Narrative Review

Autologous Platelet-Rich Plasma Applications in Chronic Pain Medicine: Establishing a Framework for Future Research - A Narrative Review

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Background: During the last decades, platelet-rich plasma has been studied for the treatment of multiple chronic pain conditions, in addition to being employed in the enhancement of healing after tissue injury.

Objective: To establish a framework for future research regarding the utilization of platelet-rich plasma in the treatment of chronic tissue injuries.

Methods: Preclinical and clinical studies from 2000-2020 relevant to applications of platelet-rich plasma for the treatment of chronic pain conditions were extracted from PubMed and Medline databases. The studies were analyzed on the basis of the study population, type of intervention, method of platelet-rich plasma preparation, the number of treatments administered, the timeframe of injections, and clinical outcomes.

Results: Although several preclinical studies and double-blind, randomized trials have shown promising results in the application of platelet-rich plasma for the treatment of multiple chronic pain conditions, various studies have also reported controversial results. Additionally, the methods employed for obtaining the platelet-rich plasma have not been standardized between studies, resulting in different concentrations of blood components between the preparations utilized. Moreover, differences between studies were also found regarding the number of injections administered per treatment.

Conclusions: Future research addressing the utilization of platelet-rich plasma in the treatment of chronic pain conditions should focus on shedding light on the following major questions: a) Is there a dose-effect relation between the platelet count and the clinical efficacy of the preparation?; b) What pathology determinants should be considered when selecting between leukocyte-enriched and leukocyte-depleted concentrates?; c) What is the role of platelet activation methods on the clinical efficacy of platelet-rich plasma?; d) Is there an optimal number of injections and time frame for application of multiple injection treatment cycles?; e) Does the addition of local anesthetics affect the clinical efficacy of platelet-rich plasma?; and f) Is there potential for future platelet-rich plasma applications for the treatment of neuropathic pain of peripheral origin?

Key words: Chronic pain, clinical research, pain medicine, platelet-rich plasma, regenerative medicine

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A human platelet-rich plasma (PRP) concentrate can be defined as a preparation of autologous human plasma with increased platelet concentration, produced by centrifugation of a larger volume of a patient's own blood (1).

Platelets contain a plethora of growth factors in their α-granules: basic fibroblast growth factor one (bFGF-1), epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor one (IGF-1), platelet-derived growth factor $\alpha\beta$ (PDGF-$\alpha\beta$), transforming growth factor β1 (TGF-β1), and vascular endothelial growth factor (VEGF). When preparing a PRP syringe, platelets are concentrated through the centrifugation process in order to then be injected in supraphysiologic concentrations to an injury site, with the final (theoretical) aim of augmenting the natural healing process (1-3).

PRP preparations have been utilized for decades with the purpose of facilitating surgical tissue repair. Thus, percutaneous injections of autologous PRP concentrates were a logical progression as a bridge between conservative modalities and invasive surgical interventions. During the last 2 decades, autologous PRP concentrates have been studied and utilized for the treatment of multiple chronic pain conditions, besides being employed in the enhancement of healing after bone or tissue reconstruction (1,2).

Although several preclinical studies and double-blind, randomized trials over the past 2 decades have shown promising results in the application of PRP preparations for the treatment of multiple chronic pain conditions, many of the studies have reported mixed results. Additionally, it should be taken into consideration that the methods employed for obtaining autologous PRP preparations have not been standardized among studies, which in turn resulted in vastly different concentrations of blood components between the preparations utilized in each study. As such, to this day, there is limited evidence to support the dissemination of PRP in pain medicine clinics around the globe, and high-quality studies with standardized methods are warranted before insurance payers, government regulators, consumers, and healthcare providers can confidently accept PRP as a potentially effective bioregenerative treatment option, as was concluded in a recent review by Navani et al (2).

In this narrative review, we aim to establish a framework for future research, which we hope may help guide clinical research initiatives through the 2020s in the field of regenerative medicine applications for the treatment of painful conditions associated with chronic tissue injury. Bearing this purpose in mind, throughout the article we discuss what we believe to be the major autologous PRP questions left unanswered to date: a) Is there a dose-effect relation between the platelet count and the clinical efficacy of the preparation?; b) What pathology determinants should be considered when selecting between leukocyte-enriched and leukocyte-depleted concentrates?; c) What is the role of platelet activation methods on the clinical efficacy of PRP concentrates?; d) Is there an optimal number of injections and timeframe for application of multiple injection treatment cycles?; e) Does the addition of local anesthetics affect the clinical efficacy of PRP?; and finally f) Is there potential for future PRP applications for the treatment of neuropathic pain of peripheral origin?

Where It All Started - Wound Healing

The premise that autologous PRP is an effective treatment for multiple chronic pain conditions is based on evidence that showed that PRP enhanced and expedited wound healing, a process usually divided into 3 different phases, each succeeding the previous one after certain local critical conditions are met (4,5). After an initial injury, the inflammatory phase typically begins within the first week with the initiation of local cell lysis, a process that releases debris and inflammatory mediators such as kinins and prostaglandins. In this first phase of healing, platelets aggregate to form a fibrin matrix that facilitates hemostasis. These platelet aggregates then degranulate, releasing cytokines that attract leukocytes, with neutrophils being the first responders to arrive, eliminating possible bacteria and cellular debris on site. Later during this first phase, growth factors released from activated and degranulated platelets attract macrophages and fibroblasts, and the activation of cyclooxygenase-2 culminates in the initial vasodilation response. Within the second week after the injury, the proliferative phase usually ensues. In this phase, macrophages cleanse the wound further, which is followed by the formation of granulation tissue and neovascularization, both promoted by local fibroblasts (4,5). The third and last phase of healing, the remodeling phase, starts 2 to 3 months after the injury and is characterized by healing through the production of collagen and scar tissue. In this phase, proteoglycan and fibronectin are replaced by type I collagen, which forms a matrix with increased tensile strength. Angiogenesis, cell proliferation, deposition of extracellular matrix (ECM), remodeling, and

