Platelet rich plasma in osteoarthritis: more than a growth factor therapy

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Osteoarthritis (OA) is the most commonly encountered degenerative joint disorder in clinical medicine and the pain and stiffness caused by osteoarthritis represents a leading cause of physical disability in individuals of retirement age. Treatment options for OA patients are limited and largely targeted to pain management. Such is the mechanism behind the most commonly prescribed OA therapies including acetaminophen, NSAIDs, opioids, and steroids. However, these palliative pain management tools do not address the underlying pathophysiology of OA disease. This leaves a significant clinical unmet need for disease modifying OA therapies. Growth factor therapies and regenerative medicine strategies are emerging as promising alternatives to palliative care because these treatments bring significant potential to control chronic inflammation, enhance cartilage repair, and restore other joint tissues to a healthy state. Interestingly, a Clinicaltrials.gov search using the terms “osteoarthritis” and “growth factor” identified 43 relevant studies. Of these, only 26% used growth factor therapies directly, including FGF-18, BMP-2, and transgenic human chondrocytes producing TGF-β1. 23% of the studies were dedicated to inhibitors of NGF. The overwhelming majority of studies (47%) used autologous blood products, especially platelet rich plasma (PRP), which constituted 33% of the total studies. This was interesting because although platelets in PRP do indeed harbor large quantities of different growth factors that can affect the status of the treated tissue, growth factors represent only a single aspect of the bioactivity of platelets. Furthermore, platelets represent only one aspect of the potential bioactivity of PRP. Depending upon the methods used for PRP generation, it may also contain other physiologically important components of the whole blood from which it is derived. Recent studies indicate non-platelet components of whole blood such as red blood cells and leukocytes are essential for normal platelet function, including growth factor release. Therefore, this narrative review discusses PRP from a physiological context that explores beyond platelet growth factors to encompass and illuminate roles of non-platelet cells in the bioactivity of PRP, and how these additional mechanisms lead to the conclusion that PRP can be much more than a growth factor therapy.

Keywords: platelet-rich plasma; osteoarthritis; growth factor therapy; leukocyte; physiology


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Introduction

Osteoarthritis (OA) is a highly prevalent, progressive and debilitating, degenerative disease that affects an ever growing percentage of the world’s population \[^1\]. Although the etiology of OA is not well understood, it is recognized that OA symptoms develop over the course of many years \[^2\]. The progression of OA pathogenesis eventually affects essentially all of the tissues and structures of the joint, ultimately leading to chronic pain and disability in many patients \[^2\]. In patients who are refractory to symptomatic relief through conservative therapy, joint replacement surgery remains the principal treatment course to relieve pain and improve long-term function. However, not all patients benefit from joint replacement, and joint prostheses have a lifespan due to mechanical wear, and revision surgeries are far more complex with greater complication rates and at times poorer outcomes than primary joint replacement surgeries \[^3\]-\[^5\]. Therefore, there is an unmet clinical need for alternative therapies that may intercept and modify OA disease processes to delay or potentially avoid joint replacement. This realization has led to an increasing clinical interest in regenerative medicine, and in particular the potential for growth factors that may stimulate tissue repair. Several anabolic growth factors, including FGF-18, TGF-β1, BMP-2 (OP-1), and GDF-5 (BMP-14) have shown success in various pre-clinical models, and some of these agents have advanced into clinical studies \[^6\] and recently reviewed in \[^7\]). However, given the heterogeneity of OA patients and the uncertain etiology and chronic evolution of OA disease, it seems unlikely that a single molecule approach will prove effective at disease modification across the spectrum of OA patients. In light of this, autologous intra-articular platelet rich plasma (PRP) injections have been gaining attention as a means for simultaneously introducing factors that are involved in controlling inflammation and stimulating tissue repair. PRP is a multi-cellular platelet concentrate derived from autologous whole blood, with platelet concentrations elevated above baseline whole blood levels \[^8\], \[^9\]. Platelets within PRP contain a high concentration of bioactive factors that are stored inside of granules that can be released to activate acute inflammation and stimulate the healing cascade \[^10\]-\[^14\]. Depending upon how PRP is generated it can contain additional blood cell types such as leukocytes (WBCs), red blood cells (RBCs), and circulating stem cells, which can all contribute to the bioactivity and regenerative potential of PRP \[^15\], \[^16\]. In fact, a current topic of contentious debate relates to the formulation of PRP and whether certain cell types, such as leukocytes (neutrophils in particular) and RBCs, should be restricted or excluded. This debate has arisen over concerns that leukocytes in leukocyte-rich PRP (LR-PRP) might exacerbate inflammation and contribute to tissue pathology, and that RBCs could leak heme-containing compounds that may lead to oxidative damage in the treated tissue \[^17\], \[^20\]. Data consistent with such concerns is largely restricted to in vitro and pre-clinical models, and these findings have not translated to clinical medicine. In fact, several pre-clinical studies that are often cited by investigators who argue that leukocyte poor PRP (LP-PRP) preparations are better suited to treat OA patients have recently come under scrutiny, leaving the conclusions of the studies in question \[^21\]-\[^23\]. Interestingly, there is currently no clinical evidence that we are aware of available in the published literature that lends support to the alleged safety concerns of LR-PRP among the numerous studies that have been conducted with PRP injections for OA of the knee (recently reviewed in \[^19\], \[^24\]-\[^27\]). To the contrary, PRP therapy for knee OA has been found to be without significant adverse effects regardless of leukocyte or RBC content (recently reviewed in \[^19\], \[^24\]-\[^27\]). Indeed, Mariani et al. recently found that LR-PRP does not induce a relevant clinical up regulation of pro-inflammatory mediators when injected into the knees of OA patients \[^28\]. Furthermore, recent studies have even suggested that LR-PRP formulations that contain high concentrations of platelets and leukocytes as well as significant levels of RBCs yield greater therapeutic effect sizes for patient reported symptom relief than formulations that do not \[^24\]. Given that many aspects of OA pathogenesis are believed to resemble the physiology of chronic or non-resolving wounds \[^29\], considering PRP therapy in terms of wound repair may provide a basic science foundation for understanding the additional benefits that non-platelet blood components may bring to the therapeutic potential of PRP in OA, and lead to the conclusion that PRP is much more than simply a growth factor therapy.

