Chapter

Isolation, Activation, and Mechanism of Action of Platelet-Rich Plasma and Its Applications for Joint Repair

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Abstract

Platelet-Rich Plasma (PRP) is a biologic therapy that uses the patient's own blood to obtain products with a higher platelet concentration than in blood. This technology provides a controlled drug delivery system of growth factors suitable for regenerative medicine. The biological effects of PRP mimic and influence biological processes such as inflammation, analgesia, and cell stimulation, providing this therapy with promising therapeutic potential. All these processes participate in maintenance, correct function, and homeostasis of the joint, where all tissues are involved. Alterations in one joint element have impact on the rest, outstanding the cellular and molecular interaction between the cartilage and subchondral bone. Therefore, the joint is an optimal therapeutic target for PRP therapy, which favors biological environment for joint repair. This chapter collects the basic concepts of joint function and the biological processes that participate in its degeneration, the definition and obtention of PRP, as well as its therapeutic potential and clinical translation.

Keywords: Platelet-Rich Plasma, growth factors, joint degeneration, cartilage, subchondral bone, intra-articular injection, intraosseous injection

1. Introduction

Despite joint degeneration, caused mainly by osteoarthritis (OA), not being a threat to life, it meets conditions that make it a real problem for both patients and health systems. This pathology is one of the leading causes of disability in the middle-aged and elderly population, and although any joint can be affected, the hip and knee are the most affected ones. This high prevalence, with 250 million people with knee OA throughout the world, represents up to 2.5% of gross domestic product for developed countries [1]. In the coming years, prevalence and costs will increase because the risk factors that favor OA are inherent to today's society such as

the aging of the population, overweight, or an uncontrolled sports practice, both by excess and by default.

Patients with OA are characterized by pain, stiffness, and limitation of function, becoming disabling in the most advanced stages [2]. Initial conservative treatments include physiotherapeutic work, nutritional supplements, and oral administration of analgesic and anti-inflammatories. In the next phases, patients can be treated with intra-articular injections of hyaluronic acid. Regardless of the success of these treatments, all of them focus on symptomatic relief without stopping or slowing the progression of the disease, and the only solution for patients with the most severe degrees of OA is total knee arthroplasty [3]. This surgical intervention not only entails the risks derived from surgery, which may be unacceptable by some patients, but also involves the majority of the cost of health systems [4, 5]. Therefore, it is necessary to develop new therapies that improve the current ones in order not only to alleviate the symptoms but also to modify the course of the pathology to slow its progression or even reverse it. This would improve the quality of life of patients, delaying or avoiding a large number of surgical interventions as well as the expense they entail.

These therapies must be based on two main pillars that sustain a new approach in joint degeneration: first, treatments based on regenerative medicine which can act on tissue biology and modify the pathophysiology of OA such as gene therapy, Platelet-Rich Plasma (PRP), or mesenchymal stem cells (MSCs). Among these treatments, PRP is currently the most widely used due to its greater ease of regulating, obtaining, and applying as well as its low cost [6, 7]. However, it is necessary to deepen their knowledge and standardize products and protocols to optimize clinical results. The second cornerstone is to understand the joint as a whole organ, taking into consideration all its elements [8]. Knowing the relationships between the different tissues that form and define the joint is key for the correct application of treatments and address degenerative pathology completely. Thus, this chapter is intended to explain the role of PRP in joint degeneration, highlighting the therapeutic potential of PRP in all the components of the joint and its clinical translation.

2. The joint as an organ

2.1 Joint components and homeostasis maintenance

All joint structures present a unique molecular and cellular composition as well as specific biomechanical properties; consequently, each element of the joint performs characteristic functions. However, they are all coordinated and related to create the biological machinery that allows the joint to have dynamic stability (**Figure 1**) [9]. This gives the joint a great adaptability to maintain a mechanical and biological balance, supporting and confronting physical forces or physiologic disorders. In a short look at the components of the joint, the periarticular muscles appear in the outermost section. This tissue presents vascular irrigation, many neuronal terminals, and high plasticity. The configuration of its extracellular matrix in a network of muscle fibers provides muscle elasticity and allows the mechanical forces generated by the muscle cells to be transmitted to the tendons, which will translate them into joint mobility [10]. However, its stability capacity is even more important than mobility in order to maintain joint homeostasis, the quadriceps muscle being key in knee anteroposterior steadiness. In addition, muscle tissue is essential in shock-absorbing, and together with the subchondral bone and ligaments, it accounts for 30–50% of the total absorbing energy [11]. Ligament composition is characterized by a high-water content and an extracellular matrix with

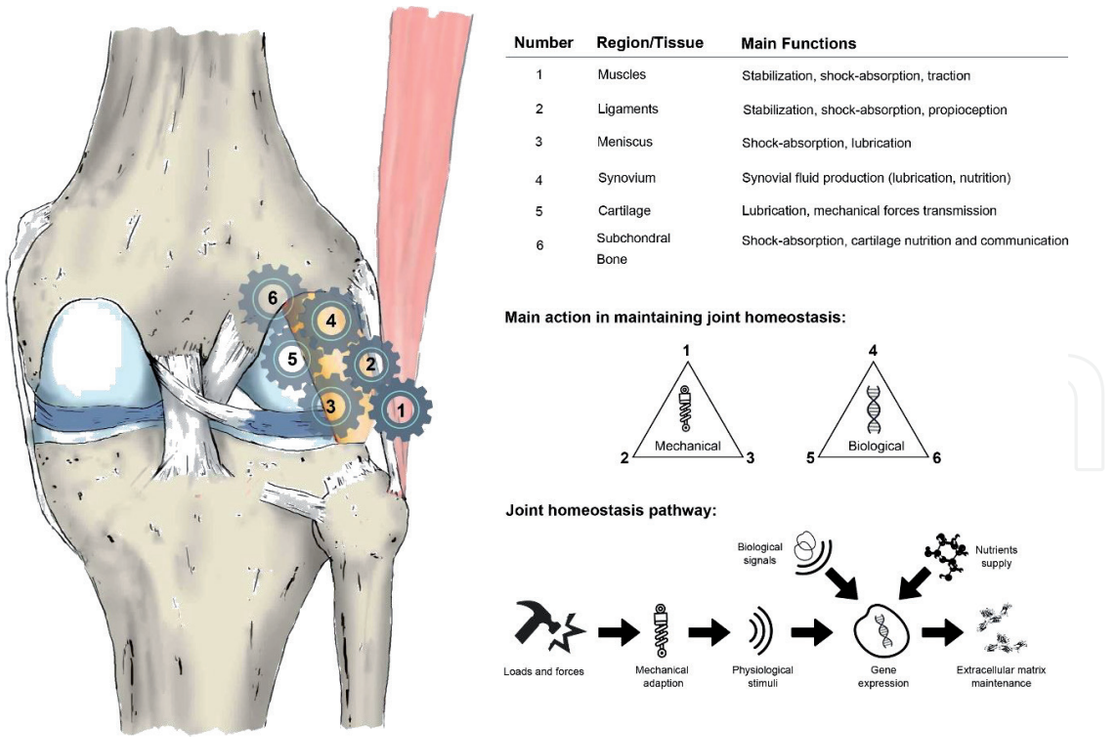

Figure 1.
Joint as an organ. All the elements of the joint participate in its correct function and in the maintenance of the homeostasis. Although they all contribute to mechanical and biological stabilization, ligament and meniscus muscles play a mainly mechanical role, whereas the synovium, cartilage, and subchondral bone have a more biological action. Correct mechanical adaptation and a favorable biological environment allow the cells to maintain a gene expression that promotes the optimal maintenance of the extracellular matrix.

