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Autologous Platelet-Rich Plasma: A Revolution in Soft Tissue Sports Injury Management?

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Abstract: Platelet-rich plasma (PRP) therapy is an emerging technology that aims to improve the process of tissue repair through local delivery of autologous bioactive agents to influence critical physiological mechanisms such as inflammation, angiogenesis, or extracellular matrix synthesis. These biological properties have prompted the therapeutic administration of PRP in orthopedics and sports medicine. Given its biocompatibility and healing properties, percutaneous injections of PRP are used in athletes to treat tendon and muscle injuries. Studies of varying levels of evidence have demonstrated the safety and beneficial effects of PRP in these applications, but standardization of the methods of plasma preparation and procedures for application is necessary for further advancements. Continued efforts to identify factors that influence the biological response to PRP treatment may yield new formulations tailored to each specific application. The growing emphasis on an evidence-based approach in the sports medicine setting demands additional research efforts before incorporating this technology in routine clinical care.

Keywords: platelet-rich plasma; sport injuries; tissue repair; growth factors

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Introduction

Platelet-rich plasma (PRP) therapy is an emerging technology that aims to improve the process of tissue repair via the delivery of bioactive agents, which will provide chemotactic, proliferative, and anabolic cellular responses and enhance recovery of tissue function.[1] Platelet-rich plasma products are easily prepared from the patients' own blood and typically involve the local injection of a set volume of PRP or the application of PRP gel form during surgery directly at the site of injury. The clinical use of PRP for promoting physiological wound healing was introduced in the 1980s for the treatment of cutaneous ulcers.[2]

Early studies on PRP examined the healing effects of purified and isolated recombinant growth factors, such as platelet-derived growth factor BB (PDGF-BB), as therapeutic molecules for wound healing. However, knowledge of healing mechanisms has led to the conclusion that isolated growth factors cannot mediate all biological aspects required for tissue repair. Thus, a more rational strategy would be the administration of a balanced combination of mediators that would act in synergy to mimic the physiological needs of the injured tissue.[1] In the 1990s, advances in oral implantology were fueled by the potential regenerative effects of PRPs in bone tissue, and observing the healing properties in soft tissues. Since then, the use of PRP has spread to many other clinical areas, including ophthalmology, orthopedics, sports medicine, cardiology, dermatology, plastic surgery, and neurology.[3]

The first reported application of PRP in sports injuries was in the arthroscopic management of an avulsion of articular cartilage in a young soccer player.[4] Further developments in PRP therapies have introduced new opportunities for tissue repair in sports medicine, such as novel therapies for the management of chronic pathologies (eg, tendinopathy and osteoarthritis).[5–7] Platelet-rich plasma has the potential to accelerate the process of healing and tissue regeneration in clinical settings. In sports medicine, this may accelerate return to play, particularly in elite and professional athletes.

Platelet-Rich Plasma Therapies and Healing Mechanisms

In the past decades, an increased understanding of the physiological role of platelets in wound healing has led to the concept of using platelets as therapeutic tools. Platelets are produced in large numbers from megakaryocytes in the bone marrow. The normal platelet concentration is 150 000 to 350 000/

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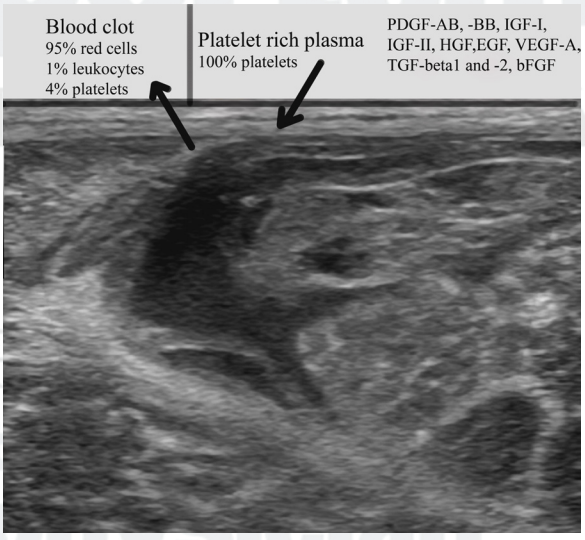
μL. Anucleate platelets circulate for 7 to 10 days and mediate primary hemostasis. On activation, platelets secrete multiple signaling proteins involved in the healing of musculoskeletal tissues. Relevant growth factors present in PRP include transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF)-AB and PDGF-BB, vascular endothelial growth factor A (VEGF-A), epithelial growth factor (EGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF)-I and IGF-II, among others.[8] These signaling proteins control cell activities by interacting with receptors located on the membrane of the target cells. This binding activates various intracellular signaling pathways that induce the synthesis of proteins needed for regenerative processes, such as angiogenesis or extracellular matrix formation. In addition to providing initial signals for local cell activation and homing of precursor cells to the injury and differentiation, PRPs contain potent adhesive substrates for cells, such as fibrin, fibronectin, vitronectin, thrombospondin, osteocalcin, and osteonectin.[8] Considering these properties, PRPs are crucial in the process of repair of tendons, muscles, ligaments, cartilage, and bone injuries.

