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REVIEW

Platelet-rich plasma for bone healing and regeneration

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ABSTRACT

***Introduction*:** Successful healing of large bone defects (LBDs) is a complicated phenomenon because the body's natural ability often fails to effectively repair the LBDs. New modalities should be utilized to increase the quality and accelerate bone healing. Platelet concentrates in different forms can be considered an attractive option for such purpose.

Areas covered: Platelets as a natural source of growth factors, cytokines, and other micro and macromolecules are hypothesized to improve bone healing. This review has covered important concepts regarding platelet-rich plasma (PRP) including mechanisms of action, preparation protocols and their differences, and factors affecting the PRP efficacy during bone healing. In addition, the most recent studies in different levels which evaluated the role of PRP on bone repair has been reviewed and discussed to clarify the controversies and conflicts, and to illustrate a future prospective and directions for orthopedic surgeons to overcome current limitations and difficulties.

Expert opinion: As the efficacy of PRP is dependent on various factors, the outcome of PRP therapy is variable and unpredictable in orthopedic patients. Therefore, it is still too soon to suggest PRP as the first line treatment option in complicated bone injuries such as LBDs and nonunions. However, combination of PRP with natural and synthetic biomaterials can enhance the effectiveness of PRP.

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1. Introduction

Many factors including compound and complicated fractures, bone tumors (e.g. osteosarcoma), necrosis, severe osteomyelitis, advanced osteoarthritis, high energy trauma, and other pathologic diseases can be the initial cause of bone defects.[1–3] In most cases, it is often necessary to stabilize the remained viable bony segments and remove the diseased bony fragments that have no proper vascular supply from the patient's body.[4] Following orthopedic surgical interventions, it is likely that large bone defects (LBDs) are developed.[5,6] Moreover, extensive fibrocartilage tissue formation following self-healing reaction may lead to development of delayed unions or nonunions in 5–10% of cases.[5] Although the bone autografts are the most effective and gold method for surgical management of such defects, their application may be restricted by several disadvantages such as donor site morbidity, limitation in quantity and availability, long operative time and further surgery for tissue harvesting.[7–10] Alternatives such as allografts and xenografts may address such limitations; however, application of these options is also limited because of disadvantages such as lack of osteogenic property, immunogenesis, and risk of disease transmission.[6,11,12] Bone graft substitutes are associated with some challenging issues such as biodegradability and biocompatibility.[10]

Bone healing, modeling and remodeling, are regulated by a variety of hormones and local factors.[6,10,13] The main responsible factors include bone morphogenetic proteins (BMPs) such as BMP-2, BMP-7, vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF) which are expressed during bone healing process.[6,10,14] These growth factors show certain shortcomings when they are used alone.[9] For instance, single use of BMPs for accelerating bone healing is associated with some limitations such as high cost, short half-life in the body, need for high concentrations to overcome low availability and loss of bioactivity, increased risk of toxicity when used in high concentrations, possible development of osteolysis, ectopic bone formation, life-threating swelling and so on.[14,15] Therefore, it may be advisable to identify a source containing multiple growth factors that can accelerate bone healing. A simple way for utilization of advantages of the aforementioned growth factors can be the application of platelet concentrates such as

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Article highlights

- Healing and regeneration of large bone defects is a challenging issue in orthopedic researches.
- Platelet-rich plasma (PRP) is considered as an ideal and cost-effective source of growth factors and bioactive compounds.
- The application of PRP as a source of growth factors may be a superior option than a single use of growth factor for promoting bone repair and regeneration.
- PRP promotes bone repair and regeneration by various mechanisms which is mainly due to its inflammatory cytokines and growth factors.
- Several factors such as time of platelet activation, platelet activators, preparation methods, platelet concentration, and methods of application may affect the efficacy of PRP during bone repair.
- Based on the basic to clinical evidences, owing to the fact that there are several controversies regarding efficacy of PRP, it is still too soon to judge and offer such therapeutic option as an appropriate modality for promoting bone repair and regeneration.

platelet-rich plasma (PRP).[16,17] Because of autogenous nature of PRP, the issues of immunogenesis and disease transmission related to grafts are eliminated.[18,19] Platelets through different growth factors and cytokines in their granules can stimulate and regulate cell proliferation and differentiation, chemotaxis, adherence, and angiogenesis; all these criteria are critical especially in early stages of bone healing.[19,20] PRP has been mostly used as a gel form often by addition of calcium chloride and thrombin resulting in a burst release of platelet contents (70% within 10 minutes and about 100% within one hour).[21,22] In addition to the soft tissues, platelet concentrates have extensively been used in the treatment of osteoarthritis,[23] oral and maxillary surgery,[24] and repair of LBDs.[16] Because bone healing is a long-term process, there is an obvious need for effective scaffolds or delivery vehicles for sustained release of PRP-derived factors into the damaged site.[22] Although positive effects of PRP have been reported in bone healing, there are also some controversies.[25] In the present review, we presented useful information regarding PRP, its mechanisms of action on bone healing, and reviewed and discussed the most recent studies regarding the effectiveness of platelets on different aspects of bone healing from basic to clinical application.

2. Search strategy

We searched *in vitro, in vivo*, clinical studies and reviews published after 1995 which used various kinds of platelet concentrations in bone regeneration and were indexed in PubMed, Scopus, and Google Scholar. Keywords and their synonyms (alone or in combination) that were used in the search included PRP, platelet, platelet concentrates, platelet gel, anticoagulant, platelet activator, PRP preparation system, growth factor, inflammatory cytokine, WBC, RBC, inflammatory cells, fracture healing, fracture repair, bone, bone regeneration, and bone remodeling. Of 535 studies we found, about 129 investigations were included in different sections of the present review. The studies included in the present review had the following criteria: (1) For *in vitro* studies, only studies were included that had a guideline for the clinical practice and their data were valuable to be translated in the clinical setting. (2) For *in vivo* studies, only those were evaluated that (a) used a clinically relevant bone injury model, (b) clearly indicated their methods of PRP preparation and platelet concentration, (c) had translational aspects so that they used clinically relevant methods of bone evaluation (e.g. histopathology, CT scan, etc.), and (d) clearly reported the main outcome of PRP therapy on bone regeneration and repair. (3) For clinical studies, we initially removed the case reports. We then included those clinical studies that indicated (a) type of the study, (b) number of patients, (c) model of bone defect, (d) clarified the materials used for bone reconstruction, (e) included exact concentration of PRP, (f) mentioned evaluation methods, and (g) added the main results and outcomes. (4) For reviews, we evaluated the most recent reviews and meta-analyses and included those studies that covered the areas of interest of this review.

3. Platelet-rich plasma

In the field of bone healing, Marx and coworkers discovered the positive effect of PRP on bone regeneration in 1998.[26] They applied PRP with concentrations of 595,000 to 1,100,000 platelets/μl in combination with the autologous bone graft for reconstructing the mandibular defects and reported an increased bone mineral density and regeneration. PRP therapy is a cost-effective, safe, reliable, and easy method for accelerating and improving tissue healing and regeneration.[20,27] PRP is defined as a small volume of the plasma obtained by centrifugation that has a platelet concentration above the baseline levels of whole blood.[20,28] PRP is prepared from peripheral blood of the veins with an autologous, allogeneic, or xenogeneic source.[29–31] If PRP is prepared from autologous blood, it is theoretically associated with minimal risk of infectious diseases transmission, immunologic reactions, and rejection.[31] Normal counts of platelets in whole blood vary from 150,000 to 350,000/μl with an average of 200,000 platelet/μl.[28,32] The benchmark for therapeutic PRP has been considered to be PRP with a

