

Platelet-rich plasma for managing pain and inflammation in osteoarthritis

Isabel Andia and Nicola Maffulli

Abstract | Osteoarthritis (OA) is a common disease involving joint damage, an inadequate healing response and progressive deterioration of the joint architecture. Autologous blood-derived products, such as platelet-rich plasma (PRP), are key sources of molecules involved in tissue repair and regeneration. These products can deliver a collection of bioactive molecules that have important roles in fundamental processes, including inflammation, angiogenesis, cell migration and metabolism in pathological conditions, such as OA. PRP has anti-inflammatory properties through its effects on the canonical nuclear factor κ B signalling pathway in multiple cell types including synoviocytes, macrophages and chondrocytes. PRP contains hundreds of different molecules; cells within the joint add to this milieu by secreting additional biologically active molecules in response to PRP. The net results of PRP therapy are varied and can include angiogenesis, the production of local conditions that favour anabolism in the articular cartilage, or the recruitment of repair cells. However, the molecules found in PRP that contribute to angiogenesis and the protection of joint integrity need further clarification. Understanding PRP in molecular terms could help us to exploit its therapeutic potential, and aid the development of novel treatments and tissue-engineering approaches, for the different stages of joint degeneration.

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Introduction

Osteoarthritis (OA) is a common disease involving cartilage damage caused by an inadequate healing response with changes in the synovium and subsequent alterations in the composition of the synovial fluid.^{1,2} Inflammation and vascular pathology, in combination with cell death, meniscal changes, bone remodelling and subchondral sclerosis, produce a vicious cycle of progressive joint degeneration. Chondrocyte senescence and loss of cartilage integrity are major features of OA, as is osteophyte development. Excessive mechanical stress and oxidative damage can also be involved in cartilage degradation.³ Moreover, under conditions of metabolic or cytotoxic stress—conditions associated with ageing—autophagy can be upregulated, further decompensating homeostatic mechanisms.⁴ Failure to understand the pathophysiology of OA hampers efforts to develop improved therapeutic strategies. How and when the condition is initiated, and what factors perpetuate the process, remain unclear, but mechanical stress and biological microenvironments seem to have a crucial role. The emergence of blood-derived products as a safe treatment modality to accelerate healing in musculoskeletal injuries has added to our understanding of tissue healing. These products could modify the biological microenvironment at different points in the disease process, and they might provide an opportunity to interfere with self-perpetuating mechanisms in OA.⁵

Three key factors have led to high demand for autologous blood-derived treatments for patients with OA. First, PRP is acceptable in principle by both patients and physicians as a potentially useful treatment. Second, PRP is simple to use, because the preparation of PRP is rapid and technically straightforward, and the administration is noninvasive. Third, PRP is likely to be safe, because the patient's own proteins are used, and bioactive molecules can be appropriately concentrated, thereby avoiding many adverse effects and drug interactions.

At present, at least 17 commercial protocols can be used to generate PRP. A different protocol can be used to generate autologous conditioned serum (ACS), which is enriched for soluble receptors, such as IL-1 receptor antagonist protein (IL-1RN), that block the proinflammatory cytokine IL-1 β and reduce joint catabolism. ACS is prepared by *in vitro* incubation of the patient's whole blood with glass beads; although the exact composition is unknown, this product contains IL-1RN as well as anti-inflammatory interleukins, including IL-4 and IL-10.^{6,7}

Numerous PRP formulations are used in experimental or clinical research, which interferes with the establishment of a consistent and successful protocol for therapeutic treatment. Different protocols can yield products with different compositions and characteristics. Delivery systems, timing, concentration, and the proteolytic condition of the PRP (which limits the half-life of many therapeutic molecules) have not been optimized. The most widely used classification system

Regenerative Medicine Laboratory, BioCruces Health Research Institute, Cruces University Hospital, Plaza Cruces S/N, 48903 Barakaldo, Spain (I. Andia). Centre for Sports and Exercise Medicine, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Mile End Hospital, 275 Bancroft Road, London E1 4DG, UK (N. Maffulli).

Correspondence to: N. Maffulli
n.maffulli@qmul.ac.uk

Competing interests

The authors declare no competing interests.

Key points

- Autologous blood products such as platelet-rich plasma (PRP) are sources of molecules that can actively participate in tissue repair
- In the joint, PRP affects local and infiltrating cells, mainly synovial cells (synoviocytes and macrophages), endothelial cells, cells involved in innate immunity, and cellular components of cartilage and bone
- PRP can alter many of the processes that are aberrant in patients with osteoarthritis (OA), including inflammation, angiogenesis, and the balance between anabolism and catabolism in cartilage
- PRP can modify the biological microenvironment that exists at different points in the disease process, and could, therefore, provide an opportunity to interfere with the self-perpetuating mechanisms of OA
- The microenvironment in joints with OA varies between patients and disease stages; the different therapeutic effects of PRP might result from the specific milieu present in the joint
- Heterogeneity in PRP formulations and the way PRP is activated can generate uncertainty in the biological effects and clinical responses

Box 1 | PRP therapies: facts and assumptions

Platelets—the key component of PRP—are small anuclear fragments of cytoplasm produced by megakaryocytes in the bone marrow. Platelets have a biconvex disc structure with an equatorial diameter of 2–3 μm . They are normally present at a concentration of $150\text{--}450 \times 10^3$ platelets per μl of peripheral blood.

