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CURRENT OPINION

Considerations for the Use of Platelet-Rich Plasma in Orthopedics

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Abstract The use of platelet-rich plasma (PRP) is expanding to numerous medical fields, including orthopedic surgery and sports medicine. The popularity of this new treatment option has prompted a rapid increase in research endeavors; however, the differences in application technique and the composition of PRP have made it difficult to compare results or make any firm conclusions regarding efficacy. The purpose of this article is twofold. First, to recommend details that should be provided in basic science and clinical PRP studies to allow meaningful comparisons between studies which may lead to a better understanding of efficacy. Second, to provide an understanding of the different PRP preparations and their clinical relevance. There are biochemical rationales for the use of PRP because it addresses several aspects of the healing process, including cell proliferation and tissue matrix regeneration, inflammation, nociception, infection, and hemostasis, all of which will be addressed. Given the current understanding of the importance the composition of PRP plays in tissue regeneration, it is likely that our future understanding of PRP will dictate 'customizing' the PRP preparation to the specific pathology of interest. The potential complications following PRP use are minor, and thus it appears to be a safe treatment option with a variety of potentially beneficial effects to injured musculoskeletal tissues.

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1 Introduction

Platelet-rich plasma (PRP) is a blood-derived plasma suspension containing variable quantities of platelets, leukocytes (white blood cells [WBCs]), and red blood cells (RBCs) [1]. The use of PRP has become prevalent in the regenerative medicine field with diverse applications from sports medicine and orthopedics to cosmetic surgery and ophthalmology [2–4]. The variability in PRP composition, use, and outcome instruments used for clinical study make the literature difficult to interpret. The purpose of this article is twofold. First, to recommend details that should be provided in basic science and clinical PRP investigations to allow conclusions on efficacy to be made from meaningful comparisons between studies. Second, to provide an understanding of how the different biologic activities of PRP (tissue regeneration, anti-inflammatory, analgesia, antimicrobial, and hemostasis) may be influenced by PRP composition and use. Complications and the effect of PRP type and activation state on their occurrence will also be addressed.

A recent meta-analysis of PRP for orthopedic indications concluded that the current evidence available does not support the enthusiasm for clinical application of PRP [2]. However, the prospective randomized controlled and cohort studies included were for 14 different indications, with 9 of the indications represented by only one publication each. This meta-analysis typifies the variability and weakness in the literature regarding reporting of the composition of PRP, use of a platelet activator, number and timing of treatments, and outcome analysis. Only 61 % of

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studies noted the preparation method used, and within those studies, nine different systems were used, and none of them reported what platelet or WBC concentration each patient received.

There are several key data points that should be reported in PRP studies to allow a more precise and accurate determination of the validity of a PRP preparation for a specific clinical application (Table 1). Considering all variables will be a learning process for investigators, but the current methodology is ineffective and will not produce reliable conclusions.

2 Defining Platelet-Rich Plasma

There is no consensus on the definition of PRP [5]. A simple definition is any plasma suspension with increased platelet concentration compared to blood. However, tremendous variability in platelet concentration is influenced by a number of factors; those of the individual (variation in response to dietary and physiological cues, exercise, smoking, and diurnal variation), differences in platelet counts within and between systems, and, in some instances, PRP is not generated after routine centrifugation according to manufacturer directions for unknown reasons [1, 6]. These issues serve to emphasize the point that absolute platelet concentration for each individual PRP preparation should be captured and reported so that it can be determined if outcome is related to platelet concentration.

PRP is also referred to as autologous conditioned plasma (ACP), reinforcing the fact that it can be produced from the patient's own blood. ACP has previously been referred to as Orthokine®, one of the trade names for the injectable autologous plasma products.

Platelet concentrates can be derived from a number of methods, including the 'standard' centrifugation technique to generate PRP. Both buffy coat and platelet apheresis have been used to this end. Each technique, however, differs in its leukocyte and platelet concentration. An in vitro study comparing platelet concentrates derived from a PRP method, buffy-coat method, and apheresis showed that buffy coat-derived platelet concentrates had both the largest platelets (in terms of mean platelet volume [MPV]) and was the most adaptable, being able to undergo shape change in the presence of EDTA. PRP had lower platelet counts and volume, while apheresis had the lowest values of these two measures [7].