platelet concentration from a three- to five-fold increase above the baseline platelet number in the whole blood.[28,33] Following the increasing importance of and attention to PRP in the treatment of musculoskeletal injuries, it has been estimated that the market for PRP will grow from $45 million in 2009 to more than $120 million by 2016.[32] There are several factors that affect the PRP efficacy in the bone healing process. Platelet activation before the application is an area of concern with PRP. For this purpose, an appropriate anticoagulant should be used to prevent the early spontaneous activation of the platelets and platelet aggregation.[34–36] Various anticoagulants have been employed for PRP preparation including heparin, citrate, acid-citrate-dextrose solution A or anticoagulant citrate dextrose-A (ACD-A), citrate-phosphate-dextrose (CPD), citrate-theophylline-adenosine-dipyrimadole (CTAD), and ethylene di-amine tetra-acetic acid (EDTA) (Table 1).[28,34–37] Regardless the selection of an appropriate anticoagulant, several manual or automated systems have been designed for preparing PRP including laboratory methods and commercial kits.[38] In addition to platelet-pheresis or autologous selective filtration technology and the cell separators, PRP is produced by single- or double-step gradient centrifugation methods.[5,20,27] However, these methods are rarely used and have limited application nowadays, this is due to their disadvantages including (1) high cost, (2) large volume of peripheral blood should be harvested from the patients, and (3) such methods may potentially damage the platelets.[27] On the other hand, the gravitational platelet sequestration (GPS) is unable to collect the RBCs and thereby they are discarded.[20] Commercially available PRP kits and devices differ in ease of use, one- or two-step centrifugation protocols, centrifugation speed, final PRP volume, platelet count and activation, platelet and growth factor concentrations, and final RBCs' and WBCs' count.[5,27,38–40] Nevertheless, the expensiveness of the kits for processing is still a challenge.[41] Moreover, lack of standardization of PRP preparations may lead to inconsistency in the literature. The number of centrifugations and centrifugation force are other important variables that may influence PRP bioactivity and efficacy.[36,42,43] Although these variables are still controversial, it has been suggested that double spine centrifugation is preferable as it easily isolates the platelets.[42] Additionally, it has been stated that double centrifugation method can prevent early activation of platelet and therefore provide higher concentrations of platelet and growth factors and it is superior to single centrifugation.[44] Regarding the centrifugation force, it has been reported that 230–270 g for 10 minutes is the most efficient speed and time of centrifugation for the first spine followed by a second spine at 2300 g for 10 minutes.[36] In another investigation, Dohan Ehrenfest and colleagues [43] reported that the range of 160–3000 g for 3 to 20 minutes is reasonable and desirable. Therefore, this variable is still an open question. Additionally, Kececi *et al*.[42] reported that the platelet concentration increased as the centrifugal force of the second spin increased from 300 g to 2000 g. On the other hand, it has been shown that high centrifugation forces may result in platelet damage, premature activation of platelets, and decrease the platelet function.[5] The selection of a suitable platelet activator before PRP application is another considerable criterion, but it has not been standardized. Platelets may be activated either endogenously or through adding an exogenous clotting factor to the PRP (Table 1).[5,18,45] In addition to the tissue collagen, centrifugation and needle-induced bleeding during PRP injection may provide endogenous clotting factors and cause platelet activation.[45] Exogenous activation lead to rapid clot formation and it is better to be delivered manually to the tissues rather than being injected, because the platelets are able to release the growth factors locally in the target site.[18]

4. Mechanisms of action of PRP on bone healing

The pro-inflammatory cytokines secreted by platelets regulate the inflammatory phase of bone healing. Platelets are recruited during the early hours and in cooperation with fibrin promote the hematoma formation.[5,10] Upon activation of platelets, they release their growth factors and cytokines thus regulate the inflammatory phase of bone healing and subsequently modulate soft and hard callus formation and bone remodeling (Figure 1).[20,46] Because of significant regulatory role of growth factors on cell migration, proliferation, differentiation, and maturation as well as matrix production and remodeling, they can effectively influence bone healing.[29] The platelet lifespan is approximately 7 to 10 days which is less than that of bone healing.[20] The active secretion of growth factors of the platelets starts since 10 minutes after blood clotting and over 95% of the presynthesized growth factors contained in the alpha-granules are secreted within the first hour.[45] Following the initial burst release of PRP-derived growth factors, during the remaining lifespan of the platelets, they continue to synthesize and secrete additional growth factors.[17,32,33] Consequently, PRP is supposed to influence early bone healing rather than late bone formation.

Table 1. Variables affecting the efficacy of platelet-rich plasma.

1- Anticoagulant	Advantages	Disadvantages
Heparin	It used in collecting whole blood for PRP production, preserves platelet function for prolonged periods	It is too expensive and activates platelets; platelets have less stability and fewer exhibited granules, early lyses and less released growth factor in heparinized plasma
Citrate or sodium citrate	It causes little and slow or no change in platelet shape and volume; preserves the normal discoid shape of platelet; and causes less pain at the injection site due to the low pH	It causes few dense granules and alpha-granules, early lyses, and high spontaneous platelet activation and aggregation and releases
Ethylene di-amine tetra-acetic acid (EDTA)	It is a strong chelator of Ca^{2+}; minimizes platelet aggregation and activation	It causes irreversible structural, biochemical, and functional damage to platelets
Acid-citrate-dextrose solution A or anticoagulant citrate dextrose-A (ACD-A)	Commonly used anticoagulant; it maintains platelet viability; causes less pain at the injection site due to the low pH; reduces platelet aggregation and spontaneous premature activation; maintains the platelet structural integrity; and stabilizes platelets	It is a weaker chelator of Ca^{2+} than EDTA
Citrate-phosphate-dextrose (CPD)	Similar to ACD-A, CPD is useful for PRP preparation	It has fewer supportive ingredients than ACD-A and is less effective in maintaining the platelet viability
Citrate-theophylline-adenosine-dipyrimadole (CTAD)	It maintains the platelet structural integrity and prevents the platelet spontaneous activation	It is a weaker Ca^{2+} chelator than EDTA
2- Platelet activation method	**Characteristics**	
Type I collagen	It results in sustained release of the growth factors; is as effective as thrombin in stimulating the growth factor release; is a safe, readily available, and inexpensive alternative to bovine thrombin in the clinical applications. Clots formed using type I collagen exhibited less retraction than those formed with bovine thrombin.	
Autogenous/bovine thrombin	It is potent stimulator of platelet and granulocyte activation; results in a reduction in total growth factor concentrations; and leads to immediate release of growth factors. Bovine thrombin stimulates antibodies against thrombin and endogenous factor V resulting in bleeding complications and anaphylactic shock.	
Calcium chloride ($CaCl_2$)	It is an inexpensive compound and causes pain and a burning sensation at the injection site; $CaCl_2$ plus thrombin produces a gel slower release of the growth factors available in PRP.	

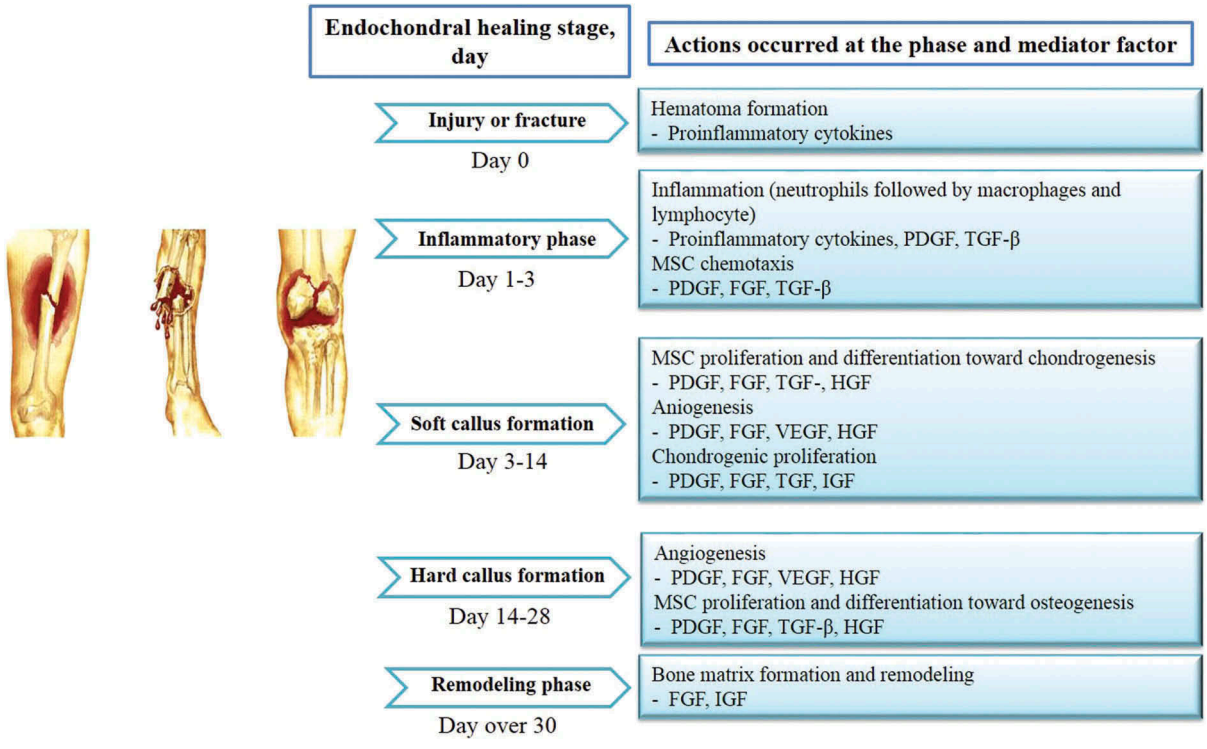

Figure 1. Typical processes of bone healing through the endochondral pathway in correlation with platelet-rich plasma contents.