- Megakaryocytes shed platelets into the bloodstream. In humans, platelets circulate for 8–10 days before being destroyed by the reticuloendothelial system, primarily in the liver and spleen, following an apoptosis-like process. From proteomic data, 1,507 unique constituents have been identified in resting platelets, including 190 membrane-associated proteins and 262 phosphorylated proteins.¹⁰⁹ Using this army of proteins, platelets affect primary haemostasis, the innate immune response and inflammation, host defences against microorganisms, wound healing and malignancy.
- In the past two decades, advances in our understanding of platelets as storage and delivery systems for proteins of interest has increased the use of PRP therapies. This natural mixture of bioactive molecules, incorporated into a formed fibrin matrix within the joint compartment, could induce the production of chondroprotective molecules by synovial fibroblasts. Once in the synovial fluid, these molecules would affect the cartilage and the joint microenvironment.

Abbreviation: PRP, platelet-rich plasma.

is based on the presence of leukocytes (owing to their large molecular contribution) in the preparation. PRPs are described as either pure PRP (P-PRP) or leukocyte-enriched PRP (L-PRP).⁸ Prior to joint injection, PRP can be activated by adding Ca^{2+} or exogenous thrombin to trigger the coagulation cascade. Injecting a developing fibrin clot instead of unmodified PRP into the joint capsule influences the molecular kinetics and the way in which molecules are presented to their cognate receptors. Activation is critical to confine PRP to the joint cavity, thereby ensuring that signalling elements are released during fibrin retraction and fibrinolysis. The superiority of one PRP formulation over another for clinical effectiveness has not been established.

Despite the widespread adoption of PRP therapies in the treatment of OA, the use of PRP is supported by scant scientific evidence.⁹ Repeated intra-articular administration of plasma has challenged existing paradigms, as recurring intra-articular bleeding episodes in patients with haemophilia cause progressive destruction of the joint.¹⁰ However, PRP therapies differ from bleeding

episodes because erythrocytes (which comprise 93% of the blood clot) are eliminated from PRP, thereby boosting cytokine availability.¹¹ Two randomized hyaluronan-controlled clinical trials^{12,13} and one placebo-controlled trial¹⁴ have been performed using PRP therapy in patients with symptomatic OA. PRP decreased pain and improved function in all three trials, suggesting some biological response to PRP, although the cellular and molecular mechanisms underlying this potential therapeutic effect remain poorly understood. Furthermore, major challenges, including interpatient variability in pathophysiology, the lack of biochemical and imaging biomarkers to improve diagnosis specificity, and the existence of different phenotypic forms of OA make refining and titrating PRP therapies difficult.

In this Review, we focus on how PRP might provide positive and negative inputs to modulate several biological processes involved in OA progression, without discussing the molecular intricacies of PRP¹⁵ or the complexity of OA in detail. We provide a brief update on PRP formulations and components, and discuss PRP therapies in the treatment of inflammation, angiogenesis, and joint protection. Accordingly, we provide a theoretical and investigational framework for improving our understanding of PRP functions in OA, and we outline gaps in information within this new field.

Basic science behind PRP therapies

Platelets are a natural source of signalling molecules with paracrine effects; treating injured or diseased tissues with PRP could, therefore, provide damaged tissues with some of these signals. The unusual morphology of blood platelets and the functional features of their secreted proteins underlie their role in the organization of tissue repair (Box 1). The presence of different platelet agonists at sites of tissue injury regulates the changes in platelet morphology required for secretion—strong and weak agonists induce different patterns of secretion. To date, more than 300 molecules have been identified in the platelet secretome.¹⁶ These molecules can be classified according to their granular source and proposed function. Dense-core granules contain primarily small molecules and ions, such as ADP, thromboxane A₂, 5-hydroxytryptamine, histamine, adrenaline and Ca^{2+} , all of which are critical for further platelet activation. Platelet lysosomes release a number of aryl sulphatases, and acidic proteases, glycosidases, and phosphatases.¹⁷ The α granules contain proteins that have a wide array of functions and can be subdivided into several functional categories.¹⁸ They include both soluble proteins, which are secreted into the microenvironment, and membrane proteins, which are released onto the cell surface. The secretion of these proteins is regulated by complex machinery, including the SNARE (soluble NSF attachment protein receptor) complex and its regulators,¹⁹ which mediate granule translocation and the membrane fusion events required for secretion. Platelet contents are determined during megakaryocyte synthesis in the bone marrow and by endocytosis (through membrane-receptor-mediated

uptake) from the plasma environment, and cannot yet be manipulated *ex vivo*.

The beneficial effects of PRP therapies are thought to be mediated by growth factors, such as platelet derived growth factor (PDGF) subunits A, B and C, transforming growth factor $\beta 1$ (TGF- $\beta 1$), insulin-like growth factor 1 (IGF-1), fibroblast growth factor 2 (FGF-2), hepatocyte growth factor (HGF) and vascular endothelial growth factor A (VEGF-A). However, growth factor signalling alone cannot account for the biological response to PRP, suggesting that additional molecules might be important mediators of tissue repair (Figure 1).²⁰

Inflammation

The secretion of chemokines occurs within seconds of platelet activation (marked by thrombin cleavage) and starts the inflammatory reaction by attracting leukocytes to infiltrate the injured tissues. The inhibition of platelet activation with antibodies to glycoprotein Ib (a component of the GPIIb-V-IX system that binds to von Willebrand factor and promotes platelet adhesion and aggregation) decreases polymorphonuclear cell influx by 50%.²¹ Platelet basic protein (PBP; also known as CXCL7) is a chemokine that contributes to leukocyte migration by interacting with CXC chemokine receptors 1 and 2 (CXCR1 and CXCR2), which are expressed on circulating neutrophils. This mechanism is tightly regulated, as the chemotactically active form of PBP is produced by proteolytic cleavage of the full-length form by neutrophils. This process, under certain conditions, can also be inhibited by plasma proteins.²² Platelet factor 4 (PF4; also known as CXCL4), a cationic chemokine that accounts for 25% of the total α granule cargo, either synergizes with or inhibits PBP in a concentration-dependent manner.²³