A more recent study [8] assessed platelet quality in terms of the following platelet characteristics: swirling, platelet count, WBC count, pH, and volume of platelet concentrate. This study also compared PRP with buffy coat- and apheresis-derived platelet concentrates and found apheresis platelet concentrates to be superior to buffy coat and PRP. Apheresis-derived platelet concentrates had better swirling (indicative of discoid morphology), higher platelet counts, and higher volume than buffy coat and PRP platelet concentrates. Moreover, although PRP- and buffy coat-derived platelet concentrates were comparable in terms of swirling, platelet count, and pH, buffy coat-

Table 1 Recommended complete data reporting for basic science and clinical platelet-rich plasma investigations

Preparation method	
Commercial	System used
	Detail any modifications to manufacturer protocol
	Detail any manufacturer options (if any) and option selected (i.e. final volume of PRP)
Manual	Volume of blood collected
	Type and final concentration of anticoagulant if any
	Centrifugation speed in gravitational (*g*) force (rpm are not appropriate—results in variable *g* force depending on centrifuge radius)
	Centrifugation time
	Number of spin cycles
	Final volume of PRP
Characterization of PRP	
Hematology	Blood and PRP platelet, leukocyte, and red blood cell concentration
	Consider reporting fibrinogen concentration
Growth factors	Consider reporting, particularly for new protocols that have not been validated to increase growth factor concentration
Storage	Fresh or frozen-thawed
Activation	
Yes	Agent (i.e. calcium chloride, autologous thrombin, bovine thrombin, etc.)
	Agent concentration
	Time to clot
	Releasate only or entire clot used
No	
In vivo models or clinical studies	
Injection	Location (intra-articular, intra-lesional, peri-lesional, etc.)
	Volume injected
	Ultrasound guidance (yes or no)
	Timing of injection relative to injury or surgery
	Re-dosing interval if any
	Post-injection rehabilitation
	Prior or concurrent treatments
Complications	Describe major and minor
	Detail number affected
	Duration post-treatment
Outcome measures	As appropriate to tissue/injury of study

PRP platelet-rich plasma, *rpm* rotations per minute

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derived platelet concentrates had greater variation in volume, which the authors suggest is due to lack of a standardized way to prepare buffy-coat platelet concentrations [8]. An older study specifically looked at platelet viability, comparing PRP and apheresis-derived platelet concentrates [9]. Unlike the aforementioned studies, this study was in vivo and involved eight subjects who underwent platelet concentrate generation via PRP and continuous flow cell-separator apheresis ($n = 4$, Group A) and intermittent flow cell-separator apheresis ($n = 4$, Group B). The results showed no difference in platelet viability between the PRP- and apheresis-derived platelet concentrates in terms of mean platelet lifespan. Furthermore, there was no difference between the two platelet-apheresis collection methods.

A recent review of platelet concentrates derived from apheresis and whole blood centrifugation (PRP) echoed the similarity of the platelets collected via these two methods [10]. The study cited additional factors such as the increased risk of viral transmission via whole blood-derived concentrates (often from multiple donors) as compared with apheresis-derived concentrates (often from a single donor). Furthermore, the risk of moderate immune reaction is 0.38 % for whole blood as opposed to 0.12 % for apheresis. The risk of severe reaction was 0.09 and 0.03 %, respectively [10]. These studies demonstrate the need for methodological procedure and collection standardization in addition to highlighting the importance of focusing on platelet collection efficiency, cost, processing times, infection rate, WBC contamination, and ease of operation [7–10].

While PRP definitions have classically relied on platelet concentration, more recent understanding of the complexity of PRP as a composite of bioactive factors from platelets, WBCs, and the plasma itself, has catalyzed the need for a more thorough classification of different PRP products. The multitude of proteins and hormones found in PRP have recently been reviewed [1]. Similar to platelets, quantifying WBC concentration is important considering laboratory work that has demonstrated inflammatory cytokine release from WBCs in PRP, and positive correlation between WBC concentration and the concentrations of interleukin (IL)-1β and matrix metalloproteinase (MMP)-9 in PRP preparations [11–19]. IL-1β is known to induce inhibition of collagen II and aggrecan gene expression, which contribute to osteoarthritis progression [20]. MMP-9 and other gelatinases cleave collagen as well as aggrecan, elastin, and cartilage link protein, thereby playing a significant role in cartilage degradation. Whether an activating agent is used is also likely to have significant biologic consequences due to differences in release kinetics of growth factors from platelets. Thrombin activation of WBC-containing PRP also results in increased release of IL-1β [21].

A classification system has been proposed in an attempt to group different PRPs based on their fundamental composition so that the optimal type of preparation for each indication could be inferred from the literature [22]. The four major categories include pure PRP (low WBC, anticoagulated), leukocyte-rich PRP (high WBC, anticoagulated), pure platelet-rich fibrin [PRF] (low WBC, coagulated), and leukocyte-rich PRF (high WBC, coagulated). Another classification scheme expanded the definition of PRP groups to include platelet concentrations greater or less than a fivefold increase over blood concentrations [23]. However, the ideal platelet concentration is likely to differ depending on tissue type and disease state and may not fit discretely into the greater or less than fivefold increase categories. For example, three different cell types cultured in the releasate of calcium chloride-activated pure PRP preparations with two platelet concentrations each had a different response with respect to proliferation and cytokine production [24]. Calcium chloride is used to activate platelets in order to release growth factors from the alpha granules. In addition, an in vivo rabbit bone regeneration study found that extreme platelet concentrations produced inferior results, while moderate concentrations were stimulatory [25]. Similar results were found when human rotator cuff fibroblasts were exposed to three concentrations of PRP, with low and moderate concentrations being optimal [26]. Further research should aim to define the ideal pathology-specific PRP for each treatment indication and further refine the current proposed PRP classification schemes.

