

# Platelet-rich plasma injections for tendinopathy and osteoarthritis

Tendinopathy and osteoarthritis are common chronic musculoskeletal conditions that are associated with frequent pain and reduced function. With the advent of autologous platelet-rich plasma (PRP), new opportunities are available to elucidate potential healing mechanisms and elaborate safe injection therapies. PRP therapy involves the use of autologous activated platelets retained in fibrin matrices as a source of growth factors and cytokines that are active in healing mechanisms, such as inflammation, angiogenesis, cell migration and proliferation. Here we provide an overview of the main components of PRP products relevant to the mechanism of action in tendinopathy and osteoarthritis, and emphasize the importance of identifying and exploiting principal molecular components in PRP for therapeutic benefit. We review the clinical applications of PRP in osteoarthritis and tendinopathy and discuss the current existing challenges.

**KEYWORDS:** angiogenesis • cytokines • growth factors • inflammation • injection therapies • osteoarthritis • platelet-rich plasma • PRP • tendinopathy

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Among the many challenges to musculoskeletal health, tendinopathy and osteoarthritis (OA) are extremely common, and can lead to frequent pain and reduced function. The problem is likely to be intensified by current trends, including the growing epidemic of injuries in sports. In truth, while the number of sports practitioners increases, the rate of sports injuries distressing the musculoskeletal system is growing and becoming a challenging problem. For example, people who participate in sports have an increased risk of joint injuries, hence OA is common among former professional athletes or sports practitioners aged over 50 years [1]. Furthermore, 30–50% of the injuries among professional and recreational athletes are overuse tendon injuries, so tendinopathy has become one of the most exasperating problems for patients and physicians in orthopedic sports medicine [2]. Accordingly, both conditions have substantial impact on quality of life, including disabilities with widespread economic consequences, as post-traumatic OA costs more than US\$12 billion annually [3]. At present, no available therapeutic options have been able to delay the progression or reverse chronic recalcitrant tendinopathy and joint damage, thus worsening prognosis and involving pessimistic social and economic implications.

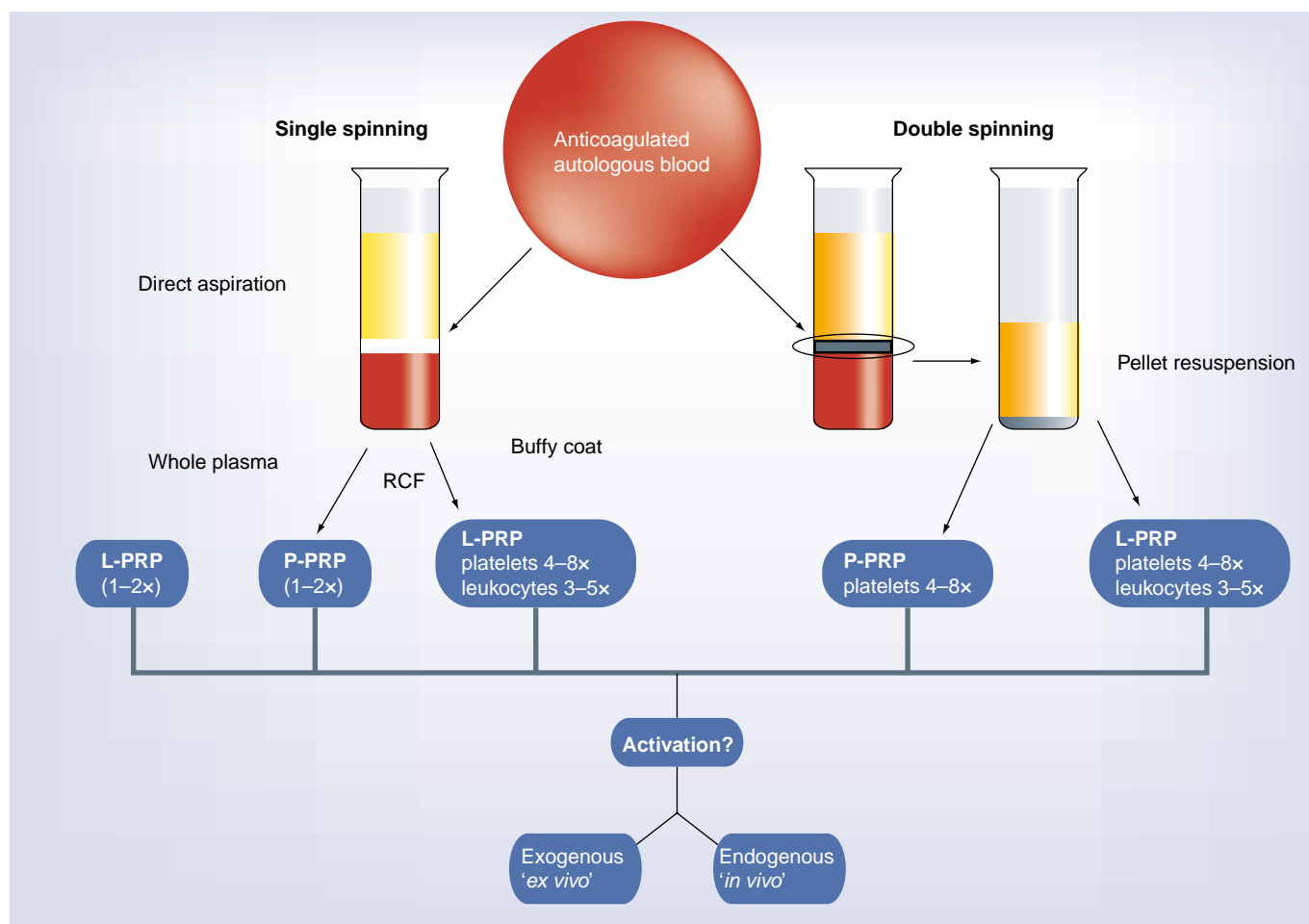
Among the emerging technologies for enhancing and accelerating tissue healing, a biocompatible and cost-effective approach, broadly referred to as platelet-rich plasma (PRP) therapy, involves the use of autologous activated platelets retained

in fibrin matrices as a source of healing molecules [4]. Nowadays, PRP therapies represent a major breakthrough in the treatment of many medical conditions and are one of the hottest topics in orthopedics owing to their presumed healing properties. The underlying principle of PRPs is to deliver a large pool of signaling proteins such as growth factors and other cytokines to the local milieu driving the tissue regeneration mechanisms [5,6]. The impact of the discoveries regarding the potential of PRP in regulating multiple biological processes, such as cell proliferation or the modulation of angiogenesis, and inflammation has fuelled the optimism regarding autologous PRP treatments. Contributions in the field have not only improved the clinical treatment of many patients with different clinical conditions but, from a multimolecular perspective, have opened the field of PRP science to cellular and molecular exploration of healing mechanisms.

This article briefly reviews the important progress that has been accomplished in the field of PRP in chronic musculoskeletal conditions in the last few years. The main components of PRP products and their impact in healing mechanisms, the most exciting applications in rheumatology and the existing current challenges will be discussed.

