Platelets

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Platelet Rich Plasma in Orthopedic Surgical Medicine.

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Abstract

There is a global interest in optimizing post-surgical tissue repair strategies, leading to better patient outcomes and fewer complications, most ideally with reduced overall cost. In this regard, in recent years, the interest in autologous biological treatments in orthopedic surgery and sports medicine has increased greatly, and the addition of platelet-rich plasma (PRP) to the surgical armamentarium is of particular note. Unfortunately, the number of PRP preparation devices has also grown immensely over the recent decades, raising meaningful concern for the considerable variation in the qualities of currently available PRP preparations. The lack of consensus on the standardization of PRP preparation and of agreement on condition specific PRP formulations is largely responsible for the sometimes contradictory outcomes in the literature. Furthermore, the full potential of PRP technology, the concept of individualized treatment protocols based on bioformulation options, and platelet dosing, angiogenesis, and antimicrobial and painkilling effects of PRP relevant to orthopedic surgery have rarely been addressed. In this review, we will discuss recent developments regarding PRP preparations and potential therapeutic effects. Additionally, we present a synopsis of several published data regarding PRP applications in orthopedic surgery for treating tendon injuries, inducing bone repair, strengthening spinal fusion outcomes, and supporting major joint replacements.

Keywords

Angiogenesis, antimicrobial, bioformulation, bone repair, leukocyte poor, leukocyte rich, O, orthopedic surgery, painkilling, platelet dose, platelet-rich plasma, spinal bone-fusion, tendon reconstruction

History

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Introduction

Autologous platelet-rich plasma (PRP) is the processed liquid fraction of autologous peripheral blood with a platelet concentration above baseline values [1].

PRP has been in clinical use for more than 35 years for a multitude of indications in a variety of medical specialties. Initially, PRP was activated with platelet agonists (e.g., calcium, thrombin, or other proteins) to create a viscous solution, which was frequently termed platelet gel (PG). This platelet coagulum was applied as a spray or as a solid gelatinous mass to soft tissues and chronic wounds and mixed with bone or synthetic bone to mimic and accelerate physiologic wound healing and regenerative tissue repair processes [2].

The first report on PRP application in a patient (an American football player) with a ruptured Achilles tendon was presented in 1999, and the first controlled study of PRP in sports medicine was published by Mishra and Pavelko in 2006 [3]. Shortly thereafter, well-known professional athletes were successfully treated with PRP. These reports triggered PRP therapies in sports medicine and orthopedics. Firstly, several commercial orthopedic companies used these positive statements to develop PRP preparation devices. The use of PRP gained popularity as a viable autologous biological treatment option, delivered either as a PRP injectate or as a PG graft.

Sheth et al. concluded in a 2012 meta-analysis that there was insufficient evidence to support orthopedic PRP applications [4]. In fact, this meta-analysis typified the weaknesses in the PRP literature. A lack of essential reporting was noted, including the composition of the PRP, the use of a platelet activator, and the number and timing of PRP treatments. This was later confirmed in another systematic review of the clinical orthopedic literature [5]. The authors reported that clinical PRP preparation protocols are highly inconsistent, and the majority of studies did not provide sufficient information to allow reproducibility of PRP preparation protocols and administration methods.

Recent advances in understanding PRP bioformulations have contributed to an increased awareness of PRP applications [6]. As a result, new studies have been initiated in pursuit of more effective orthobiological applications, such as PRP. In this review, we will discuss an adjunctive role of autologous bio cellular PRP formulations with regard to platelet dose, angiogenesis, and antimicrobial and analgesic effects, potentially contributing to significantly improved healing potential for patients undergoing orthopedic surgical procedures.

The Rationale for Platelet-Rich Plasma Applications

Autologous PRP procedures have gained greater acceptance as a contemporary treatment modality, with wide application in distinct medical fields, even though these biological therapies are marked by substantial heterogenicity of formulations, inconsistencies in nomenclature and specimen quality, and poor standardization of evidence-based preparation guidelines. The underlying rationale for PRP therapy is that concentrated platelets at (surgical) injury sites can initiate tissue repair via the release of many biologically active factors, including growth factors, cytokines, and lysosomes and adhesion proteins. An impressive

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number of platelet (and therefore PRP) constituents have direct or indirect effects on the local tissue environment, initiating the hemostatic cascade, synthesis of new connective tissue, and revascularization, to jump-start healing in chronic injuries and accelerate the repair process in acute injury [7]. A wide variety of growth factors, cytokines, and locally acting regulators contribute to most basic cell functions at all stages of the tissue repair process, via endocrine, paracrine, autocrine, and intracrine mechanisms. The main advantages of PRP include its safety and the innovative capabilities of current commercial devices in the preparation of an autologous biologic that can be used in a broad application profile [8]. Most importantly, PRP is an autologous product with no known adverse effects, in distinction to the non-autologous orthobiologics such as corticosteroids, placenta, and placenta derivates [9].

Preparations and Classification of Platelet-Rich Plasma

Fadadu et al. reviewed 33 PRP systems and protocols [10]. One of their findings was that some of these systems produced PRP preparations with platelet concentrations below that of whole blood, while dual-spin closed systems produced PRP vials with platelet counts exceeding 1.6 × 106/μL. Currently, a clinical PRP formulation is best described by its absolute platelet concentration, which is a shift from the initial definition of PRP based on a platelet concentration above the baseline value to a minimum platelet concentration of >1 × 106/μL, or an approximately five-fold increase in platelets from baseline [11]. Many modern PRP preparation systems are capable of producing high platelet concentrates, different formulations of PRP with regards to leukocyte, and erythrocyte content and platelet growth factor (PGF) concentrations [12]. However, these specific PRP formulations can only be achieved by using dual-spin preparation methods, as shown in Figure 1.

Rossi et al. have recently noted that PRP for orthobiological applications can be categorized into three groups: leukocyte-rich (LR)-PRP, leukocyte-poor (LP)-PRP, and pure platelet-rich fibrin (*P*-PRF) [13]. Although more specific than the generic PRP product definition, the LR-PRP and LP-PRP categories are notably lacking any specificity regarding the leukocyte content. LR-PRP products containing specific leukocytes can make significant contributions to immune modulation and tissue repair and regeneration, affecting the intrinsic biology of tissues and chronic lesions via their immune and host-defense mechanisms. Specifically, monocytes and macrophages play key roles in immunomodulatory processes and tissue repair mechanisms, while neutrophils are referred to as inflammatory cells in PRP products.

