# EVALUATION OF THE EFFECTS OF PLATELET-RICH PLASMA (PRP) THERAPY INVOLVED IN THE HEALING OF SPORTS-RELATED SOFT TISSUE INJURIES

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## ABSTRACT

Musculoskeletal injuries are the most common cause of severe long-term pain and physical disability, and affect hundreds of millions of people around the world. One of the most popular methods used to biologically enhance healing in the fields of orthopaedic surgery and sports medicine includes the use of autologous blood products, namely, platelet rich plasma (PRP). PRP is an autologous concentration of human platelets to supra-physiologic levels. At baseline levels, platelets function as a natural reservoir for growth factors including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-beta 1 (TGF-β1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF-I). PRP is commonly used in orthopaedic practice to augment healing in sports-related injuries of skeletal muscle, tendons, and ligaments. Despite its pervasive use, the clinical efficacy of PRP therapy and varying mechanisms of action have yet to be established. Basic science research has revealed that PRP exerts is effects through many downstream events secondary to release of growth factors and other bioactive factors from its alpha granules. These effects may vary depending on the location of injury and the concentration of important growth factors involved in various soft tissue healing responses. This review focuses on the effects of PRP and its associated bioactive factors as elucidated in basic science research. Current findings in PRP basic science research, which have shed light on its proposed mechanisms of action, have opened doors for future areas of PRP research.

## INTRODUCTION

According to the World Health Organization (WHO), musculoskeletal injuries are the most common cause of severe long-term pain and physical disability, and affect hundreds of millions of people around the world[1]. The impact such injuries have on everyday life is substantial. Thus, the primary aim of orthopaedic surgeons is a safe and full recovery for their patients with a return to pre-injury level of activity as quickly as possible. The traditional management of orthopaedic and sports related injuries includes everything from conservative "RICE" treatment and physical therapy to corticosteroid injections and surgical intervention. Recently, advances in biomedicine and biotechnology have enthused the use of cell therapy, tissue engineering, and autologous blood concentrates to enhance healing and stimulate growth in bone and soft tissue injuries.

One of the most popular methods used to biologically enhance healing in the fields of orthopaedic surgery and sports medicine includes the use of autologous blood products, particularly, platelet rich plasma (PRP). PRP is an autologous concentration of human platelets to supra-physiologic levels. It is produced from a patient's peripheral vein and centrifuged to achieve a high concentration of platelets within a small volume of plasma. It is then re-injected at a site of injury or inserted as a gel or other biomaterials during surgery.

Historically, platelets have been used to treat patients with thrombocytopenia or hemorrhage[2, 3]. Other blood products such as fibrin (in the form of surgical glue) have been utilized for wound healing[4-6]. In fact, fibrin (in the form of a clot) has been used in a few studies to augment healing in meniscal repairs[7, 8] and also to augment graft healing in ACL reconstruction[9]. Because of its limited use only for surgical procedures and the inability to modify the platelet concentration, fibrin in its various forms is not as extensively used as PRP for sports related injuries.

PRP therapy has grown in popularity over the past few years. Not only has there been an increasing number of

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**TABLE 1. Growth Factors and Cellular Effects.**

| Growth Factor | Cellular Effects |
|---|---|
| **PDGF**<br>*Platelet Derived Growth Factor* | Macrophage activation and angiogenesis<br>Fibroblast chemotaxis and proliferative activity<br>Enhances collagen synthesis<br>Enhances the proliferation of bone cells |
| **IGF-I**<br>*Insulin-like Growth Factor-I* | Chemotactic for myoblast and fibroblasts and stimulates protein synthesis<br>Mediator in growth ad repair of skeletal muscle<br>Enhances bone formation by proliferation and differentiation of osteoblasts |
| **TGF-β**<br>*Transforming Growth Factor-β* | Enhances the proliferative activity of fibroblasts<br>Stimulates biosynthesis of type I collagen and fibronectin<br>Induces deposition of bone matrix<br>Inhibits osteoclast formation and bone resorption<br>Regulation in balance between fibrosis and myocyte regeneration. |
| **PDEGF**<br>*Platelet Derived Endothelial Growth Factor* | Promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts |
| **PDAF**<br>*Platelet Derived Angiogenic Factor* | Induces vascularization by stimulating vascular endothelial cells |
| **EGF**<br>*Endothelial Growth Factor* | Cellular proliferation<br>Differentiation of epithelial cells |
| **VEGF**<br>*Vascular Endothelial Growth Factor* | Angiogenesis<br>Migration and mitosis of endothelial cells<br>Creation of blood vessel lumen<br>Creation of fenestrations<br>Chemotactic for macrophages and granulocytes<br>Vasodilation (indirectly by release of nitrous oxide) |
| **HGF**<br>*Hepatocyte Growth Factor* | Stimulates of hepatocyte proliferation and liver tissue regeneration<br>Angiogenesis<br>Mitogen for endothelial cells<br>Antifibrotic |

