Biologics: Targets and Therapy

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REVIEW

Advances with Platelet-Rich Plasma for Bone Healing

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Abstract: Despite significant advances in the understanding and delivery of osteosynthesis, fracture non-union remains a challenging clinical problem in orthopaedic surgery. To bridge the gap, basic science characterization of fracture healing provides a platform to identify and target biological strategies to enhance fracture healing. Of immense interest, Platelet-rich plasma (PRP) is a point of care orthobiologic that has been extensively studied in bone and soft tissue healing given its relative ease of translation from the benchtop to the clinic. The aim of this narrative review is to describe and relate pre-clinical in-vitro and in-vivo findings to clinical observations investigating the efficacy of PRP to enhance bone healing for primary fracture management and non-union treatment. A particular emphasis is placed on the heterogeneity of PRP preparation techniques, composition, activation strategies, and delivery. In the context of existing data, the routine use of PRP to enhance primary fracture healing and non-union management cannot be supported. However, it is acknowledged that extensive heterogeneity of PRP treatments in clinical studies adds obscurity; ultimately, refinement (and consensus) of PRP treatments for specific clinical indications, including repetition studies are warranted.

Keywords: platelet rich plasma, bone regeneration, fracture healing, fractures, ununited

Introduction

Bone healing stands as a complex and pivotal process within the realm of orthopedics, carrying with it substantial clinical and financial burdens. In 2019, the global incidence of bony fractures stood at a staggering 178 million cases, underscoring the pervasive nature of this often-debilitating health concern.[1] While the majority of fractures heal successfully, approximately 5–10% of patients encounter a formidable obstacle: non-union.[2] The Food and Drug Administration (FDA)[3] has defined a non-union as a fracture that persists at 9 months post-injury, exhibiting insufficient signs of healing over three consecutive months. This condition manifests in two distinct forms—hypertrophic non-union, due to inadequate stability at the fracture site, and atrophic non-union, attributed to a deficiency of fracture biology and bone healing.[4] The management of fracture non-union is considered on an individualized basis and involves surgical strategies to optimize stability (and strain) at the fracture site while identifying and addressing any deficits in fracture biology manifested in the bone healing response [183]. The costs associated with non-union treatment can be staggering, with tibial non-unions, for example, incurring an approximate cost of $25,556 USD compared to $11,686 USD for tibial fractures without non-union complications.[5]

The physiological process of bone healing is intrinsically sophisticated, typically progressing through three stages: the inflammatory phase, where hematoma forms and inflammatory cells infiltrate the site; the reparative phase, which involves the formation of a soft callus that gradually mineralizes; and the remodeling phase, where the callus is replaced by mature bone tissue.[6] Each of these stages is marked by a distinct cascade of cellular and molecular events, underscoring the potential for therapeutic interventions that can modulate these processes.

Historically, the treatment of bone injuries has evolved from rudimentary splinting techniques to advanced surgical interventions, reflecting a deepening understanding of bone biology and healing processes. In the quest to enhance bone healing,

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regenerative medicine has introduced several innovative therapies, of which platelet-rich plasma (PRP) therapy has emerged as a significant contender. PRP therapy, in comparison to other regenerative approaches like stem cell therapy or bone morphogenetic proteins (BMPs), offers a unique blend of autologous growth factors and cytokines, potentially reducing the risk of immune rejection and other complications associated with allogeneic or synthetic materials.[7] Initially recognized for its role in tissue sealing as fibrin tissue adhesives, PRP subsequently garnered attention for its potential to emulate the initiation of the natural healing cascade.[8] The rationale behind PRP therapy lies in its ability to release biologically active factors and adhesion proteins, offering the potential to stimulate the resolution of chronic pathological processes.[9] Specifically, PRP is replete with growth factors such as Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-beta (TGF-β), and Insulin-Like Growth Factor-1 (IGF-1), which are critical mediators in the bone healing process.[10] These growth factors and cytokines play key roles in regulating inflammation, angiogenesis, and osteoblastic activity, making them vital to the various phases of bone repair.[11–13]

Despite promising pre-clinical data supporting the potential of PRP, clinical trials have yet to unequivocally demonstrate its benefits in bone healing. Moreover, the absence of a standardized PRP injection protocol(s) hinders efforts to generalize findings or collate the data of individual studies. Dosage and timing intervals remain uncertain, and the composition of PRP varies widely in terms of leukocyte and platelet count, growth factor concentration, and red blood cell contamination due to patient characteristics and the preparation kit used.[8,14] This heterogeneity in PRP formulations further complicates its clinical application to date, given the current absence of a universally accepted PRP injection protocol. Therefore, the aim of this narrative review is to provide a comprehensive platform to evaluate the evidence regarding the use of PRP for bone healing.

Biological Activity of PRP: Influence on the Bone Regeneration Process

The pursuit of optimal strategies for bone healing has driven the exploration of PRP therapy due to its ability to serve as a concentrated source of autologous growth factors and cytokines. Our current understanding of PRP's biological activity in bone healing has been predominantly centered on three key aspects: inflammatory cytokines, growth factors, and angiogenic factors (Table 1). These factors orchestrate the intricate process of cell signaling, tissue regeneration, and angiogenesis during the bone healing process.

Table 1 Function of the Growth Factors and Cytokines Found in PRP on Bone Regeneration

Category	Factors	Roles and Function
Inflammatory Cytokines	Interleukin-1 (IL-1)	Initiates inflammation, recruits immune cells, and triggers cellular responses.
	Interleukin-6 (IL-6)	Involved in callus remodeling and mineralization, recruit's osteoblasts.
	Tumor Necrosis Factor-alpha (TNF-a)	Recruit's osteoblasts and plays a pivotal role in bone formation.
Growth Factors	Platelet-Derived Growth Factor (PDGF)	Stimulates revascularization, collagen synthesis, and bone regeneration.
	Transforming Growth Factor-beta (TGF-B)	Initiates signaling pathways in osteoprogenitor cells and supports long-term healing, bone regeneration, and remodeling.
	Insulin-Like Growth Factor-1 (IGF-1)	Influences osteoblasts and pre-osteoblasts, inhibits apoptosis, and enhances collagen synthesis and osteogenesis.
Angiogenic Factors	Vascular Endothelial Growth Factor (VEGF)	Stimulates angiogenesis, recruits endothelial cells, and supports oxygen and nutrient delivery.
	Angiogenin	Contributes to the development of collateral circulation, enhancing blood supply redundancy.
Other Bioactive Factors	Serotonin, Histamine, and Dopamine	Increases capillary permeability, facilitating the influx of inflammatory cells.
	Calcium	Promotes the formation of a stable fibrin clot.
	Adenosine	Mitigates excessive inflammation and tissue damage.

Inflammatory Cytokines

The initial phase of bone healing is characterized by inflammation, a crucial process that dictates the subsequent stages of repair, and platelets in PRP have been shown to successfully modulate this inflammatory response.[10,15] Once activated, the platelets in PRP release a spectrum of inflammatory cytokines from their alpha-granules such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF- α).[13,16] These cytokines play pivotal roles in the initiation of fracture repair by recruiting immune cells and initiating a cascade of biochemical and cellular alterations that set the stage for subsequent stages of bone repair. IL-1 stands out as a main regulator of the initial inflammatory responses in bone healing. Its release at the fracture site follows a biphasic pattern, characterized by an initial peak during the onset of the fracture healing process, succeeded by a subsequent peak during the shift from chondrogenesis to osteogenesis in the phase of endochondral maturation.[17,18] This cytokine plays a multifaceted role, influencing the recruitment of immune cells to the injury site and initiating a multitude of cellular responses necessary to the bone healing process.[19–21] Additionally, TNF-alpha also follows a biphasic pattern in its expression during the healing process and plays a pivotal role in recruiting osteoblasts to the injury site.[17,18] These bone-forming cells are crucial for the synthesis of new bone tissue and studies[20–22] have indicated that both TNF-α and IL-1β recruit osteoblasts, highlighting their collaborative role in bone regeneration. Furthermore, IL-6 is a multifunctional cytokine involved in bone repair. Studies using IL-6 knockout mice[23] have shown that this cytokine plays a role in callus remodeling and mineralization, indicating its significance in the later stages of bone healing. Additionally, IL-6 has been implicated in recruiting osteoblasts, further contributing to bone formation.[24,25] Thus, the orchestrated release of inflammatory cytokines in the early phases of bone healing is crucial for initiating the repair process and may be able to be amplified through the application and activation of PRP.

Growth Factors

PRP's effectiveness in bone healing can be attributed significantly to the rich assortment of growth factors contained in the alpha-granules of platelets. Of the numerous growth factors that have been defined in the literature,[26–29] the three that appear to play the most prominent role in bone healing include PDGF, TGF-β, and IGF-1.[10] PDGF is a critical growth factor in PRP that plays a pivotal role in the early phases of bone healing by initiating several essential processes upon release from activated platelets. It stimulates revascularization, an essential step in bone repair, by promoting the growth of new blood vessels.[11,30] This improved blood supply may facilitate the delivery of oxygen and nutrients to the injury site, accelerating the healing process. PDGF also has a profound impact on collagen synthesis, a key component of bone tissue. It encourages the production of collagen, enhancing the formation of a robust extracellular matrix (ECM) essential for bone regeneration.[11,31,32] Moreover, PDGF can directly influence mesenchymal stem cells (MSCs), inducing their migration and osteogenic differentiation.[33,34] These MSCs are crucial for generating new bone tissue, making PDGF a potent stimulator of bone formation.