3 Tissue Regeneration

The rationale for the role of PRP in regenerative therapy is based on the numerous growth factors within platelets and plasma [1]. Many of these growth factors have been investigated for their individual effects on tissue repair but at concentrations much different than those found in PRP [27]. The synergistic effect of the combination of proteins in PRP makes extrapolation of the results of growth factor therapy problematic.

Treatment of tendon and ligament injuries with PRP was one of its earliest and most popular uses in sports medicine. PRP has demonstrated anabolic effects, including increased matrix gene expression and protein production, increased chemotaxis of bone marrow cells, increased tenocyte proliferation, improved histologic organization, and increased force at failure [26, 28–36]. Growth factors in PRP have anti-catabolic effects which may be important. Transforming

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growth factor (TGF)-β inhibits the expression and release of IL-1β, tumor necrosis factor (TNF)-α, IL-6 and IL-8, and inhibits MMP activity [37–39]. Treatment of human tenocytes with IL-1β and TNF-α results in upregulation of endogenous IL-1β and TNF-α, MMP-3, -1 and -13, all without a change in tissue inhibitors of metalloproteinases leading to an overall effect of tissue degradation [40].

The clinical efficacy of PRP use during arthroscopic rotator cuff repair has yielded inconclusive results. In a systematic review by Chahal et al. [41], a meta-analysis of sorts (both randomized control trials and retrospective studies were included) was performed on the available published studies evaluating rotator cuff re-tear rate and standardized patient-reported clinical outcome measures related to shoulder symptomology before and after arthroscopic rotator cuff repair surgery for full-thickness tears. They found no statistically significant difference in re-tear rates for patients treated with PRP and those treated without PRP. However, a subgroup analysis showed a statistically significant decrease in re-tear rates for patients treated with PRP who had small and medium-sized rotator cuff tears versus those who were not treated with PRP with the same size tears ($p = 0.006$). When analyzing the subgroups further, no difference in re-tear rates was found between groups with large or at-risk tears. Furthermore, Chahal et al. [41] found that treatment with PRP did not result in significant differences in shoulder-specific outcome scores for patients undergoing rotator cuff repairs. A subgroup analysis of these outcomes was unable to be performed because shoulder-specific outcome measures were not reported for these groups.

These results were echoed by Zhang et al. [42] in their more classical meta-analysis of studies including patients undergoing arthroscopic rotator cuff repair surgery with and without PRP treatment. They found no significant difference between the PRP group and the control group in shoulder-specific outcome measures nor in overall re-tear rates. Additional subgroup analysis of re-tear rates based on initial tear size was performed and again showed a significant decrease in re-tear rates of PRP-treated patients with small and medium tears ($p = 0.03$), with no significant difference in re-tear rates for large or massive tears. Despite the lack of statistical heterogeneity in these two studies (I^2 <50 % for both), significant clinical heterogeneity, differing PRP preparations, a variety of rotator cuff repair techniques and varied postoperative rehabilitation was acknowledged by both authors to contribute to the complexity of data interpretation. This emphasizes the need for large, multicenter, randomized control trials with standardized PRP and procedural protocols to better delineate the relationship between the in vitro biological properties of PRP and its translational clinical outcomes.

In addition to investigating the clinical efficacy of PRP in arthroscopic rotator cuff repair, studies have also looked at its role in tendinopathy. Mautner et al. [43] conducted a multicenter retrospective review of the effect of PRP injections on patient-reported symptoms of all-cause chronic tendinopathy. In addition to physical examination findings, patients were required to have ultrasound or magnetic resonance imaging (MRI) findings consistent with chronic tendinopathy. While a number of tendons were treated, the three most commonly treated were the lateral epicondyle, Achilles, and patellar tendons. Of all survey responders, 82 % reported a moderate-to-complete resolution of symptoms (>50 % improvement) at a mean of 15 ± 6 months post-injection, with no significant difference in results between patients responding before 1 year post-procedure or after 1 year post-procedure. Furthermore, there was a significant improvement in visual analog scale (VAS) scores following injection, which corresponded to an average pain reduction of 75 %. Although these results are encouraging, there was no correlation or discussion of the effects of platelet concentration, leukocyte levels, platelet activation, or number of required injections on the results.