Salient roles of blood components in wound repair

Although very little is actually known about the roles of non-platelet blood components in the activity of platelets with respect to PRP therapy, there is a substantial body of literature relating to the roles of blood components in the physiology of normal wound repair (recently reviewed in \[^30\]-\[^36\]). In a normal wound repair reaction all of the components of blood have important roles in driving the healing cascade, and the co-operative physiological balance of cellular and biochemical action results in restoration of the injured tissue to a healthy state.

Human blood is comprised of plasma and three main types of cells; platelets, WBCs, and RBCs. Plasma is the fluid medium component of blood that suspends and transports blood cells and contains nutrients, endocrine factors, basal growth factors and essentially all of the clotting factors that are necessary for thrombin generation to drive hemostasis.
and platelet activation in the context of a wound. In the context of PRP, plasma is important for injectability and to provide sufficient clotting factors, especially pro-thrombin, to allow robust platelet activation.

Platelets are non-nucleated cells derived from very large pre-cursor cells called megakaryocytes that reside in the bone marrow. During their development, platelets obtain large numbers of storage granules that contain different growth factors, cytokines, and hormones required for activating acute inflammation, which is the first stage of wound repair [11, 12, 14, 37, 38]. Platelet activation is a highly regulated process that culminates in degranulation, or the release of granule contents [12, 39-43]. A fundamental concept in innate immunity, and thus in wound repair, is that platelets and WBCs circulate in an inactive state that prevents these cells from engaging in inappropriate and potentially deleterious activities. However, in the context of a wound, platelet activation is initiated by contact with components of the extravascular connective tissues that are exposed at the site of injury [44, 45]. Collagen and Tissue Factor are examples of tissue components that can initiate platelet activation [46]. Platelets in this state begin to release factors that are capable of initiating the process of RBC and WBC activation. However, robust thrombin generation is important for platelets to achieve their full potential to drive efficient activation of acute inflammation and growth factor release that can lead to wound repair. Robust thrombin generation involves an amplification mechanism that is dependent on RBCs, which will be discussed further below. Fully activated platelets release their alpha granule contents with an initial burst, and exhaust most of their stored cargoes within the first hour after activation [11, 12, 32, 41, 47, 48]. Platelets have a limited capacity to synthesize new proteins [49-51]. Therefore, their major contribution to wound healing is to activate the acute inflammatory response and program damaged tissue for debridement and repair.

