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OPTIMIZING PLATELET-RICH PLASMA (PRP) INJECTIONS: A NARRATIVE REVIEW

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ABSTRACT

Platelet-rich plasma (PRP) is an orthobiologic treatment that has gained popularity as a potential alternative treatment for various musculoskeletal conditions. The physiologic role of platelets in the healing cascade provides clarity regarding its potential as it releases various growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta-1 (TGF-β1), and vascular endothelial growth factor (VEGF). However, there are various characteristics of PRP treatments including platelet count, presence or absence of leukocytes and red blood cells, as well as the use of an activating agent that introduces heterogeneity among preparations. This aim of this article is to provide clarity, where available, regarding the optimal characteristics for PRP treatments regarding tendon and ligament injuries as well as articular and muscular pathology.

Key words: *Articular Cartilage, Platelet-Rich Plasma, PRP, Tendinopathy, Osteoarthritis*

INTRODUCTION

Platelet-rich plasma (PRP) is an autologous product derived from whole blood that contains concentrated platelets. These platelets secrete growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta-1 (TGF-β1), and vascular endothelial growth factor (VEGF) that contribute to its effectiveness for various conditions.[1] Studies have shown that over 8,000 athletes annually are treated with PRP for various musculoskeletal conditions originating from tendon, ligament, or muscular sources.[2] However, there continues to be a discrepancy among the consistency and preparation of PRP products.[3,4] Various classifications systems have been proposed to bring uniformity to these preparations and treatments.[5,6] Mautner, Malanga, Smith, et al., devised a classification system identifying the total platelet number injected, the presence of leukocytes and red blood cells, as well as if an activator was used (PLRA),

to provide clarity and consistency among clinicians and researchers.[7]

This article aims to review the different factors that can influence PRP composition and to provide clarity, where available, on the optimal preparation for use in tendon, ligament, joint, and muscle-related pathologies.

METHODS

A review was conducted of PubMed, Medline, and Google Scholar articles with search terms including osteoarthritis, articular cartilage, platelet count, leukocytes, erythrocytes, red blood cells, activation, ligament, ACL, hamstring, myotendinous, muscle, tendinosis, tendinopathy, platelet-rich plasma, PRP, randomized controlled trials (RCT), case series, case reports, animal studies, basic science investigations, systematic reviews, and meta-analyses were reviewed. Only studies investigating tendinopathy, osteoarthritis, and ligamentous or muscle injuries were included. We did not include studies investigating other orthobiologic injections such as stem cells, lipoaspirate, and bone marrow aspirate for osteoarthritis, tendinopathy, or muscle injuries. Articles chosen included both basic science and clinical research as certain areas, such as articular cartilage in the knee, have more well-designed studies compared to tendon or muscle-related research. We sought to include systematic reviews and randomized controlled trials when available.

PLATELET COUNTS

Platelets originate from the bone marrow stem cell lineage of megakaryocytes and contain bioactive proteins that play an important role in the process of healing.[3,8] The normal range for platelet count in blood is 1.5 to 4.0 x10^5 platelets/µl.[9] When preparing PRP, the system of preparation can introduce heterogeneity in the final platelet count which can affect therapeutic outcomes.[10] Magalon, Bausset, Serratrice, et al., compared five different preparations and looked at outcomes related to cellular counts as well as growth factor concentrations.[11] Their team found significant variability among the preparations which could contribute to the diverse outcomes seen in the literature. Moreover, the authors noted that platelet dose had a positive correlation with the number of growth factors delivered.

Literature has shown that the number of platelets can be increased based on a variety of factors. In particular, Davis, Boyd, McKinney et al.,[12] evaluated the effect of exercise in six patients over 12 weeks. The authors noted an increment of greater than 50% at week 12 compared to their week one values. A similar result was seen by Anz, Parsa, Romero-Creel, et al.,[13] in which 20 subjects participated in a 20-minute exercise regimen with pre and post blood samples obtained for analysis. The authors noted a 20% increase in the platelet concentration in buffy coat-based PRP, plasma-based PRP, and whole blood following exercise. Hamilton, Tol, Knez, et al., [14] investigated 10 subjects that were exposed to 1 hour of submaximal exercise with results recorded immediately after and 18 hours later. They also demonstrated comparable increases in platelet concentrations as noted in these other studies. However, there was an inverse relationship seen in the samples that were not activated with decreases seen in the concentration of PDGF-β, VEGF, and insulin-like growth factor-1 (IGF-1). In the samples that did receive activation (with calcium chloride) there was an increase in the concentration of the growth factors. Resistance exercises can also have a potential therapeutic role in the realm of PRP given its effect on cell counts.[15,16] Ahmadizad, Nouri-Habashi, Rahmani, et al.,[17] had 21 subjects participate in resistance exercises corresponding to 80% of one repetition maximum and noted a significant increase in platelet count immediately after 30-35 minutes of exercise. This effect, however, did taper off and returned to near baseline levels after 30 minutes of recovery. These studies provide some guidance when determining the role of exercise before a blood draw to optimize PRP content.

