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Application of Platelet-Rich Plasma to Disorders of the Knee Joint

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Abstract

Importance. The promising therapeutic potential and regenerative properties of platelet-rich plasma (PRP) have rapidly led to its widespread clinical use in musculoskeletal injury and disease. Although the basic scientific rationale surrounding PRP products is compelling, the clinical application has outpaced the research. *Objective*. The purpose of this article is to examine the current concepts around the basic science of PRP application, different preparation systems, and clinical application of PRP in disorders in the knee. *Evidence Acquisition*. A systematic search of PubMed for studies that evaluated the basic science, preparation and clinical application of platelet concentrates was performed. The search used terms, including platelet-rich plasma or PRP preparation, activation, use in the knee, cartilage, ligament, and meniscus. Studies found in the initial search and related studies were reviewed. *Results*. A comprehensive review of the literature supports the potential use of PRP both nonoperatively and intraoperatively, but highlights the absence of large clinical studies and the lack of standardization between method, product, and clinical efficacy. *Conclusions and Relevance*. In addition to the call for more randomized, controlled clinical studies to assess the clinical effect of PRP, at this point, it is necessary to investigate PRP product composition and eventually have the ability to tailor the therapeutic product for specific indications.

Keywords

platelet-rich plasma, variability, articular cartilage, tissue, anterior cruciate ligament (ACL)

Introduction

Musculoskeletal injuries limit the activity level and affect lifestyles of athletes at all levels. In addition, they representing a substantial health care burden.[1] Not only are acute injuries associated with immediate inflammation and pain, but chronic or poorly healing musculoskeletal injuries often are associated with increased inflammation and tissue catabolism that outpace anabolic reconstruction. The use of platelet-rich plasma (PRP) as a therapeutic treatment to control inflammation and enhance repair of musculoskeletal injuries is an approach that has recently grown in popularity.

The systematic search of PubMed performed between June 2011 and January 2013 for studies that evaluated the basic science, preparation, and clinical application of platelet concentrates revealed that although there is an abundance of published literature on the therapeutic use of PRP, clinical reports are predominantly case studies on various conditions that report mixed findings on efficacy. Taken as a whole, this body of evidence highlights a lack of standardization with respect to the devices and methods used for preparation and application of PRP across fields, including sports medicine. The absence of statistically significant randomized controlled trials makes it difficult to derive firm recommendations regarding the clinical utility of PRP therapeutics. Nonetheless, positive effects reported for PRP in multiple medical capacities, from its early use in maxillofacial and plastic surgery to sports medicine, and the underlying hypotheses for its mechanism of action are interesting, if not compelling.[2-8] It is therefore worth considering that mixed reports on efficacy relate to a lack of standardization rather than an absence of therapeutic potential. This review examines the development and continuity of thought around the use of PRP based therapeutics through the examination of the proposed mechanisms of action, differences in PRP preparations, and published clinical findings on the use of PRP for injuries and disorders of joint tissues in the knee.

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What Is Platelet-Rich Plasma?

Classification of Platelet-Rich Plasma

All PRP is not the same. Currently, PRP is a generic term used to describe a broad range of plasma products derived from a sample of whole blood. Depending on the methods and devices used to produce PRP, preparations differ significantly in cellular composition of red blood cells, platelets, leukocytes, and plasma proteins.[9] For example, the American Association of Blood Banks defines PRP as the resultant plasma fraction following a single light spin of whole blood, in which platelets are enriched in comparison with other cell types.[10] This definition refers to the methodology that produces a plasma fraction containing platelet concentrations close to those found in a whole blood sample and having greatly reduced, or undetectable levels of red blood cells and leukocytes. In stark contrast, the seminal publication by Marx *et al*.[11] describes PRP produced using a double-spin centrifugation cycle. In this case, the plasma fraction that is obtained contains a concentration of platelets approximately 5 times the baseline and a significant presence of leukocytes and red blood cells. Today, a number of commercially available PRP systems and manually prepared solutions have been used to produce therapeutic preparations of the PRP that differ greatly with respect to the relative compositions of blood constituents. It is therefore important, whether evaluating literature or choosing a PRP system, to consider the suitability of different PRP preparations within the context of the targeted indication.

In attempt to distinguish PRP products, a recent publication proposes a classification system based on the absolute number of platelets, platelet activation method and leukocyte content.[12] While this classification may prove beneficial for meta-analysis of studies and provide some guidance with respect to the choice of a PRP system, to avoid confusion, it remains reasonable for investigators to provide quality control (i.e., cellular composition and platelet concentration) of the final therapeutic PRP preparation and to examine results with regard to efficacy and tissue metabolism.

Basic Scientific Rationale

PRP products are thought to facilitate the recruitment, proliferation, and maturation of cells that participate in the regeneration of tendon, ligament, muscle, bone, and cartilage. This is based on our understanding of the normal physiological role of platelets as first responders to injury.[13] In addition to hemostatic activity, platelets are known to release biomolecules that control myriad different biological activities. Through the efforts of the platelet proteome project, more than 1,500 different proteins have been identified in platelet releasate, and many of the important growth factors (GFs), cytokines, and chemokines have been defined as critical for processes necessary for effective tissue regeneration (**Table 1**).[3,14-16]

Many of the chemical messengers and bioactive proteins released by platelets have been well studied in both *in vitro* and *in vivo* systems and their roles to coordinate chemotaxis, proliferation, and differentiation, to modulate tissue homeostasis through inflammatory responses, and to provide antimicrobial activity have been widely proposed. Recent *in vivo* studies report that the systemic concentration of growth factors, insulin-like growth factor (IGF-1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and mRNA expression of pro-inflammatory biomarkers IL-1b, IL-6, IL-8, and inducible nitric oxide synthetase (iNOS) change significantly following PRP application.[26-28] Furthermore, it has been reported that the release of growth factors from platelet granules is directly correlated with PRP platelet concentration.[8,29]

The alteration of these biomarkers indicates that PRP appears to trigger multiple biological pathways and also delivers multiple biological factors directly to the injection site. However, there is limited clinical evidence that reports optimum concentrations of the bioactive factors responsible for cytokine and chemokine expression in PRP products. Although the high level clinical evidence has yet to be developed, it is intuitive that PRP may indeed provide a unique therapy that drives physiological regeneration by activating the necessary biological pathways.

Preparation of Platelet-Rich Plasma Products

Many systems are available for the preparation of PRP: This results in significant differences in the composition of PRP products (**Table 2**). The preparation of PRP begins with collection of autologous peripheral blood containing citrate (sodium citrate, calcium citrate, or acid-citrate dextrose) to inhibit the coagulation pathway. Two basic preparation systems exist; plasma-based PRP systems and buffy-coat–based PRP systems. These systems differ based on centrifugation spin parameters that directly affect the spatial distribution of blood-cell components. The centrifugation time and speed affect the number and concentration of platelets and other cell types within the PRP and thus availability of growth factors, chemokines as well as pro-inflammatory and anti-inflammatory mediators. Several examples of different PRP systems are presented in **Table 2**.