TGF-β is also abundantly present in PRP and holds a multifaceted role in bone healing. It functions by exerting both paracrine and autocrine effects, influencing various cell types involved in long-term healing, bone regeneration, and bone modeling.[29] One of TGF-β's most crucial functions is its ability to initiate the signaling pathway of osteoprogenitor cells, which synthesize BMPs.[12] These BMPs have demonstrated the potential to play a pivotal role in regulating the expression of growth factors in bone and cartilage tissue, further promoting bone healing and regeneration.[35,36] TGF-β's influence also extends to fibroblasts and pre-osteoblasts, stimulating the biosynthesis of type I collagen and fibronectin, supporting the formation of a robust ECM.[37–39] Additionally, TGF-β promotes the deposition of bone matrix, contributing to the early stages of bone repair.[40] Furthermore, it inhibits osteoclast formation and bone resorption, tilting the balance toward bone formation over resorption.[41]

IGF-1 is another significant component of PRP that plays a vital role in bone regeneration. This growth factor is deposited in bone matrix, endothelial cells, and chondrocytes and is released during the bone regeneration process.[42] IGF-1 is responsible for orchestrating the complex interaction between bone formation and bone resorption. IGF-1's presence in platelets influences osteoblasts and pre-osteoblasts, initiating osteogenesis and inhibiting the apoptosis of bone cells.[43] Additionally, IGF-1 affects the expression of mesenchymal collagen enzymes, decreasing their degradation and enhancing collagen synthesis within the ECM. This leads to improved structural integrity and strength in the newly formed bone tissue.[43] The growth factors found in PRP, including PDGF, TGF-β, and IGF-1, work synergistically to

enhance bone healing. They promote angiogenesis, collagen synthesis, ECM formation, and osteogenesis, contributing to the regeneration and repair of bone tissue. These growth factors play distinct but interconnected roles, collectively facilitating the intricate process of bone healing and regeneration.

Angiogenic Factors

Angiogenesis plays a sustained role in delivering oxygen, nutrients, and precursor cells to the site of injury.[44–46] PRP has demonstrated the ability to serve as a potent facilitator of angiogenesis, promoting the formation of new blood vessels that are crucial for supporting the regenerative processes in bone repair. Among the angiogenic factors found within PRP, Vascular Endothelial Growth Factor (VEGF) stands out as a principal driver of neovascularization. VEGF is a signal protein and its primary function is to stimulate angiogenesis.[47] Upon the application of PRP to the bone defect site, the release of VEGF from platelets sets in motion a cascade of events. VEGF initiates a signaling cascade, acting as a potent mitogen and chemoattractant for endothelial cells, promoting their proliferation and migration to the area surrounding the bone defect.[48–50] Once recruited, endothelial cells start to organize into primitive vascular structures, sprouting and elongating to form capillaries that infiltrate the damaged tissue.[48,51] This neovascularization process serves two essential purposes in bone healing. Firstly, it ensures a continuous supply of oxygen and nutrients to the healing site, facilitating the metabolic demands of reparative cells. Secondly, it provides a conduit for the migration of osteoprogenitor cells and mesenchymal stem cells, which are crucial for the formation of new bone tissue.[10,52,53]

While VEGF primarily influences the growth of new vessels, angiogenin, another angiogenic factor found in PRP, contributes to the development of collateral circulation, which can be particularly relevant in cases where the primary blood supply to a bone defect may be compromised.[10] Enhanced blood supply increases the resilience of the healing process, ensuring that adequate resources are available to support the regenerative demands of the damaged bone tissue. In the intricate orchestration of bone healing, angiogenesis is a fundamental process that ensures the delivery of essential resources to the site of injury. PRP therapy, enriched with angiogenic factors such as VEGF and angiogenin, plays a central role in promoting neovascularization and collateral circulation. By stimulating the formation of new blood vessels and alternate circulation pathways, PRP creates an environment conducive to optimal bone regeneration.

Other Bioactive Factors

In addition to growth factors, PRP contains a diverse array of bioactive factors stored within the dense granules of platelets, including serotonin, histamine, dopamine, calcium, and adenosine.[54,55] These factors exert fundamental effects on the biologic aspects of wound healing, influencing inflammation modulation and cell function. In the context of PRP therapy, serotonin, histamine, and dopamine contribute to wound healing by increasing capillary permeability. This effect facilitates the influx of inflammatory cells to the site of injury, promoting an initial immune response and the activation of macrophages.[56,57] Furthermore, calcium is essential for blood clotting, and its release from platelet granules upon activation is crucial for the formation of a stable fibrin clot at the site of injury.[58] The clot not only prevents excessive bleeding but also provides a scaffold for cells involved in tissue repair to attach and proliferate. Additionally, adenosine receptor activation has been shown to modulate inflammation during wound healing, promoting an anti-inflammatory environment.[59] Attenuation of local inflammation may be beneficial in the early stages of bone repair, as it may help mitigate excessive inflammation and tissue damage.[10,59]

PRP Separation: Optimizing Platelet Concentration

Over the past decade, substantial efforts have been devoted to refining PRP preparation techniques, with the aim of optimizing platelet concentration—a critical factor influencing its therapeutic effectiveness in bone healing. While numerous studies have demonstrated PRP's positive effects on the differentiation and proliferation of human osteoblasts, at present, there exists no unanimous agreement on the ideal PRP dosage. Marx et al initially defined PRP as containing a minimum platelet concentration of 1,000,000 platelets/μL, however, the US Food and Drug Administration (FDA) mandates that PRP products must possess a minimum platelet concentration of 250 × 103/mL.[60] Several additional investigators[61–63] have also reported that a platelet concentration approximately two times greater than that found in peripheral blood positively affects osteoblast proliferation in vitro and significantly reduces bone healing time. However, Jovani-Sancho et al[64] reported that an optimal platelet concentration of four times that of peripheral blood was necessary for optimal results. Other studies[65–67] have indicated that

concentrations below approximately 0.85×10^9/mL had no significant effect on osteogenesis. In contrast, however, Choi et al[68] found that lower PRP concentrations, ranging from 1% to 5% of peripheral blood levels, stimulated the viability and proliferation of osteoblasts. Furthermore, it is crucial to exercise caution when considering platelet concentration, as adverse events have been observed at higher dosages.[69–71] Fernandez-Medina et al[72] indicated that cell viability and migration assays demonstrated detrimental effects on human osteoblasts when the PRP concentration exceeded 60%. Similarly, Al-Hamed et al[61] reported that platelet concentrations greater than $8.21 \pm 0.4 \times 10^9$/mL inhibited osteogenic proliferation and Graziani et al[65] observed that a platelet concentration approximately 3.5 times greater than that of native blood led to a reduction in cell proliferation. These findings underscore the complexity of determining the precise platelet concentration required for optimal bone healing, as different concentrations of PRP may produce varying effects.

Centrifugation separates individual cells within blood based on their individual density gradients, thus the overlaps and proximity of the density of platelets and leukocytes present the possibility of contamination (Figure 1). Similar to other indications, the optimal concentration of leukocytes within PRP for bone healing remains not fully understood. Proponents of incorporating leukocytes argue that the antimicrobial properties of WBCs could mitigate the risk of infection, particularly when PRP is utilized intraoperatively.[73–76] Moreover, studies by Zimmermann et al[77] have revealed that leukocytes in leukocyte-rich PRP (LR-PRP) contribute significantly to the increased variability of growth factors, such as PDGF-αβ, PDGF-β1, and VEGF, in comparison to leukocyte-poor PRP (LP-PRP). This suggests that the concentration of white blood cells can be manipulated to optimize growth factor levels, potentially influencing the healing process positively. However, critics of leukocyte incorporation argue that the existence of WBCs can result in immediate pain and discomfort post-injection, while their catabolic and proinflammatory attributes may adversely impact the process of articular cartilage recovery as a result of the increased release of proinflammatory cytokines.[78–80] Clinical investigations have further validated concerns regarding increased acute swelling and pain after intra-articular LR-PRP injection.[81,82] Nevertheless, it is noteworthy that both LR-PRP and LP-PRP have demonstrated statistically significant improvements in clinical outcomes. Recent research, however, has added to this debate by highlighting the importance of matching the type of PRP with the specific clinical context. The prevailing evidence suggests that the choice of leukocyte concentration should be guided by the injection site.[73,83] For intra-articular applications, LP-PRP appears to be more beneficial, as indicated in the treatment of knee osteoarthritis. LR-PRP has shown adverse effects on synovial cells, resulting in cell death and proinflammatory mediator production.[81,84] In contrast, for the treatment of chronic tendinopathy, leukocyte-rich PRP has demonstrated superiority over leukocyte-poor PRP.[85]