There are increasing evidences that platelet-induced inflammation plays a critical role in the early bone healing and without a proper and robust inflammatory response, no effective healing should be expected. [28,47,48] However, exaggerated inflammation is also not useful for bone repair and platelets can modulate the inflammation. Recent investigations suggest that platelets are able to increase the inflammatory response for a short period of time and also reduce its duration and do not prolong the inflammation thus being able

to enhance the reparative phase of the healing process. [14,29,48] The main pro-inflammatory cytokines include interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α.[5,9] It has been shown that the exaggerated inflammation and leukocyte recruitment could be prevented by anti-inflammatory agents such as TGF-β1, IL-4, and hepatocyte growth factor (HGF) as well as TNF-α. [29,47,48] The beneficial role of PRP in reducing the exaggerated inflammation has previously been shown at osteoarthritis model.[49]

4.1. PRP and its growth factors

After secretion from the platelet granules, the growth factors bind to trans-membrane receptors on the target cells via their ligands.[39] The intracytoplasmic region of the ligand attaches to the receptor tyrosine kinase and a series of phosphorylation and activation steps of protein kinases within the cytoplasm occur. At the final step, the translocation of the phosphorylated kinases to the nucleus activates the transcription factors necessary for the gene expression and transcription.[5] This signaling pathway can both stimulates or inhibits cell proliferation and differentiation.[5,39] The level of growth factors released from the platelets is commonly measured by enzyme-linked immunosorbent assay (ELISA). [20] These growth factors include PDGF, VEGF, HGF, the TGF-β isomers, FGF, IGF, EGF, and platelet factor-4 (PF-4) [20,29,50] (Table 2). In a study performed by Weibrich *et al*.[51], the growth factor concentrations and platelet count of 115 PRP samples were analyzed. The platelet count in PRP was five times higher than the donor blood (1,407,640 ± 320,100/μl vs. 266,040 ± 60,530). Growth factors in PRP included PDGF-AB (117,763 ng/ml), TGFb-1 (169,784 ng/ml), IGF-I (84,723 ng/ml), PDGF-BB (1078 ng/ml), and TGFb-2 (0.470.3 ng/ml).

4.2. Lipid portion of PRP

Previously, it was thought that the beneficial effects of PRP is solely attributed to its growth factors. However, this hypothesis is paradoxical as newer findings demonstrated that the chronic wound environment is highly proteolytic in nature and capable of rapidly degrading peptide growth factors.[52] Therefore, the likelihood of the demonstrated healing potential of PRP being solely due to the peptide component is low. This suggests the presence of additional factors in PRP that are resistant to proteolytic degradation and able to promote healing process. The roles for lipid signaling molecules in multiple cellular biologies have extensively been established. [53] Direct relevance for the lipid component of PRP in biological mechanisms related to wound healing has recently been demonstrated that it has significant roles on cell proliferation and migration and more importantly, it overcomes proliferative growth arrest in the presence of chronic wound fluid.[54]

A primary challenge in the treatment of chronic wounds is the persistent incidence of neutrophils in the wound bed. This maintains the wound in a permanent inflammatory state and inhibits the normal course of wound repair.[53,54] It has been shown that the elevated lipoxin A_4 (LXA_4) in the lipid fraction of PRP is relevant toward explaining the pro-healing properties

Table 2. Growth factors in platelet-rich plasma and their roles in bone healing.

Growth factor	Source cells	Target cells	Mechanism of action
Platelet-derived growth factor (PGD-AA, -AB and -BB)	Platelets, macrophages, monocytes, endothelial cells, fibroblasts, and bone matrix	Fibroblasts, macrophages, neutrophils, chondrocytes, osteoblasts, and mesenchymal stem cells	Chemotactic and mitogenic for mesenchymal stem cells, fibroblasts, chondrocytes, and osteoblasts; regulating mesenchymal progenitor cells proliferation; chemotactic for macrophages and neutrophils
Vascular endothelial growth factor	Platelets and macrophages	Endothelial cells	Stimulating angiogenesis and endothelial cell proliferation, mitogenic for endothelial cells
Transforming growth factor-β (TGF-β1, -β2 and -β3)	Platelets, extracellular bone matrix, cartilage matrix, macrophages, and neutrophils	Endothelial cells, fibroblasts, monocytes, marrow stem cells, and osteoblasts	Chemotactic and mitogenic for fibroblasts and osteoblasts; regulating cell growth and osteoblast proliferation; inducing bone matrix deposition
Fibroblast growth factor	Platelets, macrophage, monocytes, osteoblasts, chondrocytes, fibroblasts, and endothelial cells	Endothelial cells, fibroblasts, bone cells, and smooth muscle cells	Stimulating endothelial cell migration and proliferation and therefore angiogenesis; regulating chondrocyte and osteoblast differentiation and mitosis
Insulin-like growth factor (IGF-1 and IGF-2)	Platelets, bone matrix, chondrocytes, fibroblasts, macrophages, monocytes, and endothelial cells	Osteoblasts, fibroblasts, and chondrocytes	Stimulating bone matrix formation, maturation, and remodeling; regulating preosteoblast and osteoblast proliferation and differentiation
Epidermal growth factor	Platelets, macrophages, and monocytes	Endothelial cells, fibroblasts, chondrocytes, and osteoblasts	Stimulating angiogenesis and chondrocyte and osteoblast differentiation and proliferation; mitogenic for mesenchymal cells such as osteoblasts and chondrocytes
Platelet fator-4	Platelets	Fibroblasts and neutrophils	Chemotactic for neutrophils and fibroblasts; promoting blood coagulation

of PRP.[54] The LXA_4 decreases neutrophil infiltration into a wound microenvironment while at the same time enhancing the recruitment of nonphlogistic monocytes.[55] The generation of LXA_4 likely occurs via a trans-cellular biosynthesis process where LTA_4 generated by neutrophils passively diffuses into the platelets, and is, in turn, acted upon by platelet 12 lipoxygenase to produce LXA_4.[56] The process of PRP generation will bring the platelets and neutrophils into close proximity, allowing for this trans-cellular synthesis. Also, the presence of exogenous 15-hydroxyeicosatetraenoic acid (HETE) (present in abundance in PRP) enhances the synthesis of another class of lipoxins (LXB_4) by neutrophils.[56] The high levels of LXA_4 in PRP, and the additional synthesis of LXB_4, in conjunction with neutrophils present in the wound bed may be responsible for PRP-induced transitioning of a chronic wound from its chronic inflammatory state into a granulating/proliferative state.[56,57]

5. WBCs and RBCs in PRP

In addition to platelets, two important components of blood include leukocytes and RBCs. Unlike the positive immune-modulatory effects of leukocytes, their inclusion in the PRP is debated.[18] Considering the high importance of leukocytes, DeLong *et al*.[18] proposed the PAW classification system for PRP on the basis of three variables including the number of platelets, manner of platelet activation, and presence or absence of WBCs. Based on this issue, two main categories have been considered for PRP, leukocyte-poor PRP (pure PRP) and leukocyte-rich PRP. With respect to leukocytes, they included granulocytes such as neutrophils, eosinophils and basophils, and agranulocytes or mononuclear cells such as lymphocytes, plasma cells, monocytes, or tissue macrophages.[39]