basic science and clinical studies, there has also been a rising level of public awareness secondary to the use of PRP to treat high-profile athletes. In fact, one of the most cited articles to date on PRP therapy was published in the New York Times showcasing its use in two professional athletes[10].

Many clinicians feel that PRP therapy is safe given its autologous nature and long-term usage without any reported major complications. For this reason in addition to its easy availability, it is readily used in clinical and surgical settings. Despite its widespread unregulated use, the efficacy of PRP therapy has yet to be established[11-13]. There are many unanswered questions concerning the composition of PRP, individual blood product characteristics, different protocols of production, different methods of administration, and mechanisms of action exerted by PRP and its individual components on a cellular level.

The actual mechanisms of action of PRP are extensive because of the release of a myriad of bioactive factors.

There is a general consensus in PRP research that the injection of concentrated platelets, once activated, results in an exponential increase in numerous growth factors (Table 1) at the sight of injection. However, the function of many growth factors, chemokines, cytokines, and inflammatory mediators has not been elucidated, nor have the interactions between factors and their influence on neighboring cells. As such, the primary purpose of this article is provide an overview of PRP-mediated effects by discussing current research on growth factors and proposed mechanisms by which PRP exerts its downstream effects on healing muscles, tendons, and ligaments. This review will first address the natural healing process for soft tissue injures; followed by proposed mechanisms of action of PRP and associated growth in tendon and ligament injuries, and muscle contusion. Finally, current and future areas of research will be discussed to address common unanswered questions concerning the applicaiton of PRP for sports-related injuries.

## THE NATURAL HEALING PROCESS
## IN SOFT TISSUE INJURIES

Acute sports-related soft tissue injuries result from a single, traumatic event (macro-trauma) such as muscle contusion or ligament sprain/tear. Chronic soft tissue injuries often result from repetitive mechanical stress (micro-trauma) or overuse followed by inflammation and take time to develop such as in the case of tendinopathies (e.g. rotator cuff tendinopathy and Achilles tendinopathy). Regardless of the type of injury, the wound healing process is shared among all soft tissues, with differences in timing, duration of phases, and interactions between key mediators [14-16].

The general healing cascade involves four overlapping phases: (1) hemostasis; (2) inflammation; (3) cellular and matrix proliferation, which begins within days of an injury and comprises the most important phase of healing; and (4) wound remodelling, the longest phase, which may involve scar tissue formation[17, 18].

Immediately following injury, capillary leak allows for the recruitment of hemostatic factors and inflammatory mediators. The coagulation cascade is activated leading to platelet aggregation, clot formation, and development of a provisional extracellular matrix construct[19]. Platelets adhere to exposed collagen and circulating extracellular matrix proteins, which triggers the release of bioactive factors from alpha granules[20]. These bioactive actors include growth factors, chemokines, and cytokines, in addition to pro-inflammatory mediators such as serotonin, bradykinin, prostaglandins, prostacyclins, thromboxane, and histamine[21].

The inflammatory phase follows in a highly orchestrated fashion. Chemoattractant agents begin to summon neutrophils to the injured site within 1-2 hours in the early inflammatory phase. Later (around 48 – 72 hours post-injury), macrophages appear in the wound and play the leading role in wound debridement and regulation of inflammation. They are also involved in recruiting fibroblasts and endothelial cells. The last cells to enter the wound during the late inflammatory phase are lymphocytes.

