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REVIEW

A new strategy to tackle severe knee osteoarthritis: Combination of intra-articular and intraosseous injections of Platelet Rich Plasma

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ABSTRACT

Introduction: Knee osteoarthritis (KOA) is a mechanically induced, cytokine and enzyme-mediated disorder involving all the joint tissue of the knee. Rebuilding a physiological-homeostatic network at the tissue level following knee organ failure, such as in severe KOA, is a daunting task with therapeutic targets encompassing the articular cartilage, synovium and subchondral bone. Intraarticular infiltration of plasma rich in growth factors (PRP) has emerged as a promising symptomatic approach, although it is insufficient to reach the subchondral bone.

Areas covered: This review addresses current molecular and cellular data in joint homeostasis and osteoarthritis pathophysiology. In particular, it focuses on changes that subchondral bone undergoes in knee osteoarthritis and evaluates recent observations on the crosstalk among articular cartilage, subchondral bone and synovial membrane. In addition, we review some mechanistic aspects that have been proposed and provide the rationale for using PRP intraosseously in KOA.

Expert opinion: The knee joint is a paradigm of autonomy and connectedness of its anatomical structures and tissues from which it is made. We propose an innovative approach to the treatment of severe knee osteoarthritis consisting of a combination of intraarticular and intraosseous infiltrations of PRP, which might offer a new therapeutic tool in KOA therapy.

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1. Introduction

Knee osteoarthritis (KOA) is a mechanically induced, cytokine and enzyme-mediated disorder with different biochemical, inflammatory, and genetic signatures undergoing distinct phases and phenotypes, and encompassing all joint tissues, with pain and inflammation as the clinical and biochemical hallmarks of the disease.[1–3] This complex mechanical organ includes articular cartilage (AC), an avascular hydrated tissue functionally sandwiched between two highly innervated and vascularized tissues, namely, synovial membrane (SM), which produces synovial fluid (SF), and subchondral bone (SB), ligaments, capsule and periarticular muscles (PM).[4] Intraarticular joint tissues are endowed with very distinct load-bearing cellular responses, which are responsible for the organization of their specific extracellular matrix (ECM), which account for the bulk mechanical properties of the tissues in order to transfer, absorb and dissipate the mechanical forces among them in a frictionless and pain-free movement.[4,5]

Subchondral bone has always been present in the equation of the cartilage repair process and osteoarthritis (OA) [6–8] but it has suffered neglect for decades as an important player in the etiopathogenesis of OA.[8,9] There is an increasingly recognized communication between the subchondral bone and articular cartilage based on the changes that the subchondral bone undergoes in patients with severe OA, including microcracks and structural defects, vascularization of channels, nerve growth and a progressive replacement of the subchondral marrow with fibroneurovascular mesenchymal tissue.[10–12] As it is yet to be established precisely which of the joint tissues or structures is the primary driver of KOA, and therapeutic strategies targeting solely one cell or tissue target may well prove to fail [13], it is advisable that approaches to KOA treatment should be aimed at reaching several joint tissues with the objective of reducing joint inflammation, controlling pain, improving joint functionality and restoring the homeostasis of joint tissues.

A biologically inspired therapeutic approach consisting in intraarticular infiltrations of PRP has proven to substantially reduce pain in patients with KOA [14,15] and to improve joint stiffness and physical function.[16] Unlike a single growth-factor-delivered therapeutic strategy in a bolus manner, PRP conveys many bioactive mediators within an autologous fibrin network released gradually, which have been shown to exert

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Article highlights.

- Knee osteoarthritis is a mechanically induced, cytokine- and enzyme-mediated cluster of disorders affecting the whole joint.
- There is an intense molecular and cellular crosstalk among AC, SB, and SM in KOA, which establishes a catabolic loop.
- Any attempt to treat KOA should address the articular cartilage, the synovial membrane, the synovial fluid and subchondral bone as therapeutic targets.
- Platelet rich plasma is a multimolecular and safe therapy, and its clinical benefits might be attributed to trophic-anabolic, antiinflammatory and analgesic effects.
- Intraosseous infiltrations of PRP modulate SB homeostasis by antioxidative stress protection, adipogenesis suppression and improvement in bone mineralization effect.
- The combination of intraarticular and intraosseous injections of PRP might offer a new therapeutic tool to address the knee joint pathology as a whole, by reaching the SM, SF and superficial zone of AC by intraarticular injections, and the deep zones of AC, and SB through PRP intraosseous infiltrations.

This box summarizes key points contained in the article.

positive effects on reestablishing homeostasis of joint tissues through a breadth of actions such as antiinflammatory, immunomodulatory and antioxidative effects [17–24], an analgesic effect [14–16,25], and finally chondroprotective and anabolic-trophic effects.[26–29]

This review will explore some of the recent insights and observations concerning the involvement of subchondral bone in the pathophysiology of osteoarthritis and additionally will highlights the increasing understanding of knee joint homeostasis and the role that PRP therapy could play in the disease-modifying osteoarthritis treatment of the knee.

2. Joint tissue responses to mechanical stimuli: homeostasis, adaptation and inflammation

2.1. Joint homeostasis and mechanical stress

At a biomechanical level, knee components work as a network from which the joint´s functional property as an organ emerges, a property known as dynamic stability, whose equivalent at the tissular and cellular level is termed tissue and cell homeostasis. Such identities do not imply biological constancy but rather dynamic adaptability.[30] The phenotype of chondrocytes, synoviocytes, and osteoblasts is constantly adapting to its dependence on the biochemical, biophysical and mechanical loading features of their microenvironment. [31–34] Signals and ligands from extracellular matrix (ECM) drive cell responses and tightly fine tune the anabolic/catabolic balance in order to maintain or to adapt their ECM composition to the ongoing mechanical challenges,[31] thereby protecting against the deleterious effect of some supraphysiological stimuli.[35] Abnormal mechanical stress and/or biochemical mediators variously stemming from trauma, obesity, lesion or disfunction of knee components, as well as metabolic diseases break knee dynamic stability and trigger biological responses that disrupt the homeostasis of cells and tissues of the joint in a locally, sustained, low-grade inflammatory fashion leading to a matrix degradation (Figure 1).[2,36,37]

In the wake of this sterile matrix degradation of articular cartilage, there is a depletion of aggrecans and cleavage of collagen II, which leads to the erosion of cartilage, subsequently altering the nanostiffness of articular cartilage and weakening its load-bearing capacity.[4,38] Besides the release of matrix-degrading products, the ECM degradation deeply impacts the micromechanical environment of chondrocytes and changes the magnitude of dynamic compressive forces transferred from them to the underlying bone, and these aberrant new sustained (chronic) abnormal forces prompt chondrocytes and osteoblasts to respond with a pro-inflammatory gene expression through activation of the NFkB signaling pathway [32,43] and increased osteoclastogenesis, thereby increasing bone resorption and sclerosis [34,44] respectively. Nevertheless, evidence is accumulating about how alterations of subchondral bone induced by mechanical or vascular stresses might be the start point in the catabolic loop of AC degradation and extend to SM (Figure 1).[1,7,45,46] Cartilage is an avascular tissue whose cells rely on synovial fluid and subchondral plate to obtain oxygen and a supply of nutrients, having the subchondral bone account source for at least 50% of articular cartilage requirements in oxygen and glucose.[46,47]

Therefore, despite the fact that tracking down the 'first pathogenic event' responsible for the initiation of KOA still proves an elusive quest, any induced mechanical or metabolic damage to joint tissues in combination with predetermined influences such as genetic, obesity and aging, paves the way to initiating a harmful joint environment involving AC, SM and SCB, and then it is difficult to establish who was first.[8]

2.2. Synovial membrane and subchondral bone in cartilage homeostasis

In recent years, a great deal of evidence has been accumulating in favour of seeing as decisive, the contribution of synovitis and subchondral bone on articular cartilage degradation, and on the progression of OA, where AC may after all be the victim, and not the culprit of catabolic inflammatory cytokines stemming from synovial membrane and subchondral bone, and triggered by abnormal mechanical stresses.[3,4,41,42,48] Hence, cartilage integrity is highly dependent on the underlying subchondral bed and vice versa, as well as on a healthy synovium and its product the synovial fluid.[7,49]

Evidence in basic science, preclinical and clinical settings has been mounting for the role of synovium inflammation in the pathogenesis and progression of OA.[2,3] Matrix-degradation products such as fibronectin, tenascin C, high-mobility group protein B1 (HMGB1) and low molecular-weight hyaluronic acid (LWHA) among others in the SF [37,42] can act as Toll-like receptor (TLR) ligands or damage-associated molecular patterns (DAMPs) and activate TLR-2 and TLR-4 of synovial macrophages and fibroblasts, chondrocytes and osteoblasts, leading to the activation of the intracellular signaling pathway nuclear factor kappa B (NFkB) (Figure 1).[3,50] The activation of the NFkB signaling pathway mediates the expression of several inflammatory genes and the synthesis of interleukin 1beta (IL-1B), interleukin 6 (IL-6), interleukin 10 (IL-10), nitric oxide (NO), prostaglandine E2 (PGE2), tumor necrosis factor alpha