a small number of fibroblasts. Collagen is the most predominant protein, mainly organized in type I collagen fibers that adopt many directions and orientations due to several forces these structures are subjected to [12]. Apart from their stabilizing function due to their biomechanical and viscoelastic properties, they are also responsible for detecting and controlling the position and movement of the knee. In this way, the joint has a balanced biomechanical behavior that prevents the origin of mechanical problems that lead to degeneration. The meniscus plays a fundamental role in functions of mechanical nature such as stability and shock-absorbing. It is a fibrocartilaginous tissue with an abundant extracellular matrix where cells such as fibroblasts and fibrochondrocytes are dispersed and where type I collagen is the predominant molecule. The presence of vascularization and nerve terminals is limited to the external zone or meniscal wall. These intra-articular elements located between the femoral condyles and the tibial plateau help stabilize the joint and withstand compression and sharing forces. In addition, they participate in the lubrication of the joint with the synovial membrane or synovium [13].

The synovium, together with the cartilage and subchondral bone, forms an important biological triangle to maintain homeostasis of the knee. Both nerve fibers and blood vessels are abundant in the synovium, which provides nutrients not only to this structure but also to the adjacent avascular cartilage. Its cellular composition stands out mainly for synoviocytes (macrophagic cells or type A and fibroblast-like cells or type B), although immune system cells and even MSCs are also present, the synovium being a source of stem cells of increasing interest [14]. Its main function is the production of synovial fluid, which is produced by type B synoviocytes. It soaks the intra-articular space and structures, being essential in the lubrication of the joint due to its hyaluronic acid and lubricin content. The synovial fluid is also an important source of nutrients, biomolecules, and cellular signals, so it is essential for the biological balance of the joint [15]. The second element of this biological triangle

is the hyaline articular cartilage. It has a very low coefficient of friction that resists compression and shear forces and absorbs only 1–3% of the total energy. The main cellular element of this tissue is a low population of chondrocytes that is distributed along the extracellular matrix composed principally of type 2 collagen, in addition to other molecules such as proteoglycans or aggrecans. Its functions of lubrication and transmission of mechanical forces are performed thanks to a stratified tissue in different zones, from the most superficial, with a higher water content and chondrocytes, to a deeper area of calcified cartilage that is over the subchondral bone [16].

Subchondral bone, together with the osteochondral unit, completes the triad of elements with a predominant role in the biological maintenance of the joint. This structure consists of a plate of cortical bone from where the bone marrow and trabecular bone areas emerge. The importance of the subchondral bone lies in its communication with the cartilage, providing this tissue with at least 50% of the oxygen and glucose requirements. This communication not only is limited to the nutritional contribution but also covers the cellular and molecular signaling that participates in the cartilage homeostasis. Besides this, it is also a source of MSCs and participates in absorbing joint loads along with the other elements mentioned above [17].

The joint adaptability is both mechanical and biological, and it is in this last component where the action of regenerative medicine could positively influence. All the structures and tissues described above participate in joint stability by adapting to the different alterations and stimuli received, ultimately maintaining a healthy cartilage. Because of the "mechanical stabilizers" of the joint, the mechanical loads and forces that it receives become molecular and cellular stimuli that are maintained at physiological levels. These stimuli activate the chondrocyte gene expression, allowing them to synthesize proteins, such as proteoglycans, collagen, and metalloproteases, that ensure the integrity and renovation of the articular cartilage [18]. The continuous adaptation of the cells to the mechanical stimuli they receive in order to maintain the adequate extracellular matrix is based on a very delicate anabolic/catabolic balance, and any mechanical or biological alteration can break it resulting in joint degeneration [19].

2.2 Joint degeneration process

The balance present in the joint may be broken because of multiple causes (**Figure 2**). For example, injuries of the tissues involved in the mechanical stabilization of the knee could entail an abnormal load distribution. This would cause an unsatisfactory shock absorption into the joint, and the stimuli generated would exceed the physiological level [20]. Lifestyle can also have an impact on the generation of pathological stimuli. Both uncontrolled physical activity and sedentary lifestyle lead to an excess or defect of stimuli, respectively. The result is a biological and cellular malfunction and, consequently, a defective tissue renewal. In addition, pathologies and biological disorders such as inflammatory processes or those affecting the structures responsible for maintaining and nourishing the cartilage could also cause cellular failures that lead to imbalance and joint degeneration.

The multifactorial nature of this pathology makes it difficult to know the exact origin of the triggered processes as well as their sequence and timing. These events take place with special importance in the interaction between the synovial membrane, cartilage, and subchondral bone. Regardless of the original cause, one of the main consequences of this imbalance is the deterioration of the extracellular matrix and the generation of degradation products that are released to the synovial fluid [21]. The cells of the different joint tissues such as chondrocytes, synovial macrophages, osteoblasts, or fibroblast interact with these molecules, which act as Toll-like-receptors (TLRs) and damage-associated molecular patterns (DAMPs).