In the physiological process of wound healing, platelets embedded in the blood clot serve as a primary source of biologically active factors. Typically, after muscle strain or contusion, the hematoma that originates as a consequence of vessel disruption contains about 94% red blood cells, a small amount of platelets (4%), and < 1% leukocytes (Figure 1). The rationale for the use of PRP involves replacing the blood clot with PRP, thus minimizing the presence of red blood cells and increasing platelet concentration at the injury site. In doing so, supraphysiological concentrations of the growth factors accelerate the repair process by direct or indirect mechanisms (ie, enhancing further synthesis of growth factors by local cells). These unique properties of PRPs have led to the commercial development of multiple systems that offer an easy, cost-effective strategy to obtain high concentrations of factors for tissue healing and regeneration in the clinical setting.

Figure 1. Natural healing versus platelet-rich plasma therapy.



The rationale for the use of PRP involves replacing the blood clot with PRP, thus minimizing the presence of red blood cells. In this way, platelet concentration at the injury site is increased (and optionally the concentration of leukocytes is also increased), achieving supraphysiological concentrations of healing bioactive factors. **Abbreviations:** EGF, epithelial growth factor; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; PGF, placental growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

Preparation of PRP and Products

In the past few years, several semiautomatic machines have been developed for centrifugal separation of PRP for therapeutic use. The process of PRP preparation is relatively straightforward and can be performed in the clinic or operating room. It can usually be completed within minutes. The cost to both medical practitioners and patients varies widely depending on the method used to produce the PRP concentrate. The cost of a commercial kit is about $300 to $600, and in-house manual techniques cost about $20. For PRP preparation, peripheral blood is drawn from the patient under sterile conditions, with or without anticoagulants, and the plasma is prepared by centrifugation or filtration. The volume can be adapted to the extent of the size of the injury and phase of injury, ranging from 10 to 100 mL. Essentially, the methods of producing PRPs determine the composition and concentration in terms of leukocytes, erythrocytes, and platelets in a given plasma volume. There are 3 methods used to make these determinations: 1) double-spinning methods using automated machines along with commercial kits, 2) single-spinning methods using conventional laboratory centrifuges followed by manual PRP separation, or 3) selective blood filtration using commercially available technology. When using single spinning, the platelet yield is 1- to 3-fold baseline levels, while 5- to 8-fold baseline levels are achieved by double spinning. Double spinning also concentrates leukocytes.

Platelet concentrates have been categorized in pure PRP (P-PRP), in which leukocytes are purposely eliminated from the PRP, and leukocyte and platelet-rich plasma (L-PRP), which contains a high concentration of leukocytes.[9]

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Whether leukocytes have detrimental effects in particular orthopedic sports medicine applications is controversial, but basic evidence points toward a deleterious effect of neutrophils, particularly in joints and muscle injuries.[10] The improved homogeneity of P-PRP and its reduced donor-to-donor variability would support the view that some PRP production techniques are more reproducible and predictable than others.

There is little consensus regarding the dose of platelets and growth factors needed to obtain efficient clinical results. The clinical variability of different studies suggests that some techniques might not produce a sufficient number of functional platelets to produce the expected outcome. Similarly, there is no consistency between the methods of applying the therapy, the timing of treatment, the number of injections per series, or the volume of injection. This has precluded the establishment of the standards necessary to combine the results of independent studies and provide an estimate of the treatment effects. For example, the methods for PRP preparation vary widely between practitioners and the volume of plasma used. Double-spinning techniques yield a PRP concentration of around 10% of the blood volume drawn (ie, 20 mL of whole blood would result in 2 mL of PRP), in contrast to 40% to 50% of the blood volume obtained after single spinning. Also, each method leads to a different product, with varying biological properties and potential uses.[11] It is unclear whether these differences have any clinical relevance.[10] Some authors have suggested that PRP preparations containing only moderately elevated platelet concentrations induce optimal biological benefit, while other authors suggest lower platelet concentrations produce suboptimal effects, and higher ones produce inhibitory effects.[12,13] According to other authors, the therapeutic dose of PRP is $\geq$ 4 to 6 times higher than the normal platelet count.[14] To add to the discussions, the actual growth factor content does not correlate with the platelet count in whole blood or in PRP when leukocytes are present in the preparation, and there is no evidence that gender or age affects platelet count or growth factor concentrations.[15] However, age may influence the number of receptors of local cells interacting with the plasma signals.[16]

Once the PRP is separated from the whole blood, it is stable for about 8 hours. However, because these procedures are considered an autograft by regulatory organizations, the plasma should be prepared and used immediately at the point of care, and should not be stored. Prior to application, platelets can be slowly activated by initiating the coagulation cascade with the addition of calcium chloride, a necessary cofactor for prothrombin conversion into thrombin. Alternatively, coagulation and platelets can be activated by instantly adding standard solution of 1000 U/mL of bovine or human thrombin along with 10% calcium chloride to the PRP. After plasma activation, the fibrin scaffold can be formed in vivo or ex vivo; the latter is suitable for implantation in surgery or in ulcer care, and provides a gradual release of growth factors in the area where it has been applied.