maturation culminate in the healing of soft tissue and ligament, muscle, and tendon. Throughout the whole process, several different growth factors contribute to the stimulation of tissue repair, playing important roles in cell regulation, differentiation, proliferation, chemotaxis, and ECM synthesis. Of note, the overall speed of the healing process, as well as the time of initiation of each phase may differ significantly between tissues based on local conditions, the most important of which seems to be local perfusion (4-6).

The Role of Platelet-Derived Growth Factors

During degranulation, a multitude of different growth factors is released from the α-granules of aggregated platelets, each playing a critical role in one or several steps of the healing process (2,6). As they bind to specific high-affinity transmembrane receptors, these growth factors trigger different intracellular signaling pathways. Some of the most important growth factors include bFGF-1, EGF, HGF, IGF-1, PDGF-$\alpha\beta$, TGF-β1, and VEGF (Table 1). In addition, different chemokines, cytokines, and metabolites further supplement the action of these factors. Moreover, the dense granules of platelets also release different catecholamines, neurotransmitters, and other cellular and non-cellular mediators, such as adenosine, adenosine diphosphate, adenosine triphosphate, calcium, dopamine, histamine, and serotonin (2,3,7).

Besides their influence on chemotaxis and cell migration via chemical mediation, growth factors also induce mitosis, contribute to the production of ECM, as well as mediate angiogenesis, promoting proliferation, maturation, and differentiation, ultimately leading to tissue repair. Furthermore, during wound healing, activation and migration of mesenchymal stem and stromal cells (MSC) to the wound site also play an integral role in the final process together with native tissue (2,3,7).

Preparation and Types of PRP Concentrates

When preparing a PRP concentrate, the liquid and solid fractions of a whole blood sample are separated in a test tube using plasmapheresis through a single or 2-phase centrifugation process (Fig. 1) (2,3,7,8).

The initial whole blood sample is most often collected in the presence of an anticoagulant, which binds calcium and prevents the initiation of the clotting cascade through inhibition of the conversion of prothrombin to thrombin. Some studies have also reported the utilization of PRP concentrates prepared in the absence of an anticoagulant. However, it should be taken into account that in such circumstances, the time required between whole blood collection and injection must be substantially shortened (8-10).

With regards to the potential effects that different anticoagulants can have on the final PRP, Hemeda et al showed that heparin concentration was critical for the culture of MSCs in human platelet lysate media, with high concentrations of both unfractionated heparin and low-molecular-weight heparin leading to impaired cellular proliferation in a dose-dependent manner (11). More recently, Amaral et al analyzed how the choice of anticoagulant for blood collection would modulate PRP characteristics as well as its effects on MSC culture (9). The authors showed that concentrates prepared with anticoagulant citrate dextrose solution A (ACD-A) demonstrated both the lowest average platelet count and a low platelet recovery rate; concentrates with sodium citrate showed medium average count and a high platelet recovery rate; preparations with ethylenediaminetetraacetic acid (EDTA) showed the highest average count and a high platelet recovery rate. As for the effects on platelet morphology, ACD-A and sodium citrate demonstrated no effect on platelet morphology, while EDTA was shown to increase mean platelet volume (MPV), possibly indicating platelet activation. With regards to their effect on growth factor release, no significant differences were found in TGFβ-1 and VEGF concentration between the 3 anticoagulants when measured by enzyme-linked immunosorbent assay (ELISA). With respect to the effects of different anticoagulants on bone marrow-derived mesenchymal stromal cell gene expression (BM-MSC), sodium citrate was shown to provoke the least amount of change in gene expression between the 3, when compared to a control group (Table 2) (8-10).

Other variations in the preparation protocols of PRP concentrates should be taken into consideration when analyzing PRP literature, as these variations between protocols increase the difficulty in determining the relative efficacy of different systems. Currently, there are at least 40 commercial kits available on the market that claim to separate and concentrate various components of whole blood (2,12). Differences in the preparation process and the final concentrates obtained by these systems include variations in the volume of whole blood collected initially, centrifuge time, the final concentration of platelets in plasma (as compared to the baseline), the final volume of PRP in the syringe, presence or absence of leukocytes, the utilization of mechanical or biochemical platelet

Table 1. *The most important α-granule growth factors and their contributions to the process of tissue healing and repair.*