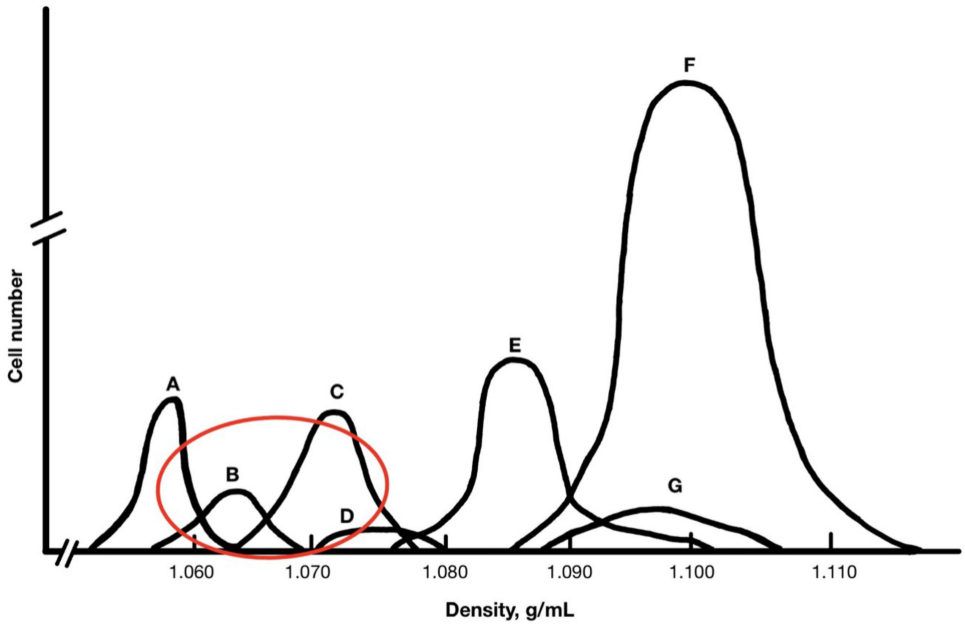

Figure 1 Density Gradients of Cells Contained within Blood Aspirate.
Notes: (A) Platelets, (B) Monocytes, (C) Lymphocytes, (D) Basophils, (E) Neutrophils, (F) Erythrocytes, (G) Eosinophils.

Unfortunately, a lack of universal preparation standardization and compositional reporting hampers our ability to collate data from individual studies and to gain consensus on findings. In most cases, blood is drawn from a patient, treated with an anticoagulant, and then centrifuged within an hour of collection. The methods then employed for isolating platelets and growth factors from whole blood can be broadly categorized based on two different distinctions: plasma vs buffy coat-based systems and single-spin vs double-spin processes. Plasma-based systems utilize a slower, shorter spin to isolate plasma and remove WBCs, resulting in platelet 2–3x's baseline levels (Figure 2A). [69] Contrary, buffy coat-based systems utilize a longer, double spin to isolate a platelet-poor layer (Figure 2B). This allows for an obtained platelet concentration of 3–8x that of baseline levels, however, because of the density it also keeps a concentration of WBCs.[69] Furthermore, single-spin processes, represented by many clinically used commercial devices, encompass variations such as low-platelet PRP (PRPLP) and high-platelet PRP (PRPHP). These one-step methods offer a more straightforward and less resource-intensive approach to PRP preparation. Conversely, double-spin processes (PRPDS) have historically been

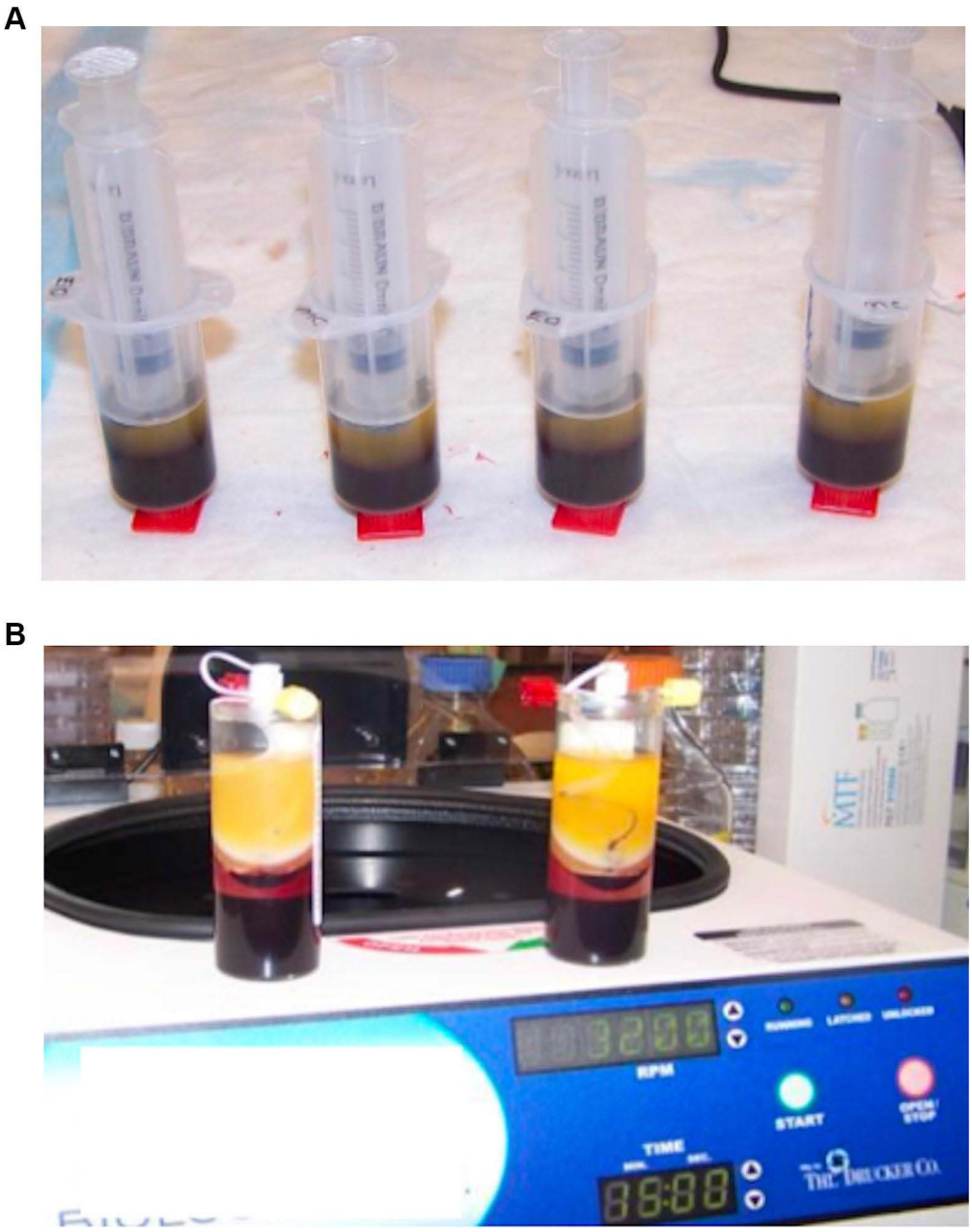

Figure 2 (**A**) Plasma-Based PRP Preparation. (**B**) Buffy Coat-Based PRP Preparation.

favored in basic science investigations due to their ability to produce PRP with higher platelet concentrations.[86] These methods often involve two sequential centrifugation steps, allowing for the separation of platelets from other blood components more effectively. However, recent studies have provided conflicting insights into the strengths and limitations of single-spin and double-spin processes. Notably, Mazzocca et al[87] demonstrated that PRPHP produced significantly higher platelet and white blood cell concentrations compared to both the single-step PRPLP and two-step PRPDS procedures. However, no significant differences were observed between PRPLP and PRPDS. Conversely, Saqlian et al[88] and Nagata et al[89] reported a greater platelet and WBC quantity following PRPDS compared to single-spin techniques. Additionally, when considering specific growth factors, Han Oh et al[88] demonstrated that PRPDS resulted in a significantly greater concentration of PDGF and VEGF whereas single-spin methods produced a significantly greater concentration of TGF and FGF. While findings by Mazzocca et al underscore the potential efficacy of one-step procedures and suggest that the increased time required for two-step procedures may not necessarily be advantageous for producing therapeutic PRP preparations, other studies still provide support to the historical superiority of PRPDS.

With the clinical advent of PRP use for bone and soft tissue indications, numerous commercial PRP preparation kits have entered the market. These devices offer the convenience of pre-packaged, standardized protocols, which can be especially beneficial for clinical applications. However, while designed to serve a common purpose, these kits exhibit noteworthy differences in multiple aspects of PRP preparation which manifests as variations in platelet, WBC, and RBC concentrations in the final PRP product. Numerous comprehensive reviews of currently available devices reveal substantial variability in their methodologies and the resultant PRP compositions. Dejnek et al[89] extensively evaluated four commonly used commercial PRP systems: Arthrex Autologous Conditioned Plasma (ACP), Mini GPS III, Xerthra, and Dr. PRP. Among the systems evaluated, Mini GPS III notably stood out, yielding significantly higher concentrations of platelets, WBCs, and RBCs compared to the other three systems. Additionally, in a systematic review of the 10 most referenced commercially available PRP systems, Oudelaar et al[83] found significant variations in platelet and leukocyte concentrations. The highest concentration of platelets was produced by the Cascade system, while the lowest concentration of platelets was generated by the ACP system. Notably, the GPS III system exhibited a significantly higher concentration of leukocytes compared to other systems. Furthermore, the study reported that the GPS III and SmartPrep systems had the highest platelet enrichment factors, while the ACP, RegenPRP, and Cascade systems showed lower platelet enrichment factors. Furthermore, when analyzing 33 different commercial systems, Fadadu et al[73] found a significantly positive correlation between maximum centrifuge spin force, platelet concentration, and PDGF concentration, however, spin time demonstrated no significant relationships. Additionally, 3 of the 33 systems resulted in a platelet count less than that of whole blood. A review by Magalon et al[90] also demonstrated that of the 36 PRP preparation systems analyzed, 11 resulted in a final product made up of more RBCs than platelets. These findings emphasize immense variability in commercially available PRP preparation systems. Thus, the choice of a specific commercial device plays a substantial role in determining PRP composition and underscores the importance of selecting the most suitable system based on the intended clinical application. Despite the profound importance of optimizing PRP's platelet concentration, the challenge of defining a singular optimal value is exacerbated by the variability in research methods employed by past studies.[87,91] Consequently, tailoring PRP to individual clinical contexts remains a dynamic process that considers the nature of the injury, the patient's unique characteristics, and the desired treatment outcomes.