Not only are there differences in PRP preparations introduced by the technological features of individual devices, but there is also wide biological variation between patients. The normal range of platelets count in human whole blood is relatively broad (150,000-350,000 platelets/μL) and can vary considerably day to day.[36,37] It is also noteworthy that the range of platelet size and density can vary depending on the size and distribution of megakarocyte precursors from which platelets are derived.[38]

Table 1. Growth Factors, Cytokines, and Bioactive Molecules Associated with Platelets.[a]

	Molecule	Biological Function
α-Granules		
Growth factors	PDGF	Chemoattraction, cell proliferation[17]
	TGF-β	Promotes matrix synthesis
	VEGF	Angiogenesis
	EGF	Cell proliferation[18]
	ECGF	Endothelial cell proliferation, angiogenesis
	bFGF	Mediates angiogenesis[19]
	IGF-I, II	Cell proliferation, maturation, bone matrix synthesis[20]
	HGF	Cell growth and motility of epithelial cells
	PDAF	Angiogenesis
Cytokines and chemokines	RANTES, IL-1β MIP-1α, MCP-3, growth-regulated ocogene-α	Chemotaxis, cell proliferation and differentiation, angiogenesis[3]
Adhesive proteins	Fibrinogen	Fibrin formation during blood clotting cascade
	Fibronectin	Binds to cell surface
	Vitronectin	Cell adhesion, chemotaxis
	Thrombospondin-1	Inhibits angiogenesis
Basic proteins	Platelet factor 4	Inhibits angiogenesis
	β-Thromboglobulin	Platelet activation, inhibits angiogenesis
	Endostatins	Inhibit endothelial cell migration and angiogenesis[21]
Proteases and anti-proteases	TIMP-4	Regulate metalloproteases and matrix degradation
	MMP-4	Matrix degradation
	α1-Antitrypsin	Inhibits proteases and enzymes
Membrane glycoproteins	CD40L	Inflammation, synthesis of interleukins, production of integrin, platelet endothelial cell adhesion, cell signaling[22]
	P-selectin	Vascular cell adhesion, binding and recruitment of leukocytes to inflamed tissue
Antimicrobial proteins	Thrombocidins	Bacterial and fungicidal properties[23,24]
Bone morphogenetic proteins	BMP-2, BMP-4, BMP-6	Initiation and maintenance of bone fracture healing, osteoinductive properties[25]
Coagulation factors	Factor V, factor XI, protein S, antithrombin	Thrombin activation fibrin clot formation
Dense granules	ADP	Promotes platelet aggregation[21]
	ATP	Participates in platelet response to collagen
	Ca^{2+}	Platelet aggregation and fibrin formation. In wound healing, modulates keratinocyte proliferation and differentiation
	Histamine	Increased capillary permeability, attracts and activates macrophages, pro- and anti- inflammatory effects
	Dopamine	Neurotransmitter, regulates heart rate and blood pressure
	Serotonin	Vasoconstriction and increased capillary permeability
	Catecholamines	Hormones released by the adrenal gland in response to stress
	Thromboxane	Vasoconstriction, platelet aggregation, clot formation

[a]This table depicts the contents and function of platelet dense granules and α-granules.

Variability of Platelet-Rich Plasma Products

Multiple studies show that the highest concentration of platelets does not necessarily stimulate and may even suppress cell proliferation and differentiation.[36,39,40] Thus, the mechanics of preparation in different devices are likely to contribute to the different outcomes seen in clinical studies. The optimal number and/or concentration of leukocytes in the final preparation that will yield the greatest benefit remains controversial as well. It has been reported that PRP containing pro-inflammatory granulocytes may contribute to the inflammatory responses beneficial for wound healing

Table 2. Platelet-Rich Plasma (PRP) Preparation Systems.[a]

Machine	Volume of Whole Blood (mL)	Centrifugation Force	Spin Time (Minutes)	Volume of PRP (mL)	PRP Platelet Concentration (Fold Change)	Activator	Leukocyte Concentration
Arthrex ACP System	9	1,500 rpm[b] single spin	5	2-4	2×-3×	None if used within 30 minutes	No
Biomet GPS	55	3,200 rpm single spin	15	6	2.07×	Thrombin $CaCl_2$	Yes (fold change: 5.4)
Cascade Platelet-Rich Fibrin Matrix (PRFM)	18	1,100 × *g* and 1,450 × *g*	6 and 15	7.5	1.6×	$CaCl_2$	No
Cytomedix Angel	40-180	3,200 rpm	15-28	2-5	4.3×	None required	Yes
Harvest SmartPReP2APC	50 or 100	3,650 rpm double spin	14	3-9 or 10-20	7.0×	Thrombin $CaCl_2$	Yes (fold change: 2.3 and 1)
Magellan	26	3,800 rpm double spin	17	6	2.8×	$CaCl_2$	Yes (fold change: 3.2)
BTI PRGF	9-72	460 × *g* single spin	8	4-32	2×-3×	$CaCl_2$	No
Plateltex	50	160-180 × *g* and 1,000-1,200 × g	10 and 10	6 and 10	1×-2×	Batroxobin for gel	Yes

[a]The information on platelet-rich plasma preparation systems was compiled primarily from references 5, 30-35.
[b]rpm = revolutions per minute; *g* = G-force.

as well as treating tendinopathy.[41,42] On the other hand, the presence of these same pro-inflammatory cells may not be desirable for addressing pathologies related to inflammation, as is the case for inflammatory arthritides,[43,44] as neutrophils secrete matrix metalloproteinase (MMP) components that have been shown to be degradative to tenocyte and chondrocyte repair following intra-articular PRP injection.[45]

Two recent articles highlight relevant findings in the platelet versus white cell debate.[8,46] Authors of the first study hypothesized that the concentration of growth factors and catabolic cytokines are dependent on the cellular composition of PRP. They compared PRP from 11 human volunteers using 2 commercial systems,[8] the PRP-I system (ACP Double Syringe System, Arthrex, Naples, FL), and the PRP-II system (GPS III Miniplatelet Concentration System, Biomet, Warsaw, IN). The PRP-I system had 1.9× platelets and 0.13× leukocytes compared with whole blood whereas PRP-II had 4.69× platelets and 4.26× leukocytes compared with whole blood. Growth factors were increased significantly in PRP-II compared with PRP-I, but catabolic cytokines (MMP-9, MMP-13, and IL-1β) also were increased significantly in PRP-II compared with PRP-I. The second study examined the effects of a single- versus double-spin preparation of PRP on anabolic and catabolic activities of cartilage and meniscal explants *in vitro*. The double-spin system had much higher platelet counts as well as white cell counts. However, the single-spin, low platelet/white cell product had significantly improved ^{3}H-proline incorporation in the cartilage explants and also increased[35] S-sulfate incorporation as an index of glycosaminoglycan incorporation.[46] More important, the gene expression of ADAMTS-4 (aggrecanase 1) was significantly increased in the high platelet/high white cell count product compared with the low platelet/low white cell count product. These results rejected the hypothesis that higher concentrations of platelets in PRP increase extracellular matrix synthesis in cartilage and meniscus and suggest that high platelet concentrations for intra-articular injection should be considered with caution.