In addition to exercise, there are other methods used to increase platelet counts. Animal studies have shown that short term hypoxia (1 to 3 days) can increase platelet counts, but this effect decreases as exposure to hypoxia continues.[18] Blood flow restriction (BFR) training has been hypothesized to increase muscle mass and function by completing an ischemic and hypoxic metabolic exercise while performing low-load resistance training for short periods.[19,20] Therefore,

BFR may be of interest to scientists and clinicians who are performing PRP treatments. A study by Bashafaa, Fallahu, Nazifi, et al., [21] evaluated the effects of cycling and blood flow restriction on hematologic factors. Their research noted a significant increase in not only platelet count, but also white blood cells, hemoglobin, and hematocrit. Though the number of studies is limited, BFR may serve as a nonpharmacologic mechanism to increase platelet count and can be of use in preparing PRP.

LEUKOCYTES AND PRP

With the evolution of PRP treatments, there have been multiple studies investigating the effects of leukocytes on PRP preparations, though the exact role is not fully understood.[22-27] Leukocytes can be broken down into 5 major subtypes including neutrophils, eosinophils, basophils, lymphocytes, and monocytes.[28] Neutrophils are the most abundant and the first to respond during infection and/or injury.[29] However, when neutrophils degranulate, they release enzymes such as collagenase, elastase, and gelatinase which cause degradation of cartilage matrix and increased production of reactive oxygen species (ROS).[30] The inclusion of neutrophils appear to be detrimental to outcomes following PRP.[31] Monocytes, however, coordinate efforts between the adaptive and innate immune response, phagocytize, and promote repair of damaged tissues by secreting cytokines and chemokines that appear to aid in tissue healing.[32,33] Monocytes differentiate into macrophages and when exposed to interleukin-4 (IL-4), an increase in levels of arginase-1 is seen, which leads to an upregulation of precursors for collagen and support extracellular matrix deposition.[33,34] The differentiation of monocytes into their M1 and M2 subtypes may have effects on PRP outcomes.[35] The M1 subtype has been theorized to act as pro-inflammatory whereas the M2 subtype is more anti-inflammatory with implications in wound healing and regeneration.[36]. Lymphocytes recognize pathogens and moderate the inflammatory response through the release of cytokines and antibodies, while also producing cytotoxic granules.[37] Eosinophils and basophils both provide cellular defense against parasites while also mediating allergic reactions.[38] Basophils also release platelet-activating factor (PAF) in response to interleukin-3 (IL-3) which increase vascular permeability.[39] The dissection of the cellular functions of leukocyte subtypes may have critical implications on the use of leukocyte rich (LR-PRP) versus leukocyte poor (LP-PRP) PRP preparations and the breakdown of the specific leukocytes may prove to be most important of them all.

RED BLOOD CELLS AND PRP

Red blood cells (RBCs) are known to have a toxic effect on chondrocytes and synoviocytes secondary to hemosiderin deposition in the synovium, irreversible apoptotic effects of RBC resorption, and viscoelastic change of articular cartilage. [40-42] In PRP preparations, the collection, and centrifugation of whole blood is an essential step to concentrate the essential cellular contents.[3,43-45] During this preparation, high shear forces can cause RBC membrane damage and hemolysis, leading to the release of hemoglobin, iron, and hemin from lysed RBCs.[46] Everts, Malanga, Paul, et al., [47] highlighted the implications of RBCs on PRP as well as other regenerative therapies. In particular, the lack of natural scavenger proteins that would normally clear the byproducts of RBC breakdown is no longer present in the processing of regenerative therapies. Therefore, the presence of plasma free hemoglobin (PFH) and other products of hemolysis create an unstable environment in which ROS are developed, tissue damage occurs, increased cellular apoptosis is noted, and potentially negative outcomes in orthobiologic procedures.

ACTIVATION OF PRP

Activation refers to processes that occur in the preparation of PRP aimed at degranulation of platelets to release growth factors from platelets as well as cleavage of fibrinogen to initiate matrix formation.[48] Activating PRP involves various substances with thrombin and calcium chloride ($CaCl_2$) being the most common.[49,50] The use of thrombin as an activating agent resulted in a greater, rapid initial release of growth factors such as VEGF and PDGF that was superior to $CaCl_2$ and was sustained over a period of 10 days.[51] Type 1 collagen is another activating agent that is particularly attractive due to its native involvement in the clotting cascade.[52] Harrison, Vavken, Kevy, et al.,[53]

compared activating PRP with thrombin or collagen. Thrombin resulted in immediate release of growth factors while PRP activated with collagen released smaller amounts over 5 days. However, the use of collagen as an activator resulted in an 80% greater cumulative release of growth factors. Various agents can influence PRP activation, without a consensus as to which is superior, so it is important to report outcomes with details of activator used.