## PRP therapy

PRP therapy is an emerging science that encompasses both the acquisition of new knowledge regarding pathogenetic mechanisms and the



**Figure 1. Methods of producing platelet-rich plasma.** The diverse methods of producing PRPs determines the composition and concentration in terms of leukocytes, erythrocytes and platelets in a given plasma volume. Basically, there are two methods: double-spinning methods using automated machines along with commercial kits; single-spinning methods using conventional laboratory centrifuges followed by manual PRP separation. When using single spinning, mostly plasma and platelet components are isolated. The platelet yield is 1–3-fold baseline levels if no plasma is discarded, while 4–8-fold baseline levels are concentrated by using double-spinning or buffy coat-based methods (single-spinning, but some plasma is discarded). Based on qualitative differences PRP products have been categorized into P-PRP, in which leukocytes are purposely eliminated from the PRP, and L-PRP, generally containing high concentration of leukocytes.

L-PRP: Leukocyte and platelet-rich plasma; P-PRP: Pure platelet-rich plasma, PRP: Platelet-rich plasma.

research necessary to link healing with PRP components. The concept of PRP therapy as a natural source of signaling molecules with paracrine effects in different cells is the basis of PRP application in different tissues and clinical conditions [7].

#### Platelet overview

Platelets comprise up to  $1.4 \times 10^{11}$  cells/l of blood and are anucleated, yet replete with secretory granules that are critical to their function. Adhesion and activation, along with fibrin formation, cause the release of intracellular stores; predominantly  $\alpha$ -granules (50–80 per platelet), dense granules (3–5 per platelet), and lysosomes [8]. Platelets contain prestored and rapidly releasable signaling proteins, such

as growth factors and chemokines, as well as small molecules such as histamine, dopamine or noradrenaline [9]. Activated platelets in PRP have the potential to rapidly modify the pericellular microenvironment and accordingly incite diverse responses in the nearby milieu. Alternatively, PRP-released growth factors and cytokines can bind to the fibrin matrix and to proteoglycans in the extracellular matrix (ECM), constituting a storage pool that can be secondarily released by metalloproteases active in the matrix [9].

#### ■ Practical insights: critical parameters in PRP elaboration

The term PRP is frequently chosen to designate the process of withdrawing peripheral blood

from the patient, with or without anticoagulants, and obtaining the plasma/fibrin fraction after centrifugation and separation under sterile conditions.

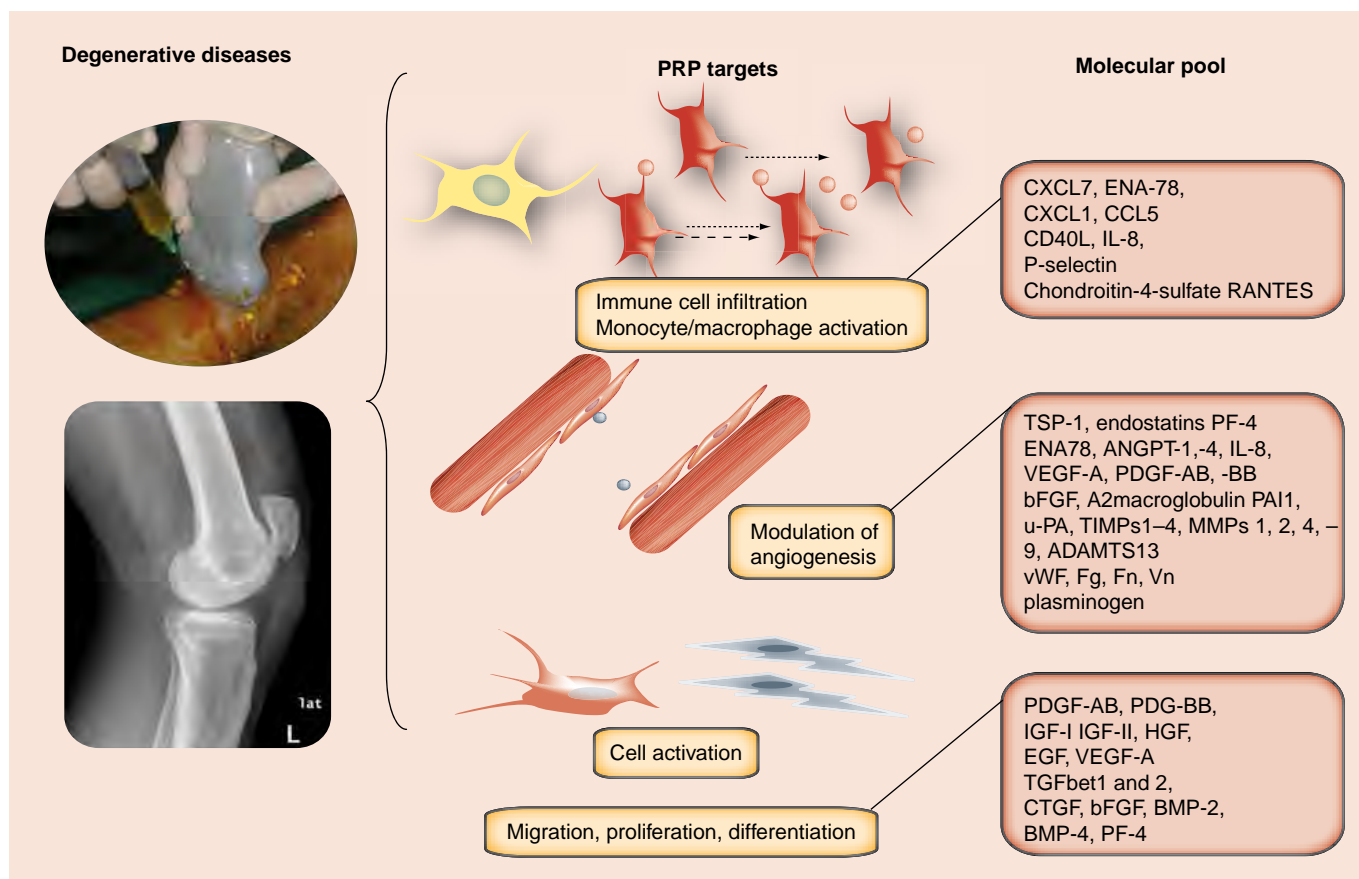
Recent investigation found that intra-donor variability in PRP production was high [10]. Many different factors such as hydration, lipemia, circadian rhythms and nonstandardized conditions of blood withdrawal (i.e., unfasting conditions) hinder reproducibility. Moreover, laboratory-generated artifacts such as platelet clumping, sample storage, shear forces during the spinning procedure or plasma manipulation compromise platelet count and ELISA measurements making interpretation of results difficult [11,12].

### Composition

Essentially, the diverse methods of producing PRPs determine the composition and concentration in terms of leukocytes, erythrocytes and platelets in a given plasma volume (see FIGURE 1). Based on qualitative differences PRP products have been categorized into pure PRP (P-PRP),

in which leukocytes are purposely eliminated from the PRP, and leukocyte and PRP (L-PRP), containing high concentration of leukocytes [13]. Further refinement of this classification is based on quantitative criteria such as platelet and leukocyte count [14]. This categorization of PRPs helps to explain nuances and biological complexities that are the key issue for better understanding and comparisons of the reported clinical effects.