Platelet-released molecules also stimulate monocyte migration in a dose-dependent fashion.²⁴ In addition to their role as macrophage precursors, monocytes influence the nature of the immune response. The part of the platelet secretome necessary for chemoattraction is complex: 32 elements, including the C-C chemokine ligand 2 (CCL2; also known as MCP1), which can augment CCL2 effects and synergistically attract monocytes,²⁵ were present in a fraction of the platelet releasate that stimulated monocyte migration.²⁶

Monocyte differentiation and macrophage polarization are critical to the resolution of inflammation. PRP therapies that encourage macrophages to differentiate into suppressor cells could be useful to treat patients with OA. PF4 is a key contributor to monocyte survival and differentiation, and induces a unique macrophage transcriptome that is distinct from, but overlapping with, the classical and inflammatory transcriptomes.²⁷ Furthermore, activating PRP with potent agonists of platelet receptors, such as collagen and thrombin, induces a massive shedding of microparticles that have immunomodulatory properties, and polarize monocytes to become resident M2 cells.²⁸

Remarkably, activated platelets mediate proinflammatory signalling during fibrin clot formation by synthesizing IL-1 β .²⁹ Indeed, although platelets are anucleated,

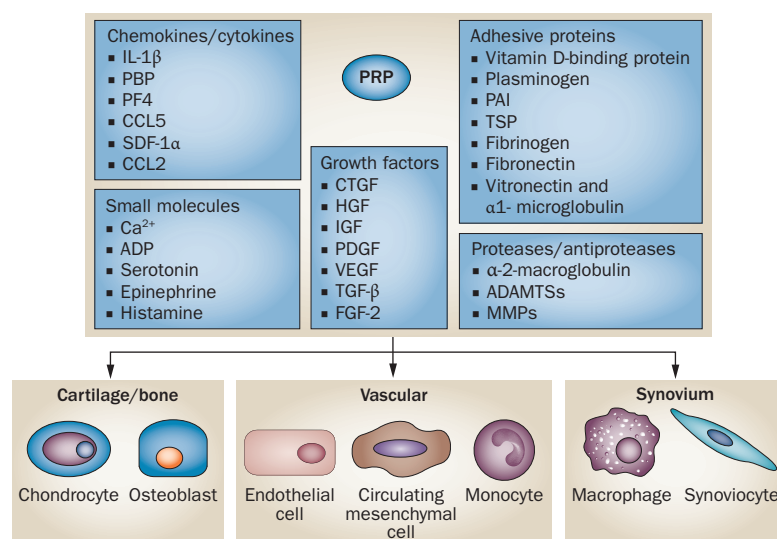


Figure 1 | PRP components and main target cells. Signalling proteins (including growth factors, chemokines and other cytokines), adhesive proteins and proteases, together with small molecules and ions that are secreted by platelets, are found at high levels in PRP and interact with multiple cell types. PRP components interact with cells involved in the immune response and inflammation, angiogenesis, cell migration and differentiation, and anabolism and catabolism of the extracellular matrix. Changes preventing these cells from maintaining homeostasis of the joint could lead to OA. PRP also affects cells in the meniscus and ligaments. This list of PRP elements is not comprehensive. Abbreviations: ADAMTSs, a desintegrin-like and metalloproteinase with thrombospondin motifs; ADP, adenosine diphosphate; CCL2, C-C chemokine ligand 2; CCL5, C-C chemokine ligand 5; CTGF, connective tissue growth factor; FGF-2, basic fibroblastic growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; MCP-1, monocyte chemoattractant protein-1; MMPs, metalloproteinases; PAI, plasminogen activator inhibitor; PBP, platelet basic protein; PDGF, platelet derived growth factor; PF4, platelet factor 4; PRP, platelet-rich plasma; SDF-1 α , stromal cell-derived factor 1 α ; TGF- β , transforming growth factor β ; TSP, thrombospondin; VEGF, vascular endothelial growth factor.

several transcripts are constitutively present in their polysomes, providing a mechanism for rapid, signal-dependent protein synthesis. Furthermore, CD40 ligand (CD40L), a membrane-bound member of the TNF ligand superfamily, is released from platelets into the plasma as soluble CD40L after metalloproteinase (MMP) cleavage, and binds to CD40 on neutrophils, monocytes, endothelial cells and fibroblasts,³⁰ inducing inflammatory effects.³¹ Platelets also store antimicrobial peptides as part of their role as the first line of defence against invading pathogens.³² Further research into how platelets affect the onset and resolution of inflammation could result in greater control of PRP therapies.

Angiogenesis

PRPs influence angiogenesis but their precise role remains elusive. Paradoxically, a granules contain both proangiogenic proteins (for example, stromal cell-derived factor 1 α [SDF-1 α], VEGF, PDGF, TGF- $\beta 1$, and FGF2) and established inhibitors of angiogenesis (such as thrombospondin-1 [TSP1] and PF4). VEGF and FGF2 stimulate angiogenesis, although TSP1 could interfere with their mitogenic effect by preventing them from binding to their receptors.³³ Also, PF4

Box 2 | Factors that can influence the effectiveness of PRP

Three main technical features can influence the effectiveness of PRP: platelet count; the balance between catabolism and anabolism; and the way PRP is activated. Evidence supporting a therapeutic difference between PRP formulations that are moderately or highly enriched for platelets is scarce. On the basis of a granule content, PRP platelets contain proteins with opposing activities: coagulation factors and anticoagulants; proteins that encourage and those that discourage angiogenesis; and proteases and their inhibitors.