Platelet Dosing

The therapeutic actions of PRP are related to the many factors that are released after platelet activation and these platelet specific elements are involved in tissue repair and regeneration. Platelets can be instantly activated by several agents, including a calcium chloride-thrombin mixture, collagen, fibronectin, thromboxane A2, epinephrine, and adenosine diphosphate, and by contact with materials used for PRP delivery, or negatively charged surfaces such as glass. Activated platelets will form a platelet plug that acts as a temporary extracellular matrix, allowing cells to proliferate and differentiate [1].

It is reasonable to suppose that PRP preparations with higher platelet dosages will promote higher release of bioactive factors and have an effect on outcomes. Several studies have indicated that cells respond to PRP in a dose-dependent manner. In this regard, Mautner et al. were the first to include the absolute PRP platelet count in a detailed PRP classification system [14]. As can be expected, conflicting results regarding platelet dosing have been published from both clinical studies and in-vitro cell culture studies using specific cells and tissue types [15,16] . Marx was the first to show that the enhancement of bone and soft tissue healing required a minimum platelet count of $1x10^6$/μL, and similar results were confirmed in a study on PRP in transforaminal lumbar fusion study, demonstrating a significantly higher fusion rate when the platelet dose exceeded 1.3 x 10^6/μL [17]. Recently, Park and associates reported that a more than 5-fold increase in PRP platelet levels above baseline was required to induce a positive outcome after spinal fusion [18], and in an in-vitro study, a dose of 1.5 × 10^6 platelets/μL was needed for tissue repair mechanisms to induce a functional angiogenic response through endothelial cell activity [19].

Noteworthy, apart from any dose-dependency, the therapeutic effects of PRP on cell activity appear to be highly time-dependent. In an in-vitro study, short-term exposure to human platelet lysates stimulated bone cell proliferation and chemotaxis, while exposure of more than 48 hours resulted in a decrease in mineral formation and alkaline phosphate activity [20].

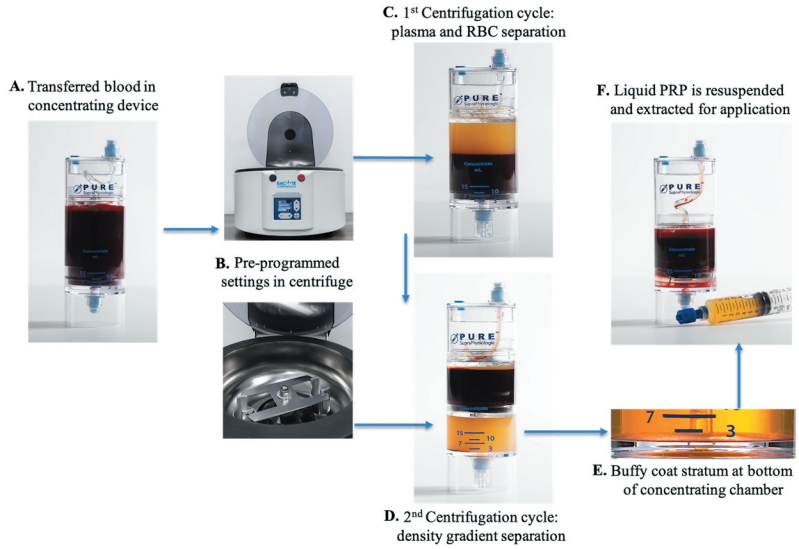

Figure 1. A flow diagram depicting a dual spin PRP preparation method.

54 ml of anticoagulated peripheral whole blood is transferred in the dual spin concentrating device (PurePRP-SP® device, EmCyte Corporation, Fort Myers FL, USA) (A). The device is placed in a dedicated centrifuge for cellular density separation (B). After the first centrifugation procedure for 1,5 minutes at 3800 RPM (2,3 RCF) (C), the whole blood is separated in 2 basic layers, the platelet (poor) plasma suspension and the RBC layer. In between a minimal buffy coat cell layer. In D, the second centrifugation step has been completed for 6 minutes, following a preparation protocol that minimizes the neutrophil content by approximately 90%. In E, in this typical example, the concentrated cell stratum is settled at the bottom of the concentrating chamber, containing high concentrations of platelets, monocytes, lymphocytes, following density gradient centrifugation. Finally, a needed amount of PRP volume is extracted after resuspending it with the remaining plasma fraction, F.

At this writing, no PRP platelet dose response curves have been established for treatment of orthopedic (or any other) conditions. However, tissue culture and several clinical studies with focus on bone growth, have shown a platelet dose-dependent proliferation of cells treated with PRP with platelet counts of at least $1x10^6/\mu L$. On-going and future clinical studies should confirm these findings, despite differences in preparation protocols and the ultimate PRP bioformulations. Important considerations are the RBC contamination and WBC count and types, because inflammatory neutrophils reduce a platelet dose induced cellular proliferation [21].

Platelet-Rich Plasma and Angiogenesis

Angiogenesis is a vivacious process involving the sprouting and organization of micro vessels from preexisting blood vessels, mediated by endothelial cell migration, proliferation, differentiation, and cell division. A pre-requisite for tissue healing after surgery is the restoration of blood flow in order to support the high metabolic activity of the tissue repair processes. The outgrowth of preexisting blood vessels is essential for the delivery of oxygen and nutrients and the removal of by products from the treated tissues [22]. The PRP preparations that are used in orthopedic, and other applications, allow for the delivery of biomolecules released by high concentrations of platelets that are activated at specific tissue sites. Consequently, various cascades are initiated that contribute to on-site angiogenetic and inflammatory processes to promote healing and tissue repair [23]. Explicitly, angiogenic activities are modulated by stimulatory pro-angiogenic platelet-derived vascular endothelial growth factor (VEGF) and by anti-angiogenic factors. Together with VEGF, several other platelet-soluble mediators in PRP, including basic-FGF and TGF-β, stimulate endothelial cells to produce new blood vessels [22]. Landsdown and Fortier reported on the various outcome effects related to PRP constituents, including intra-platelet sources of numerous angiogenic modulators, and concluded that an increase in angiogenesis contributes to healing in areas of poor vascularization [24]. The application of PRP with a high dose of platelets at high concentrations will provide high levels of platelet-derived growth factors, exerting controlled pro-angiogenic and stimulatory effects to induce angiogenesis, vasculogenesis, and arteriogenesis [25]. Interestingly, Richardson et al. have demonstrated that the synergistic activity of proangiogenic VEGF and platelet-derived growth factor-bb (PDGF-BB) results in more rapid formation of a mature vascular network than the individual activities the growth factors [26]. Therefore, adequate platelet dosing with PRP is critical for the induction of angiogenesis, which will then contribute to the therapeutic efficacy of a number of applications, including tissue restoration and wound healing.

