

# PRP Injections in Orthopaedic Surgery: Why, When and How to Use PRP Dynamic Liquid Scaffold Injections in Orthopaedic Surgery

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Mikel Sánchez, Diego Delgado, Ane Garate, Pello Sánchez, Jaime Oraa, Ane Miren Bilbao, Jorge Guadilla, Beatriz Aizpurua, Nicolás Fiz, Juan Azofra and Sabino Padilla

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## Abstract

Platelet-rich plasma (PRP) products can be described as any autologous blood platelet concentrate within a plasma suspension. PRP products include plasma and twofold or greater increases in platelet concentrations above baseline levels. The injection of activated PRP in its liquid formulation delivers growth factors locally and simultaneously mimics and amplifies the spontaneous healing response in injured areas and in special cell niches, which would otherwise be inaccessible. This *in situ* generated transient three-dimensional scaffold will gradually release growth factors and maintain their concentration at the site of the scaffold formation. The combination of liquid PRP with surgical techniques in orthopaedic surgery allows a wide range of therapeutic strategies in the management of injuries in the field of orthopaedics and sports medicine. The use of different therapeutic elements, including PRP as biological stimuli and rehabilitation and physiotherapy treatments as mechanical stimuli, provides extremely favourable synergies that will help fulfil the physician's objective, to stop the progression of disease and to improve function in the shortest period of time

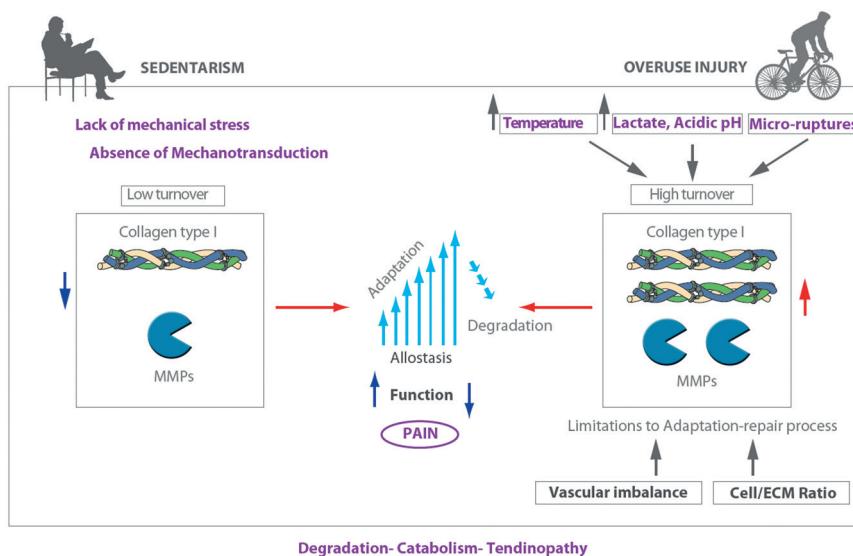
**Keywords:** platelet-rich plasma, orthopaedic surgery, injections

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## 1. Introduction

Virtually, all the cells of the musculoskeletal tissues are mechano-sensitive and experience mechanical stress through the distortion of the extracellular matrix (ECM) complex. The exposure of musculoskeletal cells to nonphysiological stimuli, either mechanical or biochemical, leads to swings of the tissue pendulum towards profound alterations of components of the ECM both cellular and acellular as well as the physical and chemical properties of the ECM. Such stimuli lead to cell microenvironment damage, non-resolving inflammation and disease. In addition to specific features of each tissue (vascularization, innervation and type of cells), abnormal biomechanical loading as obesity, a sedentary lifestyle leading to metabolic disorders, joint injury or high intensity and prolonged sports activities make musculoskeletal tissues vulnerable to injury. Through such overuse or disuse, these nonphysiological stimuli may well produce a consequential disruption in tissue homeostasis (**Figure 1**).

A new innovative approach to the treatment of acute and chronic sports injuries uses engineering biology assisted by the application of platelet-rich plasma (PRP) in its different formulations. Generally, PRP products can be described as any autologous blood platelet concentrate within a plasma suspension. PRP products include plasma and twofold or greater increases in platelet concentrations above baseline levels: not insignificantly, their concentration of leukocytes and erythrocytes varies widely [1] from a complete absence of these cells to a high concentration of them. In this chapter, the described PRP has a platelet concentration between



**Figure 1.** Both an excess and insufficiency of physical activity associated with factors such as vascular imbalance, intrinsic hereditary risk factors and a novel environment may disrupt the fragile homeostasis maintained by the tenocyte, stromal fibroblast and tissue-resident macrophages. A localised, predominantly catabolic context associated with a high temperature, ECM fragments and acidosis with building up of lactic acid may be the root cause of inflammation-degeneration of the ECM.

2- and 2.5-fold higher than blood and no leukocytes (PRGF®-Endoret®, BTI-Biotechnology Institute, Vitoria-Gasteiz) [2]. PRP can be activated with  $\text{CaCl}_2$  offering a variety of autologous formulations whose versatility endows this technology with a myriad of applications in orthopaedics [3–5].

This chapter addresses the following questions: Why would surgeons want to harness the biological features of PRP in the operating theatre? When are PRP injections indicated as an adjuvant to? How should the injections be introduced to obtain beneficial outcomes in surgery?