The balance between growth factors and catabolic cytokines is influenced by the cellular composition of PRP. Platelets and plasma contain molecules that promote anabolic signalling, and the presence of leukocytes increases the concentration of catabolic and proinflammatory signalling molecules in PRP formulations.^{110–112} Leukocytes also increase the concentration of proteases such as elastases and MMPs in PRP. Notably, some L-PRP formulations, which contain high numbers of leukocytes, could mimic an inflammatory fluid, vastly exceeding the threshold of leukocytes (2,000 cells per μl) that defines inflammatory synovial fluid.¹¹³ In a randomized trial¹¹⁴ comparing P-PRP with an L-PRP formulation moderately enriched in leukocytes in patients with OA, no clinical differences (in pain, function or sports activities) were observed after 6 months. L-PRP was safe, although more injection swelling and pain were present after L-PRP treatment than after P-PRP treatment.¹¹⁴ Knowledge of the effectiveness of different types of PRP in clinical practice can be extremely useful, as information on which components of PRP are important in modulating the biological response comes from both the similarities and the differences between different formulas.

Abbreviations: L-PRP, leukocyte-rich PRP; MMPs, metalloproteinases; OA, osteoarthritis; P-PRP, pure PRP; PRP, platelet-rich plasma.

could directly interact with VEGF and FGF2, inhibiting ligand binding, receptor dimerization and the mitogen-activated protein kinase (MAPK) pathway.³⁴ HGF and TGF- β 1 signalling are also critical. The effects of TGF- β 1 signalling are biphasic (signalling through ALK1 [activin receptor-like kinase 1; also known as SKR3] and SMAD4/SMAD5 results in the activation of angiogenesis and signalling through ALK5 [also known as TGFR1] and SMAD2/SMAD3 subsequently results in angiogenesis resolution) and depend on the concentration and microenvironment.³⁵ Finally, substances released from dense granules (for example, prostaglandin E2, 5-hydroxytryptamine, histamine, ADP, ATP and Ca^{2+}) can alter the permeability of blood vessels—an early stage of angiogenesis. The different angiogenic mechanisms can operate in parallel or cooperatively, depending on the site and the microenvironment.

Adhesive proteins, such as fibrinogen, fibronectin, vitronectin and TSP1, are also secreted by platelets, as are fibrinolytic agents such as plasminogen activator inhibitor and carboxypeptidase β 2. Fibrinolysis is important for clot remodelling, and can precisely regulate the pericellular proteolytic environment, thereby controlling cell migration and matrix remodelling.³⁶ For example, urokinase plasminogen activator has the potential to affect the motile programme on multiple levels (such as attachment, detachment and motility), providing the opportunity to therapeutically manipulate migration of human vascular cells in pathophysiological settings.³⁷ Moreover, other components of the PRP, such as HGF, have important roles in cell motility. For instance, mesenchymal stem cells (MSCs) express the high-affinity tyrosine kinase receptor for HGF (Met), which, when activated, promotes their migration towards sites of injury.³⁸

The optimal composition of PRP therapies has not yet been determined. Compositional heterogeneity renders PRP therapies unpredictable, especially when leukocytes are included (Box 2). The mechanisms through which platelets and their molecular contents function and interact with each other are also incompletely understood. However, the list of secreted elements, their multiple cell targets and biological functions suggest that PRP could be critical in establishing the microenvironment around intracapsular tissues.

Role for PRP therapies in OA Inflammation

Synovitis is associated with cartilage degradation in patients with OA, and early synovial inflammation could precede structural changes.³⁹ Low-grade inflammation sets the stage for chronic disease.³⁹ Targeting inflammation is daunting in cases of early OA as well as established OA—deciphering which cells interact with PRP (considering all joint components including cartilage, synovium, menisci, ligaments and subchondral bone), and how their bidirectional interactions are modified by PRP in the different stages of OA, is challenging.

Cartilage degradation and oxidative damage are hallmarks of OA. Joint stress causes oxidative damage, which, in turn, causes the release of molecular fragments of the extracellular matrix. Moreover, stressed cells (apoptotic or necrotic) release endogenous molecules that can trigger inflammation in the absence of infection. These stress-related stimuli are collectively known as damage-associated molecular patterns (DAMPs), and they promote articular inflammation by binding to pattern-recognition receptors,⁴⁰ primarily Toll-like receptors (TLRs), expressed by phagocytes and other cell types in the joint. Stimulation of these receptors can lead to the activation of nuclear factor κB (NF κB), an essential and ubiquitously expressed transcription factor that, together with other pathways including the MAPK pathway, mediates inflammatory and catabolic events in OA (Figure 2).⁴¹ Likewise, accumulation of advanced glycation end products leads to the activation of NF κB in OA chondrocytes,⁴² providing a mechanism for the progression of age-related OA.

Given its role in inflammatory signalling, and its involvement in the development and processing of pathological pain, NF κB has long been considered an attractive target for therapeutic intervention in OA.⁴³ Silencing the expression of the p65 subunit of NF κB (through specific RNA interference *in vivo*), for example, has been shown to reduce the induction of IL-1 β and TNF in synovial fluid, synovial inflammation and cartilage degradation in the early phases of experimental OA.⁴⁴ The anti-inflammatory effects of PRP are partially attributable to reduced canonical NF κB signalling (Figure 2).^{45–47} The precise nature of the connection between PRP signalling and downstream NF κB pathway molecules is not fully established, but the underlying mechanisms are becoming increasingly understood. For example, in cultured human chondrocytes, CaCl_2 -activated PRP reduces the expression of

the NF κ B transcriptional targets cyclooxygenase 2 (COX2; also known as prostaglandin G/H synthase 2) and chemokine receptor CXCR4.⁴⁵ Additionally, HGF from PRP enhances the expression of NF κ B inhibitor α (I κ B α), which reduces NF κ B signalling.⁴⁵ PRP releasate (the soluble fraction extruded from PRP clots) stimulates the OA synoviocytes to produce additional HGF.^{48,49} Moreover, TGF- β 1, within the context of PRP, abolished CXCR4 expression in monocytes in a NF κ B-dependent manner,⁴⁵ thus impeding monocyte migration towards its specific ligand, SDF-1 α , in the presence of TNF (which was used to recreate inflammatory conditions in the cell culture).