Antimicrobial Effects of Platelet Rich Plasma Preparations

Clinical PRP preparations have been widely studied for their effects on tissue repair and regeneration mechanisms and on postsurgical outcomes, exercising beneficial effects by relieving postsurgical discomfort and reducing infection rates, suggesting that PRP possesses anti-inflammatory and antimicrobial properties [27]. However, the anti-microbial effects of PRP are rarely discussed, even though growing evidence suggests that PRP has important anti-microbial properties that may help to prevent or even to treat challenging bone infections [28]. The anti-microbial properties of a PRP product depends on its bioformulation.

Leukocyte Poor PRP

In LP-PRP, platelets are the only cells with anti-bactericidal activities. In the event of a post-surgical infection, platelets are among the first cells to detect endothelial injury and microbial pathogens and endothelial injury as they gain access or invade the bloodstream or tissues. Platelets aggregate and promote the release of the platelet agonists ADP, thrombin, and von Willebrand Factor, leading to platelet activation with a rapid accumulation of platelets at the tissue injury site. The innate immune system is a group of proteins and phagocytic cells that are genetically programmed to detect tissue trauma, as in surgical wounds, and foreign invaders, such as bacteria, and viruses. The innate immune system identifies well-preserved features of the pathogens and quickly activate the immune response to help destroy the invaders, even if the host has never been previously exposed to a particular pathogen [29]. Neutrophils, monocytes, and dendritic cells are the most common innate immune cells in the blood [30]. Their recruitment is required for an adequate early-phase immune response. When PRP is used in orthopedic surgery, platelet-leukocyte interactions regulate inflammation, wound healing, and tissue repair. Platelets express a wide range of bacterial receptors, acting as sentinels of the circulatory system with the ability to internalize bacteria and the capability to release a broad assortment of molecules that provide an array of host defense functions [28]. Platelets are well recognized for their role in the host defense system, which results from the release of granules containing antimicrobial peptides (AMPs) and from inducing expression of AMPs by other cells [31,32]. In LP-PRP, in addition to the release of AMPs, platelets can also generate reactive oxygen species, bind and internalize microorganisms, and participate in antibody-dependent cellular cytotoxicity [33] .

Leukocyte Rich PRP

LR-PRP buffy coat preparations, apart from being enriched in platelets, also contains a high concentration of viable WBCs, including neutrophils. These cells play an important role in the innate immune defense against infections [34]. Activation of neutrophils results in the so-called respiratory burst, during which the highly bactericidal hypochlorous acid is formed through the action of myeloperoxidase (MPO), an enzyme produced mainly by neutrophils and monocytes [35]. Previous research suggested that this oxidative killing, compared to the nonoxidative killing that is also present, accounts for the largest part of the antibacterial effect of neutrophils and that MPO plays an essential role in this process [36]. As discussed above, platelets have a direct role in recognizing, sequestering, and neutralizing invading pathogens. Additionally, platelets have an indirect role in bacterial phagocytosis, as they recruit neutrophils, resulting in neutrophil degranulation and formation of neutrophil-extracellular traps (NETs) [37]. NETs are comprised of the neutrophil nucleus and other neutrophil intracellular contents that trap bacteria and kill them by NETosis. The formation of NETs is an essential killing mechanism of neutrophils at infection and inflammation sites [38]. Remarkably, the antibacterial effects of PRP are evident immediately upon activation [39]. PRP can act synergistically with antibiotics and can be considered as an adjunct treatment for infections once the pathogen has been identified, especially in cases involving antibiotic-resistant bacteria [40]. The effect of the leukocytes in PRP on its antibacterial properties remains controversial. There is no doubt that leukocytes are important in the host defense mechanisms, which suggests that the presence of leukocytes in PRP should augment the antibacterial properties of PRP. The potential contribution of the antimicrobial properties, particularly of LR-PRP, to post-surgical

wound healing is an attractive addition to the proven tissue repair and regenerative potential of autologous prepared PRP.

Analgesic Effects of Platelet Rich Plasma

Activated platelets release many pro- and anti-inflammatory mediators that can induce pain, but can also reduce inflammation and pain. Once PRP is applied to the tissues, platelet dynamics change the microenvironment by initiating pathways that lead to tissue repair, including cell proliferation and differentiation, and stem cell regulation. This attribute has led to the implementation of PRP application in an assortment of clinical conditions commonly associated with chronic pain, including sports injuries, bone and joint diseases, spinal disorders, and complex chronic wounds, and for acute post-surgical pain. Everts et al. were the first to report on the analgesic effects of an autologous buffy-coat PRP formulation activated with autologous thrombin following shoulder decompression surgery [41]. The authors reported significant reductions in visual analogue scale (VAS) pain scores and opioid analgesics use, and consequently, a more successful post-surgical rehabilitation. The authors discussed the analgesic effects of activated platelets and hypothesized about the role of platelet-released peripheral serotonin (5-HT) following platelet activation and release of intracellular α- and dense granules [6]. PGF, cytokines, and platelet lysosomes proliferate at surgical sites treated with activated PRP. More specifically, when the dense granules release their contents, an abundance of pain-modulating 5-HT will be discharged [42]. In high dose PRP, the platelet concentration is 5 to 7-fold higher than in peripheral blood. Therefore, the release of 5-HT from the platelets is astronomical, potentially interfering with nociceptive transmission at peripheral wound and tissue sites through specific 5-HT receptors [43].

