Sports Medicine Applications of Platelet Rich Plasma

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Abstract: Platelet rich plasma (PRP) is a powerful new biologic tool in sports medicine. PRP is a fraction of autologous whole blood containing and increased number of platelets and a wide variety of cytokines such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and transforming growth factor beta-1 (TGF-B1), fibroblast growth factor (FGF), Insulin-like growth factor-1 (IGF-1) among many others. Worldwide interest in this biologic technology has recently risen sharply. Basic science and preclinical data support the use of PRP for a variety of sports related injuries and disorders. The published, peer reviewed, human data on PRP is limited. Although the scientific evaluation of clinical efficacy is in the early stages, elite and recreational athletes already use PRP in the treatment of sports related injuries. Many questions remain to be answered regarding the use of PRP including optimal formulation, including of leukocytes, dosage and rehabilitation protocols. In this review, a classification for platelet rich plasma is proposed and the in-vitro, preclinical and human investigations of PRP applications in sports medicine will be reviewed as well as a discussion of rehabilitation after a PRP procedure. The regulation of PRP by the World Anti-Doping Agency will also be discussed. PRP is a promising technology in sports medicine; however, it will require more vigorous study in order to better understand how to apply it most effectively.

Keywords: Platelet rich plasma, classification, sports medicine, tendinopathy, PRP, tennis elbow.

INTRODUCTION

Biologic engineering is a rapidly evolving field in sports medicine and orthopaedic surgery. The ideal biologic tool would be effective, simple to use, inexpensive, safe and available immediately at the point of care. Platelet rich plasma (PRP) meets many of these criteria. PRP is autologous, therefore, potential for adverse reactions and transmission of infection is very low. It has been used for almost 20 years in several medical specialties without reports of significant complications. PRP is simple to produce from small amounts of peripheral blood in short period of time (15 minutes or less) using desktop sized equipment obviating the need to send the material out for processing or culturing. PRP is inexpensive to produce compared to stem cells or genetically engineered proteins.

Athletes of all competition levels are early adopters of novel treatment methods. They are driven to find less invasive methods of injury management and faster means of returning to their sports. In their quest to find these methods, they sometimes choose treatments that have little or no published peer reviewed evidence of efficacy. It takes years for new treatments to be fully validated by large, prospective, randomized, controlled trials.

The lay press also influences athletes and practitioners. High profile athletes have been treated with PRP for a variety of injuries and disorders and their treatments have been chronicled in publications, newspapers and broadcast media [1, 2]. This has fueled speculation about PRP as either a miracle cure or a treatment that lacks any value. It is important that scientists and physicians conduct and interpret the data so that practice is driven by sound clinical judgment rather than media hype. Speculation about PRP being a form of blood doping has also arisen although it does not meet the definition. The actions of the World Anti-Doping Agency regarding PRP and its rulings will be examined in this review. PRP has evolved in the setting of media, athlete and, in some cases, practitioner hype as well as regulatory uncertainty. This article will attempt to review the science and current state of practice and suggest a reasoned path forward.

The concept of using a component of whole blood to augment healing was first promoted by Ferrari in 1987 as an autologous transfusion component after an open heart operation to avoid homologous blood product transfusion [3]. The clinical value of PRP was also demonstrated by Marx in the 1990s in the oral and maxillofacial literature [4]. Mishra and Pavelko were the first to publish a human pilot trial for a sports medicine application [5]. Over the last several years, many more basic science and clinical investigations have been published supporting the use of PRP for sports related injuries and disorders [6-21]. It is difficult to directly compare studies because there is a lack of a coherent nomenclature or classification system for PRP. Few clinical studies have quantified the actual components of what is used as a treatment and often formulation or dosage of PRP is not recorded.

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WHAT IS PRP?

By definition, PRP must contain a higher concentration of platelets than baseline, however, there are many other variables in the make-up of PRP that will lead to different biologic and physiologic properties; and possibly effectiveness. An increase in platelets is an incomplete and simplistic description of PRP. There are multiple parameters in addition to platelet concentration which should be considered.

In addition to platelet concentration, the inclusion of leucocytes in the PRP preparation, the use of anticoagulant, and the use of activating agents also needs to be considered. PRP containing white blood cells will have different biologic activity than PRP without. PRP containing increased platelets with WBCs is the most extensively studied form of PRP to date [5, 7, 22]. It is important to remember, however, that there are many types of white blood cells including neutrophils, monocytes/macrophages and lymphocytes. Their roles in tissue healing vary considerably. Neutrophils contain hydrolytic enzymes and are phagocytic. This may be of value in chronic tendinopathy but this release of proteases may lead to secondary damage when applied to an acute muscle injury. Macrophages are the tissue form of the circulating monocytes. Their role is the removal of debris and they are primarily phagocytic. They also have role in balancing the pro-inflammatory and anti-inflammatory aspects of healing [23-25]. Finally, lymphocytes initiate cell-to-cell interactions and also modulate tissue healing *via* release of bioactive molecules. Although WBCs are important in preventing infection, platelets alone *in vitro* and *in vivo* enhance the immune response [26]. Leukocytes in their various forms are powerful cells and play a crucial role in many forms of healing. Their role in different mechanisms and applications of PRP needs to be studied in greater depth.

When whole blood is drawn, many PRP kits will use an anti-coagulant to prevent it from clotting. Most kits use anticoagulant citrate dextrose (ACD) to inhibit clotting. ACD binds calcium preventing the coagulation proteins from using it and initiating the clotting cascade. The addition of citrate to the blood prevents clotting, but also makes it more acidic than is physiologic. Some growth factors can be influenced by the pH of the tissue, thus some protocols recommend buffering the PRP back to a physiologic range prior to injection.