## 2. Reasons to use PRPs as repair process enhancers in orthopaedic surgery: the scientific rationale behind it

The physicochemical features of PRP liquid formulation, once activated, make it appropriate to reach wide areas of soft and hard tissues such as the tendons, muscles, ligaments, menisci, cartilage and bone. Platelet growth factors and fibrin, together with plasmatic growth factors (HGF, IGF-1) present within PRP, stimulate in a pleiotropic manner cell proliferation and migration, angiogenesis, synthesis and deposition of ECM components and tissue remodelling in the musculoskeletal tissues [6–8]. The surgical site is opened in the normal manner, and in the following 2: 4 minutes, the liquid-activated formulation must be injected as a solution into soft tissues. Because of its local and gradual activation and homogeneous distribution and interaction with the ECM of different tissues, it is converted into a matrix-like malleable transient structure [2]. There is a direct interplay between components of a tissue's ECM (collagens, glycosaminoglycans and adhesive proteins) and the adhesive proteins and growth factors released gradually from the degrading fibrin clot which will influence cellular growth, differentiation and morphogenesis [9]. Therefore, the injection of PRP in its liquid formulation delivers growth factors locally and simultaneously mimics and amplifies the spontaneous healing response in injured areas and in special cell niches, which would otherwise be inaccessible. This *in situ* generated and moulded plastic nano-scaffold of fibrin interacts with ECM proteins and cells, binding to fibronectin [10], generating a transient three-dimensional scaffold, which will gradually release growth factors and maintain their concentration at the site of the scaffold formation (**Figure 2**)

The fibrin molecules, together with growth factors, influence and govern the repair mechanisms to reconstruct structures and restore function, both by harnessing local or resident cells and by stimulating cell migration and proliferation, thereby regulating angiogenesis, modulating inflammation, chemoattracting circulating progenitor cells and guiding tissue remodelling [11, 12]. PRP *in situ* generated nano-scaffold of fibrin offers a biologically active cell-matrix landscape where adhesive proteins, namely, fibrinogen, fibronectin, vitronectin and thrombospondin (TSP-1), facilitate cell adhesion, migration, proliferation and differentiation. Furthermore, by the release of stromal cell-derived factor 1 (SDF-1) which has been entrapped in the fibrin network, the nano-scaffold mediates the chemotaxis of CD34 progenitor cells and mesenchymal stem cells (MSCs) [7, 13, 14]. Once recruited, MSCs or pericytes [15] adhere to a fibrin network and may exert several functions such as tissue organisation, regulating the fate of other circulating and resident progenitor cells [16] and serving as

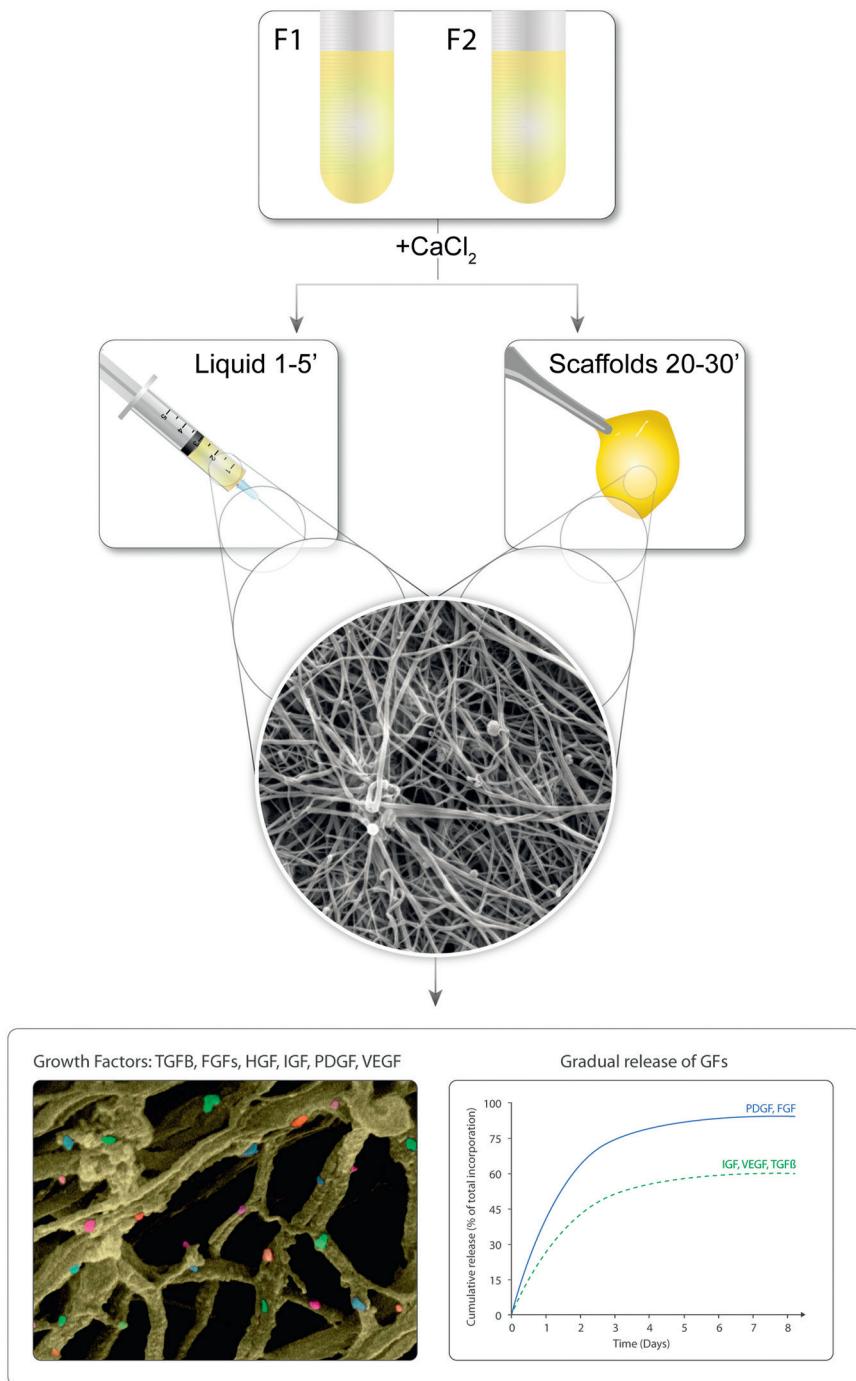


Figure 2. PRP niche therapy approach: injectable dynamic scaffold for molecular intervention.

progenitor cells that replace the damaged tissue, prevent scar-forming cells from entering the damaged area and exerting immunomodulation activities [7, 14].