The 5-HT system represents a powerful mechanism that can modulate the intensity of pain after noxious stimulation. Peripheral regulation of nociceptive signaling and alterations in the 5-HT system have been reported in chronic pain patients. Intriguingly, Sprott et al. observed substantial pain reduction following acupuncture, and measured a significant decrease in platelet-derived 5-HT concentrations, with a subsequent increase in 5-HT plasma levels [44]. Earlier published clinical studies on the nociceptive and analgesic effects of PRP found little to no pain relief in patients treated for tendinoses or rotator cuff tears [45]. In other, mostly later studies, PRP relieved or even eliminated pain in patients suffering from tendinosis, osteoarthritis (OA), and plantar fasciitis and other foot and ankle disorders [46,47]. Notably, Kuffler has addressed the potential of PRP for pain relief in patients with mild to severe chronic neuropathic pain secondary to a damaged non-regenerated nerve. The objective of Kuffler's study was to find out whether promotion of axonal regeneration and target reinnervation by surgical PRP application would lead to relief or cure of neuropathic pain [48]. Strikingly, successfully treated patients remained pain-free or had less pain for a minimum of six years after the procedure. Furthermore, pain relief began within three weeks after the surgical PRP application in all patients. Similar analgesic PRP effects have been observed in post-surgical wound and skin care. Interestingly, the authors reported the physiological aspects of wound pain related to vascular injury and skin tissue hypoxia and highlighted the importance of angiogenesis in optimizing oxygenation and nutrient delivery. Their controlled study confirmed the potential analgesic properties of PRP and recorded significantly increased angiogenesis in the PRP-treated patients compared to the controls [23]. Finally, Johal et al. have presented a systematic review and meta-analysis and concluded that PRP reduces pain in orthopedic applications [49]. Unfortunately, they were not able to present data on the PRP bioformulations or the use of platelet-activating agents, as it is known that these variables affect the overall effectiveness of PRP.

The optimal PRP platelet concentration that leads to pain relief in clinical settings is yet unknown. In an animal model, complete pain relief was facilitated with a platelet concentration of $1.0 \times 10^6/\mu L$, whereas PRP with half this platelet concentration was associated with significantly less pain relief [50]. In the literature, platelet dose and bio-cellular PRP composition have been identified as key factors contributing to consistent posttreatment analgesic effects. Other variables that have been cited are PRP delivery methods, application techniques, the use of platelet activation protocols, the bioactivity levels of the released PGFs and cytokines, the types of tissues to which PRP was applied, and the type of post-surgical injury.

Indications for Platelet Rich Plasma and Platelet Gel in Orthopedic Surgery

Although robust clinical trials are yet missing for some indications, the use of PRP and PG has been rapidly growing in popularity within orthopedic surgery and sports medicine, and the true efficacy for several orthopedic surgical indications must be fully established. Multiple meta-analyses have been conducted on the topic, showing sometimes contradictory results. Several studies find that PRP application to injury sites does not provide significant benefits with respect to clinical outcomes, whereas many others report the contrary, with emphasis on pain reduction. In this section, we focus on PRP or PG applications in orthopedic surgery. Moreover, we critically review, if available, the most recent clinical evidence for specific patient outcomes after implementation PRP or PG in tendon repair, support of bone repair, augmentation of spinal fusions, and support for better outcomes in major joint replacements.

Tendon Reconstructions Augmented by PRP

Anterior Cruciate Ligament Repair

Anterior cruciate ligament (ACL) rupture is a common knee injury. The gold standard for restoration of knee stability and function is surgical reconstruction of the ACL. Orthopedic surgeons have been increasingly using PRP as a biological adjunctive tool during ACL reconstruction to augment the integration of the soft tissue graft or support the bone-patellar tendon-bone ingrowth and to reduce donor-site morbidity. In general, outcomes after ACL reconstruction have shown high rates of return to regular activities of daily living and preoperative sports activities, with low revision rates [51,52].

Recently, Davey et al. published a systematic review of randomized controlled trials (RCTs), evaluating the efficacy of PRP in 13 studies including a total of 765 patients included [53]. The collected trials were executed between 2009 and 2018, with a mean follow up time of 18 months. The analysis included incorporated 3 subgroups as different graft types were used.

Hamstring Tendon Autograft. This subgroup included 7 RCTs. One demonstrated a significant difference in ACL density, with a more structured ACL graft, in patients who received PRP compared to controls. One showed that at 3 months that there was less tunnel widening in the PRP group, and one found a significant difference in the tunnel diameter between the postoperative and 1-year follow-up visits in the PRP group compared with the control group [54].

Autograft Bone-patellar Tendon-bone Grafting

Four RCTs evaluated the use of PRP as an adjunct to ACL reconstruction with a BPTB autograft. There was no statistically

significant difference between groups with regard to graft integration and filling defects. Postoperative VAS scores were not significantly different. Two studies used Victorian Institute of Sport Assessment (VISA) scores to assess pain levels. One reported a statistically significant reduction in postoperative pain levels at 12 months post-ACL reconstruction was seen in patients receiving PRP.

Allograft

In 2 trials of PRP as an adjunct to ACL reconstructions using allografts, there were no significant radiologic differences in graft thickness, intensity, and uniformity.

Anterior Cruciate Ligament Graft Maturation. According to this recent review, there is some evidence that the addition of PRP could be a synergic factor in acquiring ACL graft maturity more quickly than without PRP. It must be remembered that three different reconstructive techniques were used, with both autografts and allografts. Furthermore, in 13 RCTs, 7 different PRP preparation devices were used and 2 PRP preparation methods were not even specified, meaning that potentially 9 different PRP formulations were used. In 85% of all RCTs, calcium chloride was used to activate the PRP, but there was no consistency in the bioformulation of the PRP applications as LP-PRP and LR-PRP were used in the same ratio. Platelet dose was mentioned only in 3 studies, and ranged from 504 x 10^6/μL to 1,185 x 10^6/μL. Without a doubt, the lack of standardized methods for PRP preparation and application will not support consistently positive outcomes. Furthermore, the use of autologous PRP products in combination with allografts warrants another discussion with regard to efficacy and significant outcome differences.

Achilles Tendon Repair

There are no systematic reviews or meta-analyses available regarding Achilles tendon rupture repair using PRP to augment the biological healing process and addressing functional improvements. However, the strong positive effects of PRP augmentation in acute Achilles tendon rupture have been reported in a number of animal and immunohistochemical studies [55,56]. As yet, compelling clinical evidence is lacking to suggest improved functional outcomes after PRP augmentation in Achilles tendon repair is lacking. Sanchez and colleagues reported on a group of athletes with surgically repaired acute Achilles tendon ruptures. They found a faster return to sport in the group treated with platelet-rich fibrin matrices, compared with a retrospective matched surgical control group without biological augmentation. However, given the study size, further studies are warranted to validate this finding.