Another tendon study from Brazil by de Almeida et al. analyzed clinical and radiographic outcomes of PRP on the healing of patellar tendons following anterior cruciate ligament (ACL) reconstruction with patellar tendon grafting [44]. This study attempted to standardize some of the PRP protocol and procedural elements that could contribute to heterogeneity and inconsistency by using the same type of cell separator for platelet apheresis, having a single surgeon perform all surgical procedures, and having a single blinded radiologist evaluate all MRI imaging. The results showed a statistically significant reduction in gap area of the patellar tendon harvest site in the PRP-treated patients compared with those not treated with PRP ($p = 0.046$), as well as a significantly improved VAS score ($p = 0.02$) in the PRP group within 24 h of surgery, indicating less immediate postoperative pain for those patients. There was no difference between groups in terms of patellar tendon thickness and length. Despite significant improvements in VAS scores in the immediate postoperative period, there were no significant differences in questionnaire or isokinetic outcomes between groups [44]. No biopsies were taken of the patellar tendon to correlate the MRI and VAS findings with mechanical and histological properties of the tendon, necessitating additional studies on the effect of PRP on these characteristics.

The importance of PRP WBC concentration and the implications for the effect of inflammatory and catabolic mediators on tendon and ligament homeostasis was exemplified in an in vitro equine tendon and ligament study [30]. This study found a positive correlation between WBC

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concentration in various biologics, including PRP, and expression of catabolic mediators. More recent work further supported these findings; treatment of tendon explants with low WBC PRP resulted in decreased IL-1β and TNF-α gene expression compared with explants treated with high WBC PRP [28]. This suggests that low WBC or pure platelets would be best suited for the purpose of stimulating tendon regeneration. Finally, activation of PRP for injection into tendons and ligaments remains controversial. Beneficial healing results have been achieved without exogenous activation of PRP, since activation presumably occurs following injection upon exposure to collagen [32]. Further work is needed to determine whether exogenous activation offers any biologic benefit over allowing endogenous activation to occur following injection.

Interest in PRP treatment of joint disorders has increased, particularly cartilage lesions and osteoarthritis. Growth factors in PRP have each demonstrated positive effects on joint biology, including chemotaxis and differentiation of mesenchymal cells, chondrocyte proliferation, matrix production, and suppressed catabolism [45–48]. Currently, the regenerative effect of PRP on the various cell types within joints has not been widely studied. The majority of studies have used the releasate from activated PRP diluted to varying degrees in cell culture. The application of PRP releasate to osteoarthritic human chondrocytes has been shown to increase cell proliferation, and increase gene expression of aggrecan and SOX-9 [49]. Inactivated PRP resulted in beneficial effects in a swine rheumatoid arthritis model [50]. There was a return of Safranin-O and collagen II staining of cartilage to baseline, staining for IL-6 and vascular endothelial growth factor (VEGF) was reduced in the synovium and cartilage, and synovial fluid concentrations of IL-6, VEGF, insulin-like growth factor (IGF)-1, and IL-1 returned to baseline levels. Chondrocytes suspended in agarose gel with inactivated pure PRP had increased proliferation, differentiation, and integration with native cartilage [51]. Many questions remain regarding the ideal PRP composition for cartilage regeneration; however, it would seem reasonable to exclude WBCs from PRP for joint injection given the possibility of catabolic mediator release.

4 Anti-Inflammatory

Significant overlap exists between the role of PRP as an anabolic/anti-catabolic therapy and an anti-inflammatory agent. Evidence supporting PRP as an anti-inflammatory therapeutic stemmed primarily from its use in osteoarthritis. In studies on chondrocyte cultures and IL-1β-exposed chondrocytes, supernatant from activated PRP resulted in decreased nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) transactivation, while non-activated PRP did not, and a reduction in NF-κB to baseline levels, respectively [52, 53]. The mechanism of action was attributed to hepatocyte growth factor released from WBCs in activated PRP [52]. The effect of PRP on chondrocyte NF-κB activation is important because it is a major regulator of inflammation; however, the joint is an organ made up of multiple tissues, including the synovium and subchondral bone, which may be more significant sources of inflammation [54]. Synoviocytes cultured in leukocyte-rich PRP significantly increased production of MMP-1 and -3 compared with cells cultured in platelet-poor plasma (PPP), platelet-derived growth factor BB (PDGF-BB), or saline [12].

In a rheumatoid arthritis model in the pig, intra-articular injection of non-activated PRP (1×10^6 platelets/μl) resulted in significant anti-inflammatory effects, including reduction in synovial hypertrophy and decreased leukocyte infiltration [50]. Current evidence supports injection of activated PRP releasate to yield the greatest anti-inflammatory effect, although clinical confirmation is needed. It is not known whether the use of releasate from pure PRPs would be beneficial compared with releasates from leukocyte-rich PRPs. Intra-articular injection of large numbers of WBCs is counter-intuitive given the propensity for inflammatory mediator and destructive protein release from WBCs.