Activation of PRP: Unleashing the Healing Potential

The activation of PRP is an important phase in its therapeutic application, as it serves to transform concentrated platelets into a biologically active state, primed to effectively stimulate the regenerative process. This activation process encompasses two key elements. Firstly, it involves the degranulation of platelets, liberating GFs from α-granules. Secondly, it triggers the cleavage of fibrinogen, initiating matrix formation—a clotting process that facilitates the development of a platelet gel, effectively constraining the secretion of molecules to the designated site.[92,93] Consequently, the choice of activator during PRP preparation becomes a critical determinant of its efficacy, influencing both the quantity and release kinetics of GFs from platelets within PRP. Research on the activation of PRP has unveiled a complex interplay of factors that significantly influence its clinical efficacy and therapeutic potential and activation methods have undergone significant development, with multiple techniques devised to unlock the potential of growth factors and other bioactive molecules.

One traditional method of activation involves the addition of bovine thrombin to PRP. Thrombin serves as a rapid activator of platelets, promoting degranulation and facilitating the conversion of fibrinogen into fibrin, resulting in the formation of a stable clot that effectively traps platelets at the target location.[94] This entrapment promotes immediate degranulation and the release of growth factors and cytokines. Prior studies have demonstrated that the rapid action of thrombin resulted in an immediate release pattern of approximately 70% of stored growth factors within 10 minutes and nearly 100% released within 1 hour.[29,95] While this method offers swift and substantial growth factor activation, it comes with a challenge—released growth factors are swiftly cleared, precluding their long-term stimulatory effects on cells. This concept has been supported by studies that have found that the rapid activation triggered by bovine thrombin results in a reduction in the overall quantity of growth factors accessible at the tissue location over time.[69,93] If not promptly utilized upon release, GFs risk degradation before additional tissue receptors become available.[95,96] Additionally, bovine thrombin has been shown to carry the potential for complications arising from the generation of antibodies that may result in immune-mediated coagulopathy.[86]

An alternative approach utilizes calcium chloride to convert autologous prothrombin to thrombin, resulting in platelets being trapped in a fibrin matrix. Numerous studies[97,98] have demonstrated that using calcium chloride as an activator can lead to higher concentrations of specific GFs, such as TGF-β(1) and PDGF-AB. Additionally, calcium-based activators have been shown to induce a gradual and extended process of platelet activation, leading to the progressive release of platelet content.[93,95] This sustained activation results in the gradual accumulation of endogenous thrombin, facilitating a slower and more extended release of growth factors spanning several days.[99] Consequently, this extended-release pattern addresses the need for sustained growth factor delivery necessary for the prolonged nature of bone regeneration.[100–102] Additionally, calcium chloride activation was observed to have advantages beyond its release kinetics. Zhuang et al[97] demonstrated that calcium chloride can avoid the complications related to antibody formation and immune-mediated coagulopathy associated with bovine thrombin due to the autologous formation of thrombin from prothrombin. However, calcium chloride activation is not without potential shortfalls as well. An excess of calcium has been shown to trigger the swift activation of the clotting cascade, inducing rapid degranulation.[102] Additionally, elevated calcium levels may enhance the activity of protein C, protein S, and antithrombin III, potentially destabilizing the fibrin clot and consequently shortening the therapeutic window for platelets.[102,103]

Fufa et al[104] initially proposed the concept of Type-1 collagen as a safe and effective alternative to bovine thrombin for clot activation in PRP. Their initial findings supported this notion by demonstrating a reduction in clot retraction and comparable levels of PDGF-AB and VEGF release. However, recent research has cast some uncertainty on its efficacy. While numerous studies[8,95] have observed a more sustained cytokine release pattern with Type-1 collagen compared to bovine thrombin, a contrasting perspective emerged from Cavallo et al,[93] indicating that collagen's platelet-activating capabilities were relatively weak, leading to a lack of clot formation and notably lower GF release compared to bovine thrombin and calcium chloride. While this approach holds promise, further investigations are imperative to elucidate the genuine activation potential of Type-1 collagen in PRP applications.

In some cases, PRP may be applied without exogenous activators. During local infusion, the presence of the natural clotting factor, thrombin, often suffices to activate platelets effectively.[105,106] This simplified activation process, however, may lead to variations in growth factor release contingent upon the specific clinical context. Additionally, in a recent meta-analysis[99] comparing activated and non-activated PRP, it was observed that non-activated PRP did not yield any substantial clinical improvements in terms of pain relief or functional scores when compared to a placebo. The choice of activator, whether it be calcium chloride, thrombin, collagen, or others, has a profound impact on clot formation, release kinetics, and the therapeutic potential of PRP. Understanding these factors is essential for tailoring PRP preparations to specific clinical needs and optimizing their effectiveness in various medical applications. In the context of bone healing, achieving sustained and controlled release of growth factors is often desirable as this aligns with the gradual and intricate nature of bone regeneration. Ongoing research continues to shed light on this dynamic field, enhancing our ability to harness the therapeutic potential of PRP for improved patient outcomes.

Delivery: Tailoring Application Methods

The manner in which PRP is delivered to the target site also plays a role in optimizing PRP's therapeutic potential for bone healing. Clinically, PRP is often given through direct injection, topical application, or in combination with a surgical procedure

and remains a widely employed and versatile clinical delivery method for bone healing. By injecting PRP directly into the affected site, clinicians can promote a concentrated release of growth factors precisely where they are needed most. Thus, this method expedites the regenerative process by providing a high concentration of growth factors directly to the injury site. Precision of delivery to the targeted tissue using ultrasound may also enhance clinically efficacy.[107–109] Additionally, topical application of PRP has gained recognition as an effective clinical approach for surgical or wound site(s), promoting tissue repair, reducing inflammation, and accelerating the healing process.[110–112] Furthermore, in addition to standalone PRP delivery, clinicians frequently combine PRP with surgical procedures involving bone grafts. This approach aims to optimize the integration of graft materials and enhance the overall success of the surgical intervention. For instance, PRP may be mixed with bone graft materials such as autografts, allografts, or synthetic grafts before implantation. However, this combination has shown contradicting effects on the enhancement of the graft's osteogenic potential and ability to accelerate bone healing and reduce the risk of graft rejection.[113–116]

To address the need for sustained growth factor release, researchers have employed scaffolds as delivery vehicles for PRP. In the context of bone healing, scaffolds can play a critical role in maintaining the integrity of the injury site, preventing migration of PRP, and enabling controlled and sustained release of growth factors.[117] Scaffolds such as hydrogels, sponges, and nanofiber-based structures offer the ability to tailor the release kinetics of PRP-derived growth factors.[118] Thus, the choice of scaffold material can influence factors like degradation rate, which, in turn, affects the release profile of PRP components. Hydrogels and sponges, composed of materials such as alginate and gelatin, have demonstrated their efficacy as delivery systems for PRP in bone regeneration. These systems offer the advantage of tailorable scaffold degradation, which affects the release of incorporated factors, making them ideal for sustained delivery and enhanced bioavailability of growth factors at the injury site.[119] In support of this, Lin et al[120] incorporated PRP into an alginate hydrogel, demonstrating that the growth factors released from the hydrogel stimulated the osteogenic differentiation of human MSCs in vitro. Lu et al[121] further investigated the growth factor release kinetics of PRP-incorporated alginate hydrogels, showcasing the varying release profiles based on carrier type and the potential of these factors to promote osteoblast-like cell proliferation and activity. In addition to alginate, gelatin, a denatured collagen derivative, has gained attention as a base material for scaffolds in bone healing. Gelatin shares functional groups with collagen, the primary organic component of bone, yet is easier to obtain and less expensive making it an attractive option.[119,122] Animal studies by Hokugo et al[123,124] have demonstrated that PRP growth factors can be immobilized within gelatin hydrogels, leading to growth factor release correlating with hydrogel degradation. Such studies highlight the potential of hydrogels and sponges to offer controlled and sustained delivery of PRP-derived growth factors, contributing to enhanced bone healing both in vitro and in vivo. Incorporation of bioactive inorganic calcium phosphates, such as carbonated hydroxyapatite (CHA), into PRP-based scaffolds holds significant promise for bone healing and regeneration. Kaur et al[125] conducted a study in which they explored the combination of PRP and CHA, finding that this hybrid scaffold yielded significantly enhanced histological bone formation. This suggests that the integration of CHA into PRP delivery systems can enhance the osteogenic potential, potentially accelerating bone healing. Additionally, a study by Liu et al,[126] focusing on the inclusion of platelets in calcium phosphate cement, indicated promising outcomes for angiogenesis and osteogenesis. Furthermore, an animal study by Qiu et al[127] adds to the growing body of evidence supporting the positive impact of PRP in combination with calcium phosphate cement on bone regeneration by demonstrating favorable results in minipigs. These studies underline the versatile applications of PRP, especially when combined with calcium-based materials, in promoting both vascularization and bone tissue formation. PRP has also been covalently or ionically bonded onto plasma polymers, showcasing enhanced scaffold properties.[128] Specifically, it has been reported that the application of poly-ε-caprolactone (PCL) nanofibers coated with PRP substantially enhances the survival and growth of human MSCs.[128] These findings emphasize the diverse strategies available for optimizing PRP delivery systems and their potential to enhance bone healing through various approaches, including surface modifications and the development of novel biomaterials.