### 3. The use of PRP infiltrations in orthopaedics: surgical applications

Although it is not within the scope of this chapter to address the wide range of therapeutic strategies in the management of injuries in the field of orthopaedics and sports medicine, only a holistic approach will fulfil the objective of surgeons, namely, to stop the progression of disease and to improve function in the shortest period of time. In this respect, and as a clinical application of cell mechanotransduction, a rehabilitation programme, which included the employment of PRP in a synergistic manner would play a crucial role in both promoting the repair or remodelling of injured tissue and avoiding the degradation and atrophy of structures such as the bone, periarticular muscles, tendons and ligaments with the goal of full recovery of function [17].

#### 3.1. PRP infiltrations in tendon surgery

There is increasing evidence showing that tendon and ligament adaptation, injury and repair processes share several intracellular pathways, and although it is difficult to draw the line between the cellular and molecular responses that lead to either tissue adaptation or tissue damage, inflammatory processes appear to be at the interface of tendon adaptation and damage [18–20]. Repetitive mechanical loading, as is the case in early stages of tendinopathy, and tendon overuse induce the activation of NF- $\kappa$ B in stromal fibroblasts and thereby the synthesis of matrix metalloproteinases (MMPs), two isoforms of cyclooxygenase (COX)-1 and COX-2 and PGE2 by inflammatory tenocytes and stromal fibroblasts, mast cells and other immunocompetent cells [18, 21–23]. PGE2 is a major systemic and local inflammatory mediator that decreases the production of collagen and causes aberrant differentiation of TDSCs into adipogenic and osteogenic lineages [23], which might partially account for the presence of fibrocartilage, calcifications and adipose tissue in injured and chronic degenerative tendons [18, 23, 24].

An excellent series of in vitro and in vivo studies demonstrated that blood-derived BDDT induced tenocyte proliferation, stimulated the synthesis of type I collagen and neovascularization [9] and promoted differentiation of TDSCs into active tenocytes, but, significantly, the addition of leukocytes into the release increased the synthesis of PGE2 and the gene expression of MMP-1, MMP-13 and IL-1 $\beta$  and decreased the expression of alfa-SMA as a marker of active tenocytes. Among the myriad mediators conveyed by blood-derived BDDT, HGF and lipoxin A4 (LX4) have been shown to exert an anti-inflammatory and pro-resolution of inflammation effect on injured tendons [21–23].

##### 3.1.1. Surgical treatment of acute ruptures of tendons

In the case of tendons such as the Achilles, patellar or quadriceps, the volume of blood extracted is approximately 60–70 mL (six to nine tubes). Blood is taken a few minutes prior to surgery, before any fluid or drugs are administered to the patient, in the operating theatre

itself. PRP should be prepared, while the patient is being prepared in the operating theatre and applied by injection immediately after activation ex vivo (**Figure 2**).

The injury site is accessed via a medial approach [25], the hematoma is evacuated and the necrotic tissue is debrided. Then, the tendon is repaired using non-reabsorbable material previously soaked in liquid-activated PRP. The PRP liquid freshly activated is infiltrated into the healthy tendon and the tendon/bone repair zone. Both the repair zone and the proximal and distal end stumps are injected (**Figure 3**). The use of small syringes means that large pressures are exerted on the ECM of the tissue during infiltration. We therefore recommend the use of 10 mL Luer lock-type syringes with 21G needles. Upon infiltration, the needle should be oriented as closely as possible parallel to, and longitudinal with, the tendon as possible for an optimal diffusion of PRP (**Figure 3**). Repair concludes with closure of the peritenon. The peritendinous regions are also infiltrated in order to recruit mesenchymal stem cells, pericytes and endothelial cells [15]. Approximately 12 mL of PRP is used during this phase. Before closing the overlying skin, the affected area is covered with a fibrin scaffold. Once closed, the subcutaneous tissue is irrigated with freshly activated PRP. An ultrasound examination of the Achilles tendon is performed in week 3, and, if healing problems, especially intratendon cyst formation, are detected, liquid-activated PRP is infiltrated under ultrasound guidance in the outpatient manner.

It is mandatory to coordinate and integrate functional recovery in light of the changes to biological mechanisms. Achieving a shorter immobilisation time allows physiotherapy to be speeded up because of the formation of a more efficient repair tissue [4, 25, 26].

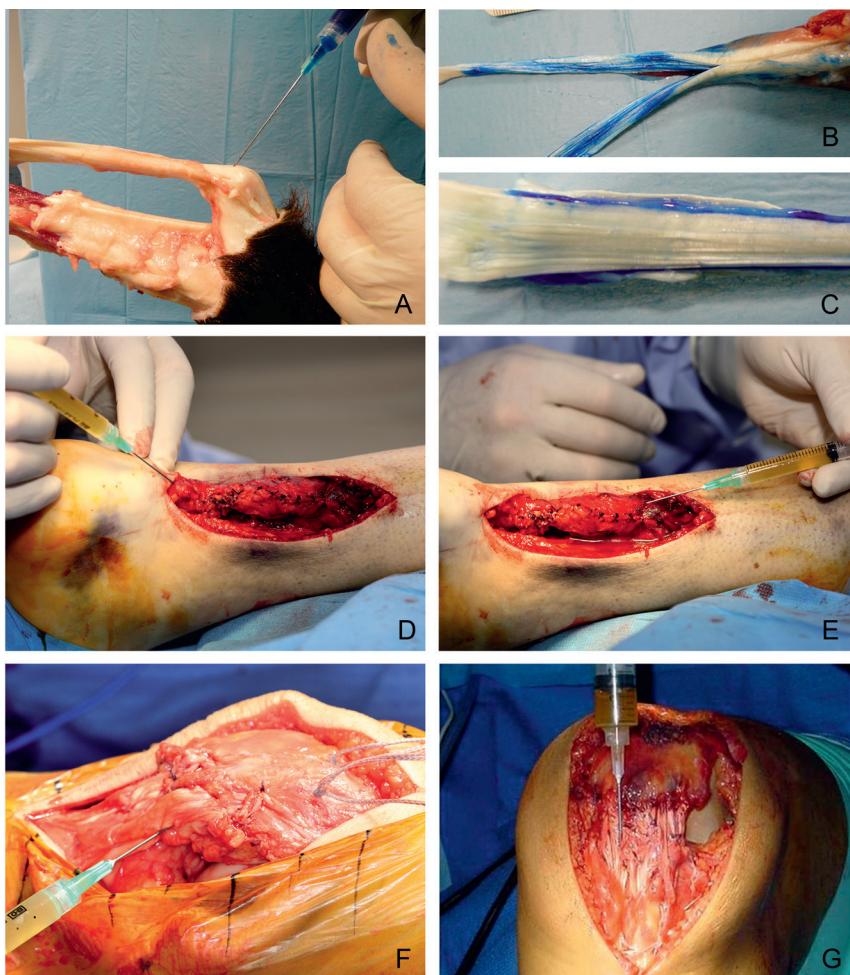
The application to ruptures of major tendons at other sites follows the same methodology. The sequence in which the PRP liquid is applied is the same, with the volumes being varied in each individual case (**Figure 3**). Two maxims must always be followed: the use of suture systems and repair techniques that respect the tendon's native biology as far as possible and the achievement of mechanically stable configurations that allow early rehabilitation.