Technical Aspects of Plate-Rich Plasma Application

For PRP to stimulate biological action, degranulation and release of growth factors from platelet α-granules at the site of application must occur. Although no evidence was provided, it has been suggested that PRP can be stored in its anticoagulated state for approximately 8 hours; however, once activated, immediate application is recommended.[47] The activation can occur in the preparation step before application via exogenous activation or *in vivo* from the endogenous inflammatory environment of the damaged tissue.

Bovine thrombin is often used as an activator to initiate clot formation before application. One potential complication presented in earlier literature was the potential to develop coaguopathies when using bovine thrombin, but

this risk has been largely obviated with the use of highly purified bovine thrombin. However, because of continued perception of a safety risk, methods to activate PRP that do not rely on thrombin have been explored.

Dugrillon *et al*.[48] demonstrated that calcium chloride generates the clotting mechanism, activates the platelets in PRP and stimulates the production of native thrombin once PRP is applied, causing an increase in the release of growth factors.[49,50] Calcium chloride in combination with thrombin has also been used in the development of a platelet-rich fibrin matrix that, in turn, produces a cascade effect whereby the release of growth factors is less than maximum, but sustained for a longer period of time at the application site.[11,51,52]

Type I collagen can also be used for PRP activation and may be beneficial as endogenous collagen is already present in the environment of PRP application. In some *in vitro* studies, collagen has been found to be equally and in some cases more effective than thrombin activation, as thrombin activates platelets to release growth factors immediately, activation by added collagen results in a gradual accumulation of growth cytokines at the site of PRP application.[53,54]

Another factor that must be considered is the duration of bioactive molecules at the injection site or interface once released from the platelet α-granules. While this can depend on the specific interface and the activation method, a recent study evaluated the kinetics of growth factor release in dental implants following PRP application.[55] Comparing the spatiotemporal relationship between implants treated with activated and nonactivated platelet-rich plasma and platelet-poor plasma, it was reported that activation and subsequent clot formation resulted in greater duration of growth factors at the interface. Furthermore, it was noted that the concentration of PDGFs at the interface was significantly higher in samples where activated-PRP was applied, but this concentration became negligible after 2 to 4 days. The authors concluded that prior to PRP application, products should be activated and allowed to clot to permit the adequate duration of bioactive factors at the injection site. Although this kinetic relationship examined in dental implants remains relevant, further investigation into the activation of PRP products and time-dependent release of growth factors must be considered in the application of PRP, particularly with intraoperative application when the injury site is copiously irrigated.

Rationale for Clinical Application of Platelet-Rich Plasma

The benefits seen early in maxillofacial and plastic surgery led to the use of PRP in myocardial surgery, treatment of ulcers, reproductive pathology, and orthopedics. As happens with novel medical advancements, when beneficial results were demonstrated, the clinical use of PRP expanded and outpaced the research behind the product. However, the application of PRP is experiencing widespread growth in this field and an increasing number of clinical trials as well as well-designed controlled studies are being performed to determine optimal application of PRP in tendon, muscle, ligament, bone, and cartilage injuries.

Basic Scientific Rationale of Platelet-Rich Plasma Use in Articular Cartilage Degradation and Repair

On a molecular level, results have indicated a beneficial effect of PRP on chondrocytes and mesenchymal stem cells. Increased cell proliferation and synthesis of proteoglycans and collagen type II has been demonstrated when cell cultures of chondrocytes[56,57] and mesenchymal stem cells[58] are treated with PRP compared with controls treated with platelet-poor plasma or fetal bovine serum. PRP also promotes differentiation of subchondral bone progenitor cells. Kruger *et al*.[59] not only demonstrated that PRP significantly stimulated the migration of human progenitor cells in chemotaxis assays, but also showed that histological staining revealed increased cartilage matrix formation in cells treated with PRP compared with untreated progenitor controls. Furthermore, Anitua *et al*.[60] reported that synoviocytes from patients with osteoarthritis (OA) cultured in PRGF demonstrated an increase in hyaluronan (HA) production. The authors proposed that intra-articular administration of PRP might be beneficial in restoring hyaluronic acid concentration and serve as an endogenous source of chondroprotection and joint lubrication. As previously discussed, the synthesis of collagen and glycosaminoglycan in equine articular cartilage explants was shown to be superior with low-platelet product compared with high-platelet product.[46]

Clinical Application of Platelet-Rich Plasma Use in Articular Cartilage Degradation and Repair in the Knee

Osteoarthritis

In an uncontrolled prospective study, 14 patients with knee OA were treated with PRP produced using anticoagulated blood processed in the GPS III system.[61] These authors reported reduced pain and improved functional outcome after a series of injections with PRP. They also reported improvement in the ultrasonographic measurement of femoral articular cartilage thickness in 6 out of 13 patients at 6 months and in the satisfaction survey at the 1-year follow-up examination, 8 of 13 patients reached their goals at 1 year (**Table 3**). Another study showed that 261 patients presenting with OA and treated with a series of 3 PRGF injections demonstrated statistically significant improvement in knee outcome assessment scales, including visual analog

Table 3. Published Human Studies of Platelet-Rich Plasma (PRP) Clinical Application in Osteoarthritis.