A Review of Pre-Clinical and Clinical Studies

Vitro Pre-Clinical Studies

This comprehensive review identified 24 in vitro studies that investigated the effects of PRP or a related derivative, on various cell types.[31,32,61,67,72,129–147] The diverse spectrum of cell types included osteoblasts, fibroblasts, osteocytes,

myocytes, tenocytes, human umbilical vein endothelial cells, bone marrow mesenchymal stem cells, marrow stromal cells, and human osteosarcoma cell lines. Among these studies, 14 (58%)[32,61,67,129,136–140,142–144,146,147] of the 24 studies reported PRP increased cell proliferation, 7 studies (29%)[67,132,134,135,139,141] reported PRP increased expression of bone-related genes and growth factors, and 5 studies (21%)[31,132,143,146,147] reported PRP increased cell migration. Notably, several of these investigations highlighted the effects of PRP were dose-dependent with differing cell responses at different concentrations.[61,67,72,142,144,146] In addition, 2 studies (8%)[140,141] provided evidence suggesting that PRP has the potential to facilitate osteogenic differentiation of pluripotent stem cells. Furthermore, 1 study (4%)[137] reported PRP's ability to induce tubular formation in human umbilical vein endothelial cells (HUVECs). Another study (4%)[145] indicated an increase in osteoblast viability and adhesion following PRP exposure. Conversely, 2 studies (8%)[130,131] did not discern any notable impact of PRP on cell behavior. Lastly, 1 study (4%)[72] found that concentrations of PRP exceeding 60% decreased cell viability and migration. A summary of the main details of all in-vitro pre-clinical studies can be found in Table 2.

Table 2 Effects of PRP on Cell Behavior in vitro

Author (Year)	Cell Type(s) Used	Control Group(s)	PRP Effect on Cells
Kinoshita et al (2020)[31]	Human osteoblasts	Cell media only	Fresh and freeze-dried PRP increased osteoblast proliferation
Kanno et al (2004)[129]	Human osteosarcoma cell lines HOS and SaOS-2	Cell media only	PRP increases HOS and SaOS-2 proliferation in dose-dependent manner
Fernandez-Medina et al (2019)[72]	Human osteoblasts	Thrombus (clot)	Reduced cell viability and migration above concentrations of 60%
Ferreira et al (2005)[142]	Human osteoblasts	None	PRP increases osteoblast proliferation in dose-dependent manner up to 50% concentration
Steller et al (2019)[143]	Osteoblasts and oral fibroblasts	Cell media only	PRP and PRF increased proliferation and migration of osteoblasts and fibroblasts, counteracting the negative effects of zoledronic acid
Ogino et al (2016)[144]	Human osteosarcoma cell line SaOS-2	Platelet poor plasma (PPP)	PRP increases cell proliferation in dose-dependent manner
Vahabi et al (2019)[45]	MG-63 osteoblast-like cells and human fibroblasts	Cell media only	PRP increases viability and adhesion of osteoblast like cells and fibroblasts
Celotti et al (2015)[146]	Human osteosarcoma cell line SaOS-2	Cell media only	PRP increases cell proliferation and migration in dose-dependent manner
Wang et al (2018)[147]	Human osteoblasts	Cell media only	iPRF more so than PRP, promoted osteoblast proliferation and migration
Graziani et al (2006)[61]	Human osteoblasts and fibroblasts	Cell media only	PRP increases cell proliferation of both osteoblasts and fibroblasts in dose dependent manner up to 50% concentration above which caused reduced cell proliferation
Vahabi et al (2017)[130]	MG-63 osteoblast-like cells and human fibroblasts	Cell media only	PRP did not show significant increase in cell proliferation
Casati et al (2015)[30]	Human osteosarcoma cell line SaOS-2	Cell media only	PRP stimulates cell migration
Slapnicka et al (2008)[131]	Human osteoblasts	Cell media only	PRP did not significantly increase cell proliferation
Martinotti et al (2014)[132]	Human osteosarcoma cell line SaOS-2	Cell media only	PRP promotes cell migration and induces a mixed osteoclastic/osteogenic gene expression

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Table 2 (Continued).

Author (Year)	Cell Type(s) Used	Control Group(s)	PRP Effect on Cells
Gaßling et al (2009)[133]	Human osteosarcoma cell line SaOS-2, human osteoblasts, and human fibroblasts	Cell media only	PRP led to increased growth factor secretion compared to PRF
Herrera et al (2012)[134]	Human osteosarcoma cell line SaOS-2	Cell media only	PRP increases osteoblast activity and cytokine release
He et al (2009)[135]	Rat osteoblasts	Cell media only	PRF led to gradual and sustained release of cytokines compared to PRP
Mazzocca et al (2012)[136]	Human osteocytes, myocytes, and tenocytes	Cell media only	All forms of PRP increased cell proliferation of all cell types
Mooren et al (2010)[137]	Rat osteoblast-like cells and human umbilical vein endothelial cells (HUVECs)	Cell media only	PRP promotes proliferation of osteoblast-like cells and promotes tubular formation in endothelial cells in a dose-dependent manner
Garcia-Martinez et al (2012)[138]	Human osteoblasts	Cell media only	PRP increases cell proliferation and altered expression of cell-surface markers
Zou et al (2014)[140]	Rabbit bone marrow mesenchymal stem cells (BMSCs)	Cell media only	PRP can promote proliferation and osteogenic differentiation of BMSCs
Bi et al (2010)[139]	Goat marrow stromal cells (MSCs)	Cell media only	Cell growth and alkaline phosphatase activity greater on the TCP +PRP composite compared to TCP and cell media alone control.
Chen et al (2013)[67]	Rate Bone marrow mesenchymal stem cells (BmMSCs)	Cell media only	Greater cell proliferation in high and medium concentration PRP. Higher alkaline phosphatase activity in low and medium concentration PRP, but inhibited activity in high concentration PRP.
Qi et al (2015)[141]	Bone marrow mesenchymal stem cells (BmMSCs)	Cell media only	Increased expression of collagen I, collagen III, tenomodulin, and osteocalcin genes, increased alizarin red staining, and increased alkaline phosphatase activity in PRP group suggestive of ability of PRP to promote osteogenic differentiation

Vivo Pre-Clinical Studies

A total of sixty pre-clinical in vivo animal studies were identified which investigated the impact of PRP on bone healing.[63,67,123,139,141,148–202] The animal models employed in these studies exhibited a notable variation in usage, with rabbits being the most commonly utilized model in 25 studies (42%), followed by rats in 12 studies (20%) and sheep in 8 studies (13%). Conversely, the less frequently employed animal models included goats in 2 studies (3%), pigs in 2 studies (3%), and mice in 1 study (2%). Regarding the bones studied, the tibia was the most frequently examined bone in 23 studies (38%), followed by the femur in 18 studies (30%) and the radius in 11 studies (18%). In contrast, the skull/forehead was among the least studied bone in 3 studies (5%), along with the fibula and metatarsal, each studied in 2 studies (3%).

Out of the 43 in vivo animal studies that incorporated scaffolds-based delivery methods, a variety of scaffold types were employed. Examples included calcium phosphate, bone autograft, bone allograft, gelatin hydrogels, titanium mesh, collagen, ceramic-coated hydroxyapatite, and coral. In several instances, studies compared the effectiveness of PRP delivery with and without a scaffold, with the most favorable outcomes generally observed when PRP was administered alongside a scaffold.[123,150,154,161,168,175,176,178] Notably, the dose-dependent response of PRP observed in in vitro studies was also echoed in some of the in vivo experiments.[67,190]

Of the 45 pre-clinical animal studies that evaluated radiographic bone healing, 36 studies (80%) reported improvements when PRP was employed, whereas 7 studies (16%) did not reveal any radiographic improvement, and 2 studies (4%) even indicated reduced radiographic bone healing. Similarly, out of the 58 pre-clinical animal studies assessing histopathologic bone healing, 43 studies (74%) reported positive outcomes when PRP was applied. Conversely, 13 studies (22%) did not detect any histopathologic improvement, and 2 studies (3%) reported reduced histopathologic bone healing in association