### *3.1.2. Surgical treatment of chronic tendinopathy*

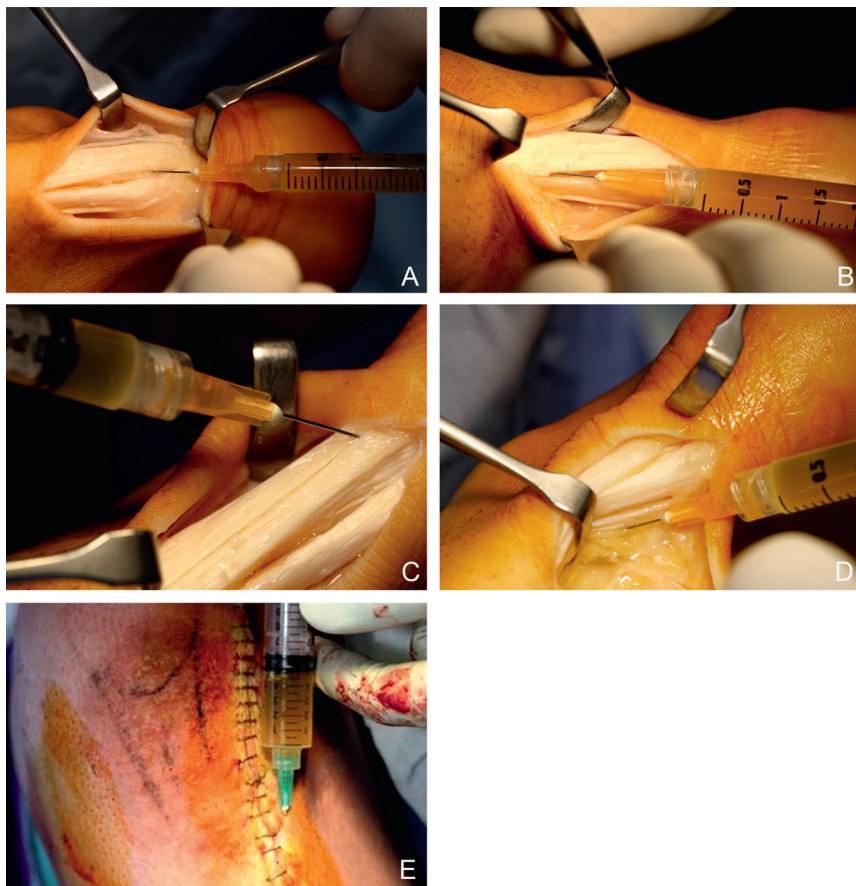
In patients with a tendinopathy in whom conservative management, including percutaneous infiltration of PRP, has failed, surgery may be indicated [5]. This procedure, which is based on longitudinal tenotomy with removal of the area of failed healing response together with the application of PRP, is intended to remove the degenerative tissue, induce neovascularization and provide the tendon with a physical support and three-dimensional structure where local and neighbouring cells (e.g., from the paratendon) can proliferate and synthesise both blood vessels and ECM. We have summarised the process in the following steps (**Figure 4**):

1. The tendon injury sites are located and excised. Longitudinal tenotomies should be performed throughout the whole thickness of the tendon in the same direction as the fibres. The aim of this process is twofold: to access the whole tendon in order to remove all the degenerative tissue and to generate a repair stimulus in the injured tendon.

2. Liquid-activated PRP (8–10 mL) is then injected into the tendon fibres at both the excision site and the proximal and distal ends of the injury site following the procedure depicted previously for tendon ruptures.
3. Once the subcutaneous tissue has been closed, it is infiltrated with freshly liquid-activated PRP.



**Figure 3.** Injection at the site distal and proximal to the rupture in the direction of the tendon fibres (A, B). PRP is injected into the healthy tendon as well. To determine the effect of infiltration angle, and the size of syringe and diameter of the needle on the diffusion, PRP stained with methylene blue was injected in Achilles tendon of the sheep (C, D, E). The optimal diffusion of the PRP was obtained when the needle was oriented as closely as possible parallel to, and longitudinal with, the tendon. Complete rupture of the quadriceps tendon (F, G).



**Figure 4.** After having performed longitudinal scarifications in the tendon, liquid-activated PRP (8–10 mL) is injected into the tendon fibres at both the excision site and the proximal and distal ends of the damaged area (A–D). Infiltration of the subcutaneous tissue with freshly liquid-activated PRP (E).

### 3.1.3. Management of postsurgical Achilles tendon complications with PRP

PRP application in combination with surgery meets the criteria for treatment of major complications from Achilles tendon rupture and repair, namely, versatility, biocompatibility, bio-safety and efficacy. After having carefully cleaned the necrotic area, we proceeded to apply PRP. We injected 3 mL of the activated liquid both in the distal and proximal tendon stumps as well as in healthy areas of the tendon as described previously. In addition, the paratendon construct was richly injected with PRP. In one case, an autologous semitendinosus tendon was used to fill the Achilles tendon gap. Before the graft was anchored, we injected PRP liquid into the newly formed tissue (during week 3 after the first operation), among the tendon fibres of the graft and into the reconstructed tendon [27].