Growth Factor	Role in Tissue Healing and Repair
bFGF-1	1. May contribute to stimulate angiogenesis; 2. Mediates cell migration; 3. Stimulates proliferation of capillary endothelial cells; 4. Influences fibroblasts to produce collagenase; 5. Contributes to the production of granulation tissue.
EGF	1. Mitogenic factor; 2. Stimulates the proliferation and differentiation of fibroblasts, epidermal and epithelial cells; 3. May play an enhancer role on osteogenic differentiation of mesenchymal stem cells (by increasing ECM mineralization).
HGF	1. Mitogenic factor; 2. Promotes angiogenesis (particularly after ischemia); 3. Accelerates healing by promoting the dedifferentiation of epidermal cells; 4. Regulates cell growth, cell motility, and morphogenesis.
IGF-1	1. Expressed mainly in the early inflammatory phase; 2. Anabolic effects; 3. Stimulates protein synthesis, the proliferation of myoblasts and fibroblasts; 4. Enhances collagen and ECM matrix; 5. May contribute to early modulation of edema.
PDGF-AB	1. Expressed in the early stages of tendon repair; 2. Facilitates the proliferation of other growth factors; 3. Attracts mesenchymal stem cells and leukocytes; 4. Stimulates angiogenesis; 5. Contributes to tissue remodeling.
TGF-β1	1. Proinflammatory type 2 cytokine (antibody response enhancer); 2. Immunosuppressant during the early inflammatory phase; 3. Aids in cell migration and fibronectin binding; 4. Augments production of tendon sheath fibroblasts; 5. Improves tendon mechanics during the healing process; 6. Controls angiogenesis and fibrosis.
VEGF	1. Expression peaks in the late inflammatory and proliferative phases; 2. Promotes angiogenesis (neovascularization).

Legend: bFGF-1, Basic fibroblast growth factor one; ECM, Extracellular matrix; EGF, Epidermal growth factor; HGF, Hepatocyte growth factor; IGF-1, Insulin-like growth factor 1; PDGF-Aβ, Platelet-derived growth factor AB; TGF-β1, Transforming growth factor β1; VEGF, Vascular endothelial growth factor.

activation methods, and pH. Among others, this was analyzed in detail by Castillo et al (12). In their study, the authors compared the composition of single-donor autologous PRP produced by 3 commercially available PRP separation systems and concluded that although there were no significant differences in mean PRP platelet, red blood cells (RBC), active TGF-β1, or fibrinogen concentrations among the 3 PRP separation systems; there were significant differences in platelet capture efficiency, concentrations of leukocytes, PDGF-αβ, platelet-derived growth factor ββ (PDGF-ββ), and VEGF. Moreover, given the differences found between leukocyte concentrations, the authors noted that only one of the systems produced leukocyte-depleted PRP, while the other 2 produced leukocyte-enriched PRP concentrates (Tables 3, 4, and 5) (12).

One other key difference to take into account when analyzing different PRP concentrates is the presence or absence of leukocytes in the preparations. After centrifugation, the whole blood sample separates into a clear plasma layer on top, a buffy coat layer consisting of leukocytes and platelets in the middle, and RBC at the bottom. Because the inclusion of leukocytes in the final preparation may affect the potency and efficacy of the final product, the more generic term "PRP" does not distinguish between different products with different specificities. For this reason, the more specific term "leukocyte-rich PRP" (LR-PRP) has been used to describe a PRP preparation with a leukocyte count superior to the patient's baseline, while the term "leukocyte-poor PRP" (LP-PRP) has been utilized to describe a PRP preparation with a leukocyte count inferior to the patient's baseline. This classification system has allowed for a better description of the type of end product, as well as a better categorization of its specific biological effects (8,13).