At present, a major debate in PRP therapies focuses on the optimal level of platelets, and on whether leukocytes should be present in certain applications. Initially, it was accepted that PRP effects were platelet/growth factor-driven; however, although this concept has been rigidly adhered to for many years, at present it is undermined by a lack of correlation between PRP composition in terms of growth factors and clinical efficacy. Establishing the physiologic importance of a particular protein in healing is challenging as platelets contain hundreds of soluble proteins that are released into the pericellular space after



**Figure 2. Insights into how platelet-rich plasma-therapies influence healing mechanisms in osteoarthritis and tendinopathy.**

PRP therapies provide a multifunctional microenvironment by releasing a myriad of molecules involved in the healing mechanisms. For this reason they target multiple cell phenotypes and modulate various biological processes including inflammation, angiogenesis, cell migration and proliferation and the anabolism/catabolism (synthesis and remodeling) of extracellular matrix. PRP: Platelet-rich plasma.

**Box 1. PRP-associated positive and negative regulators of angiogenesis.****PRP-associated angiogenesis stimulators**

- VEGF
- bFGF
- PDGF
- EGF
- HGF
- IGF-1, IGF-2
- ANGPT1
- MMP-2 and -9
- LPA
- SIP
- SDF-1, CXCL12
- Heparanase
- Factor V/Va, Factor XI
- Deoxyribose-1-phosphate
- CD40-L
- Tissue factor
- IL-8, CXCL8
- CXCL12

**PRP-associated angiogenesis inhibitors**

- Angiostatin
- Endostatins
- Platelet factor 4
- $\beta$ -thromboglobulin
- PAI-1
- TGF- $\beta$
- TSP-1
- TIMP-1,-2,-3,-4
- Fibronectin
- Vitronectin
- $\alpha$ 2-macroglobulin
- $\alpha$ 2-antiplasmin
- Osteonectin
- TFPI
- High-molecular-weight kininogen
- Antithrombin

**Downregulators of vessel permeability**

- Angiopoietin-1
- Serotonin
- Sphingosin-1-P

**Increasesors of vessel permeability**

- VEGF
- Histamine
- Noradrenaline
- Dopamine

The list of PRP molecules has been compiled from refs [9,77–80] although the authors have made additions. It should be noted that some of these molecules may have both pro- and anti-angiogenic potential depending on the situation at the time of their release and/or the expression of cryptic sites.

PRP: Platelet-rich plasma.

PRP application [15]. Moreover, diverse proteins, as well as some proteins found in  $\alpha$ -granules, are also present in plasma, and tissue outcome may depend on the balance between plasma and platelet proteins. In fact, IGF-I, IGF-II and HGF, primarily present in plasma, are critical to musculoskeletal tissue healing.

Not only do platelets count in a given volume of plasma, but the presence and concentration of leukocytes may change the properties of PRP. Whether leukocytes have detrimental effects in orthopedic applications is still controversial, but basic evidence point towards a deleterious effect of neutrophils by synthesizing prostaglandins, reactive oxygen intermediates, elastases and other proteases that may reduce the activity of growth factors and cytokines, and destabilize the fibrin matrix [16,17].

**Activation**

Before injecting PRP into the injured tissue, plasma can be activated *ex vivo*. Activation of the coagulation cascade is a critical step in stimulating growth factor and cytokine release from PRPs. In fact, the PRP fibrin that forms upon coagulation serves as a reservoir for cytokines that are bound within the fibrin matrix and are released overtime during fibrin retraction or during fibrinolysis. Thus, the kinetics of fibrin formation/retraction is crucial in signaling and cellular functions. For instance, PRP activated *ex vivo* with thrombin induces rapid clot formation/retraction and a sudden burst of signals compared with  $Ca^{2+}$  or collagen [18–20]. Alternatively, the clotting cascade can be activated *in situ* by tissue factor, the initiator of the host response to injury. Recent experimental *in vitro* work shows that inactivated PRP has better healing properties than thrombin-activated PRP [19]. In fact, thrombin activates PAR-1 and PAR-4 nonspecifically, inducing a contemporary release of antagonistic factors (PAR-1 for VEGF and PAR-4 for endostatin) [20]. The differences in the effect may be attributed to the way the growth factors were presented to the cells. At present, most commercial systems avoid the use of immunogenic bovine thrombin. A common strategy for activation is progressive  $Ca^{2+}$ -induced generation of endogenous thrombin. *In situ* activation of platelets, by local tissue factors, is another commonly used strategy for achieving favorable outcomes. In a recent study in an animal model of diabetes, Scherer *et al.* [19] found that nonactivated platelets stimulated wound healing more efficiently than activated platelets, by enhancing fibroblast differentiation

and contractile function [19]. In this study, non-activated platelets were used for their ability to interact with the environment, as a cell therapy, rather than be exploited for their GF content. This is an important topic that deserves new and more in-depth research.

In addition, laboratory experiments have demonstrated that thrombin induces an immediate release in contrast to a more sustained release pattern achieved with local collagen [21]. The kinetics of cytokine release is important as most cellular responses are widely influenced not only by cell surface receptors but also by the method by which their cognate ligands (growth factors or cytokines) are delivered. For example, a receptor may be acutely activated on an immediate increase in ligand concentration (as mimicked by most drugs), but often in physiology, a constitutively secreted factor needs to accumulate over time to reach a threshold set by the affinity of the receptor. Moreover, when the ligand is secreted from a distant source the responding cells may experience a gradual increase in the concentration of the ligand. This mechanism of action is supported by experimental evidence showing that when local fibroblasts (from tendon or synovium) are treated with PRP, these cells synthesise further amounts of VEGF and HGF [22].

### ■ Insights into how PRP works

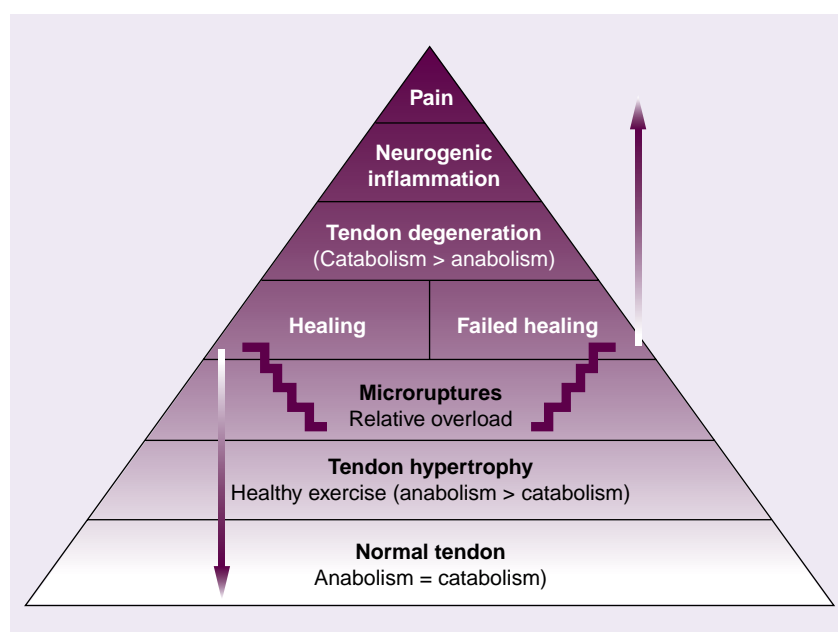
No simple rule can be generalized to describe how PRP works for the treatment of tendinopathy and OA. However, it is considered that any PRP, however platelet concentrate or moderate, must be capable of interfering with various healing mechanisms – that is, influencing the inflammatory response, inducing cell migration and proliferation, and modulating angiogenesis. Given the redundancy and pleiotropy of the PRP cytokine network, the specific actions of individual cytokines and the molecular mechanisms behind their functions have not yet been identified. To examine the theoretical links between PRP biology and chronic musculoskeletal conditions this report briefly analyzes the following relevant concepts: inflammation, angiogenesis and stem cell migration, and proliferation (see FIGURE 2).