Authors	Diagnosis	Design	Purpose	PRP Preparation	Outcome Measurements	Results
Sampson *et al.* (2009)[61]	Knee osteoarthritis	Prospective, preliminary study; 14 patients	Evaluate potential efficacy of PRP injection in treatment of osteoarthritis	GPS system	At 2-, 5-, 11-, 18-, and 52-week follow-up Brittberg-Peterson VAS, KOOS, and cartilage ultrasound	BP VAS: At final follow-up significant reduction in moving pain, resting pain, and bent knee pain KOOS: At final follow-up significant increase in pain and symptom relief scores Ultrasound: No significant changes in cartilage thickness with small sample size
Wang-Saegusa *et al.* (2011)[62]	Knee osteoarthritis	Nonrandomized prospective study; 261 patients	Assess quality of life and functional capacity following a series of PRP injections	PRGF	VAS, SF-36, WOMAC, Lequesne Index pretreatment and 6 months following treatment	Total % improvement at 6 months: 67.2% Average assessment and % improvement at 6 months: VAS: 3.32, 73.4% SF-36: mental 52.87, 52% physical 42.28, 64.6% WOMAC: pain 4.69, 65.5% stiffness 2.13, 48.2% function 15.31, 67.4% Lequesne: pain 2.97, 59.8% distance 1.58, 36.6% DLA 2.94 53.7%
Napolitano *et al.*, (2012)[63]	Degenerative joint disease of the knee	Prospective clinical study; 27 patients	Evaluate the effect of 3 PRP as a treatment for arthritis and degenerative cartilage disease	System not reported 8 mL whole blood, 3,100 rpm 8 minutes	NRS and WOMAC scales pretreatment, 7 days posttreatment and at 6-month follow-up	NRS arthritis: pretreatment: 8.1 ± 1.7, posttreatment: 3.4 ± 2.5 WOMAC arthritis: pain: pretreatment 10.4 ± 3.9, posttreatment 17 ± 2.5, 6 months 17.9 ± 2.8. Stiffness: pretreatment 4.9 ± 2.2, posttreatment 7 ± 0.9, 6 months 7.4 ± 0.9. Function: pretreatment 36.3 ± 11.8, posttreatment 58.9 ± 9.9, 6 months 60.7 ± 7.6 NRS cartilage disease: pretreatment: 6.8 ± 1.7, posttreatment: 2.3 ± 2.1 WOMAC cartilage disease: pain: pretreatment 13.0 ± 4.8, posttreatment 18 ± 2.5, 6 months 18 ± 2.8. Stiffness: pretreatment 5.1 ± 2.2, posttreatment 6.8 ± 1.0, 6 months 6.8 ± 1.3. Function: pretreatment 46.2 ± 13, posttreatment 61.0 ± 4.7, 6 months 63.1 ± 4.3.
Gobbi *et al.* (2012)[64]	Symptomatic knee osteoarthritis	Prospective case series; 50 patients treated with 2 PRP injections, 25 of who had undergone previous operative intervention	Determine efficacy of intra-articular PRP injections and compare outcomes in patients with and without previous surgery	RegenACR 9 mL peripheral blood, 3,500 rpm for 9 minutes, 4 mL PRP used	VAS, IKDC subjective, KOOS, and Tegner scores at pretreatment, 6 and 12 months	VAS: Baseline operated: 3.2 ± 1.4; nonoperated: 4.4 ± 2.7, 6 months operated: 1.9 ± 1.7; nonoperated: 2.4 ± 1.9, 12 months operated: 1.2 ± 1.1; nonoperated 1.3 ± 1.4. Significant improvement, no significant difference between groups.
Kon *et al.* (2010),[65] Filardo *et al.* (2011)[66]	Degenerative cartilage lesions and osteoarthritis of the knee	Prospective clinical trial; 91 patients received series of 3 PRP injections, 90 available at 2 years	Investigate the continued outcomes of PRP injection in degenerative cartilage of the knee	System not reported 150 mL whole blood, 1,800 rpm 15 minutes and 3,500 rpm 10 minutes, 20 mL PRP (15 mL used)	IKDC objective and subjective, EQ-VAS evaluated pre- and posttreatment, at 6, 12, and 24 months	IKDC: Baseline operated: 48.6 ± 12.1; nonoperated: 49.0 ± 14.9, 6 months operated: 64.5 ± 10.6; nonoperated: 64.9 ± 9.9, 12 months operated: 64.0 ± 22.9; nonoperated 76.3 ± 16.8. Significant improvement, no significant difference between groups. KOOS: Significant improvement in all subgroups at 6 and 12 months with no significant difference in operative vs. non-operative or in shaving vs. microfracture.

(continued)

Table 3. (continued)

Authors	Diagnosis	Design	Purpose	PRP Preparation	Outcome Measurements	Results
						Tegner: Baseline operated: 2.7 ± 1.7; nonoperated: 3.0 ± 1.3, 6 months operated: 3.8 ± 1.7; nonoperated: 3.7 ± 1.5, 12 months operated: 4.8 ± 2.3; nonoperated 4.9 ± 1.8. Significant improvement, no significant difference between groups. IKDC objective: Normal or nearly normal knees; 46.1% pretreatment, 78.3% posttreatment, 73.0% 6 months, 66.9% 12 months, 59% 24 months IKDC subjective: 40.5 ± 10.4 pretreatment, 62.5 ± 15.9 posttreatment, 62.6 ± 18.6 6 months, 60.6 ± 18.9 12 months, 51 ± 20 24 months EQ-VAS: 50.3 ± 16.4 pretreatment, 71.2 ± 15.2 posttreatment, 70 ± 17.5 6 months, 69.6 ± 17.4 12 months, ~59 12 months Length of PRP action: mean 11 ± 8 months, median 9 months
Patel *et al.* (2013)[67]	Bilateral knee osteoarthritis	Randomized, controlled trial; single injection of PRP (50 knees) vs. series of PRP injections (52 knees) vs. saline injection (46 knees)	Compare the clinical outcomes of patients treated with 2 injections of PRP vs. 1 injection of PRP vs. a saline injection	White blood cell–filtered PRP	WOMAC pretreatment, 6 weeks, 3 months, and 6 months	Mean total WOMAC: Single PRP injection; 49.86 baseline, 27.18 final follow-up Series of PRP injections: 53.20 baseline, 30.48 final follow-up Saline injection: 45.54 baseline, 53.09 final follow-up Statistically significant improvement in both PRP groups compared with no significant improvement in saline group.
Filardo *et al.* (2012)[68]	Degenerative cartilage lesions and osteoarthritis of the knee	Clinical comparison study; 72 patients single-spin PRP product series injections vs. 72 patients double-spin PRP product series injections	Compare the safety and clinical efficacy of 2 different PRP preparation methods	Single-spin PRGF: 4 tubes 9 mL whole blood 580 rpm 8 minutes, 5 mL PRGF Double-spin PRP product: 150 mL whole blood, 1,800 rpm 15 minutes and 3,500 rpm 10 minutes, 20 mL PRP	IKDC, EQ-VAS, and Tegner at baseline, and 2, 6, and 12 months	Significant improvement in both groups with no significant difference between groups IKDC subjective: PRGF: 45.0 ± 10.1 to 59.0 ± 16.2, 61.3 ± 16.3, 61.6 ± 16.2 at 2, 6, and 12 months. PRP; 42.1 ± 13.5 to 60.8 ± 16.6, 62.5 ± 19.9, 59.9 ± 20.0 at 2, 6, and 12 months EQ-VAS: PRGF and PRP groups showed significant improvement at 2 months with respect to basal level. Maintained at 6 and 12 months Tegner: PRGF and PRP groups showed at 2 months significant improvement, at 6 months further improvement, at 12 months improvement
Sánchez *et al.* (2008)[69]	Osteoarthritis of the knee	Retrospective, preliminary clinical study; series of 3 intra-articular injections 30 PRP vs. 30 hyaluronan	Compare the preliminary clinical outcomes of patients following intra-articular injection of PRP and hyaluronan	PRGF	WOMAC preinjection and 5 weeks	Pain subscale 5 weeks: Achieved 33.4% PRGF group vs. 10% hyaluronan group

(continued)

Table 3. (continued)