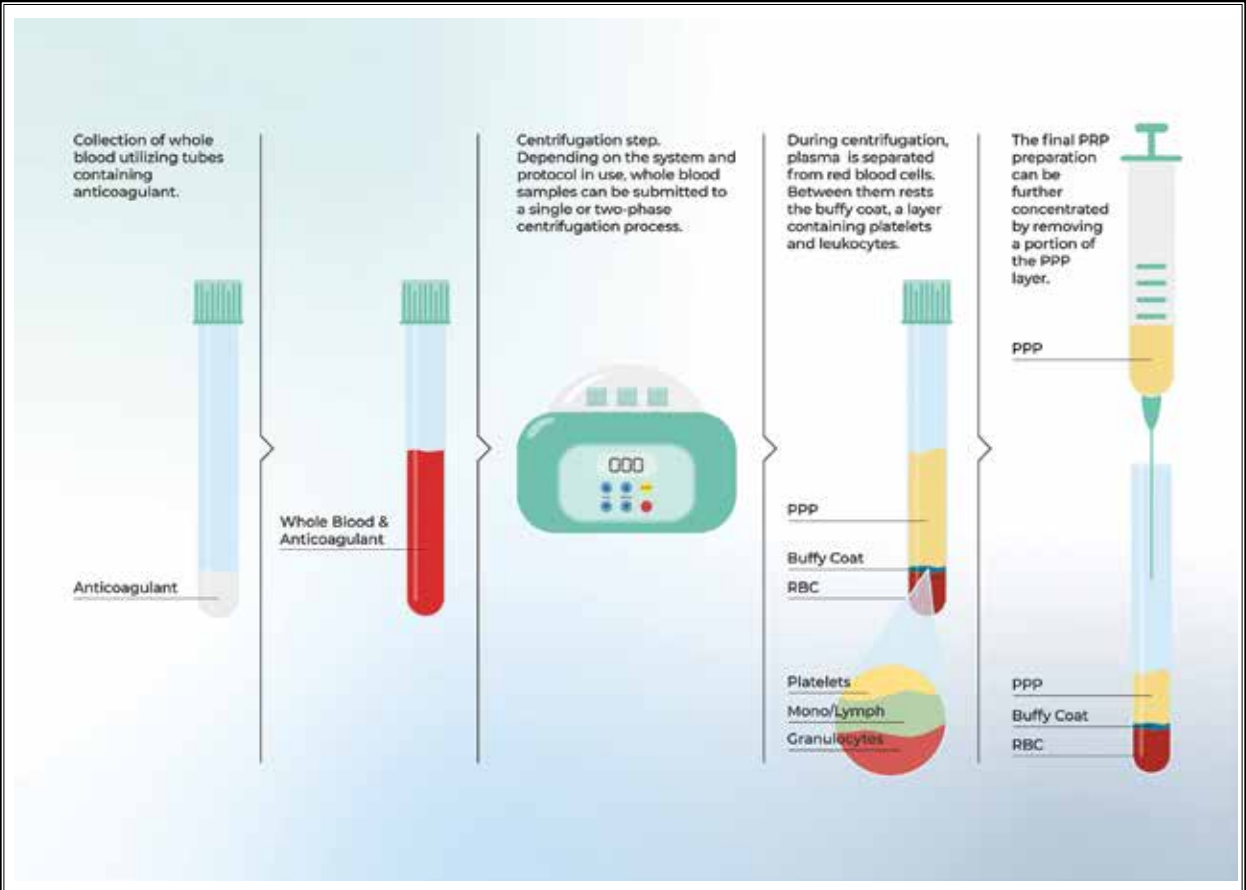

Fig. 1. *Platelet-rich plasma separation process in a test tube using plasmapheresis, through a single or two-phase centrifugation process. During the first centrifugation phase, or "soft spin," plasma and platelets are separated from red blood cells and leukocytes. A second phase, or "hard spin," may be performed in order to further concentrate and separate the platelet-rich and platelet-poor components.*

Table 2. *Anticoagulants utilized for platelet-rich plasma concentrate preparation and their effects on platelet count and recovery, morphology, platelet morphology, α-granule growth factor release, and bone marrow-derived mesenchymal stromal cell gene expression.*

Anticoagulant	Platelet Count / Recovery Rate	Platelet Morphology	Growth Factor Release	BM-MSC Gene Expression
ACD-A	Lowest average count / 45% of total	No influence on MPV	No significant differences in TGFβ-1 and VEGF concentration	30% different from control group
EDTA	Highest average count / 76% of total	MPV increase, likely indicating platelet activation		47% different from control group
Sodium Citrate	Medium average count / 81% of total	No influence on MPV		25% different from control group

Legend: ACD-A, Anticoagulant citrate dextrose solution A; BM-MSC, Bone marrow-derived mesenchymal stromal cells; EDTA, ethylenediaminetetraacetic acid; MPV, Mean platelet volume; TGF-β1, Transforming growth factor β1; VEGF, Vascular endothelial growth factor.
Adapted from: Amaral RJ, da Silva NP, Haddad NF, et al. Platelet-rich plasma obtained with different anticoagulants and their effect on platelet numbers and mesenchymal stromal cells behavior in vitro. Stem Cells Int. 2016; 2016:7414036.

Lastly, PRP separation protocols can also vary in the platelet activation method utilized. The term "activation" refers to 2 key processes that are initiated during PRP preparation: first, the degranulation of platelets to release growth factors from their α-granules, and second, the cleavage of fibrinogen to initiate matrix

Table 3. *Head-to-head comparison of the protocols for platelet-rich plasma separation from 3 different commercial systems.*

Commercial Systems	Whole Blood Volume (mL)	Anticoagulant Utilized	Centrifuge Force (g) / Centrifuge Time	Final Volume of PRP (mL)
Cascade (MTF Sport Medicine, Edison, New Jersey)	18	Sodium citrate, 2 mL	1100 / 6 min	7.5
GPS III (Biomet Orthopedics Inc, Warsaw, Indiana)	55	ACD-A, 5 mL	1100 / 15 min	6.0
Magellan (Arteriocyte Inc, Cleveland, Ohio)	26	ACD-A, 5 mL	1200 / 17 min	6.0

Legend: ACD-A, Anticoagulant citrate dextrose solution A; PRP, Platelet-rich plasma.
Adapted from: Castillo TN, Pouliot MA, Kim HJ, Dragoo JL. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. *Am J Sports Med* 2011; 39(2):266-271.

Table 4. *Comparison of mean platelet concentration and platelet capture efficiency, red blood cell, white blood cell, and fibrinogen concentrations between three different platelet-rich plasma separation systems.*

Commercial Systems	Platelet Concentration (x103/μL)	Platelet Capture Efficiency Rate (%)	Red Blood Cells (x103/μL)	White Blood Cells (x103/μL)	Fibrinogen (mg/dL)
Cascade (MTF Sport Medicine, Edison, New Jersey)	443.8 ± 24.7	67.6 ± 4.1	0.1 ± 0.1	1.1 ± 0.2	283.8 ± 34.2
GPS III (Biomet Orthopedics Inc, Warsaw, Indiana)	566.2 ± 292.6	22.6 ± 11.8	1.5 ± 1.7	34.4 ± 13.6	286.0 ± 42.7
Magellan (Arteriocyte Inc, Cleveland, Ohio)	780.2 ± 246.5	65.5 ± 19.6	0.5 ± 0.3	11.0 ± 8.2	277.4 ± 30.5

Adapted from: Castillo TN, Pouliot MA, Kim HJ, Dragoo JL. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. *Am J Sports Med* 2011; 39(2):266-271.