The cellular and matrix proliferation phase is arguably the most important phase of wound healing; in part, because the cells involved serve as a metabolic engine driving tissue repair[22]. These cells originate from pluripotent progenitor cells in adjacent tissues including infrapatellar fat pad for anterior cruciate ligament, muscle derived stem cells[23] and satellite cells[22] for muscle. After 2-3 days of wound healing, macrophages and chemotactic, mitogenic, and angiogenic growth factors recruit fibroblasts and epithelial cells to infiltrate the site of injury[22]. Once in the wound, fibroblasts synthesize collagen and change to their myofibroblast phenotype to facilitate wound contraction. Angiogenesis and the formation of granulation tissue are also important aspects during the proliferative phase of healing.

The final phase of the healing process involves wound maturation and remodelling. During this phase, growth factors such as PDGF and TGF-β, and fibronectin stimulate fibroblasts proliferation, migration, and synthesis of the components of extracellular matrix[24, 25]. The remodelling phase is tightly regulated to maintain the balance between degradation and synthesis. Type I collagen replaces Type III collagen, proteoglycan, and fibronectin through a process referred to as "creeping substitution" to form a more robust matrix with increased tensile strength[26]. The maturation phase varies in duration depending on the extent of the wound pathology, individual characteristics, as well as specific tissue healing capabilities of the tissue involved[27, 28]. Additionally, pathophysiological and metabolic factors can affect wound healing. They include local causes such as ischemia, tissue hypoxia, infection, and growth factor imbalance, as well as systemic causes such as metabolic disease and nutritional status. In such unfavorable environments, PRP has been shown to be a viable therapeutic adjunct for soft tissue injuries[29].

## PRP: DEFINITION, PROPERTIES,
## PREPARATION AND COMPOSITION

Platelet-rich plasma (PRP) is an autologous concentration of human platelet to supra-physiologic levels. Platelets are irregularly shaped, non-nucleated cytoplasmic bodies derived from fragmentation of megakaryocyte precursors. They circulate in the blood of mammals expressing glycoproteins on their cell membranes and play a pivotal role in hemostatis and wound healing via the formation of fibrin clots[30, 31]. At baseline levels, platelets function as a natural reservoir for growth factors including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-beta 1 (TGF-β1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF-I), to name a few[32-36]. Such growth factors are released from the alpha granules of activated platelets and are involved in important cellular processes including mitogenesis, chemotaxis, differentiation, and metabolism[37]. Hence, the rationale for increasing platelet concentration in compromised (or injured) tissue lies in the belief that additional platelets will result in the exponential release of multiple bioactive factors, and subsequently, enhance the natural healing process.

Many clinical studies have demonstrated the beneficial effects of PRP therapy in oral-maxillofacial, plastics, and orthopaedic surgery. In fact, autologous blood

TABLE 2. Commercially Available PRP Systems. Vol, volume; CaCl$_2$, Calcium chloride; NS, not specified.

| Commercial System | Blood Vol (ml) | Centrifugation (No. of Spins) | PRP Vol (ml) | Platelet Concentration | Activator (+ / −) | Leukocytes (+ / −) |
|---|---|---|---|---|---|---|
| *Cascade®* | 9-18 | 1 | 4-9 | 1-1.5x | CaCl$_2$ | - |
| *GPS III* | 60 | 1 | 10 | 9.3x | Trombin | + |
| *ACP®* | 9 | 1 | 3 | 2-3x | None | - |
| *Smart PRP2®* | 20-120 | 2 | 3-20 | 4-6x | Trombin | + |
| *PRGF®* | 9-72 | 1 | 4-32 | 2-3x | CaCl$_2$ | - |
| *Magellan®* | 30-60 | 2 | 6 | 3-7x | CaCl$_2$ | + |
| *Angel®* | 40 | 2 | 4 | 1-18x | None | +/- |
| *Genesis CS®* | 30-60 | 1 | 4-10 | 9x | CaCl$_2$ | NS |
| *Sequire®* | 50 | 2 | 5 | 1.6x | Trombin Bovine | NS |
| *Platelex®* | 50 | 2 | 4-6 | NS | Batroxobin | + |
| *Symphony II PCS®* | 55-110 | 1 | NS | 3-6x | Thrombin Bovine + CaCl$_2$ | NS |

products including platelets were first popularized in the 1990s in oral and plastic surgery[38-42]. Despite the increasing evidence of PRP as an augmenter of natural healing, its clinical efficacy in the management of sports-related injuries is still a matter of debate[11-13].