Authors	Diagnosis	Design	Purpose	PRP Preparation	Outcome Measurements	Results
Kon *et al.* (2011)[70]	Osteoarthritis of the knee	Prospective, controlled, comparative study; 50 PRP, 50 high and 50 low molecular weight hyaluronan	Compare the efficacy of PRP injection to hyaluronan injection	150 mL whole blood, 1,480 rpm 6 minutes and 3,400 rpm 15 minutes, 20 mL PRP	2- and 6-month clinical evaluation IKDC and EQ-VAS	2 months: Low molecular weight hyaluronan and PRP higher results 6 months: PRP higher results
Cerza *et al.* (2012)[71]	Gonarthrosis in the knee	Prospective, randomized, comparative study; 60 ACP injections weekly for 4 weeks vs. 60 hyaluronan injections weekly for 4 weeks	Compare the clinical efficacy of a series of ACP injections vs. a series of hyaluronan injections in patients with knee gonarthrosis	Arthrex ACP	WOMAC clinical assessment pretreatment, 1, 2, and 6 months	Mean WOMAC scores ACP group: pretreatment 76.9 ± 9.5, significant improvement at 1 month 49.6 ± 17.7, continued improvement at 2 months 39.1 ± 17.8, continued improvement at 6 months 36.5 ± 17.9. Significant improvement at all follow-ups and significant improvement at 2 and 6 months compared with hyaluronan group. Mean WOMAC scores hyaluronan group: pretreatment 75.4 ± 10.7, significant improvement at 1 month 55.2 ± 12.3, slight worsening at 2 months 57.0 ± 11.7, sharp worsening at 6 months 65.1 ± 10.6
Sánchez *et al.* (2012)[72]	Symptomatic osteoarthritis in the knee	Randomized, controlled, double-blinded clinical trial; 176 patients treated with intra-articular PRP injection vs. intra-articular hyaluronan injection	Evaluate short-term clinical outcome and effectiveness of PRP injection vs. hyaluronan injection	PRGF	WOMAC and pain response rate	14.1% higher clinical assessment scores in PRP-treated group compared to hyaluronan-treated group No statistically significant difference between groups
Filardo *et al.* (2012)[73]	Osteoarthritis of the knee	Randomized, controlled, double-blinded clinical trial; 54 PRP injections weekly for 3 weeks vs. 55 hyaluronan injections weekly for 3 weeks	Compare the clinical efficacy of a series of PRP injections versus a series of HA injections for treatment of osteoarthritis in the knee	150 mL whole blood, 1,480 rpm 6 minutes and 3,400 rpm 15 minutes, 20 mL PRP	IKDC, EQ-VAS, Tegner, and KOOS scores at baseline, and 2, 6 and 12 months	IKDC: PRP group: basal 50.2 ± 15.7, 2 months 62.8 ± 17.6, 6 months 64.3 ± 16.4, 12 months 64.9 ± 16.2. Hyaluronan group: basal 47.4 ± 15.7, 2 months 61.4 ± 16.2, 6 months 61.0 ± 18.2, 12 months 61.7 ± 19.0. Significant, sustained improvement from baseline in both groups with no significant intergroup difference EQ-VAS: PRP group and hyaluronan group significant improvement from baseline to each follow-up. No significant difference between groups Tegner activity level: PRP group; from 2.9 ± 1.4 at basal to 3.8 ± 1.3 at 12 months. Hyaluronan group; from 2.6 ± 1.2 at basal to 3.4 ± 1.6 at 12 months KOOS: Both groups significant improvement in all subcategories compared to baseline, but no significant difference between groups at any follow-up

EQ-VAS = EuroQoL visual analog scale; IKDC = International Knee Documentation Committee; KOOS = Knee Injury and Osteoarthritis Outcome Scores; NRS = Numerical Rating Scale; SF-36 = Health Survey Scoring Demonstration; VAS = visual analog scale; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index.

scale (VAS), Health Survey Scoring Demonstration (SF-6), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and Lequesne Index, 6 months following the final intra-articular injection (**Table 3**).[62] Napolitano *et al*.[63] reported that patients with knee arthritis or degenerative cartilage disease of the knee who received 3 manually prepared PRP injections at weekly intervals showed significantly improved outcome parameters (Numerical Rating Scale (NRS) and WOMAC Index). While parameters improved immediately after treatment, the greatest improvement was in assessment scores was at the 6-month follow-up (**Table 3**). In a prospective, uncontrolled case series, Gobbi *et al*.[64] treated 50 patients with knee OA, 25 of whom had undergone previous operative treatment for cartilage lesions, with a series of 2 intra-articular PRP injections at a 1-month interval (**Table 3**). The authors reported significant improvement in clinical outcome using International Knee Documentation Committee (IKDC), Knee Injury and Osteoarthritis Outcome Score (KOOS), VAS, and Tegner assessment scores at 6 and 12 months compared with pretreatment scores, with no significant difference between patients with previous operative treatment and nonoperative treatment, between cartilage shaving and microfracture, or between genders (**Table 3**).

Kon *et al*.[65] treated a series of 91 patients with knee pain and radiographic evidence of degenerative chondral lesions, early, or advanced OA, using a series of 3 PRP injections (**Table 3**). The authors noted substantial improvement in IKDC and EuroQoL (EQ-VAS) scores at the 6-month and at the 12-month evaluation; however, the scores were highest at the 6-month follow-up. At the 2-year follow-up, the authors reported that, although the patients still showed improved outcome measurements above pretreatment levels, a decrease of clinical outcome scores over time was observed (**Table 3**).[66] The best results were observed in cases with a lower degree of articular cartilage damage and in younger patients. It was concluded that treatment with PRP was effective in improving pain, function, and quality of life in the short term, but more controlled research into the long-term effects of PRP on OA is necessary. It must also be noted that there was no control group by which to compare the improvement observed in these studies. Still, in a recent randomized controlled trial Patel *et al*.[67] reported the clinical efficacy of white blood cell (WBC)–filtered PRP intra-articular injections compared with a saline control injection in 78 patients (156 knees) who presented with bilateral knee OA (**Table 3**). They reported significant improvement in WOMAC scores from baseline within 2 to 3 weeks lasting until the final 6-month follow-up following a single injection PRP injection or a series of 2 PRP injections compared with no significant improvement in patients who received a single saline injection. Again, the best results were observed in patients with a lower degree of cartilage degeneration and it was noted that outcome scores were slightly decreased at the 6-month follow-up, although they were still significantly improved from baseline scores.

In a study comparing 2 different PRP preparation approaches, Filardo *et al*.[68] examined the safety and efficacy of intraarticular injection of PRP products for treatment of cartilage degeneration of the knee (**Table 3**). Patients in both treatment groups showed significant improvement in all clinical outcome scores at all follow-up assessments compared with baseline regardless of production method, with better results in younger patients with a lower degree of articular cartilage degeneration. There was no significant difference in IKDC, EQ-VAS, and Tegner scores between the 72 patients treated with a single-spin PRGF product versus the 72 patients treated with the double-spin PRP product, however, increased swelling and a greater pain reaction were demonstrated following the PRP injections.