Table 5. *Comparison of mean growth factor concentrations between 3 different platelet-rich plasma separation systems.*

Commercial Systems	PDGF-αβ (ng/mL)	PDGF-ββ (ng/mL)	TGF-β1 (ng/mL)	VEGF (ng/mL)
Cascade (MTF Sport Medicine, Edison, New Jersey)	9.7 ± 3.6	14.8 ± 2.5	0.1 ± 0.08	0.3 ± 0.3
GPS III (Biomet Orthopedics Inc, Warsaw, Indiana)	18.7 ± 12.8	23.1 ± 10.1	0.1 ± 0.08	2.4 ± 1.1
Magellan (Arteriocyte Inc, Cleveland, Ohio)	34.4 ± 10.7	33.0 ± 8.2	0.2 ± 0.1	1.2 ± 0.8

Legend: PDGF-αβ, Platelet-derived growth factor αβ; PDGF-ββ, Platelet-derived growth factor ββ; TGF-β1, Transforming growth factor β1; VEGF, Vascular endothelial growth factor.
Adapted from: Castillo TN, Pouliot MA, Kim HJ, Dragoo JL. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. *Am J Sports Med* 2011; 39(2):266-271.

formation, a clotting process that allows the formation of a platelet gel, and therefore confines the secretion of molecules to the injection site (1,13). Several of the commercial kits available on the market already include an activation step before PRP administration, commonly by adding thrombin and/or calcium chloride. However, external activation may not be necessary, as PRP activates when in contact with fibrillar collagen. For this reason, some commercial systems do not add any activator to their concentrates before injection, preferring to rely on the spontaneous platelet activation occurring after exposure to the native collagen present in the connective tissues (1,13).

Considering all the above-mentioned variations between commercial PRP separation systems, in addition to operator variability, the success or failure of a specific PRP product for a specific condition cannot be universally applied to all PRP products. This limits the interpretation of available data and the ability to draw meaningful conclusions.

From Interpersonal Variations in Whole Blood Samples to a Working Definition of Platelet Concentrate

One important aspect to take into consideration when addressing the standardization of PRP separation protocols is the variation of components in whole blood collected from 2 different individuals, both in absolute number and relative proportions. Moreover, the same variation may also occur in whole blood samples collected from the same patient at different times (14).

While the normal range of platelets in the whole blood of a healthy individual is 150,000 to 350,000 platelets/μl, past consensus published in the literature has agreed on a working definition of platelet concentrate (PC) as a preparation with a minimum of 1.0 x 10^6 platelets/μl. In other words, for a preparation to be classified as PRP, the respective concentration of platelets should be increased by 3 to 5 fold over the normal baseline (14,15).

Variations in platelet concentrations among individuals, in addition to the daily variation in platelet parameters observed within individuals, can further affect the consistency, efficacy, and clinical outcomes of the final product (14-16). As discussed previously, the final platelet concentration of any PRP preparation is based on the initial volume of whole blood collected, the platelet recovery efficiency of the technique used, and the final volume of plasma used to suspend the concentrated platelets. As such, changing any of the aforementioned variables will proportionally change the final platelet concentration of the preparation (14,15).

Biological Effects of PRP

Although initially utilized in the early 20th century for the treatment of dermatologic and maxillofacial conditions, interest in the clinical applications of PRP grew exponentially in the last 2 decades with the development of the field of regenerative medicine. In addition to being employed as an adjunct to surgical reconstruction, PRP has been widely studied and utilized for the treatment of several chronic pain conditions, including osteoarthritis (OA), chondropathy, muscle and/or ligament tear, and tendinopathy (2-4). More recently, several studies have also looked at possible applications of PRP for the treatment of chronic neuropathic pain related to peripheral nerve injury (17-19).

As discussed earlier, proteins such as bFGF-1, PDGF-αβ, and VEGF can be detected in high concentrations in autologous PRP preparations. This led several researchers to postulate that PRP may have a beneficial effect on the process of tissue healing. In addition, local injection of PRP gel has also been shown to promote a systemic inductive effect, triggering a transient increase in serum levels of IGF-1, VEGF, and basic fibroblast growth factor 2 (bFGF-2) (20,21). Conversely, other proteins present in PRP concentrates are known for their inhibitory effects, including TGF-β1, which may lead to variable clinical results in applications in different local tissues and conditions. Overall, although growth factors are key components that mediate tissue healing through effects on cell migration, proliferation, differentiation, as well as deposition of ECM, remodeling, and maturation, their exact activities in situ are unknown to date (2,20).

Question Left Unanswered 1: Is There a Dose-Effect Relation Between the Platelet Count and the Clinical Efficacy of the Preparation?

Findings to Date: Platelet count does not show a direct relation with the concentration of α-granule growth factors released nor with the clinical efficacy of the concentrate.

From 2000 to 2020, at least 30 clinical studies (retrospective, single-blind and double-blind prospective trials, and systematic reviews with meta-analysis) were published on the utilization of PRP for the treatment of several chronic pain conditions of the musculoskeletal system (bone, joint, muscle, tendon, and ligament). In these studies, the platelet count of the autologous PRP concentrates utilized varied from 4 to 10 times the baseline count (2).

Taking the above-mentioned into consideration, coupled with the fact that the effects of PRP concentrates are thought to derive mainly from the release of growth factors stored in α-granules, it would be logical to assume that the studies in which PRP preparations with higher platelet count were utilized showed overall better clinical outcomes. Nonetheless, not only did this not happen, but some of the studies that reported better outcomes utilized PRP concentrates with platelet counts of 4 to 6 times the baseline count. This may be explained by the fact that concentrations of platelets and growth factors do not exhibit a directly proportional relationship (13,22). Recently, Hsu et al concluded that a higher concentration or the absolute number of platelets within the PRP concentrate might not necessarily lead to an enhanced effect on tissue healing (22). In addition, Kobayashi et al demonstrated that leukocyte concentrations could also have direct effects on both growth factor and catabolic factor concentrations within the PRP solution (13).