Whether or not the PRP is activated prior to injection is another parameter that requires further discussion. Many human protocols do not activate the PRP prior to use since tissue collagen is a strong activator of platelets [5, 22, 27]. However, PRP can be activated exogenously by thrombin, calcium chloride, or mechanical trauma. Once the PRP is activated, a fibrin network will begin to form and the plasma will begin to solidify creating a fibrin clot or membrane. If PRP is activated too strongly, the fibrin network that forms will be a bivalent, unstable network. If it is activated in a more physiologic manner a tetramolecular stable network will form which enhances enmeshment of cells and growth factors [28]. Although this can be useful for surgical procedures, it is undesirable to have the PRP too viscous when injecting into soft tissue.

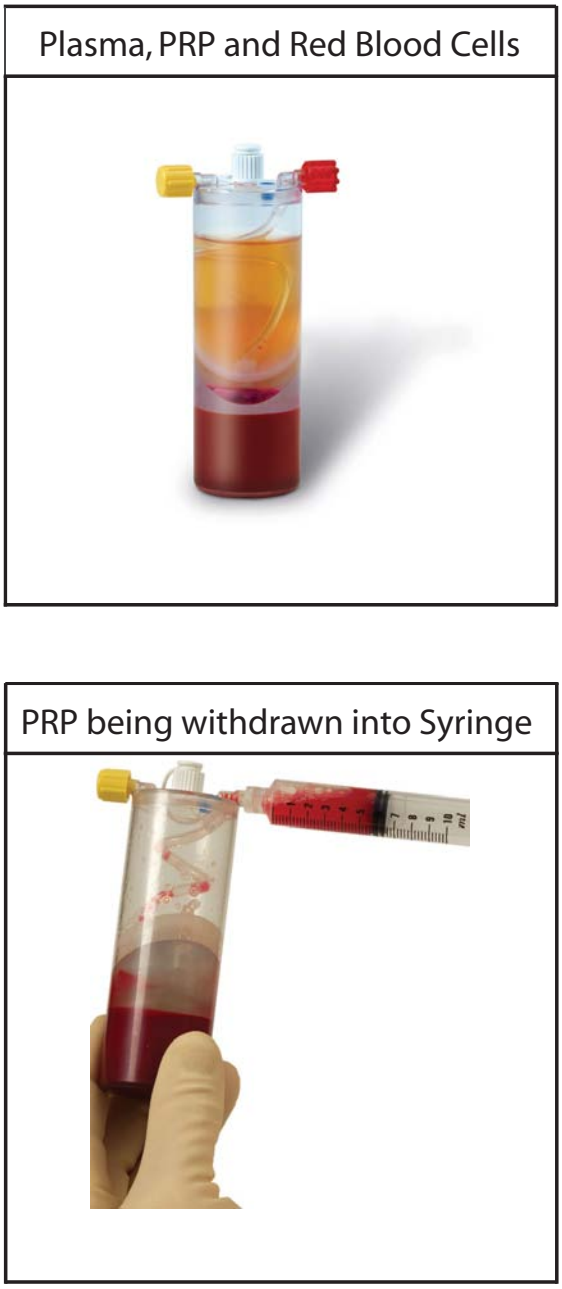

Fig. (1). Separation of whole blood into PRP.

Activation results in rapid release of the growth factors in any form of PRP. Ninety percent of the prefabricated growth factors will be released in the first 10 minutes. Many growth factors have very short half-lives, so for them to be most effective it would appear that they should be activated at the time of injection or just prior to injection. The variable half-lives of different growth factors will also lead to a differential make-up of the PRP depending on how soon after it is activated it is used. Most commercial PRP kits for use in soft tissue do not activate PRP. Some will use replace the calcium that was bound by the ACD in order to return it to a more physiological state. Employing unactivated PRP results in a more physiologic activation by the tissue into which it is injected or applied.

When this paper discusses human trials, the formulation of PRP will be reported whenever possible. A new sports medicine and orthopaedic surgery classification scheme is proposed based on platelet concentration, the presence or absence of white blood cells, and whether or not the PRP has been activated with exogenous thrombin or calcium chloride. This framework will allow the critical reader to draw more accurate conclusions. Classification will lead to improved study comparison and ultimately a better understanding of how to use PRP in sports medicine.

SPORTS MEDICINE PLATELET RICH PLASMA CLASSIFICATION SYSTEM

Four types of platelet rich plasma are identified in this new classification (see Fig. (**2**)). Type 1 PRP contains an increased concentration of platelets and white blood cells over baseline and is not activated by an exogenous activator such as thrombin or calcium. Type 2 is activated with thrombin and or calcium and contains both increased platelets and white blood cells. This type of PRP is also known as *platelet-leukocyte gel*. Type 3 PRP contains only an increased concentration of platelets without any white blood cells and it is not activated prior to application. This type is sometimes known as *platelet concentrate*. Type 4 is activated with thrombin and or calcium and contains only an increased platelet concentration. This type of PRP may also be called *platelet gel* in the literature. Subtype A contains an increased platelet concentration at or above five times baseline. Subtype B contains an increased platelet concentration less than five times baseline. If no concentration is reported, no subtype is noted. Further classification may be done if the white blood cells are fractionated.

	White Blood Cells	Activation?	Platelet Concentration
Type 1	Increased	No Activation	A, 5x or > B, < 5x
Type 2	Increased	Activated	A, 5x or > B, < 5x
Type 3	Minimal or No WBCs	No Activation	A, 5x or > B, < 5x
Type 4	Minimal or No WBCs	Activated	A, 5x or > B, < 5x

Fig. (2). Sports medicine classification of platelet rich plasma.