Platelet-rich plasma is produced from blood obtained by phlebotomy, which is centrifuged to achieve a high concentration of platelets within a small volume of plasma. The platelet-rich product is then re-injected at a site of injury or prepared as a gel or other biomaterial and inserted during surgery. There are numerous protocols and commercial systems for producing PRP. Traditionally, two centrifugation steps are used to isolate the erythrocyte fraction from the buffy coat (plasma containing platelets, leukocytes, and clotting factors). The second step separates the platelet-poor plasma (PPP) from the platelet-rich fraction. Single-step systems are also available. Centrifugation itself is likely the most critical step during the PRP preparation process, as centrifugal forces in spins greater than 800x gravity (g) may result in significant loss of granular loads of platelets, which would subsequently dilute growth factor concentrations[43].

A major limitation in evaluating the clinical effects of PRP is variation in established preparation protocols. Many different commercial systems are available that utilize different centrifugation machines and protocols (Table 2). Platelet and growth factor concentrations can vary depending on the system used[44]. Furthermore, the presence or absence of leukocytes, which produce VEGF[45] and possess antimicrobial properties[46], and activating factors such as calcium chloride or thrombin further influence the quality of PRP and resulting effects.

Additional variation in PRP products results from patient differences in age, medical comorbidities (particularly hematologic disorders), and healing capabilities. In essence, the effects of PRP are influenced by substantial differences in the content of platelet concentrates as well as individual characteristics, which likely contributes to its variable findings in the literature.

## ORTHOPAEDIC APPLICATIONS OF PRP

PRP is commonly used in orthopaedic practice for sports-related injuries of skeletal muscle, tendons, and ligaments. It is thought to exert its effects through many downstream events secondary to release of growth factors and other bioactive factors from its alpha granules. These effects may vary depending on the location of injury and the concentration of important growth factors involved in various soft tissue healing responses.

**Skeletal Muscle**

Skeletal muscle injuries are common causes of severe long-term pain and physical disability, accounting for up to 55% of all sports injuries[47]. Contusions and strains are the most frequent muscular lesions, representing more than 90% of all sports related injuries[48]. Muscle injury represents a challenging problem in traumatology, as injured muscles heal very slowly and often with incomplete functional recovery[23]. Furthermore, barriers to complete muscle recovery following injury are scarring and fibrosis suggesting that scar formation may be at the expense of muscle regeneration[49-52]. For this reason, regulation of fibrosis is one of the goals of PRP therapy in the management of muscle lesions.

One study evaluating the healing process in skeletal

muscle injury and the impact of inflammation on injured mice gastrocnemius cells found that prostaglandin E2 (PGE$_2$) can regulate the level of fibrosis by decreasing the expression of TGFβ1[53]. TGFβ1 is an inducer of extracellular matrix (ECM) protein synthesis and fibroblast proliferation[54, 55]. As a particularly strong inducer[56], TGFβ1 has been implicated in the fibrogenesis of various tissues including kidney, lung, and skin[57]. It also appears to play a role in muscle injury as an inflammatory modulator[53].

Abolishing the fibrotic effects of TGFβ1 appears to allow for full recovery of skeletal muscle function. In a currently unpublished study evaluating the effects of PRP on skeletal muscle healing, PPR injections in combination with Losartan, an angiotensin II receptor blocker (ARB), were found to decrease fibrogenesis and increase angiogenesis in a rat muscle-contusion model[58]. When exposed to angiotensin II, fibroblasts increase their production of TGFβ1[59]. As such, the inhibition of angiotensin II with an ARB resulted in decreased formation of fibrosis following traumatic muscle injury[59]. In combination with PRP, Losartan further inhibited fibrosis formation while also increasing angiogenesis and myofiber regeneration in a murine model[58]. Additionally, combined PRP/Losartan injections resulted in improved muscle strength, as measured in peak twitch and tetanic forces. These findings suggest that combination treatment with PRP/Losartan can improve muscle healing by exerting anti-fibrosis, pro-angiogenesis, and pro-myogenesis effects through inhibition of TGFβ1.