The use of anticoagulants in preparation of PRP may also influence growth factors and cell counts. Giraldo, Álvarez, and Carmona investigated the effects of sodium citrate (SC) and acid citrate dextrose (ACD) anticoagulants on equine PRP preparations.[54] The authors noted that in all preparations, there was no significant impact on cell counts. The authors did find that PDGF-β was higher in the SC group while ACD group had superior concentrations of TGFβ-1, but differences were not significant. This supports findings by Lei, Gui, and Xiao [55] who also evaluated the effects of heparin, SC, ACD, and citrate-theophylline-adenosine-dipyridamole (CTAD) as anticoagulants for PRP. They noted a similar increase in TGFβ-1 concentration in the ACD and CTAD groups compared to heparin and SC. The rate of spontaneous activation was significantly lower in the ACD group based on an increased number of alpha and dense granules. Although the research is limited, we do know that the selection of anticoagulants and activators should be considered.

PRP FOR TENDONS AND LIGAMENTS

Platelet Count

Preparation of PRP for tendinopathy should begin with an assessment of platelet concentration on outcomes. There are various commercially available kits, with platelet concentration reported to be 1.5x to as high as 9x baseline values.[11,56,57] Regarding tendinopathy, clinicians must be cognizant of the optimal range to ensure the best outcomes. We now know that more is not always better when looking at the effect of PRP on tenocytes. Giusti, Rughetti, D'Ascenzo, et al., [58] noted that in concentrations ranging from 1.5 – 2 x 10^6 platelet/μL, tenocytes were induced to proliferate and migrate. However, platelet concentrates below or above that range were noted to have lower angiogenic and cell proliferation potentials. Another in vitro study investigated the effect of tenocyte stem cells (TSCs) from rabbits cultured in growth media of different PRP concentrations.[59] The authors cultured TSCs in growth media containing various concentrations (0%, 2%, 5%, 10%, 20%) of LR-PRP or LP-PRP. They noted that TSCs placed in media of 10% LR-PRP or LP-PRP showed greater cell differentiation as well as a dose-dependent proliferation response compared to the other concentrations. The platelet concentrations (10% group) in both LR-PRP and LP-PRP were about 3x higher than whole blood. The authors also noted a similar outcome as Giusti in which higher concentrations, such as 20%, did not yield better rates of differentiation or proliferation. Deal, Smith, Heard, et al., used LR-PRP for partial ulnar collateral ligament tears (UCL) in the elbow.[60] The authors reported successful reconstitution of the ligaments in 22 out of the 23 patients in the study with a platelet count reported being 1.228 ± 312 x 10^6 platelet/μL. When evaluating the literature on PRP for tendinopathy and/or ligamentous injury, it is imperative to report the actual platelet counts in preparations. This may play a role in whether we would expect a good outcome from the procedure as too low and too high platelet counts may adversely affect the healing of these tendons.

LEUKOCYTES

Leukocytes can influence the outcome of PRP for tendinopathy. Mishra, Skrepnik, Edwards, et al.,[61] evaluated 225 patients with lateral epicondylosis in which 112 patients received LR-PRP. The authors found that compared to the control group who received bupivacaine with needle fenestration, the treatment group continued to see significant improvement in pain 6 months from the date of injection. Similar results were seen by Peerbooms, Sluimer, Bruijn, et al., [62] who compared LR-PRP to corticosteroid injection (CSI). They also noted an improvement in visual analog pain (VAS) scores and functional measures in the PRP group that exceeded the effect at 1 year. The authors subsequently reported continued improvement in the PRP group at the 2-year mark as well.[63] PRP for gluteus medius/minimus tendinopathy has been an area investigated with the use of PRP. Fitzpatrick, Bulsara, O'Donnell, et al., compared LR-PRP for gluteus medius/minimus tendinopathy and CSI in

80 patients. They noted a clinically and statistically significant improvement in hip function and pain in the PRP group at 12 weeks.[64] They followed these individuals to the 2-year mark and the PRP treatment group maintained improvement whereas the CSI group did not maintain improvement beyond 24 weeks.[65]