Phagocytosis of dead cells, necrotic tissue and debris by phagocytic cells such as neutrophils and macrophages, is an essential step during the early inflammatory phase of the healing process.[45] Macrophages also provide some growth factors and pro-inflammatory cytokines involved in the repair process that include TGF-β1, PDGF, IGF, EGF, FGF, and ILs.[58] In addition, macrophages by producing serine and metalloproteinases allow the inflammatory cells and other cells involved in the healing process to migrate into the defect site via degrading the provisional matrix.[45] Therefore, the exclusion of leukocytes particularly macrophages may be associated with reduced blood vessel density, delayed bone formation, and impaired remodeling.[18,45]

Although it seems that lymphocytes are important in tissue healing due to their role in triggering the inflammatory reactions, they may direct the healing process toward the remodeling reaction rather than inflammatory reaction via regulating the macrophages function.[17,47] In fact, T-helper lymphocyte type I and II regulate the macrophage type I and II, respectively.[10] Macrophages type I has more phagocytic activity than the type II and their elevation in the injured area is suggestive of an inflammatory reaction which is responsible for acute degradation of the implanted bone substitutes and acute rejection of the bone grafts. In contrast, promotion of the remodeling and acceptance of the bone grafts and bone graft substitutes have been suggested to be due to the activation of macrophages type II.[10,29] Overall, it seems that macrophages are the most important cells among leucocytes due to their modulatory effect on cell behavior through releasing several growth factors and cell stimulators.[29] Although neutrophils can interfere and destroy infectious agents, they may also induce local tissue destruction.[45,52] The high population of neutrophils in PRP may act as an antagonist for promoting the healing process because neutrophils can release reactive oxygen species resulting in a respiratory burst that damage the tissue.[59]

The primary and secondary granules of neutrophils contain proteases such as elastase, cathepsin G, proteinase 3, MMP-8, as well as MMP-9 resulting in extracellular matrix degradation. This allows cellular migration through the damaged tissue that is critical in tissue remodeling.[59,60] It is possible that neutrophils interfere with tissue regeneration by exaggerating local inflammation that can reduce the possibility of success with PRP therapy.[59]

WBCs can enhance the growth factor concentrations of PRP by releasing their own growth factors or by stimulating release of platelet growth factors.[58] Since leukocytes are important component of the immune system, they can negatively affect bone regeneration via triggering immunological responses for a long time.[61] Additionally, the high concentration of the pro-inflammatory mediators such as IL-1 present in leukocytes may increase the harmful effects of the inflammatory process such as swelling and pain.[61]

Based on the above explanations, it seems that high concentrations of leucocytes retard bone healing by inducing an exuberant inflammatory reaction so that the acute inflammation may be changed to a chronic state which is not beneficial for bone healing.[10] In contrast, because proper robust inflammatory response is important for a desirable bone healing, low concentration of leukocytes in PRP may not be able to induce a

proper inflammatory response at early healing stage. [10] Therefore, moderate concentrations of leukocytes in PRP may be desirable for bone healing [62,63]; however, this hypothesis should be further investigated in the near future. In addition to the above variables, RBCs should be eliminated in the PRP products because of their destructive capabilities due to the free radical production and induction of the spontaneous platelet clumping and aggregation.[29,31]

6. *In vitro* application of PRP

The beneficial role of PRP on cellular behavior has extensively been shown at *in vitro* level. For instance, PRP has been shown to induce proliferation of the bone marrow stem cells and their osteogenic differentiation *in vitro*.[44] Mesenchymal stem cells (MSCs) are multipotent stem cells that are able to differentiate into other cell types such as fibroblasts, chondrocytes, and osteoblasts as the main soft and hard connective tissues producing extra cellular matrix; therefore they can efficiently be used in healing and regeneration.[44,64] Given this fact that PRP is able to induce cell migration, differentiation, proliferation, and maturation, some studies have investigated these properties and concluded that combination of PRP with undifferentiated bone marrow-derived (BMSCs) and adipose tissue-derived stem cells (ASCs) could be an effective approach in increasing new bone formation and mineralization. [44,64,65] They showed that addition of PRP into the culture media containing MSCs can effectively enhance adhesion, migration, proliferation, and differentiation of the stem cells. These properties could contribute to the growth factors released from the PRP. Interestingly, in the *in vitro* study carried out by Huang and Wang,[66] the influence of PRP-derived growth factors on proliferation and osteogenic differentiation of the muscle satellite cells was assessed in rats showing the PRP-derived growth factors significantly increased osteogenic differentiation of these cells, represented excellent cell compatibility and enhanced cell proliferation. The authors suggested that the PRP-derived growth factors are appropriate for stem cell growth factors delivery and bone tissue engineering approaches. Nevertheless, some studies reported inconclusive results. For instance, Choi *et al*.[67] examined the effect of PRP concentrations on the viability and proliferation of alveolar bone cells *in vitro* and showed that viability and proliferation of bone cells can change in a concentration-dependent manner, so that the low concentrations of PRP (1 and 5%) are stimulator, whereas the high concentrations (10, 20, 30, 50 and 100%) can result in a decreased viability and proliferation of the bone cells. Although *in vitro* studies are important line of researches showing the cellular mechanisms of PRP, the *in vivo*-based studies are more valuable because of giving this opportunity to have a better understanding regarding the role of PRP on different aspects of bone healing.

7. *In vivo* experimental animal studies

The efficacy of PRP on healing of various models of bone defects have extensively been investigated in large numbers of experimental animal studies (Table 3). PRP can be used in different forms such as liquid or solution (inactivated), gel, hydrogel, sponge, or nanofiber.[22,68] On the other hand, PRP can be used either alone [69] or in combination with autograft,[26] allograft,[25] synthetic implants, and bone graft substitutes containing polymers and ceramics [8,67,70] in order to improve bone healing process. Most preclinical and *in vivo* studies are in agreement that the application of PRP is effective in stimulating and enhancing the healing processes of the bone defects. Nevertheless, there is no complete consensus on the beneficial effect of PRP on bone fractures, delay unions and nonunions, and some researchers believe that it cannot be effective in enhancing new bone formation.[24,71] Kim and colleagues [72] evaluated the effect of the grafts based on the autologous PRP, platelet-rich fibrin, and concentrated growth factor (as the experimental groups) on the healing of a skull defect model in rabbits. Based on micro-CT analysis, they showed that the experimental groups are associated with significantly greater bone mineral density and bone volume compared with the groups without any graft. However, the difference among the experimental groups was nonsignificant. Moreover, histomorphometric evaluation confirmed more new bone formation in the experimental groups than that in controls. They showed comparable osteogenic effects by PRP, platelet-rich fibrin (PRF), and concentrated growth factor (CGF).

In 35 *in vivo* animal studies investigated the role of PRP on bone healing (Table 3; Source: PubMed), several animal models have been used including rabbit (n = 14), rat (n = 5), dog (n = 2), sheep (n = 10), goat (n = 2), and minipig (n = 2). These studies used PRP as an adjunct to the treatment of calvarial (n = 7), radial (n = 5), mandibular (n = 6), cranial (n = 2), tibial (n = 4), femoral (n = 2), maxillary sinus (n = 2), ulnar (n = 1), calcaneal (n = 1), osteochondral (n = 1), sternal (n = 1), and talar (n = 1) defects. One study used PRP as a primary management strategy for a tibial inflammatory osteomyelitis model, whereas another study used it in a muscle pouch model. A total of 25 studies (about

Table 3. Effects of PRP on bone healing in animal experimental studies.