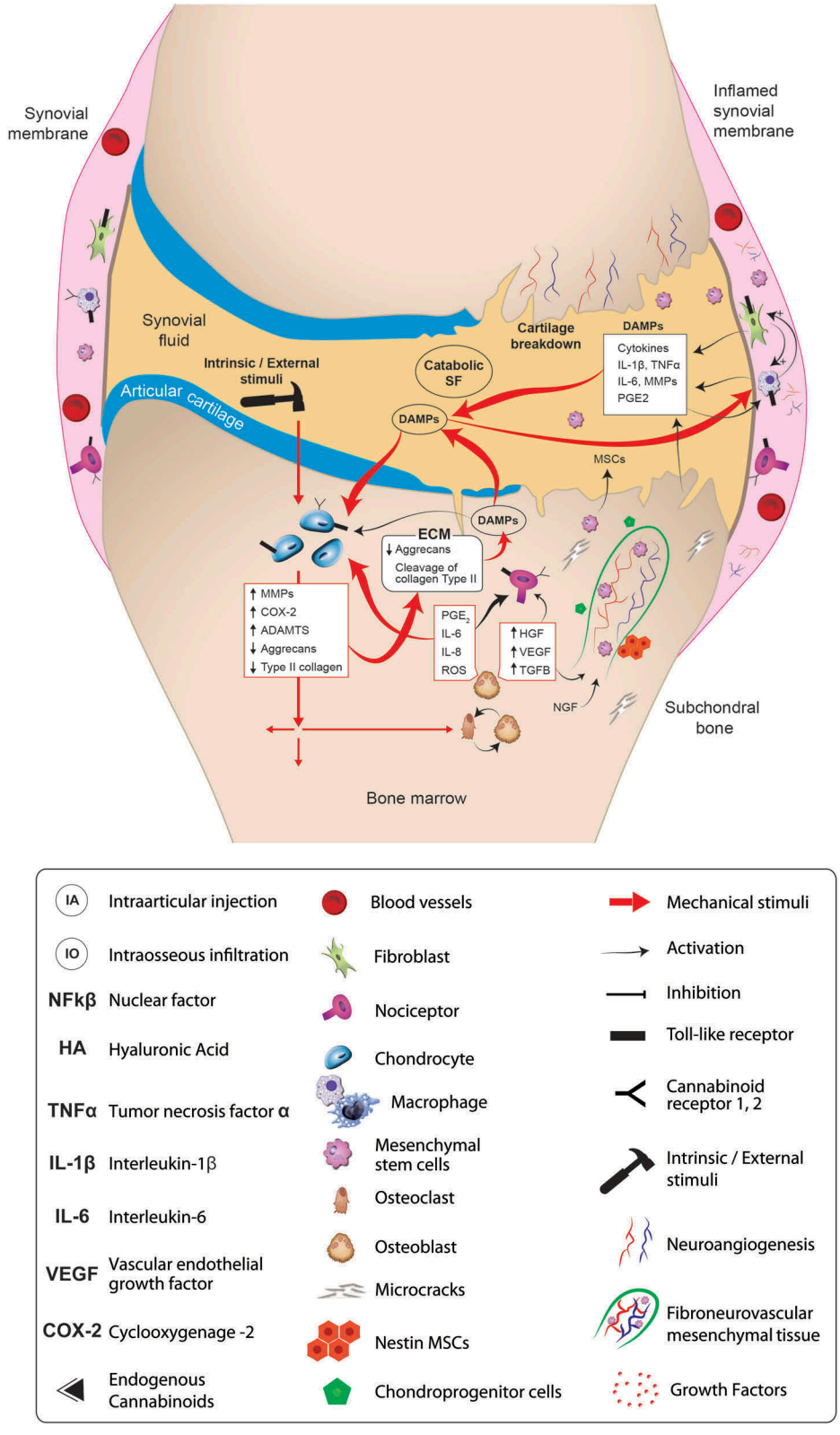

Figure 1. Abnormal distribution of mechanical loading across joint cartilage breaks the homeostasis of articular cartilage and provokes adaptive or catabolic cell responses, which leads to an increased synthesis of matrix metalloproteinases (MMPs) and aggrecanases (ADAMTS), expression of proinflammatory cytokines and mediators such as interleukin-1B (IL-1B) and cyclooxygenase-2 (COX-2), high levels of reactive oxygen species (ROS), disruption of water tissue distribution and matrix fragments.[4,38–40] Proinflammatory cytokines involved in OA, such as IL-1B and TNF-a are major players in the destruction of AC by inhibiting the synthesis of aggrecans and collagen type II while at the same time stimulating the synthesis of MMPs in chondrocytes.[41] It has been reported that activation of TLRs of synovial macrophages and fibroblasts, and monocytes by DAMPs present in an inflammatory SF, is an important pathway in promoting synovitis in OA through the NFkB pathway [3], cells that respond with the production of MMP-1, MMP-3, and MMP13, IL-1B, TNFa and IL-6 among other catabolic mediators, promoting synovitis in OA.[3,41,42]

(TNF-a), interferon gama (IFN-j) and nerve growth factor (NGF) among other inflammatory cytokines (Figure 1).[3,39,41,50,51] Moreover, NFkB transcription factor has been postulated as a functional connection among the mechanobiological, developmental programming and stress-inflammatory responses of AC, SM and SB, making the NFkB signaling pathway a potential multi-faceted target in OA disease.[13,32,50] Another pathway involved in OA synovitis is the activation of complement as it has been shown by Wang et al. [52] who reported that the expression and activation of complement is abnormally high in the human OA joint, where the presence of some products of dysregulated cartilage remodeling such as fibromodulin, cartilage oligomeric matrix protein (COMP), and osteoadherin in synovial fluid and membranes might account for this activation.[3]

Important clinical features of the inflamed synovium (synovitis) are pain, swelling and stiffness,[42] whereas histopathological changes are characterized by an uneven, abnormal cell infiltration and an aberrant proliferation of macrophages, fibroblasts, and blood and lymphatic endothelial cells that lead to a neofibroangiogenesis.[42] SM and SB are highly vascularized and innervated tissues endowed with heat receptors, chemoreceptors and mechanoreceptors from where nociceptive stimuli, coming from a microenvironment undergoing non-physiological mechanical loading and/or pro-inflammatory cytokines and damage-associated molecular patterns (DAMPs), might initially lead to peripheral and eventually both peripheral and neuropathic pain by mechanisms yet to be fully identified. [3,53] In addition, proinflammatory cytokines may contribute to pain by stimulating hyperalgesia and sensitizing joint nociceptors to other stimuli [3,42] thereby perpetuating a catabolic vicious circle among SM, AC and SB.

2.3. Joint inflammation and mesenchymal stem cells

Aggression and inflammation to AC, SM, menisci and ligaments has been reported to bring about an increase of MSCs in SF,[54,55] which is commonly interpreted as a tissue response to injury [56,57] equivalent to the response of migratory chondrogenic progenitor cells from SB to injured cartilage.[58,59] Moreover, several studies have reported that the accumulation of SF MSCs increases with the severity of osteoarthritis, joint damage and the disease duration. [55,60,61] Healthy human and osteoarthritic cartilage and SF contain a population of cells with characteristics of mesenchymal progenitor cells [56,62] with migratory and chondrogenic potential.[56,58] According to these observations, endogenous mesenchymal stem cells have been postulated as a reservoir of repair cells and immunomodulatory drugstore cells to dampen inflammation.[63] Although the source of MSC increase has yet to be determined, the most likely origin may be the SM, [55,56] the breakdown zone of superficial AC,[62] and the SB.[10,12,58,59] However, the SB origin of SF MSCs is less likely to occur for as some authors have suggested, the marrow of patients with severe OA is almost depleted in MSCs and the remaining MSCs are functionally deficient.[60]

Bone, like cartilage, responds to mechanical stress in an intensity-dependent manner and a tight regulation between the sequential processes of deposition and resorption at the same site. These processes are carried out by the coupling of osteoblast and osteoclast-metabolic activities [43] and unlike cartilage, when damaged regenerates spontaneously due mainly to its high elevated vascular and cellular network. Evidence is accumulating not only about the involvement of bone, and more particularly SB in the development and progression in OA but also about how these SB changes might even precede changes in AC of OA joints.[7,8,12,33,64]