Despite the above studies showing positive benefits of LR-PRP on tendinopathy, there is animal data and basic science studies that suggest results might be superior with LP-PRP. Rubio-Azpeitia, Bilbao, Sánchez, et al.,[66] examined the differences in paracrine signaling of three different preparations – LR-PRP, pure PRP, and platelet-poor plasma (PPP) on tendinopathic cells. The authors noted that LR-PRP and pure PRP was superior to PPP for chemotactic and proliferative properties. PRP and PPP stimulated matrix anabolism the most whereas LR-PRP was the most proinflammatory. Zhou, Zhang, Wu, et al.,[59] also found that LR-PRP induces catabolic and inflammatory effects on tendons. Yan, Gu, Ran, et al., conducted animal studies investigating the effect of intratendinous LP-PRP compared to LR-PRP and saline.[67] After injecting achilles tendons of rabbit models with collagenase, they administered 200μL of either LP-PRP, LR-PRP, or saline. After 4 weeks, they noted decreased signal intensity on MRI, significantly better scores on histological grading, as well as transmission electron microscopy showing larger fibril diameters in the LP-PRP group. Cross, Cole, Spatny, et al.,[68] evaluated the in vitro effect of LP-PRP compared to LR-PRP on torn human rotator cuff tendons. Diseased supraspinatus tendons were placed in 6-well plates and cultured with either LP-PRP, LR-PRP, or a control for 96 hours. In moderately degenerated tendons, the authors noted a greater promotion of a normal collagen matrix as well as decreased concentrations of both TGFβ-1 and MMP-9 in LP-PRP media compared to LR-PRP, which are both associated with inflammation and matrix degradation. Mozzocca, McCarthy, Chowaniec, et al., also reported results that supported the use of LP-PRP with the significant proliferation of tenocytes noted compared to control.[4] As far as human RCTs utilizing LP- PRP, Behera, Dhillon, Aggarwal, et al.,[69] compared LP-PRP to bupivacaine for recalcitrant lateral epicondylosis in 25 patients. Their study noted that compared to bupivicaine, LP-PRP provided superior relief in VAS and function at 6 months in addition to the 1-year mark.

There does appear to be variability in outcomes regarding the use of LR-PRP as well as LP-PRP though no head to head tendinopathy studies have been performed to know if this is clinically significant. Also, it is important to realize that the reference to leukocytes does not clearly define which cell type(s) among this cell lineage is represented.[70] Based on preliminary basic science research and limited clinical studies, reducing leukocytes, especially neutrophils, may provide the optimal outcome. Further research carefully examining leukocyte subtypes is needed.

RED BLOOD CELLS

RBCs can have a detrimental effect on PRP procedures.[47] However, when we look at the cascade of healing and compare the role of platelets and erythrocytes, platelets may have a greater effect on molecular cascades and potentially greater influence on PRP outcomes. Jacobson, Fufa, Abreu, et al.,[71] evaluated the effect of platelets and erythrocytes and their role in anterior cruciate ligament(ACL) healing. The ACL fibroblasts cultured in scaffolds had a collage-fibrin matrix base that was either high in platelet concentration, contained only collage-fibrin, or contained both platelets and RBCs (1.5×10^9 RBCs/ml). The authors noted an increase in cytokine release in the concentration that was high in platelets compared to the other two groups. Also, the scaffolds containing the RBCs significantly inhibited human ACL fibroblast proliferation. Harrison, Vavken, Murray, et al., compared RBC concentrations on provisional scaffolds and their effects on ACL fibroblasts.[72] The three groups were divided in RBC concentrations that were physiologic (1×10^9 RBCs/ml), subphysiologic (1×10^8 RBCs/mL), and supraphysiologic (1×10^{10} RBCs/ml). The authors noted that subphysiologic concentrations of RBCs stimulated greater fibroblast proliferation. Also, the concentrations of RBCs above whole blood (physiologic) showed suppression of cell proliferation over time but interestingly had a higher rate of DNA production at the 3-week mark compared to the other samples. Given these results and the known toxic effect of RBC breakdown products, it is best to avoid RBCs in PRP.

ACTIVATION

Various activating agents have been used in the treatment of tendinopathy, including thrombin, $CaCl_2$, and soluble collagen. [48] For tendinopathy, studies have been performed in which the outcomes of PRP for lateral epicondylosis and gluteus medius have shown favorable responses, regardless of activation.[57,64,65,69] Given the heterogeneity in outcomes and the fact that the injected PRP is immediately exposed to collagen, exogenous activation appears unnecessary.[48] Endogenous activation also appears to be more gradual with a longer and more sustained release of growth factors.[53] The PLRA summary for tendons and ligaments is shown in Figure 1.