5 Analgesia and Return of Function

A primary objective of PRP therapy is to gain improved and long-lasting functional outcomes. Improved function is intimately related to decreased pain. Pain can result from a variety of stimuli, and there are several complex pathways involved in transmission and perception of pain. The concept of PRP having antinociceptive properties is in its infancy. Current evidence indicates that PRP affects many molecules involved in inflammation, and an anti-inflammatory mechanism may explain clinical perception of PRP-related analgesia.

PRP has been used to treat rotator cuff, patellar, elbow, and Achilles tendinopathies, and for augmenting ACL repair. A recent review outlined the clinical studies assessing PRP effectiveness for the treatment of these injuries [55]. Excluding ACL repair, PRP decreased pain and improved function in seven of nine investigations, with earlier return to function and increased range of motion for as long as 2 years. Less success has been recognized for PRP-augmented ACL repair, with no significant improvements in analgesia or function scores [55]. Platelet concentration was only reported in four studies, some did not activate PRP, and others used different combinations of

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activators [55]. The use of PRP to augment arthroscopic rotator cuff repair has also produced disappointing results and will not be discussed further [56, 57]. Recent investigations on PRP treatment of lateral epicondylitis mostly used similar leukocyte-rich, non-activated PRP preparations delivered in a similar manner. Pain and function outcomes were somewhat equivocal, with PRP being no different from autologous blood in two studies, improved compared with bupivacaine in one study, and corticosteroids produced contradictory results in two studies (Table 2) [55–62]. Further studies using different types of PRP, different administration techniques, or activation states may be useful to determine if outcomes can be improved. Patellar tendinopathy treatment with PRP appears promising, with positive outcomes in three recent publications; however, only one study compared results with a control (Table 2) [63–65]. These investigations emphasize the need for detailed controlled studies before evidence-based decisions can be made.

Intra-articular injection of PRP for treatment of early cartilage degenerative lesions and osteoarthritis is showing promise. Table 3 outlines recent studies on the use of PRP for the treatment of knee osteoarthritis. Leukocyte-poor and leukocyte-rich PRP were each evaluated in three instances, while two studies did not characterize the PRP used. Filardo et al. [66] compared pure PRP with leukocyte-rich PRP, and there was no difference in pain and function scores. In fact, all investigations demonstrated positive results despite differences in PRP activation, control treatment, and dosing regimen (Table 3) [67, 68]. Interestingly, two studies demonstrated effective outcome measures, with a single dose lasting approximately 6–8.8 months [69, 70]. A common feature of many studies was a superior outcome in younger patients and those with more acute lesions [66, 70, 71]. Additionally, in their study of the effect of PRP on chronic tendinopathy, Mautner et al. [43] included a brief discussion of the number of PRP injections. Their algorithm for determining the number of injections was predicated on the patient's reported global improvement and trajectory of improvement, with 80 % being the threshold below which an additional injection was recommended. In this study, 60 % of patients received one injection only, 30 % received two injections, and 10 % received three or more injections. These numbers translated into 83 % of patients reporting moderate-to-complete resolution of symptoms with one injection, 82 % with two injections, and 76 % with three or more injections. While the authors question the utility of administering more than three injections, the significance of the response trend is not discussed, nor was the frequency of injection.

Another study specifically evaluating the effect of PRP injections on patient-reported clinical outcomes for patients with bilateral osteoarthritis found evidence suggesting that more PRP injections were not necessarily more effective than a single injection [69]. This study compared three groups of patients who received either one injection of PRP, two injections of PRP 3 weeks apart, or a single injection of normal saline. The groups receiving PRP had significantly improved VAS and Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores compared with the placebo group, and there was no significant difference between PRP groups, suggesting that one injection was as effective as two for this study. These studies highlight the need for further investigation of the frequency, dose, and preparation of PRP products, as well as emphasize the need for clear indications for PRP treatment to determine what patient demographic and what specific lesions might respond to PRP treatment. Moreover, the relationship between dose and frequency of injections to cell signaling pathways must be explored.

6 Antimicrobial

The increase in antimicrobial-resistant bacteria has prompted the medical community to seek new means of preventing and treating surgical site infections. PRP has been proposed to have antimicrobial activity primarily based on the known antimicrobial activity of WBCs. Currently, it is unknown how leukocytes function after being removed from the circulation for PRP preparation and directly applied to tissue, bypassing the migration phase of activation. Intracellular calcium also plays a role in neutrophil granule release, and activation with calcium chloride may have some effect on leukocyte activation [72].

