Procedures.

Platelet Rich Plasma

In the last decades, the number of clinical studies involving the experimental use of another type of PC has been increasing, defined as Platelet Rich Plasma (PRP). In the American *clinical trials* (www.clinicaltrials.gov) platform, PRP (platelet-rich plasma) is the subject of study in about 300 clinical trials in orthopedic, plastic, maxillofacial surgeries, severe skin burns, chronic ulcers, osteoarthrosis, repair of tendons and ligaments. The non-transfusional clinical use of PRP is based on its ability to maximize the tissue regeneration process at the cellular level. PRP has the ability to accelerate the vascularization of grafts, reduce postoperative morbidity, stimulate tissue regeneration, reduce scar formation and recruit and activate cells involved in the inflammation/regeneration process[11].

PRP can be defined as a small volume of plasma containing a high concentration of platelets obtained by centrifugation of peripheral blood in the presence of anticoagulant substances. PRP preparation protocols include venous blood collection, followed by a double centrifugation. In the first step, the platelets are separated from other blood cells, and harvested with the plasma above the buffy coat interface, and they may or may not include mononuclear leukocytes. In the second centrifugation, the recovered platelets are concentrated in a small volume of plasma, raising the platelet rate 3 to 5 times higher than in the peripheral blood[12,13]. Ehrenfest et al.[14,15] classify the result of this processing as P-PRP (Pure-PRP) or L-PRP (PRP with Leukocytes), according to the presence or absence of the leukocytes in the final product.

Two characteristics differentiate the PRP from the PC produced in hemotherapy centers. The first is the platelet concentration - since, by definition, the PRP should concentrate at least 1 x 10^6 platelets/microliter in 5 mL of plasma[12], representing from 3x to 5x of the basal blood concentration. The second is the purpose of the use: unlike hemo-therapeutic platelet concentrates, which propose supplementation of platelets already existing in the patient's bloodstream, the application of PRP aims to enhance the local regeneration of injured tissues that have difficulties in repairing naturally or lack direct blood supply. This enhanced regeneration is due to the exceptional load of growth factors and cytokines derived from the activation of platelets, concentrated in the PRP.

Among the factors released during platelet activation, the most significant and already identified in the PRP are: (a) Tumor Growth Factor family (TGF-b 1 and 2); (b) Platelet Derived Growth Factors (PDGF); (c) Insulin Growth Factor (IGF), (d) Fibroblast Growth Factor (FGF), (e) Epidermal Growth Factor (EGF), and (f) Vascular Endothelial Growth Factor (VEGF). This set of factors (Table 1) plays an important role in cell proliferation and differentiation, chemotaxis, and angiogenesis[13,16,17]. In addition to these factors present in the a-platelet granules, platelets have dense granules that contain serotonin, histamine, dopamine, as well as calcium and adenosine. All these compounds have fundamental implications in the biological aspects of tissue healing[18], modulating the inflammation process, stimulating the vascularization and synthesis of collagen, and mediating the healing of the tissue injury[6,11].

In the PRP, the addition of anticoagulants prevents the early activation of platelets and, consequently, the formation of the fibrin network during centrifugiation[14]. The activation of platelets in the PRP and the release of regenerative factors can occur in two ways: (1) while handling the centrifuged plasma, with the addition of thrombin and/or of Ca^+ ions[13,17], or (2) directly upon its application to the patient, in which trauma caused by needle insertion and/or contact with tissue collagen macromolecules triggers the immediate activation of platelets and the release of regenerative factors. The association of PRP with organic and/or mineralized matrices is also possible, generating the 3D structures that will provide the support to regeneration of complex structures through bioengineering.

Table 1. Factors present in PRP.