PRP FOR ARTICULAR CARTILAGE

Platelet Count

There is a lack of definitive literature when discussing the optimal platelet count for intraarticular treatment of PRP. Various systematic reviews evaluating the use of PRP for knee osteoarthritis (KOA) and hip osteoarthritis (HOA) have been conducted with reported platelet concentrations ranging from 2–10× physiologic levels.[73,74] We will further dissect these studies based on those that are RCTs. (Table 1)

Many of the studies listed in Table 1 do not document or reveal their total platelet count. Those that did provide some clarity in certain trends. The majority of studies that used a platelet concentration that was 4x-6x that of whole blood did report results that showed the superiority of PRP compared to various controls or treatments including saline, HA, and ozone.[75,76] However, Filardo, Kon, Di Martino, et al.,.[77] compared the use of LR-PRP versus HA for KOA over one year. The authors reportedly used PRP with platelet counts around 5x of whole blood values, but at the one-year mark, there was no significant difference compared to HA. It is also worth mentioning that the authors did freeze and subsequently thaw their autologous PRP which introduces the possibility of this product being platelet lysate rather than actual PRP.

Cole, Karas, Hussey, et al.,[78] compared PRP to HA for KOA. Their PRP product featured a platelet concentration of 1.73 ± 0.05× that of whole blood. The authors did not find PRP was superior to HA for KOA at the one-year mark. On the other hand, Patel, Dhillon, Aggarwal, et al.,[79] noted that a 10x concentration of platelets did not yield superior results past 6 months. However, other studies showing 9-13x concentrations did show positive results.[80] The RCTs for HOA were less in number and inconsistent with PRP preparation results compared to KOA. [81-84] Further studies are needed for HOA. Given the variability in patient to patient baseline platelet counts, it is difficult to make absolute conclusions. Better research with true delivered platelet counts is needed to determine the optimal platelet counts for articular cartilage.

Leukocytes

The presence of leukocytes in PRP for intraarticular use has been a debated topic in the literature.[73,76,85,86] A meta-analysis by Riboh, Saltzman, Yanke, et al., identified 9 studies that either had level 1 or 2 evidence comparing the efficacy of LR-PRP versus LP-PRP for KOA.[87] The studies included a total of 1055 patients that showed no difference in adverse effect rate between LR-PRP versus LP-PRP. However, outcomes such as WOMAC and the International Knee Documentation Committee (IKDC) were significantly better in the LP-PRP group compared to HA or placebo. No difference was seen with LR-PRP group when compared to HA or placebo. PRP preparations with LR-PRP often contain RBCs which may confound outcomes and contribute to its inferior efficacy. Smith [88] conducted an RCT involving 30 patients, evaluating the safety and efficacy of LP-PRP for KOA. The group noted a significant improvement in WOMAC scores in the LP-PRP group at a year mark when compared to the placebo (saline) group, along with no adverse effects. Similar findings were noted when Simental-Mendía, Vilchez-Cavazos, Pena-Martinez, et al., compared oral acetaminophen (500mg every 8 hours) to 3 intraarticular injections (once every 2 weeks) of LP-PRP for KOA.[89] The 33 patients who were treated with the series of

FIG. 1 PLRA summary for tendons and ligaments.

P	L	R	A
1.2 – 2 × 10^6 platelets/μL	-	-	-

**The values listed in the table above are the recommendations of the authors based on the available data from the research presented.*

LP-PRP showed sustained significant improvements in VAS, WOMAC, and Short-Form 12 (SF-12). The absence of leukocytes, specifically neutrophils, in PRP for intraarticular treatments appears to provide the best outcome although a direct head to head study should be done to confirm this.

Red Blood Cells

The presence of RBCs with PRP injections can be detrimental to the intraarticular chondrocytes due to their breakdown products [40-42]. Braun, Kim, Chu, et al., compared the effects of LR-PRP, LP-PRP, RBC concentrate, and PPP on human fibroblast-like

TABLE 1 Randomized Controlled Trials of PRP for Articular Cartilage

	Number of Patients	Amount/ Type of PRP	Number of PRP Injections	Platelet Count	Leukocyte & Neutrophil Count	Red Blood Cell	Activator	PRP Superior?	Location
Cerza et al.,[108]	120 Patients ⇒ 60 PRP ⇒ 60 HA	5.5 mL – exact type of PRP not reported	4 Total (administered weekly)	NR	NR	NR	NR	Yes	Knee
Cole et al.,[78]	99 Patients ⇒ 49 PRP ⇒ 50 HA	4 mL of LP-PRP	1	1.73 ± 0.05× Whole blood concentration	- Neutrophil: NR	NR	None	No	Knee
Dallari et al., [81]	111 Patients ⇒ 44 PRP ⇒ 36 HA ⇒ 31 PRP + HA	5 mL – exact type of PRP not reported	3 Total (administered weekly)	NR	NR	NR	$CaCl_2$	Yes	Hip
Di Sante et al.,[82]	43 Patients ⇒ 21 PRP ⇒ 22 HA	4 mL of LP-PRP	3 Total (administered weekly)	1-1.5× Whole blood concentration	-	NR	NR	No	Hip
Doria et al.,[83]	80 Patients ⇒ 40 PRP ⇒ 40 HA	5 mL – exact type of PRP not reported	3 Total (administered weekly)	NR	NR	NR	NR	No	Hip
Duymus et al.,[80]	102 Patients ⇒ 33 PRP ⇒ 34 HA ⇒ 35 Ozone	3-4 mL of LR-PRP	1	9-13× Whole blood concentration	+ Neutrophil: NR	Minimal	None	Yes	Knee
Filardo et al.,[85]	183 Patients ⇒ 94 PRP ⇒ 89 HA	5 mL of LR-PRP	3 Total (administered weekly)	4.6 ± 1.4× Whole blood concentration	+ Neutrophil: NR	NR	$CaCl_2$	No	Knee