WBCs are important effector cells of the innate immune system that decontaminate a wound and prevent infection. WBCs also debride the wound of dead and damaged tissue and ultimately deposit and activate growth factors that direct the conversion of a fibrin clot into vascularized and viable tissue [30, 31, 34, 36, 41, 52-54]. There are two main classes of WBCs: granulocytes and mononuclear cells. Granulocytes include neutrophils that engulf foreign bodies, produce immune regulating cytokines, lipids, and proteases, and can release antimicrobial granules and oxidative toxins [53, 55-58]. Neutrophils are the most abundant WBCs in humans, and if improperly regulated these cells can indeed cause significant damage to healthy tissue. Because of the existential risk of excessive neutrophil activation, an evolutionary fail-safe mechanism has developed to control their activity. In the circulation neutrophils are actively maintained in a resting state [59]. This quiescent state requires two different activating signals to cause neutrophils to launch an inflammatory response [59-65]. The first signal is a “priming signal” that “wakes up” the resting neutrophil, and this priming signal must precede an “activating signal” that makes the neutrophil competent to launch a robust and potentially damaging inflammatory response. The priming signal is usually a chemotactic factor such as IL-8, or an inflammatory cytokine like IL-1 or TNF-α, while the second or activating signal is typically a microbial specific product or a foreign body. Regardless, the activating signal must either be biochemically different from the priming signal or be of significantly greater magnitude than the same original priming signal [61]. In the context of a PRP injection, this priming signal can be provided by platelets that have been activated by contact with extravascular tissue [61]. Following the priming signal, neutrophils become competent to engage with their environment and gain the ability to generate and release an array of cytokines, growth factors, bioactive lipids, and proteases that can modulate the activity of other cells [58, 60, 61, 66-71]. Interestingly, new evidence shows that the priming reaction is actually reversible, and priming does not necessarily lead to activation of inflammatory responses [62, 64, 72, 73]. In the case of a PRP injection, neutrophils have been harvested along with platelets from the circulating blood and are thus in a resting state when they are introduced to the tissue. Therefore, neutrophil priming can occur upon collagen-mediated platelet activation within the treated tissue [52, 70, 74]. In this case, the complex “soup” of factors within the tissue being treated simultaneously become part of the platelet-driven leukocyte priming reaction. Thus, no separate activating signal would exist in the tissue to drive the injected neutrophils to an excessive inflammatory state. This theory of neutrophil priming without subsequent activation can begin to explain why LR-PRP injections are not associated with significant safety risks that could be expected if full neutrophil activation did indeed occur, or why LR-PRP therapy did not exacerbate inflammatory cytokine levels when used in osteoarthritic joints [8, 19, 24, 42, 43, 53, 75-79]. Thus, one could question the logic and the motivation driving concerns over the use of LR-PRP in OA as the current clinical evidence actually suggests that these formulations may provide even greater benefit to treated tissues than LP-PRP [24, 78].

The primary role of RBCs is to carry oxygen to tissues to support metabolism, and carry carbon dioxide waste away to prevent acidification. However, in wound repair RBCs act very early during the hemostasis reaction in an amplification loop to increase the activation and release of bioactive factors from platelets [50-83]. ADP (adenosine di-phosphate) and LPA (lyso-phosphatidic acid) are platelet-derived factors that can
activate RBCs to release large amounts of ADP. ADP is a platelet agonist that reinforces platelet aggregation and amplifies platelet recruitment into the forming clot \([81-84]\). Activated RBCs also accelerate and amplify thrombin generation \([80-84]\). This is important because thrombin is the most powerful natural platelet activation signal, and without robust thrombin generation platelet activation and growth factor release from PRP is deficient \([85-88]\). This RBC-driven activity stems from the exposure of phosphatidyl serine (PS), a negatively charged lipid that is normally stored inside of the RBC, on the surface of the activated RBC \([82-84, 89]\). This is a particularly powerful feed-back amplification mechanism to ensure platelet activation and subsequent growth factor and cytokine release. Because robust platelet activation initiates acute inflammation and provides the initial stimulus for the healing cascade, deficiency at this initial stage of wound repair may lead to aberrant healing responses \([90, 91]\).

Interestingly, because PS on the cell surface also functions as a hallmark of apoptosis it serves as a very powerful efferocytosis or “eat me” signal for debridement by professional phagocytes like neutrophils and macrophages. This is important because phagocytes that are engaged in PS-mediated debridement preferentially express and release anti-inflammatory cytokines such as IL-10 and TGF-β1 and pro-resolving lipid mediators like lipoxins and resolvins \([92, 93]\). Therefore, it stands to reason that in the context of PRP, inclusion of RBCs may provide mechanisms to ensure complete platelet activation and to limit the magnitude and duration of platelet-driven inflammation.