Previously, Giusti et al had already suggested that the most efficacious platelet concentration for tissue healing is 1.5 x 106 platelets/uL (23). In their work, the authors postulated that the dose-response curve of PRP is not linear, and a saturation effect occurs that is accompanied by the activation of an inhibitory cascade once a sufficiently high concentration of platelets is achieved. Given that platelets seem to exert the great-

est influence on healing during or early after the inflammatory phase of an injury, some authors, like Hsu et al and Rodeo et al, have suggested that the timing of the administration of PRP might have a greater influence on healing than the absolute number of platelets. Other authors have stressed that the accurate location of injections may be the primary factor determining clinical efficacy (22,24).

Moving forward: Future studies should elucidate the dose-response curve of PRP, as well as determine the most efficacious platelet concentration for tissue healing.

Question Left Unanswered 2: What Pathology Determinants Should Be Considered When Selecting Between Leukocyte-Enriched and Leukocyte-Depleted Concentrates?

Findings to Date: LR-PRP has shown more promising results in the treatment of tendinopathy and might be detrimental when injected into joints. LP-PRP appears more efficacious when administered into joints exhibiting mild to moderate arthritic changes.

When cultured with tendon stem cells (TSC) isolated from the healthy patellar tendons of rabbits, both LR-PRP and LP-PRP induced similar TSC differentiation into active tenocytes. Nonetheless, while LR-PRP induced predominantly catabolic and inflammatory changes in differentiated tenocytes, such as increased matrix metalloproteinase (MMP) 1, MMP-13, IL-1β, IL-6, and TNF-α, LP-PRP induced predominantly anabolic effects such as increased gene expression of anabolic genes, alpha-smooth muscle actin, and collagen types I and III (24-26). This may help explain, at least in part, why LR-PRP has shown more promising clinical results in the treatment of tendinopathy when compared to LP-PRP. Although in the acute phase, LR-PRP may exert detrimental effects due to its catabolic activity, the same catabolic activity coupled with the proinflammatory changes appears vital in its role in the treatment of chronic tendinopathy (2,25-28). Conversely, the use of LP-PRP in soft tissue injury may result in excessive scar formation due to the strong potential of inducing inordinate anabolic effects, especially when administered in the acute phase (25,26).

When cultured with synovial fibroblasts isolated from patients with osteoarthritis undergoing joint surgery, LR-PRP induced a greater increase in the proinflammatory factors IL-1β, IL-8, and bFGF-2, while at the same time decreasing anti-catabolic factors in chondrocytes, such as HGF and tissue inhibitor of MMP-4 (25,29). This may help explain why LP-PRP has shown superior in vivo results when administered to patients exhibiting signs of mild to moderate OA due to its predominant anti-inflammatory and anabolic effects (2,25,27-29).

More recently, the perception of the noxious role of neutrophils in PRP concentrates has begun to shift, as researchers demonstrated that the interaction between neutrophils and activated platelets could release anti-inflammatory products. Parrish et al showed that activated platelets release arachidonic acid, which is absorbed by neutrophils and converted into leukotrienes and prostaglandins, both proinflammatory in nature (30). Nonetheless, the authors also demonstrated that activated platelets in association with neutrophils could take up leukotrienes and convert them into lipoxins, a potent anti-inflammatory protein that limits neutrophil activation and prevents diapedesis, driving the resolution phase of the healing cascade. This production of lipoxins through activated platelets is only possible in the presence of leukotrienes, which in turn require the presence of leukocytes to be produced (25,30,31).

Macrophages (both M1 and M2), through their plasticity properties, may also play a critical role in the promotion of the inflammatory process that is required to initiate the healing cascade, a process which Lana et al termed "regenerative inflammation" (25). In addition, as discussed earlier, macrophages are indispensable for the proliferative and remodeling phases of the healing process.

The effect of LR-PRP and LP-PRP on the healing process of several types of injuries and pathologies remains to be investigated. Furthermore, to our best knowledge, there have been no clinical studies published comparing the use of LR-PRP and LP-PRP, and several of the available in vivo studies in the literature do not detail the type of PRP used.

Moving Forward: Future studies should elucidate what conditions benefit from leukocyte-enriched concentrates as opposed to leukocyte-depleted PRP preparations and in what stage of the healing process the therapeutic effect of each type of concentrate is optimal.

Question Left Unanswered 3: What Is the Role of Platelet Activation Methods on the Clinical Efficacy of PRP Concentrates?

Findings to Date: The choice of activation method was shown to influence the physical form of PRP concentrates, the amount of platelet-derived growth factors released, and the kinetics of the release process.

To date, several different physiological/biochemical

and mechanical methods of in vitro platelet activation have been tested (coagulation with calcium chloride, activation with adenosine diphosphate, fibrillar collagen type I, thrombin, thrombin receptor activating peptide, or zeolite) (1). As stated earlier, several of the commercial systems available on the market already include an activation step in their PRP separation protocol, most commonly by adding thrombin and/or calcium chloride (2).