Growth factor	Effects
Tumor growth factor (TGF-β 1 and 2)	Regulates the mitogenic effect of other growth factors; stimulates the proliferation of undifferentiated mesenchymal cells; fibroblasts and osteoblast. Vascular stabilizer and regulator of collagen synthesis and collagenase secretion. It stimulates angiogenesis and endothelial chemotaxis, inhibiting the proliferation of macrophages and lymphocytes.
Fibroblast growth factor (FGF)	Mitogenic effect for mesenchymal cells, chondrocytes and osteoblasts. Stimulates the growth and differentiation of cartilage and bone.
Platelet-derived growth factor (PDGF)	Stimulates the chemotaxis and mitosis of fibroblasts, smooth muscle cells and glial cells. Regulates collagenase secretion and collagen synthesis, mitogenic to mesenchymal cells and osteoblasts. It stimulates the chemotaxis of macrophages and neutrophils.
Epidermal growth factor (EGF)	Stimulates mitosis of mesenchymal cells. It regulates the secretion of collagenase. It stimulates chemotaxis and angiogenesis of endothelial cells.
Vascular-endothelial growth factor (VEGF)	Stimulates endothelial cell mitosis. It increases angiogenesis and vessel permeability.
Insulin-like growth factor (IGF)	Stimulates the differentiation and mitogenesis of mesenchymal cells and lining cells. It stimulates the proliferation of osteoblasts and the production of collagen type I, osteocalcin and alkaline phosphatase.

Adapted from Moshiri and Oryan[19].

We can see that the main mechanism of action of PRP is not in the platelets per se, but in the content of active molecules released by their platelet granules during their activation (secondary function of the platelets). Since in these protocols the platelets or their products will not be introduced through blood vessels, the application will not be orthologous, their action may be considered distinct from that performed in the patient's bloodstream.

Platelet-derived products like PRP have been used experimentally since the 1970s, and they have become rather common since the 1990s. Even so, reviews of scientific literature on "platelet concentrates" like PRP are still difficult because of the absence of clear terminology in the literature[13,20]. Many PC were classified as PRP without the proper characterization of concentration of their components or content, with different production protocols. Only a few studies actually described the PRP content quantitatively and qualitatively. Platelet concentrates are difficult to characterize, since they are not traditional pharmaceutical preparations like antibiotics or anti-inflammatory drugs. Platelet concentrates do not represent only an association of several growth factors. They are associated with blood clots, which concentrate factors and cytokines that orchestrate and modulate tissue regeneration[20]. The individual biological differences of each donor, as well as their age and gender, make the PRP variable as to the composition and dosage of its factors and their regenerative effect.

Even the time of platelet activation (pre or post-application) may influence the release of short-lived inflammatory cytokines. Another factor that must be taken into account is the time elapsed between obtaining it and its clinical application, since it may compromise the presence and performance of cytokines initially obtained in PRP[21].

PRP clinical applications

The first clinical application of PRP was in the study of Ferrari *et al* (1987), in which PRP was used as an additional element to transfusion in a heart surgery. Ever since then, the application of PRP has been shown to be safe and used mainly for: orthopedics, sports medicine and dentistry, but also neurosurgery, ophthalmology, urology and even in facial and aesthetic surgeries. Evidences support that PRP has effects on inflammation and postoperative infections, as well as on the regeneration of bones and connective, epithelial and muscular tissues[23]. Although the use of PRP is a safe method, there are some conditions in which the application should be considered with caution, such as clinical signs of thrombocytopenia, platelet dysfunction, septicemia, hypofibrinogenemia, fever, anemia, cancer, dermal lesions close to the application area, use of corticosteroids and anti-inflammatory drugs, in addition to active infections[11,24].

It is important to emphasize that the lack of standardization of well-established protocols for PRP generates many controversial results. Studies with unsatisfactory results are often associated with the poor quality of the material obtained, both in the concentration of platelets and in their integrity and effectiveness of activation[11].

The method to obtain PRP was elaborated and standardized in the 1970s[18,25] and the clinical applications of PRP occurred in oral and maxillofacial surgeries in the 1990s[26]. In 2003, the first report of the human use of PRP in orthopedics appeared in the non-traumatic avulsion of the articular cartilage of the knee, in the form of a case report[27]. Since then, several papers have been published using PRP also in bone tissue engineering, in the treatment of joint cartilage lesions, muscle and tendon injuries, where most PRP studies are concentrated[28]. Table 2 presents

Table 2. Clinical trials and PRP application in orthopedics.