Reference	Injury model	Materials used	Animal model	Platelet concentration or count	Evaluation methods	Main outcomes
[73]	Maxillary sinus floor elevation	Autogenous PRP	12 sheep, 4 and 12 weeks	218–1751 × 10^6/ml in PRP vs. 6–268 × 10^6/ml in whole blood	Histology and histomorphometry	The newly formed bone was not significant with PRP after 4 and 12 weeks and PRP had poor regenerative capacity
[74]	Calvaria critical-sized defects	Autogenous PRP without any latency or with 5 days latency	16 sheep, 6 weeks	4 to 5 times higher than whole blood	Radiography and 3D-quantitative computer tomography	New bone was generated in all groups. PRP only had effect on bone regeneration when used immediately after the distraction gap formation
[75]	Maxillary sinus defects	DFDBA and CCFDBA with or without autogenous PRP	10 sheep, 3 and 6 months	2 to 5 times higher than whole blood	Histology and histomorphometry	PRP did not enhance and accelerate bone regeneration as combined with bone allograft
[76]	Tibial defects	Autogenous PRP-loaded collagen scaffold	16 sheep, 12 weeks	3.5-fold higher than whole blood	Radiography, CT, biomechanics, and histology	PRP did not enhance new bone formation
[77]	Mandibular defects	Autogenous mandibular bone, FHA alone, PRP-FHA, MSCs-PRP-FHA	8 minipigs, 3 months	1 × 10^6 /mL platelets	Histology and histomorphometry	MSCs-PRP-FHA were associated with higher percentage of newly formed bone compared with others
[78]	Calvarial defects	Autograft bone combined with 50, 100, and 150 µl autologous PRP	25 rats, 30 days	2611.80 ± 313.34 × 10^3 platelets/µl in PRP vs. 465.803 ± 93.46 × 10^3 platelets/µl	Radiology, histology, and immunohistochemistry	The proportion of autogenous bone graft influenced bone healing so that the best results obtained with AB/PRP-100 indicating high PRP concentrations could be deleterious
[79]	Tibial defects	CPG and autologous PRP	16 minipigs, 6 weeks	4.4-fold concentration of platelets in PRP	Radiology, histology, and histomorphometry	PRP combined with CPG promoted bone formation and regeneration but did not led to a solid fusion of tibiae
[80]	Critical-sized defects in the forehead (frontal bone)	Autogenous particulate cancellous bone plus deproteinized bovine bone mineral (Bio-Oss) particles ± autogenous PRP	20 goats, 2, 6, and 12 weeks	4 to 5 times higher than in whole blood	Histology and histomorphometry	PRP did not enhance the early and late bone healing
[81]	Bony mandibular defects	Allograft of frozen rib, rhOP-1® (rhBMP-7), allograft + rhOP-1 and autogenous PRP	15 sheep, 2 months	NA	Radiography, histology, histomorphometry, and immunohistochemistry	In the PRP group, there was no bone formation. Allograft plus rhOP-1 was superior to other groups followed by rhOP-1 in terms of new bone formation
[82]	Sternal defects	Autologous PRP, PRGF	24 sheep, 9 weeks	NA	Histology	New bone formation was accelerated in the PRGF group
[83]	Critical-sized tibial defects	Mineralized collagen, BMSC and ASC and ASC-human (xenogenous) PRP	20 sheep, 6 months	4–5-fold increase	Radiography and histology	ASCs were inferior to BMSCs in terms of osteogenic potential, but it was partially compensated by PRP
[84]	Critical-sized tibial defect, fixed using plate	Artificial bone graft (Coragraft®) and autologous PRP	12 rabbits, 11 weeks	2.62–4.61 (mean of 3.27 ± 1) times higher than the baseline	Radiography and histology	PRP + Coragraft showed the best bone healing, improved the quality of healing compared to PRP or Coragraft alone
[85]	Circular bicortical critical-sized cranial defect	HA/β-TCP ± autologous PRP	18 rabbits, 6 weeks	1,140,500 platelet/µl, based on similarity of hematologic profile between human and rabbit	Histology and histomorphometry	PRP had no effect on bone healing and there was no significant difference in new bone formation between two groups
[86]	Critical-sized radial defects	CDHA scaffold either + VEGF-transfected bone marrow stem cells or + allogeneic PRP	30 rabbits, 16 weeks	10.05 × 10^8/ml ± 3.2 × 10^8 vs. initial 1.9 × 10^8/ml ± 0.39 × 10^8 ml	Radiography, micro-CT scan, histology, and immunohistochemistry for blood vessels	Overall, bone healing was better with PRP than VEGF; VEGF + BMSC promote vascularization better than BMSC + PRP but similar to CDHA + PRP + BMSC
[87]	Osteochondral defects	Autologous PRP or CBMA with a biphasic collagen/GAG osteochondral scaffold	24 sheep, 26 weeks	3.9 to 33.9 times higher than in whole blood	Biomechanics, histology, and immunohistochemistry	Cyst formation was reduced by the addition of CBMA and PRP to the scaffold. Hyaline cartilage-like tissue formed in the PRP/scaffold group was more

(*Continued*)

Table 3. (Continued).

Reference	Injury model	Materials used	Animal model	Platelet concentration or count	Evaluation methods	Main outcomes
[25]	Muscle pouches for examining the effect of PRP on the osteoinductivity of DBM	Allogeneic DBM ± autologous PRP	6 rabbits, 3 weeks	NA	Histology and histomorphometry	PRP had negative effect on the early phase of osteoinduction by DBM
[15]	Critical-sized defect in the radial diaphysis	Xenogeneic/human PRP plus hydroxyapatite	36 rabbits, 8 weeks	2422×10^3/μl compared with 239×10^3/μl in the PRP and whole blood, respectively (more than 10-fold increase)	Radiology, histopathology, and biomechanical analyses	The hPRP in combination with hydroxyapatite enhanced bone healing
[49]	Tibial osteomyelitis	Autologous L-PRP gel, vancomycine	40 rabbits, 6 weeks	$217 \pm 91 \times 10^6$/ml and $4.8 \pm 1.3 \times 10^6$/ml in whole blood vs. $1561 \pm 702 \times 10^6$/ml and $24.2 \pm 24.2 \times 10^6$/ml in L-PRP, for platelets and leukocytes, respectively	Radiology, microbiology, and histology	L-PRP gel plus vancomycine eliminated infection and increased bone repair, L-PRP could be used as a favorable antimicrobial agent
[88]	Osteotomy of the femoral diaphysis after distraction osteogenesis	Injection of autogenous PRP on days 0, 10, and 20	20 sheep, 40 days	590.240 platelets/μl, 4.6 times higher whole blood	Radiology, CT, and histology	PRP showed no significant effect on bone formation in the early phases of distraction osteogenesis
[89]	Standardized circular calvarial defects	Bioactive glass (BG) and autologous PRP	10 rabbits, 12 weeks	NA	Radiography, histology, and histomorphometry	Both PRP and BG + PRP groups showed higher bone density and new bone formation
[90]	Alveolar bone defects	Allogeneic PRP, PRGF, simvastatin	18 rats, 45 days	NA	Histology	Simvastatin plus PRP and simvastatin plus PRGF showed nonsignificantly the mature and immature bone tissue. In fact, simvastatin did not improve PRP and PRGF effects
[91]	Osteochondral defects of the talus	Allogeneic DBM with autologous PRP	16 goats, 24 weeks	The median concentration of 1511×10^9 platelets/l	Micro-CT, histology, histomorphometry, and fluorescence microscopy	There were no significant difference between the groups and no beneficial effect of DBM ± PRP on the healing of the caprine talus was observed
[92]	10 mm diameter bone defect in calvarium	Autologous PRP gel in combination with BCP ceramics containing rhBMP-2	18 rabbits, 6 or 12 weeks	About 2.32-fold compared to the initial concentration	Radiography, histopathology, and histomorphometry	PRP and rhBMP-2 showed synergistic effects on new bone formation
[93]	Bilateral large radial defect	Allogeneic PRP + MSC + DPB, MSC + DPB, PRP + DBP, DPB alone	24 rabbits, 4, 8, and 12 weeks	4.1-fold higher amount of platelets in the whole blood ($12.87 \pm 0.848 \times 10^6$/μl vs. $3.172 \pm 0.4864 \times 10^6$ /μl)	Radiography, histology, histomorphometry, and immunohistochemistry	PRP + MSC + DPB led to the best bone regeneration and vascularization than others; a synergetic effect existed between allogeneic PRP and autologous MSCs; allogeneic PRP was associated with great healing efficacy with negligible immunogenicity
[94]	Circular mandible bicortical bony defects	BPBM and BGM ± autologous PRP	54 rabbits, 12 weeks	4.19–4.43-fold to that of the whole blood	Histology and pathology	New bone formation was seen at BPBM/BGM grafts ± PRP, with PRP, osteoblast activity, bone defects filling, and bone density were increased
[95]	Critical size calvarial defects (1 cm)	HA-TCP scaffold (Skelite™) and lyophilized allogenic PRP gel	24 rabbits, 8 and 16 weeks	3×10^6 platelet/μl	Micro-CT and histopathology	PRP plus Skelite enhanced late stage bone healing, progenitor cell recruitment, collagen, and bone matrix deposition in two durations
[69]	48 mandibular premolar defects	Autologous PPP, PRP, and PRF	12 dogs, 4 and 8 weeks	$18.6 \pm 2.2 \times 10^4$/μl in whole blood, $1.8 \pm 0.8 \times 10^4$/μl in PPP, $55.6 \pm 7.4 \times 10^4$/μl in PRP	Radiography and histology	Bone maturation was more with PFR and PRP, growth factor concentration was higher with PRP, but under more severe conditions, growth factors had a negative effect on bone formation
[96]	Ulnar defect	Gelatin hydrogels incorporated with SEW2871, a macrophage recruitment agent and autologous PRP	Rat, 6 weeks	Autologous PRP, concentration was not present	Radiography, micro-CT, histology, and RT-PCR	Hydrogels incorporating SEW2871 and PRP but no SEW2871 alone promoted bone regeneration to a greater extent
[97]	Eighty critical-sized cancellous bone defects	Autogenous PRP combined with a BCP vs. autograft	20 sheep, 4 weeks	393 ± 34 in whole blood vs. 1039 ± 127 in PRP	Radiography, micro-CT, histology, histomorphometry, and fluorochrome bone labels	All doses of PRP were more effective than the BCP alone for new bone area and bone ingrowth depth. PRP-induced new bone formation in a dose-dependent manner

(*Continued*)

Table 3. (Continued).