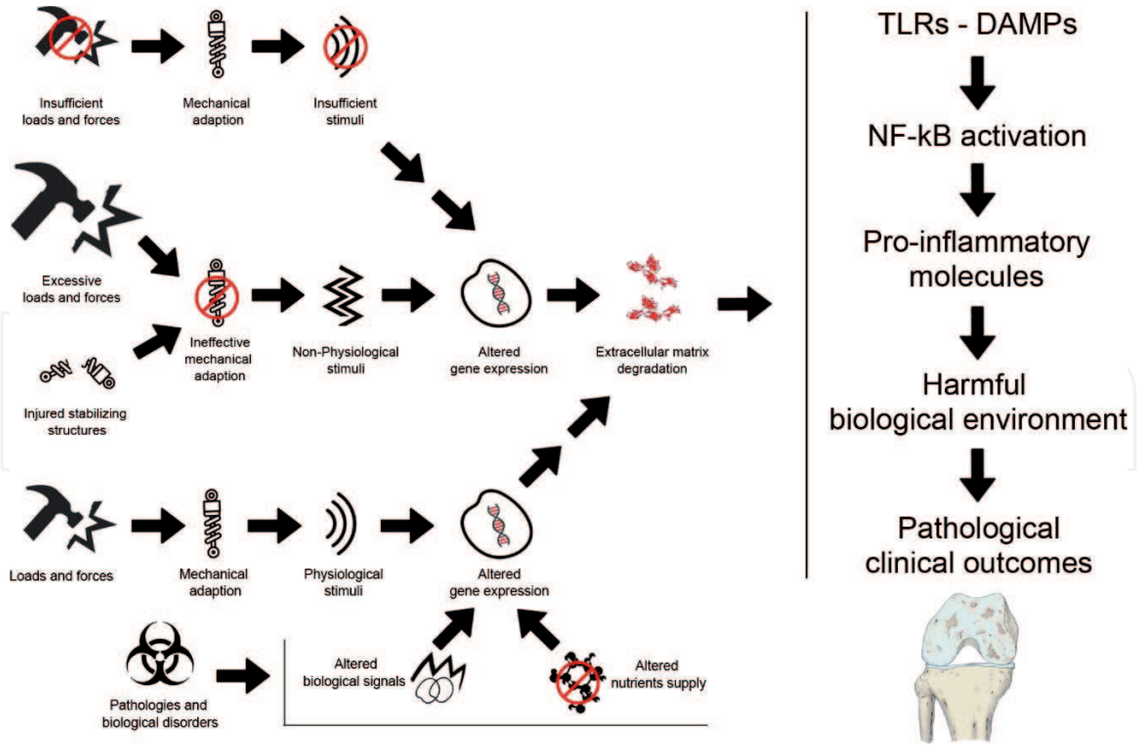

Figure 2.
Joint degeneration processes. Different causes such as abnormal mechanical loads, injuries of stabilizing structures, or pathologies and biological disorders cause nonphysiological stimuli that modify the gene expression of cells. As a consequence, the extracellular matrix degenerates, activating pro-inflammatory pathways that create a harmful environment and joint degeneration.

As a consequence of these interactions, the intracellular pathway of the nuclear factor kappa β (NF-kB) is activated, connecting the mechanobiological program and the inflammatory response. The gene expression of the affected cells shifts to an inflammatory pattern synthesizing molecules, namely, interleukins (IL-1b, IL-6, IL10), prostaglandins (PEG-2) and other pro-inflammatory biomolecules, and cytokines (necrosis factor alpha (TNF-α), interferon gamma, or nerve growth factor (NGF)). Pathological levels of these molecules also interfere in physiological repairing responses. For instance, the action of MSCs from the bone marrow is altered by high levels of transforming growth factor beta (TGF-β), compromising their modulating and repairing functions [22].

All the harmful biological environment generated by this event cascade leads to pathological outcomes in the cartilage, synovium, and subchondral bone. Chondrocytes of cartilage turn into a much more active state, forming cell clusters and increasing their proliferation. They also increase the synthesis of both extracellular matrix proteins and enzymes, causing an altered remodeling of the matrix with hypertrophy and calcifications [19]. Concerning the synovium, inflammation occurs in the early stages together with macrophage infiltrates and an increased synovitis in the advanced stages [23]. Communications between the cartilage and subchondral bone are increased due to the presence of fissures and microcraks, in addition to the remodeling of this tissue with fibroneuroangiogenesis because of the overexpression of molecules like TGF-β and vascular endothelial growth factor (VEGF) [24].

Moreover, the negative effects arising from joint degeneration can affect other tissues as well [8]. For example, studies conducted in the meniscus of patients with arthrosis showed a tissue with increased vascularization and nerve terminals, with the unstructured extracellular matrix, abnormal cell organization, and cell death [25]. Likewise, ligaments with osteoarthritic patients also showed calcifications and

disorganized collagen fibers [26]. Finally, muscle tissue is also affected by inflammation produced in joint degeneration, showing fibrosis, collagen depositions, and muscle wasting [27]. Considering all this, it is clear that joint degeneration is not a sole cartilage disease. Instead, it affects all the elements present in the joint, and, therefore, it should be clinically tackled taking into consideration all of them in order to reverse or slow down the degenerative progression.

3. Platelet-Rich Plasma

3.1 Platelets as a source of bioactive molecules

PRP is an autologous biological therapy framed in the regenerative medicine whose basic principle is to obtain a fraction of blood plasma that contains platelets at a higher concentration than in the blood. From the pharmacological perspective, it is very difficult to define it since the PRP presents a large number and variety of active substances, even often antagonistic. Its therapeutic potential lies both in the biomolecules present in the plasma and in the platelet and its content that is the core element of this therapy.

The platelets are produced by the megakaryocytes of the bone marrow, which migrate to the endothelial barrier after maturation and project their prolongations releasing into the bloodstream the proplatelets or precursors that will generate the platelets [28]. Platelets are discoid and anucleated blood elements with a diameter of 2–3 μm; their blood concentration is 150.000–400.000 platelets/μL with a life span of 7 to 10 days. Platelets are limited by an external plasma membrane that contains a large network of receptors that trigger intracellular signals that allow platelets to perform their numerous functions. Among them glycoprotein Ib (GPiB) and glycoprotein VI (GPVI) receptors can be found, which are involved in functions related to homeostasis, the main function of platelets. GPIb and GPVI bind to von Willebrand factor (VWF) and collagen when there is a discontinuity in the endothelial barrier that exposes the extracellular matrix. These interactions cause conformational changes in platelets and allow them to bind to fibrinogen, tissues, and other platelets to form the thrombus that will participate in tissue repair. In addition, this platelet activation also causes the release of their internal content that has regenerative abilities and justifies the use of PRP.

The internal content of platelets is stored in different granules called dense granules, α-granules, and lysosomes. The material present in these granules may have been synthesized by the original megakaryocyte as well as captured by platelets by endocytosis. The α-granules are those that have a higher content of active biomolecules related to tissue repair. Hundreds of these molecules have been identified, including adhesive proteins, fibrinolytic and coagulation factors, antimicrobial molecules, cytokines, and growth factors, among others. These last two groups of molecules participate in tissue repair and regeneration processes such as angiogenesis, chemotaxis, migration, or cell proliferation [29]. When platelets are activated, not only these molecules are released but also other elements such as platelet microparticles, which are involved in anti-inflammatory processes, or exosomes. Exosomes are small vesicles of 100–400 nm that carry several proteins in addition to other biomolecules as genetic material. Although not much is known about these platelet exosomes, it has been found that they are very important in cellular communication [30].

The activation of the PRP platelets causes the release of platelet content related to tissue repair to the outside, and it joins to the circulating biomolecules in the plasma. Thus, the levels of many growth factors will depend on the platelet concentration of

the PRP. Among these platelet growth factors, there is platelet-derived growth factor (PDGF), which is a potent chemotactic for several cell types and has an important effect on tissue repair over tissues such as cartilage and meniscus. Another growth factor with a large presence in platelets is TGF-β, whose effects are varied and can be of different nature depending on the molecules and cells with which it interacts. It influences early responses in tissue repair, on the differentiation processes of mesenchymal stem cells, and on the maintenance of cartilage and subchondral bone. Other regulatory factors in tissue repair are VEGF, epidermal growth factor (EGF), or basic fibroblast growth factor (bFGF) with key roles in cell migration, proliferation, differentiation, or angiogenesis. In addition, circulating molecules such as insulin-like growth factor type I (IGF-I) or hepatocyte growth factor (HGF) have also crucial importance in the effect of PRP; they are growth factor enhancers of regeneration processes as well as modulators of inflammatory processes [31].