3. The role of SB in pathophysiology and clinical symptoms of osteoarthritis

3.1. The subchondral bone-articular cartilage functional unit

Subchondral bone has always been present in the equation of OA pathogenesis and more than 40 years ago, partially inspired by the 1827 proposal by surgeon Dr. P.P. Physick on the SB as an effective shock absorber. Radin et al. [7,65] suggested a cause-effect connection among mechanical loading, subchondral bone sclerosis and osteoarthritis. Subchondral bone is the layer of bone which lies immediately below the calcified cartilage (Figure 2),[66] and consists of two different anatomical entities, one called subchondral or cortical plate which is nonporous and poorly vascularized cortical bone, and the SB which contains bone marrow (fatty) and trabecular bone.[47,67] Together with the AC, it forms the osteochondral functional unit, which undergoes mechanical stresses that trigger adaptive cell responses and establish a crosstalk among them to adjust their architecture to ongoing physical and biochemical challenges.[12,68] In the functionality of the osteochondral unit, articular cartilage provides an elastic, gliding, smooth frictionless surface, while subchondral bone, a very low viscoelastic structure, together with periarticular muscles and ligaments, acts as shock absorber structures, accounting for 30 and 50% of the total absorbing energy and only 1–3% for the AC.[4,47] Besides the pivotal shock absorbing function, SB is a source of vessels whose perfusion rate enables an important nutritional route for AC but any damage to this microvasculature affects venous bony circulation thereby altering cartilage and chondrocyte function.[10,46,47]

3.2. SB turnover and structural changes in OA

The osteochondral unit in an OA joint undergoes several structural changes including loss of articular cartilage, development of inflamed synovium, calcified cartilage thickening and tidemark duplication, undermineralization of bone, sclerosis and stiffness of SB, bone marrow lesions (BMLs), cysts, osteophyte, and a localized bone marrow replacement by fibroneurovascular tissue.[10–12,33]

Despite the high turnover of SB in OA, an uncoupling between bone formation and resorption at the same site leads to an increase in bone volume without a concomitant increase in bone mineralization pattern.[8,33,43] This SCB sclerosis is characterized by an increase of the osteoid volume and a decrease of calcium bind to collagen fiber, and is associated with a gain of trabecular thickness, loss of trabecular number, and a trabecular

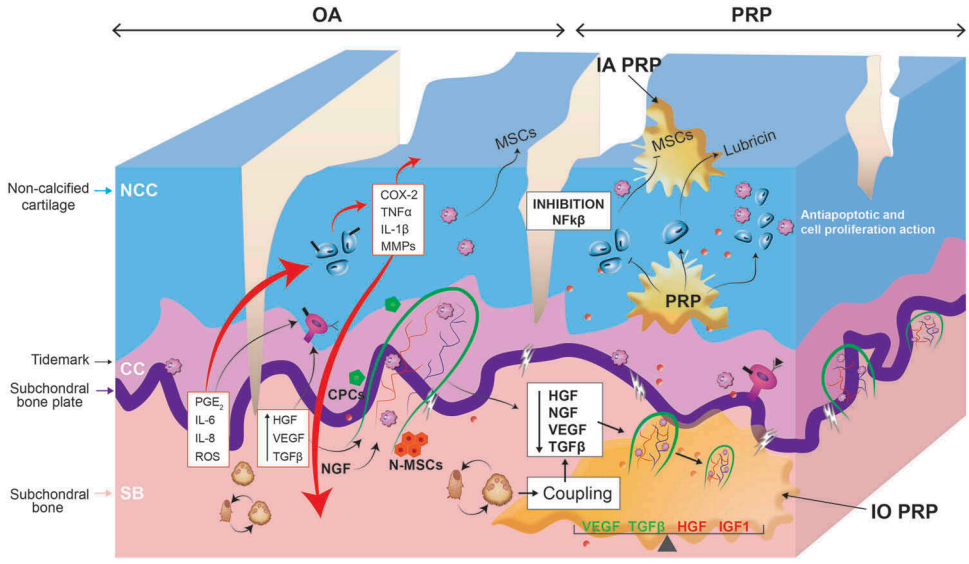

Figure 2. SB. Targeting the osteoarthritic subchondral bone with Intraosseous infiltration of PRP. This schematic drawing illustrates the outside-in (AC-SB) and inside-out (SB-AC) flow of mediators and cells. SB as a point of egress of morphogens and cells, through the channels and vessels breaching the osteochondral junction, partially recruited by the osteoarthritic synovial fluid.[8,12] This cartilage cell invasion might be facilitated by the loss of aggrecans, collagen II cleavage, and disruption of water tissue distribution [38] of the articular cartilage as well as by the secretion by MSCs of fibrinolytic enzymes.[66] The excessive presence of TGFB1 and VEGF in OA subchondral bone [8,12,69] could be a driving factor for changes in osteoblast-osteoclast coupling thereby leading to a bone remodeling imbalance.[8,64,70] NGF expression,[71] and fibroneurovascular growth changes that additionally might well contribute to overlying cartilage degradation, [64,69] pain [12,67,68] and an osteoarthritic joint.[64,69]

network more separated and less interconnected.[43,64] It has been suggested that sclerotic subchondral bone, localized at subchondral plate, could decrease the load transfer to the underlying bone tissue leading to osteoporotic-like changes.[10] Moreover, SB can undergo microdamage, such as microcracks and clefts, that modify SB stiffness and reduce the shock-absorbing capacity of SB, thereby chronifying a microdamage context and perpetuating an accelerated bone remodeling, which impairs normal mineralization of bone once it has been deposited, most likely by a modified osteoblastic phenotype.[10,67,72] Magnetic resonance imaging (MRI) has helped to detect subchondral bone marrow edema-like lesions (BMLs), which have been found to be associated with pain and disease progression in KOA,[73] and together with bone attrition, are strong indicators of a structural deterioration in KOA.[10] Several studies conducted in human knee and hip OA paralleling MRI bone marrow edema lesion studies with histological analysis of SB retrieved at the time of joint replacement, revealed microfractures and increased bone remodeling, subchondral ingrowth of fibrovascular tissue and increased vascularity, as well as various types of bone marrow fibrosis.[73–75] These observations were confirmed in rodent models of OA.[12,76] The increased activity of osteoclasts in OA cause channels to extend from SB to AC, passing across the calcified tissues into the noncalcified articular cartilage.[68] The neurovascular invasion of those newly formed channels is accompanied by a new fibroneurovascular mesenchymal tissue within the channel along with cells such as macrophages, osteoclasts, osteoblasts and endothelial cells, which interact to stimulate angiogenesis and growth of sympathetic and sensory nerves [12] and reach the noncalcified cartilage, a finding which has been supported by animal models of OA (Figure 1).[12]

3.3. Cellular interactions and molecular crosstalk in osteochondral unit in OA

There is now good evidence that even in a non-diseased joint, naturally occurring pores and holes enable communication between SB and AC through diffusion of small molecules. [11,70,77] This communication may be exacerbated by structural changes seen early in the osteochondral unit in OA. The increased osteoclastic activity in the OA subchondral plate [33] may increase the permeability of bone–cartilage interface by inducing channel formation in the tidemark, in addition to the existent aberrant fibroneurovascular tissue and vasculature, and mechanical stress-induced microcracks.[12,67,78] Reinforcing this view, Pan et al. [77] have demonstrated the diffusion of small-size molecules between SB and AC by utilizing the FLIP method (Imaging method based on fluorescence loss, which quantifies diffusivity of small molecules) with sodium fluorescein in the distal femur of mice, and this communication is greatly increased in osteoarthritic joints of the mice model.[11] Therefore, the presence of these connections enables an elevated crosstalk among chondrocytes, osteoblasts, osteoclasts and MSCs through biological factors and signaling pathways.