Product Safety

There remain safety concerns about the routine use of PRP. Any concerns regarding transmission of diseases such as human immunodeficiency virus, hepatitis, or Creutzfeldt-Jakob disease, or of immunogenic reactions (a concern with allografts or xenografts), are by definition not applicable, given the autologous nature of PRP.[17] However, some systems use purified bovine thrombin to activate the platelets. This may produce coagulopathies, and most authorities now use human recombinant thrombin.

Some authors have raised the issue of genetic instability, and hypothesized that the use of PRP may lead to the development of neoplasms. Growth factors act on receptors located on the cell membranes rather than on the cell nucleus, and activate normal gene expression via intracellular signaling proteins, which promote normal, not abnormal, gene expression.[18] Growth factors are not directly mutagenic, and their activities in normal wound healing are highly regulated by various feedback control mechanisms. Furthermore, until now, no systemic effect on circulating growth factors has been shown after PRP application.[19] Some antimicrobial activity of PRP (platelet-leukocyte gel) against *Staphylococcus aureus* has been shown in vitro[20] and in vivo, although this antimicrobial activity is not comparable with systemic antibiotic treatment.[21]

Therapeutic Application of PRP in Sports-Related Injuries

The therapeutic administration of PRP extends to the treatment of multiple musculoskeletal injuries in orthopedics and sports medicine. Its widespread clinical use has been popularized following its use in high-profile athletes. Orthopedists, primary care sports medicine physicians, and rheumatologists are among the practitioners using PRP in the management of tendon, ligament, muscle, nerve, bone, and joint injuries.

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Table 1. Studies on Platelet-Rich Plasma and Soft Tissue and Articular Pathologies

Study	Tendon Pathology/ Intervention	Type of PRP (Platelet Concentration) Activation-Clotting	Study Design	Results	Level of Evidence
Mishra and Pavelko[34]	Epicondylitis/injections	Buffered L-PRP (6–8x); no activation	Cohort study	Reduction of visual analog pain score (93% of treated patients)	III
Peerbooms et al[35]	Epicondylitis/injections	Buffered L-PRP (6–8x); no activation	RCT	DASH score improved both groups but much better in PRP group	I
Sánchez et al[30]	Achilles tears/surgery	Pure PRP (2–3x); $CaCl_2$ activated and clotted	Case-control	Enhanced healing and functional recovery, less CSA in PRP group	III
de Vos et al[32]	Achilles tendinopathy/ injection	Buffered L-PRP (6–8x); no activation	RCT	Outcome was not different between both groups	I
Kon et al[37]	Patellar tendon/injections	L-PRP (6–8x); $CaCl_2$ activated	Case series	Improvement in Tegner, EQ, VAS, SF-36 scores	IV
Filardo et al[38]	Patellar tendon/injections	L-PRP (6–8x); $CaCl_2$ activated	Cohort study	Improvement in Tegner, EQ, VAS scores	III
Everts et al[39]	Subacromial decompres sion/open surgery	L-PRP (6–8x); thrombin clotted	Cohort study	Less pain medication, earlier functional recovery	III
Randelli et al[36]	Rotator cuff/arthroscopic surgery	L-PRP (6–8x); autologous thrombin clotted	Case series	Decrease in VAS, increase in UCLA, constant scores	IV
Weber and Kauffman[40]	Rotator cuff/arthroscopic surgery	PRFM (1–2x); $CaCl_2$ clotted	RCT	No differences in SST, UCLA, ASES	I
	Ligament Pathology/ Intervention				
Mei-Dan et al[48]	Elbow medial ligament complex/injections	Pure PRP (2–3x); $CaCl_2$ activated	Case report	Accelerated functional recovery	IV
Sánchez et al[41]	ACL tears/arthroscopic surgery	Pure PRP (2–3x); $CaCl_2$ activated	Case-control	Better graft remodeling	III
Vogrin et al[44]	ACL tears/arthroscopic surgery	L-PRP; thrombin clotted	RCT	Improved knee stability (KT200) at 6 months	I
Radice et al[42]	ACL tears/arthroscopic surgery	L-PRP (4–6x); thrombin clotted	Case-control	Enhanced maturation of the graft	III
Silva and Sampaio[46]	ACL tears/arthroscopic surgery	L-PRP (4–6X); thrombin clotted	Case-control	No difference in bone tunnel healing	III
Orrego et al[43]	ACL tears/arthroscopic surgery	L-PRP (4–6x); thrombin clotted	RCT	Better graft maturation, no difference in bone tunnel healing	II
Nin et al[45]	ACL tears/arthroscopic surgery	L-PRP (2x); $CaCl_2$ clotted	RCT	No clinical or biomechanical differences	I
Figueroa et al[47]	ACL tears/arthroscopic surgery	L-PRP (4–6x)	Case-control	No difference in graft maturation and bone tunnel healing	III
	Articular Pathology/ Intervention				
Sánchez et al[5]	Cartilage avulsion/ arthroscopic surgery	Pure-PRP (2–3x); $CaCl_2$ activated	Case report	Cartilage healing and functional recovery	IV
Sánchez et al[50]	Knee osteoarthritis/3 injections	Pure-PRP (2–3x); $CaCl_2$ activated	Case-control	Reduction in overall WOMAC and in function and pain subscales at 5 weeks	III
Kon et al[51]	Knee osteoarthritis/3 injections	L-PRP (4–6x); $CaCl_2$ activated	Case series	IKDC, EQ, and VAS improvement at 6–12 months	IV
	Muscle Injuries/ Intervention				
Loo et al[54]	Strain/injections	Pure PRP; $CaCl_2$ activated	Case report	Acceleration of healing Reduced pain	IV
Wright-Carpenter et al[52]	Strain/injections	Autologous conditioned serum	Cohort study	Faster regression of oedema and return to competition	III