Therefore, PRP is a cocktail of thousands of biomolecules from plasma and platelets that regulate hemostasis, coagulation, tissue repair and regeneration, inflammation, cellular behavior, or defense against microorganisms, among other biological processes. All this therapeutic potential depends largely on its composition, which may vary according to the method used to obtain it. As a result, there is a wide variety of PRP products as will be explained below.

3.2 Obtaining process

3.2.1 Blood collection

As stated previously, the PRP obtaining technique is used to achieve a fraction of blood plasma with higher levels of platelets than blood. The first step consists in the collection of a small volume of peripheral blood from the patient using tubes with anticoagulant—to prevent blood clotting. Different types of anticoagulants can be used such as sodium citrate and ethylenediaminetetraacetic acid (EDTA), which chelate calcium and prevent the coagulation cascade, or heparin that inhibits thrombin. However, sodium citrate is the most recommended anticoagulant since it ensures a better preservation of platelets [32]. It also causes less secretion of microvesicles that are the result of platelet activation, which is increased when EDTA and heparin are used as blood anticoagulants [33].

3.2.2 Blood fractionation and Platelet-Rich Plasma obtention

After blood collection, a centrifugation process is performed, whose force and time vary according to the methodology and, hence, the PRP formulation to be obtained. Centrifugation has to generate sufficient force to create a gradient that separates the blood into different fractions but without damaging its components (**Figure 3**). These centrifugations can be single or double, with a centrifugal force of between 350 and 2000 g and a centrifugation time of 3 to 15 minutes depending on the method used. Thus, the blood is divided into a lower fraction of red blood cells, a thin layer of leukocytes or buffy coat, and finally the plasma fraction with platelets, which gradually decrease their number in the uppermost areas. This last layer will constitute the PRP, and depending on the centrifugation process, the number of platelets may vary. However, a higher number of platelets are not strictly linked to an improved effect of PRP. In fact, several studies have reported that an excessive concentration of platelets may have inhibitory effects on cell proliferation or differentiation in populations such as tenocytes or adipose tissue-derived stem cells. Thus, the optimal platelet concentration for an optimized function is considered two- to threefold compared to blood levels [34, 35].

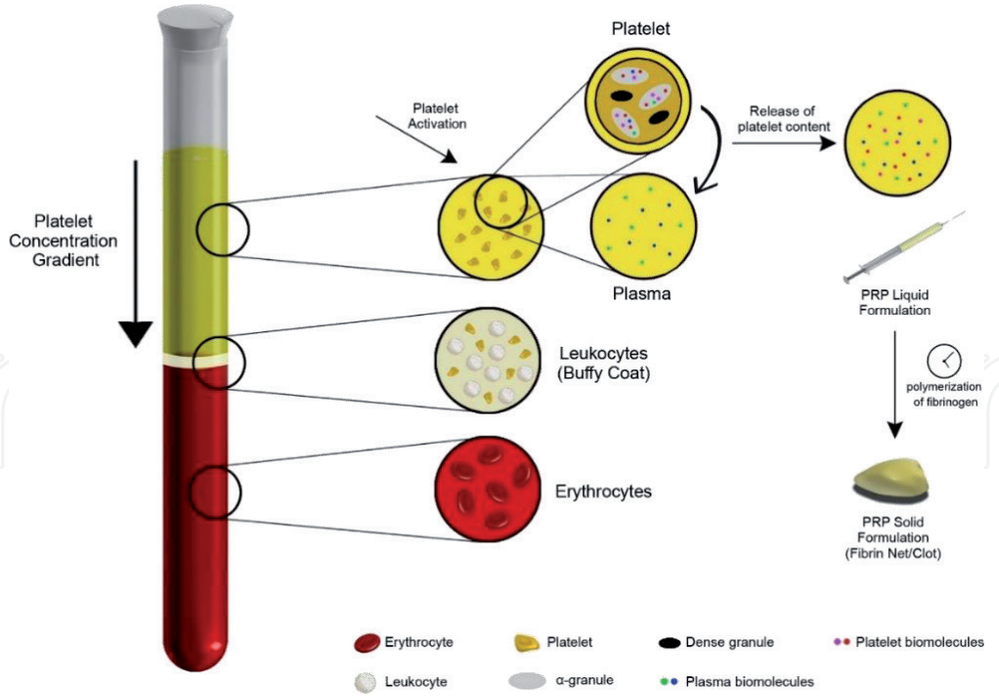

Figure 3.
Obtaining Platelet-Rich Plasma. After blood fractionation, the platelet-enriched plasma fraction is obtained. The activation of this fraction causes the release of the platelet content that together with the plasma molecules constitutes the effector biomolecules of the PRP. It also generates the polymerization of the fibrinogen that will create a network of fibrin where these biomolecules will be trapped, and that will be released progressively.

When separating the PRP from the rest of the blood fractions, there is the option to include or not the leukocyte layer, thus obtaining different PRP products, which will be detailed below. Although in some musculoskeletal disorders the use of Leukocyte-Rich PRP (LR-PRP) need further research, there is an increasingly broad consensus by which the use of leukocyte-poor PRP (LP-PRP) preparations is recommended for joint degeneration [36]. The inclusion of leukocytes in the PRP generated pro-inflammatory molecules that had negative effects on cell proliferation and chondrogenic differentiation as well as a worse regeneration of articular cartilage [37]. However, the fraction of red blood cells must be discarded in order to avoid the presence of erythrocytes in the PRP. The presence of erythrocytes in the PRP entails their own degradation processes such as hemolysis and eryptosis. As a result, products that promote inflammation and cellular stress are generated, which would hinder the beneficial action of PRP [38].

3.2.3 Platelet-Rich Plasma activation

The last step in the process to obtain PRP is the activation of platelets, through which its platelet content not only is released but also triggers the polymerization of fibrinogen in a fibrin mesh that traps the molecules. Thus, a controlled release system that delivers the molecules as it degrades is obtained. Activation can be exogenous either by physical methods such as freeze–thaw cycles or by the addition of certain substances (calcium chloride, thrombin). Some methods propose endogenous activation in which PRP is administered without prior activation and platelets are physiologically activated inside the body [39]. However, the use of exogenous activation allows a more versatile PRP, and depending on the time that has elapsed since the activation, different formulations are achieved at the point of care. The addition of calcium chloride as an activation method avoids the use of

exogenous biological elements such as thrombin. It also prevents local hypocalcemia that can be caused by the calcium-chelating anticoagulants previously used in blood collection to prepare the PRP. Thus, PRP can be used as an injectable liquid formulation immediately after activation or as a fibrin membrane-clot minutes after adding the activator. In this case, and due to its consistency, this formulation can be used as a biological and autologous scaffold in surgical interventions that promote tissue repair [40].

3.3 Types of Platelet-Rich Plasma

As mentioned above, many variables may be involved in the obtaining process. It is not the intention of this chapter to delve into the large number of PRP types that exist both in the market and in the scientific literature. However, it is important to mention the variables that condition not only the type of PRP and therefore the different biological effects but also the classification systems (**Table 1**).