Recently, Cavallo et al compared the effects of different methods of PRP activation (calcium chloride, thrombin, calcium chloride with thrombin, and fibrillar collagen type I) on the content of both platelet-derived growth factors and cytokines, as well as their release kinetics (32). In their study, the authors reported that the choice of activation method influenced PRP clot formation, leading to differences not only in the physical form of the concentrate but also in the amount and release kinetics of platelet-derived growth factors. In specific, PRP activated with calcium chloride, thrombin, and calcium chloride with thrombin formed clots detected from the 15-minute evaluation, whereas in samples activated with fibrillar collagen type 1, no clot formation was noticed. Furthermore, samples activated with fibrillar collagen type 1 produced an overall lower release of platelet-derived growth factors. In addition, thrombin, calcium chloride with thrombin, and collagen type I activated PRP samples showed an immediate release of PDGF-$\alpha\beta$ and TGF-β1 that remained stable over time, whereas VEGF showed an increasing trend from 15 minutes up to 24 hours. In contrast, samples activated with calcium chloride induced a progressive release of growth factors from 15 minutes and increasing up to 24 hours (32).

The influence of different activation methods on platelet-derived growth factor release kinetics may also affect clinical efficacy. As was shown by DeLong et al (33), rapid activation has been associated with a decrease in the total amount of growth factors available at the tissue site over time. As growth factors possess a short half-life (minutes to hours), they might be degraded before additional tissue receptors become available, if not immediately used, upon release from platelets (32-34). From a clinical perspective, this may help explain some of the less promising results reported by studies analyzing the effects of different PRP concentrates on musculoskeletal tissue regeneration (32,34).

The effect of different activation methods on the physical form of PRP concentrates, which can range from liquid to solid gel, is also an important aspect to consider for the successful application of PRP in chronic pain conditions. As shown by Cavallo et al, PRP concentrates activated with fibrillar collagen type 1 exhibited far less aggregation than concentrates activated by other methods, with no visible clots formed for up to 24 hours (32). Although the lack of a clot might not be a problem in the treatment of OA, where a liquid PRP preparation allows for all articular tissues to be targeted without the risk of dispersion from the closed joint cavity, a PRP preparation in liquid form may be unsuitable for other applications (32,35).

Moving Forward: Future studies should elucidate the clinical efficacy of PRP concentrates activated by different methods in different tissues and whether different activation methods and PRP physical forms are more appropriately selected for specific target tissues.

Question Left Unanswered 4: Is There an Optimal Number of Injections and Time Frame for Application of Multiple Injection Treatment Cycles?

Findings to Date: Current literature suggests that, within a 6-month interval, a single PRP injection may be as effective as multiple (2 or 3) PRP injections with regards to pain improvement in patients with knee OA. Multiple injection cycles may show superior long-term results in the treatment of chronic tendinopathy, although single PRP injections seem to provide better pain control in the short term.

In a recently published systematic review and meta-analysis, Vilchez-Cavazos et al analyzed 5 clinical trials (a total of 301 patients) on the clinical effectiveness of single versus multiple PRP injections in the treatment of knee OA (36). The authors' main findings suggested that within a 6-month interval, a single injection was as effective as multiple (2 or 3) PRP injections with regards to pain improvement and that 3 injections were more effective than a single injection in functionality improvement (36). However, several important differences between the trials included in the meta-analysis led to high heterogeneity and should be taken into consideration when analyzing the results in detail. Firstly, although all the studies analyzed knee OA, its severity varied among the trials; in addition, the platelet count between the preparations utilized also differed between studies. Lastly, while Patel et al and Simental-Mendía et al utilized an activated LP-PRP preparation, Görmeli et al and Kavadar et al employed an activated LR-PRP concentrate, and Uslu et al used a non-activated LR-PRP solution (37-41).

Multiple (2 or 3) injection cycles may show superior long-term results in the treatment of chronic tendinopathy. In a systematic review and meta-analysis on the effectiveness of nonsurgical treatment options for patellar tendinopathy, Andriolo et al compared the clinical outcomes of different therapeutic modalities, including single and multiple PRP injections (15 PRP studies in total, including prospective case series and prospective comparative trials). The authors reported that while a single PRP injection led to better short-term results (< 6 months) with regards to pain improvement, multiple PRP injection cycles demonstrated higher pain improvement at longer follow-up times (> 6 months) (42). However, as was the case with PRP trials concerning knee OA, differences found in platelet count, presence or absence of leukocytes, activation methods employed, storage procedures, number of injections, and the interval between injections also led to high heterogeneity between the tendinopathy studies analyzed.

Several authors have proposed different explanations as to why a single PRP injection may provide better short-term pain relief when compared to multiple injection cycles. Zayni et al (43) argued that single injection treatments might have a faster onset of action than multiple injections, as the latter is generally associated with more protracted injection-related discomfort and require a delay in resuming physical activities. In contrast, a single PRP injection may be less efficient in obtaining the desired biological benefits since most platelet-derived growth factors are relatively short-lived, and the benefit of their local infiltration may dissipate over time (42,43).

Current literature concerning an optimal time frame for the application of multiple PRP injection cycles is limited. To our best knowledge, to date, there have been no head-to-head trials published comparing the efficacy of short (< 1 month) versus long intervals (> 1 month) between consecutive PRP injections for the treatment of chronic pain conditions. Based on the short half-life of platelet-derived growth factors, authors like Zayni et al among others argued that the period between injections (in a multiple infiltration treatment cycle) should be relatively short, between 1 and 2 weeks (42,43).