In a prospective study of Zou et al. PRP served for biological augmentation in the treatment group [57]. PRP was injected into the paratenon sheath and around the ruptured tissue after the tendon was repaired. PRP treated patients had increased isokinetic muscle strength, achieved higher SF-36 and Leppilahti scores, at 6 and 12 months. Furthermore, at 24 months, the PRP group had an improved ankle range of motion, when compared to the control group. Their LR-PRP had a platelet concentration of approximately 6 times the baseline value.

Recently, the PATH-2 trial group conducted a randomized, placebo controlled, two-armed trial with blinded participants [58]. The study was executed at 19 hospitals in the United Kingdom to determine whether a single, standardized, PRP injection combined with standard rehabilitation care, improved patient functional outcomes after acute Achilles tendon rupture. In this large and well-designed trial, PRP treated patients did not show significant better outcomes with regard to pain management, muscle tendon function, patient reported function, or quality of life, when compared to the placebo group. In all patients, the Magellan Autologous Platelet Separator and PRP kits were used (Arteriocyte Medical Systems, Hopkinton MA, USA) to produce a LR-PRP specimen. From the initially prepared 8 ml of PRP, with an average platelet concentration of 4.1 times the baseline whole blood platelet count, 4 ml was injected in the ruptured tendon. No data were presented regarding the WBC differentiation.

Rotator Cuff Repair

Rotator cuff tears are the most common cause of shoulder disability, with a prevalence in the aging population ranging from 20% to 40%, and both surgical and non-surgical options as treatment modalities [59]. Laboratory research and clinical trials have largely found that that PRP has beneficial effects for tendinous injuries. The primary goal of PRP application in rotator cuff repair is to reduce the re-tear rate and support the tendon-to-bone ingrowth in highly hypovascularized areas [60]. An analysis of 10 level 1 studies, evaluating the role of PRP to augment surgical rotator cuff repair, concluded that that there was no significant difference in the rate of persistent tears in all but 1 of the RCTs. Strikingly, in all these 9 studies the PRP formulation did not meet a platelet dose of 1 x 10^6/μL, or higher [61].

A Cochrane review showed inconsistent results after surgical repair with regard to pain and VAS scores [62]. Interestingly, the patients in the PRP group had a 45% lower risk of re-tear at the 1-year follow-up [63]. A lower re-tear rate was also observed by Vavken et al., with a subgroup analysis revealing that patients in the PRP group with tears <3 cm had a significantly reduced risk of re-tear (40%). However, this difference was not observed for tears larger than 3 cm. The effects of PRP on re-tear rate and functional outcomes were assessed in a 2020 systematic review and meta-analysis that showed a significant decrease in re-tear rates and improved function, measured as Constant score and short-term UCLA activity score [64]. The authors concluded that the application of PRP to the bone-tendon interface during arthroscopic rotator cuff repair is beneficial, with a significant short term decreased VAS score. Additionally, a fibrin-platelet matrix graft (FPMG), enriched with a high dose of platelets, can be incorporated at the tendon-to-bone repair site, as this insertion point is biomechanically, compositionally, and structurally a complex area (Figure 2). The FPMG is a PRP-matrix, potentially facilitating a sustained platelet growth factor release over a longer period during the healing phases, as platelet growth factors have been identified to play key roles during rotator cull healing processes in humans [65,66]. However, the biological effects may be conditional of the platelet dose in the FPMG.

Bone Repair and Grafting Support with Platelet Rich Plasma

Repair of bone defects caused by failed fracture healing, trauma, infection, or tumors, and the effects of compromised blood supply near fracture sites, which can result in critical long-bone defects leading to nonunion or delayed union, are clinically challenging conditions necessitating bone grafting, reconstructive techniques, and biomaterials [67]. Commonly used bone graft biomaterials include autografts, allografts (human cadaver bone), xenografts (animal bone), and synthetic biomaterials. Of these, autologous bone or autografts are still considered the clinical gold standard and the most effective method for bone regeneration.

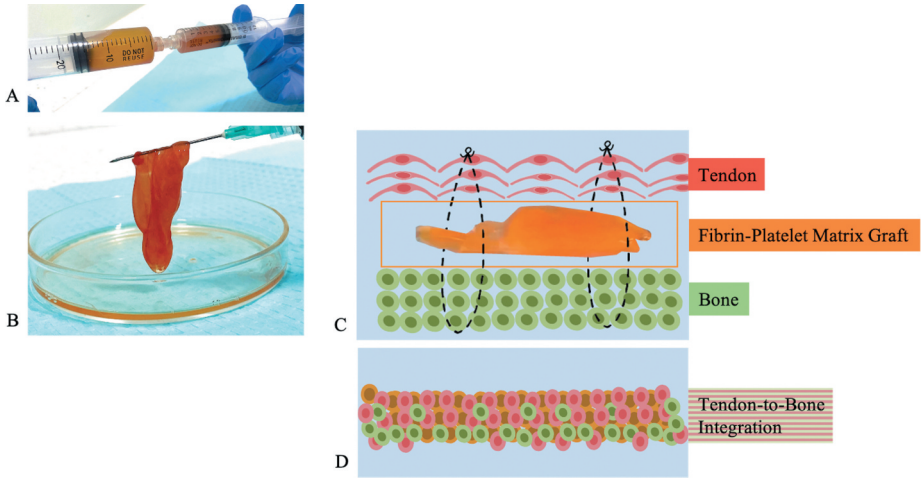

Figure 2. Autologous fibrin-platelet matrix graft preparation to reinforce surgical repair site.