**Cooperative action between platelets and neutrophils interrupts the inflammatory cycle**

The platelet: WBC ratio is a metric that has gained attention as an important parameter for the activity of PRP, although the influence of this ratio remains controversial \([14, 33]\). To the contrary, cooperative mechanisms of critical importance for controlling inflammation and promoting wound repair involving platelets and neutrophils have been characterized \([91, 94, 95]\). For example, activated platelets and primed neutrophils cooperate in a trans-cellular lipid metabolism pathway that can limit the magnitude and duration of inflammation \([91, 94, 95]\). More specifically, the attachment of activated platelets to neutrophils allows neutrophils to take up arachidonic acid (AA) that is released by activated platelets. Neutrophils can convert this platelet-derived AA to prostaglandins (PG) and leukotrienes (LT) \([96]\). Platelets can quickly take up LTs and convert them to lipoxins (LX) \([96]\). Lipoxins are very potent anti-inflammatory molecules that play important roles in limiting neutrophil activation and preventing their migration from vessels into tissues, and in driving the resolution of inflammation \([91, 94, 95, 97]\). It is important to note that platelets lack the ability to synthesize lipoxins without the LT intermediates produced by neutrophils \([96]\). In addition, neutrophils can be induced in an autocrine fashion by the PGs they generate to switch from pro-inflammatory LT synthesis directly to LX generation \([98, 99]\). This neutrophil switch from pro-inflammatory lipids to anti-inflammatory/pro-resolving lipids can also prevent further neutrophil recruitment and activation while simultaneously activating resolution pathways that can accelerate repair \([91, 95, 96, 99]\). Such cooperative regulation of the magnitude and duration of inflammation forms a foundation for the importance of platelet-leukocyte ratios and suggests that physiological ratios should emerge as important parameters in the therapeutic efficacy of platelet-rich plasma products \([18, 53, 76, 86, 100, 101]\).

**Role for proteases in tissue repair**

Protease activity in the OA joint has been historically viewed as destructive, contributing to the degeneration of cartilage matrix and accelerating disease progression. However, protease cleavage has also been determined to play integral roles in the inactivation of pain mediators and catalytic inflammatory cytokines, and is imperative for the activation of anabolic growth factors for proliferation, angiogenesis, and new matrix synthesis. For example, key growth factors such as IGF-1, TGF-β1 and VEGF depend on extracellular protease activity for their bioavailability \([102-104]\). Moreover, protease cleavage also targets the destruction of key inflammatory mediators like IL-1β and MCP-3 (CCL7), and as such functions to accelerate resolution of inflammation and promote tissue repair \([57, 104, 105]\). Therefore, non-selective protease inhibitor strategies such as α2-macroglobulin therapy that aim to preserve cartilage in OA may inadvertently increase pain and interfere with tissue repair \([102, 104]\).

Interestingly, the matrix metalloproteinase MMP-2 (also known as neutrophil gelatinase A) appears to play key functional roles in promoting wound repair \([102]\). MMP-2 can directly inactivate inflammatory mediators like IL-1β and MCP-3 and represents a key mechanism to release critical growth factors like TGF-β1 and IGF-1 from their tissue inhibitors \([55, 68, 102-104, 106, 107]\). The catalytic activity of MMP-2 also unlocks the potent anti-inflammatory activity of TGF-β1 \([55, 68, 103, 106]\). In addition, MMP-2 can also inactivate powerful neuro-inflammatory mediators of chronic pain such as Nerve Growth Factor (NGF) and Calcitonin Gene-Related Peptide (CGRP) \([108, 109]\). NGF is a member of the neurotrophin family of signaling molecules that has an important role in driving peripheral pain sensitization and pain hypersensitivity in OA \([109]\). Antibody therapy targeted against NGF is currently in clinical trials, and although this strategy has indeed been shown to deliver very potent pain relief in OA.
patients it may be associated with potentially serious side effects that may limit its general clinical utility \[109\]. Similarly, CGRP is thought to play an important role in the chronic pain of osteoarthritis by causing vasodilation that contributes to effusion \[108\], and by causing persistent sub-acute synovitis and hyperalgesia \[110\]. It is therefore noteworthy that recent data indicate that levels of active MMP-2 are greater in leukocyte-rich PRP compared with leukocyte poor \[111\]. This finding should not be considered surprising since neutrophils represent a rich source of MMP-2, also known as neutrophil gelatinase A.

**Conclusions**

Treatment options for OA patients are limited and largely targeted to pain management \[1\]. These palliative care tools do not address the underlying pathophysiology of OA to intercept disease progression, or drive repair of the degenerating joint structures \[1\]. This significant clinical unmet need has led to the exploration of growth factor therapies and biologics approaches that are beginning to emerge as potential future therapeutic alternatives. Autologous PRP is one such approach that has gained significant attention over the last decade. Although autologous PRP therapy has demonstrated an excellent safety profile and has been shown effective for symptomatic OA relief in numerous clinical studies regardless of leukocyte or RBC content (recently reviewed in \[19, 24-27\]), future studies are needed in order to investigate whether PRP can provide a regenerative effect leading to disease modification.