Several *in vitro* and *in vivo* studies have demonstrated that osteoblasts from sclerotic subchondral bone show an altered phenotype. In an *in vitro* study, Westacott et al. [79] reported that osteoblasts in OA-affected bone exhibited a different phenotype, whose activity can degrade articular cartilage *in vitro*. Supporting this observation,[80] Hilial et al. reported that osteoblasts from OA subchondral bone have an abnormal metabolism with increased levels of PGE2 and TGFβ (Figure 1 and 2). Using a co-culture model of OA subchondral bone osteoblasts with

chondrocytes, Sanchez et al. reported that osteoblasts induced a catabolic response of chondrocytes including a decrease in aggrecan, type II collagen and SOX-9, and an increase of MMP-3 and MMP-13 among other mediators.[81,82] Moreover, osteoblasts from scletoric subcondral bone have an elevated TGFβ expression [43] and under cyclical compression express proangiogenic factors such as VEGF, FGF and IL-8.[34] Hepatocyte growth factor (HGF) is a pleiotropic morphogen present in articular cartilage but produced by osteoarthritic subchondral bone osteoblats, osteoclasts and MSCs,[69,83,84] with likely implications in both the chondrocyte anabolic state and the proliferation of an invasive fibroneurovascular tissue in SB,[10,12,69] the latter when an uncoupling osteoclast–osteoblast activity may lead to an overexpression of HGF (Figure 1 and 2).[83] The excessive presence of TGFB1 and VEGF in OA subchondral bone [12,71] could be a driving factor for changes in osteoblast–osteoclast coupling thereby leading to a bone remodeling imbalance,[10,64] NGF expression,[85] and fibroneurovascular growth changes that additionally might well contribute to overlying cartilage degradation,[64,71] pain [12,67,68] and an osteoarthritic joint.[64,71] In a recent study, Zhen et al. showed that by inhibiting TGF-β signaling in a specific population of MSCs present at the SB (Nestin positive MSCs), the severity of OA was reduced, a change associated with improvement of bone parameters, cartilage structure and joint function without affecting TGFB signaling in AC.[71] In fact, previous studies have shown that the decrease of MSCs in the synovial fluid, in low degree OA, suggests clinical improvement.[55] MSCs from osteoarthritic bone marrow have been reported to be substantially reduced in yield and proliferative activity besides showing a weakened chondrogenic and adipogenic activity and increased osteogenic activity.[60] However, *in vitro* studies indicate that the inclusion of growth factors, as a supplementary culture medium, can be beneficial in reverting their chondrogenic activity.[86]

4. Plasma rich in growth factors as an effective and safe therapeutic approach to treat OA

4.1. PRP as an emergent and promising knee osteoarthritis treatment

Despite important advances made in the development of treatments to reduce pain and inflammation, and in spite of endeavors to develop an efficacious and early disease and structure-modifying therapeutic intervention, the path to osteoarthritis treatment remains elusive. Among the emerging biologic interventions to target the clinical and biochemical hallmarks of OA, namely joint pain and inflammation, platelet rich plasma stands out.[87]

4.2. Platelet-rich plasma preparation and content

4.2.1. What is platelet-rich plasma?

Drawing on the regenerative potential of platelets, plasma biomolecules and fibrin matrix,[88] a plethora of systems to produce PRPs have been developed to enhance the natural regenerative capacity of damaged tissues.[89,90] Platelet rich plasma is an autologous platelet concentrate within a plasma suspension whose cell and plasma composition are determined by the method used to obtain it. PRP products include plasma and twofold or more increases in platelet concentrations above peripheral blood levels and the concentration of leukocytes and erythrocytes varies widely, from a complete absence to a high concentration of them.[89] There is a wide range of PRP products obtained by different blood-spinning preparation protocols (number of centrifugations and centrifugation speeds and time, the type of anticoagulant and platelet activation methods),[89,91,92] and consequently, the different biological effects that necessarily result, produce very distinct clinical outcomes, which produce a confusing picture of efficacy.

4.2.2. Plasma rich in growth factors (PRGF) preparation

PRGF, one of the multiple autologous platelets- and plasma-derived products, which is included in PRPs, is produced as follows. Briefly, peripheral venous blood from the patient is withdrawn into 9 ml tubes containing 3.8% (wt/vol) sodium citrate as anticoagulant. Blood is centrifuged at 580 *g* for 8 min at room temperature. The 2 ml plasma fraction located just above the sedimented red blood cells is collected in a tube without aspirating the buffy coat (F2). The remaining upper volume of plasma is deposited in another tube (F1). The activation of PRGF is carried out by adding calcium chloride (10% wt/vol).[14] Additionally, PRPs can be manufactured by using other standardized or commercial systems whose protocol heavily influences the composition of the final product (platelet concentration, the presence of leukocytes and erythrocytes, the level of platelets activation).[89,93]

4.3. Platelet-rich plasma rationale

Plasma rich in growth factors (PRP) consists of a pool of autologous growth factors (GFs) and other bioactive mediators stemmed from platelets and plasma. Once PRP is activated, plasma fibrinogen polymerizes into a three-dimensional transient fibrin scaffold, which contains heparan sulfate binding domains for growth factors (PDGF, FGF, HGF, BDGF, VEGF, IGF and TGF-B), cytokines (TNF-a, IL-2,3,4,5), chemokines (PF4), ECM components (Fibronectin, thrombospondin and tenascin), cell adhesion (L-selectin and N-CAM), acute phase proteins and proteins related to lipid metabolism.[94,95] By sequestering several growth factors, microparticles, and other biomolecules released from the degranulation of platelets and plasma [95–97] this biocompatible and biodegradable scaffold provides plastic-elastic stiffness and generates growth factor gradients that are essential cues for cell proliferation, differentiation, migration and correct orientation in the nascent tissue.[98] Once infiltrated into the joint and subchondral bone, this liquid-to-gel 3D injectable scaffold is converted into a matrix-like viscous and malleable structure, which adheres to SM, AC and SB and covers them (Sanchez et al. 2014; Figure 3).[99] When fibrinolysis begins, a gradual, sustained release of GFs and other biomolecules occurs, in contrast to a bolus delivery modality.[96,100] Such a gradual yet sustained release of GFs influence on cells, mimics the biological repair process,[96,97,100] which is the topic of a review published in this journal.[101]

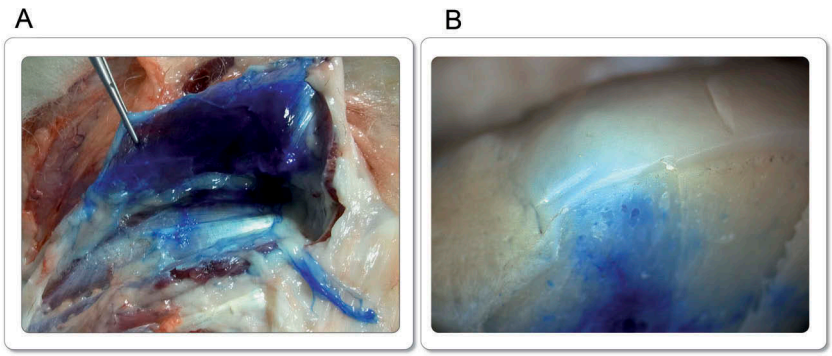

Figure 3. Infiltration of activated PRP previously stained with methylene blue was performed in sheep`s joint to ascertain its diffusion across the joint. Once the animals were put down and the joint opened, we infiltrated the femoral condyle as well and took these picture in which the PRP liquid-to-gel ·3D injectable scaffold was converted into a matrix-like viscous and malleable structure, which adhered to synovium (A) and covered it as it diffused across the condyle (B) (figure 2 unpublished data).

4.4. Some pitfalls in the application of PRPs on tissue repair

Despite the care and seriousness with which medical staff may elaborate and apply PRPs in different medical fields, the poor standardization, which mainly pivots around the controversial presence/absence of leukocytes, the modalities of application and the donor-related variabilities, are three elements that contribute to drawing misleading conclusions about the clinical efficacy of PRPs.[90,93,102] In a sterile inflammatory repair scenario such as musculoskeletal injuries including KOA, leukocytes may aggravate tissue damage and promote a proinflammatory microenvironment by releasing TNF-α, IL-6, IFN-γ cytokines, which then induce the over-expression of MMPs, elastase and cathepsin G, as well as reactive oxygen species among others, thereby breaking down the ECM and exacerbating the original lesion.[40,103] Several research groups have highlighted the detrimental effect that the presence of leukocytes within PRP may exert on synoviocytes, chondrocytes, human subchondral MSCs [93,104–106] as well as on clinical symptoms.[15] With regard to the modality of application, PRP cannot be considered a magic bullet applied as a kind of single scatter shot. Rather, a biological approach is most productive with distributed infiltrations: infiltrating several times and including healthy peripheral tissue which surrounds the injury, with the aim of recruiting, activating and mobilizing resident MSCs and influencing macrophages and endothelial cells as well. Finally, the Spanish Agency of Medical Devices (AEMPS) has defined PRP as a human-use medicinal product and framed PRP outside the category of advanced-therapy medicinal products. Therefore PRP therapy can be applied intraoperatively and on an outpatient basis.

There is no doubt that the challenges to fulfill the requirements of safety and efficacy are daunting, and these must be demonstrated by further clinical trials. Moreover, the heterogeneity of PRPs is hindering their regulation and several gaps will be filled in the coming years particularly regarding PRPs medical indications.[107] As the body of research about the regenerative effects of PRPs skyrockets, expansion of their applications is inevitable. Several unanswered questions remain, some regarding molecular mechanisms involved in the clinical benefits and others encompassing aspects of dosage, such as how many injections would be ideal, the interval between them and the suitability of combining PRP with stem cells to enhance the healing power of PRPs.