Corroborating these findings, PRP releasate completely reversed the IL-1 β -induced inflammatory response of chondrocytes isolated from patients with OA, including the increase in NF κ B activation.⁴⁶ IL-1 β -activated NF κ B in chondrocytes turns off almost all anabolic pathways, including type II collagen and aggrecan synthesis; treatment with PRP rescued the synthesis of these molecules.^{46,47} Similarly, PRP treatment counteracted the IL-1 β -induced transcription of the genes encoding a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) and COX2.⁴⁷ However, the IL-1 β -mediated production of metalloproteinases (notably MMP1, MMP3 and MMP13) and nitric oxide were not affected by PRP treatment in chondrocytes⁴⁶ or synoviocytes⁴⁸ from patients with OA. Likewise, exposing endothelial cells pretreated with TNF and interferon- γ to P-PRP suppressed the expression of inflammatory markers, including cell adhesion molecules (such as E-selectin) and the HLA class II histocompatibility antigen HLA-DR.⁵⁰ These findings, although not necessarily predictive of results in patients, provide preclinical support for the concept that PRP deactivation of NF κ B could reduce chondrocyte inflammation, restoring anabolic activity and hindering monocyte migration.

As mentioned above, growth factors present in PRP might be responsible, at least in part, for the anti-inflammatory effects of PRP. IGF-1 or homodimers of PDGFB inhibit I κ B kinase α (IKK α), thereby suppressing the activation of NF κ B and the transcription of its downstream targets involved in inflammation, cartilage degradation or chondrocyte apoptosis and reversing the antianabolic effects of IL-1 β .⁵¹ In addition, HGF treatment decreases the production of the proinflammatory cytokine IL-6 and increases that of the anti-inflammatory cytokine IL-10 in macrophages stimulated by lipopolysaccharide.⁵² Many chemokines and cytokines activate NF κ B in different cell types; thus, administering PRP can potentially affect numerous overlapping pathways simultaneously. Studies using a single cell type do not recapitulate the true interactions and influences between cells (macrophages, chondrocytes and synoviocytes) in this network of cytokine signalling. Nevertheless, despite our incomplete knowledge of its mechanisms of action, PRP is a suitable treatment for established inflammatory conditions, and attenuates immunogenic arthritis of the knee in a porcine model.⁵³

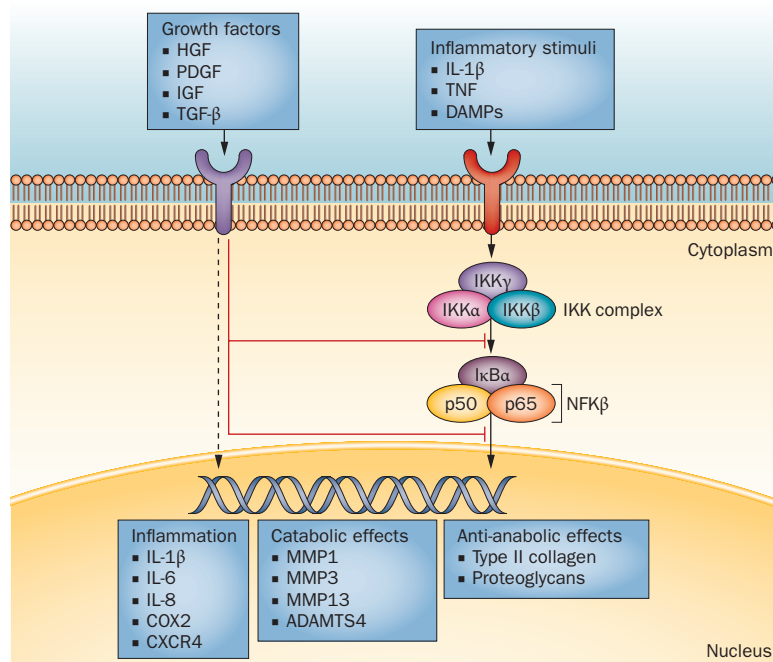


Figure 2 | Anti-inflammatory activities of PRP in chondrocytes. Sterile inflammation, caused by DAMPs and mediated by IL-1 β or TNF in the absence of infection, involves canonical NF κ B activation and proinflammatory signalling. In OA, molecules from cartilage breakdown and intracellular components of dying cells (DAMPs) spread to all joint tissues through the synovial fluid, and interact with pattern recognition receptors, mainly Toll-like receptors, expressed by synoviocytes, macrophages and chondrocytes. NF κ B is also activated by proinflammatory cytokines such as IL-1 β and TNF binding to their receptors. Once activated, NF κ B translocates into the nucleus and binds to the promoter region of target genes, thereby upregulating the transcription of proinflammatory mediators and molecules that promote tissue remodelling.^{110–112} PRP inhibits the NF κ B cascade, preventing the release of NF κ B from I κ B α , thereby trapping NF κ B in the cytoplasm and preventing the induction of NF κ B target genes.^{39,42} Not all NF κ B target genes are affected equally by PRP treatment. Abbreviations: ADAMTS, a disintegrin-like and metalloproteinase with thrombospondin motifs; COX-2, prostaglandin G/H synthase 2; CXCR4, C-X-C chemokine receptor type 4; DAMPs, disease activating molecular patterns; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; I κ B α , inhibitor of κ B α ; IKK, I κ B kinase; IL-1R, interleukin 1 receptor; MMP, metalloproteinase; NF κ B, nuclear factor κ B; OA, osteoarthritis; PDGF, platelet derived growth factor; PRP, platelet-rich plasma; TGF- β , transforming growth factor β .