The three main variables that condition the obtaining of PRP, namely, number of platelets, presence or not of leukocytes, and activation, generate many different products under the PRP term which are necessary to differentiate. Not only a wide variety of products have emerged but also several classification systems that have attempted to clarify the inconsistency that accompanies the term PRP. Initially, the main difference was the presence or not of leukocytes. In the first classification of Dohan et al., PRPs could be distinguished in leukocyte-poor PRP and Leukocyte-Rich PRP, besides contemplating the fibrin presence [41]. Subsequently, Mishra [42] and DeLong [43] took into consideration the number of platelets and the activation of PRP. In the following classifications, the presence of erythrocytes [44] was also mentioned, and in recent years aspects such as recovery efficiency or centrifugation and application methods were addressed [45, 46], trying to classify as much as possible the different PRP products (**Table 1**).

As if that were not enough, new denominations are being coined in products derived from blood but that share the fundamental principles of PRP. This is the case of the Platelet-Rich Fibrin. These types appeared initially in the Dohan classification and refer to the fibrin clots that are formed either by centrifuging the blood without anticoagulants or by activating the liquid PRP and waiting for the formation of fibrin net, as mentioned above. A product derived from this is the hyperacute serum that is obtained with a procedure similar to that of the PRP but without using anticoagulants. Thus, after centrifugation of the blood, the upper fraction is a fibrin clot (Platelet-Rich Fibrin), which is squeezed to obtain the hyperacute serum [47]. It contains all the plasma and platelet biomolecules without coagulation proteins such as fibrinogen, namely, the product obtained is almost identical to the exudate gradually released from the fibrin net achieved after the activation of PRP. However, many growth factors present in the hyperacute serum will be eliminated quickly after its injection into the affected area due to its short half-life, whereas if they are released in a controlled manner as in the activated PRP, its time of action will be longer [48].

The lack of standardization is one of the main limitations in the application of PRP. Although all these products are called PRP, their composition may differ from many others and as a consequence their biological effects and clinical results. For instance, the presence of leukocytes determines the levels of pro-inflammatory molecules, and the activation or not of platelets affects the biomolecule release kinetics. Therefore, the comparison of PRP studies, assuming that it is the same product, yields contradictory data, so it is necessary to specify the type of PRP used in these works [49].

Variable type	Dohan [41]	Mishra [42]	PAW [43]	PLRA [44]	DEPA [45]	MARSPILL [46]
Composition	Leukocytes Fibrin	Platelets Leukocytes	Platelets Leukocytes Neutrophils	Platelets Leukocytes Neutrophils Erythrocytes	Platelets Leukocytes Erythrocytes	Platelets Leukocytes Erythrocytes
Activation	—	Activation	Activation	Activation	—	Activation Light
Others	—	—	—	—	Efficiency	Method Image guided Spin

Table 1.
Variables analyzed in the different classification systems.

4. Platelet-Rich Plasma and joint degeneration

4.1 Therapeutic potential of PRP in joint degeneration

As PRP is a product that contains a large number of bioactive molecules, it is wrong and impossible to define the effect of the PRP based on the isolated actions of each molecule. The biological effect of PRP depends not only on its molecules but also on its synergistic effect, which also considers interactions between molecules. Indeed, many PRP molecules are activated in the presence of others, or on the contrary many have antagonistic effects, conditioning the final effect of PRP.

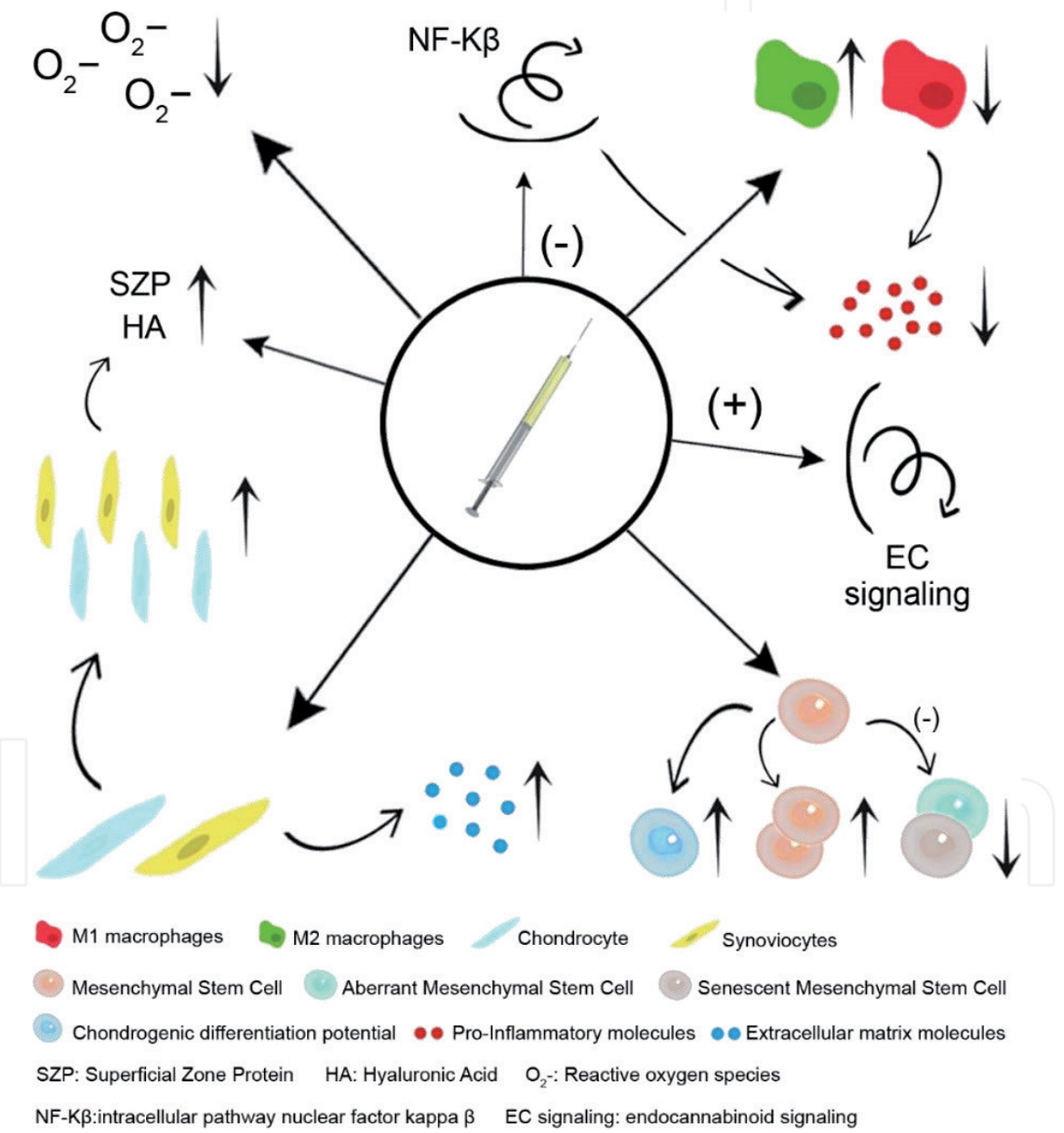

Figure 4.
Biological effects of Platelet-Rich Plasma on joint degeneration. The inhibition of the intracellular signaling pathway NF-Kβ, the reduction of reactive oxygen species, and the promotion of M2 macrophages cause the drop of pro-inflammatory molecule levels, achieving an anti-inflammatory effect. In addition, this decrease in pro-inflammatory molecules such as prostaglandin E2 achieves an analgesic effect, which is also favored by the activation of endocannabinoid systems. On the other hand, PRP modulates the cellular response, stimulating the proliferation of chondrocytes and synoviocytes, which increase the production of the substances responsible for lubrication. This modulation also affects mesenchymal stem cells, which increase their chondrogenic potential and decrease their aberrant and senescent forms.