A retrospective study comparing the effectiveness of 30 patients who received PRP injections versus 30 patients who received HA injections for the treatment of knee OA, yielded encouraging results.[69] Three injections were administered over a 3-week period and by week 5, the success rate for the WOMAC pain subscale reached 33.4% for the PRP group versus 10% for the HA group ($P = 0.004$; **Table 3**). More recently, a 150 patient study of knee cartilage degenerative lesions and OA compared the efficacy of PRP and viscosupplementation of low and high molecular weight HA intra-articular injections across a broad age range of 26 to 81 years.[70] At 6 months, PRP treatment showed superior and longer improvement in pain reduction and recovery of articular function compared to HA injection (**Table 3**). The results also demonstrated improved efficacy of PRP in younger and more active patients with less degenerative articular cartilage degeneration compared to older patients with a higher degree of articular cartilage degeneration. In a recent randomized controlled trial, Cerza *et al*.[71] compared the clinical outcomes of 120 patients with articular cartilage degeneration of the knee treated with either 4 intra-articular injections of the PRP (Arthrex ACP Double Syringe System) or 4 intra-articular HA injections. Patients who received the ACP treatment showed significantly improved and sustained WOMAC scores at follow-up times compared with the HA treatment group (**Table 3**). Furthermore, ACP treatment was associated with significant improvement of all grades of gonarthrosis, whereas HA treatment had no effect on patients presenting with grade III gonarthrosis. Similarly, in a multicenter, randomized, controlled, double-blinded trial of 176 patients with symptomatic knee OA, Sánchez *et al*.[72] reported that the PRGF treatment group demonstrated outcome scores, including pain response rate and WOMAC scores, that were 14.1 % higher compared to those in the HA treatment group. Although the response rate and short-term results were superior in patients that received PRGF, there was no significant difference

between the treatment groups (**Table 3**). Filardo *et al.*[73] also reported that while both treatment groups exhibited significant clinical improvement at the 2-, 6-, and 12-month follow-up evaluations compared with baseline, there was no significant difference in IKDC, EQ-VAS, Tegner, or KOOS scores between 54 patients who received a series of 3 weekly PRP intra-articular injections versus 55 patients who received 3 weekly HA injections for knee OA (**Table 3**). In fact, patients treated with the cycle of PRP injections presented with significantly higher postinjection pain reaction. It was noted though, that there was a favorable trend ($P = 0.07$) for improvement in the PRP group in patients who presented with low-grade articular degeneration of the knee (Kellgren–Lawrence < 3) compared with the HA treatment group. Collectively, the methods of preparation, modes of application, patient demographics, and degree of articular cartilage degeneration still vary from study to study, making it difficult to draw overall conclusions, but these studies indicate that stratification must occur with regard to age, degree of chondropenia, and PRP product.

Intraoperative Use of Platelet-Rich Plasma in Cartilage Repair

PRP has also been investigated as a treatment option for cartilage repair and its application to chondral defects using both animal and human models has been evaluated in a clinical setting. In an ovine model, treatment of 15 chronic full-thickness chondral lesions of the knee using microfracture supplemented with PRP and fibrin glue resulted in improved outcomes compared with the microfracture-alone controls.[74] The PRP product consisted of 60 mL of autologous blood centrifuged in a 2-step process (2400 rpm 3 minutes, 3000 rpm 12 minutes), resulting in 6 to 8 mL of PRP. Similarly, Sun *et al.*[75] found positive results in 48 rabbits when osteochondral defects, created in the femoropatellar groove, were treated with a double-centrifuged PRP product (800 rpm 15 minutes, 2000 rpm 15 minutes) in a polylactic-glycolic acid compared with those treated with polylactic-glycolic acid alone and those left untreated.

Basic Scientific Rationale of Intra-Articular Use of Platelet-Rich Plasma in Meniscal Injuries

Damage to meniscal tissue presents unique challenges because of the absence of healing at the avascular zone, the accelerated degeneration of articular cartilage and increased rate of knee OA that can occur following a meniscal injury.[76] Application of PRP represents a potential therapeutic technique to stimulate proliferation and enhance the healing process of menisci. Although there are limited published studies evaluating the efficacy of PRP application in meniscal injuries, Ishida *et al.*[77] examined, *in vitro*, monolayer lapine meniscal cell cultures to assess the proliferation, extracellular matrix synthesis and mRNA expression that occurred following exposure to a PRP product. Meniscal cell cultures showed increased deoxyribonucleic acid synthesis, extracellular matrix synthesis, and greater mRNA expression of biglycan and decorin in the presence of PRP. In addition, the same study evaluated the *in vivo* application of PRP. PRP combined with gelatin hydrogel was applied to defects at the avascular region of the meniscus and compared with platelet-poor plasma with gelatin hydrogel and gelatin hydrogel alone through histological examination at 4, 8, and 12 weeks after surgery. Histological scoring indicated significant improvement in the PRP-treated defects at 12 weeks compared with baseline and the 2 other treatment groups. Further histological findings showed that the gelatin hydrogel was present at the defect site as long as 4 weeks.

Basic Scientific Rationale of Intra-Articular Use of Platelet-Rich Plasma in Ligaments

Several *in vitro* studies in equids suggest promising results regarding the use of PRP to enhance ligamentous healing. Smith *et al.*[78] reported that 10% PRP treatment demonstrated a significant increase in total protein synthesis and cartilage oligomeric matrix protein production compared with the control culture in suspensory ligament fibroblast cultures. A study performed by Schnabel *et al.*,[79] using PRP and acellular bone marrow treatments on equine suspensory ligament explant cultures, yielded similar results. Despite differences in the study designs, including the treatment concentrations and the type of culture, it is important to note that stimulation of matrix synthesis was observed in the ligament cultures treated with PRP.

Perhaps of more interest is the effect of PRP on anterior cruciate ligament (ACL) cells. ACL rupture is a common injury that often requires surgical reconstruction, but the absence of normal knee kinematics and premature osteoarthritic changes that can result following operative treatment[80] and may in part be a result of incomplete healing, call for the investigation into therapeutics that augment the repair process, such as PRP. Cheng *et al.*[81] examined the viability and metabolic activity of ACL cells cultured in collagen with platelets, platelet-poor plasma, and PRP. Not only did ACL cells combined with PRP show a reduced rate of apoptosis and enhanced metabolic activity, but they also exhibited increased collagen gene expression compared with the controls. The authors concluded that PRP stimulates collagen synthesis in ACL fibroblasts, providing a biologically plausible mechanism by which PRP may enhance the functional healing of the ACL following operative reconstruction. In fact, in a recent study performed in a canine model, significant changes in the mRNA expression

collagen type I and II, MMPs, biglycan, and TGF-β1 occurred following ACL reconstruction when ACL grafts were augmented with PRP compared with ACL grafts treated with saline.[82]

In another study, Yoshida and Murray[83] examined the effect peripheral blood mononuclear cells (PBMCs), a byproduct that includes monocytes and lymphocytes but excludes polymorphonucleocytes and often is removed from PRP products along with other WBC components on ACL fibroblasts cultured on collagen scaffolds with and without platelets and plasma. When exposed to PBMCs in the presence of platelet products, ACL fibroblasts demonstrated increased collagen gene expression, collagen protein expression, and cell proliferation. Not only do these results indicate the positive effect of some leukocyte components in PRP products, but this interaction between PBMCs and platelet products may also be beneficial in ACL reconstruction where collagen production by fibroblasts is desired.

Clinical Application of Intra-Articular Use of Platelet-Rich Plasma in Ligaments

Intraoperative Use of Platelet-Rich Plasma in Anterior Cruciate Ligaments

There are several studies that support the use of PRP products to augment ACL reconstruction in animal models, particularly when PRP is used in combination with a collagen-based carrier.[84,85] Still other studies show these combined PRP products to be ineffective. The difference in results may be explained, at least in part, by the different methods of preparation of PRP derivatives used in these studies. Still, the resultant controversy leads to a lack of consensus.[86-88]

Mixed results and various manufacturing systems are also factors in evaluating human ACL reconstruction studies. In ACL reconstructions the tibial and femoral graft-to-bone healing and ligamentization of the tendon graft represent the primary biological processes that occur postoperatively.[89-92] Measurements of these processes are used as indicators of healing following ACL reconstruction.