(*continued*)

TABLE 1 Randomized Controlled Trials of PRP for Articular Cartilage (*continued*)

Gormeli et al.,[75]	162 Patients ⇒ 39 multiple PRP injection ⇒ 44 single PRP injection ⇒ 39 HA ⇒ 40 Control (saline)	5 mL – exact type of PRP not reported	3 Total in multiple PRP injection group (administered weekly) 1 in the single PRP injection group	5.3 × in multiple PRP injection group 5.2× in single PRP injection group	NR	NR	$CaCl_2$	Yes	Knee
Kraeutler et al.,[84]	30 Patients (32 hips) ⇒ 18 PRP ⇒ 14 HA	NR	3 Total (Administered weekly)	NR	NR	NR	NR	Yes	Hip
Patel et al.,[79]	⇒ 74 Patients (148 knees) ⇒ 25 (50 knees) multiple PRP injections ⇒ 26 (52 knees) single PRP injection ⇒ 23 (46 knees) control (saline)	8 mL of LP-PRP	2 Total in multiple PRP injection group (given 3 weeks apart) 1 in the single PRP injection group	10× normal concentration	-	NR	$CaCl_2$	Yes	Knee
Paterson et al.,[109]	19 Patients 10 PRP 9 HA	3 mL of LR-PRP	1	NR	+ Neutrophil: NR	NR	UV light	No	Knee
Raeissadat et al.,[76]	139 Patients ⇒ 77 PRP ⇒ 62 HA	4-6 mL of LR-PRP	1	4-6× normal concentration	+ Neutrophil: NR	NR	None	Yes	Knee

(*continued*)

TABLE 1 Randomized Controlled Trials of PRP for Articular Cartilage (*continued*)

Sanchez et al.,[94]	153 Patients ⇒ 79 PRP ⇒ 74 HA	2 mL of LP-PRP	1	NR	-	NR	$CaCl_2$	Yes	Knee
Smith[88]	30 Patients ⇒ 15 PRP ⇒ 15 Control (Saline)	4-7 mL of LP-PRP	3 Total (administered weekly)	NR	-	NR	NR	Yes	Knee
Vaquerizo et al.,[92]	90 Patients ⇒ 48 PRP ⇒ 42 HA	8 mL of LP-PRP	3 Total (administered weekly)	NR	-	NR	$CaCl_2$	Yes	Knee

NR = Not reported.

synoviocytes (FLS).[90] FLS is found in normal synovium and can play a role in cell signaling that contributes and mediate cartilage catabolism. The authors noted that RBC concentrate resulted in significantly greater cell death when compared to saline. Furthermore, measurements of IFN-γ levels were the greatest in RBCs, which is known to play a role in inducing cell death.[91] Removing RBCs in PRP products for any joint injection is recommended to eliminate the potential of harm caused by these cells.

Activation

Both exogenously and endogenously activated PRP has shown promise in the outcomes of intraarticular injections. Vaquerizo, Plasencia, Arribas, et al., compared 3 injections of activated PRP with $CaCl_2$ to HA for KOA in a RCT and noted a superior effect of PRP regarding pain and function.[92] This result is also consistent with other studies that used $CaCl_2$ as an activating agent for KOA.[79,93,94].

Other studies have shown success with endogenous activation for KOA. Gobbi, Karnatzikos, Mahajan et al., conducted a case series of patients treated with 2 intraarticular injections with autologous PRP and noted an increase in IKDC scores at 6 and 12 months despite not using an exogenous activating agent.[95] PRP without exogenous activation was also used by other researchers for KOA, with a decrease in pain and functional improvements reported.[76,96,97] Successful outcomes have been reported with endogenous and exogenous activating agents for articular cartilage.