### Modulation of inflammation

The role of platelets in inflammation has been carefully studied in atherosclerosis, a chronic inflammatory disease of blood vessels; however, outside the blood stream platelet actions are under-researched. Platelets release a broad

range of inflammatory mediators that support endothelial cell activation, leukocyte adhesion and transmigration, and monocyte maturation. Platelets modulate the magnitude and duration of inflammation by secreting high levels of chemokines (a subset of small, diffusible cytokines) required to control trafficking, and the further accumulation of leukocytes and monocytes in the injured tissue [23]. For example, platelets are considered the major source of  $\beta$ -tromboglobulin (CXCL7), a strong chemoattractant and an activator of neutrophils. Together, platelet–neutrophil interactions can induce hyperactivation of neutrophils to produce increased proinflammatory molecules. This concept has implications for the development of PRP formulations, as we may infer that L-PRP will attract more neutrophils from the blood stream than P-PRPs.

Platelets also augment the local concentration of other relevant chemokines, including CXCL1/GRO, ENA78/CXCL5, MCP-1, CCL5 or IL-8 [24]. In fact in PRP, MCP-1 collaborates with CCL5 to recruit monocytes in a dose-dependent fashion [25]. PRP also promotes significant changes in monocyte-mediated proinflammatory cytokine/chemokine release. Furthermore, platelet factor 4 (PF4), the most abundant cytokine in the PRPs, prevents monocyte apoptosis and promotes macrophage differentiation [26]. Further basic knowledge could help in refining PRP therapies in rheumatology;



**Figure 3. Prevailing mechanical hypothesis of tendinopathy.** The pathogenesis of tendinopathy is a continuum from physiology to overt clinical presentation. This sequence of events can be compared with a pyramid, having several thresholds, pain being the top of the pyramid.

Table 1. Platelet-rich plasma therapies and osteoarthritis: clinical studies.

Author (year)	Affected joint (intervention)	Type of PRP (platelet enrichment) [volume]	Study design (n)	Outcome index	Follow-up	Results	Level of evidence	Ref.
Spakovà et al. (2012)	Knee (3 injections 1-week interval)	L-PRP (4.5 x Plt 3.6 x leukocytes) [3 ml]	Prospective comparative, L-PRP vs HA (60 each group)	VAS, WOMAC	3 and 6 months	Significant improvement with both treatments. PRP significantly better than HA at 3 and 6 months	II	[59]
Kon et al. (2011)	Knee (3 injections 2-week interval)	L-PRP (4-6x) CaCl <sub>2</sub> activated [5 ml]	Prospective comparative, PRP (50 vs high- and low-MW HA; 50 each group)	IKDC, VAS	2 and 6 months	Similar at 2 months, at 6 months PRP better than HA in pain and function	II	[58]
Filardo et al. (2012)	Knee (3 injections 3-week interval)	L-PRP (4.7x) vs Pure-PRP (1.5x) Both CaCl <sub>2</sub> activated [5 ml]	Prospective comparative, L-PRP vs Pure-PRP (72 each group)	VAS, IKDC, Tegner, KOOS	2, 6 and 12 months	Significant improvements with both pure PRP and L-PRP but more swelling and pain after L-PRP injections	II	[33]
Mei-Dan et al. (2012)	Osteochondral lesions in the talus (3 injections, 1-week interval for HA and 2-week interval for PRP)	Pure-PRP (1.5-2x) CaCl <sub>2</sub> activated [2 ml PRP vs 2 ml HA]	Prospective comparative study, PRP (15 patients/ OCLs vs HA 14 patients/15 OCLs)	VAS, AHFS	4, 12 and 28 weeks	Significant improvement with both PRP than HA	I	[51]
Sánchez et al. (2008)	Knee (3 injections 1-week interval)	Pure-PRP (1.5-2x) CaCl <sub>2</sub> activated [8 ml PRP vs HA]	Case-control (30)	WOMAC	6 months	Significant differences in function and pain at 5 weeks	III	[59]
Wang-Saegusa et al. (2011)	Knee (3 injections 2-week interval)	Pure-PRP (1.5-2x) CaCl <sub>2</sub> activated [ml]	Case series (261)	WOMAC, VAS, SF-36, Lequesne	6 months	Significant improvement in all scores	IV	[52]
Sampson et al. (2010)	Knee (3 injections 4-week interval)	L-PRP(4-6x) thrombin activated [6 ml in supra-patellar bursa]	Case series (13)	KOOS, VAS, cartilage thickness US	2, 5, 11, 18 and 52 weeks	Significant improvement in KOOS and VAS; 6/13 increased femoral cartilage thickness	IV	[53]
Gobbi et al. (2012)	Knee (2 injections 1-month interval)	L-PRP (3.5x) CaCl <sub>2</sub> activated [4 ml]	Case series, PRP + surgery vs PRP (20 in each group)	IKDC and VAS	6 and 12 months	Significant improvement in patients with and without previous surgery	IV	[54]
Sanchez et al. (2012)	Hip (3 injections 1-week interval)	Pure-PRP (1.5-2x) CaCl <sub>2</sub> activated [8 ml]	Case series (40)	WOMAC, VAS, HARRIS	6 months	Significant improvement in pain and function in 40% of patients	IV	[49]
Napolitano et al. (2012)	Knee OA and pre-OA (3 injections/ 1-week interval)	PRP (0.17-5.2x) Ca glucuronate [5 ml]	Case series (14) pre-OA (13) OA	VAS, WOMAC	7 days, 6 months	Improvement in pain, and WOMAC better results in pre-OA	IV	[56]
Kon et al. (2010), Filardo et al. (2011)	Knee (3 injections 2-week interval)	L-PRP (4-6x) CaCl <sub>2</sub> activated [5 ml]	Case series (91)	IKDC and VAS	2-6 months 12-24 months	Significant improvement for up to 12 months but declined at 24 months	IV	[55,57]

HA: Hyaluronan; IKDC: International Knee Documentation Committee; KOOS: Knee injury and Osteoarthritis Outcome Score; L-PRP: Leukocyte platelet-rich plasma; MW: Molecular weight; OA: Osteoarthritis; P-PRP: Pure platelet-rich plasma; PRP: Platelet-rich plasma; SF-36: Quality of life and health survey; US: Ultrasound; VAS: Visual Analogue Scale; WOMAC: Western Ontario and McMaster Universities Index of Osteoarthritis.



for example, inducing ‘classical’ macrophage activation while avoiding ‘innate’ activation may produce an anti-inflammatory environment. Minimal research has been conducted to investigate PRP formulation at this level, but microarray technology has shown that PF4 induces a unique macrophage transcriptome distinct from the known activation patterns (classical and inflammatory) [27].