### 3.1.4. Surgical treatment of rotator cuff tears

The three factors that cause tissue damage of connective tissue in the musculoskeletal system coincide in the aetiology of rotator cuff injuries: (1) mechanical factors, (2) overuse-related micro-/macrotraumas and (3) the vascular decompensation inherent to this structure. Indeed, biopsies have shown a structure with a disproportionately low degree of vascularization and cellularity for its high level of functional demands. This tissue undergoes constant demands where the cell phenotypes cannot adapt themselves to the high level of motor demands and where stromal fibroblasts are chronically activated. The commitment and fragility become even more evident in gliding tendons in which the part of the tendon that is in contact with the bone develops an avascular fibrocartilaginous tissue in response to the compression forces. During the surgery, we infiltrate approximately 8–10 mL of liquid-activated PRP, distributing it as follows [28]:

1. Into the body of the damaged and sutured tendon in order to promote a chemotactic and angiogenic effect in it.
2. Into the myotendinous junction, where the majority of healthy cells are present, and the subacromiodeltoid bursa, a likely source of multipotent cells.
3. Into the tendon/bone region and into the cancellous bone of the humerus in order to stimulate mesenchymal stem cells in the cancellous bone.
4. Finally, we inject a further 8–10 mL of the remaining PRP into the subacromial space in order to bathe the entire sutured region.

Rotator cuff injuries tend to have a poor prognosis as more than 50% of sutured tendons may not heal. This fact highlights the importance of strictly observing the PRP protocol. An ultrasound examination is performed at week 3 and week 6, and the tendon suture and subacromial space are infiltrated again (8–10 mL of liquid-activated PRP).

## 3.2. PRP injections in cartilage diseases

In spite of advances in pharmacological and surgical techniques, the treatment of cartilage injuries is still a challenge. Articular cartilage is a tissue that is remarkably resilient to compressive and shearing forces. Yet, it is highly fragile to alterations of the synovial membrane and subchondral bone, two well-vascularized tissues from where systemic and local inflammation insults arise. These aggressions are mediated by pro-inflammatory cytokines and inflammatory macrophages and synoviocytes, which damage articular cartilage as in the case of rheumatoid arthritis or osteoarthritis [29]. However, synovial membrane and subchondral bone are also the egress point and source of nutrients and MSCs for mounting a chondrogenic reparative response, which is driven by the recruitment and chemotactic homing of synovium and bone marrow-derived stem cells mediated by SDF-1, TGF- $\beta$  and fibronectin. This is the case in microfracture techniques and in the combinatorial strategy using intraarticular (IA) and intraosseous (IO) infiltrations of blood-derived BDDT such as PRP [30]. In doing so, PRP tackles the four synovial joint tissues and acts as a dynamic autologous liquid scaffold that, in a sustained and gradual manner, conveys chemotactic endogenous MSC homing and chondrogenic factors

such as SDF-1, TGF- $\beta$  and fibronectin [31, 32]. In addition, PRP dampens inflammatory stress at the level of joint tissues, by both inhibiting the NF- $\kappa$ B on chondrocytes and macrophages [33] and upregulating the antioxidant response element NF-E2-related factor 2 (NrF2-ARE) pathway in osteoblasts [34]. Improvements of clinical outcomes of patients with knee and hip OA were reported applying this strategy [35, 36] which might primarily be mediated by HGF, CTGF, IGF-1 and PDGF, among others [33, 34, 37], thereby paving the way to cartilage regeneration; however, elusively, it remains.

### *3.2.1. PRP and chondral surgery*

In joint diseases, the whole joint is affected: cartilage, subchondral bone, synovium, ligaments, neural tissue, etc. Thus, all components of the joint are essential to maintain homeostasis, and both genetic and acquired or environmental factors can break this balance, causing degeneration of cartilage, subchondral bone and other joint components and becoming a clinical problem [36]. The use of PRP as treatment in joint pathology is based on its capacity to restore homeostasis joint, to have inductive and protector effects on chondrocytes and to act on the synovial membrane, stimulating the production of hyaluronic acid and other molecules. All these properties contribute to the promotion of a biological environment that is conducive to slowing the joint cartilage degeneration and relieving clinical symptoms [37].

### *3.2.2. Fracture/avulsion and osteochondritis dissecans*

The first step is to debride the wound bed and to separate the fragment carefully. The bony surface of the said fragment is refreshed to achieve an appropriate appearance. When a bleeding bed is obtained by spongialization, an intraosseous infiltration of 3 mL of liquid-activated PRP is conducted. Next, the osteochondral fragment is fixed into its original niche and its stability is endured. Finally, 2 mL of PRP is infiltrated into space between the wound bed and the fragment using a fine needle. When the fragment is reinserted, the region around all edges is filled and sealed.

### *3.2.3. Osteochondral injuries with an inviable fragment*

In this case, the subchondral bone is debrided removing all damaged tissue as in the osteochondritis dissecans. Therefore, a spongialization is conducted in order to achieve a bleeding bed. The Pridie procedure or microfractures are performed to drill the bone, and a trocar is introduced in order to infiltrate liquid-activated PRP. Consequently, MSCs are stimulated, generating cell and molecular signals that promote the repair processes of joint cartilage. Moreover, a three-dimensional fibrin matrix is formed from PRP, which traps the cells that have come to the lesion area. As a result, the synthesis of the new tissue is promoted, performing a similar mechanical function as the original.