Moving Forward: Future studies should focus on shedding light on the clinical efficacy of single versus multiple PRP injection treatment cycles in different clinical settings and tissues. In addition, future research should also focus on determining the optimal time frame for the application of multiple injection cycles in different target tissues.

Question Left Unanswered 5 - Does the Addition of Local Anesthetics Affect the Clinical Efficacy of PRP?

Findings to Date: Recently published in vitro studies have shown that the addition of local anesthetics to PRP concentrates can result in a significant decrease in platelet function. The addition of epinephrine may partially inhibit the negative effect of lidocaine on platelet aggregation capacity.

Local infiltrations of PRP concentrates have been found to be painful in several different clinical settings, which in turn has prompted some clinicians to add local anesthetics to their PRP concentrates in order to minimize post-procedural discomfort (44,45). However, the effect that the addition of local anesthetics can have on the clinical efficacy of PRP remains a controversial and poorly understood topic in the literature (44,45).

Recently, Bausset et al studied the in vitro effects of lidocaine with or without epinephrine (10 mg/mL, 1/200.000) and ropivacaine (7.5 mg/mL) on platelet aggregation capacity and platelet-derived growth factor (PDGF-$\alpha\beta$ and TGF-β1) release after platelet activation (44). The authors reported that, although the addition of local anesthetics did not interfere with the release of platelet-derived growth factors when analyzed by ELISA, it resulted in a significant decrease in platelet functionality when assessed by light transmittance aggregometry. The use of epinephrine combined with lidocaine enhanced platelet aggregation when compared to lidocaine alone (44).

Previously, Carofino et al had already reported that the addition of either lidocaine (1%) or bupivacaine (0.5%) to PRP had an in vitro inhibitory effect on the proliferation of human tenocytes treated in a culture medium enriched with 10% fetal bovine serum (45).

One important consideration to have in mind when analyzing the results of the above-mentioned studies in detail is that the in vitro behavior of platelets may not mimic the in vivo environment. Several other difficulties also arise when trying to reproduce environment injection conditions in vitro, especially pertaining to the local concentrations of anesthetics: immediately after an in vivo injection, the surrounding fluids quickly dilute the local anesthetics present in the preparation (44,45).

Moving Forward: Future studies should focus on

elucidating the in vivo effects of local anesthetics on the clinical efficacy of PRP concentrates in different target tissues.

Question Left Unanswered 6 - Is There Potential for Future PRP Applications for the Treatment of Neuropathic Pain of Peripheral Origin?

Findings to Date: In preclinical studies, PRP was shown to improve neuropathic pain by triggering enhanced inflammation and its resolution, including the regenerative process resulting in axon regeneration and local reinnervation.

Peripheral neuropathic pain most often results from trauma-induced nociceptive neuron hyperexcitability and subsequent generation of spontaneous ectopic activity. Typically, the pain persists until the trauma-induced cascade of events runs its full course, resulting in complete tissue repair, including the nociceptive neurons recovering their normal biophysical properties, ceasing to be hyperexcitable, and stopping having spontaneous electrical activity. However, the injury site may undergo insufficient or too much inflammation, which leads to the development of chronic neuropathic pain (46,47).

In the past, PRP has been applied to reduce neuropathic pain caused by arthroplasty, transgluteal decompression of the pudendal nerve, and to the surgical site following tonsillectomy, among others (46,48). In addition, PRP concentrates have been shown to induce axon regeneration in several preclinical studies performed in animal models, and it may be through axonal regeneration that PRP might assist in reducing neuropathic pain. (46,49,50). In one preclinical study in a rat model, PRP was shown to induce axonal regeneration following a crush injury to the sciatic nerve. The authors noted that this promotion of axonal regeneration was likely due to the local increase of IGF-1, a neurotrophic factor, and VEGF, an angiogenic factor that has also been previously shown to stimulate axonal outgrowth and enhance Schwann cell proliferation (46,49).

PRP concentrates also contain multipotent MSC, which have previously been shown to enhance axon regeneration when applied to the end of transected peripheral nerves, likely resulting from their secretion of nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF), as well as their promotion of local angiogenesis (46,50-52). Another set of recent preclinical studies suggested that MSC present in PRP concentrates may promote axon regeneration through differentiation into a Schwann cell phenotype, which was shown to induce axonal regeneration equivalent to that of Schwann cells in vitro and in vivo (52-54).

Taking the above-mentioned into consideration, PRP may play an important role in assisting with neuropathic pain by triggering enhanced inflammation and its resolution, followed by the full cascade of the injury healing process, including the regenerative process resulting in axon regeneration and target reinnervation. These in turn may allow axons to uptake target-released factors that eliminate nociceptive neuron hyperexcitability, thereby decreasing/eliminating neuropathic pain.

Moving Forward: Future studies should focus on elucidating the therapeutic effects of PRP concentrates in neuropathic pain of peripheral origin in clinical scenarios.

Conclusions

Although autologous PRP concentrate have shown overall promising results regarding its therapeutic effect in multiple chronic pain conditions, including possible future applications for the treatment of neuropathic pain of peripheral origin, study heterogeneity and lack of standardization between PRP separation protocols undermine the interpretation of available data and the ability to draw meaningful conclusions for clinical practice.

By addressing what we believe to be the biggest questions still unanswered regarding the therapeutic applications of autologous PRP concentrates in chronic pain conditions, we hope that our work may help in establishing a theoretical framework for future research. By doing so, we aim to contribute to the ultimate goal of achieving an international consensus on the optimal protocol for autologous PRP separation, as well as its definitive applications in Pain Medicine.

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