Table 3 Effects of PRP on Bone Healing in Pre-Clinical in vivo Animal Models

Author (Year)	Animal Model (Bone)	Scaffold Used	PRP Group(s)	Control Group(s)	Endpoint	Blinded Evaluation?	Radiographic Outcome	Histopathologic Outcome	Biomechanical Outcome
Rai et al (2007)[164]	Rat (femur)	Polycaprolactone tricalcium phosphate (PCL-TCP)	PCL-TCP + PRP	PCL-TCP	12 weeks	X-rays	Increased bone formation in PRP group by x-ray and micro-CT	Similar qualitative outcomes between PRP group and control group	PRP group stiffer but no difference in yield and maximum torque
Cho et al (2013)[177]	Dog (tibia)	None	PRP	Untreated	16 weeks	None	Bone activity index on nuclear scan greater at 4 weeks in PRP group, but less in PRP group at weeks 8, 12, and 16	Bone-to-implant contact (BIC) was higher for the PRP group	None
Dallari et al (2006)[178]	Rabbit (femur)	Freeze-dried bone allograft (FDBA)	PRP Bone marrow stromal cells (BMSCs)+PRP FDBA+PRP BMSCs+FDBA +PRP	Untreated	12 weeks	Histology	None	Increased bone healing in all experimental groups compared to control. Increased bone healing in FDBA+PRP, and BMSCs +FDBA+PRP compared to PRP alone	None
Guzel et al (2015)[196]	Rat (femur)	None	PRP	Untreated	9 weeks	Histology and biomechanics	None	Increased bone healing in the PRP group	Higher ultimate failure load in PRP group
Hakimi et al (2010)[197]	Mini pig (tibia)	Autologous bone graft	Autologous bone graft+PRP	Autologous bone graft	6 weeks	None	Similar rates of osseous bridging on x-rays	Superior bone formation in central and cortical defect zone in PRP group	None
Hokugo et al (2005)[123]	Rabbit (ulnar)	Gelatin hydrogel	Gelatin Hydrogel+PRP Fibrin+PRP PRP	Gelatin hydrogel Untreated	4 weeks	None	Greatest rate of bone healing in Gelatin hydrogel+PRP group followed by the Fibrin +PRP group	Greatest rate of bone healing in Gelatin hydrogel+PRP group followed by the Fibrin +PRP group	None
Jungbluth et al (2010)[198]	Mini pig (tibia)	Calcium phosphate granules (CPG)	CPG+PRP	CPG	6 weeks	X-rays	Semi-quantitative analysis showed slightly more osseous bridging in PRP group	Greater new bone formation in PRP group in central and cortical defect zones	None

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Kanthan et al (2011)[150]	Rabbit (tibia)	Artificial bone graft (ABG)	ABG+PRP PRP	ABG Untreated	11 weeks	X-rays	Greatest healing in ABG +PRP group compared to all other groups. PRP alone better than untreated control but no different to ABG alone	Greatest healing in ABG +PRP group. ABG better healing than control. PRP did NOT have better histologic healing than untreated control.	None
Kasten et al (2008)[151]	Rabbit (radius)	Calcium-deficient hydroxyapatite ceramic scaffold (CDHA)	CDHA+PRP CDHA+PRP +mesenchymal stem cells (MSCs)	CDHA Untreated	16 weeks	None	Bone formation greater in CDHA+PRP and CDHA+PRP+MSC groups compared to CDHA alone, as measured by micro-CT	Bone formation greater in CDHA+PRP and CDHA+PRP+MSC groups compared to CDHA alone	All test groups were stiffer than untreated control. No difference in stiffness between test groups
Kroese-Deutamn et al (2008)[152]	Rabbit (radius)	Titanium fiber mesh (TFM)	TFM +autologous bone+PRP TFM autologous bone	TFM	12 weeks	None	Bone healing seen in all test groups but not in all control groups	Greater bone formation in TFM+autologous bone +PRP than all other groups	None
Kurikchy et al (2013)[153]	Rabbit (femur)	Xenogeneic bone graft (XBG)	XBG+PRP	XFG Untreated	4 weeks	None	None	Increased number of osteocytes, osteon diameter, and lamellar thickness in XBG+PRP groups compared to all other groups	None
Lin et al (2013)[154]	Rabbit (femur)	Nanohydroxyapatite-type I collagen beads (CIB)	CIB+BMSCs +PRP CIB+PRP PRP+BMSCs PRP	Untreated	8 weeks	Histology	Greater bone healing in all experimental groups compared to untreated control as assessed on micro-CT	Increased bone formation in CIB +BMSCs+PRP and PRP +BMSCs groups	None
Lysiak et al (2008)[155]	Rabbit (femur)	Collagen	Collagen+PRP	Untreated	12 weeks	None	None	Greater bone formation in experimental groups compared to control group	None

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Table 3 (Continued).

Author (Year)	Animal Model (Bone)	Scaffold Used	PRP Group(s)	Control Group(s)	Endpoint	Blinded Evaluation?	Radiographic Outcome	Histopathologic Outcome	Biomechanical Outcome
Malhotra et al (2014)[156]	Sheep (tibia)	Biphasic calcium phosphate (BCP)	BCP+PRP	BCP Untreated	4 weeks	X-ray and histology	Greater bone healing in BCP+PRP compared to all other groups via micro-CT Greater bone healing in BCP+PRP group compared to untreated but no different to BCP alone on x-ray.	Greater bone formation in BCP+PRP compared to BCP alone and untreated control.	None
Manitha et al (2009)[158]	Goat (femur)	Tri-phasic ceramic-coated hydroxyapatite (HASi)	HASi+BMSCs +PRP	HASi	8 weeks	None	Greater bone formation in the experimental group compared to HASi alone	No significant difference in bone formation between all groups	None
Niemeyer et al (2010)[160]	Sheep (tibia)	Collagen sponges (CS)	CS+Adipose-tissue derived stems cells (ASCs)+PRP	CS	26 weeks	None	No significant difference in bone formation between test group and control assessed on x-ray	No significant difference in bone formation between test group and control assessed on histology	None
Parizi et al (2012)[162]	Rabbit (radius)	Coral	Coral+PRP	Coral Untreated	8 weeks	Gross evaluation	Improved bone healing in the coral and coral+PRP groups compared to untreated control	Improved bone healing in the coral and coral+PRP groups compared to untreated control	PRP with coral group had higher ultimate load than the negative control group, whereas coral group alone did not
Simman et al (2008)[166]	Rat (femur)	None	PRP	Untreated	4 weeks	Fully blinded analysis	Higher callus to cortex width ratio in PRP group	No difference in BMP2 or total TGF-B expression between the groups	Increased strength in PRP group
Souza et al (2012)[167]	Dog (radius)	None	PRP	Untreated	8 weeks	Fully blinded analysis	Greater healing in the PRP group	PRP group showed new bone formation superior to control group	None
Sugimori et al (2006)[168]	Rat (tibia)	Apatite foam (AF)	AF+PRP PRP	AF Untreated	12 weeks	None	None	AF+PRP has more bone formation than all other groups	None

Zhang et al (2013)[169]	Rabbit (radius)	Deproteinized bone matrix (DBM)	DBM+PR DBM+PRP +MSCs	DBM	12 weeks	None	Greater bone formation in all experimental groups compared to control, and greater bone formation in DBM +PRP+MSC compared to DBM+PRP	Greater bone formation in all experimental groups compared to control, and greater bone formation in DBM +PRP+MSC compared to DBM+PRP	None
Chaput et al (2007)[148]	Rabbit (femur)	Beaded metal implant (BMI)	BMI+PRP	BMI	5 weeks	None	None	No difference in bone growth between groups	None
Hernandez-Fernandez et al (2013)[149]	Sheep (femur)	None	PRP	Untreated	6 weeks	Fully blinded analysis	No difference in bone growth between groups	No difference in bone growth between groups	None
Molina-Minano et al (2009)[157]	Rabbit (tibia)	Autologous bone graft (ABG)	ABG+PRP PRP	ABG Untreated	8 weeks	Fully blinded analysis	No difference in bone growth between groups	No difference in bone growth between groups	None
Nather et al (2012)[159]	Rabbit (tibia)	Autologous bone graft Allograft	Allograft+PRP	Allograft	24 weeks	None	None	Allograft+PRP had more osteocytes than allograft alone. The greatest new bone formation, callus encasement index, and osteocyte count was seen in autograft compared to all other groups	None
Rabillard et al (2009)[163]	Dog (ulnar)	Calcium phosphate ceramic granules (CaP)	CaP+PRP	CaP	16 weeks	None	None	No difference in bone growth between groups	None
Sarkar et al (2006)[165]	Sheep (tibia)	Collagen matrix (CM)	CM+PRP	CM	12 weeks	None	No difference between groups on x-ray or CT	No difference between groups	No difference between groups
Lopez et al (2019)[63]	Dog (radius/ ulnar and tibia/fibula)	None	PRP	Untreated	24 weeks	Fully blinded analysis	Faster rate of bone healing in the PRP group	None	None

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Table 3 (Continued).

Author (Year)	Animal Model (Bone)	Scaffold Used	PRP Group(s)	Control Group(s)	Endpoint	Blinded Evaluation?	Radiographic Outcome	Histopathologic Outcome	Biomechanical Outcome
Orth et al (2018)[161]	Mouse (femur)	Microcalcite (MCA)	MCP+PRP PRP	Untreated	5 weeks	None	Bone volume higher in MCRP+PRP group compared to controls	Smaller callus formation in MCP+PRP group compared to control	Polar moment of inertia (PMOI—used as surrogate for mechanical stability) higher in MCRP+PRP group compared to controls
Szponder et al (2018)[173]	Rabbit (tibia)	Tri-calcium phosphate (TCP)	External fixator or intramedullary nail with TCP +PRP	None	12 weeks	None	Bone formation observed in both ex-fix and IMN group	Bone formation observed in both ex-fix and IMN group	None
Canbeyli et al (2018)[170]	Rabbit (femur)	None	PRP	Untreated	12 weeks	X-rays	Increased union rate in PRP group	Greater cortical callus formation, woven bone percentage area, fibroblast proliferation, and mature bone formation in PRP group	None
Kim et al (2014)[172]	Rat (ulna)	Gelatin hydrogel	PRP+SEW2871 (macrophage recruiter) PRP	Untreated	6 weeks	None	Greater bony healing and bone density observed in the PRP +SEW and PRP groups compared to controls as assessed by micro-CT	Greater bony healing observed in the PRP +SEW and PRP groups compared to controls	None
He et al (2015)[171]	Rabbit (radius)	Poly (lactic-co-glycolic acid) with calcium phosphate cement (PLGA-CPC)	PLGA-CPC +PRP	PLGA-CPC	12 weeks	None	No difference in healing at 12 weeks between groups. However, micro-CT showed more bone healing in PRP group compared to control	More bone formation in PRP group compared to control	None
Shafiei-Sarvestani et al (2015)[174]	Rabbit (radius)	None	PRP	Untreated	8 weeks	Histology	More bone growth and union in PRP group	Greater bony healing in PRP group	Greater ultimate strength in PRP group
Weibrich et al (2004)[189]	Rabbit (femur)	None	PRP	Untreated	4 weeks	None	None	Higher platelet concentration in PRP group but no difference in bone healing	None