Many reports of the use of PRP alone in humans are associated with positive outcomes in ACL reconstruction.[93,94] Sánchez *et al*.[93] assessed graft morphology and histology and showed that applying PRP to the donor site of an autologous graft potentially enhanced tissue regeneration and reduced donor-site morbidity. The osteoinductive effects of PRP appeared to limit tunnel widening and facilitate the fusion of bone and graft in the femoral and tibial tunnels created during ACL reconstruction. It was also reported that greater graft integration occurred within the tunnels when PRP was used at the tendon insertion site of both hamstring and bone–tendon–bone grafts.

In a single-blinded controlled study, Radice *et al*.[94] evaluated, through MRI, the results of ACL grafts treated with PRP over time. They found that grafts treated with the PRP gel, obtained from the GPS system, showed complete homogeneity at 179 days compared with 369 days for standard ACL reconstruction (**Table 4**). Orrego *et al*.[95] reported significantly increased graft maturation in ACL grafts treated intraoperatively with PRP prepared from the GPS II system compared with untreated control ACL grafts, as 100% of patients who received PRP, had graft classified as low-intensity grafts (similar to native tissue) when examined at the 6-month MRI assessment. Still, although this outcome measurement was significant, the MRI assessment revealed the absence of an osteoligamentous interface after 6 months, indicating a lack of complete graft integration (**Table 4**). Another double-blind clinical trial demonstrated significantly higher vascularization in the osteoligamentous interface zone in grafts treated with PRP produced from the Magellan system compared with the control at 3 months, but no difference in the vascularization of the intra-articular portion of the grafts in either group (**Table 4**).[96] In a study of 20 patients, Ventura *et al*.[97] evaluated graft maturation of 10 ACL grafts treated intraoperatively with a GPS PRP product compared with 10 ACL grafts without PRP (**Table 4**). Through computed tomography assessment, PRP-treated ACL grafts showed a significantly greater native appearance compared to control grafts that appeared heterogeneous in nature; however, there was no significant difference between clinical outcome scores between the treatment groups.

In another clinical study, at the 12-month follow-up examination, Cervellin *et al*.[98] found complementary clinical outcome results of increased Victorian Institute of Sports Assessment and VAS scores for those subjects who had PRP produced by the GPS II system applied to the tendon and bone plug harvest sites compared with those who were not treated with PRP during ACL reconstruction (**Table 4**). The authors also found that the percentage of satisfactory bone filling was greater in PRP-treated patients. However, even though these results suggest clinical improvement using PRP, only the Victorian Institute of Sports Assessment endpoint measurements were significantly different in treated versus untreated patients.

On the other hand, some clinical studies have shown no beneficial effect of PRP use during ACL reconstruction. Silva and Sampaio[99] failed to find a significant difference in MRI assessment of the fibrous interface of the reconstructed ACL hamstring graft and the femoral tunnel treated with GPS system–produced PRP at 3 months (**Table 4**). Also, in a study on 50 patients following ACL reconstruction, Figueroa *et al*.[100] found no significant difference in ACL graft remodeling or integration at the bone–tendon interface between those treated with PRP produced by the Magellan system and those treated without PRP (Table 4). Another

Table 4. Published Human Studies of Platelet-Rich Plasma (PRP) Clinical Application in Ligament Injuries.

Authors	Diagnosis	Design	Purpose	PRP Preparation	Outcome Measurements	Results
Radice *et al.* (2010)[94]	ACL tear	Prospective clinical study, 25 reconstruction with PRP vs. 25 reconstruction without PRP, all patients same posttreatment protocol	Determine effectiveness of PRP application in ACL reconstruction	GPS system	MRI at 3 to 9 months for PRP group and 3 to 12 months for control	MRI: PRP group heterogeneity was 1.14 vs. control group heterogeneity of 3.25 Mean time to complete homogeneity of graft in PRP group was 177 days vs. 369 days in control group
Orrego *et al.* (2008)[95]	ACL tear	Randomized controlled trial, 108 patients with 4 treatment groups	Evaluate the effect of PRP on the outcome of hamstring graft	GPS system	MRI at 3 and 6 months	88% in the PRP group and 70% in the PRP-BP group showed no osteoligamentous interface compared with 67% in the control group 89% in the BP group and 81% in the PRP group showed no tunnel widening compared with 59% in the control group MRI 6 months: 100% in PRP group mature, low-intensity graft signal compared with 78% in control group
Vogrin *et al.* (2010)[96]	ACL tear	Randomized clinical trial, 25 treated with PRP vs. 25 no PRP	Evaluate effect of PRP application on graft revascularization in ACL reconstruction	Magellan system 60 mL whole blood + calcium citrate, 6 mL PRP	MRI at 4 to 6 weeks following reconstruction	Osteoligamentous interface zone: Level of vascularizttion in PRP group was 0.33 vs. 0.16 in control group Intra-articular graft portion: No evidence of revascularization in either group
Ventura *et al.* (2005)[97]	ACL tear	Randomized, controlled trial; 10 treated with PRP product intraoperatively compared with 10 treated without PRP	Examine the effect of PRP on ACL graft maturation following ACL reconstruction	GPS system	KOOS, KT-1000, Tegner, and CT assessment of graft maturation at 6 months	No significant difference in KOOS, KT-1000, or Tegner assessments between PRP and control groups at 6 months CT: ACL grafts treated with PRP significantly greater native appearance compared with control grafts
Cervellin *et al.* (2012)[98]	ACL tear	Randomized controlled clinical study, 20 treated with PRP vs. 20 treated without PRP	Evaluate the effect of PRP application to bone plug and tendon harvest sites during ACL reconstruction	GPS system	VISA and VAS scores and MRI at 12 months	VISA: Patients treated with PRP 97.8 ± 2.5 vs. patients treated without PRP 84.5 ± 11.8 VAS: Patients treated with PRP 0.6 ± 0.9 vs. patients treated without PRP 1.0 ± 1.4 MRI: satisfactorily filled defect in 85% of PRP group vs. 60% of control group

(continued)

Table 4. (continued)