4.5. Inflammation and oxidative stress

In vitro and in vivo studies (Table I) have reported that PRP and GFs within it such as HGF, IGF-1, PDGF and TGFB, and platelet microparticles have proven to exert an immunomodulatory effect and promote an antiinflammatory environment. HGF and platelet microparticles have been reported to polarize macrophages from M1 to M2 phenotype. [20,108,109] IGF-I, PDGF, HG and PRP releasate modify the inflammatory status of chondrocytes by suppressing the NF-kβ signaling pathway [17–19] (Figure 2), which might lead to the decreased presence of IL-β, and TNF-∝ and other pro-inflammatory cytokines in synovial fluid [3,110,111] (Figure 4). Reinforcing this interpretation, Anitua et al. reported that LPS-treated osteoblasts and fibroblasts which had been cultured in the presence of releasates obtained from PRP without leukocytes, showed an increased expression of Ikβ∝, an antiinflammatory protein that anchors the transcription factor NFkβ to the cytoplasma and inhibits its activation, whereas releasates obtained from leukocyte-rich PRP induced a NFkβ activation.[112] In one recent study, Xie et al. [113] reported that PRP attenuated the multiple-cyclic tensile strain mediated MMPs, NO and PGE2 synthesis in chondrocytes, suggesting that PRP may protect chondrocytes from mechanically induced injury. Connective tissue factor (CTGF), one of the most abundant growth factors released by platelet activation [114] was reported to protect chondrocytes from age-related degenerative changes and from cellular stress, the latter mediated through NFkβ.[115] On the other hand, synovial fibroblasts from osteoarthritic patients cultured in 20% PRP supernatant produced a significant amount of HGF, even in the presence of IL-1β, which is known to inhibit the NFkβ on macrophages [20] and to mediate the antiinflammatory effects of PRP on fibroblasts.[57] In a recent work, Assirelli et al. [105] observed that L-PRP (leukocyte-rich PRP)-treated human synoviocytes sustained a long-term upregulation of IL-β, IL-8 and FGF-2, together with a down-regulation of HGF

Table 1. Summary of *in vitro* and *in vivo* effects of platelet-rich plasma and growth factors.

Cell type/animal model	Intervention	Outcome	Reference
Immortalized human chondrocytes	PRP releseate after thrombin and $CaCl_2$ activation and single centrifugation	Reduction of transactivating activity of NFκB, decreased COX-2 and CXCR4 expression	[17]
Human monocytic tumor cell line	PRP releseate after thrombin and $CaCl_2$ activation and single centrifugation	Decreased chemotaxis	[17]
Human osteoarthritic chondrocytes	10% PRP releseate after $CaCl_2$ activation	Decreased IL-1β-related inflammation, inhibition of NFκB activation	[18]
Primary canine chondrocytes	Medium supplemented with HGF and IGF-1	Inhibition of IL-1β-mediated activation of NFκB, decreased apoptosis in chondrocytes	[19]
Mouse bone marrow derived macrophages	Medium supplemented with HGF	Decreased IL-6 production, increased IL-10 production, reduction of transactivating activity of NFκB	[20]
Human osteoarthritic synoviocytes	Autologous conditioned plasma	Decreased TNF-α concentration, decreased MMP-13 expression, increased HAS-2 expression	[21]
Human osteoarthritic chondrocytes	Autologous conditioned plasma	Decreases TNF-α concentration, increased cartilage synthetic activity	[21]
Primary human osteoblast and osteoblast-like cell line	5% and 10% PRP releseate after activation and single centrifugation	Increased antioxidant response element activity, increased Nrf2 accumulation, increases VEGF gene expression	[22]
Human adipose-derived stromal cells	PRP releseate after thrombin activation	Increased cell proliferation, ALP activity and mineralization	[23]
Aged mouse bone marrow stem cells and adipose derived stem cells	PRP activated with bovine thrombin and single centrifugation	Increased cell proliferation, colony formation and osteogenesis, decreased adipogenesis, restoration cell senescence markers, resistance oxidative stress	[24]
Young-senescence-accelerated prone mouse strain (SAMP38) mice	PRP activated with bovine thrombin and single spin; injection into the tibia bone marrow	Delayed mice aging, improved survival and body weight, recovered cellular potential of stem cells	[24]
Human keratinocyte cell line	PRP releseate after freeze-thaw cycle activation and single centrifugation	Increased endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) production	[25]
Mouse model of acute inflammatory pain induced	PRP releseate after freeze-thaw cycle activation and single centrifugation	Reduced nociceptive behavior	[25]
Immortalized human chondrocytes cultured in a collagen scaffold	PRP activated with bovine thrombin and single centrifugation	Decreased IL-1β and TNF-α production, restored collagen type II and chondrogenesis	[26]
Human osteoarthritic chondrocytes	5% PRP releseate after double freeze-thaw cycle activation and single centrifugation	Increased cell proliferation, proteoglycan synthesis, Sox-9 and aggrecan expression, and chondrogenic differentiation proteins production	[27]
Human osteoarthritic synoviocytes	20% PRP and 20% PRP releseate after $CaCl_2$ activation	Increased hyaluronic secretion and HGF production	[28]
Human synoviocytes, chondrocytes and anterior cruciate ligament-derived cells	Autologous conditioned plasma	Increased cell proliferation and superficial zone protein production	[29]
Human subchondral mesenchymal progenitor cells	Different PRP formulations	Modulated chondrogenic differentiation by PRP formulation	[93]
Human type B fibroblast-like synoviocytes	Different PRP formulations	Increased cell death and IL-1 β, IL-6 and TNF-α production by formulations contained leukocytes and red blood cells	[104]
Huma osteoarthritic synovial fibroblasts	Leukocyte-rich PRP	Increased FGF-2, IL-1β and IL-8 production, decreased HGF and TIMP-4 production	[105]
Human osteoarthritic chondrocytes	Different PRP formulations	Stimulated cell proliferation and chondrocyte anabolism by PRP, stimulated catabolic pathway by leukocyte-rich PRP	[106]
Mouse macrophages cell line	Different formulations of human and mouse PRP	Decreased nitric oxide, TNF-α and inducible NO synthase	[108]
Human acute monocytic leukemia THP-1 cells	Platelet-derived micropartilces	Promoted monocytes towards a resident phagocytic phenotype	[109]
Primary human gingival fibroblast and primary human alveolar fibroblast	Leukocyte-rich PRP	Increased NFκB activation, decreased cell proliferation, increased pro-inflammatory cytokines production	[112]
Bovine chondrocytes	PRP releseate after $CaCl_2$ activation and single centrifugation	Increased type II collagen and aggrecan messenger RNA expression, decreased cyclic tensile strain-mediated catabolic and inflammatory response	[113]
Human nasoseptal chondrogenic cells and human bone marrow mesenchymal stromal cells	PRP releseate after $CaCl_2$ activation and single centrifugation	Promoted chondrogenic differentiation and their recommitment	[119]
Rabbit chondrocytes	Pool of rabbit PRP loaded in hydrogel scaffold	Increased cell viability and cannabinoid receptor CB1 and CB2 expression	[120]

(*Continued*)

Table 1. (Continued).

Cell type/animal model	Intervention	Outcome	Reference
Male 4-month-old New Zeland white rabbits with induced articular cartilage defect in the groove of femur	Pool of rabbit PRP loaded in hydrogel scaffold	Enhanced cell proliferation and maturation of joint chondrocytes	[120]
Human cortico-cpongius progenitor cells	PRP releseate after freeze-thaw cycle activation and single centrifugation	Stimulated cell migration, increased cartilage matrix formation, promoted chondrogenic differentiation	[121]
Human subchondral progenitor cells in polyglicol acid-hyaluronan scaffolds	PRP releseate after freeze-thaw cycle activation and single centrifugation	Induced collagen type II and IX, aggrecan and cartilage oligomeric matrix protein expression	[122]
Human tenocytes	Different PRP releseate after $CaCl_2$ activation supplemented with PDGF and TGF-β1	Modulated cell proliferation and collagen type I, HGF and VEGF production by TGF-β1 addition	[123]
Primary human keratocytes and conjunctival fibroblasts	PRP releseate after $CaCl_2$ activation and single centrifugation	Stimulated cell proliferation and migration, inhibited TGF-β1-induced myofibroblast differentiation	[124]
Human tenocytes	PRP releseate after $CaCl_2$ activation and single centrifugation	Stimulated cell proliferation and HGF and VEGF production	[125]
Human tenocytes and synoviocytes	PRP releseate after $CaCl_2$ activation and single centrifugation	Stimulated cell migration	[126]