In a controlled laboratory study, either PRP or PPP was injected into contraction-induced strained muscles in rats at 0, 3, 5 and 7 days post-injury[60]. PPP, commonly used as a research control, is produced by high-speed centrifugation separating plasma from platelets and erythrocytes. Compared to the PPP control, the PRP treated group showed significant improvement at multiple time points in the small strain model[60]. Such improvement was attributed to the effects of PRP on muscle regeneration. In a similar study, autologous conditioned serum (ACS, Arthrex, Florida, USA), a PRP equivalent containing high concentrations of TGFβ-1 and FGF-2, was injected into gastrocnemius muscles in mice at 2, 24, and 48 hours post-injury[61]. Compared to the saline injection control, histological examination of the ACS-treated mice muscles resulted in an 84% increase in satellite cell activation at 30 and 48 hours post-injury[62]. Additionally, there was a significant increase in centrally nucleated myofibers and in the proportion of large-diameter fibers, suggesting myofiber regeneration.

The aforementioned studies have shed light on the importance of specific growth factors involved in myogenesis including IGF-1, FGF-2, HGF, and TGFβ-1[63-65].

The premise for the improvement in muscle regeneration and satellite cell activation following PRP treatment appears to lie in the tight regulation of platelet-released growth factors.

**Tendon**

Tendon injuries are very common disabling conditions in athletes. Tendon injuries include acute or chronic degeneration as well as partial or complete tendon ruptures[66]. Full recovery requires a long, complex healing process, particularly in the case of tendon rupture and or retraction. Even so, healed tendons are comprised of scar tissue that is mechanically inferior to normal tendon tissue. This, in turn, impairs normal tendon function and joint kinematics, predisposing the patient to further tendon injury. Although biopsies in a case of chronic tendinopathy show an absence of inflammatory cell infiltration, conservative treatments include anti-inflammatory agents (non-steroidal anti-inflammatory drugs and corticosteroids) and local anesthetic injections[67]. The efficacy of these treatment modalities remains in question[67-71]. Thus, recent orthopaedic basic science and clinical research has focused on PRP as an alternative modality in the management of tendon injuries[11, 32, 72, 73].

Several laboratory studies have shown the beneficial effects or PRP on the tendon healing process. Previous in vitro studies using cultured human tenocytes have shown that PRP (in a clot releasates form; PRCR) stimulates cell proliferation and total collagen production compared to those cultures treated with PPP clot releasates[74, 75]. An additional study evaluating PRP effects on tendon healing found that PRCR also promoted differentiation of human tendon stem cells into active tenocytes with high proliferation rates and collagen producing capabilities[32]. Collagen gene expression levels were also increased in response to PRP in equine flexor digitorum superficialis tendons including enhanced type I collagen, type III collagen, and oligomeric matrix protein expression without concomitant increases in catabolic metalloproteinase expression[76]. In addition to stimulating tendon stem cell (TSC) differentiation, tenocyte proliferation, and increasing collagen expression, PRP was also found to induce VEGF and HFG production in human tendon cells.

VEGF contributes to angiogenesis or new blood vessel formation [77]. In an animal study evaluating the safety of PRP injections, autologous PRP was injected into normal patellar tendons of New Zealand White rabbits[78]. Histological analysis at 6 and 12 weeks post-injection revealed a robust angiogenic response. In another study, locally injected PRP into a wounded rat patellar tendon resulted in an increased number of circulation-derived

cells involved in the tendon healing[79]. Circulation-derived cells (inflammatory cells responsible for secreting growth factors and cytokines) and fibroblast-like cells that synthesize extracellular matrix were significantly increased in response to PRP treatment at 3 and 7 days post-injection[79]. Though chemotaxis was not directly observed in this study, the authors hypothesized that chemotaxis of circulation-derived cells was likely induced by PRP.

Though angiogenesis plays an important role for the initial phase of wound healing by facilitating cell and inflammatory mediator chemotaxis, it may also be detrimental to ligament and tendon healing via the upregulation of matrix metaloproteinases (MMPs) [80, 81]. Angiogenesis has been shown to alter the material properties of the extracellular matrix [82]. The injection of VEGF into rat Achilles tendons has been shown to reduce biomechanical strength assessed by tendon stiffness and ultimate load[83]. In anterior cruciate ligament (ACL) reconstruction, angiogenesis contributes to remodeling of the autologous tendon graft, but has also been shown to induce proteolysis of the extracellular matrix, hindering the mechanical stability of the maturing graft[84]. The above controversial findings suggest that tendon healing may either be impaired *or* facilitated by angiogenesis, which highlights the importance of tight VEGF regulation.