### *3.2.4. Extensive osteochondral injuries and necrosis*

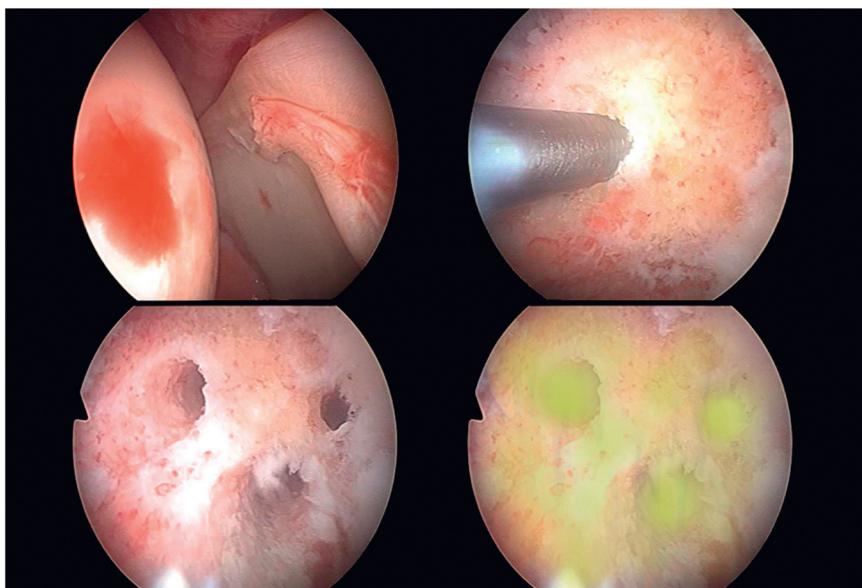
First, the injured tissue has to be debrided in order to achieve a bleeding spongy bone. Next microfractures are conducted and serum is aspirated by intraarticular wash. Finally, liquid-activated PRP is administrated by an intraosseous (3–5 mL) and an intraarticular (8 mL) injections.

The use of autologous osteochondral grafts are recommended when subchondral bone is affected by osteonecrosis of the medial condyle of the knee. Infiltrations of liquid-activated PRP help to integrate such graft. These infiltrations are conducted into the bed and bone osteochondral graft and in the interface where the allograft is implanted. At the end of surgery, serum is aspirated, intraarticular space washed and PRP infiltrated in an intraarticular manner. During the post-operative period, three intraarticular injections of PRP are performed on a weekly basis. Initially, the patient has to walk assisted by crutches with minimal load.

#### 3.2.4.1. Avascular osteonecrosis of the hip

This condition is the final point of several factors. Below, we describe the steps and times for this surgery and the use of PRP [37]. This arthroscopic protocol describes the “light bulb” technique and the biological support to achieve satisfactory results during a mean follow-up of 14 months (**Figure 5**).

1. Both diagnosis and treatment of associated intra-articular damages are addressed by arthroscopy.
2. During stages I and II A, arthroscopic vision allows to perform several image-guided perforations in order to decompress the necrotic cephalic region. With a trocar, liquid-activated PRP is administrated into this region and into the surrounding healthy bone with it. When the femoral head is deformed, an osteoplasty is conducted and PRP infiltrated.



**Figure 5.** Arthroscopic diagnosis of associated damage. Creation of perforations down to the necrosis bed. Intraosseous infiltration with PRP.

3. In stages IIA and IIB, where the condition curses with cystic and sclerotic changes, a debridement and removal of necrotic tissue are performed with trephines, curettes and a synoviotome. When the healthy bone is available, autologous bone graft is impacted into the femoral in order to adapt it properly. The preparation of graft is carried out by using the ipsilateral iliac crest bone graft and liquid-activated PRP. When the size of the injury allows it, a demineralized bone matrix/PRP mixture can be applied.
4. Finally, 8 mL of liquid-activated PRP is injected into the joint. In the next weeks, infiltrations are repeated (three or four times) under ultrasound guide.

### **3.3. PRP infiltrations in bone damage**

When a fracture occurs, the first tissue-based phenomena to be manifested are tissue destruction, vessel rupture and cell necrosis. This results in bleeding that stimulates and activates defence systems to prevent excess bleeding and contamination of the injury site (both of which may be life-threatening). Although the fracture site tends to be hypoxic, with an altered pH and mechanical instability (the local reaction attempts to isolate the fracture site to prevent infection), cells such as platelets, endothelial cells and macrophages are responsible for orchestrating a cell-based response by releasing growth factors such as PDGF, TGF- $\beta$ , IGF I and IGF II, FGFs, VEGF, BMPs, IL-1, IL-6, TNF- $\alpha$  and PGE2 [38]. This group of bioactive molecules promotes the attraction/migration of osteogenic and MSCs from the periosteum and bone marrow, as well as fibroblasts from the surrounding soft tissue to the fracture site, where they form an extensive network based on fibrin and other plasma proteins.

These growth factors play a key role in the initial phases of recruitment/migration, MSC mitogenesis and angiogenesis, which are essential. Simultaneously, the development of new blood vessels is stimulated and under the influence of angiogenic factors such as angiopoietin 1 and VEGF. MSCs and osteoprogenitor cells continue to express BMPs, which induce chondro-/osteogenesis and ECM synthesis. These MSCs initially form aggregates that express transcription factors sox9 and col2 (to express cartilaginous proteins) and then differentiate into chondroblasts (by the third or fourth day). The TGF- $\beta$  expressed by both platelets and endothelial cells during the initial stages of callus formation, and subsequently by chondrocytes and osteoblasts, appears to be key to both MSC chemotaxis and proliferation and the chondrogenesis and formation of endochondral bone [30, 39].

#### *3.3.1. Treatment of fractures assisted with PRP*

We have developed a set of basic guidelines for the application of PRP during the minimally invasive treatment of bone fractures (**Figure 6**) [5].

1. Once the whole context of the fracture has been assessed to determine the most appropriate treatment, 36 mL of peripheral venous blood is withdrawn. Occasionally, due to the type of bone and fracture, it may be necessary to extract further amounts of blood.
2. Reduction and percutaneous osteosynthesis of the fracture under radiographic guidance. If the fracture does not require osteosynthesis, the process can be performed under radiographic control ensuring optimal sterility.