Abbreviations: ACL, anterior cruciate ligament; ASES, American Shoulder and Elbow Surgeons ratings scale; CSA, cross-sectional area; DASH, Disabilities of the Arm, Shoulder, and Hand; EQ, measure of quality of life; IKDC, International Knee Documentation Committee; L-PRP, leukocyte and platelet-rich plasma; PRFM, platelet-rich fibrin matrix; PRP, platelet-rich plasma; RCT, randomized controlled trial; SF-36: Short-Form 36; SST, simple shoulder test; UCLA, University of California Los Angeles score; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

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However, PRP use should be considered experimental in all applications of sports medicine. Studies of varying levels of evidence have demonstrated the safety and beneficial effects of PRP in some of these applications, but adequate level I randomized trials needed to perform meta-analyses are lacking. Moreover, advances in the field of clinical research on the use of PRP are hindered by the lack of standardization of the various formulations and administration regimens and modalities. In this section, we focus on the use of PRPs in soft tissue injuries (Table 1).

Tendon Injuries

One interesting application of PRP therapies is the management of tendon injuries. Tendon injuries are a major cause of musculoskeletal morbidity affecting professional and recreational athletes in various anatomical locations. Tendinopathy is associated with a failed healing response. The tendon cell-mediated process involves increased turnover and remodeling, and gradual transformation in the quality and quantity of extracellular matrix that habitually precedes tendon rupture. Tendon cells (tenoblasts and tenocytes) have a central role in the repair and maintenance of extracellular matrix, synthesizing new proteins and producing the enzymes that degrade them.

The activities of tendon cells are likely to be influenced by external growth factors and cytokines released from PRPs.[22] This is the core hypothesis of PRP application that is supported by recent advances in basic laboratory research providing a more detailed understanding of the biological mechanisms influenced by PRPs. In an animal study, Kajikawa et al[23] showed the chemotactic action of PRPs in tendon injury. In addition, PRPs stimulated cell proliferation and the synthesis of angiogenic factors, such as VEGF and HGF, which act in a paracrine manner on endothelial cells, promoting angiogenesis.[24–26] Moreover, PRPs induced the synthesis of molecules of the extracellular matrix, such as collagens or hyaluronan.[27] However, PRPs should be combined with an appropriate loading regimen to enhance extracellular matrix organization in the short term. Indeed, injections of PRP 1 week postoperatively increased tendon regenerate strength after 4 weeks if combined with early therapy.[28] A placebo-controlled experimental trial in 6 horses reported less inflammation and increased metabolic activity and maturation, higher strength at failure, and elastic modulus in tendons treated with PRP.[29]

In a case series study including 12 athletes who were undergoing surgical repair of Achilles tendon tears, Sánchez et al[30] applied basic research findings to a clinical investigation. In their study, they applied P-PRP with a moderate concentration of platelets (2–3 times the concentration of platelets compared with whole blood) clotted ex vivo and injected it in liquid form, activated with calcium chloride. Controls were treated with an identical surgical procedure performed by the same surgeon, but they did not receive PRP during surgery. The authors reported an enhancement in the range of motion and a faster return to sporting activities in the group that received PRP during surgery. The cross-sectional area of these Achilles tendons had fewer differences compared with the contralateral tendon after 18 months, indicating a more physiological repair of the PRP-treated tendon.