Multiple actions are attributed to the PRP in the treatment of pathologies of the musculoskeletal system. However, this chapter will be limited to highlighting the effects that have the greatest impact on improving joint degeneration (**Figure 4**).

4.1.1 Anti-inflammatory effect

Due to the complex OA pathophysiology, inflammation can be both the cause and consequence of other pathological processes. Because of this, it is important to reverse the pro-inflammatory environment of this pathology and restore homeostasis of the joint to promote tissue repair. Many of the PRP molecules participate in the regulation of these inflammatory processes, which are key in the progression of the pathology. The anti-inflammatory effect of PRP is achieved through the action of its biomolecules at different levels. Molecules such as IGF-1 or HGF restore the original acquiescent state of cell populations from an inflammatory state because of joint degeneration. This effect occurs through the inhibition of the intracellular signaling pathway NF-Kβ by these molecules, and, as a result, the generation of pro-inflammatory molecules such as IL-β or TNF-α is reduced [50, 51]. The use of PRP rich in leukocytes can be especially important in this mechanism of action since, instead of inhibiting this inflammatory pathway, they activate it due to the presence of certain pro-inflammatory molecules in this type of PRP [37]. This inhibitory effect not only is limited to chondrocytes but also affects other cell populations such as fibroblast, osteoblasts [52], or macrophages [53]. The consequence of silencing this pathway in the different cell types of the joint is the drop in the inflammatory molecular levels of the synovial fluid, relieving the inflammatory environment [54].

Within its anti-inflammatory action, PRP also acts on macrophages by changing its phenotype. This effect may be indirect due to the decrease in pro-inflammatory molecules or direct by a direct action on the PRP components such as the microparticles produced by platelet apoptosis. The result is a phenotype shift of the macrophages from inflammatory (M1) to reparative (M2) phenotype, where the reduction of inflammation is favored and tissue repair is stimulated [55]. This effect is especially important in macrophages present in the synovial membrane. The increase in anti-inflammatory macrophages to the detriment of pro-inflammatories results in a decrease in inflammation of the synovial membrane, which is a hallmark of OA [56]. This polarization towards a reparative state may be due to the action of the interleukin 1 receptor antagonist, present in the PRP, which in addition to avoiding the inflammatory effect of IL-1 promotes the repair phenotype M2 of macrophages [57].

The inflammatory environment in the osteoarthritic joint is also potentiated by the increased presence of reactive oxygen species (ROS), which participate in the OA pathogenesis through synovium inflammation, cartilage degradation, or subchondral bone dysfunction [58]. PRP activates the antioxidant response element through the intracellular signaling pathway NrF2-ARE in osteoblasts [59]. This achieves an antioxidant and protective effect in these cell populations, avoiding damage caused by ROS increment.

The interaction of PRP biomolecules in the mechanisms that trigger inflammation results not only in a decrease in the levels of pro-inflammatory molecules and ROS but also in a promotion of gene expression related to anti-inflammatory action. It has recently been shown that gene expression of enzymes related to aggrecan destruction and metalloproteinase modulation, namely, metalloproteinase with thrombospondin motifs-5 (ADAMTS-5) and tissue inhibitor of metalloproteinases-1 (TIMP-1), are decreased in cartilage and synovium under the presence of PRP. However, gene expression related to the formation of collagen 1 and aggrecan is increased [60].

4.1.2 Analgesic effect

Pain is one of the most characteristic symptoms of OA and one of the most limiting factors for the patient, affecting its functionality and quality of life. One of the main causes of pain associated with joint degeneration is the inflammation that occurs. Solving the inflammatory problem would partly relieve the pain of the OA patient. This relief is one of the most observed effects in clinical studies since it is the most studied variable. However, it is necessary to deepen the mechanisms of action by which the PRP achieves the analgesic effect. During the inflammatory processes, molecules are generated by resident macrophages outstanding prostaglandin E_2 (PGE_2), which is one of the main causes of the inflammatory pain [61]. As mentioned earlier, PRP favors the change in macrophages from pro-inflammatory to anti-inflammatory phenotype as a consequence of the production of PGE2 and other pro-inflammatory molecule reductions [56]. In addition, the action of the PRP over the NF-Kβ pathway could also reduce the levels of substances that stimulate the nociceptors of the joint synovitis [62]. Therefore, inhibition of the synthesis of these substances is one of the mechanisms of action by which PRP reduces pain.

Although the action on inflammation may be the most predominant mechanism in pain relief, the implication of other pathways has been studied, namely, the peripheral endocannabinoid-mediated mechanism, which could be a promising therapeutic target in the synovial tissue of OA patients [63]. The influence of PRP on this signaling system is associated with the stimulation that occurs in the cells located in inflammatory environments. In the presence of PRR, these cells would generate analgesic substances such as anandamide and 2-arachidonoylglycerol, which are agonists of cannabinoid receptors 1 and 2. This effect is observed both in vitro and in vivo, with a lower nociceptive response in treated animals [62].

4.1.3 Biolubricanting effect

One of the problems associated with osteoarthritis is the lack of lubrication and therefore the increased friction in the joint. In a healthy joint, the synovial fluid has a natural lubricant function due to the presence of hyaluronic acid. The alteration of the components of the synovial fluid worsens the lubrication, deteriorating the cartilage. In addition, this layer of lubricant decreases progressively as the disease worsens, creating a vicious circle [64]. Restoring joint lubrication is one of the priorities to improve the course of the disease, and it is the purpose of intra-articular hyaluronic acid infiltrations [65].

The application of PRP also may restore joint lubrication through several mechanisms. First, it has a stimulating effect on the chondrocytes and synoviocytes, due to the fact that it not only enhances its proliferation but also increases the production of hyaluronic acid, improving the lubricating capacity of the synovial fluid [66–68]. Secondly, PRP also influences lubrication through the superficial zone protein (SZP) or lubricin. This protein synthesized by chondrocytes and synoviocytes acts as a chondroprotective barrier against direct contact in joints. PRP improves lubrication both directly, since it contains endogenous SZP, and indirectly by stimulating the SZP secretion by articular cartilage and synovium [69].