and TIMP-4 expression, two anti-catabolic mediators in cartilage, the former indicating a proinflammatory and procatabolic response. These observations were not present when the culture medium was obtained by P-PRP (Pure PRP) or PPP (Poor PPP), a notable signal that suggests there is indeed an impact of leukocytes on the biologic effects of PRP. This repertoire of antiinflammatory responses induced by PRP may break the catabolic loop, and dampen inflammatory response in SM and AC when these cells are exposed to proinflammatory cytokines and to abnormal mechanical stress and DAMPS, which is the significant OA context (Figure 2 and 4).[37] One cellular process that accentuates the catabolic state of the AC and SB is the oxidative stress resulting from the imbalance between levels of reactive oxygen species (ROS) relative to antioxidant, which is amplified by aging.[35,116,117] Osteoblasts cultured in the presence of PRP supernatant showed an upregulation of NrF2-ARE pathway and subsequent activation of antioxidant response element (ARE), an important mechanism involved in detoxifying ROS and protecting chondrogenic and osteogenic precursor cells.[22] Moreover, intraosseous infiltrations of PRP in mice can revert the decreased expression of SIRT1 in bone-marrow derived stem cells from aged animals, making stem cells more resistant to oxidative stress and maintaining their stemness, suppressing adipogenesis within the bone marrow and improving osteogenesis and bone mineral density.[23,24] Hence, PRP might additionally play a role as an anti-aging factor by stabilizing AC and protecting SB against oxidative stress.[22–24,115] However, as aging is one physiological risk factor for developing OA,[35,117] there are some age-related changes in the composition of PRP, such as the reduction of IGF-1 and PDGF in elderly people, two important chondrogenic mediators,[118] that might account for some contradictory outcomes in the application of this therapy.

4.6. OA and pain

Pain is considered the clinical hallmark of KOA and several clinical trials have been conducted to assess the efficacy of intraarticular injections of PRP for both pain and function of the knee. There are several relevant studies using the same type of PRP product (PRGF) demonstrating a significant pain reduction and an improvement in knee joint physical function [16] in patients with KOA treated by 3 weekly infiltrations of PRP.[14–16,127] The mechanism/s causing osteoarthritis pain remain yet to be fully identified [53] as do the proposed mechanisms of PRP effectiveness. Two mechanisms might likely link the pain reduction to PRP treatment. The first is the suppression of NFkβ on intraarticular inflamed cells, which leads to the reduction of proinflammatory cytokines that otherwise, might contribute to pain by stimulating hyperalgesia and sensitizing joint nociceptors to other stimuli.[3,42] The second is the reported significant amount of endogenous cannabinoids within PRP [25] that might act as ligands for cannabinoid receptor 1 (CB1) and 2 (CB2) of chondrocytes, synovium cells and bone cells [128] of OA patients, thereby supporting both a pain and inflammation reduction by targeting the endogenous cannabinoid systems (Figure 2 and 4).[25,128]

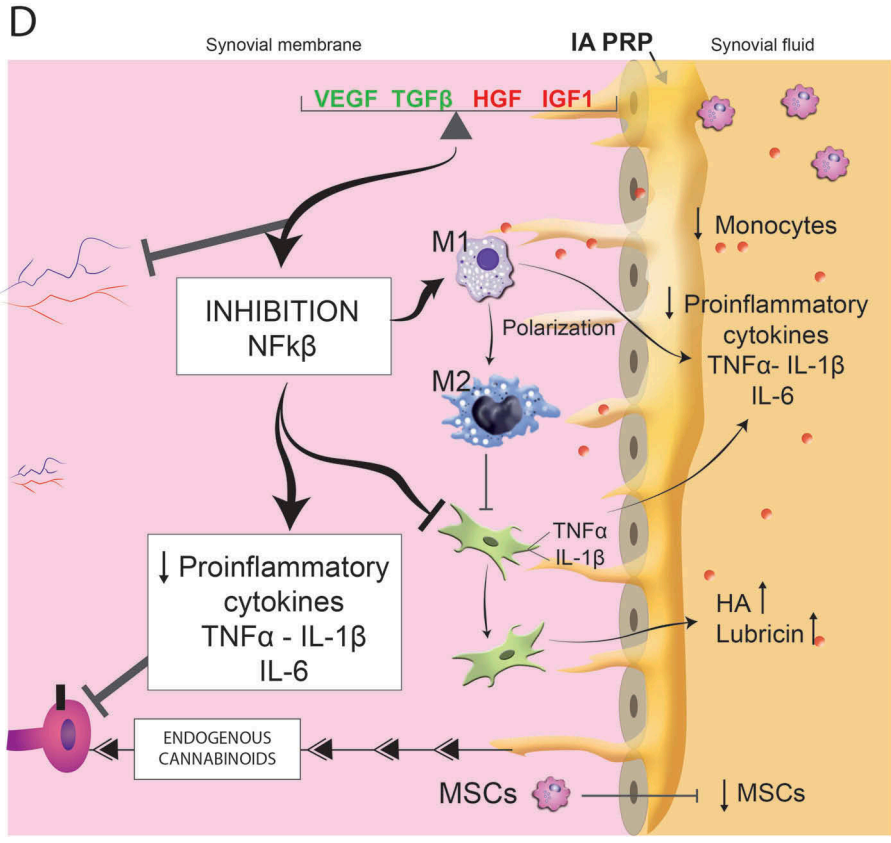

Figure 4. SM. This repertoire of antiinflammatory responses induced by PRP may break the catabolic loop, and dampen inflammatory response in SM and AC when these cells are exposed to proinflammatory cytokines and to abnormal mechanical stress and DAMPS, which is the significant OA context.[1,3,40] This sterile disruption of ECM homeostasis in osteoarthritic joint and an early inflammatory response has been suggested to resemble a chronic injury.[3]

4.7. Trophic and anabolic effects

PRP has been shown to have a consistent *in vitro* proliferative effect on cultured human chondrocytes in a dose-and time-dependent manner [27,29,119] and on rabbit chondrocyte when GFs are delivered in a sustained manner through the upregulation of CB1 and CB2 receptors.[120] Moreover, an *in vitro and in vivo* anabolic effect of PRP on chondrocytes has been reported by increasing the synthesis of proteoglycan and collagen type II [26] or decreasing catabolism by reducing MMP-13 expression and TNF-□ concentration in synoviocyte and cartilage co-cultured systems with PRP media.[21] Another chondroprotective effect is based on the visco-inducing effect of PRP, which stimulates the synthesis of hyaluronic acid and lubricin by synoviocytes and chondrocytes respectively,[21,28,29] which help restore the SF homeostasis and function (Figure 5), the latter preventing chondrocyte apoptosis, synovial cell overgrowth, cartilage breakdown, and inhibition of the MSC release and migration.[29,111,121] On the other hand, platelet rich plasma obtained by apheresis, and characterized by a low platelet concentration and very few leukocytes has been shown to exert positive effects on migration, proliferation and chondrogenic differentiation of cultured human subchondral mesenchymal progenitor cells.[93,121,122] Several soluble morphogens embedded in a fibrin network such as IGF-I and -II, PDGF, SDF-1, TGF-β, CCL5 and fibronectin, among other biomolecules, have been shown to be involved in the recruitment and homing, and in a chondrogenic-differentiation effect of PRP on chondroprogenitor or MSCs from subchondral mesenchymal progenitor cells.[121,129] Last but not least, uncontrolled angiogenesis and fibroneurovascular tissue proliferation are two histological features of osteoarthritic SM and SB. Despite the fact that PRP contains proangiogenic and profibrotic growth factors (VEGF, FGF, PDGF and TGFβ) several *in vitro and in vivo* studies have reported no increase in the level of VEGF and TGFβ [123] nor were tissular fibrosis or an aberrant angiogenesis induced.[123,124,130,131]

5. Targeting subchondral bone as one important tissue in the knee OA treatment

5.1. Subchondral bone as a tissue target in OA treatment

The realization of the biological and mechanical connection between AC and SCB has lead to numerous *in vivo* animal studies that have shown that targeting SB with some drugs can have protective structural effects on cartilage.[9] Blocking or limiting the bone remodeling with alendronate, [132] zoledronic acid [133] or improving the microstructure and quality of subchondral bone in osteoarthritic and osteoporotic rabbits with parathyroid hormone, [9] prevent cartilage degradation and OA progression. Moreover, Sagar et al. [134] reported a reduction in pain behavior after a subcutaneous treatment with osteoprotegerin in a monosodium iodoacetate (MIA) rat model of OA pain, and Pelletier et al. [135] demonstrated that