Buffering and the use of an anticoagulant are other parameters to consider when reporting the type of PRP. In order to keep the classification simple and reproducible, only three variables are reported—the presence of white blood cells, activation status and platelet concentration.

There are multiple forms of PRP being applied for a wide variety of conditions. Most have not been vigorously tested *in vitro* or *in vivo*. The combination of growth factors and cytokines present in each formulation interact with each other. The ultimate value of each type of PRP must be tested both *in vitro* to better understand the basic science and with prospective randomized clinical trials.

Regulatory Discussion

The World Anti-Doping Agency (WADA) was initially formed in 1999 in response to doping scandals in the world of cycling. Over the next five years the International Standards and Code was developed [29]. WADA's mission is to promote, coordinate and monitor the fight against doping in sport in all its forms. WADA creates a list of substances and practices which are prohibited for use. It bases its decision on whether or not something is prohibited based on its Code. A substance will be considered for the prohibited list if it meets two of the following three criteria:

1. Medical or other scientific evidence, pharmacological effect or experience that the substance/method has the potential to enhance sports performance.
2. Medical or other scientific evidence, pharmacological effect or experience that the use of the substance/method represents an actual or potential health risk to the athlete.
3. WADA's determination that the use of the substance/method violates the spirit of sport [29].

Anti-doping organizations, international sport federations (IFs), national anti-doping organizations (NADOs) such as the US Anti-Doping Agency, and major games organizers such as the International Olympic Committee (IOC) become signatories to the WADA code [30]. In doing so they agree to implement and enforce the rules of the Code. There is significant international support for major sporting entities to comply with the code.

The use of autologous growth factors had not been specifically considered by WADA, prior to 2010. Although previous prohibited lists have included hormones and related substances such as growth hormone (GH), IGF-1, and MGFs which were specifically mentioned, there was not delineation between exogenous and endogenous hormones. Because of this ambiguity, IFs and NADOs were uncertain regarding the status of platelet rich plasma that delivers endogenous growth factors to the site of injury. Unlike blood doping, however, the amount of growth factor delivered is not adding any additional growth factor to the body and is unlikely to be ergogenic. Because of the uncertainty, some athletes applied for a Therapeutic Use Exemption (TUE). A TUE is special permission to use a prohibited substance for medical reasons based on substantial medical documentation [30]. Depending on the level of the athlete and which competition they are competing in, the granting authority of the TUE can change.

In 2009 the WADA Scientific Committee reconsidered PRP and the 2010 rules were amended. Platelet-derived preparations administered by the intramuscular route was prohibited; however, other routes of administration required only a Declaration of Use. A Declaration of Use documents use of PRP and the medical circumstances which it is used under but does not require approval. Given that there is no documented evidence of significant harmful effects with the use of PRP, WADA's concern rested with the possibility that PRP is ergogenic, beyond normal healing. In muscle the primary concern for WADA is likely IGF-1 and mechano growth factor (MGF) both growth hormones which were specifically named in the 2009 code. MGF, which is an isoform of IGF-1, is known to stimulate terminal differentiation of muscle cells into myotubes and promote muscle regeneration and hypertrophy [31]. Although high doses of MGF may enhance muscle growth, it is highly unlikely that PRP in injured muscle has ergogenic effect. Creany *et al*. reviewed IGF-1 and MGF found in PRP and determined that,

- The unbound IGF-1 half-life (10 minutes) is too short to be able to exert systemic effects. Therefore, any effect of the PRP would be at the site of injection.
- The iso-form (subtype) of IGF-1 in PRP is not the iso-form (MGF) principally responsible for skeletal muscle hypertrophy.
- The doses of IGF-1 in PRP are subtherapeutic in terms of producing a systemic anabolic effect by a factor of 500 [31].

In injured muscle, the hope is that PRP will accelerate the formation of new muscle and inhibit the formation of fibrosis. There is no evidence that PRP injected into uninjured muscle is ergogenic. The prohibition of PRP injected intramuscularly was deleted for 2011. [32, 33]

While there are many signatories to the WADA Code, there are many organizations that develop independent doping programs. Most professional organizations have their own system. Use of PRP in the National Football League (NFL), National Basketball Association (NBA), and Major League Baseball (MLB) in the United States is frequent and not controversial. Intramuscular use is relatively common in the professional ranks, where days shaved off recovery can translate to millions of dollars. It is not considered ergogenic in these cases. The National Collegiate Athletic Association (NCAA) has not taken a position on the use of PRP. As the successes of PRP are documented in the press more and more recreational athletes and sedentary people with tendinopathy or other problems are seeking out and utilizing this new technology.

The science of PRP continues to evolve, and a more complete picture regarding whether and in what settings it is effective and if there should be concern for it giving an athlete an unfair competitive advantage will emerge. At this point research does not support PRP being ergogenic.

IMAGING

Ultrasound and MRI are commonly used to image musculoskeletal injuries. Ultrasound can be used to evaluate injury, accurately apply treatment, and follow outcomes. Musculoskeletal ultrasound (MSK US) is a point of care technology which can be easily applied in the office. Ultrasound has higher resolution for tendon fibrils than MRI, is less expensive, and obviates the need for a return visit. Tendinopathy can be seen as hypoechoic, thickened tendon with a loss of the normal fibrillar architecture. Treatment can then be targeted directly to the pathogenic area.