4.1.4 Cellular modulating effect

All the effects described above are generated through the interaction between growth factors and cell membrane receptors, triggering intracellular pathways and affecting gene expression that generates the biological effects. In addition to these, which are the most influential in the asymptomatic relief of OA, namely,

inflammation, pain, and lubrication, there are other trophic and regulating effects that, although they do not have such a drastic clinical outcome, are necessary to promote tissue repair and reverse or slow down the disease.

PRP has demonstrated its biological effect over the chondrocytes of articular cartilage and its consequent impact on cartilage repair. Fice et al. published a systematic review including numerous in vitro and in vivo studies that showed the action of PRP on the cellular response [70]. On the one hand, it acts on cellular behavior, increasing growth, migration, and proliferation rates and reducing negative effects such as apoptosis. On the other hand, PRP enhances the synthesis of glycosaminoglycans (GAGs), proteoglycans, and collagen, improving the production of extracellular matrix.

In addition, stimulation of cartilage repair is also conditioned by the action of MSCs. They are able not only to differentiate into cells with specialized functions such as chondrocytes, osteoblasts, and adipocytes but also to release molecules and cellular signals that regulate the repair processes [71]. The behavior of MSCs in OA is modified, increasing in number in synovial fluid as the severity of the disease increases [72]. These MSCs come from resident joint niches such as the synovial membrane, the surface of the articular cartilage, and the subchondral bone, once again confirming the involvement of all the joint structures in the development of OA [17]. In this pathological environment, these cells have their function altered, losing their restorative activity [73]. Bearing this in mind, mesenchymal stem cells are considered a therapeutic target for the PRP to modulate its behavior and restore its physiological functions. Muiños-López et al. observed a decrease in MSCs in synovial fluid of patients with severe OA after the application of PRP directly into the subchondral bone [74]. The regulatory capacity of PRP on MSCs may be due to the direct action on their cellular response as well as the improvement of the biological environment in which the cells reside. Bone marrow-derived MSCs treated with PRP showed an increase in proliferation and chondrogenic capacity [75]. This increased proliferation was also observed in human adipose-derived stem cells, although their chondrogenic differentiation potential was retained [76]. Restoring tissue homeostasis where MSCs reside, for instance, by decreasing inflammation by inhibiting pro-inflammatory molecules, also improves the action of these cells. The attenuation of a TGF-β-mediated signaling excess in the subchondral bone during OA restores the dysfunction of the MSCs, preventing cartilage degeneration [77]. Liu et al. observed that intraosseous infiltrations of PRP promoted MSC cell proliferation and osteogenesis in an in vivo study, whereas adipogenesis, senescence, and oxidative stress were decreased [78].

4.2 Clinical translation

The transfer of the PRP from the laboratory to the clinical application has been very fast with extensive worldwide expansion. This has occurred in part because of its ease of obtaining and its high safety as an autologous product, and, as a consequence, more and more clinical studies are being published on the use of PRP in OA. It is not the intention of this chapter to analyze all these studies but to highlight the most relevant aspects of this translation. The latest published meta-analyses concluded that the use of intra-articular PRP infiltrations achieves effects on symptoms such as pain relief or improved function better than the use of hyaluronic acid or placebo especially for the long term [79–81]. Based on these data, it could be accepted that the PRP has evolved from being a promising alternative to a real option for clinicians and patients.

However, it should not be forgotten that it is necessary to continue to carry out high-quality clinical studies to clarify possible doubts and achieve the ideal

protocol for both obtaining and applying PRP products [82]. Some of the clinical studies carried out have attempted to elucidate this type of questions, the presence of leukocytes being one of the most critical issues. Several authors have studied the clinical effect of including leukocytes in the PRP. Mariani et al. studied the pro-inflammatory effect that intra-articular infiltrations of Leukocyte-Rich PRP could have. Surprisingly, and contrary to the in vitro studies, patients who received this treatment did not experience an increase of pro-inflammatory molecules in the synovial fluid or plasma in the short or long term [83]. These data were confirmed in a meta-analysis where there were no differences in adverse reactions between PRP with and without leukocytes, being very rare and local such as pain and inflammation. However, as far as efficacy is concerned, this same work carried out by Riboh et al. showed that PRP poor in leukocytes had significantly better results than those obtained by hyaluronic acid and placebo, whereas this difference did not occur in PRP rich in leukocytes. Therefore, according to the studies carried out in this matter, the inclusion of leukocytes in the PRP does not affect the safety of the product but does diminish its effectiveness in the treatment of knee OA [36]. In spite of these advances, it is necessary to continue studying the rest of the composition variables that may condition the clinical response of the PRP, such as platelet concentration. Recent studies suggest that a concentration below fivefold blood platelet concentration is recommended [84].

Not only the variables related to the obtaining or composition of the PRP products condition the clinical effect of this therapy but also the different methods of application. Several clinical studies addressed the effect of a single or repeated administration of intra-articular infiltrations of PRP. A first group of studies focused on analyzing the differences between a single infiltration of PRP and several repeated infiltrations every 1 or 2 weeks. These studies demonstrated that PRP obtained better results than control treatment and, in addition, patients who received repeated intra-articular infiltrations of PRP achieved better clinical response on items such as pain, symptomatology, and function [85–87]. Other studies analyzed the effect of applying several cycles of PRP infiltrations, referring to a cycle as a series of repeated infiltrations in a short period of time. Gobbi et al. compared the efficacy of administration of one PRP cycle against two PRP cycles separated by 1 year, one cycle being three intra-articular infiltrations of PRP in 1 month. In both groups, there was an improvement in patients 1 year after the first cycle, which was accentuated at 18 months after the application of a second cycle [88]. Vaquerizo et al. conducted a similar study comparing patients treated with one PRP cycle with patients treated with two PRP cycles separated by 6 months, one cycle being three weekly intra-articular PRP infiltrations. The results showed that although there were no significant differences in pain improvement, patients who received two cycles had better symptoms and functionality 1 year after treatment [89]. Thus, the different studies that analyze this variable recommend the application of repeated PRP injections instead of isolated ones.