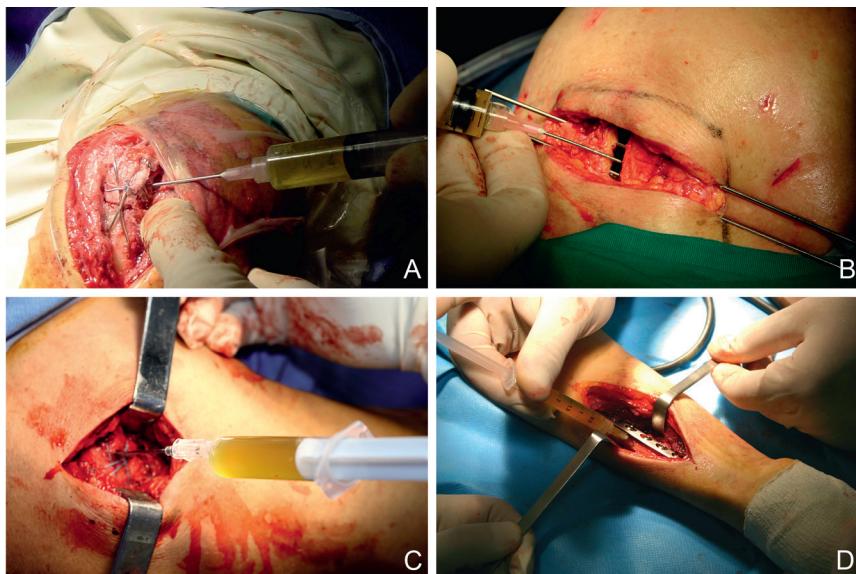
**Figure 6.** Infiltration of liquid PRP at the fracture site, after stabilisation, under radioscopic control: Colle's fracture (A), Bennett's fracture (B) and a distal finger/hand phalanx fracture (C, D).

3. Liquid PRP is then activated for injection at the previously reduced and stabilised fracture site, under radiological control, to form the fibrin clot that is responsible for sustained release of the cell signals that induce the biological repair programme.
4. The volume of PRP infiltrated depends on the size of the fracture, although it is normally around 8 mL.
5. Healing of the fracture is then monitored clinically and radiographically. If signs of consolidation delay are detected, liquid PRP is injected a second time between weeks 4 and 6 in the same manner as the first infiltration (radiographic guidance and between 6 and 8 mL).

### 3.3.2. Surgical fracture treatment

Generally, irrespective of the type of osteosynthesis material expected to be used (always on the basis of the most appropriate surgical indication), this biological bone regeneration therapy is used with PRP in either its liquid form or as a fibrin membrane or clot during surgical fracture repair. Our group has developed a set of basic guidelines for the application of PRP during the surgical treatment of fractures [5].

The fracture to be treated is assessed to determine the amount of PRP required and therefore the volume of blood to be extracted. If surgical treatment of the fracture site does not require the use of allo- or autografts, the fracture is reduced/stabilised. Sound stabilisation of the fracture site is a key factor in the subsequent repair process. Liquid-activated PRP is infiltrated at the fracture site and at its bony ends, using a Luer Lock syringe fitted with a needle of the appropriate gauge (**Figure 7**).



**Figure 7.** Infiltration of liquid PRP at the fracture site, after stabilisation and osteosynthesis, into an olecranon fracture (A), into the os acromiale (B), into a fracture of the humeral head (C) and (D) at the radial fracture.

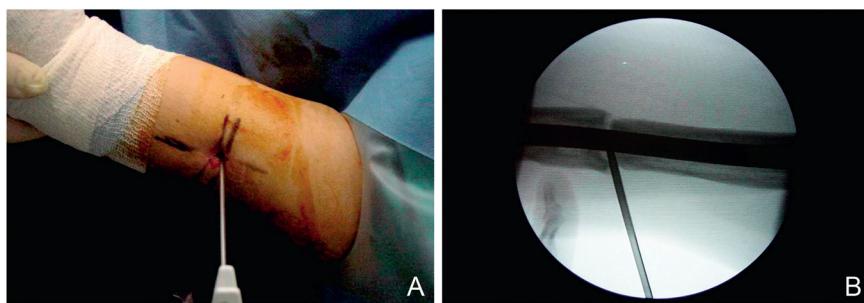
It is particularly important to stress that in the case of fractures of the fibula, calcaneus and other sites where the skin tends to heal poorly, we apply PRP on the skin margins of the surgical wound to enhance spontaneous epithelisation and to induce a bacteriostatic and anti-inflammatory effect. If fracture repair presents signs of delayed consolidation, a further percutaneous infiltration is performed at the fracture site following the same basic steps as described previously.

### 3.3.3. Treatment of nonunions

When the nonunions present a stable fracture area and appropriate osteosynthesis, the procedure consists in a percutaneous infiltration under anaesthesia. It is important to locate and infiltrate the edges of the bones accurately by using an image amplifier. In these cases, a trocar is used to perform the injection to allow several controlled perforations in the injured site. The fracture region and contiguous bone areas are infiltrated with 6–8 mL of liquid-activated PRP. This treatment is repeated in a weekly basis up to a total of three infiltrations (Figure 8).

When an adequate fixation is not reached, debriding and bleeding of the bony edge fragments are conducted in the nonunion area. Next, the region is stabilised using appropriate osteosynthesis material. Liquid-activated PRP is infiltrated into the edges of the bone fragments as in the previous case. When the nonunion presents a bone defect, a bone graft (auto- or allograft) is used together with liquid-activated PRP.

If the criteria described before are followed, treated patients should evolve favourably, presenting clinical and radiographic results that show full resolution in between 2 to 6 months [40]. Similar outcomes have been achieved by other authors such as Seijas et al. [41].



**Figure 8.** Percutaneous infiltration of liquid-activated PRP at the area of a humeral nonunion (A) under radiological control (B).