Local fibroblasts (synovial or tendon), another crucial target cell for PRP therapies, contribute to the resolution of inflammation by normalizing chemokine gradients, thereby allowing infiltrating leukocytes to undergo apoptosis or to leave the tissue through the draining lymphatic.

PRPs may terminate inflammation by restoring local cells to a noninflammatory phenotype. This effect could be mediated by various growth factors, including HGF, VEGF and TGF- $\beta$ , which protect the function of the endothelial barrier [28–30]. For example, TGF- $\beta$  could be of special interest in neutralizing PAR-2 (proteinase activated receptor), a major mediator of articular inflammation. In addition, HGF treatment induces an anti-inflammatory cytokine profile in endothelial cells, specifically by suppressing E-selectin [30]. Coudriet *et al.* in a model of inflammatory activation (lipopolysaccharide stimulation of macrophages), showed that when HGF was present in the milieu, there was a decrease in the proinflammatory cytokine IL-6 and an increase in the anti-inflammatory cytokine IL-10 [31]. HGF is primarily found in plasma, and very little is found in platelets, hence the importance of considering the balance between plasma and platelet proteins when formulating PRPs. These insights on the role of PRP in inflammation may lead to tailored and targeted formulations able to discriminate between the beneficial and harmful effects of this relationship between PRP and inflammation.

The efficacy of PRP therapy in nonsystemic inflammatory conditions is proven (from positive effects on tendon and cartilage pathologies). Nevertheless, particular formulations are more anti-inflammatory than others; for example, recent studies confirm that leukocyte-poor PRP injections are more anti-inflammatory and less painful than leukocyte-rich PRPs [32,33].

Likewise, PRP is suitable for established inflammatory conditions. For example, PRP restored type II collagen and proteoglycan synthesis via the inhibition of IL-1 $\beta$  and TNF- $\alpha$  in an *in vitro* model of arthritis [34]. Furthermore, PRP treatment led to attenuation of immunogenic arthritis of the knee in a porcine model [35].

However, data regarding PRP efficacy in human arthritic joints are still lacking.

### Modulation of angiogenesis

Angiogenesis is a critical element in wound healing, as well as in pathological processes such as tendinopathy and OA. Platelets have been presumed to contribute to these angiogenic-dependent processes by providing many pro- and anti-angiogenic proteins (see Box 1), but their regulatory role is incompletely understood.

These angiogenic activators collectively promote vessel wall permeability and recruitment and the growth and proliferation of vascular cells. On the other hand, PRPs release a repertoire of angiogenesis inhibitors. Therefore, molecular paradoxes designate platelets as crucial regulatory cells in angiogenesis.

### Stem cell migration & cell proliferation

The cell type that platelets influence the most is not known, but PRP has emerged as an autologous stimulator of stem cell differentiation and growth. Activated platelets express on their surface and subsequently secrete CXCL12 (or SDF-1). SDF-1 is a powerful chemoattractant for stem cells and is essential for both homing (migration, retention and development) and trafficking of CD34<sup>+</sup> progenitor cells to bone marrow or peripheral blood respectively [36]. Moreover, platelets provide additional cues, such as PDGF-B, bFGF and CXCL5, for the homing of precursor cells to the tissue. Representing 25% of the content of  $\alpha$ -granules, platelets are the major source of PF4 [37], which, in cooperation with PDGF and CXCL7, activates fibroblast migration. PRP also targets the niche of local stem cells. For example, PRPs affect the fate of tendon stem/progenitor cells residing in between the long parallel chains of collagen fibrils [38]. In a controlled laboratory study Zhang and Wang demonstrated that PRP releasate induced differentiation of tendon stem cells into activated tenocytes that proliferated quickly and produced abundant collagen to repair injured tendons that had lost cells and matrix [39]. Recent studies show that the combination of tendon stem cells and PRP has synergistic effects on tendon healing under both loaded and unloaded conditions [40,41]. In a laboratory model, PRP enhanced migration of corticospongius progenitors from subchondral bone and stimulated chondrogenesis [42]. Furthermore, periodical PRP injections after microfractures promoted better and more durable reparative response than microfractures

alone [43]. Distinct mesenchymal progenitor cell subsets have been identified in the synovium [44], but their association with PRP products needs to be explored.

### Osteoarthritis

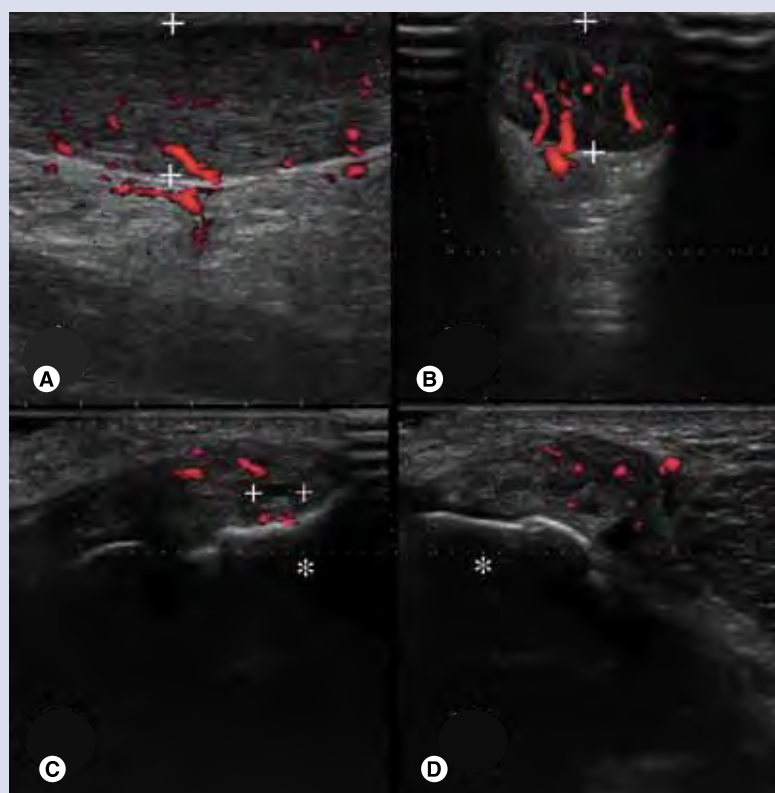
OA is a progressively degenerating joint disease with immense unmet need and no disease-modifying strategies on the market. None of the therapeutic options available have been shown to reverse joint damage. The barriers to treatment development and research include the insufficient understanding of the pathology and the fact that the physiopathology of OA may not be identical for all patients. The prevailing paradigm suggests that OA results from a failure of the damaged cartilage repair process due to biomechanical and biochemical changes in the joint. Namely, OA is considered a disease of the entire joint involving subchondral bone changes with

increased metabolism and sclerosis, chondrocyte death and extracellular matrix catabolism, as well as primary or secondary changes in the synovium, including endothelial cell proliferation, macrophage infiltration and inflammation, with subsequent alterations in the molecular composition of the synovial fluid [45,46]. The clinical signs associated with these changes include pain, rigidity and decreased functionality [47]. PRP therapies can modify joint pathology by interfering with early catabolic and inflammatory events and by subsequently promoting anabolic responses [6,48]. Essentially, local injection in the joint space is of special interest to reach a proper concentration of anabolic molecules, as well as modulators of inflammation and angiogenesis at the joint site without systemic exposure, thus avoiding adverse effects and drug interactions. However, the ongoing controversy surrounding PRP therapies for OA goes beyond the science of matrix metabolism, inflammation and angiogenesis, and involves questions regarding the clinical efficacy of these preparations, as well as economic arguments over the ever-increasing costs of these therapies.