Authors	Type of injury	Type of study	Number of patients (or surgical site)	Experimental groups	Main clinical outcomes
Campell et al., 2015[29]	Knee osteoarthritis	Systematic review	3,278 knees	PRP and control (hyaluronic acid or placebo)	There was a significant difference in the clinical improvement of patients treated with PRP - 2 and 12 months after treatment, but it was not possible to define the ideal number of applications.
Meheux et al., 2016[30]	Knee osteoarthritis	Systematic review (6 studies)	817 knees	PRP and control (hyaluronic acid or placebo)	There was a significant difference in the clinical improvement of the patients treated with PRP - 3 and 12 months after treatment (assessed by the Womac index).
Kanchanatawan et al., 2016[31]	Knee osteoarthritis	Systematic review (551 studies evaluated)	-	PRP, hyaluronic acid (HA) and placebo)	In short-term results (≤1 year), PRP injection improved functional outcomes (assessed by Womac, IKDC and EQ-VAS indices) when compared to HA and placebo, but there was no statistically significant difference in adverse events when compared with HA and placebo. This study suggests that PRP injection is more effective than HA and placebo in reducing symptoms and improves function and quality of life. The study suggests that PRP treatment has the potential for patients with mild to moderate osteoarthritis of the knee who did not respond to conventional treatment.
Dai et al., 2017[32]	Knee osteoarthritis	Systematic review (10 studies)	1,069 patients	PRP and control (hyaluronic acid or placebo)	There was no significant difference in clinical improvement (pain and joint function) of patients treated with PRP or hyaluronic acid at 6 months after treatment (assessed by the Womac index). However, at 12 months there was a significant difference in clinical improvement (both pain and joint function) of patients treated with PRP in relation to hyaluronic acid (assessed by the Womac index). PRP does not increase risks of adverse events.
Kuang et al., 2016[33]	Total knee arthroplasty	Systematic review (12 studies)	1,234 patients (1,333 knees)	Autologous platelet gel and control (placebo)	Autologous platelet gel treatment improves pain control (assessed by the VAS scale), but there is no difference between blood loss, length of stay, and postoperative recovery.
Li et al., 2017[34]	Total knee arthroplasty	Systematic review	1,316 patients	PRP and control (placebo)	Treatment with PRP significantly increases motor function (assessed by the ROM index) at 30 days and 3 months post-surgery. There was no significant difference in clinical improvement (assessed by the Womac index) between the two experimental groups at 24 hours, 48 hours, and 7 days post-surgery. There is no significant difference between the two groups in the occurrence of infection.
Warth et al., 2015[34]	Rotator cuff rupture	Systematic review (11 studies)	-	PRP and control	There was no significant difference in clinical improvement (assessed by the ASES index, Constant, VAS) of patients treated with PRP or controls. However, there is a significant increase in the Constant scale when PRP is placed at the tendon-bone interface rather than when it is placed directly on the lesion surface.
Di Matteo et al., 2016[36]	Anterior cruciate ligament	Systematic review (23 clinical studies)	-	PRP, PRP with stem cells and control	There is evidence that PRP improves the maturation of the implant and its bone integration.
Everhart et al., 2017[37]	Patellar tendinopathy	Systematic review (15 clinical studies)	-	PRP and controls	Treatment with PRP expedites patient recovery.
Chiew et al., 2016[38]	Plantar fascitis	Systematic review (1,126 scientific articles)	455 patients	PRP and control (steroids)	Treatment with PRP was superior to treatment with steroids and did not cause any type of adverse reaction or clinical complications.

Continue

Continuation

Filardo et al., 2016[39]	Tendon lesions	Systematic review of Level I, II, III and IV clinical studies	19 scientific papers on the patellar tendon, 24 on the Achilles tendon, 29 on the lateral tendon of the shoulder, 32 on the rotator cuff	PRP and control	PRP was beneficial for the treatment of the patellar tendon, but not the Achilles tendon. For the treatment of lateral shoulder tendinopathy, clinical improvement was observed in most of the more advanced studies, but there was a lack of evidence of superiority in conventional treatments. In rotator cuff disease, most studies have reported lack of evidence of the beneficial effects of PRP over traditional therapies.
Roffi et al., 2017[40]	Bone defects (pseudoarthrosis fractures)	Systematic review (45 preclinical and 19 clinical articles)	-	PRP and controls	The benefits of PRP are observed in 91.1% of the preclinical studies; in histological analyses, the positive results appear in 84.4% of the studies and the biomechanical and radiological results are observed in 72.5% and 75% of the studies, respectively. The results of clinical trials are still inconclusive.
Pas et al., 2015[41]	Hamstring injury	Systematic review	526 patients	PRP and control	The meta-analysis showed superior efficacy for rehabilitation exercises. Injection of PRP had no effect on acute hamstring injury. Limited evidence has been found that agility and stem stabilization can reduce re-injury rates. Limitations identified in most ACEs should improve the conception of new ACEs.