Joint injections done without image guidance are frequently inaccurate [34-43]. Cortisone diffuses through tissue planes and therefore injections may not need to be accurately placed for effectiveness. Biologics are relatively more expensive and the goal is to deliver them as accurately as possible to the site of injury. Ultrasound is helpful for this in most cases. For deeper tendons which may be difficult to target such as the hamstrings or external rotators this may be particularly important or when potentially dangerous structures are in close proximity to the targeted tendon such as the femoral artery to the illiopsoas tendon. In addition, MSK US can be used to accurately and cost-effectively inject the hip and other deep joints which generally require some sort of image guidance (fluoroscopic, CT) [44].

Lesions can be followed over time. The size of a partial tendon lesion can be measured *via* ultrasound and recorded (see Fig. (**3**)). Limited data presently exists but as PRP studies progress, ultrasound will be able to provide clinicians and patients with an objective endpoint in addition to subjective pain and clinical scoring systems. MRI is likely to remain the best option to evaluate cartilage lesions before and after PRP treatments.

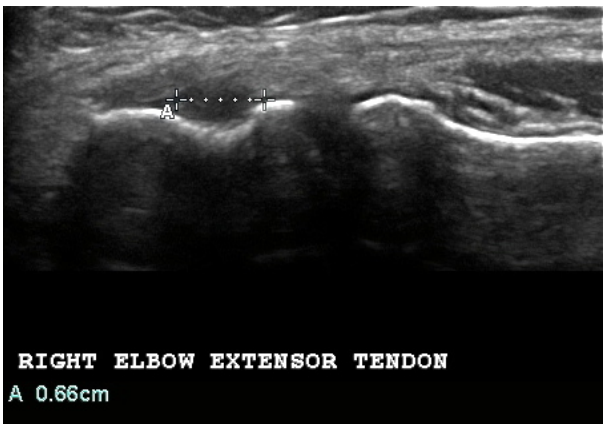

Fig. (3). Elbow extensor tendinopathy.

PRP AND TENDINOPATHY

The use of PRP in chronic tendinopathy appears promising. As the pathogenesis of tendinopathy is better understood as degenerative, therapies which attempt to initiate the bodies own healing mechanisms make sense. Preclinical and human cell culture studies support the use of PRP for the treatment of tendon injuries and disorders [10, 15, 45]. Specifically, Schnabel *et al*. found PRP enhanced gene expression of matrix molecules with no concomitant increase in catabolic molecules [14]. Other authors have suggested that PRP could improve tendon healing with less fibrosis [45]. This may mimic embryonic scarless healing. Animal studies confirmed that PRP improves force to failure in a rat Achilles tendon repair model in addition to other biomechanical improvements [13, 29, 46]. Kajikawa *et al*. demonstrated enhanced type 1 collagen synthesis in PRP treated patellar tendons compared to controls. Importantly, the authors also showed how PRP is an activator of circulation-derived cells and how these cells become incorporated into healing tendons [47]. Other investigators have found PRP improves tendon fiber alignment when measured by computerized ultrasound in an equine tendon study [46]. Immunohistochemical evidence has further demonstrated PRP enhances and accelerates the tendon healing process [9].

ELBOW TENDINOPATHY

Mishra and Pavelko published the first human use of PRP (Type 1A) for chronic severe elbow tendinosis, in a prospective, controlled pilot study [5]. They found a 60% improvement in pain scores for PRP treated patients versus a 16% improvement in control patients 8 weeks after treatment. ($p = 0.001$) At final follow-up (mean, 25.6 months; range, 12-38 months), the PRP patients reported 93% reduction in pain

compared with pretreatment scores. This initial study was limited by its study design including a lack of full randomization and small patient numbers. It did, however, have an average of over two year follow-up and demonstrated an excellent safety profile for Type 1A PRP as a potential treatment option for sports related injuries.

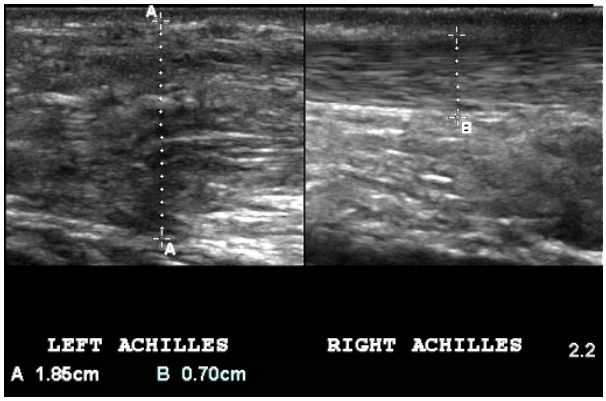

Fig. (4). Hypoechogenic thickening of left achilles tendon.

Using the same type of PRP (Type 1A) and methodology, Peerbooms *et al*. found significant improvements in pain and disability scores in a prospective double-blind randomized controlled trial of patients with elbow tendinopathy [7]. This study enrolled 100 patients and measured PRP versus cortisone as a treatment after failure of a variety of treatments including physical therapy. The initial visual analog pain scores of the patients in the PRP treated group and control group were 70.1 and 65.8 respectively. These patients had significant pain that had failed physiotherapy and were seeking further intervention. This is an important distinction to studies where subjects had not exhausted other treatment options [22]. The patients in the PRP group after six months reported 53.5% (70.1 to 32.6) improvement in pain scores compared to 14% (65.8 to 50.1) improvement in the corticosteroid group ($p < 0.001$). At one year, the PRP patients reported 63.9% (70. 1 to 25.3) compared to 24% (65.8 to 50.1) in the steroid group ($p < 0.001$). Similar changes in a functional evaluation (DASH Score) were noted. No complications were reported in this study.