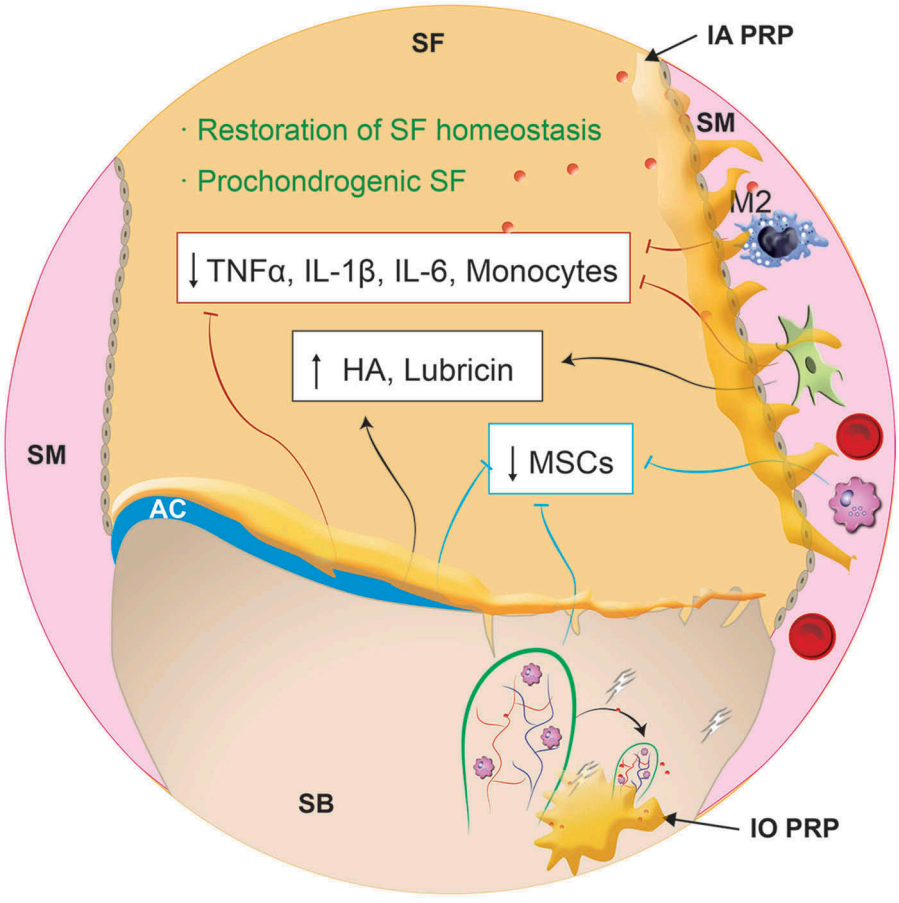

Figure 5. Intraarticular infiltration of PRP helps restore SF homeostasis by stimulating the synthesis of hyaluronic acid and lubricin by synoviocytes and chondrocytes respectively,[21,28,29] dampening inflammation and suppressing the concentration chemoattractan cytokines in SF, which might contribute to the inhibition of the MSC release and migration.[3,95,96] PRP might favour a homing and chondrogenic-differentiation effect on MSCs of subchondral mesenchymal progenitor cells and SF-MSCs.[88,108,111].

an oral strontium ranelate treatment in an experimental osteoarthritic dog model reduced the progression of structural changes including the subchondral bone. Despite the fact that the translation of these promising observations in preclinical research to human clinical trials has often failed, as indicated by a recent metaanalysis of clinical trial with risedronate in knee osteoarthritis,[136] recent clinical trials are raising expectations. For instance, using zoledronic in patients with clinical KOA associated with bone marrow lesions (BMLs) assessed by MRI, Laslett et al. [137] reported a beneficial effect on pain and on BML evolution at 6 months. In participants from the osteoarthritis initiative, Laslett et al. [138] demonstrated significant pain reduction during the first 3 years of treatment with biphosphonates. Two more clinical trials have shown positive structural effects of strontium ranelate on KOA, one improving the joint space narrowing [139] and the other reducing the loss of cartilage volumes concurrent with the decrease of BMLs at 3 years of follow up.[140]

5.2. Intraosseous infiltrations of PRP

Infiltrations of PRP into the BM cavity of femur of young and old ovariectomized-SAMP8 age-related osteoporotic female mice have been reported to up-regulate osteogenesis and down-regulate adipogenesis.[23] The increase of fat tissue mass in BM is correlated with decreased bone mineralization in aged SAMPS8 mice,[23,24] bone demineralization that occurs in osteoarthritic subchondral bone together with cysts.[67] Moreover, improvement of bone mineral density in PRP-treated osteoporotic mice concurred with both histological sections of the bone samples showing more trabecular bone areas and more intense calcium staining and a suppression of bone resorption process as evidenced by the decrease of RANKL transcript.[23] In a trial on 13 healthy volunteers, Philippart et al. [141] reported fatigue on the first day as the only clinical adverse effect after a self-stimulation of BM of the iliac crest by injected autologous platelet-rich plasma.[141] Figure 5 shows the histological analysis of cartilage and SB from a patient suffering from severe KOA who underwent intraosseous infiltrations of PRGF. Eight months later, the patient had not improved clinically and underwent a knee replacement. During the surgery, we took this sample of cartilage and subchondral bone from the femoral condyle in which 5 cc of PRGF had been infiltrated intraosseously. Part of the biopsy showed a good gross appearance, with pearly areas similar to the original hyaline cartilage, though histological study revealed a fibrocartilage repair tissue. Another area showed nearly exposed bone.

In light of the aforementioned research and others not mentioned here because of space limitation, and the significant clinical improvement obtained in some but not all patients