Platelets have primary antimicrobial activity. Microbicidal proteins have been purified from rabbit platelets and were show to have dose-dependent microbistatic and microbicidal activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans* [73]. Antimicrobial proteins from resting platelets have greatest activity at pH 5.5, while thrombin stimulation releases antimicrobial proteins with extended action (pH 5.5–7.2) [73]. Microbicidal proteins purified from thrombin-stimulated human platelets elicited bactericidal effects against *B. subtilis*, *E. coli*, *S. aureus*, and *Lactococcus lactis*, and fungicidal effects against *Cryptococcus neoformans* [74]. In addition to the release of antimicrobial proteins, platelets are capable of phagocytosis, and generation and release of reactive oxygen species [75].

Comparisons of PPP with PRP in antimicrobial activity are not well-documented. An in vitro study by Burnouf et al. [76] compared unaltered (native) and complement-inactivated (via heat) PPP, PRP, platelet gel, and solvent/detergent-treated platelet lysate (S/D-PL) in their activity

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Table 2 Recent tendon and ligament platelet-rich plasma injection protocols and complications

Location	PRP type	Activation	Injection	Control	Endpoint	Outcome	Complications	Comments	Reference
Chronic lateral elbow epicondylitis	GPS® III (L-PRP)	No	'Peppering' intra-lesional injection × 1	Autologous blood	6 months	No difference except PRP improved VAS at 6 weeks	Local pain		[64]
Chronic lateral elbow epicondylitis	Biomet Recover® (L-PRP)	No	'Peppering' intra-lesional injection × 1	Corticosteroid	24 months	PRP improved VAS, DASH	None		[65]
Chronic lateral elbow epicondylitis	Manual single spin	No	Intra-lesional injection × 2 (q1 month)	Autologous blood	6 months	No difference		U/S guided	[60]
Chronic lateral elbow epicondylitis	GPS® (L-PRP)	No	'Peppering' intra-lesional injection × 1	Bupivacaine	12 and 24 weeks	PRP improved VAS at 8 and 24 weeks, local tenderness at 4, 12, and 24 weeks, overall success at 24 weeks. Both improved PRTEE	Local pain—18 % control, 19 % PRP	Buffered with sodium bicarbonate	[61]
Chronic lateral elbow epicondylitis	GPS® II (L-PRP)	No	'Peppering' intra-lesional injection × 1	Saline, triamcinolone	12 months	PRTEE improved in all at 3 months, triamcinolone improved by 1 month. Triamcinolone better color Doppler and tendon thickness	Local pain 2–3 weeks	U/S guided. Buffered with sodium bicarbonate	[62]
Chronic patellar tendinopathy	Double-spin manual	$CaCl_2$	Intra-lesional × 3 (q2 weeks)	None	48.6 months	Blanzina, VISA-P, EQ-VAS, Tegner all improved		U/S guided. 2 tx frozen-thawed PRP	[63]
Chronic patellar tendinopathy	MyCells® Autologous Platelet Preparation System	No	Intra-lesional × 2 (q1 week)	Focused extracorporeal shockwave therapy	12 months	PRP improved VISA-P and VAS at 6 and 12 months, and modified Blazina at 12 months	Local pain		[64]
Chronic patellar tendinopathy	Biomet Recover® (L-PRP)	No	Intra-lesional × 1	None	18.4 months	Improved VISA-P and VAS, prior treatments decreased outcome		Mixed with sodium bicarbonate, epinephrine, bupivacaine	[65]

PRP platelet-rich plasma, *L-PRP* leukocyte-rich PRP, *$CaCl_2$* calcium chloride, *VAS* visual analog scale, *DASH* Disabilities of the Arm, Shoulder, and Hand Score, *PRTEE* Patient-Rated Tennis Elbow Evaluation, *EQ-VAS EQ-VAS* EuroQuol-visual analog scale, *VISA-P* Victorian Institute of Sport Assessment-Patella, *U/S* ultrasound, *tx* treatment, *q1 month* every month, *q1 week* every week, *q2 weeks* every 2 weeks

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Table 3 Platelet-rich plasma injection protocols and complications for osteoarthritis of the knee