Reference	Injury model	Materials used	Animal model	Platelet concentration or count	Evaluation methods	Main outcomes
[98]	Endosseous defect model (integration of bone implants in the femur with methylcellulose gel vehicle	PDGF, IGF-I, and autologous PRP	8 rabbits, 12 days	An average of 50,000–70,000 platelets per µl	Histopathology and immunohistochemistry	A combination of PDGF and IGH-I were more effective than PRP in bone regeneration
[65]	Three-wall intrabody defects around dental implants with a HA scaffold	Human BMMSCs and autologous PRP	4 dogs, 12 weeks	1×10^6 platelets/µl	Histology and histomorphology	The highest bone density and bone maturation was obtained with HA + BMMSCs + PRP but it was nonsignificant
[70]	Segmental radial and femoral defects	Allogeneic PRP, with PLGA/CPC scaffold	Rabbit, 12 weeks	$1.13 \pm 0.06 \times 10^9$, in a range of 1.07×10^9 to 1.16×10^9/ml vs. $0.34 \pm 0.03 \times 109$/ml	Histopathology, radiography, and micro-CT	PRP effectively accelerated wound healing and new bone formation at the early stage of implantation
[99]	Critical size effect of 10 mm in the radial diaphysis	Human/xenogeneic PRP, percutaneous injection into the defect	24 rabbits, 8 weeks	10.1-fold compared to normal blood; 2422×10^9/l in PRP vs. 239×10^9 platelets/l in the whole blood	Histology, biomechanics, and radiology	Significantly promoted bone regeneration after 8 weeks
[100]	Calvarial defects	BCP-mixed HA-gel hydrogel ± autologous PRP loaded into a BCP sponge scaffold	18 rats, 4 and 8 weeks	Not mentioned, only growth factors in PRP: PDGF-BB: 12.26 ± 0.64 and TGF-β1: 366.91 ± 48.59	Micro-CT and histology	In *in vitro* study, the cell count, proliferation, and survival were higher with PRP, but in the *in vivo* study, the scaffold containing PRP was not superior to the one without PRP
[64]	Calvarial defect model	ASCs and allogeneic PRP admixture	50 rats, 4 and 8 weeks	19.1×10^4/ml in whole blood vs. 180×10^4/ml in PRP (nine-fold increase)	Micro-CT, histology, and immunohistochemistry	Showed dramatic effects on bone regeneration and ASCs directly differentiated into osteogenic cells

PRP: platelet-rich plasma; DFDBA: demineralized freeze-dried bone allograft; CCFDBA: cortical cancellous freeze-dried bone allograft; FHA: fluorohydroxyapatite; MSCs: mesenchymal stem cells; CPG: calcium phosphate granules; rhBMP: recombinant human bone morphogenetic protein; rhOP: recombinant human osteogenic protein; NA: not available; PRGF: plasma rich in growth factors; BMMSC: bone marrow-derived mesenchymal stem cells; ASC: adipose-derived stem cells; HA/β-TCP: hydroxyapatite/β-tricalcium phosphate; CDHA: calcium-deficient hydroxyapatite; VEGF: vessel-endothelial growth factor; CBMA: concentrated bone marrow aspirate; GAG: glycosaminoglycan; BMSC: bone-marrow stem cells; DBM: demineralized bone matrix; L-PRP gel: leukocyte- and PRP gel; BG: bioactive glass; PRGF: platelet-rich growth factors; BCP: biphasic calcium phosphate; DPB: deproteinized bone matrix; BPBM: bovine porous bone mineral; BGM: bio-guide membrane; PRF: platelet-rich fibrin; PLGA: poly(lactic-co-glycolic acid); CPC: calcium phosphate cement; BCP: biphasic calcium phosphate; PDGF: platelet-derived growth factor; IGF-I: insulin-like growth factor-I; HA-Gel: hyaluronic acid-gelatin.

71.4%) described the effect of autologous PRP on bone repair. Of these, 15 (60%) studies concluded that PRP improves the histologic appearance of the bone, whereas one report (4%) showed harmful effects of PRP on bone healing and nine studies (36%) were associated with nonsignificant positive effects. Seven studies (approximately 20%) used the allogeneic PRP, of those five studies showed promising results, and in two cases it was effective but nonsignificant. In addition, the effect of human/xenogenous PRP in radial defect model was assessed by three studies (approximately 8.6%) that interestingly led to promising results.

Few *in vivo* animal studies evaluated the effect of PRP on bone formation and regeneration after utilizing xenogeneic-based human PRP to treat critical-sized calvarial and long bone defects in animal models.[16,30,99,101] Although it is likely that PRP with a xenogenous origin stimulate immune response and increase the possibility of inflammatory reaction, Parizi *et al*.,[101] and Shafiei-Sarvestani *et al*.[30,99] showed that xenogeneic PRP has promising therapeutic effects on healing of the bone defects in rabbits. In line with these new findings and regarding the role of platelet concentrates on tissue healing, Moshiri *et al*.[48] demonstrated that two mechanisms may be involved including the growth factors and the role of PRP in the quality and rate of inflammatory response. They confirmed that the xenogenous platelet gel increases the inflammatory reaction for a short period of time but does not prolong the inflammation; these criteria may enhance the fibroplasia and remodeling phase of wound healing. In other words, PRP increases the acute inflammation but reduces the duration of chronic inflammation; in the former, the wound healing is accelerated because the acute inflammation is a part of healing process while in the latter, the wound healing is retarded because the enzymatic degradation continuously degrades the extracellular matrix and does not let the healing cells to do their normal physiological duties. Although most of the studies presented in Table 3 have shown the efficacy of PRP particularly in combination with other effective biomaterials and grafts, their use in treatment of bone defects in human practices remains in debate.

8. Human clinical studies

PRP has been used in the soft and hard tissues including tendon, ligament, bone, and the maxillofacial injuries in the field of orthopedics and sports medicine to help hemostasis and musculoskeletal healing.[21] Several clinical studies have applied PRP in patients with different conditions of bone defects. A total of 27 human clinical studies performed between 2012 and 2015, regarding the effects of PRP on bone healing and regeneration are presented in Table 4. Most of these studies (96%) have used PRP with an autologous origin in which 22 studies (81%) yielded excellent outcomes. The results obtained from two studies showed that PRP in combination with other material and scaffolds led to nonsignificant improvement in bone regeneration and the treatment in one study had no positive effect on bone healing after 12-week follow-up. Some clinical studies used allogenous form of PRP and to the knowledge of the authors, no study have still used the xenogenous PRP for treatment of bone defects. For instance, Antonello Gde *et al*.[102] estimated the effect of allogeneic PRP on alveolar bone defects in 25 patients during 6 months. The results obtained from this study confirmed the promising efficacy of the allogeneic PRP. Most of the studies (Table 4) evaluated the efficacy of PRP on bone healing by radiographic assays, and only five studies used histologic and histomorphometric examinations. In a study conducted by Golos *et al*.,[103] the efficacy of PRP in the treatment of delayed union of long bones was assessed in 132 participants with long bone fracture between 2009 and 2012. After PRP administration, bone union was established in 108 patients (81.8%) and 24 patients (18.2%) showed no improvement and resulted in nonunion, respectively. The highest efficacy was related to delayed union of the proximal tibia, whereas the lowest one was associated to that of the proximal humerus and therefore, they concluded that the PRP efficacy depends on the fracture location. In addition, they showed the effect of the material used for fracture fixation on PRP efficacy, so that open reduction and plate fixation led to a much more incidence of delayed union than closed reduction with intramedullary nail fixation. Although, most studies have reported the effectiveness of PRP in enhancing and improving bone healing (Table 4), the beneficial effects of PRP in clinical applications still remains controversial and doubtful because of the limitations in the methodologies, follow up and study design.