These authors also reported the use of P-PRP in 2 configurations (clotted ex vivo and calcium chloride-activated liquid) to treat complications derived from the surgical repair of the Achilles tendon.[31] In a recent randomized clinical trial, de Vos et al[32] treated patients with Achilles tendinopathy with eccentric exercises and 1 injection of buffered L-PRP (6–8 times the concentration of platelets compared with whole blood) in the experimental group (although leukocytes play a significant role in the biology of these products, their concentration was not reported), while the control group was managed with eccentric exercises and 1 injection of saline. The patients were followed-up at 6, 12, and 24 weeks. Buffered L-PRP injection did not improve pain or activity for patients who were all treated with a concurrent eccentric exercise regimen. However, if testing a combination therapy, the optimal study design to address the buffered L-PRP hypothesis has to consider various options for control groups. In fact, a third arm including patients who do not receive any active therapy (ie, needling trauma) could have produced more reliable results. To note, this trial used only 1 injection of L-PRP: most practitioners report benefits in Achilles tendinopathy, and tendinopathy as whole, after 2 to 3 injections.[33]

It is possible that there are clinical differences in the effects of PRP injections between anatomical locations. Preliminary studies in wrist extensor and flexor tendinopathy have been favorable to PRP treatment. Mishra and Pavelko[34] reported the effects of buffered L-platelet concentrate injections in a small group of patients and found a significant improvement in pain after 8 weeks. More recently, in a randomized clinical trial (level I), Peerbooms et al[35] also reported their findings after

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administering buffered L-PRP in the experimental group and corticoids in the control group of patients with chronic tennis elbow. The group treated wtih corticosteroids appeared to recover initially, but improvement eventually declined, whereas the L-PRP group progressively improved. An observational case series study (level III) reported significant functional improvement on arthroscopic rotator cuff repair in 14 patients followed up at 12 and 24 months postoperatively.[36] Another prospective observational study[37] (level IV) reported decreased pain and enhanced functional recovery after PRP application in 20 athletes with chronic patellar tendinopathy (jumper's knee). In a prospective case-control study (level III), Filardo et al[38] administered 3 injections of activated L-PRP (6 times the concentration of platelets compared with whole blood, leukocyte concentration was not evaluated) in the patellar tendon, with a significant functional improvement after 6 months.

The effects of PRP on rotator cuff pathology have been mixed. Everts et al[39] reported better functional recovery and less pain in a prospective cohort study using L-PRP open subacromial decompression. However, no group differences were found at 2 years. It can be suggested that PRP could bring forth an early biological and clinical response that becomes less pronounced in the long term. In a recent randomized clinical trial, Weber and Kauffman[40] reported that platelet-rich fibrin matrix (Cascade®; Cascade Medical Enterprises, Inc., Wayne, NJ) applied during rotator cuff surgery had the same outcomes as controls. Recently, some presentations at the AAOS 2010 meeting reported no benefit of applying the platelet-rich fibrin matrix (Cascade®) in rotator cuff surgery.[40] All of these studies, however, concur on the safety of the plasma products.

Ligament Injuries

Bone-tendon-bone patellar grafts and hamstring grafts are both used in anterior cruciate ligament (ACL) reconstruction. The goal of this surgery is to obtain rapid tendon ligamentization (tendon transformation into a ligament-like structure) and rapid bone-bone or tendon-bone healing. In an observational case series study (level III), Sánchez et al[5] applied P-PRP to both types of grafts, and evidenced enhanced functional recovery. Recently, the same authors[41] reported enhanced ligamentization at histological examination of tendon grafts treated with P-PRP injections. Confirming these findings, in a case-control study (level III), Radice et al[42] reported enhanced ligamentization after evaluating 100 ACL grafts by magnetic resonance imaging (MRI) using ex vivo clotted L-PRP. Orrego et al[43] found better graft maturation evaluated by MRI signal intensity, without any significant effect in the osteoligamentous interface or tunnel widening evolution (level II). In a recent prospective randomized clinical study (level I), Vogrin et al[44] reported better anteroposterior knee stability at 6 months postsurgery in the ex vivo clotted L-PRP (platelet gel) group. However, Nin et al,[45] in a randomized, case-controlled trial, and Silva and Sampaino[46] and Figueroa et al,[47] in case-controlled studies (level I and III, respectively), could not find any improvement after applying ex vivo clotted L-PRP. Differences in the results could be attributed to both the PRP product and the procedures of application. Some researchers in our group (OMD, GM) have just completed a randomized controlled trial in professional athletes with anterior tibiofibular ligament tears (high ankle sprain). Injection of P-PRP under ultrasound guidance resulted in quicker return to play when compared with untreated controls (unpublished data). Mei-Dan et al[48] reported on an Olympic medalist judoka who won the gold medal world championship < 6 months after sustaining a complete tear of the elbow medial ligamentous complex, which was then injected twice with P-PRP.