ASES: *American Shoulder and Elbow Surgeons*; Constant: *Constant Score*; DASH: *Disability of the Arm, Shoulder and hand;* KJOC: Kerlan: *Jobe Orthopedic Clinic*; PRP: platelet rich plasma; ROM: Range of Motion; VAS: *visual analogic scale;* Womac: *The Western Ontario McMaster Universities Osteoarthritis Index Bellamy.*

some of the most recent results described in the scientific literature on the use of PRP for orthopedic diseases.

Although the major PRP clinical studies focus on the area of orthopedic diseases[18], many others have been exploring the regenerative potential of PRP for other areas. In dermatological and esthetic procedures, PRP has been used in the form of topical facial injections to fill and regenerate facial wrinkles (anti-aging effects)[42], acne scars or depressed or photo-damaged areas. PRP applications have been performed with positive results in conjunction with facelift procedures, adipose tissue grafts[43], fractioned laser and alopecia[42,44,45]. Other studies have also evaluated the potential of PRP in chronic ulcers[23,42], muscle injuries[46,47] and even in ocular lesions[48].

Regulatory aspects of the use of PRP for non-transfusion therapeutic purposes

Legislation in Brazil

The preparation of the PRP for clinical use follows the regulations defined by Brazilian Health Regulatory Agency (Anvisa) in Technical Note n. 64, of July 14, 2015, as follows:

> The processing of the PRP with an autologous purpose through a closed system may be done in healthcare establishments, in accordance with the provisions of RDC n. 63/2011, which provides for the requirements of Good Operating Practices for Health Services[49].

It is further specified that:

> the products used to process the PRP must be regularized by Anvisa, according to RDC n. 185 of October 22, 2001, which approves the Technical Regulation that deals with the registration, alteration, revalidation and cancellation of medical products and must be used according to the manufacturers' instructions/recommendations[50].

The preparation in the open system has the following definition:

> The processing of the PRP by means of an open system should be done in Cell Processing Centers, according to the provisions of the recently published RDC n. 214, of February 7, 2018[51], which addresses the Good Practices in Human Cells for Use Therapeutic and clinical research.

RDC n. 214/2018 was drafted based on public consultation n. 270/2016, which defined the procedures to be used in the production of the PRP[52].

The non-transfusional use of PRP follows Technical Note n. 064/2015 GSTCO/GGPBS/Sumed/Anvisa[49] on the "Use of Platelet Rich Plasma - PRP for non-transfusion therapeutic purposes", which specifies: "The clinical indications and therapeutic purpose for the use of PRP should be recognized and regulated by the respective Professional Councils".

In Resolution n. 2.128, of July 17, 2015, the Federal Council of Medicine (CFM) considers the practice of using PRP as an experimental tool in the treatment of musculoskeletal and other diseases[53]. The same resolution still restricts the use of PRP to clinical experimentation, within the protocols of the Committee for Research Ethics (CEP)/National Commission of Research Ethics (Conep). CFM Resolution n. 1998 prohibits physicians to provide therapeutic treatment services that are not recognized by the scientific community. Therefore, clinical trials using PRP should be previously approved by the CEP/Conep system.

On the other hand, the Federal Council of Dentistry, through Resolution CFO n. 158/2015, regulates the use of Platelet Rich Plasma and Platelet Rich Fibrin, called Autologous Platelet Aggregates, for non-transfusion purposes within the scope of Dentistry[55]. PRP is considered to be the portion of blood that contains platelet components, without the addition of any product, including anticoagulants or coagulants.