9. Safety of PRP

Considering the autologous nature of PRP, safety concerns are minimal.[128] Transient pain and inflammation at the injection site have been reported when PRP is used in an injection form.[128] The risk of morbidity, infection, injury to the blood vessels or nerves, scar tissue formation, and calcification at the injection site has been reported as rare side effects of PRP injection.[31,128] In addition, activation of platelets by $CaCl_2$ and

Table 4. Effects of PRP on bone healing in human clinical studies.

Reference	Type of the study	Number of patients	Bone defect model	Material used	PRP concentration	Evaluation methods	Main results
[60]	Clinical study	6 patients, 4-year follow-up	Large femoral bone cysts	Demineralized freeze-dried bone allograft, autologous L-PRG	NA	Radiography, X-rays, and DXA	Combination of the substances was not efficient
[104]	Clinical study	15 patients, 6 months follow-up	Mandibular third molar defects	Autologous PRP gel vs. blood clot	30,000–45,000/μl vs. 170,000 to 240,000 platelet/μl in PRP	Radiography	PRP accelerated alveolar bone regeneration especially in men
[105]	Clinical study	14 patients, 6 months follow-up	Intrabony defects	β-TCP ± autogenous PRP	Not mention	Radiography	β-TCP without or with PRP was effective, but it was nonsignificant between two groups
[106]	Single-site, randomized and controlled clinical trials	16 patients, 3-month follow-up	Extraction socket preservation graft before implant placement	MGCSH with autologous PRP vs. CRP	NA	Radiography and histomorphometry	MGCSH with PRP significantly showed greater vital bone volume and bone healing enhancement than collagen graft without PRP
[107]	Observational prospective cohort study	35 patients, 12-week follow-up	High tibial osteotomy	Bone chips and autologous PRP	NA	CT scanning	PRP decreased bone density under the wedge and it did not have any positive effect on bone healing
[108]	Clinical study	14 patients, 7 months follow-up	Atrophic maxillary sinus	Algae-derived hydroxyapatite AlgOss/C Graft/Algipore (1:10 ratio)-autologous PRP-thrombin	259,600 ± 58,000/μL vs. 1,484,972 ± 1,198,865/μl	Radiography and histology of biopsies	With PRP resorption of the biomaterial and new bone formation was increased
[109]	Randomized double-blinded, controlled clinical trials	90 defects in 54 patients, 9-month follow-up	3-wall intrabony defects	Autologous PRF vs. PRP gel	NA	Radiography	Autologous PRF or PRP-enhanced bone fill and reduced intrabony defect depth
[71]	Prospective cohort study	254 patients, 12, 24 and 72-month follow-up during a period of 7 years	276 type III, intra-articular calcaneal fractures	101 autograft, 90 allograft, and 85 allograft + autologous PRP	Platelet concentration 420% (mean: 780,000 platelets/μl)	Radiography and 3D-CT	PRP augmented the favorable outcome of allograft, PRP + allograft obtained promising results similar to autograft group at 24 and 72 months
[102]	Prospective clinical study	25 patients, 6-month follow-up	Alveolar bone defect following extraction of impacted third molar	Allogeneic PRP	1.2–1.8 million platelets/cm^3	Radiography	PRP-treated sockets were well-healed and PRP accelerated alveolar bone repair
[110]	Prospective, randomized, double-blinded controlled clinical trials	42 patients, 9-month follow-up	Mandibular degree II furcation defect	24 OFD + autologous PRF, 25 autologous PRP + OFD, and OFD alone	NA	Radiography	There was not any difference between PRF + OFD and PRP + OFD, PRP and PRF both were effective in the treatment of furcation defects
[111]	Prospective clinical study	19 patients, 5-month follow-up	Atrophic maxilla	Autogenous corticocancellous bone (onlay, from iliac crest) graft with autologous PRP vs. cortical block bone	NA	Radiography	There was nonsignificant greater marginal bone alteration after 1 year than 5 years in PRP presence
[112]	Case series	40 patients, 3-month follow-up	Mandibular defect due to removal of third molar	Porous hydroxyapatite crystal ± autologous PRP	3 to 4 times more than the normal	Radiography	Early bone formation and maturation were observed with PRP, pain severity was equal with and without PRP
[113]	Prospective clinical study	20 patients, 3- and 6-month follow-up	Oral and maxillofacial defects	Autogenous nonvascularized bone graft ± autologous PRP	NA	Radiography, CT, and histology of the biopsies	More matured bone was formed with PRP and the compact bone was thick in PRP group, PRP was safe, biocompatible, effective, and did not carry any transmissible disease

(*Continued*)

Table 4. (Continued).

Reference	Type of the study	Number of patients	Bone defect model	Material used	PRP concentration	Evaluation methods	Main results
[114]	Clinical study	10 patients, 6–10 years follow-up	Frontal sinus disease and other related cranial osseous derangements	Autogenous PRP covered with a PPP membrane and a periosteal flap	Mean increase concentration of 5.03-fold	Radiography, CT scan, and MRI	All patients had a good recovery with bone formation and no complications or recurrences over the years
[115]	Clinical study	20 patients, 6-month follow-up	Cystic bone defects of the mandible	Highly purified bovine xenograft (Laddec®) and autologous PRP	NA	Radiography, histology, and histomorphometry	A good bone regeneration was showed with Laddec and PRP and there are significant presence of new bone tissue and vessels near anorganic bone particles
[116]	Randomized split-mouth clinical trials	48 patients, one-year follow-up	Noncontained intrabony periodontal defects	DFDBA plus autologous PRP	NA	Radiography	DFDBA + PRP improved clinical and radiographic measurements such as bone fill, defect resolution, and alveolar crest resorption
[117]	Prospective clinical study	18 patients, 6-month follow-up	Intrabony mandibular defect following extraction of third molars	HA and resorbable collagen membrane with autologous PRP	NA	Radiography	Amount of radiographic density was significantly higher with the PRP compared to HA alone
[118]	Randomized, controlled, masked clinical trials	28 patients, 4-month follow-up	Edentulous ridge defect	Cancellous allograft ± autologous PRP	NA	Clinical, radiography and histology	PRP-enhanced bone regeneration and increased bone gain and vital bone percentage
[119]	Comparative controlled clinical trials	10 patients with 20 defects, 12-month follow-up	Bilateral intrabony defects in localized aggressive periodontitis patients	HA and autologous PRP	More than three times increase from the baseline	Radiography	Defects were filled by greater amount of bone in the HA + PRP group than the HA group
[120]	Randomized clinical trials	20 patients, 3 and 6 months follow-up	Periodontal intrabony defects	Autologous PRP ± DFDBA	NA	Radiography	PRP + DFDBA led to significant improvements in bleeding, hard tissue filling, and bone-depth reduction
[121]	Level 1 of evidence of the randomized controlled trials	20 patients, 28 (24–34) months mean follow-up	Bilateral tibial osteotomy, small size	BMAC plus autologous PRP, injection	NA	Radiography	Improved bone healing in osteogenesis of the tibia with more callus regeneration – complicated with superficial infection
[122]	Case series	7 patients, 34 months follow-up (range 9–27 months)	Forearm fracture and nonunion	Autologous PRP with acumed ulnar and radial rod and Thalon elastic nail	NA	Radiography and clinical examination	Complete recovery and excellent clinical outcomes
[123]	Case series	11 patients, 6 months follow-up	Bone defects due to cysts and tumor of the jaws	Autologous PRP	Platelet enrichment of more than 300% (mean 318%)	Radiography	A nonsignificant more rapid healing was observed with PRP
[124]	Case series, level IV of evidence	52 patients, 1- and 2-year follow-up	Chondral defects in the knee	PGA-HA implant plus autologous PRP	832.1 × 10^3 platelets/µl	Histology of biopsies	PRP showed the potential to regenerate hyaline-like cartilage
[125]	Randomized clinical study	41 patients, 8 weeks follow-up	Premolar defects freeze-dried bone allograft	Collagen plug, FDBA/β-TCP/collagen plug, FDBA/β-TCP/PRP/collagen plug, FDBA/β-TCP/rh-PDGF-BB/collagen plug	Not mention	Radiography, histology of biopsies, and histomorphometry	Inclusion of PRP and rh-PDGF-BB-enhanced bone quality, healing within sockets, reduced residual bone graft particles and reduced the healing time before dental implant placement
[126]	Clinical trial	10 patients, 12 months follow-up	Periodontal intrabony defects	DFDBA-allograft ± autologous PRP	Not mention	Radiography	DFDBA-allograft plus PRP was more effective than DFDBA-allograft alone
[127]	Single-blind randomized clinical trials	24 patients, 9 months follow-up	Idiopathic bone cavity	Autologous PRP gel	Not mention	Radiography	PRP could enhance bone formation in dental cavities