Proinflammatory effects

Certain components of PRP can induce synovitis, suggesting that PRP is not unequivocally anti-inflammatory (Figure 3). Three plasma proteins (α -2-macroglobulin, α -1-microglobulin, and vitamin D-binding protein [also known as group-specific component globulin]) activate macrophages via the innate immune receptor TLR4 and induce the expression of TNF, IL-1 β , IL-6, and VEGF.⁵⁴ Other plasma elements, including fibrinogen, fibronectin and hyaluronan, can induce sterile inflammation by acting as DAMPs and stimulating TLR4 and TLR2;⁵⁴ PRP might, therefore, promote inflammation. Moreover, the platelet-derived bioactive lipid, platelet-activating factor, along with leukotriene B4 and high mobility group protein B1 might independently act as TLR ligands.⁵⁴ These findings are clinically relevant because TLR2 and TLR4 are specifically upregulated in areas of cartilage with lesions,⁵⁵ and TLR activation

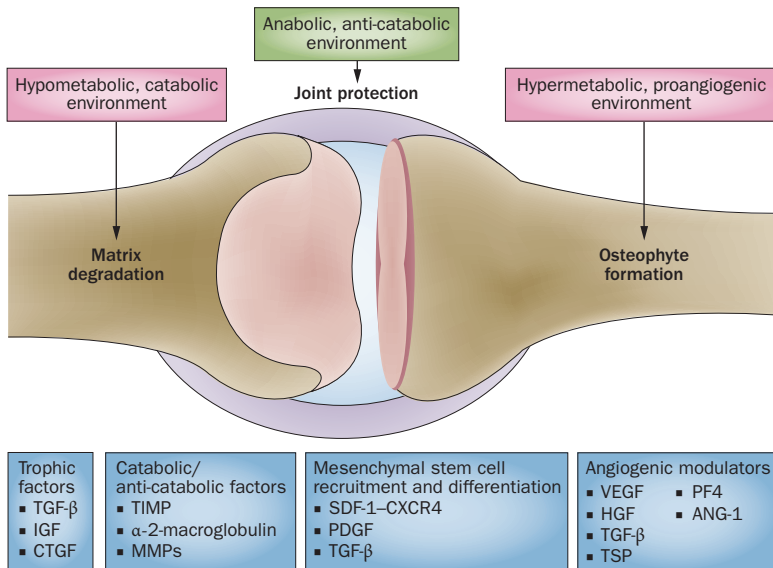


Figure 3 | Synthetic and degradative processes can be modified with autologous blood products. PRP can elicit healing but also has potentially adverse consequences. PRP contains molecules that affect multiple aspects of joint function and homeostasis. Trophic factors can positively modify cell metabolism and activate anabolic pathways. These anabolic processes could be age-dependent. PRP can alter the ratio between catabolic and anabolic factors. The degree to which angiogenesis is promoted within the joint can be altered by PRP components. PRP influences mesenchymal stem cell migration and self-renewal, and promotes their differentiation in specialized joint cells. Conversely, trophic and proangiogenic factors (such as TGF- β and VEGF) can promote osteophyte formation. The effects of PRP-released cytokines, and whether these cytokines are beneficial or detrimental to overall joint health, therefore depend on the timing of release, the dose and the context. Analysing these conflicting roles might help to improve PRP therapies. Abbreviations: ANG-1, angiopoietin-1; CTGF, connective tissue growth factor; CXCR4, CXC chemokine receptor 4; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; MMPs, metalloproteinases; PDGF, platelet-derived growth factor; PF4, platelet factor 4; SDF-1, stromal cell-derived factor; TGF- β , transforming growth factor β ; TIMP, tissue inhibitor of metalloproteinases; TSP1, thrombospondin 1; VEGF, vascular endothelial growth factor.

increases cartilage breakdown.⁵⁶ If leukocytes are also present in PRP (as in L-PRP), additional inflammatory elements are present, and MMP1 and MMP3 are produced by synoviocytes from patients with OA cultured with L-PRP.⁵⁷ Cell signalling in osteoarthritic joints is, therefore, complex. The multiple effects of PRP might arise not only from differences among formulations but also from the specific microenvironment present in each experiment and in each patient.

Angiogenesis

Factors present in osteoarthritic joints promote angiogenesis.^{58,59} Cartilage degeneration is associated with the presence of VEGF receptors,⁶⁰ the invasion of blood vessels from subchondral bone into the cartilage and the formation of osteophytes at the joint margin, a process mediated by VEGF-induced endochondral ossification.⁶¹ Moreover, VEGF is highly concentrated in the synovial fluid, reflecting increased vascular turnover and local synthesis.⁶² *In vitro*, synovial fibroblasts from patients with OA constitutively produce VEGF; PRP does not induce additional synthesis.^{48,49}

PRP contains many angiogenic modulators. However, the secretion of these modulators by platelets can be context-dependent: platelets can release specific sets of molecules from subpopulations of a granules, and secretion patterns are agonist-specific.⁶³ Secretion of different types of granules occurs after the selective engagement of proteinase-activated receptors 1 and 4 (PAR-1 and PAR-4).⁶⁴ For example, PAR-1 activation induced the release of large quantities of SDF-1 α and VEGF but small quantities of PF4 and endostatin; by contrast, PAR-4 activation triggered substantial PF4 and endostatin release.⁶⁵ This observation suggests that different environmental stimuli evoke distinct secretion patterns of proangiogenic and anti-angiogenic factors.