PRP type	Activation	Injection	Control	Endpoint	Outcome	Complications	Comment	Reference
Manual double-spin	$CaCl_2$	IA × 3 (q2 weeks)	LMW-HA HMW-HA	6 months	PRP improved IKDC and EQ-VAS	None	PRP more effective in younger patients and earlier lesions	[71]
PRGF (P-PRP)	$CaCl_2$	IA × 3 (q3 weeks)	Manual double-spin PRP (L-PRP)	12 months	Both improved IKDC, EQ-VAS, Tegner	More pain and swelling with L-PRP	Both preparations more effective in younger with earlier lesions	[66]
Magellan autologous platelet separator (L-PRP)	No	IA × 1	No	12 months	VAS and IKDC improved out to 6 months, effect declined 9–12 months, mean relapse pain 8.8 months	Mild swelling and pain (63 %)	PRP less effective with increasing age and joint degeneration	[70]
Single-spin manual and leukocyte filtration (P-PRP)	$CaCl_2$	IA × 1 or IA × 2 (q3 weeks)	Saline × 1	6 months	WOMAC and VAS improved to 6 months both PRP groups, start return of pain. Control WOMAC and VAS worsened	Dizziness and nausea. Pain and stiffness 2 days—significant increase with platelet concentration	Severe OA excluded. Large volume blood collected	[69]
ACP (P-PRP)	No	IA × 4 (q1 week)	HA × 4 q1 week	24 weeks	4-week WOMAC HA better than PRP, after 4 weeks to 24 weeks PRP improved and HA declined		Severe OA excluded	[67]
Regen ACR-C®	No	IA × 2 (q4 weeks)	No	12 months	IKDC, VAS, KOOS, Tegner, Marx scores all improved	No	50 % patients prior surgery. No effect of prior surgery or degree of OA	[68]

PRP platelet-rich plasma, *P-PRP* pure PRP, *L-PRP* leukocyte-rich PRP, *IA* intra-articular, *LMW-HA* low-molecular weight hyaluronic acid, *HMW-HA* high-molecular weight hyaluronic acid, *HA* hyaluronic acid, *IKDC* International Knee Documentation Committee, *VAS* visual analog scale, *EQ-VAS* EuroQuol-visual analog scale, *WOMAC* Western Ontario and McMaster Universities Index of Osteoarthritis, *KOOS* Knee Injury and Osteoarthritis Outcome Score, *ACP* autologous conditioned plasma, *ACR-C* autologous cellular rejuvenation-classic, *OA* osteoarthritis, *q1 week* every week, *q2 weeks* every 2 weeks, *q3 weeks* every 3 weeks, *q4 weeks* every 4 weeks

against common bacteria found in wounds. PRP in this study was generated through platelet apheresis and platelet gel was activated with calcium chloride. Samples originated from two donors. The results of this study showed strong inhibition of *E. coli* by all native plasma and platelet materials. Additionally, there was stronger inhibition of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with native PPP, PRP, and S/D-PL than with platelet gel. No native plasma or platelet materials inhibited *Enterococcus cloacae*, *B. cereus*, *B. subtilis*, *S. aureus*, or *S. epidermidis*. Complement-inactivated plasma and platelet materials did not inhibit any bacteria [76].

Because complement-inactivated products did not inhibit any bacteria, the authors posit that complement and/or other heat-sensitive compounds are the primary negotiators of antimicrobial activity in platelet products. Moreover, because S/D-PL more strongly inhibited *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* than platelet gel, they

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suggest that calcium chloride activation and coagulation cascade-induced activation of fibrin may consume complement or other inhibitors or may support bacterial proliferation by releasing other factors [76]. While these results suggest that antimicrobial activity against *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and *S. aureus* is mediated not by platelets or WBCs but by plasma or other heat-sensitive components, other animal and human in vivo studies have continued to show the significant antimicrobial activity of PRP.

In vivo evidence supports the antimicrobial actions of PRP. Surgical wound infection rates in patients undergoing cardiac surgery were significantly lower in wounds treated with PRP at the time of incision closure compared with untreated controls [77, 78]. PRP demonstrated antimicrobial activity against bacteria isolated from an infected wound, and negative cultures were obtained from the wound 5–6 days after treatment [79]. An in vitro study evaluating the antimicrobial spectrum of PRP obtained from 20 donors found that PRP was active against methicillin-sensitive and methicillin-resistant *S. aureus* (MRSA), and *E. coli* [80]. However, PRP from two individuals did not demonstrate antimicrobial activity against the strains tested. There was no antimicrobial activity of PRP against *K. pneumoniae* or *E. faecalis*, and PRP potentiated the growth of *P. aeruginosa* [80]. Finally, in a rabbit tibial MRSA osteomyelitis model, debridement with systemic vancomycin and local injection of PRP gel resulted in superior clearance of infection and bone defect repair compared with all other treatments, including debridement and systemic vancomycin [81]. All of these studies used activated leukocyte-rich PRP preparations.