L-PRG: leukocyte and platelet-rich plasma gel; NA: not available; DXA: dual-energy X-ray absorptiometry; PRP: platelet-rich plasma; β-TCP: beta-tricalcium phosphate; MGCSH: medial-grade calcium sulfate hemihydrate; CRP: collagen resorbable plug; PRF: platelet-rich fibrin: 3D-CT: three-dimensional computed tomography; OFD: open flap debridement; PPP: platelet-poor plasma; MRI: magnetic resonance imaging; DFDBA: demineralized freeze-dried bone allograft; HA: hydroxyapatite; BMAC: bone marrow aspirate concentrate; PGA-HA: polyglycolic acid-hydroxyapatite; FDBA: freeze-derived bone allograft; rh-PDGF-BB: recombinant human platelet-derived growth factor-BB.

bovine thrombin containing bovine factor V may result in development of antibodies against bovine clotting factor V which led to coagulopathies.[20,31,68] However, Wang-Saegusa and colleagues [23] in their study on 808 patients with osteoarthritis of the knee reported no adverse effects following injection of PRP into the knee joint during a period of six months. The contraindications for PRP application include preexisting coagulopathies, pregnancy, active infection, hypersensitivity to bovine thrombin when used for platelet activation, malignant neoplasms particularly hematopoietic, or those originated from bones. Possible induction of neoplastic diseases, muscle tissue fibrosis, and finally infection at the injection site could be listed as the contradictory adverse effects of PRP application. [9,39,93]

10. Expert opinion

Several factors such as type and size of the bone defect, type and nature of the bone implant, bone graft substitute and bone fixation device, volume of whole blood and final volume of PRP, final platelet and growth factors concentration, methods that PRP is produced, activator agents, presence or absence of leukocytes and RBCs, the origin of PRP used (autologous, allogeneic or xenogeneic), and surgical approach of bone fixation can affect the efficacy of PRP.[29,71,107] These variations may help to explain different outcomes resulting from PRP application in orthopedic practices. Most of the human clinical studies, referred in the present study, used an autologous form of PRP. Such PRP induces no or less immune reaction and provides more efficient results as compared to allogeneic form of PRP. Application of allogeneic PRP for bone tissue engineering purposes has rarely been investigated so far; therefore, its immunogenicity in such applications remains unknown. There are some limitations associated with PRP administration. The first one is that an optimal dose range of PRP has not been well-defined as yet. It has previously been shown that a platelet concentration of two to seven times more than the normal blood platelet concentration is effective *in vivo*. Nonetheless, it has been claimed that much higher concentrations may exhibit negative effects on healing.[17,18] Lack of standardized protocols to produce and evaluate PRP in the literature can explain the inconsistent clinical and experimental results. It is possible to avoid the problems and concerns in future by standardizing the PRP production systems followed by clinical trials to study the effect of PRP on tissue healing. More importantly, the conflicting results among the orthopedic studies probably may be due to nonstandardization of the blood centrifugation processes. The literature has shown that there is no uniformity among various methods of centrifugation and the resultant growth factors and other efficient agents; therefore, various production methods could led to different outcomes in patients. The criteria such as the effective concentration of platelets in PRP and similarities or differences in their mechanisms of action, for different animal species and humans, have to be defined and be carefully interpreted. Only the PRP preparation of human is a partly standardized procedure, whereas PRP preparations from animal blood have not been standardized yet. Plachokova *et al*.[33] compared the effect of PRPs derived from rat, goat, and human inserted into cranial critical-sized defects in immune-deficient rats, using citrate-phosphate dextrose as an anticoagulant. The platelet concentration was 10-fold in the human PRP, while it was three- and six-fold in rat and goat, respectively. They showed that following platelet activation, more growth factors were released from hPRP than from the rat and goat PRP; therefore, the effect of hPRP was more significant. No effect of bone formation was detected in the animals that were treated by the rat and goat PRP. They showed that human PRP exhibits higher concentrations of TGF-β1, PDGF-AA, -AB and -BB than those of rat and goat. Such findings may be reliable reasons for the positive and promising outcomes obtained from the human (xenogeneic) PRP in bone regeneration, previously stated in rabbit models. [16,30,99,101] From the viewpoint of the vehicles for PRP delivery into the damaged site, there are different material and scaffolds that can influence the effectiveness of PRP. Combination of PRP with autografts can enhance bone regeneration.[71] On the other hand, it has been shown that addition of PRP in the gel form with MSCs, as osteoblast progenitor cells, can serve as a suitable alternative option to autografts.[91] In addition, PRP can be combined with natural and synthetic bone graft substitutes including bioactive calcium phosphates such as hydroxyapatite (HA), tricalcium phosphate (TCP),[85] other calcium phosphates,[92] bioactive glasses,[89] and chitosan [129] to reduce the need for autologous bone grafting. However, the combination of PRP with HA and TCP in an investigation carried out by Faratzis *et al*.[85] showed no effect on bone regeneration in a cranial defects of rabbit. PRP gel in combination with rhBMP-2 incorporated biphasic calcium phosphate ceramics exhibited synergistic and positive effects on new bone formation.[24] Penteado *et al*.[89] showed an improvement in bone formation in a calvarial defect model in rabbits by application of PRP with bioactive glass. However, bioactive glasses have not been used popularly with PRP.

Hydrogels or sponges composed of gelatin [96] and collagen [117] can serve as a good carrier system for PRP in which the delivery and bioavailability of growth factors to the defect site is sustained and enhanced. [48] These biomaterials or biopolymers are commonly used as carriers and delivery vehicles for different biomolecules and PRP in bone regeneration and repair, because of their biodegradability, biocompatibility, bioactivity and nonimmunogenicity nature.[22] Combination of PRP with these biomaterials can enhance the effectiveness of the PRP and bioimplants.[22] However, addition of PRP into the allogeneic demineralized bone matrix (DBM), used in muscle pouches of rabbit, had negative effect on the osteoinductivity of DBM.[25] There are several methods for incorporating PRP including addition of PRP to the base biopolymer solution before gelation, adding PRP incorporated micelles within the scaffold, and soaking the biopolymer hydrogels or sponges in a PRP solution.[22,48] The studies presented herein can be found helpful for the researchers who are studying PRP and its contents in tissue engineering and drug delivery for different purposes particularly bone regeneration and formation. However, to date, there is no ideal delivery method for PRP to treat bone defects.

In conclusion, there is little doubt that PRP has the potential to be beneficial for bone healing and regeneration. However, the effectiveness depends on several variables including PRP preparation and activation method, anticoagulant agent, platelet count and concentration, biomaterial used with PRP, the origin of the PRP (auto-, allo- or xenogenous), presence or absence and also the concentrations of RBCs and WBCs within the PRP, the implantation site, and the surgical approach of fractured bone fixation. Furthermore, one important challenge is to retain the growth factors in a physiologic and active manner at the damaged site. However, the application of PRP alone and without any additional components does not seem to be effective in bone healing and cannot be recommended these days. Finally, owing to the fact that there are several controversies regarding efficacy of PRP, it is still too soon to judge and offer this therapeutic option as an appropriate modality in the orthopedic surgery and regenerative medicine with the aiming to promote healing of LBDs, treat nonunions and delayed unions and manage the patients associated with osteomyelitis particularly in the clinical setting.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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