Adjuncts for Cartilage

Some adjuncts may be beneficial for intraarticular injections. One is the nano dosing of dexamethasone. Although corticosteroids at high doses (>3 mg/dose) have been shown to have a deleterious effect on cartilage,[98] lower doses may have a positive effect on chondrocytes. In vitro studies have found that dexamethasone, when used at a dose of 100 nanometers (nM), can upregulate gene expression of cartilage matrix components such as aggrecan, dermatopontin, and collagen type XI.[99] Li, Wang, Kopesky, et al.,[100] noted that cytokines interleukin-1 (IL-1), as well as tumor necrosis factor-α (TNF-α), play important role in signaling chondrocyte apoptosis in posttraumatic osteoarthritis (PTOA). When PTOA human cartilage cells were harvested from knee joints and exposed to 100nM doses of dexamethasone in vitro, the apoptotic effects of IL-1 and TNF-α were inhibited, thus rescuing these chondrocytes from cell death.

Frank, Hung, Krishnan, et al.,[101] investigated the effect of microdoses of triamcinolone acetonide on cartilage. The authors used articular cartilage discs from the femoropatellar grooves of 1-2 week old calves and assigned them to three different media (5-6 disks per group) after incubation: "normal cartilage" with just the serum-free medium alone, "inflamed cartilage" which was medium supplemented with TNF-α with or without interleukin-6 (IL-6), or "inflamed and injured cartilage" which was the same as the "inflamed cartilage" but with compression to cause injury. The groups were treated with varying doses

of triamcinolone ranging from 100 picometers (pm) to 100 micrometers (μm). The authors ultimately noted that triamcinolone can have a dose-dependent protective effect in the inflamed and injury groups. Specifically, a dose of 10 μm showed a trend towards protection in the inflamed cartilage group. A dose of 10 μm as well as 100 nM was protective against glycosaminoglycan loss in all three groups.

Another adjunct that may be of interest is doxycycline, a tetracycline antibiotic that has traditionally been used for various bacterial infections.[102] Animal models have noted a potential role for this medication as an adjunctive treatment because it downregulates various MMPs and collagenases.[103,104] It has been theorized that MMP-13 plays an important role in the progression of degradation of articular cartilage in osteoarthritis.[105,106] Lee, O'Malley, Friel et al.,[107] evaluated the in vivo effect of doxycycline on human mesenchymal stem cell (hMSC) chondrogenesis. They noted that hMSCs cultures treated with 2 μg/mL resulted in reduced MMP-13 mRNA expression and proteins at 21 days. Although the literature is sparse when it comes to clinical uses, the basic science literature that is available does indicate a possible role of doxycycline as an adjunctive treatment. More clinical studies are needed with this as well as other adjuncts to determine their precise role in treating joints with PRP. The PLRA summary for articular cartilage is shown in Figure 2.

PRP FOR MUSCLE

Platelet Count

PRP may have a role in the treatment of certain myopathic conditions, though there is much less available evidence compared to tendons and cartilage.[110]

FIG. 2 PLRA summary for articular cartilage.

P	L	R	A
$6 \times 10^5 - 2.4 \times 10^6$ platelets/μL‡	-	-	+/-

‡ *The value is based on our interpretation that platelet concentration 4-6x that of normal levels (150,000 – 400,000) may be the ideal range for articular cartilage*

**The information listed in the table above are the recommendations of the authors based on the available data from research presented*

Li, Usas, Poddar, et al.,[111] investigated the role of PRP in muscle cell proliferation. The study reported that LP-PRP, with a platelet count of 2×10^6 platelet/μL, significantly enhanced the cell proliferation of human muscle-derived progenitor cells (hMDPCs) in vitro. Other basic science animal studies did show improvements in muscle wound and strain healing with subsequent strength in those treated with PRP compared to saline.[112,113] However, the exact platelet counts were not reported. Hamilton, Tol, Almusa, et al., conducted a double-blinded, RCT to determine if there is a role of PRP in enhancing return to play after acute hamstring injuries.[114] The authors compared PRP, platelet-poor plasma (PPP), or no injection for imaging confirmed hamstring injuries in 90 professional athletes. The authors concluded that there was no significant difference in PRP or PPP when compared to no injection at all for these injuries. Similarly, Rettig, Meyer, Bhadra, et al., compared LR-PRP to rehabilitation for acute grade 2 muscle/myotendinous injuries in football players. They noted a median return to play (RTP) was 20 days versus 17 in the PRP and control groups, respectively. The platelet count was not mentioned.[115] Hamid, Mohamed, Yusof et al., conducted a similar study in soccer players comparing LR-PRP with a platelet count of 1.29×10^6 to rehabilitation for acute grade 2 hamstring injuries and noted the median RTP was 27 days in the PRP versus 43 in the control.[116]

Miroshnychenko, Chang, and Dragoo [117] compared the use of LP-PRP to PPP for enhancing the differentiation of skeletal myoblasts. The mean platelet count in the PRP group was 3.71 ± 1.26x that of whole blood. The authors noted an increase in proliferation with PRP, but no evidence of differentiation. However, the PPP group had significant induction of myoblast differentiation when compared to PRP. Although PPP contains a very low concentration of platelets, it still contains growth factors such as PDGF, IGF-1 that may explain why it has enhanced differentiation capabilities with myoblasts. Much of the literature available investigating the role of PRP in muscle injuries have looked at outcomes of acute muscle injuries. The role of PRP in chronic injuries has not been reported to our knowledge and may have different outcomes.