HGF, one of the major growth factors released from activated platelets, is primarily involved in hepatocyte regeneration and proliferation[85, 86] and fibroblasts proliferation (Hannafin, Attia, Warren, & Bhargava, 1999). Additionally, HGF has also been shown to exert anti-inflammatory effects in human abdominal aortic aneurysm (AAA) tissue via modulating the cytokine profile[87]. HGF-mediated regulation of pro- and anti-inflammatory cytokines has also been reported in animal models of acute kidney injury[88], endotoxemia[89], cardiac allograft transplantation[90], and myocarditis[91].

Based on the findings of the aforementioned studies, PRP-mediated effects on tendon healing could be secondary to improved vascularity (with careful consideration of the potential degradative properties of angiogenesis) and/or the anti-inflammatory effects of growth factors known to increase with exogenous administration of platelets. For instance, PRP has been shown to suppress cyclooxygenase (COX)-1, COX-2, and membrane prostaglandin E synthase (mPGES) expression *in vitro*[92]. All three enzymes are involved in the inflammatory pathway. *In vivo* injection of PRP into injured mouse tendons resulted in decreased PGE$_2$ production at multiple time points post-treatments[92]. Reduced COX-1, COX-2 and mPGES expression in conjunction with decreased production of PGE$_2$ suggest that PRP exerts anti-inflammatory effects on healing tendons and may contribute to pain reduction following injection[93-97]. Repeated exposure to PGE$_2$ has previously been shown to disrupt collagen fiber uniformity and normal tissue architecture in rabbit tendons [98]. In this case, it follows that hindering production of PGE$_2$ would be beneficial in tendon healing. However, other studies have found that PGE$_2$ inhibition has actually resulted in decreased collagen synthesis and delays in tendon healing[99-102]. These contradictory findings, again suggest the importance of regulation. PGE$_2$ production must be tightly controlled to achieve a favorable balance bewteen pro- and anti-inflammatory effects during tendon healing.

## Ligaments

Besides tendon injuries, acute ligament sprains rank among the most common orthopaedic injuries. Most ligamentous injuries can be treated conservatively; e.g. isolated medial collateral ligament (MCL) ruptures[103]. Other injuries, such as the anterior cruciate ligament (ACL) need surgical reconstruction given its relatively low healing potential[104-106]. Recently, autologous blood products including PRP and growth factors (isolated or in combination) have been used to treat ligamentous injuries with the goal of accelerated healing and an earlier return to activity[107]. One characteristic of the MCL that substantiates its healing capacity is increased vascularity[103, 108, 109] compared to low-healing capacity ligaments such as the ACL. Hence, the rationale behind the use of autologous blood products is to increase blood flow and the delivery of inflammatory mediators. As previously mentioned, VEGF plays an important role in healing as a potent growth factor of angiogenesis[77, 110-112].

In a laboratory study evaluating the role of VEGF on MCL healing, murine muscle derived stems cells (MDSCs), which have been shown to promote healing via neoangiogenesis[113-115], were retrovirally transduced to express (1) VEGF (MDSC-*VEGF*); (2) a soluble fms-like tyrosine kinase-1 (*sFLT1*; a VEGF-specific antagonist) gene (MDSC-sFLT1); and (3) *nLacZ* promoter gene to track the fate of the donor cells after transplantation (MDSC). The control group was injected with phosphate-buffer saline (PBS) only. Rat VEGF (rVEGF) expression was significantly higher in the MCL cells transduced with MDSC-*VEGF*. In MCL cells transduced *MDSC-sFLT1*, rVEGF was strongly suppressed[116]. Additionally, biomechanical strength of the injured MCLs was significantly higher in the MDSC-*VEGF* group compared to both the MDSC-*sFLT1* group and the PBS control group. There was no significant difference in failure load between the MDSC-*VEGF*, MDSC, and normal MCL groups. The authors conclude that though supplemental VEGF does not further improve healing, inhibiting VEGF