ACHILLES TENDINOPATHY

A well-designed prospective, randomized trial evaluated 54 patients with moderate Achilles tendinopathy using PRP (Type 1A) vs saline injections in combination with eccentric exercise as a treatment protocol [22]. The authors specifically excluded any patients who had previously performed an eccentric exercise program. The baseline VISA-A scores for the PRP group and control group were 46.7 and 52.6 respectively. The inclusion criteria required only two months of Achilles pain, included a broad age range (18 – 70y) and did not require previous failure of any type of treatment such as immobilization or formal physical therapy. A VISA-A score of ~50 places the patient population group in the moderate zone of severity. The patients were then given injections of either buffered PRP or saline under ultrasonic guidance. Eccentric exercises were initiated at one week after the treatment in the form of a 12-week daily program of 180 repetitions. The patients were allowed to return to sports activities after four weeks. At 24 weeks after treatment the PRP group had improved 21.7 points from baseline compared with 20.5 points for the control group. The differences were not statistically different.

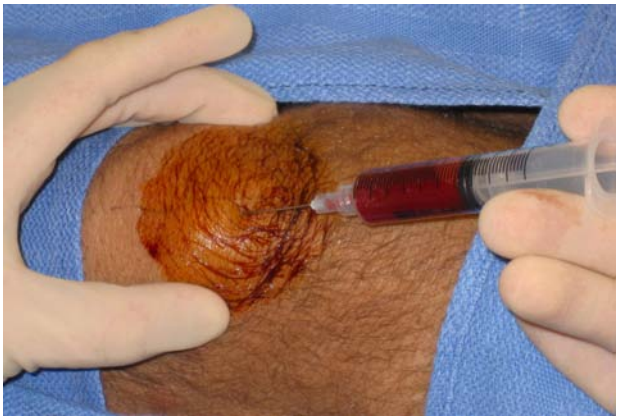

Fig. (5). Platelet rich plasma injection into quadriceps tendon.

This investigation had some weaknesses including the relatively small number of patients and broad age range. In addition, the saline group had the needle inserted into the tendon twelve times according to the study protocol. Tenotomy alone has been shown to be 60% effective in the treatment of tendinopathy [49, 50]. In a study of an almost demographically identical group (mean age ~50y, mean VISA-A ~50) comparing eccentric exercises, extracorporal shock wave therapy (ESWT) and a wait-and-see approach, eccentric exercises and ESWT groups each improved 20 points suggesting many different interventions can be effective in this group. Clearly less invasive methods of treatment should be considered prior to using PRP; however, when other treatments have failed, PRP may still be effective.

Monto treated 30 patients with Achilles tendinopathy patients who had failed eight months of non-operative treatment including immobilization and physical therapy with an ultrasonically guided type 1 PRP injection. All patients were considering operative intervention [51]. Pretreatment AOFAS scores averaged 34 indicating significant pathology. Six months after treatment, the AOFAS scores averaged 92. Ninety-three percent (28/30) patients were satisfied. Resolution of MRI or ultrasound abnormalities was also noted and no complications were reported using this technique. This data supports the use of PRP for chronic, severe Achilles tendinopathy that as failed non-operative treatment. In the future, objective endpoints such as the width of the Achilles tendon measured *via* ultrasound before and after treatment should be reported.

In another study comparing athletes undergoing Achilles tendon surgical repair**,** patients treated with PRP (Type 4B) at the time of surgery recovered range of motion faster than the control group [8]. Subjects in the PRP group also returned to their pre-injury activity levels at an average of 14 weeks compared to 22 weeks in the control group. This study was small and not a randomized group. Further evidence in a prospective, randomized manner is needed to clarify the potential value of using PRP to help athletes with Achilles tendon tears to return earlier to competition.

Patellar Tendinopathy

In a pilot study Volpi *et al*. used PRP (Type 1) to treat chronic patellar tendinosis in elite athletes. The patients had been recalcitrant to conservative measures and were considering surgical treatment. The authors found significant improvements in VISA scores ($p < 0.001$) (39 to 75) after PRP injection under ultrasonic guidance. They also demonstrated reduced irregularity within the tendon by MRI [52]. Kon *et al.* reported statistically significant better scores (IKDC and VAS) in 20 male athletes treated with PRP for refractory jumper's knee using manually extracted Type 1 PRP [53]. Filardo *et al.* had good results with the use of PRP for chronic patellar tendinopathy in 15 athletes with patellar tendinopathy (manual Type 1) [54]. This data suggest PRP may be helpful for athletes with chronic severe patellar tendinosis who have failed other treatment options. A prospective, randomized trial of elite athletes of PRP vs surgery vs continued conservative care for this difficult problem will help better answer the question of PRP's value.

ROTATOR CUFF

There are no studies examining the effectiveness of PRP in the non-operative treatment of rotator cuff and shoulder injuries. There are some studies which examine the use of PRP intraoperatively. Randelli *et al.* was the first to discuss the use of PRP in conjunction with arthroscopic rotator cuff repair in a case series [55]. They found it to be a safe and effective technique. This same group reported a prospective randomized trial of 53 patients (PRP 26, Control 27). The PRP treated patients reported less pain at 3, 7, 14 and 30 days after surgery ($p = <0.05$). Strength in external rotation, simple shoulder test, Constant and UCLA shoulder scores were all higher in the PRP treated group compared to the control group at three months ($p < 0.05$). Strength in external rotation was higher at six and twelve months in patients with tendon retraction ($p < 0.05$) [56]. Weber and Kaufmann reported on a prospective randomized trial of patients treated with a different PRP technique (Type 4 PRP Concentrated Platelets activated with calcium). In their study of 60 patients, undergoing arthroscopic rotator cuff repair, there were no differences at three months in terms of residual defects or perioperative morbidity [57]. Suarez *et al.* reported no clinical differences in a small study (PRP 8, Control 15) of patients who underwent traditional open rotator cuff repair compared to repair augmented with PRP [58]. Collectively, this work on the use of PRP to augment rotator cuff repair is conflicting. Larger studies comparing types of PRP and or techniques will be required to clarify the data should lead to specific recommendations.