The situation in A shows the mixing of concentrated platelet-poor plasma with PRP, containing a high dose of platelets, in order to maintain a therapeutic dose of platelets above 1 x 106/μL. The mixture is then mixed in the consolidating syringe with a combination of CaCl2 10% and thrombin. This will activate platelets and convert soluble fibrinogen into insoluble fibrin, which polymerizes to create a gelatinous clot, which is then placed in a sterile Petri-dish (B). The biological active FPMG is removed from the Petri-dish, placed between the tendon and bone structures, and incorporated in suture lines, or anchors, as part of the surgical reconstruction (C). The 3-dimensional extracellular matrix graft acts as a conduit with cellular infiltration at the complex tendon-to-bone interface. A sustained delivery of platelet derived growth factors, cytokines, and lysosomes will facilitate synergistical growth factor effects over a longer period of time, potentially augmenting tendon-to-bone integration (D), as adequate ingrowth of hypovascularized tendons to bone is critical to gain functional recovery and reduce re-tear rate.

Multiple bone repair-specific essentials are needed for bone regeneration. These include osteoconductive scaffolds, osteoinductive growth factors, and cells that possess the capacity for osteogenicity for graft vascularization [68]. The basic elements for bone tissue repair and engineering are signaling molecules, cells, and matrices [69]. The process is similar to normal hemostatic mechanisms, including clot formation and platelet activation. In 1998, Marx reported on the beneficial use of PRP in bone regeneration in patients with mandibular defects, and this ignited an increased interest in and use of PRP, first within the oral and maxillofacial surgical fields, and later followed by expanding use across an increasing variety of surgical fields, including its use as an adjunct to support autografts in orthopedic reconstructive procedures (Figure 3). Many clinical studies have

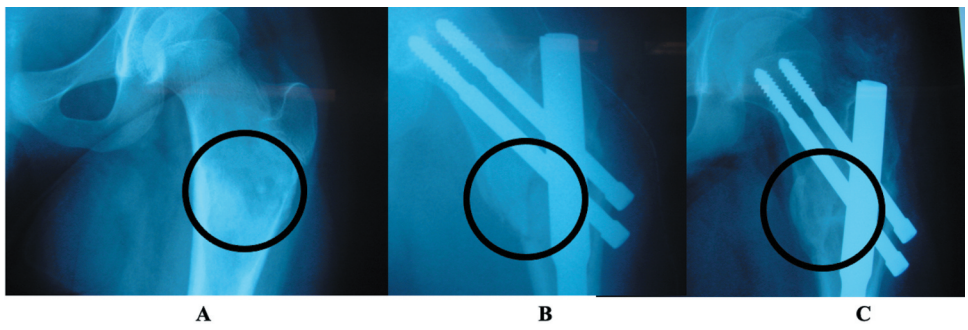

Figure 3. Successful bone – platelet gel grafting procedure in a patient with polyostotic fibrous dysplasia.

Polyostotic fibrous dysplasia in the neck-shaft angle in the proximal femur (A). Repair consisted of curettage and placement of a standard intramedullary rod with the application of 50 ml of bone which was mixed with PG (B). After 6 weeks a good bone consolidation was seen (C).

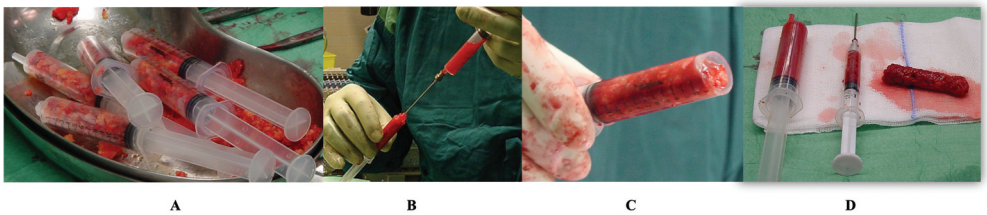

Figure 4. Bone – platelet gel graft preparation.

Autologous bone is harvested from the iliac crest and sized in smaller cortical and spongious fragments before it is placed in 10 ml syringes (A). Each syringe is injected with a mixture of PRP and CaCl2 (B). After 1–2 minutes all bone fragments are consolidated by the platelet gel (C). The bone graft is removed from the syringe and can applicated in-situ (D).

obtained good outcomes in terms of high consolidation rates by using bone-PG grafts (Figure 4). Nevertheless, despite positive results, autografts continue to carry the risk of donor site morbidities, persistent pain, and limited supply [70,71].

Correspondingly, in spite of increasing use of combined PRP and autograft procedures, conflicting outcomes have begun to emerge in concert with variable PRP terminologies and product preparations [4], hindering the progression of PRP applications [72,73]. The development of advanced and innovative biomaterials for bone regrowth, has contributed to alternative bone repair approaches by means of concentrated bioactive factors on a variety of scaffolds (ceramic, polymers, composite materials) that produce an optimized microenvironment for bone tissue regeneration [74]. Although several studies have indicated that most biomaterials have limited bioactivity, numerous studies have also demonstrated synergistic effects when biomaterials are combined with autologous biologics such as PRP, bone marrow aspirate concentrate, or adipose tissue, to induce bone regeneration [75–77]. Combining PRP or PG with bone graft substitutes has the potential to reduce or eliminate the need for bone autografts. Currently, PRP is being used and investigated for a variety of orthopedic applications that are directly related to bone repair, as well as in various bone-related regenerative medicine applications (Table I).

Platelet Rich Plasma in Spinal Fusion Surgeries

Improvements in life expectancy over the past century and innovations in spinal instrumentation have contributed to an increase in the number of neuro-orthopedic spinal fusion surgeries to treat spinal instability and deformities resulting from various spinal pathologies [78,79]. Spinal fusion is now a common procedure, and the results have improved because of advances in surgical technique and spinal implants [80], albeit that despite these advanced techniques, pseudarthrosis with failure of bony implant-fusion remains as a serious complication. Therefore, while autografting remains the gold standard in spinal fusion, additional materials and biologics, including PRP, are employed to enhance the fusion rate [81,82]. Ceramics, demineralized bone matrix, and bone morphogenetic proteins (BMPs) have also been used in lumbar fusion surgeries to avoid serious complications including infection, hematoma, fracture, and wound healing disturbances [83]. Notably, the US Food and Drug Administration does not recommend the use of BMP-2 and BMP-7 in cervical fusion surgery.

PRP was initially used successfully in enhancing spinal fusion in animal spinal surgical models. PRP promoted bone formation and shortened the time required for spinal fusion in posterolateral lumbar intertransverse process arthrodesis (PLIF) (Figure 5) and anterior lumbar interbody fusion (ALIF), as demonstrated in Figure 6 [84,85]. However, the clinical results confirming these PRP advantages have been conflicting, as indicated by the development of lumbar pseudarthrosis and the failure of post-surgery pain relief. In two independent studies, the nonunion rate was

Table I. Bone tissue related pathologies that have been augmented by platelet-rich plasma or platelet gel applications.