Importantly, a granules constitute the largest rapidly releasable reservoir of TSP1 in humans ($3.5 \pm 0.5 \mu\text{g}$ per 10^8 platelets).⁶⁶ TSP1, which inhibits endothelial cell proliferation by preventing VEGF from binding to receptors on the cell surface, could have a role in an experimental model of OA.⁶⁷ Intra-articular gene transfer of *THBS1*, which encodes TSP1, reduces microvessel density and inflammation, and suppresses OA progression relative to controls. Angiogenesis might, therefore, be modulated by PRP, and could be an important target in the treatment of OA.

Extracellular matrix

Synovial fluid—a dialysed form of blood plasma that contains hyaluronan, lubricin, plasma low-molecular-weight proteins, and cytokines produced by the joint—enables reciprocal communication between multiple cell types in the joint and contributes to the observed phenotype at the organ level. PRP injections might directly modify synovial fluid composition or indirectly alter the local synthesis of synovial fluid by synoviocytes. Indeed, synoviocytes isolated from patients undergoing total knee arthroplasty and cultured with PRP produced and secreted more hyaluronan than synoviocytes cultured with platelet-poor plasma.⁴⁸ Synoviocytes treated with IL-1 β also secreted elevated levels of hyaluronan, and combined treatment with P-PRP and IL-1 β had a synergistic effect on hyaluronan levels, suggesting that P-PRP induces chondroprotection and joint lubrication, even in the presence of inflammation.⁴⁸ By contrast, synoviocytes produce MMPs when treated with L-PRP.⁵⁷ Identifying factors in PRP that modulate synovial secretion could improve the formulation.

PRP is assumed to be chondroprotective for cartilage because it has high levels of IGF-1 and TGF- β 1—molecules that mediate cell survival and extracellular matrix deposition. However, the activities of these proteins are regulated at multiple levels. Physical association with other proteins (such as IGF binding protein 3 and latency-associated peptide) can, in certain contexts, inhibit IGF-1⁶⁸ and TGF- β 1.⁶⁹

Furthermore, proteases present in synovial fluid affect both angiogenesis and immune responses.⁷⁰ The activities of growth factors are also regulated by the number and avidity of their cognate receptors. For example, during ageing or the development of OA, chondrocytes

progressively lose the type II TGF- β receptor with a subsequent decline in the phosphorylation of SMAD2 and downstream signalling.⁷¹ Moreover, without SMAD2/SMAD3 signalling, chondrocytes exit their quiescent state and undergo abnormal terminal differentiation through alternative SMAD1/SMAD5/SMAD8 signalling pathways, leading to chondrocyte hypertrophy and increased production of MMP13.⁷¹ Likewise, the capacity of IGF-1 to stimulate cartilage matrix synthesis is reduced in aged and osteoarthritic cartilage; elevated levels of reactive oxygen species alter IGF-1 signalling intermediates,⁷² resulting in inadequate chondrocyte biosynthetic and growth responses to IGF-1 stimulation. Theoretically, PRP therapy is proanabolic; however, molecular changes that occur with ageing, or during the development of OA, could lead to growth factor dysfunction.⁷³ Therefore, if anabolic growth factor stimulation is impaired with increasing age, PRP might be most effective when administered to young patients with traumatic injuries or early OA.⁷⁴

In low-grade chronic inflammation, excessive or inappropriately expressed MMPs can contribute to cartilage destruction, and can also affect a wide range of nonmatrix extracellular proteins, such as growth factors, cytokines, chemokines, receptors and other proteins on the cell surface. For example, connective tissue growth factor (CTGF, which is found in PRP at levels 20-fold higher than any other growth factor) mediates collagen deposition and promotes cartilage regeneration⁷⁵ but is degraded by MMP13,⁷⁶ providing one plausible explanation for the inability of CTGF to orchestrate cartilage repair in OA. Importantly, plasma is a major source of potential MMP inhibitors, including α -2-macroglobulin and the aggrecanases ADAMTS4 and ADAMTS5.⁷⁷ However, although PRP also contains tissue metalloproteinase inhibitors, their concentrations are low enough to be irrelevant.⁷⁸

Using a sheep model, PRP therapy was used in combination with subchondral drilling, and recruitment of repair cells to damaged tissues was enhanced by PRP treatment.^{79,80} SDF-1 α is a chemoattractant for stem cells (which are involved in repair) and is a component of PRP. Further investigation into the role of SDF-1 α as a pivotal regulator of stem cell trafficking might uncover a mechanism through which these cells can be recruited.^{81,82} The repair of articular cartilage in early OA could ultimately rely on tissue-engineering strategies involving MSCs and their association with PRP.⁸³

PRP-augmented cartilage engineering

Preclinical data

PRP-augmented cartilage engineering refers to efforts to improve the outcome of cartilage tissue engineering. PRP is a powerful research tool to study the molecular and cellular processes of cartilage regeneration. PRP increases chondrocyte viability^{84,85} and proliferation, and enhances the deposition of extracellular matrix proteins (including proteoglycans, glycosaminoglycans and type II collagen).⁸² PRP has been shown to decrease type II collagen synthesis and promote chondrocyte dedifferentiation in

several studies.^{84,86,87} However, these results were not consistent with those of other studies in which PRP increased the differentiation of MSCs into chondrocytes;^{82,88,89} this differentiation state was maintained through multiple cell passages in PRP cultures relative to cultures grown in foetal calf serum.⁹⁰ These disparities could be attributed to different culture models and to variations in the composition of PRP, including the presence of platelet lysates (obtained through repeated cycles of freezing and thawing) or PRP releasates. The gross and histological appearance of the repaired cartilage^{91–93} was improved after PRP-augmented cartilage engineering compared with cartilage engineering in the absence of PRP, except with one construct (PRP with a collagen–hydroxyapatite scaffold).⁹⁴ MSCs from different sources, including bone marrow, adipose tissue,⁸³ skeletal muscle,⁹⁵ and synovial membrane,⁹⁶ seeded within PRP scaffolds, differentiate into cartilage and could successfully resurface cartilage defects.