Severe tendinopathy of the hamstrings, quadriceps, peroneal tendons, and posterior tibialis have also been treated with anecdotal clinical success; however, there are no published studies documenting results.

These preliminary studies suggest PRP may be effective in some settings. Whether there are certain tendons which will respond more readily to treatment with PRP remains to be answered. Questions also remain regarding timing and frequency of injections. PRP should be reserved for patients who have failed eccentric exercise treatment and have severe tendinopathy. Studies comparing the efficacy and cost-effectiveness of treatment with PRP vs surgical treatment should be undertaken.

While it appears that the use of PRP for tendon-related disorders and injuries may have value direct comparisons among types of PRP and variable effectiveness are lacking. Type I PRP (Increased platelet and WBC concentration applied in an unactivated manner) has been used most frequently in existing clinical studies. Level one evidence suggests chronic tennis elbow is more effectively treated with Type I PRP than cortisone Other level one evidence suggests mild to moderate Achilles tendinopathy is better treated with eccentric exercise prior to considering PRP. Further prospective randomized trials will be required to confirm and guide other treatment protocols.

MUSCLE INJURIES AND PRP

PRP is also being used for the treatment of acute muscle injuries. Muscle strains and contusions are common in sport and account for significant time loss [23, 59-61]. Although injury to muscle is usually self-limited, in elite athletes return to play just a week or two earlier than would have otherwise been expected can mean the difference betweens wins and losses and have significant economic impact for both teams and athletes. The use of PRP in muscle is less well-studied than in tendinopathy, however, there remains sound theoretical basis for use. Initial interest in healing muscle examined the use of individual growth factors to enhance and speed repair [60-67]. More recently there has been interest in the use of platelet rich plasma (PRP) to enhance healing in muscle injury.

When muscle is injured, there is capillary rupture and bleeding which initiates hemostasis which is followed by an inflammatory reaction. The complete healing response in muscle is characterized by three phases, 1) degeneration and inflammation, 2) regeneration, and 3) fibrosis [60, 68]. The extent of muscle injury is dependent on the size of the initial injury, the vascularity of the tissue, and secondary damage caused by the respiratory burst [69, 70]. The ultimate quality and function of the repair is dependent on the delicate interplay of cytokines, cellular factors, and growth factors. The goal of PRP therapy is to influence this intricate process to favor the formation of functional muscle rather than fibrosis.

When muscle is injured, bleeding occurs and a platelet plug is formed providing hemostasis. As the platelets are activated by the collagen and thrombin, they provide not only hemostasis but release growth factors and cytokines causing a cascade of events. The initial phase of healing, the degeneration/inflammatory phase is characterized by the necrosis of the injured myofibrils and the invasion of inflammatory cells. 1 – 2 hours after the injury, circulating neutrophils are the first leukocytes to arrive. Neutrophils have a phagocytic function, and also contain hydrolytic enzymes that lead to the creation of oxygen free radicals and proteases, the respiratory burst. This release of toxic molecules from the neutrophils leads to a secondary damage to the muscle [25, 69-71]. Peak damage in an injured muscle occurs 24 hours after the initial injury at the same time as peak neutrophil concentration [69, 72]. There is debate as to whether or not neutrophils play any type of beneficial role in muscle injury, but it is clear they may be detrimental [25, 69,

73]. Because neutrophils may be detrimental, PRP which is neutrophil free may be a better formulation for muscle injuries.

Macrophages are another type of white blood cells which arrive during the inflammatory/degenerative phase. Macrophages have many roles and do not appear to mediate secondary damage to the muscle. Their role is primarily phagocytic and they likely have a role in moving the inflammatory phase toward regeneration [25, 71]. Because it is not possible to fractionate different types of white blood cells out of PRP, it may be that the absence of macrophages is more detrimental to healing muscle than any secondary damage inflicted by neutrophils. More study is needed in this area.

The second phase of healing, the regeneration phase of the healing of muscle involves a delicate balance between the creation of new muscle and the formation of fibrosis. Satellite cells are inactive in uninjured muscle. When muscle is injured, they transform into myoblasts which are muscle precursor cells or myofibroblasts which produce fibrotic scar tissue. The myoblasts will fuse under the right conditions and form mature, multinucleated muscle fibers [73]. The growth factor milieu is critical in influencing the fate of the satellite cells. In particular, Insulin-like Growth Factor-I (IGF-1) appears to play a critical role in enhancing muscle regeneration by stimulating myoblast proliferation, differentiation, and myofiber protein synthesis and hypertrophy [74]. Conversely, Transforming Growth Factor Beta-1 (TGF-B1) appears to encourage the formation of fibrosis by stimulating the production of extracellular matrix proteins and inhibiting their degradation [75, 76]. Because in PRP the growth factors are provided in physiologic proportions, the hope is that this will lead to a balance of proliferative and inhibitory effects.

The final phase of muscle healing is the remodeling phase. The maturation and innervation of muscle fibers as well as the retraction of scar tissue occur during this phase [77]. Fibrosis is the key inhibitor of complete muscle healing. Fibrotic scar tissue can prevent the stumps of immature myofibers from rejoining and may inhibit the reinnervation of muscle. Muscle which is not innervated will atrophy [31, 73, 75, 77].