Arthrodesis
Avascular osteonecrosis
Avulsion fractures
Bone Fracture
Bone infection
Chondral pathologies
Delayed union
Endoprosthesis loosening
Fibrous dysplasia
Nonunions
Open-surgical fracture treatment
Osteochondritis dissecans
Osteoporosis
Pseudarthrosis
Traumatic bone loss
Traumatic open fractures

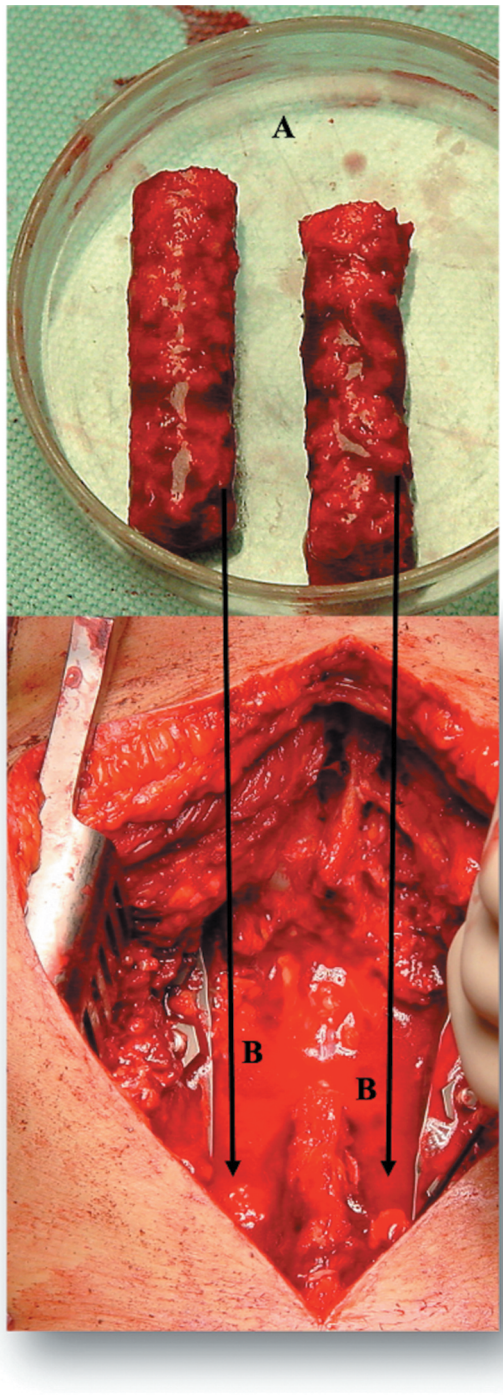

Figure 5. Posterior lumbar interbody fusion with pedicular screws fixation and plate fixation at L4-L5 level, in a 66-year male.

In two 10 ml syringes autologous bone grafts are created (A). Each syringe contained approximately a volume of 6,5 ml bone fragments that are mixed with 3,5 ml CaCl2/thrombin activated PRP. In B, the bone grafts are placed into the interbody space, and along the side of the back of the vertebra. After the plate has been placed, 4 ml of PG is injected on top of the bone grafts.

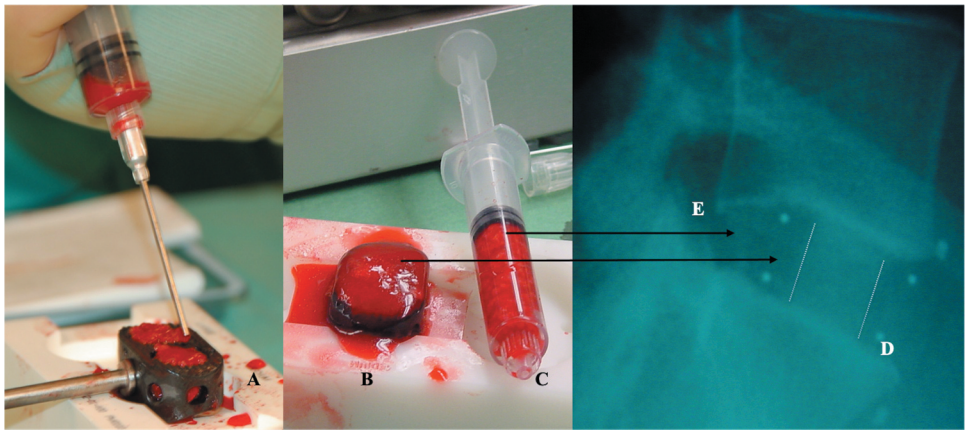

Figure 6. Anterior lumbar interbody fusion with an intervertebral cage at level L5-S1 in a 58-year-old patient.

In A, a radiolucent cage was filled with autologous bone fragments and injected with activated PG (B). The syringe holds a 5 ml autologous bone-PG graft, 3 ml : 2 ml ratio respectively (C). The cage (white 4 dots) is anteriorly placed to fuse the S5-L1 vertebrae (D). Between the dashed lines bone is visible in the cage, 7 weeks after surgery. The PG impregnated bone graft is placed at the posterior part of the vertrebral body, behind the cage, for additional fusion support (E).

higher in the treatment groups when compared to the control groups [86,87]. In both studies, autologous growth factor (AGF) technology was used. Primarily prepared PRP was concentrated through a filter to remove the water fraction of the PRP plasma and increase the platelet concentration to higher concentrations. Strangely, no platelet data were reported in either of the studies employing the AGF device. In more recent studies, dual spin prepared PRP was activated with platelet agonists to produce a PRP-gel viscous biological product, which was then mixed with autologous bone, as a safe alternative strategy to improve the rate and extent of bone fusion in postero-lateral lumbar and interbody fusions [79,88]. Interestingly, in these radiographically confirmed successful studies, the average platelet dose was on average 8.0 x the baseline platelet value.