Following the public consultation of Anvisa n. 270/2016, biological products consisting of human cells or their derivatives, not chemically defined, autologous or allogeneic, which perform in the recipient a function other than that performed in the donor, shall be considered as products of advanced cell therapies, the use of which will have its own regulations[52]. This definition applies to the use of PRP in non-transfusion procedures, which should then be adapted to the new legislation.

Examples of international legislations

PRP is classified by the Food and Drug Administration (FDA), an agency of the United States Department of Health and Human Services, as an autologous and minimally handled material obtained from blood, primarily used locally or topically[56,57]. The devices used in PRP preparation are subject to FDA authorization and are considered low risk. They must be at least as safe and effective as an already legally marketed device[56]. PRP may be part of a set of procedures, as associated with bone graft materials.

Currently, the use of PRP in the United States is considered a medical procedure, not subject to FDA regulation. The FDA does not regulate the practice of medicine, so clinicians may use products in "undescribed use" or off label[58], provided they have the responsibility to be well informed about the product and base its use on rational scientific and medical evidence, in addition to keeping records of the use of the product and its effects[56,59].

In Europe, the regulatory framework for the blood system is currently governed by Directive 2002/98/EC, which lays down rules on the quality and safety standards to collect, control, process, preserve and distribute human blood and its components, recognizing internal regulations in the various member States[60]. Blood components may be considered as products or medicines. Depending on the amount, processing and clinical protocol, they may be used in a less restrictive manner, under the prescription and control of a physician[61].

In Italy, procedures classified as "topical application" are those in which blood components are not applied to patients by transfusion, but directly in the affected area, such as intra-articular or intra-tissue injection in orthopedics, or cutaneous and subcutaneous injection in dermatology and plastic surgery. Blood handling is restricted to Blood Transfusion Services, but PRP preparation may occur on an outpatient basis[61].

The Spanish Agency of Medicines and Medical Devices (Aemps) prepared a comprehensive report and a resolution regulating the use of PRP as a medical product for human use, defining the composition of the PRP, its mechanisms of action and medical guides for use[62]. Medical products include any substance or combination of substances that may be used in or administered to humans, with the function of restoring, correcting or modifying physiological functions, exerting a pharmacological, immunological or metabolic action or making a medical diagnosis[59]. The use of PRP may be prescribed by physicians or dentists. It must be handled with appropriate equipment and instruments in authorized health centers in accordance with regional regulations.

The equipment must be registered with the EC medical device, indicating that it complies with the European directives and should be used according to the manufacturer's instructions. In terms of efficacy, the use of PRP can be classified into three categories, depending on the evidence available: (a) pathologies in which there is evidence to recommend treatment, (b) those in which there was a negative balance between risk and benefit and will not be recommended for use and (c) those requiring further evidence. Physicians will have to adopt specific measures of control, supervision and traceability to prevent the transmission of infectious diseases. They should immediately notify the drug surveillance authority of any suspected adverse reaction. All medicines must have a summary in which the characteristics of the product are detailed and a package leaflet with basic information and instructions for the patient. One should expose the pros and cons compared to other treatments and any potential risks and/or side effects.

To date, there is no clear gold standard protocol for the manufacturing of PRP, as there is still little characterization performed on the products and lack of regulation and standardization. To characterize the components that play a key role in tissue regeneration and formulate a preparation appropriate to each pathophysiological situation, a simple and well-defined procedure is required, where centrifuging conditions are critical to high quality PRP[63].

CONCLUSIONS

The PRP used in non-transfusion procedures represents a chemically undefined product. It has been used in a non-orthologous manner in the areas of orthopedics, dermatology, dentistry and ophthalmology. According to the new proposals of Anvisa, therefore, it will be considered an Advanced Cell Therapy. Like in the United States and Europe, regardless of whether PRP is prepared in open or closed systems, it will be necessary to better define its composition and its therapeutic action. These parameters should have their minimum specifications determined according to their indications of use. These also need to be described, in each area, according to the degree of scientific evidence that corroborates their use. Furthermore, it is necessary to establish quality control criteria like traceability, efficacy and drug-surveillance, so that it is properly controlled by the responsible regulatory agencies, allowing greater security in its use.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.