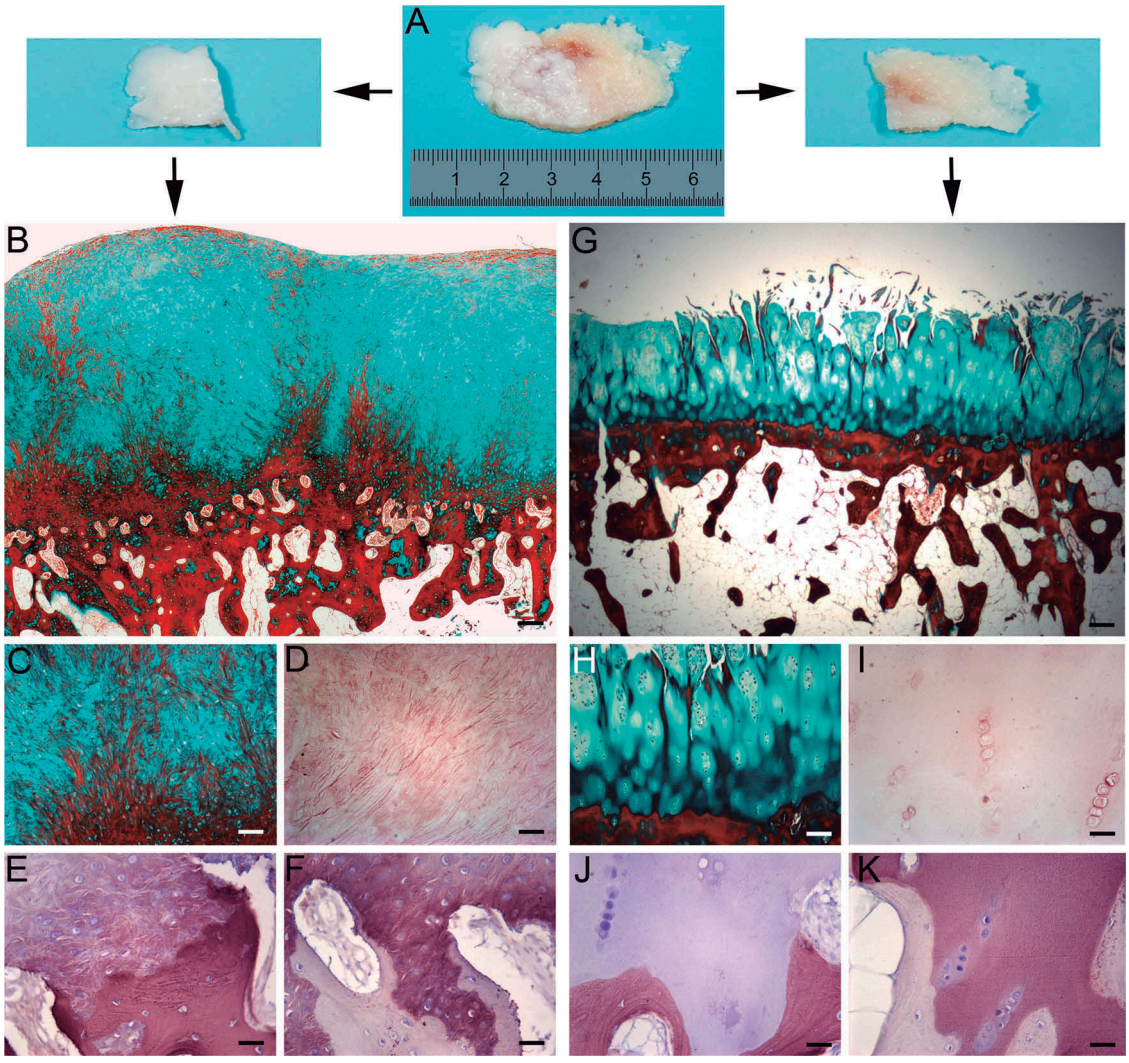

Figure 6 Fibrocartilage repair tissue after intraosseus PRGF infiltrations in the treatment of human knee osteoarthritis: a histological study. (A) Macroscopic morphology of the sample. The sample was divided into two pieces. The fragment on the left-hand corresponds to fibrocartilage repair tissue (B to F) while the right-hand fragment shows osteoarthritic cartilage (G to K). B and G show panoramic images of the sample (Masson's trichrome staining). In photomicrographs C and H, details of the structure of articular cartilage are observed (Masson's trichrome staining). The presence of elastic fibers is demonstrated by Orcein staining (D and I). These fibers can be seen in D, while they are absent in I. An immunohistochemical study was performed to detect the presence of type I (E and J) and type II (F and K) collagen. In all samples (E to K), both subchondral bone (always positive for type I and negative for type II collagens) and cartilage are observed. In fibrocartilage (E and F) both types of reactivity are observed, while in the degenerated cartilage, only type II collagen positivity is shown (K). Histologically, the pearly area (the left-hand side of the sample) is fibrocartilage repair tissue, while the right-hand side of the sample displays an osteoarthritic area with loss of cartilage surface integrity.

with KOA treated with intraarticular infiltrations of PRP [14–16,142]. Our group arrived at the strategy of combining another drug delivery route, namely, the intraosseous infiltrations combined with intraarticular infiltrations of PRP.[99,143]

We have already conducted a phase II clinical trial combining intraarticular and intraosseous infiltrations of PRP for severe KOA. The first treatment included one PRP intraarticular infiltration and two PRP intraosseous infiltrations (in femoral condyle and tibial plateau). The procedure is carried out in the operating room under a 4–5 degree of sedation of the patient. In addition, local anesthesia is conducted into the periosteum of condyle and tibial plateau by injecting 2 ml of 2% mepivacaine. Intraosseous infiltrations are performed with a 13G trocar used for bone biopsy, and the control of trocar placement is facilitated using a fluoroscope.[144] Two more weekly intraarticular infiltrations were performed. After a 6 month follow-up, a significant pain reduction and decrease of MSC and CFU-F in synovial fluid with no adverse effects were reported.[14,99,143] We have been performing intraosseous infiltrations of PRGF since 2003 applying them regularly at the condyle and tibial tunnels in the arthroscopic reconstruction of anterior cruciate ligament, and in osteochondral injuries and osteonecrosis of the hip and knee.[144]

6. Conclusions

There is a substantial and growing body of evidence indicating that subchondral bone is a crucial target, which should be included in KOA therapy. PRP molecular intervention positively influences SB, SF, AC and SM homeostasis, adaptation, and metabolism in addition to reducing joint pain and inflammation, and providing a circuit breaker in KOA, thereby acting as a symptomatic and structure-modifying OA therapy. However, many unanswered questions remain, regarding

molecular mechanisms, dosage aspects and whether combining PRP with stem cells might enhance the efficacy of PRP.

7. Expert opinion

Intraarticular delivery is an alternative modality to deliver PRP in patients with KOA and it has been shown to be safe and efficacious in improving clinical symptoms.[14–16] This route of drug delivery reaches the SM and the AC, which is sometimes inefficiently targeted by systemic drug delivery. Intraarticular delivery circumvents systemic toxicity and its side effects, offers an excellent bioavailability and does not present molecular size limitation, in contrast to the systemically delivered molecules entering the joint through capillaries of the subsynovium.[145,146] Nevertheless, intraarticular therapy faces other challenges when treating chronic nonsystemic sterile-inflammatory conditions as in the case of KOA. One significant challenge is a short joint dwell time of drugs, as the lymphatic drainage clears proteins in a few hours. This is not the case of PRP, as it acts as a dynamic liquid scaffold with a fibrin network from where GFs are gradually released into the tissue.[96,100] Moreover, the increasingly recognized role of SB in the pathophysiology of OA [8,12,33,67] might make the intraarticular route insufficient to tackle all the joint tissues involved in KOA.

Intraosseous delivery strategy for local, prolonged and sustainable release of GFs has been proven to be efficacious in some musculoskeletal pathology, non-union fractures, osteoporosis and bone fracture healing among them.[143,147,148] Over the past 30 years, surgical experience in cartilage defect has revealed that only when the subchondral bone is involved through bone marrow stimulating procedures such as transcortical Pridie drilling and microfractures, is a temporary functional fibrocartilage tissue synthesized, with no serious adverse reported.[5] There is good *in vitro* and *vivo* evidence that events in the subchondral bone concur with and have a direct effect on the overlying articular cartilage.[9,43,45,46] Moreover, although the titles and much of the text of Liu et al. [24] and Philippart et al. [141] papers are not focused on osteoarthitis, these studies shed important light on the role that intraosseous infiltrations of PRP might play in subchondral bone homeostasis by targeting both osteoblast-osteoclast coupling and mesenchymal stem cells responses, as well as in its safety.

The combination of intra-articular and intraosseous injections of PRP is an *in situ* local biological 'joint-centric' approach to treat severe KOA addresses the SM, SF and superficial zone of AC by intraarticular injections of PRGF, and deep zones of AC and SB through PRP intraosseous infiltrations.[99] These PRP infiltrations convey a mimetic biomaterial embedded with a pool of growth factors acting as a smart scaffold [149] which might sustain a gradual delivery of growth factors at the dysfunctional and deregulated tissues as a niche therapy. Rebuilding a physiological-homeostatic network at knee organ failure tissue level, as is the case of severe knee OA, must be an orderly process, which entails a daunting therapeutic task. Our hypothesis is that the concurrent presence and a balanced ratio between platelet-secreted TGFB-1 and VEGF, and plasma growth factors such as IGF-1 and HGF, [105,124–126] all conveyed by PRP intraosseous infiltrations, might reduce or buffer the excess of TGFB in SB and restore HGF activity synthesized by subchondral bone cells. This modulatory effect of PRP on TGFB-1 signaling pathway might shrink the fibroneurovascular tissue that replaces the bone marrow of OA subchondral bone, an explanation which parallels the antifibrotic mechanism already reported to be exerted by the PRP on several cell phenotypes. [105,124,126] This new reestablished homeostatic balance between TGFB1 and HGF [71,78] would reduce the synthesis of NGF, VEGF and other inflammatory mediators thereby contributing as well to modulate the aberrant fibroneurovascular tissue and to alleviate pain and hyperalgesia.[150]

However we do not forget that '*the aim of science is not to open the door to infinite wisdom but to set a limit to infinite error*' (Bertolt Brecht), and many questions and uncertainties still persist unanswered in the field of PRPs and inflammation. When the concept of inflammation defined as a cooperative and amplifying protective multicellular response, orquestrated both locally and remotely, that is intended to eliminate the original insult and their toxic consequences, thereby initiating the repair process, [30] there are some difficulties applying it to tissue damage brought about by mechanical stresses, which is the case of most sterile inflammation pathologies such the KOA.

In spite of a wealth of preclinical and clinical publications on PRP, many uncertainties remain regarding the ultimate molecular mechanism/s, the variability in its composition mainly because of the presence/absence of leukocytes, the platelet concentration, the donors age and the manner in which PRPs are applied to the damaged tissues.[90] Moreover, we need to delve into the systemic effect that this procedure might entail as few studies on human have been carried out regarding PRP treatments and systemic effects.[151,152]

The restoration of TGFβ and other extracellular matrix GFs balance by the application of PRP deserves a deeper research and opens the door to explore the analgesic, antiinflammatory and immunomodulatory, and trophic-anabolic effects of PRP through a systems biology approach. In addition, we cannot rule out a systemic effect of intraosseous infiltrations as suggested by studies carried out in animal model, which should be explored. And finally, we still do not know how to combine PRP with rehabilitation programs and exercise in a synergistic application with the goal of full recovery of knee function.[31]

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Declaration of interest

E Anitua is the Scientific Director of and S Padilla, R Prado and G Orive are scientists at BTI Biotechnology Institute, a dental implant company that investigates in the fields of oral implantology and PRGF-Endoret technology. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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•• of considerable interest

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