Authors	Diagnosis	Design	Purpose	PRP Preparation	Outcome Measurements	Results
Silva and Sampaio (2009)[99]	ACL tear	Prospective cohort study, 40 patients subdivided into 4 groups	Assess tendon–bone integration of hamstring in ACL reconstruction	GPS system	MRI at 3 months posttreatment	MRI showed no difference in tendon–bone integration between groups
Figueroa *et al.* (2010)[100]	ACL tear	Randomized, controlled, blinded clinical trial; 30 patients treated with PRP compared with 20 patients treated without PRP	Evaluate the efficacy of PRP on graft maturation and graft–bone interface healing following ACL reconstruction	Magellan system	MRI assessment of graft maturation and graft–bone interface healing at 6 months after ACL reconstruction	MRI graft assessment: PRP group; 63.2% hypointense grafts. Control group; 42.11% hypointense grafts. No significant difference between groups (P = 0.316) MRI graft–bone interface assessment: PRP group; no synovial fluid at interface in 86.6% of patients. Control group; no synovial fluid at interface in 94.7% of patients. No significant difference between groups (P = 0.720)
Nin *et al.* (2009)[101]	ACL tear	Prospective, randomized, double-blind controlled clinical study, 50 PRP vs. 50 control	Evaluate the clinical and inflammatory outcomes of PRP in ACL reconstruction	40 mL whole blood, 3,000 rpm 8 minutes and 1,000 rpm 6 minutes, 4 mL PRP, $CaCl_2$ activator	IKDC score, MRI, inflammatory parameters (C-reactive protein) at mean 24-month follow-up	IKDC: No difference between groups MRI: Mean diameter of graft in PRP group 9 mm vs. 8 mm in control group. Signal intensity of the graft showed a mean of 230 in ROIs in the PRP group compared to 190 in ROIs in the control group CRP (mg/dL): control group 1.22 vs. PRP group 1.14 CRP protein 2 (mg/dL): control group 0.85 vs. PRP group 0.88

ACL = anterior cruciate ligament; CRP = C-reactive protein; CT = computed tomography; IKDC = International Knee Documentation Committee; KT-1000 = Knee Arthrometer; ROI = region of interest; VAS = visual analog scale; VISA = Victorian Institute of Sports Assessment.

study reported that a PRP product produced by the GPS III system caused no accelerating effect on bone–tendon integration or prevention of tunnel widening at a 2-year follow-up (**Table 4**).[101]

Three different products with differing cellular components and various outcome measurements were used between the ACL studies, making it difficult to compare the overall efficacy of treatment (**Table 4**). Moreover, the limited sample size resulted in a beta error that may account for equivocal results reported in some studies. Although some results suggest that PRP can enhance graft remodeling and contribute to improved interface healing, collectively, the outcomes of the effect of PRP on ACL reconstruction both short term and long term is still in need of further exploration.

Further Considerations

The promising potential of PRP has led to its rapidly expanding use in sports medicine. The inconsistent results between studies, however, demonstrate that, although the groundwork is laid, the true efficacy of PRP is yet to be

determined. The use of PRP in sports medicine is supported in some clinical studies, showing a clear trend toward patient or lesion improvement; but, there are also well-designed controlled studies that fail to demonstrate any significant effect of PRP.

A recent meta-analysis that evaluates the use of PRP in orthopedic indications highlights the inconsistencies across studies that must be remedied to determine the efficacy of PRP.[102] This systematic review quantitatively assessed methodological quality and functional outcome measurements of 33 randomized controlled trials or prospective cohort studies. Of these, only 22 studies (61%) reported the manufacturer of the platelet separation system used, only 20 were considered of high methodological quality, and 27 different functional outcomes were used. The authors concluded that considerable uncertainty about the benefit of PRP remains and that future studies must address the deficiencies found in the current body of literature and the cases presented in this review. Thus, it remains difficult to draw conclusions in comparing current studies; for not only is the very definition of what constitutes PRP unclear, but the machine, processing, volume, concentration, contamination with other blood cells, treatment interval and frequency, posttreatment therapy, and many other factors, vary between reports.

Since the preparation methods and PRP products are not the same, it seems reasonable to first standardize the preparation of PRP to study its effect. Individual patients blood counts likely contribute to the effectiveness of PRP and these variable counts in both humans and animals, may, in turn, ultimately lead to optimization of individual treatment. Although an optimum concentration of PRP is yet to be determined, certain studies have demonstrated that positive outcomes may be achieved when platelet concentrations fall within a certain range.[36,39,103,104] In the study performed by Torricelli *et al*.,[103] horses with PRP platelet counts greater than 751,000 platelets/μL showed more effective clinical outcome and improved lameness evaluation compared with horses with lower platelet counts. However, the highest absolute platelet count or greatest platelet concentration may not generate the most effective PRP product.[36,104] These authors surmised that perhaps high concentrations of PRP growth factors may overload receptors, halting further function of anabolic processes and, in some cases, actually cause inhibitory effects rather than beneficial ones. Accordingly, the opposite may also be true; platelet concentration in the PRP product that is too low may fail to induce any significant effect. More studies are needed to determine favorable concentration ranges for optimal outcomes, but a machine must have the capacity to correct for individual variation.

In addition to variation of blood cell components contained within each product, an individual's health, age and comorbidities may also reflect the effectiveness of PRP. These factors cannot be overlooked, especially in the bone-healing process. For example, circulating estradiol or testosterone levels may account for some of the varying results between age groups, and should be corrected in order to optimize the effects of PRP growth factors on bone.

Furthermore, application of PRP to different sites of injury requires different techniques in processing and delivery. In a study to determine whether shear force on platelets during the injection of PRP has a significant effect,[105] a comparison of injection of equine PRP with high pressure, using a small-bore needle (25 guage), and injection with a 21-gauge needle was conducted. The authors concluded that shear force did not have a significant effect on growth factor concentration. Products also are applied to the region of interest using multiple methods; PRP is injected as bolus, applied with a scaffold, or administered in microinjections. Superiority of a certain administration process over another or the optimal number of injections has not been determined and may depend on the microenvironments of the target lesion.[106]

Following the application of PRP, the posttreatment protocol must be optimized as well. The mobility, stretching, and activity levels after treatment play an important role.[107] The protocol that is prescribed, and followed by the patient, varies between studies and patients respectively.

The variety of injuries seen in sports medicine encompasses multiple tissue types and different microenvironments. The environment of the lesion influences the outcome of treatment and tissue-specific requirements that promote optimal conditions for the healing process. Available oxygen, hydrostatic pressure, and the pH in the region of interest should be considered in determining treatment.[107] Kalen*et al*.[108] actually demonstrated that the release of PDGF and TGF-β from platelet concentrates is pH dependent, for in an acidic environment, such as the hematoma stage of the healing process, a more sustained growth factor release is stimulated compared to a neutral environment.

Furthermore, acute and chronic injuries respond differently to the present metabolic state of the lesion. For instance, generally, tendinitis is characterized by an acute inflammatory response, whereas tendinosis does not necessarily refer to the inflammation of tendon, but rather, microtears in tendon pathology. Although they may be concomitant injuries, they are categorized under the umbrella of tendinopathies, and will differ in their healing processes and response to platelet components. Therefore, not only are the components that are administered important in PRP but also the milieu of bioactive factors already present within the site of injury. As mentioned before, the leukocyte concentration in PRP has recently attracted much attention, and while leukocytes may be beneficial in some injuries because of their antimicrobial components, WBCs, in particular neutrophils, have also demonstrated deleterious potential in many tissue types.[8,109]

In fact, it is recommended that for soft tissue engineering scaffolds, such as PRP, a ratio of 2,000:1 platelet to WBC is prepared.[109] This ratio may be critical in instances where PRP and other mixed regenerative cell concentrate products provide growth factors and act as a scaffold.[110] Not only is the variation in blood cell components important but also the variation in growth factor concentration. Currently, clinicians are conducting studies to determine the optimum ratio of the cellular components contained in PRP with respect to the site of injury and degree of tissue degeneration.

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Ethical Approval

This study was approved by our institutional review board.

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