7 Hemostasis

Platelets play a major role in coagulation, first by forming the initial platelet plug and then by participating in the conversion of soluble fibrinogen to fibrin matrix. Therefore, the use of PRP to minimize hemorrhage at surgical sites would seem logical. Following total knee arthroplasty, PRP has been primarily used as a hemostatic agent at the time of closure. Postoperative bleeding may lead to a variety of complications, including hematoma or seroma formation, increased pain, arthrofibrosis, and the need for blood transfusion and associated complications [82, 83]. The literature contains only a handful of studies on this specific subject, and results are conflicting. Three studies found no significant effect of PRP gel on postoperative hemoglobin concentration [82, 84, 85]. However, another study commented that the use of suction drains may have resulted in loss of PRP and consequently reduced effect [83]. This study found a positive effect of PRP gel for hemostasis following total knee arthroplasty, with significantly smaller decreases in postoperative hemoglobin, decreased narcotic use, increased range of motion at discharge, and earlier hospital discharge. The authors specified that a tourniquet and electrocautery were used and tissues thoroughly dried prior to PRP application. Different systems were used in each study, and there was no characterization of PRP composition. Therefore, recommendations on the optimal PRP product cannot be made.

8 Imaging

The radiological impact of PRP injections was evaluated by de Almeida et al. [44] in their randomized control trial comparing patients receiving PRP for patellar graft donor site healing following ACL repair with controls receiving no PRP following repair. Grafts were harvested from the central third of the patellar tendon, and apheresis-derived PRP platelet gel was applied to the harvest site. Their MRI results showed a significantly smaller patellar gap area for the PRP group ($p = 0.046$) and no difference between groups for cross-sectional area of the patellar tendon or patella height at 6 months postoperative.

Focusing their efforts on radiologically assessing the effect of PRP on osteoarthritis, Halpern et al. used MRI to assess the effects of a single PRP injection on progression of osteoarthritis of the knee [20]. Patients aged 30–70 years with Kellgren grade 0–II osteoarthritis confirmed by MRI and knee pain were given a single injection of PRP. They were evaluated at 6 months and 1 year post-procedure by clinical outcomes and at 1 year post-procedure by MRI. The results showed significant and sustained reduction in mean baseline VAS scores at 6 months and 1 year, as well as significant improvements in WOMAC pain, stiffness, and ADL scores over the same time frames. MRI results showed no significant worsening of patellofemoral osteoarthritis in 80 % of knees and no change in the appearance of lateral femoral and tibial compartment osteoarthritis in 83.3 % of knees. There was a non-significant lack of change in medial compartment osteoarthritis in 73.3 % of cases, and one knee with medial compartment osteoarthritis actually improved in appearance after 1 year [20].

These studies suggest that PRP may play a role in improving clinical outcomes in patellar tendon healing and early-onset osteoarthritis in the 6 months to 1 year post-procedural period. Interestingly, PRP was prepared differently in each study, with one study using platelet apheresis and the other using PRP derived from whole blood. There were additional differences in dose and no mention of leukocyte concentration or activation status in the osteoarthritis study. These differences make it difficult to correlate the biochemical, clinical and radiological effects of PRP.

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9 Complications

Rare and predominantly minor complications have been reported following PRP use. The most frequently reported complications following intra-articular injection include swelling, tenderness, joint pressure, and local pain, which are typical following intra-articular treatments due to distension of the joint causing pressure and pain [86, 87]. Patel et al. [69] reported significantly more post-injection pain with higher platelet concentrations. Another study comparing single-spin PRP with double-spin PRP injection for knee osteoarthritis found that complications of pain and swelling were significantly more common in the double-spin group which had higher platelet and WBC concentrations [66]. This difference suggests that the composition of PRP may impact patient comfort. Local pain at the injection site is the main complaint reported for treatment of tendons and ligaments, although little detail has been provided in many studies (Table 2).

A final potential complication is related to activation of the platelets in PRP. Potential side effects of thrombin activation include immune reaction, development of antibodies to human coagulation proteins, and coagulopathy [5]. Based on these risks, it would be prudent to use autologous thrombin or calcium chloride alone for platelet activation.

10 Conclusion

PRP has numerous advantages as an autologous biologic for treatment of musculoskeletal injuries. It is accessible, easily prepared, has minimal complications, and has a broad range of potential therapeutic actions. There are numerous types and application methods described. However, fully detailed basic science and clinical prospective randomized clinical trials must be performed to improve our understanding of the optimal composition and use of PRP. The major disadvantages of PRP use include the high variability in PRP research, making it difficult to counsel patients regarding efficacy, particularly as treatment can represent a significant out-of-pocket expense.

Currently there is insufficient literature to support a consensus on the optimal PRP preparation for each indication, dose volume, dosing interval, and whether activation is necessary (and if so, by what method). Until defined algorithms and evidence-based protocols are available, the clinician should consider the biology of the condition being treated and the intended goal for PRP therapy when choosing the type of PRP and injection method. Also, patients should be informed that while PRP has several theoretical advantages with minimal complications, the use of PRP is still investigational.

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