**TABLE 3. Opportunities for future research.**

| Unanswered Questions in PRP Research | Opportunities for Future Research |
| --- | --- |
| Recommendations for administration of PRP? | Assessment of timing, dosing, and frequency of doses. |
| Recommendations for use of an activator? Use of a cell scaffold? Addition of leukocytes? Addition of stem cells? | Evaluating histological, biomechanical, and clinical differences in outcomes for various situations: +/- platelet activators, leukocytes, scaffold, or stem cells. |
| What is the optimal concentration of platelets? Does this optimal concentration vary depending on the injury site? | Evaluation of required platelet concentrations by injury site and wound characteristics. |
| Where should PRP be administered? What technique for administrating yields the greatest results? | Evaluating histological, biomechanical, and clinical differences in outcomes based on site of administration. For instance, application over the bone, over the tendon; injection within the tendon, within the ligament, or intra-articular. |
| Are all PRPs created equally? | Evaluation of the influence of patient variation on autologous blood product composition and quality. |
| At what phase of the healing cascade does PRP exert the greatest effect? | Evaluation of histological, biomechanical, and clinical differences in outcomes based timing of injection from original injury. |
| How do different centrifugation speeds affect platelet behavior? Growth factor concentrations? | Assessment of platelet behavior under various conditions and at different stages of the healing cascade. |
| Do platelets and released growth factors stay localized at an injury site? | Assess localization of PRP using immunostaining techniques, isotopic labeling, and imaging modalities. |
| Side effects of PRP? | Evaluation of the potential adverse effects of PRP therapy |

interferes with the regeneration process by delaying ligament repair[116].

PRP has been shown to instigate the aggregation of inflammatory cells via release of chemotactic, angiogenic, and mitogenic growth factors released from activated platelets[22]. In the case of an MCL injury, PRP is thought to function during the early phases of healing, thus assisting in the recruitment of pro-inflammatory cells such as macrophages. During the proliferative and remodeling phases of healing, macrophages accumulate in an injured site and express either a pro-inflammatory or anti-inflammatory phenotype. M1 macrophages are involved in phagocytizing cellular debris, recruiting additional reparative cells (e.g. myofibroblasts), and assisting in pro-inflammatory cytokine release. M2 macrophages promote angiogenesis, matrix remodeling, and ultimately assist in scar tissue formation by releasing anti-inflammatory cytokines[26, 117]. Both phenotypes play important roles in ligament healing, and in conjunction with platelet-released growth factors, macrophages help regulate angiogenesis, fibroblast differentiation, and collagen production. In MCLs, non-specific inhibition of macrophages using clondronate (a bispohosphonate) can help subdue excessive granulation tissue formation, but impairs early matrix formation and ligament strength[26]. In ACL reconstruction; however, inhibition of macrophages at the bone-tendon junction resulted in improved healing[118]. The reasons behind the opposing findings are multifactorial with innate differences in healing capacity likely playing a role.

With increasing attention being paid to ACL graft maturation, PRP has been used to augment healing in the case of animal ACL repair and reconstruction. In a porcine model of suture repair after ACL transection, ligaments treated with a collagen-PRP hydrogel showed significant improvement in biomechanical properties including load and linear stiffness at 4 weeks post-repair compared with untreated repairs[119]. Furthermore, a collagen-PRP hydrogel was shown to improve ACL wound site filling in a canine ACL injury model[120]. Both fibrinogen and fibronectin were restored in the wound site treated with collagen-PRP hydrogel. Additionally, procollagen I (a marker for collagen formation) and vWF (a marker for revascularization) were restored in the wound site. These findings support PRPs role in fibroblasts chemotaxis and adherence to extracellular matrix structures[121] and also demonstrate that the collagen-PRP scaffold stimulates ingrowth of fibroblast and endothelial cell invasion, mimicking the natural function of the fibrin clot[9, 120].

The effects of PRP on clinical outcomes of ACL reconstruction are also being evaluated. One study evaluated the ability of collagen-platelet composite (CPC), or blood platelets added to a collagen scaffold, to restore

knee function following ACL reconstruction in a caprine model[122]. The authors assessed the effect of platelet composition on post-operative knee laxity and graft structural properties. They found that anteroposterior (AP) laxity significantly decreased in the CPC group compared to the control, but no significant differences in failure load or linear stiffness were observed between groups. When evaluating both groups combined, a higher systemic platelet count positively correlated with improvements in anteroposterior (AP) knee laxity at 30° and 60° of knee flexion as well as linear stiffness and graft strength[122]. This study finding had the greatest implications with respects to the use of PRP in ACL reconstruction. As previously mentioned, administration of PRP results in the localization of platelet-released growth factors including IGF-1, TGFβ-1, VEGF, PDGF, and FGF-2[123]. Each of these growth factors –and likely many more – play a role in tissue healing. Pertaining to ACL reconstruction, TGFβ-1 (pro-inflammatory) has been shown to stimulate ACL cell migration in vitro[123], and PDGF-AB and FGF-2 can stimulate ACL proliferation in a 3D collagen scaffold[124].