PRP Employment in Major Joint Replacements

Primary major joint arthroplasties are commonly performed in patients suffering from osteoarthritis, rheumatoid arthritis, and osteonecrosis. Total knee arthroplasty (TKA) and total hip arthroplasty (THA) are established and frequently executed procedures that can effectively reduce pain and improve function and quality of life. To a lesser extent, shoulder and revision joint replacements are performed. Some of the complications associated with joint replacements are infection, bleeding requiring allogeneic blood product transfusion, wound healing disturbance, and postoperative impairment of joint function, loss of prosthesis, and deep vein thrombosis [89].

The postoperative recovery after total shoulder arthroplasty (TSA) might be hindered by a number of complications, as mentioned above. In a RCT, Zavadil et al. utilized $CaCl_2$/thrombin activated PRP and PPP during primary TSA [90]. PG was sprayed in the intermedullary canal of the humerus before the humeral stem implant was placed to support bone ingrowth into the prosthesis. An additional PG volume was placed extra-articularly and during wound closure activated PPP was sprayed on capsule and subcutaneous tissue. In PRP treated patients, the VAS scores were significantly decreased, and patients received less opioid pain medication. The internal rotation index improvement factor was increased, the postoperative hemoglobin content was higher, and hospitalization time was decreased by 9 hours, compared to control patients.

A number of strategies are proposed for avoiding or minimizing the need for allogeneic blood transfusions and the related risk of serious posttransfusion complications in TKA and THA, particularly for patients with general or local risk factors for developing serious postoperative complications [91]. These strategies for decreasing blood loss and the risk of subsequent allogeneic blood transfusion during major joint arthroplasty include intra-articular and intravenous administration of tranexamic acid, autologous re-transfusion of shed blood, and use of fibrin tissue adhesives [92–94].

Later research has focused on the effectiveness and safety of PRP or PG in the management of perioperative blood loss as well as for pain relief and restoration of joint function. Thus far, the outcomes are inconclusive with regard to significantly reduced postoperative blood loss or allogeneic transfusion [95].

Interestingly, Everts et al. evaluated not only activated PG during wound closure, but also the platelet-poor plasma (PPP) split/waste product (Figure 7) [96]. The PPP was concentrated to eliminate the water fraction and increase the fibrinogen concentration, which was then activated and sprayed on the intra articular structures, capsule, and subcutaneous tissues. From all studies, the combination of platelet therapy and concentrated PPP seems to have a more pronounced effect on hemostasis.

In a recent systematic review and meta-analysis including six RCTs evaluating PRP as an adjunct to TKA, Tram reported that all of the studies showed a significantly lower drop in hemoglobin, and 4 studies showed better pain control, in patients treated with PRP [97]. These promising results after PRP or PG application in TKA procedures should encourage further well-planned high powered RCTs. Furthermore, the most effective overall blood conservation method should be established in both primary and revision joint replacement procedures in order to decrease pain and the use of analgesics and minimize the risk of complications directly related to blood transfusion and, ultimately, the costs.

Conclusion

Our most important finding of this review was the wide range of PRP preparation protocols used in orthopedic surgical medicine. Furthermore, the clinical characterization of PRP formulations was rarely mentioned in the reviewed literature. Remarkably, all products were classified under the term “PRP”. The neglect of these PRP fundamentals constitutes a challenge in assessing the effectiveness of PRP outcomes in both controlled individual and

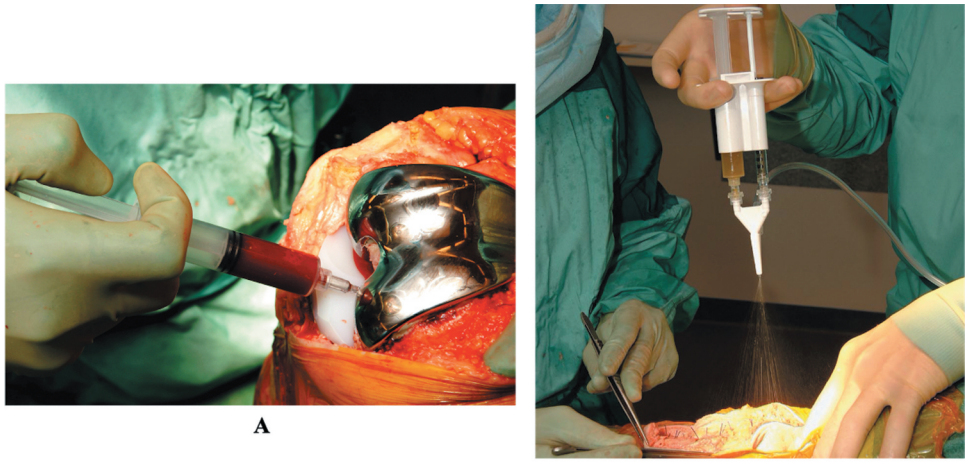

Figure 7. Activated PRP and platelet-poor plasma application in a TKA patient.

In A, the 10 ml of activated PRP (PG) was injected in the back of the knee cavity, the posterior recess, the gutters and the raw exposed surfaces of the femur and tibia after the prosthesis was placed. The wound area was retracted and activated PPP was applied to the dried tissues by a double syringe technique and delivered by aerosol topical spraying at a distance of 15 cm (B), thereafter the wound was closed in layers (Technique is described in reference 95).

comparative studies, despite the technological enhancements in PRP devices.

The heterogeneity in PRP composition, combined with an absence in platelet dosing formulations, have an impact on the tissue cellular environment wherein PRP is applicated and is likely to determine its overall effect on tissue healing and repair. These circumstances represent the main reason for the reported diverse outcomes in the PRP literature. It is fair to assume that the full potential of using PRP in countless clinical applications, exploiting various bioformulations and dosing regimen, has yet to be determined.

Consensus on PRP preparation standards might not be a realistic goal as there are numerous parameters to be considered with even more differentials and options. However, acceptance of PRP platelet dosing regimen for different indications might be a more attainable quality indicator. Calculating the total deliverable platelet dose (total injected volume multiplied by platelet concentration per unit of volume) is most pertinent, as this indicator indicate accurately the total number of platelets that were applied to patients.

Sufficiently powered and well-documented clinical studies are needed to determine the full potential and thus therapeutic effects of different PRP bioformulation and platelet dosages, based on patient specific pathologies.

Declaration Of Interest

The corresponding author (PE) is also the Chief Scientific Officer of EmCyte Corporation. All other authors have nothing to declare.

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