Because PRP can be prepared using various methods that will either maintain physiological ratios of blood components (leukocyte rich PRP containing RBCs), or remove specific components such as RBCs and WBCs (leukocyte poor PRP), or even just RBCs, it may become more tangible to approach questions of disease modification as devices that generate PRP become more reliable in the formulations they produce. In addition, formulations and treatment regimens that produce symptom relief may not be optimal to promote repair of joint structures and intercept disease progression. From this lens, it seems pertinent that the greatest potential for success may be found in PRP formulations that leverage the full array of inflammation control and wound repair mechanisms that exist in the precursor whole blood. For example, platelets have been considered as the active component in PRP, and in particular the growth factors contained in their storage granules \[92, 78, 112, 113\]. However, platelet growth factors represent only one facet of platelet bioactivity \[54, 101, 114\]. Platelets activate inflammation that triggers wound repair in response to injury. In this context, platelets work with RBCs and leukocytes to execute the repair process, and their cooperative engagement helps to direct the magnitude and duration of the inflammatory response.

Although neutrophils are classically described as “inflammatory cells”, primed neutrophils that have not been activated have enhanced phagocytic activity and can generate large quantities of anti-inflammatory mediators such as TGF-β1, IL-10, and IL-1 receptor antagonist (IL-1RA) that can directly combat chronic inflammation \[56, 58, 98, 115\]. In addition, primed neutrophils are a rich source of MMP-2 (neutrophil gelatinase-A), a tissue remodeling enzyme that provides protease activity to inactivate inflammatory and chronic pain mediators like NGF, CGRP, IL-1β, and MCP-3 and to release critical growth factors like TGF-β1 and IGF-1 from their tissue inhibitors \[104, 107\]. Furthermore, neutrophils cooperate with platelets to generate bioactive lipid mediators that restrict recruitment of activated neutrophils from the circulation and promote active resolution of inflammation \[96\]. With respect to RBCs, engagement with activated platelets causes an amplification loop for robust thrombin generation and this mechanism helps ensure platelet degranulation to optimize growth factor and inflammatory mediator release \[80-84\]. Therefore RBCs have an important role in determining biochemical composition and regenerative potential in wound repair. It is important to note that these physiological mechanisms to control platelet inflammatory action and growth factor deposition that depend on non-platelet blood cells are lacking in LP-PRP formulations that restrict RBC and WBC inclusion \[85-88\]. It seems logical then to conclude that the complete physiological repertoire of blood components, rather than any individual part, should be considered the “active component” of PRP \[86\]. Until human clinical studies advance to the level of sophistication that allows for the elucidation of “optimal formulations” of PRP, maintaining the physiological context of platelets with respect to other blood cells may be one way to ensure robust and balanced PRP activity. It has become evident that PRP is much more than just a growth factor therapy for OA, requiring further clinical studies to elucidate the full spectrum of potential benefits that physiologically balanced PRP may provide.

**Conflicting interests**

WRP and BR are employees of DePuy Synthes Mitek Sports Medicine. WRP is a co-inventor on patent applications regarding the preparation and use of blood components (US 9,555,171 and US 13/250,086)

**Disclaimer**

The views expressed in this review article are the scientific position of the Authors and do not represent an
official position of DePuy Synthes Mitek Sports Medicine or the Johnson and Johnson Family of Companies.

Abbreviations

OA: Osteoarthritis; nSAID: non-steroidal anti-inflammatory drug; PRP: platelet-rich plasma; LR-PRP: leukocyte-rich PRP; LP-PRP: leukocyte-poor PRP; FGF-: fibroblast growth factor; BMP: bone morphogenic protein; TGF-: transforming growth factor; NGF: nerve growth factor; CGRP: calcitonin gene-related protein; IL-: interleukin; IGF-: insulin-like growth factor; VEGF: vascular endothelial growth factor; MCP-: macrophage chemotactic protein; MMP-: matrix metalloproteinase; WBC: white blood cell; RBC: red blood cell; ADP: adenosine di-phosphate; AA: arachidonic acid; PG: prostaglandin; LT: leukotriene; LX: lipoxin.

Author contributions

WRP conceived manuscript arguments. BR and WRP drafted manuscript, and critically revised for content. BR and WRP read and approved the final manuscript.

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