Osteoarthritis and Cartilage

Damage to the knee ACL, cartilaginous tissue, or meniscus in an early stage of life can lead to osteoarthritis (OA) later. Post-traumatic or secondary OA is a relatively common condition for athletes with a history of joint injury. In a laboratory study, PRP application can improve the quality of synovial fluid by inducing the endogenous secretion of hyaluronic acid by synovial cells.[49] In a retrospective cohort study (level III), Sánchez et al[50] reported decreased pain and enhanced function, as assessed using the WOMAC scale, after intra-articular injection of activated P-PRP in knee OA compared with intra-articular hyaluronic acid. The same group is using activated P-PRP injections in hip OA with promising preliminary results. Recently, in a case series (level IV) involving 115 knees of young patients with a low degree of articular degeneration, Kon et al[51] reported reduced pain and improved function after L-PRP treatment.

Muscle Injuries

Athletes often experience muscle strains and contusions, which temporarily disable them from training and competition. Applying the rest, ice, compression, and elevation protocol shortly after injury relieves pain and minimizes swelling. Thereafter, the combined injection of Traumeel®/Actovegin®

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(the former is a homeopathic formulation and the latter an amino acid mixture) for the management of acute muscle strains is popular in many countries. The use of autologous plasma preparations might be a safe alternative to this treatment. In a nonrandomized and nonblinded pilot study (level III), Wright-Carpenter et al[52] compared both treatments, assessing the time needed to resume full sports activities after moderate strains in 18 patients receiving 5 mL of autologous conditioned serum (ACS) every second day versus 11 patients receiving the same volume of Traumeel®/Actovegin®. Autologous conditioned serum incubates whole blood with glass beads; it contains signalling proteins including interleukin-1β (IL-1β), TNF-α, IL-7, fibroblast growth factor-2 (FGF-2), IL-1Ra, HGF, PDGF-AB, TGFβ1, and IGF-1. The mean time needed to resume full competition was shorter for the ACS group. Moreover, regression of edema/bleeding was faster in the ACS group as monitored by MRI.

In the Second World Congress of Regenerative Medicine, Sánchez et al[53] reported activated P-PRP injections in 21 muscle injuries of varying severity and anatomical locations. These athletes, who played in division 1 teams of the Spanish Soccer League, resumed normal training activities after half the time needed by matched historical controls. Using the same leukocyte-free PRP preparation, Loo et al[54] reported good outcome after application to adductor longus strain in a single case report. More recently, in a laboratory-controlled study, Hammond et al[55] injected either L-PRP or PRP in 2 models of muscle strains in the tibialis anterioris of rats (8 animals per group). The authors found enhanced myogenesis and improved contractile function with PRP.

Doping Concerns

Because PRP contains growth factors, its use may go against anti-doping rules. In 2008, the World Anti-Doping Association (WADA) and the International Olympic Committee (IOC) organized an international meeting to discuss possible conflicts with the WADA Code. The resulting position paper, the Aspetar Consensus, discussed the use of PRP in muscle injury in relation to evidence-based medicine and doping. The recommendations from the Aspetar Consensus were equivocal and left the decision to further research. However, therapeutic use exemption when wishing to use growth factor technologies in elite athletes was recommended. Currently, in Section (S.2.6) of the current 2010 Prohibited List, intramuscular injections of PRP are prohibited, while other applications require a declaration of use. In the same Section (S.2.5), the growth factors explicitly mentioned in connection with PRP are IGF-1, PDGF, FGFs, VEGF, and HGF. The concentration of all these factors, while present in PRP, is in the physiological range. Moreover, in these preparations, HGF and IGF-1 are not readily available because they are bound to proteins that regulate their physiological actions.

Finally, after more discussion in 2010, guidance has emerged from the WADA clarifying that PRP formulations (as they exist currently) do not increase muscle growth beyond return to a normal physiological state. Hence, because the use of PRP injections for therapeutic purposes does not violate the spirit of sports, the prohibition for intramuscular injections of PRPs has been removed from the 2011 prohibited list.

Summary

Clinical interventions in musculoskeletal and sports medicine aim to achieve predictable, rapid tissue repair through the deposition of new, well-organized extracellular matrix to facilitate and enhance wound healing that will restore the high mechanical performance and functional levels of noninjured tissue in the shortest period. In this respect, PRP technology may be a remarkable step forward. However, much remains to be learned about how variations in the number of platelets or leukocytes, volume of injection, or timing of treatment influence the clinical response. Researchers in this field should undertake appropriately powered level I studies with adequate and relevant outcome measures and clinically appropriate follow-up to assess the efficacy of various PRP treatment modalities.

Conflict of Interest Statement

Omer Mei-Dan, MD, Giuseppe Lippi, MD, Mikel Sánchez, MD, Isabel Andia, PhD, and Nicola Maffulli, MD, MS, PhD, FRCS(Orth) disclose no conflicts of interest.

References

1. Anitua E, Sanchez M, Nurden AT, Nurden P, Orive G, Andia I. New insights into and novel applications for platelet-rich fibrin therapies. *Trends Biotechnol*. 2006;24(5):227–234.
2. Margolis DJ, Kantor J, Santanna J, et al. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care*. 2001;24(3):483–488.
3. Anitua E, Sanchez M, Orive G, Andia I. The potential impact of the preparations rich in growth factors (PRGF) in different medical fields. *Biomaterials*. 2007;28:4551–4560.