### 3.4. PRP and meniscal surgery

The abundant ECM (between 60 and 70% of tissue weight) presented in meniscus determines the reparation of this tissue. Cells such as fibrochondrocytes and fibroblast are dispersed throughout the ECM. The peripheral area or meniscal wall presents the tissue vascularity (limited to 10–30%), the largest number of cells, and it receives nerve endings [42]. Due to these characteristics, the recovery capacity of meniscus is highly influenced by this outer portion, since that is where the repair stimuli are generated [43]. Meniscus participates in the stability to the knee and in the support of compressive, traction and shearing forces. In addition, it absorbs part of the mechanical stress received by the knee and takes part in the lubrication of the knee. Thus, injuries in this structure compromise joint function, and it is recommendable to enhance its limited regenerative capacity to achieve an optimal repair. Because promising results are showed by PRP on meniscal cells in laboratory experiments [44], it has been emerged as a novel technique for treating meniscal tears [45].

#### 3.4.1. Meniscectomy

Bearing in mind the special conditions of the meniscal wall, liquid-activated PRP needs to be infiltrated into this structure during a partial meniscectomy. The injection is conducted in an extra-articular way (from outside to inside). However, when the posterior horn of the external meniscus is infiltrated, the injection is performed from inside to avoid vascular or nerve damage. A 21G needle and a 3 mL syringe are used in order to spread the PRP into the meniscus, since a high pressure is required because of the high density of this tissue compared with other structures. Finally, an intraarticular infiltration is performed with 8 mL of liquid-activated PRP is infiltrated in an intraarticular manner.

The maintenance of the meniscal wall is a key element to achieve a partial repair and healing process of the meniscus, and it should be maintained whenever possible. This region presents the cellularity and vascularization needed to generate the biological stimuli for repair and regeneration.

### 3.4.2. Meniscal sutures

The meniscal sutures are a suitable technique to preserve the structure of the knee and consequently reach greater stability and protection of cartilage. The infiltration protocol is similar to that described in the meniscectomies, but in this case, PRP infiltration is applied not only into the meniscal wall but also into the suture region. An intraarticular infiltration of PRP is carried out when the whole process is finished. After 14 days other intraarticular injections of PRP could be conducted to improve the repair process, depending on the evolution of patient.

## 3.5. PRP in the management of neuropathies

PRP products hold an important therapeutic potential as a neuroprotective, neurogenic and neuroinflammatory therapeutic modulator system [46–50] and as enhancer of sensory and motor functional nerve-muscle unit recovery [51–53]. They are applied either as a filler of nerve conduits or vein-muscle grafts across nerve gaps post-trauma by ultrasound-guided perineural and intraneuronal infiltrations or as scaffolds to bridge or wrap the injured nerve stumps [54–56]. Moreover, there are non-traumatic peripheral injuries such as compression, adhesion and fibrosis [46], where this novel approach may diminish undesirable consequences such as fibrotic scars and denervated organ atrophy, since this adjuvant therapy can speed up the functional recovery of the nerve-muscle unit [55–58]. The therapeutic potential of PRP for nerve repair lies in the prolonged and gradual delivery system of biomolecules and in its function as a transient guidance scaffold for axonal sprouting [51, 57]. Considering Schwann cells (SC) as key in the nerve repair process, they are an idoneal target for the synergic action of neurotrophic and neurotropic factors of PRP. Thus, the release of biomolecules from the fibrin matrix at the beginning of regeneration process would induce several biological effects of SC aimed to repair [58–60].

In surgical repair by PRP as in the case of end-to-end neurorrhaphy, nerve compression and nerve entrapment, we recommend combining intraneuronal and perineuronal infiltrations of liquid PRP with the application of a PRP membrane as scaffold, which wraps the injured tissue.

## 4. Guidelines for the appropriate use of PRP infiltrations

Good treatment commences with a correct overall diagnosis that entails the highest number of factors implicated in the disease and considers all the best options.

1. It should be remembered that inactivated PRP can be stored for 3–4 hours without losing its efficacy. However, once activated, it must be used immediately, in the ensuing 2–3 minutes after activation. This aspect provides us with room for manoeuvre when scheduling its use in the theatre room.
2. The volume of the infiltration syringe and the diameter of the needle used will affect the diffusion of PRP within the tissues. The use of small syringes means that large pressures are exerted on the ECM structures during infiltration, thereby accounting for local disruption of the components.

3. Upon infiltration, the needle should be oriented as closely as possible, parallel to and longitudinal with the tendon. This results in the optimal diffusion of PRP while allowing the position of the needle to be controlled as closely as possible with ultrasound guidance.
4. The application of PRP should not alter the surgical technique commonly used for their repair. The main result of combining PRP with surgical treatment is to shorten and lessen the intensity of the initial defence phase and to accelerate the proliferative and trophic phases during the tissue repair process.
5. It is fundamental to combine the application of PRP with other rehabilitation treatments and physiotherapy as mechanical stimuli. Indeed, the combination of different therapeutic elements provides extremely favourable synergies.
6. Given the heterogeneous composition and products of PRPs, it is difficult to ascertain general guidelines in order to optimise them. Rehabilitation and other systemic factors such as nutritional imbalance, overuse or disuse of tissues and life style may account for the majority of degenerative processes.

## Conflicts of interest

SP is a researcher of BTI (Biotechnology Institute), a dental implant company that investigates in the fields of oral implantology of PRGF-Endoret technology.

## Author details

Mikel Sánchez<sup>1\*</sup>, Diego Delgado<sup>2</sup>, Ane Garate<sup>2</sup>, Pello Sánchez<sup>2</sup>, Jaime Oraa<sup>1</sup>, Ane Miren Bilbao<sup>1</sup>, Jorge Guadilla<sup>1</sup>, Beatriz Aizpurua<sup>1</sup>, Nicolás Fiz<sup>1</sup>, Juan Azofra<sup>1</sup> and Sabino Padilla<sup>3</sup>

\*Address all correspondence to: mikel.sanchez@ucattrauma.com

1 Arthroscopic Surgery Unit, Hospital Vithas San José, Vitoria-Gasteiz, Spain

2 Advanced Biological Therapy Unit, Hospital Vithas San José, Vitoria-Gasteiz, Spain

3 University Institute for Regenerative Medicine and Oral Implantology – UIRMI (UPV/EHU-Fundacion Eduardo Anitua), Vitoria-Gasteiz, Álava, Spain

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