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Wiltfang et al (2004)[190]	Mini pig (forehead)	Autologous bone graft Tricalcium-phosphate granules (Cerasorb) Bovine spongious blocks (BioOss) Bovine bone-inducing collagenous sponge (Colloss)	4 different scaffolds (listed left) with PRP (2 different concentrations)	4 scaffolds without PRP	12 weeks	None	PRP increased bone healing in the autologous bone group but not the other bone scaffold groups	PRP did not change bone healing	None
Thorwarth et al (2006)[188]	Pig (skull)	Autologous bone graft Deproteinized bovine bone matrix (DBBM)	2 scaffolds with PRP (2 different concentrations)	Autologous bone alone DBBM alone	26 weeks	None	No significant difference in bone mineralization	No difference in expression of bone-related gene expression	None
Bi et al (2010)[139]	Goat (tibia)	Tricalcium phosphate/chitosan composite (TCP)	TCP+PRP	Untreated	16 weeks	None	Improved bone healing in the TCP+PRP group	Higher rate of newly formed bone in the TCP +PRP group	TCP+PRP biomechanically equivalent to TCP alone
Kon et al (2010)[184]	Sheep (femur)	Hydroxyapatite-collagen nanocomposite scaffold	Hydroxyapatite-collagen nanocomposite scaffold+PRP	Untreated	24 weeks	Histology	Scaffold+PRP had worse bone regeneration than scaffold alone	Scaffold+PRP had worse bone regeneration than scaffold alone	None
Oryan et al (2012)[186]	Rabbit (radius)	Hydroxyapatite scaffold	Hydroxyapatite +PRP	Untreated	8 weeks	N/A	Scaffold+PRP and scaffold without PRP had equal bone formation but better than negative control	Scaffold+PRP and scaffold without PRP had equal bone formation but better than negative control	Ultimate strength greater in scaffold +PRP group compared to untreated defect control
Neves et al (2013)[185]	Rabbit (fibula)	None	PRP Hyperbaric oxygen + PRP	Untreated	8 weeks	None	None	Hyperbaric oxygen and PRP together or alone showed increased bone formation	None
Kasten et al (2012)[183]	Rabbit (radius)	Calcium-deficient hydroxyapatite (CDHA)	CDHA+PRP	CDHA alone	16 weeks	None	CDHA+PRP had greater bone formation	CDHA+PRP had greater bone formation	None

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Table 3 (Continued).

Author (Year)	Animal Model (Bone)	Scaffold Used	PRP Group(s)	Control Group(s)	Endpoint	Blinded Evaluation?	Radiographic Outcome	Histopathologic Outcome	Biomechanical Outcome
Chen et al (2013)[67]	Rat (femur)	None	PRP clot with low, medium, and high PRP concentrations	Untreated	8 weeks	Histology and radiology	Medium concentration PRP has increased bone healing	Medium concentration PRP has increased bone healing	Ultimate strength higher in medium concentration PRP
Gumieiro et al (2010)[181]	Rat (tibia)	None	PRP	Untreated	12 weeks	None	None	Increased bone formation in the PRP group	None
Filardo et al (2014)[180]	Sheep (metatarsal)	Biomorphic silicon carbide (BioSiC) scaffold	BioSiC+PRP	BioSiC alone	16 weeks	None	No radiographic difference in the PRP group	Increased bone formation in the PRP group	None
Velev et al (2015)[179]	Rabbit (tibia)	Calcium phosphate cement (CPC)	CPC+PRP	CPC alone	4 weeks	None	None	Increased bone formation in the PRP group	None
Zhong et al (2014)[192]	Dog (tibia)	Tricalcium phosphate (TCP)	TCP+PRP	TCP alone	12 weeks	None	Increased bone formation in the PRP group	Increased bone formation in the PRP group	Ultimate strength higher in TCP+PRP group
Qi et al (2015)[141]	Rat (femur)	Calcium phosphate particles (CPP)	CPP+PRP	CPP alone	4 weeks	None	Increased bone formation in the PRP group	Increased bone formation in the PRP group	None
Yilmaz et al (2014)[191]	Pig (tibia)	Tricalcium phosphate (TCP)	TCP+PRP	TCP alone	12 weeks	None	None	Increased bone formation in the PRP group	None
Hakimi et al (2014)[182]	Mini pig (tibia)	Calcium phosphate granules (CPG)	CPG+PRP +bone marrow concentrate (BMC)	CPG alone	6 weeks	None	Increased bone formation in the CPG +PRP+BMC group	Increased bone formation in the CPG +PRP+BMC group	None
Chen et al (2016)[176]	Rabbit (radius)	Calcium sulfate (CS)	CS+PRP PRP alone	CS alone	10 weeks	None	Increased bone formation in the CP +PRP group	Increased bone formation in the CP +PRP group	None
Bölükbaşı et al (2013)[175]	Sheep (tibia)	Biphasic calcium phosphate (BCP)	BCP+PRF PRF alone	Untreated	6 weeks	None	Increased bone formation in the BCP +PRF group	None	None

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Schneppendahl et al (2015)[187]	Rabbit (tibia)	Autologous bone graft	Autograft+PRP	Autograft	6 weeks	N/A	Increased bone formation in the Autograft+PRP group	Increased bone formation in the Autograft+PRP group	None
Batista et al (2011)[194]	Rabbit (tibia)	Tricalcium phosphate (TCP)	TCP+PRP	TCP+bone marrow concentrate	4 weeks	None	Similar bone formation in the TCP+PRP group on x-ray but increased bone formation in the TCP+PRP group on micro-CT	Increased bone formation in the TCP +PRP group	
Park et al (2016)[200]	Dog (femur)	None	PRF	Untreated	4 weeks	None	None	Increased bone formation in PRF group	None
Sindel et al (2017)[202]	Rat (skull)	None	PRF	Untreated	3 weeks	None	None	Increased bone formation in PRF group	None
Dulgeroglu et al[73] (2017)[195]	Rat (femur)	None	PRF	Untreated	4 weeks	Histology	Increased bone formation in PRF group	Increased bone formation in PRF group	None
Akyildiz et al (2018)[193]	Rat (tibia)	None	PRF	Untreated	6 weeks	Radiology and histology	Reduced bone formation in PRF group	Reduced bone formation in PRF group	None
Raafat et al (2018)[201]	Rat (tibia)	None	PRF Simvastatin +PRF	Untreated	8 weeks	None	Increased bone formation in simvastatin +PRF group	Increased bone formation in simvastatin +PRF group	None
Lucarelli et al (2005)[199]	Sheep (metatarsal)	Allograft	Allograft+bone-marrow-derived stromal stem cells (BmMSCs)+PRP +collagen	Allograft alone	16 weeks	Histology	Increased bone formation in PRP group	Increased bone formation in PRP group	Higher extraction torque values in the PRP group

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Table 4 Effects of PRP on Bone Healing in Clinical Studies

Author (Year)	Study Design	PRP Delivery	Bone(s) Studied	PRP Group (s)	Control Group(s)	Sorting Method	Number of Patients	Follow-Up	Outcome
Namazi et al (2016)[217]	Prospective randomized control trial	Intra-articular PRP injection	Radius	CRPP+PRP injection	CRPP	Non-blinded randomization	30	6m	PRP group shows decreased pain and increased function
Wei et al (2012)[225]	Prospective randomized control trial	Allograft bone+PRP	Calcaneus	ORIF+allograft +PRP	ORIF +allograft Autograft	Non-blinded randomization	175	72m	Better radiographic outcomes for allograft+PRP and autograft groups compared to allograft alone
Namazi et al (2016)[218]	Prospective randomized control trial	Intra-articular PRP injection	Scaphoid	Casting+PRP injection	Casting	Non-blinded randomization	14	6m	PRP group had decreased pain at rest and increased total function
Griffin et al (2013)[210]	Prospective randomized control trial	Fracture site PRP injection	Femur	CRPP+PRP injection	CRPP	Participant blinded randomization	200	12m	PRP reduced length of hospital stay, but risk of revision and clinical outcomes were equivalent
Rodriguez-Collazo et al (2015)[226]	Retrospective case series	Demineralized bone matrix (DBM)+PRP	Tibia/ fibula	Ilizarov fixator +DBM+PRP Ilizarov fixator +DBM +concentrate bone marrow aspirate (cBMA)	Ilizarov fixator+DBM	None	20	18m	Faster radiographic healing with PRP and cBMA compared to control
Samy et al (2016)[220]	Prospective randomized control trial	Fracture site PRP injection	Femur	CRPP+PRP injection	CRPP	Non-blinded randomization	60	12–48m	Faster radiographic healing with PRP group, no difference in functional outcomes
Chiang et al (2007)[206]	Prospective case series	Bone graft and autologous platelet gel at fracture site	Femur and tibia	Internal or external fixation, ± soft tissue reconstruction	None	None	12	24–40m	Possible benefit of using PRP to treat non-unions
Lee et al (2014)[213]	Prospective randomized control trial	Bone marrow aspirate concentrate (BMAC)+PRP at fracture site	Tibia	External fixator (limb lengthening)	External fixator alone	Non-blinded randomization	20	24m	Significant improvement in bone formation in PRP +BMAC group