Following with the PRP administration modality, it is important to remember that the mechanism of action of PRP biomolecules is cell stimulation and improvement of the biological environment to favor tissue repair. Furthermore, as explained at the beginning of this chapter, OA is an alteration of the whole joint and not just a few elements. Considering these two assumptions, it would be advisable to act on the majority of the tissues involved in the joint and especially on those that perform a more predominant biological function. When PRP is intra-articularly administered, it soaked the articular space, reaching and acting on the cells present both in the synovial membrane and on the articular surface. However, this route of administration does not reach the subchondral bone which communicates with the cartilage, especially in OA case, and it is fundamental both in the maintenance

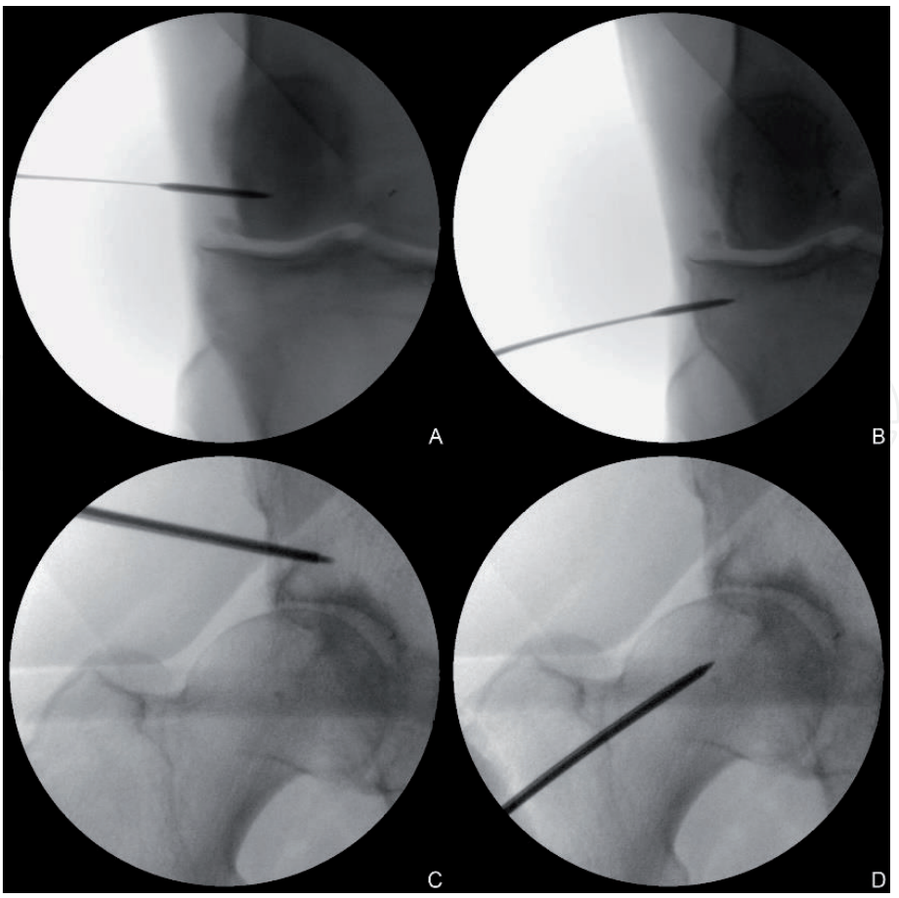

Figure 5.
Intraosseous administration of PRP. Intraosseous PRP administrations allow the subchondral bone to be reached and its therapeutic effect to be extended. Intraosseous infiltrations are applied in the femoral condyle (A) and tibial plateau (B) in patients with knee OA and in the acetabulum (C) and femoral head (D) in cases of hip OA.

of homeostasis and in the pathophysiology of joint degeneration [17]. In order to extend the range of action of the PRP and also act on the subchondral bone, Sánchez et al. described the technique of PRP intraosseous infiltrations (**Figure 5**). This method of application combines conventional intra-articular injection of PRP with intraosseous infiltrations into the subchondral bone of the femoral condyle and tibial plateau in severe cases of knee OA [90]. Afterward, this technique was adapted to treat advanced cases of hip OA, combining intra-articular infiltration with intraosseous infiltrations into the femoral head and acetabulum [91]. In both cases, intraosseous administration must be assisted by imaging, ultrasound, or fluoroscopy, to ensure correct delivery in the required area.

The first published works carried out using this technique provided promising results. In a pilot study performed with patients who presented knee OA of grades 3 and 4 according to the Ahlbäck scale, pain was significantly reduced, and an increase in joint function was observed at 6 months after receiving the combination of intra-articular and intraosseous PRP injections. In addition, the number of MSCs present in the synovial fluid decreased after this treatment [92]. This finding was not observed in patients treated only with intra-articular infiltrations, suggesting the importance of the subchondral bone in the modulation of cellular response in joint degeneration [74]. Following the same trend, an observational study compared the intra-articular administration of PRP versus the combination of intra-articular and intraosseous injections in patients with severe knee OA. The results of this study showed that although there was no difference between both groups at 2 months after treatment, patients who received the PRP intraosseously showed clinically superior results at 6 and 12 months [93]. Su et al. conducted a

clinical trial in which, in addition to comparing intra-articular against intraosseous injections, they used hyaluronic acid as a control treatment. The patients enrolled in this study presented knee OA of grades 2 and 3 according to the Kellgren-Lawrence scale. The results achieved with treatment based on intraosseous infiltrations of PRP were superior to those obtained with both intra-articular PRP and hyaluronic acid [94]. No severe adverse effects were reported in any of these studies, and they were limited to pain after infiltrations. One of the characteristics of the subchondral bone in patients with knee OA is the presence of fibroneurovascular proliferation. Although the PRP contains proangiogenic and profibrotic molecules, no basic or clinical study showed the uncontrolled induction of this effect after the application of intraosseous PRP [22].

Finally, intra-articular injections of MSCs derived from various sources associated with PRP were analyzed in some studies. The vehiculization of MSCs in PRP could entail an improvement in cell viability and may be translated into better clinical results. Although studies performed with both bone marrow [95, 96]- and stroma fraction [97, 98]-derived MSCs showed improvement in these patients after the application of this therapeutic combination, the association of PRP with the MSCs did not lead to a greater clinical improvement in patients. However, the therapeutic potential of the synergy of both therapies justifies further research in this field.

5. Conclusions

Joint degeneration is a pathology that affects a large part of the population, deteriorating their quality of life being disabling in many cases. It is also related to aging and unhealthy lifestyle habits; thus it is expected that its prevalence will increase in the coming years, assuming a great cost to health systems. Current conventional treatments focus on symptomatic relief without addressing the cause of the disease. Because of this, new treatments based on regenerative medicine are emerging in order to expand the therapeutic arsenal and delay or prevent joint replacement, which is currently the only definitive solution for patients. Moreover, in order to achieve an optimal treatment for joint degeneration, it must be understood that the joint works as a whole organ. All elements of the joint participate in the maintenance of homeostasis, the synovial membrane, cartilage, and subchondral bone being key for biological balance.

This balance could be maintained or restored by means of several biological therapies such as PRP that is a cocktail of plasma and platelet biomolecules, and it is obtained after fractionating small blood volumes by centrifugation. PRP has a great versatility since it allows its use through different types of formulations, being able to be applied both in outpatient infiltrations and surgical interventions. The therapeutic potential of PRP in joint degeneration lies in its ability to modulate inflammation, lubrication, and pain, acting on different cell populations to create a biological environment conducive to tissue repair. However, the variety in the composition of PRP products leads to different biological effects and consequently contradictory clinical results. It is, therefore, necessary to identify and characterize the PRP used in order to advance both research and clinical practice.

The success of the PRP also depends on the method of clinical application. The administration of PRP has to cover the main joint tissues so that the biological effects of PRP act over the cells of in order to reverse the course of the pathology. Although the safety and ease of obtaining PRP have allowed a quick transfer from the laboratory to the hospital, much is still unknown about this therapy, and further basic and clinical research is needed.

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