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4. Sánchez M, Azofra J, Anitua E, et al. Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. *Med Sci Sports Exerc*. 2003;35(10):1648–1652.
5. Sánchez M, Azofra J, Aizpurua B, et al. Use of autologous plasma rich in growth factors in arthroscopic surgery. *Cuadernos de Artroscopia*. 2003;10:12–19.
6. Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of play. *Br J Sports Med*. 2008;42(5):314–320.
7. Sánchez M, Anitua E, Orive G, Mujika I, Andia I. Platelet-rich therapies in the treatment of orthopaedic sport injuries. *Sports Med*. 2009;39(5):345–354.
8. Nurden AT, Nurden P, Sanchez M, Andia I, Anitua E. Platelets and wound healing. *Frontiers in Bioscience*. 2008;13:3532–3548.
9. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*. 2009;27(3):158–167.
10. Tidball JG. Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol*. 2005;288(2):R345–R353.
11. Mazzucco L, Balbo V, Cattana E, Guaschino R, Borzini P. Not every PRP-gel is born equal. Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet, RegenPRP-Kit, Plateltex and one manual procedure. *Vox Sang*. 2009;97(2):110–118.
12. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. *Clin Oral Implants Res*. 2006;17(2):212–219.
13. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg*. 2004;114(6):1502–1508.
14. Alsousou J, Thompson M, Hulley P, Noble A, Willett K. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. *J Bone Joint Surg Br*. 2009;91(8):987–996.
15. Weibrich G, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. *J Craniomaxillofac Surg*. 2002;30(2):97–102.
16. Vavken P, Saad FA, Murray MM. Age dependence of expression of growth factor receptors in porcine ACL fibroblasts. *J Orthop Res*. 2010;28(8):1107–1112.
17. Landesberg R, Moses M, Karpatkin M. Risks of using platelet rich plasma gel. *J Oral Maxillofac Surg*. 1998;56(9):1116–1117.
18. Marx RE. Platelet rich plasma (PRP): what is PRP and what is not PRP. *Implant Dent*. 2001;10(4):225–228.
19. Banfi G, Corsi MM, Volpi P. Could platelet rich plasma have effects on systemic circulating growth factors and cytokine release in orthopedic applications? *Br J Sports Med*. 2006;40(10):816.
20. Moojen DJ, Everts PA, Schure RM, et al. Antimicrobial activity of platelet-leukocyte gel against Staphylococcus aureus. *J Orthop Res*. 2008;26(3):404–410.
21. Jia WT, Zhang CQ, Wang JQ, Feng Y, Ai ZS. The prophylactic effects of platelet-leucocyte gel in osteomyelitis. An experimental study in a rabbit model. *J Bone Joint Surg Br*. 2010;92B(2):304–310.
22. Andia I, Sanchez M, Maffulli N. Platelet rich plasma therapies and tendon healing. *Expert Opinion Biol Therap*. 2010;10(10):1415–1426.
23. Kajikawa Y, Morihara T, Sakamoto H, et al. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. *J Cell Physiol*. 2008;215(3):837–845.
24. Anitua E, Andia I, Sanchez M, et al. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. *J Orthop Res*. 2005;23(2):281–286.
25. Anitua E, Sanchez M, Nurden AT, et al. Reciprocal actions of platelet-secreted TGF-beta1 on the production of VEGF and HGF by human tendon cells. *Plast Reconstr Surg*. 2007;119(3):950–959.
26. Anitua E, Sanchez M, Nurden AT, et al. Autologous fibrin matrices: a potential source of biological mediators that modulate tendon cell activities. *J Biomed Mat Res A*. 2006;77(2):285–293.
27. Anitua E, Sánchez M, Zalduendo MM, de la Fuente M, Prado R, Orive G, Andia I. Fibroblastic response to treatment with different preparations rich in growth factors. *Cell Proliferation*. 2009;42(2):162–170.
28. Virchenko O, Aspenberg P. How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. *Acta Orthop*. 2006;77(5):806–812.
29. Bosch G, van Schie HT, de Groot MW, et al. Effects of platelet-rich plasma on the quality of repair of mechanically induced core lesions in equine superficial digital flexor tendons: a placebo-controlled experimental study. *J Orthop Res*. 2010;28(2):211–217.
30. Sánchez M, Anitua E, Azofra J, Andía I, Padilla S, Mujika I. Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. *Am J Sports Med*. 2007;35(2):245–251.
31. Sánchez M, Anitua E, Cole A, et al. Management of post-surgical Achilles tendon complications with a preparation rich in growth factors: a study of two-cases. *Injury EXTRA*. 2009;40:11–15.
32. de Vos RJ, Weir A, van Schie HT, et al. Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. *JAMA*. 2010;303(2):144–149.
33. Sanchez M, Anitua E, Azofra J, et al. Local injection of endogenous growth factors for Achilles tendon pathology. In: Maffulli N, Oliva F, eds. *Achilles Tendon*. Corso Trieste, Rome; CIC Edizioni Internazionali: 2009;14:92–100.
34. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med*. 2006;34(11):1774–1778.
35. Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T. Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. *Am J Sports Med*. 2010;38(2):255–262.
36. Randelli PS, Arrigoni P, Cabitza P, Volpi P, Maffulli N. Autologous platelet rich plasma for arthroscopic rotator cuff repair. A pilot study. *Disabil Rehabil*. 2008;30(20–22):1584–1589.
37. Kon E, Filardo G, Delcogliano M, et al. Platelet-rich plasma: new clinical application: a pilot study for treatment of jumper's knee. *Injury*. 2009;40(6):598–603.
38. Filardo G, Kon E, Della Villa S, Vincentelli F, Fornasari PM, Marcacci M. Use of platelet-rich plasma for the treatment of refractory jumper's knee. *Int Orthop*. 2009;34(6):909–915.
39. Everts PA, Devilee RJ, Mahoney CB, et al. Exogenous application of platelet-leukocyte gel during open subacromial decompression contributes to improved patient outcome. A prospective randomized double-blind study. *European Surg Repair*. 2008;40(2):203–210.
40. Weber SC, Kauffman JI. Platelet-rich fibrin matrix in arthroscopic rotator cuff repair. A prospective, randomized study. Presented at: American Academy of Orthopaedic Surgeons Meeting; March 9–13, 2010; New Orleans, LA.
41. Sanchez M, Anitua E, Azofra J, Prado R, Muruzabal F, Andia I. Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. *Arthroscopy*. 2010;26(4):470–480.
42. Radice F, Yanez R, Gutierrez V, Rosales J, Pinedo M, Coda S. Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet-derived growth factors. *Arthroscopy*. 2010;26(1):50–57.
43. Orrego M, Larrain C, Rosales J, et al. Effects of platelet concentrate and a bone plug on the healing of hamstring tendons in a bone tunnel. *Arthroscopy*. 2008;24(12):1373–1380.