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Calori et al (2008)[205]	Prospective randomized control trial	PRP injection at fracture site	Various	Surgical fixation + PRP	BMP-7 injection at fracture site	None	120	9–25m	Lower rate of clinical and radiographic union in PRP group compared to BMP-7 group
Liebergall et al (2013)[214]	Prospective randomized control trial	Demineralized bone matrix (DBM), mesenchymal stem cells (MSCs), and PRP injected into fracture site	Tibia	Surgical fixation + DBM+MSCs+PRP	Surgical fixation alone	Non-blinded randomization	24	12m	The PRP group decreased time to union
Bielecki et al (2008)[204]	Prospective case series	Platelet-leukocyte rich gel (PLRG) injection at fracture site	Tibia/ fibula	PLRG injection to fracture site	None	None	32	9m	Possible benefit of using PRP to treat non-unions
Peerbooms et al (2012)[219]	Prospective randomized control trial	PRP and bone chips at fracture site	Tibia	PRP and bone chips	Bone chips alone	Non-blinded randomization	41	3m	PRP group had lower bone density
Mariconda et al (2008)[216]	Prospective case series (compared to historical control group)	PRP injection at fracture site	Various	PRP and external fixator	External fixator alone	None	20	9m	PRP showed equal union rates compared to controls
Dallari et al (2007)[207]	Prospective randomized control trial	PRP and bone chips at fracture site	Tibia	PRP and bone chips PRP+bone chips +bone marrow stromal cells	Bone chips alone	Non-blinded randomization	33	12m	Higher rates of osseointegration in both PRP groups compared to control
Sanchez et al (2009)[221]	Retrospective case series	PRP and bone graft at fracture site at time of surgery, then repeated PRP injections into fracture site post-operatively	Various	PRP and bone graft	None	None	15	8m	Possible benefit of using PRP to treat non-unions
Malhotra et al (2015)[215]	Prospective case series	PRP injection at fracture site	Various	PRP injection	None	None	94	4m	Possible benefit of using PRP to treat non-unions
Galasso et al (2008)[208]	Prospective case series	PRP injection at fracture site	Various	Intra-medullary nail and PRP at fracture site	None	None	22	13m	Possible benefit of using PRP to treat non-unions
Say et al (2014)[222]	Prospective case series	PRP injection at fracture site	Various	PRP injection at fracture site	None	None	20	12m	Possible benefit of using PRP to treat non-unions

(*Continued*)

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Table 4 (Continued).

Author (Year)	Study Design	PRP Delivery	Bone(s) Studied	PRP Group (s)	Control Group(s)	Sorting Method	Number of Patients	Follow-Up	Outcome
Tarallo et al (2012)[224]	Retrospective case series	Bone graft+PRP	Ulna	Surgical fixation with bone graft +PRP	None	None	10	3–36m	Possible benefit of using PRP to treat non-unions
Golos et al (2014)[209]	Prospective case series	PRP injection at fracture site	Various	PRP injection	None	None	132	4m	Possible benefit of using PRP to treat non-unions
Bibbo et al (2005)[203]	Prospective case series	Autologous platelet concentrate (APC)	Various	APC+autograft APC alone	None	None	62	2m	Possible benefit of using PRP to treat high risk fractures
Kitoh et al (2007a)[211]	Retrospective case series	Bone marrow cells (BMCs)+PRP at distraction osteotomy site	Femur/ tibia	Distraction osteogenesis BMC+PRP	Distraction osteogenesis alone	None	20	N/A	Faster union rate in BMC +PRP group
Kitoh et al (2007b)[212]	Retrospective case series	Bone marrow cells (BMCs)+PRP at distraction osteotomy site	Femur/ tibia	Distraction osteogenesis BMC+PRP	Distraction osteogenesis alone	None	46	N/A	Faster union rate in BMC +PRP group
Sys et al (2011)[223]	Prospective randomized control trial	Autograft+PRP to posterior lumbar interbody fusion site	Lumbar spine	Autograft+PRP	Autograft alone	Non-blinded randomization, Radiologists were blinded	38	24m	No improvement in autograft+PRP compared to autograft alone

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with PRP. Biomechanical properties also displayed favorable trends, with 11 out of 13 pre-clinical animal studies (85%) reporting improvements in bone healing when PRP was employed. Only 2 studies (15%) did not observe any biomechanical improvement, and none indicated a reduction in biomechanical properties when PRP was used.

In summary, pre-clinical in vivo animal studies generally demonstrate overall positive effects of PRP on bone healing. However, the substantial variability in study designs and protocols makes direct comparisons challenging. Moreover, several studies combined PRP with other factors like stem cells or scaffolds, complicating the isolation of PRP's specific effects. Additionally, many studies compared interventions to untreated negative controls, which may not be ideal, and a few studies lacked control groups entirely. Furthermore, subjective evaluations and a lack of statistical comparisons were observed in several studies.[152,154,155,158,167,171,198] Lastly, the use of blinded analysis of specimens was inconsistent, with only 20 studies (33%) reporting its implementation. Table 3 presents a concise overview of the key information pertaining to all in-vivo pre-clinical studies.

Vivo Clinical Studies

There were 24 clinical studies that evaluated PRP to treat fractures in human patients (Table 4). [203–226] Among these studies, 11 were prospective randomized control trials, 8 were prospective case series, and 5 were retrospective case series. The bones predominantly examined were the tibia in 9 studies (38%) and the femur in 5 studies (21%). In terms of PRP delivery methods, 11 studies (46%) utilized PRP injection alone at the injury site, while 9 studies (38%) incorporated PRP with a scaffold, such as bone graft. Four studies (17%) involved the injection of PRP in combination with other substances like bone marrow aspirate or stem cells. The average number of patients per study was 52±52, with a range of 10 to 200 patients, and an average follow-up period of approximately 16±15 months (range 2–72 months). Of the clinical studies, 19 (79%) reported favorable clinical outcomes associated with the use of PRP to improve bone healing. Three studies (13%) demonstrated equivocal outcomes, while two studies (8%) indicated negative effects of PRP on bone healing. As with the pre-clinical studies, there is considerable variability among clinical studies, making it challenging to draw direct comparisons between outcomes. Notably, 9 studies (38%) lacked a control group, rendering it impossible to draw definitive conclusions due to the absence of a comparative baseline. Additionally, 14 studies (58%) did not employ any form of randomization in patient assignment to different treatment types within the study. It is worth noting that, to the best of our knowledge, there are no published double-blinded randomized control trials of PRP in the context of bone healing. Considering the existing body of research, which encompasses a reasonable number of patients and follow-up periods, future clinical investigations should prioritize the use of double-blinded randomized control trials to ascertain the true efficacy of PRP in promoting bone healing.

Conclusion

Recent evidence gathered in this extensive review of in vitro pre-clinical, in vivo pre-clinical, and clinical studies underscores the growing significance of PRP as a valuable adjunct in the domain of bone healing. In vitro investigations have demonstrated PRP's potential to stimulate various cell types, promoting proliferation, gene expression, and migration, thereby substantiating its regenerative potential at the cellular level. Pre-clinical animal investigations, despite the inherent diversity in experimental models and methodologies, affirm the positive impact of PRP on radiographic, histopathologic, and biomechanical aspects of bone regeneration. However, the landscape of pre-clinical research reveals the complexity of direct comparisons due to the variable inclusion of adjunctive factors. In the clinical arena, a majority of studies extend support for the beneficial role of PRP in bone healing yet emphasize the demand for more rigorous methodologies to delineate its precise therapeutic potential. Furthermore, investigations delving into dose-dependent PRP effects and the differentiation between PRP formulations concerning platelet concentration and leukocyte content also represent areas meriting further exploration.

Overall, PRP has emerged as a promising adjunctive tool in the context of bone healing, offering multifaceted advantages that encompass augmented cellular responses, accelerated tissue restoration, and potential expedited rehabilitation. However, advancing its integration into evidence-based medical practice necessitates meticulous and standardized clinical investigations, encompassing larger and more diverse patient cohorts, and employing well-defined outcome measures. These endeavors are poised to deepen our comprehension of PRP's therapeutic implications, particularly in the dynamic field of regenerative medicine, offering renewed optimism for individuals seeking enhanced musculoskeletal recovery.

Abbreviations

PRP, Platelet-Rich Plasma; LR-PRP, Leukocyte-Rich PRP; LP-PRP, Leukocyte-Poor PRP; PRPLP, Low-Platelet PRP; PRPHP, High-Platelet PRP; PRPDS, Double-Spin PRP; FDA, Food and Drug Administration; GF, Growth Factor; IL-1, Interleukin-1; IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-alpha; PDGF, Platelet-Derived Growth Factor; TGF-β, Transforming Growth Factor-beta; IGF-1, Insulin-Like Growth Factor-1; ECM, Extracellular Matrix; MSCs, Mesenchymal Stem Cells; BMPs, Bone Morphogenetic Proteins; VEGF, Vascular Endothelial Growth Factor; WBC, White Blood Cell; ACP, Autologous Conditioned Plasma; CHA, Carbonated Hydroxyapatite; PCL, Poly-ε-Caprolactone; HUVEC, Human Umbilical Vein Endothelial Cell.

Disclosure

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