#### ■ Clinical data: what to expect from present PRP therapies

Conservative management of OA with PRP is becoming increasingly popular; however, clinical evidence is preliminary and limited to few studies (seven case series, one retrospective and four prospective cohorts) (Box 1). Most studies focus on knee OA, but there are two case series on hip OA [49,50] and a prospective cohort on osteochondral lesions in the ankle [51]. These studies were merely faced with the burden of demonstrating a clinically meaningful result (e.g., pain relief and functional improvement) have used patient-reported outcomes as end points (WOMAC, KOOS, IKDC and VAS). Importantly, clinical studies performed thus far have strongly supported the safety of PRP, in other words, no infections, worsened outcomes, or serious complication have been reported. The fact that PRP is not a potentially harmful experimental treatment is corroborated by clinical studies in other conditions and medical fields.

All observational studies have concluded that PRP treatment can decrease pain and improve function [49,50,52–56]. For example, in a case series involving 115 knees, mostly in young adults with low degrees of articular degeneration (50% KL 0; 28% KL I–III; and 22% KL IV), patients reported symptom relief and functional improvement for up to 12 months



**Figure 4. Ultrasound appearance of degenerated Achilles tendon and elbow extensors.** (A) Longitudinal and (B) transverse typical power Doppler images of a thickened Achilles tendon in a 65-year-old male. Images demonstrated a hypoechoic and dishomogeneous tendon with loss of the normal fibrillar pattern and intratendinous neovessels entering from the ventral portion of the tendon. (C) Longitudinal (D) and transverse images of elbow extensors in a 40-year-old male with an intratendinous partial thickness tear detected as focal hypoechoic discontinuity (calipers) with irregular margins, and new vessels within the tendon. \*Humeral bone.



[55]. Afterwards, patients showed a progressive worsening as measured at 24 months [57]. Another large case series of knee OA in more than 250 young adults has found significant reduction in pain and functional disabilities, as well as improved quality of life at 6 months [52]. Furthermore, PRP is also indicated in young adults that underwent previous knee surgery [54]. Comparative studies in knee OA, using hyaluronan (HA) injections as a control, have shown superior outcomes but have not always been statistically significant. Thus, in a retrospective cohort study of knee OA, a third of the PRP-treated patients achieved at least 40% pain reduction in contrast with a tenth of the HA-treated patients [58]. In another knee study, both treatments showed similar improvements at 2 months, but PRP had longer efficacy as measured at 6 months [55]. Remarkably, PRP was superior to HA in young patients with a low degree of cartilage degeneration [59]. Similar findings were reported in young active patients with osteochondral lesions in the talus [51]. Unfortunately, a comparison between these studies is difficult due to different affected joints, differences between products, protocols and outcome measures.

Subsequently, when discussing PRP therapies, differences between the PRP products and the readministration procedures used should be acknowledged. For example, P-PRP [49,51,52,60] and L-PRP [53–55,57–59] formulations are not comparable with each other in terms of leukocyte content, platelet count and plasma volume. Moreover, the platelet and leukocyte concentration of the final L-PRP product can vary by as much as 100% [10]. Whether the differences in the clinical results are secondary to the differences in the formulation requires clarification. To evaluate differences in the clinical outcome between PRP and L-PRP, a recent prospective comparative study has been conducted by Filardo *et al.*; at the end point, they reported similar improvements in pain and function in both groups, albeit L-PRP caused more swelling and post-injection pain [33]. It is worth mentioning that in these studies, storage of L-PRP introduces additional variability of the final product [33,55,56].

Regarding administration procedures, the volume and number of injections is empirically determined for each study (see Box 1). Although most studies involve three injections, the period between the injections is variable, ranging from 1 to 4 weeks. In knee OA, PRP is generally injected into the femorotibial compartment,

although Sampson injected PRP into the suprapatellar bursa and reported increased cartilage thickness in 6 out of 13 patients (46%).

Theoretically, PRP application would be much more efficacious in patients with early post-traumatic OA before the radiographic signs become severe, but this needs further confirmation. In patients with significant irreversible bone and cartilage damage, the effect of PRPs would most likely be less impressive, even so, PRP therapy probably would still improve the patients' quality of life. Whether frequent PRP administration can delay OA progression and replacement surgery in patients with advanced OA may be a plausible hypothesis, but long-term studies using surrogate end points such as WOMAC reduction and refined imaging and biochemical markers potentially predictive of the delay of OA progression are required.

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## Tendinopathy

The term tendinopathy is used to describe the clinical syndrome characterized by a combination of longstanding activity-related pain, swelling (diffuse or localized) and impaired performance [61]. The process develops along a continuum from physiology to overt clinical presentation, so there is a threshold of loading frequency and magnitude that, once overcome, changes the tendon response from beneficial to pathological (FIGURE 3) [62]. For instance, long-term, well-structured exercise, within a 'physiologic window', reinforces tendon structure, stimulating the production of new collagen fibers (anabolic factors > catabolic factors). Thus, in optimal conditions, a well-ordered sequence of biological events results in a larger and stronger tendon, with increased tensile strength and elastic stiffness [63,64]. Quite the reverse, tendon homeostasis is lost when tissue breakdown exceeds the rate of tissue healing or when the capacity of tissue healing is impaired. This may occur in overloaded tendons or idiopathically predisposed tendons [62].

In addition to mechanical overwork, chemical or physical stresses can provoke an abnormal healing response, which brings forth biochemical changes in the microenvironment. These include overexpression of inflammatory mediators (i.e., IL-1 $\beta$ , TNF- $\alpha$ , prostaglandins and neuropeptides) that along with degradation products initiate the pathological cascade leading to tendinopathy [65]. Likewise, low oxygen tension appears to evoke activation of HIF-1 $\alpha$  [66], followed by expression of downstream

Table 2. PRP therapies and tendinopathy: clinical studies.