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44. Vogrin M, Rupreht M, Crnjac A, Dinevski D, Krajnc Z, Recnik G. The effect of platelet-derived growth factors on knee stability after anterior cruciate ligament reconstruction: a prospective randomized clinical study. *Wien Klin Wochenschr*. 2010;122(suppl 2):91–95.
45. Nin JR, Gasque GM, Azcárate AV, Beola JD, Gonzalez MH. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? *Arthroscopy*. 2009;25(11):1206–1213.
46. Silva A, Sampaio R. Anatomic ACL reconstruction: does the platelet-rich plasma accelerate tendon healing? *Knee Surg Sports Traumatol Arthrosc*. 2009;17(6):676–682.
47. Figueroa D, Melean P, Calvo R, et al. Magnetic resonance imaging evaluation of the integration and maturation of semitendinous-gracilis graft in anterior cruciate ligament reconstruction using autologous platelet concentrate. *Arthroscopy*. 2010;26(10):1318–1335.
48. Mei-Dan O, Carmont M, Kots E, Barchilon V, Nyska M, Mann G. Early return to play following complete rupture of the medial collateral ligament of the elbow using preparation rich in growth factors: a case report. *J Shoulder Elbow Surg*. 2010;19(5):e1–e5.
49. Anitua E, Sánchez M, Nurden AT, et al. Platelet-released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. *Rheumatology*. 2007;46(12):1769–1772.
50. Sánchez M, Anitua E, Azofra J, Aguirre JJ, Andia I. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. *Clin Exp Rheumatol*. 2008;26(5):910–913.
51. Kon E, Buda R, Filardo G, et al. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. *Knee Surg Sports Traumatol Arthrosc*. 2010;18(4):472–479.
52. Wright-Carpenter T, Klein P, Schäferhoff P, Appell HJ, Mir LM, Wehling P. Treatment of muscle injuries by local administration of autologous conditioned serum: a pilot study on sportsmen with muscle strains. *Int J Sports Med*. 2004;25(8):588–593.
53. Sánchez M, Anitua E, Andia I. Application of autologous growth factors on skeletal muscle healing. Second International Congress on Regenerative Medicine. http://www.plateletrichplasma.com/pdf/Orthopedic-PRP/Sports%20Medicine/66-SanchezRegMed2005.pdf. Accessed December 1, 2010.
54. Loo WL, Lee DY, Soon MY. Plasma rich in growth factors to treat adductor longus tear. *Ann Acad Med Singapore*. 2009;38(8):733–734.
55. Hammond JW, Hinton RY, Curl LA, Muriel JM, Lovering RM. Use of autologous platelet-rich plasma to treat muscle strain injuries. *Am J Sports Med*. 2009;37(6):1135–1142.

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