There have been a limited number of studies on the use of PRP in muscle injuries. *In vitro*, PRP has been shown to stimulate cell migration and myofibroblastic differentiation [78] Carda *et al.* examined the use of PRP in surgically injured sheep muscle and demonstrated accelerated healing [79]. Wright-Carpenter *et al*. investigated the effectiveness of autologous conditioned serum (ACS) in muscle injuries in mice and human skeletal muscle [80]. ACS is not PRP. ACS is serum containing released growth factors, essentially activated PRP and produces a lower yield of growth factors than most PRP. In rats, there was an 84% increase in satellite cell activation and a 27% increase in the regeneration of myofibers compared to controls [80].

Human studies are more difficult to conduct. Muscle injury in humans is heterogeneous and difficult to quantify both in terms of the initial injury as well as outcome. One study in professional sportsmen treated with ACS suggested PRP was effective in speeding return to play [81]. Eighteen athletes with a variety of muscle strains treated with ACS improved at 16.6 days vs. 22.3 days for the control group. The control group was a retrospectively analyzed group of demographically similar athletes treated with a homeopathic anti-inflammatory substance (Traumeel) and a deproteinised dialysate of bovine blood (Actovegin). The injection of Traumeel and Actovegin is common in parts of Europe but poorly studied [81]. In addition, Sanchez demonstrated in a case series of 20 professional athletes with hamstring muscle injuries who received PRP therapy that functional recovery was regained in half the expected time [82].

Whether PRP is beneficial in muscle injury remains to be seen. As opposed to a chronically degenerative tendon, the muscle is an actively healing, acutely inflammatory entity and there remains potential to affect both the timing and the quality of repair adversely as well in a beneficial manner. All PRP is not the same and it may prove that some types of PRP are more beneficial or harmful than others. Lastly, the timing and quantity of injections required are also in question. Acute muscle injuries are typically self-limited and will heal if given enough time although re-injury is a frequent occurrence. Finally, PRP can be expensive and the cost may prohibit all but the most serious athletes from utilizing this treatment.

Ligament

The use of PRP in acute ligament injuries has not been studied in humans, however, there are basic science and animal studies which would suggest that it may have a role. There is increased matrix synthesis in ligaments exposed to PRP *in vitro* [83] and PRP has also been shown to enhance human ligament cell adhesion, proliferation, and differentiation [84]. In addition, it has been demonstrated that not only does PRP increase ligament cell proliferation, the increase in collagen synthesis is not solely due to transforming growth factor beta and platelet-derived growth factor suggesting there are other important growth enhances in PRP [85-88].

The use of PRP for MCL injuries in humans has been reported in the lay press but no study has been published in the medical literature on the topic. A rabbit medial collateral ligament (MCL) healing study found platelet-derived growth factor-BB enhanced ultimate load and energy absorbed to failure [89]. Use of ultrasound to measure MCL thickness especially in comparison to the opposite uninjured MCL and comparing this objective measurement to validated clinical scoring systems may be a valuable outcome measure for future studies (see Fig. (**6**)).

The use of PRP to augment ACL repairs has been studied in both animal and human models. A collagen-PRP scaffold in a dog model was shown to enhance anterior cruciate ligament healing [90]. There have been a number of conflicting studies in humans; however, differences in technique, type of PRP, and outcomes make direct comparison difficult. Radice *et al*. applied PRP (Type 1) in combination with Gelfoam to the grafts of ACL reconstruction patients. This was a prospective single-blinded study of 100 patients who were followed with MRI to assess graft heterogeneity. The PRP treated grafts needed 179 days to achieve a completely homogeneous signal by MRI compared to 369 days for the control group ($p < 0.001$) [91]. Orrego *et al* has also reported

improved graft maturity using PRP compared to controls at six months [92].

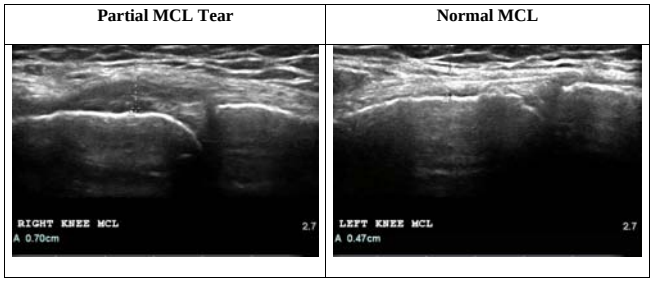

Fig. (6). Ultrasound of partial MCL tear and normal MCL.

There have been studies that do not support the use of PRP to augment ACL healing. Silva reported no differences in graft appearance at three months using type 1 PRP. This study, however, did not use a scaffold [93]. Nin *et al*. applied PRP (Type 4) on allografts used for ACL reconstruction and found no difference in clinical or biomechanical effects at two years [94]. Future studies will help to determine if and how PRP can be used to augment ACL repair.

There are other ligamentous injuries which are being treated with the application of PRP such as the ulnar collateral ligament injuries of the elbow, acromioclavicular joint, sternoclavicular joint, and partial tears of the scapholunate ligament. The use of PRP for sports hernias, generally agreed to be weakness of the posterior inguinal wall with associated changes to the conjoint tendon, insertion of the rectus abominus and adductors is gaining popularity, especially in other countries. Studies are needed to confirm there is any clinical benefit.

Cartilage

The use of PRP to stimulate cartilage repair is a particularly exciting application. Osteoarthritis represents a large burden on quality of life, productivity, and health care costs and its incidence is forecast to rise considerably over the next decade. It is thought that PRP can stimulate chondral anabolism, reduce catabolic processes, and may improve overall joint homeostasis reducing synovial membrane hyperplasia [53]. The work of Mishra *et al*. supports this demonstrating that PRP (Type 1) stimulates mesenchymal stem cell proliferation *in vitro* [20]. Messenger RNA (mRNA) levels of Sox-9 and aggregan were elevated compared to control suggesting chondrogenic differentiation.