Author (year)	Tendon (intervention)	Type of PRP (volume)	Study design (n)	Outcome index	Follow up	Results	Level of evidence	Ref.
Volpi <i>et al.</i> (2007)	Patellar (1 injection)	L-PRP (4–8x) buffered pH:7.4 (3 ml)	Case series (8)	VISA, MRI	4	91% VISA improvement; reduction in irregularity in 80% of treated tendons (MRI)	IV	[71]
Kon <i>et al.</i> (2009)	Patellar (3 injections 2-week interval)	L-PRP (6x) CaCl <sub>2</sub> -activated (5 ml)	Case series (20)	Tegner, EQ-VAS, SF-36	6	Improvement in EQ-VAS (+22), SF-36 and Tegner (sport return); 80% of patients were satisfied	IV	[73]
Gaweda <i>et al.</i> (2010)	Achilles (1 injection)	L-PRP (4x) (3 ml)	Case series (14)	VISA, AOFAS, US evaluation	18	Improved AOFAS (+41) and VISA (+72); reduction of tendon thickness and hypoechoic areas (ultrasonography)	IV	[74]
Barret <i>et al.</i> (2004)	Plantar fascia (1 injection)	P-PRP (3 ml)	Open (9)	VAS, US evaluation	12	Improved echotexture (reduced plantar fascia thickness) and pain resolution in 78% of patients	IV	[75]
Finnoff <i>et al.</i> (2011)	Several sites (1 injection + needling)	P-PRP (4x) No activation (2.5–3.5 ml)	Open (41)	VAS (pain and function), satisfaction, US evaluation	14	Improvement in function (+68%), pain (-58%), echotexture (84%), intratendinous calcifications (-64%), and neovascularity (-82%); 83% of patients were satisfied	IV	[76]
Volpi <i>et al.</i> (2010)	Several sites (1 injection)	L-PRP (4–8x) No activation buffered pH:7.4 (3 ml)	Open (15)	VISA, MRI	24	Improvement in VISA score (+37) and reduction of abnormalities in 80% of treated tendons (MRI)	IV	[72]
Mishra <i>et al.</i> (2006)	Elbow (1 injection)	L-PRP (4–8x) No activation buffered pH:7.4 (3 ml)	Cohort study PRP, (15) Controls, (5)	VAS, Mayo Elbow score	24	PRP group better than control at 8 weeks (+60% vs +16%) In PRP, at 24 months +93% in VAS score and function	III	[77]
Peerbooms <i>et al.</i> (2010)	Elbow (1 injection)	L-PRP (4–8x) No activation buffered pH:7.4 (3 ml)	RCT PRP, (51) vs 4 ml Corticosteroid, (49)	VAS, DASH	12 and 24	PRP group was more often successfully treated than the corticosteroid group	I	[82,83]
Creaney <i>et al.</i> (2011)	Elbow (2 injections 4-week interval)	L-PRP (2.8x) (1.5 ml)	RCT PPR, (80) vs 1.5 ml ABI, (70)	PRTEE	6	Success rate: PRP (+66%) vs ABI (+72%), p = ns	I	[84]
Thanasas <i>et al.</i> (2011)	Elbow (1 injection)	P- PRP (5x) No activation (3 ml)	RCT PPR, (14) vs 3 ml ABI, (14)	VAS, Liverpool elbow score	6	Significant improvement in VAS score only at 6 weeks (PRP: 3.8 vs ABI: 2.5, p < 0.05); no differences in function	I	[85]

ABI: Autologous Blood Injection; AOFAS: American Orthopedic Foot and Ankle Society; SF-36: Quality of life and health survey; L-PRP: Leukocyte platelet-rich plasma; P-PRP: Pure platelet-rich plasma; PRP: Platelet-rich plasma; PRTEE: Patient-Related Tennis Elbow Evaluation; RCT: Randomized controlled trial; US: Ultrasound; VAS: Visual Analogue Scale; VISA: Victoria Institute of Sports Assessments.

Table 2. PRP therapies and tendinopathy: clinical studies (cont.).

Author (year)	Tendon (intervention)	Type of PRP (volume)	Study design (n)	Outcome index	Follow up	Results	Level of evidence	Ref.
De Vos et al. De Jonge et al.	Achilles (1 injection)	L-PRP (4–8x) No activation 4 ml	RCT PRP, (27) vs 4 ml saline, (27)	VISA, Satisfaction, Return to sports, US evaluation	6 and 12	No differences in VISA and ultrasonographic improvement; 59% of patients were satisfied in both groups	I	[87,88]
Filardo et al.	Patellar (3 injections 2-week interval)	L-PRP (6x) CaCl <sub>2</sub> -activated (5 ml)	Non RCT PRP, (15) vs Controls, (16)	Tegner, EQ-VAS	6	No statistically significant differences for EQ-VAS and pain level among groups; in PRP, Tegner score improved at significant level (+39 vs 20%)	III	[89]

ABI: Autologous Blood Injection; AOFAS: American Orthopedic Foot and Ankle Society; DASH: XXX; SF-36: Quality of life and health survey; L-PRP: Leukocyte platelet-rich plasma; P-PRP: Pure platelet-rich plasma; PRP: Platelet-rich plasma; PRTEE: Patient-Related Tennis Elbow Evaluation; RCT: Randomized controlled trial; US: Ultrasound; VAS: Visual Analogue Scale; VISA: Victoria Institute of Sports Assessments.

molecules, including VEGF [67], that collectively alters tendon metabolism towards a catabolic response. Frequently, neovascularization of the injured tendon occurs, and is associated with its innervation by fine unmyelinated sensory nerves [68]. In this phase, also called 'neurogenic inflammation', the patient may still be asymptomatic until algogenic molecules reach a threshold [69]. This condition is not self-limiting and can lead to a tendon rupture if not adequately diagnosed and treated.

Eventually, several factors may enhance the vulnerability to tendinopathy, for example extrinsic (i.e., heavy sport activities, environmental adverse conditions and training errors) and intrinsic factors (i.e., advanced age, osteoarticular pathologies and systemic diseases affecting microcirculation or collagen metabolism), as well as genetic susceptibility (FIGURE 3) [70].

### ■ Clinical studies

The rationale for using PRP therapy in tendinopathies is to provide blood-derived healing factors to otherwise deprived tissue. Certainly, by delivering a physiological pool of growth factors and cytokines we can modify the biological conditions and influence multiple cell activities. In doing so, we can switch chronic nonhealing injuries into acute injuries with healing potential [5].

Several studies on the therapeutic potential of PRP have been performed including open studies [71–76] and prospective randomized controlled trials [77–84]. Some studies have been disappointing [80,82,83], while other studies have reported good results in refractory tendinopathies [71–76] and a superiority of PRP against other therapeutic options (Box 1) [78,79,81,84]. Although the better results have been observed in elbow tendinopathies [78,79,81], the PRP therapy should be suitable for all tendons in patients suffering from recalcitrant tendinopathies after the failure of conservative treatments. In this section, on the basis of the available literature, practical issues on the use of PRP in tendinopathies are discussed.

The optimal PRP formulation as well as the protocol for treating patients with tendinopathies is unknown. The volume and the number of injections are still unclear. As shown in Box 1, there is a large variability of these parameters; volume: 1.5–4 ml; platelet concentration: four- to eight-times; leukocyte concentration: not detected to five-times; number of injections: 1–3; plasma activation: *ex vivo* with Ca<sup>2+</sup>/no activation (local tissue factors); and time interval between injections: 1–4 weeks. It is the

authors' opinion that some of these parameters, such as the volume, number of injections and interval between injections, should be tailored to each patient, taking into account the injury location and clinical response.