## FUTURE DIRECTIONS OF RESEARCH

Major strides have been made in PRP research with findings influencing the management of orthopaedic injuries. Despite the multitude of clinical and basic science studies published within the last year, many important questions remain unanswered including those concerning the dosing, timing, and frequency of PRP injections; different techniques for delivery and location of delivery (over the injured tissue, within the injured tissue, or intra-articularly); optimal physiologic conditions for injections; and the concomitant use of recombinant proteins, cytokines, additional growth factors, biological scaffolds, and stems cells (Table 3).

In a recent systematic review evaluating clinical outcomes of PRP use in orthopaedic injuries, the authors concluded that one of the most challenging barriers to critical evaluation is lack of standardization in preparation and dosage of autologous blood concentrates[125]. Specific alterations in growth factor concentration, supplementation of platelet activators, leukocytes, and or other cellular components decrease the generalizability of clinical and basic science findings, rendering any results difficult to interpret.

Individual patient characteristics also contribute to the observed variation in PRP content and quality as well as the cellular responses to autologous blood products. Age or skeletal maturity has been shown to influences ACL cell metabolic activity, apoptotic rate, collagen production, and response to PRP in a porcine model[126]. The diversity of the human population can affect physiologic responses to PRP. Thus, future research studies should account for individual differences in age, gender, healing capabilities and whole blood characteristics.

Another important topic for consideration in PRP research is defining the optimal dose or concentration of platelets and growth factors in PRP. Although a correlation was found between increased platelet count and increased ACL graft strength[122] – "more" platelets and growth factors may not necessarily be more effective. In a study using a porcine model to assess optimal platelet concentration in PRP, increased concentration did not seem to have an effect on functional outcomes of primary ACL repair[127]. PRP containing 5x the baseline systemic platelet count and PRP containing 3x the baseline systemic platelet count did not compromise ligament stiffness and resulted in similar biomechanical outcomes despite histological differences[127]. The 5x group ACLs had increased cellularity, more organized collagen bundles, and more elongated fibroblasts. The finding of similar biomechanical outcomes despite differences in histology raises another important question in PRP research: is there a relationship between cellular findings following PRP treatment and actual clinical implications? Previous animal studies have demonstrated that increased cellularity during early wound healing may have a positive impact on ligament [128] and tendon [129]; however, others have found no such relationship[130, 131]. From a basic science standpoint, elucidation of specific cellular responses to PRP involves the evaluation of the inflammatory cells involved, their interactions, and behavior in an altered cellular environment. This response will likely vary depending on the injury site and thus, provides another avenue for continued PRP research.

To our knowledge, there are no studies reporting the negative effects of PRP. The assumption that autologous products are intrinsically safe should be critically evaluated. When cells are in their natural environment, they behave "naturally" meaning many of their activities, interactions, and released bioactive factors can be predicted. However, when cells and cell fragments are exposed to unnatural environments, for instance, a high-speed centrifugation process, the resulting products and exerted effects may be less predictable. Furthermore, there is no guarantee that platelets (in the form of PRP) will remain localized at the site where they are injected. Dissemination may result in unexpected results in surrounding tissues or even systemically. As such, the safety of autologous blood products – especially in the presence of cell, growth factor, and platelet activator adjuncts – proves yet another aspect of PRP therapy warranting research.

## CONCLUSION

There is not one overarching mechanism of action of PRP given the differences in platelet content, resulting growth factor concentrations, and varying downstream cellular responses. What is known is that PRP contains a high concentration of platelets and that these platelets, once activated, release numerous growth factors into the surrounding environment. The resulting cellular effects are both pro-inflammatory and anti-inflammatory in nature and appear to be dependent on many different factors including the stage of the natural healing process, the site of the injury, and cellular environment. The interplay between individual growth factors and their resulting effects are currently being evaluated through well-designed basic science studies. Despite the volumes of basic science research supporting the use of PRP for sports related injuries, clinical evidence for PRP therapy has not been well established due to many of the unanswered questions addressed above. Continued basic science research elucidating the downstream effects of PRP can help drive clinical research. Strong scientific and clinical findings can then be used in conjunction to help develop clinical recommendations for the use of PRP in the management of sports related injuries.

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