The number of investigations evaluating the effects of PRP or PPP on muscle recovery is limited, but it appears that PPP has a role in enhancing recovery of muscles from injury while promoting differentiation towards myoblasts and not fibroblasts.

Leukocytes

LP-PRP has shown to enhance proliferation, but not differentiation of skeletal myoblasts.[117] Literature is sparse regarding the exact role of leukocytes when used in PRP or PPP injections for muscle-related pathologies. When we evaluate the phases of muscle healing in animal studies, we are aware that neutrophils are key players as they induce oxidative damage and inhibit the body from resolving contraction-induced muscle injuries.[118] Therefore, it is important to be aware of the timeline on when to possibly use LR-PRP for muscle injuries. Halpern, Chaudhury, and Rodeo have recommended that for the first 24 hours, LR-PRP likely should not be used or consideration should be given for PPP at that time.[119] Researchers have also reported that the relationship between LR-PRP and TGF-β1 may harm the outcome of studies.[25] TGF-β1 can play a role in inducing fibrosis development in skeletal muscle, but also inhibiting myogenic differentiation.[120] It is important to understand that although basic science studies are noting the above changes, there is a paucity of high-level clinical research available for us to truly evaluate the effect of leukocytes on PRP for muscle pathology at this time.

Red Blood Cells

The effect of RBCs on outcomes of PRP for muscle-related injuries is limited. As noted earlier, the presence of PFH, heme, and iron after RBC damage can create a toxic environment and a negative outcome for PRP and other orthobiologic procedures.[47] Studies note that the presence of RBCs contribute to an increase in cell death and a decrease in anti-inflammatory cytokines such as IL-4.[90] Available research appears to recommend against the inclusion of RBCs, but further high-level research is needed.

Activators

The studies evaluating the use of activating agents for muscle-related pathology are not as robust as tendons and cartilage. Studies have noted positive results with animal studies when thrombin was used as an activating agent.[111,112] Hamilton, Tol, Almusa, et al., chose not to activate their PRP for acute hamstring injuries.[114] Though the evidence is scarce, natural activation via direct exposure to collagen is likely sufficient for muscle pathology. Further research is warranted in this area.

Adjuncts

Losartan as an antifibrotic agent may be of interest as an adjunct to PRP therapy to improve muscle healing.[121] Losartan can inhibit pathways involved in TGF-β1 signaling[122,123], which may be useful as TGF-β1has known negative implications in muscle recovery.[120,124] Terada, Ota, Kobayashi et al., investigated the effect of tibialis anterior muscle contusion recovery with control, PRP with and without oral losartan, as well as losartan alone in animal models.[125] It was noted that 20 μL of PRP, with reported 5.5× increase in platelet concentration, was injected into the mice one day after injury in the PRP groups with and without losartan. The losartan groups received an oral dose of 10 mg/kg/day at day 3 after injury and then daily until the end of the study. At conclusion, the authors noted that the PRP and PRP with losartan groups resulted in accelerated muscle regeneration at two weeks compared to control. Groups with losartan had significantly reduced the area of fibrotic scar tissue compared to those without. These findings are similar to results reported Bedair, Karthikeyan, and Quintero in which the use of angiotension II receptor blockers resulted in reduced fibrous tissue formation in a dose-dependent manner.[126] Although animal models, these studies demonstrate that losartan may be considered as an adjunct for PRP to reduce muscle fibrosis but further studies are needed. The PLRA summary for muscles is shown in Figure 3.

FIG. 3 PLRA summary for muscles.

P	L	R	A
$<1.5 \times 10^5$ platelets/μL	-	-	+/-

**The information listed in the table above are the recommendations of the authors based on the available data from research presented*

CONCLUSIONS

PRP preparations currently have a vast amount of variability that needs to be standardized to effectively make decisions about their therapeutic efficacy. Several factors can influence platelet and growth factor that is released from platelets including diet, exercise, etc. The decision on the type of PRP used should be made based upon the area of tissue pathology. RBCs should be removed from the PRP product regardless of the target tissue. However, optimal platelet counts, presence or absence of leukocytes, and the use of activators need to be clearly defined in future studies to determine the best options for optimal outcomes. We highlight the use of the PLRA system of classification to guide as a means of standardization and guidance for specific tissue pathology.

AUTHORS CONTRIBUTION

Conception and design: All authors.
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Provision of study materials or patients: All authors.
Collection and assembly of data: All authors.
Data analysis and interpretation: All authors.
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