Before injection, some rules must be observed: 1 week before, the patient must avoid platelet-inhibiting medications (such as nonsteroidal, anti-inflammatory drugs or aspirin); moreover, 6 h before blood extraction, eating fatty food is forbidden. The use of local anesthetics is controversial, as PRP is sensitive to changes in pH and it can also be diluted. Similarly, the addition of either anesthetics or corticosteroids alters PRP effects by decreasing tenocyte proliferation and cell viability [85]. Hence, some physicians do not use it at all [73,84], while others anesthetize only the skin and subcutaneous tissues [80,82]. PRP protects tenocytes from adverse side effects of dexamethasone and ciprofloxacin. Ciprofloxacin and dexamethasone have differing adverse effects on human tenocytes, with ciprofloxacin inducing cell death while dexamethasone primarily induces senescence. Zargar *et al.* showed that PRP can

protect cultured human tenocytes against cell death or senescence induced by these drugs [86]. These findings suggest the potential for local administration of PRP will enhance tendon healing in patients undergoing glucocorticoid or fluoroquinolone treatment.

The technique of PRP injection is another critical point. Different injection modalities have been reported; for instance, barely in the injured site [80], or alternatively, with the peppering technique, in a clock like manner, to achieve a more expansive zone of delivery [72,73,81,82]. It seems that the injection of the 'healthy' peripheral tissue can stimulate further resident cells to synthesize additional amounts of GFs and cytokines [5]. Moreover, PRP can be also injected around the tendon (between tendon and paratenon) and in the tendon sheath. Otherwise, before the injection, the degenerated area can be repetitively fenestrated, until the needle goes freely through all aspect of the pathologic region (dry needling), disrupting fibrils and causing internal bleeding; this is known to promote the healing response [76]. When a selective delivery is preferred, real-time ultrasound

## Executive summary

### **Injectable platelet-rich plasma: critical parameters**

- To prepare platelet-rich plasma (PRP), peripheral blood is withdrawn from the patient and the volume of blood is tailored to each particular condition. The choice of anticoagulant is critical to preserve platelet integrity and functionality; the preferred are sodium citrate or acid–citrate–dextrose. The choice of centrifugation method determines cellular composition in PRP. Single spinning is used to obtain pure PRP with a moderate concentration of platelets (one-to-two-times baseline levels); double spinning in general produces leukocyte-PRP, plasma with a high concentration of leukocytes and platelets (four-to-ten-times). Discontinuous aphaeresis is an alternative method used in blood banks, but is out of reach of the clinician as it involves higher production costs, a highly controlled logistic system and delayed availability of PRP compared with bedside devices. Plasma can be activated *ex vivo* with Ca<sup>2+</sup> or thrombin or, alternatively, it can be activated *in situ* by tissue factors and collagen.
- A major goal for the optimization of PRP formulations lies in establishing the cascade of events and the molecular and cellular hierarchy that produce the therapeutic effects of PRPs.

### **PRP: biological activity**

- No simple rule can be generalized to describe how PRP works for the treatment of tendinopathy and osteoarthritis.
- Platelets modulate the magnitude and duration of inflammation by secreting the high levels of chemokines required to control trafficking and the further accumulation of leukocytes and monocytes in the injured tissue.
- PRPs release a repertoire of proangiogenic proteins, as well as angiogenesis inhibitors. Platelets also modulate vascular permeability.
- The cell type that platelets influence the most is not known, but PRP has emerged as an autologous stimulator of stem cell differentiation and growth. PRP also targets the niche of local stem cell.

### **Osteoarthritis**

- Conservative management of osteoarthritis is popular, but clinical evidence is limited to few studies. However, PRP treatment improves algo-functional indexes for osteoarthritis.
- PRP targets multiple tissues within the joint, mostly the synovial membrane, cartilage and subchondral bone.
- PRP modulates inflammation and substitutes the chondro–destructive environment with high levels of anabolic cytokines.

### **Tendinopathy**

- The rationale for using PRP therapy in tendinopathies is to provide blood-derived healing factors to otherwise deprived tissue.
- PRP therapy should be suitable for all tendons in patients suffering from recalcitrant tendinopathies after the failure of conservative treatments.
- The volume of plasma, number of injections and interval between injections should be tailored to each patient, taking into account the injury location and clinical response.

imaging is mandatory to visualize the proper needle position, and to display the precise anatomical location of PRP, which becomes evident as a hyperechoic image. Otherwise, without imaging guidance, PRP could be delivered away from the affected areas, reducing the efficacy of the treatment. When multiple injections are considered, decision regarding further injections has to be carried out after an adequate monitoring period (usually 1–2 weeks following the last injection).

After the injection, the patient is instructed to lay prone for 10 min. A temporary worsening of the symptoms may occur, with expected pain relief within a few days after injection. The authors apply cold therapy for at least 10–15 min (2–3-times daily) to avoid excessive fluid production within the joint, and a simple dressing and compressive wrap is placed over the procedure site. At home, the patient must limit physical activities for at least 24 h, and avoid the use of platelet inhibiting medications for 2 weeks after the procedure; only rescue medication, such as acetaminophen (500 mg up to 2 g daily), is allowed. An individual-specific rehabilitation program, based on eccentric training and strengthening, must be started, as mechanical stimuli seem to enhance PRP efficacy [85,87,88]. Being an autologous injection, the risk of adverse reactions and transmission of infection are very low.

In addition to pain evaluation (visual analogue scale), the patients can be monitored by using specific functional scales (i.e., general health status: EQ, quality of life and health survey, patient's satisfaction; for the elbow: Mayo, DASH, Patient-Related Tennis Elbow Evaluation, Liverpool scores; for the patellar: Victoria Institute of Sports Assessments, Tegner; for the achilles: Victoria Institute of Sports Assessments, American Orthopedic Foot and Ankle Society) [89]. Imaging studies (ultrasound and MRI) can be used to evaluate structural changes, even if, in most of cases, a good clinical response does not correspond to synchronous imaging findings and vice versa [76].

The main indications for PRP injections presented in this review are OA and tendinopathy. The clinical impact has been demonstrated in less-controlled settings and in a few prospective comparative studies (BOX 1 & TABLE 1). Although observational studies describe outcomes in patients in which selection criteria are not restrictive, they provide insights into the clinical practice and realities encountered in chronic and recalcitrant pathologies. The common

outcome of improvement for all studies is pain reduction; it is well accepted that reducing pain has clinical significance in terms of improving patient quality of life. In general, differences in function are less obvious and objective imaging outcomes are lacking.

Overall, clinicians suspect that PRP intervention may have utility, at least, in specific stages of the pathology. In fact, PRP appears to convert many recalcitrant tendinopathies into actively evolving ones. However, the reason why some patients respond and others do not is not currently known.

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### Future perspective

PRP therapies are multitargeted approaches that may symptomatically treat patients with chronic musculoskeletal diseases. The PRP therapies described herein have improved clinical outcomes in certain patients suffering OA or tendinopathy. However, current PRP therapies sometimes fail or produce only partial responses. Effective PRP treatments for OA and tendinopathy rest on the understanding of the disease pathways, as well as how PRP is involved in the molecular mechanisms we have discussed. Therefore, advances in PRP science encompasses both the acquisition of new knowledge regarding pathogenetic mechanisms and the research necessary to link tissue healing with PRP biology. We must elucidate how PRP interferes with the mechanisms driving the various progressive disorders underlying tendinopathy and joint pathology that contribute substantially to reductions in the quality of life of numerous people. Ultimately, we must strive to develop optimized autologous therapies that will transform the notion of OA and tendinopathy as progressive degenerative diseases.

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