Kon *et al.* injected PRP (Type 4) into 115 knees with documented degeneration. Significant improvement of all clinical scores was found at 6-12 months compared to baseline ($p < 0.0005$). They found PRP injections to be safe and had the potential to reduce pain and improve knee function [95]. This data may help support the use of PRP to treat athletes with early degenerative changes. Other reports have documented the use of PRP to enhance the surgical treatment of displaced osteochondral lesions [96]. Unpublished data suggests PRP may be helpful to augment microfracture surgery by improving the fill of the defect. (Mishra, A., data not published).

PRP has also been used to enhance meniscal repair and shoulder labral surgery. Published data, however, is not yet available. Pilot studies, prospective cohorts and randomized trials are needed in all of these applications to understand if PRP can be helpful for these common clinical problems.

Bone

It is beyond the scope of this review to discuss the use of PRP to enhance bone in detail. It should be noted, however, that PRP has the potential to help difficult fractures heal faster. Certain fractures such as a Jones' fracture may be an ideal one to study. In general, should athletes with fractures be treated with PRP as a means to accelerate return to play. Presently, it is an unanswered question.

Rehabilitation

Rehabilitation protocols after PRP procedures have not been well outlined in the literature. Some studies have suggested a stretching and strengthening protocol after injections for chronic tendinopathy [5]. Verchenko and Aspenberg have shown that loading of a tendon after applying PRP improves biomechanical properties in an acute injury model [13]. This has lead most protocols to endorse early controlled loading of a tendon after a PRP procedure. The rehabilitation process should be customized to the athlete, his or her sport and the severity of the pathology. As the literature matures, however, some standardization of tissue loading and return to sports will be require to best understand how to use PRP.

DISCUSSION

Platelets contain many powerful cytokines within their alpha granules such as platelet-derived growth factor, transforming growth factor beta and vascular endothelial growth factor. Eppley *et al.* demonstrated the concentration of these growth factors and others increases linearly with platelet concentration [97]. The dense granules within platelets contain serotonin, histamine, adenosine, and calcium. Upon platelet activation the proteins and cytokines within these granules are released. Everts and his colleagues demonstrated electron microscopic imaging of this activation and release [98, 99]. In isolation, some of these proteins have been shown to enhance proliferation and differentiation of a variety of cell types. Woodall *et al*. has further demonstrated how PRP suppresses macrophage proliferation and IL-1 production *in vitro* in the first 48-72 hours [100, 101]. This potential inhibition of a macrophage derived inflammatory response has important implications for the use of PRP in a variety of orthopedic injuries and disorders.

Tissue injury occurs in predictable phases after injury. It begins with the inflammatory phase where cells flow into an area of damage. This is followed by the proliferation and maturation phases of healing. Remodeling is the final phase of tissue repair. There is mounting evidence that PRP may initiate, enhance, or accelerate healing of connective tissue. Its value has been most clearly defined in chronic, severe

tendinopathy but there is also considerable interest in using PRP for acute muscle injuries [102]. How PRP coordinates a proliferative, inflammatory, or remodeling response is not yet fully known. Understanding the mechanisms by which PRP affects specific tissue types *in vitro* and *in vivo*, however, is paramount. By better determining how PRP works, more precise formulations and applications could be developed. Accomplishing these goals will require detailed *in vitro* assays and vigorous animal investigations. Reporting of platelet and white blood cell concentrations in all studies should also lead to improved understanding of PRP's value. Rehabilitative protocols in humans such as when and how to load a tendon, muscle, or ligament after a PRP treatment need more evaluation. A plausible mechanism of action is outlined in Fig. **7**.

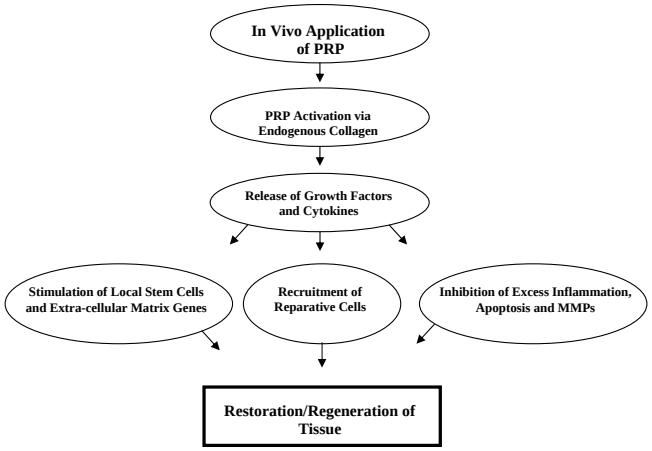

Fig. (7). PRP mechanism of action.

FUTURE DIRECTIONS

The optimal formulation of PRP is not yet known and may differ based on the specific clinical indication. What may work for a chronic tendon lesion may be detrimental in an acute muscle injury. Combining PRP with gels, scaffolds and/or stem cells are exciting applications for exploration.

In sports medicine, athletes and the media will continue to drive the use of platelet rich plasma as a treatment for a plethora of acute and chronic problems. In the long run, prospective, randomized trials will supplant anecdotal results and help clinicians develop specific treatment guidelines. It will be crucial as this evidence evolves to demand reporting of the type of PRP used, the rehabilitation process and objective outcome measurements whenever possible. Basic science and clinical research are the keys to realizing the promise of PRP and has the potential change the way musculoskeletal medicine is practiced.

CONFLICT OF INTEREST

None declared.

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None declared.

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