Clinical data

One-step surgical approaches

Addition of PRP to microfracture treatment of chondral defects enhanced the mechanical qualities of the repaired cartilage,^{79,80} and thus PRP could be used to improve a number of approaches to tissue engineering. For example, bone-marrow-stimulating techniques (for example microfracture, which involves drilling into the subchondral bone) that induce the invasion of mesenchymal progenitor cells from the subchondral bone into the lesion have been successfully augmented with PRP in a cohort study of patients 40–50 years of age, with early-stage OA.⁹⁷ Promising modifications of microfracture surgery involve sealing the PRP-treated lesions with a membrane, which can confine cells to the lesion.⁹⁸ Moreover, after microfracturing, polyglycolic acid–hyaluronan implants immersed in L-PRP have been used to fill the focal cartilage lesions, leading to improvements in clinical symptoms and anatomy.⁹⁹

Cartilage engineering using PRP injections

Nonsurgical approaches are of increasing interest. Preliminary evidence of improvements in cartilage by radiography and whole-organ MRI score has been observed after injection of adipose-tissue-derived MSCs from the infrapatellar pad combined with PRP.¹⁰⁰ Likewise, the injection of the stromal vascular fraction of adipose-tissue-derived MSCs, combined with PRP or hyaluronic acid, decreased pain for ≥ 1 year in patients with chondromalacia patellae.¹⁰¹

The simplest PRP-based tissue-engineering approach is an intra-articular injection of PRP whereby PRP clots within the joint cavity. Indeed, PRP provides two of the main components required for tissue engineering—a 3D scaffold and cell signalling factors. Ideal candidate patients for PRP injections tend to be young and have osteochondral lesions¹⁰² or early post-traumatic OA, but this patient profile needs further confirmation.^{103–106} The specifics of PRP treatment, including the volume of plasma, number of injections, and interval between

injections, have not yet been optimized. Up to nine injections in 1 year did not promote significant cartilage regeneration in patients with grade II or III chondromalacia.¹⁰⁷ Frequent PRP administration could plausibly delay OA progression and joint arthroplasty. However, high-quality evidence for the use of PRP injections is limited to a few randomized trials focused on pain and function with ≤ 6 months of follow-up.^{12–14} Unambiguous answers will require prospective, controlled randomized trials using surrogate end points such as refined imaging and biochemical markers that are potentially predictive of OA progression. In patients with substantial and irreversible bone and cartilage damage, PRP will not cause osteophytes to regress or cartilage and meniscus to regenerate. Nevertheless, PRP is a potentially beneficial, nonsurgical option because it could improve quality of life, even for patients with advanced OA.¹⁰⁸

In clinical studies to date, PRP is safe, with no infections, worsened outcomes, or serious complications reported. Minor adverse events associated with repeated intra-articular injections of PRP have been moderate pain, swelling and mild effusion that lasted a few days.^{12–14,102–107} Thus, clinical acceptance of PRP therapies and marketing of devices for preparing PRP have occurred quickly, because of both the safety profile and the low amount of manipulation involved in the preparation. However, extensive clinical use is not supported by high-quality evidence of a clear clinical improvement.

Conclusions

Stimulating endogenous joint repair mechanisms with PRP could be rational and realistic—local administration of PRP is an appealing, integrated, multiple-target strategy to manipulate the intensive crosstalk and temporospatial relationships between joint components, thereby creating a microenvironment conducive to preventing OA progression. However, PRP formulations are

complex, and understanding how they affect a joint with OA is not straightforward.

PRP therapies produce context-dependent results. Thus, intercellular and intracellular signalling events could be modified differently by PRP in the various stages of OA. A clearer understanding of the situations in which PRP is effective could help to explain which processes (for example, inflammation, angiogenesis or cell metabolism) are primarily affected by PRP treatment.

Research efforts are currently underway to provide a comprehensive description of the relationships between PRP components and major pathogenic mechanisms. The identification of new formulations that can be used safely and effectively to manipulate the biological environment would be valuable and would hopefully deliver clues on the processes involved in OA pathogenesis. The success of PRP-augmented tissue engineering will depend on defining which candidates are best suited to each approach (surgical or conservative). Opportunities abound for researchers to use existing knowledge and bridge the gaps that currently constrain the therapeutic use of PRP.

Review criteria

The information in our Review is primarily based on PubMed and Web of Knowledge searches using the term “platelet rich plasma” in combination with “osteoarthritis”, “cartilage”, and “synovium”. This search was completed by a manual search of these articles for additional relevant material. English language publications were selected based on authors’ views of their relevance to the concepts discussed. We mainly included papers published within the past 5 years, with the addition of highly regarded older papers, up to June 2013. We also included some review articles as comprehensive overviews of areas beyond the scope of this article.

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Author contributions

Both authors researched data for the article, discussed content, and wrote, reviewed and edited the article.