CHAPTER 7

The Scientific Rationale to Apply Plasma Rich in Growth Factors in Joint Tissue Pathologies: Knee Osteoarthritis

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SUMMARY

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. In the daunting task of rebuilding a physiological-homeostatic network at the tissue level in synovial joint organ failure, as in severe KOA, a biologically inspired therapeutic approach consisting in intra-articular infiltrations of PRP has proven to substantially reduce pain in patients with KOA and to improve joint stiffness and physical function.

This chapter is an attempt to shed more light on the molecular and cellular data in joint homeostasis, pathophysiology, and to discuss some mechanistic aspects that have been proposed which provide the rationale for using PRP in KOA.

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E. Anitua et al. (eds.), *Platelet Rich Plasma in Orthopaedics and Sports Medicine*,
https://doi.org/10.1007/978-3-319-63730-3_7

1. INTRODUCTION

The treatment of synovial joint injuries remains daunting despite advances in pharmacological management of the pain and inflammation, the refinement of surgical procedures and techniques, and the paramount contribution of the field of regenerative medicine. Synovial joint is a complex mechanical organ that 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)[1]. These tissues are highly specialized mechano-sensitive and/or load-bearing tissues whose homeostasis relies on the precise interaction between biomolecules and cells when the latter are subjected to physiological loading[2,3]. 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[1,4]. 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[5-7].

A biologically inspired therapeutic approach consisting in intra-articular infiltrations of PRP has proven to substantially reduce pain in patients with KOA[8-10] and to improve joint stiffness and physical function[11]. 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 positive effects on reestablishing homeostasis of joint tissues through a breadth of actions such as antiinflammatory, immunomodulatory, and antioxidative effects[12-19], an analgesic effect[8,9,11,20], and finally chondroprotective and anabolic-trophic effects (figure 1)[21-24].

This chapter addresses current molecular and cellular data in joint homeostasis and pathophysiology, some mechanistic aspects that have been proposed, and provides the rationale for using PRP in KOA.

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[25]. The phenotype of chondrocytes, synoviocytes, and osteoblasts is constantly adapting to its dependence on the biochemical, biophysical and mechanical loading features of their microenvironment[3,26-28]. 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[3], thereby protecting against the deleterious effect of some supraphysiological stimuli[29]. Abnormal mechanical stress and/or biochemical mediators variously stemming from trauma, obesity, lesion or disfunction of knee components, as well as from metabolic diseases, break knee dynamic stability and trigger biological responses that disrupt the homeostasis of cells and tissues of the joint in a local, sustained, low-grade inflammatory fashion leading to a matrix degradation (figure 2)[6,30,31].

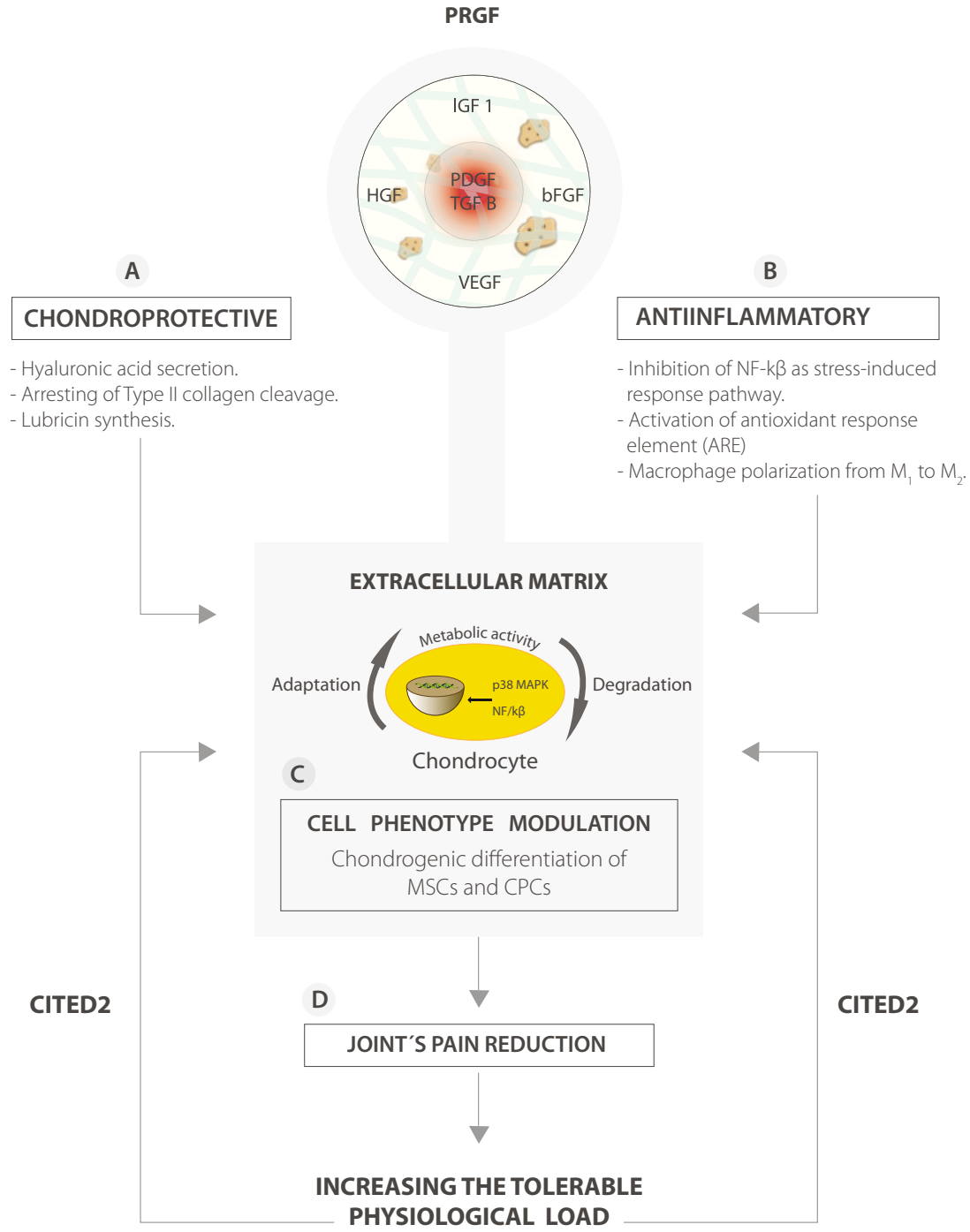

FIG. 1
The overall outcomes in basic science, preclinical, and clinical studies suggest four synergetic effects of PRGF application on the osteoarthritic joint. By modulating gene expression and gene products, PRGF may well influence cell behaviour which is conducive to maintaining the homeostatic state of the joint tissues, thereby reducing pain and improving joint function and motion[76]. (Reprinted with permission from Anitua et al.[76])

In the wake of this sterile matrix degradation of AC, 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[1,32]. 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 signalling pathway[26,33] and increased osteoclastogenesis, thereby increasing bone resorption and sclerosis[28,34] respectively (figure 2). Nevertheless, evidence is accumulating about

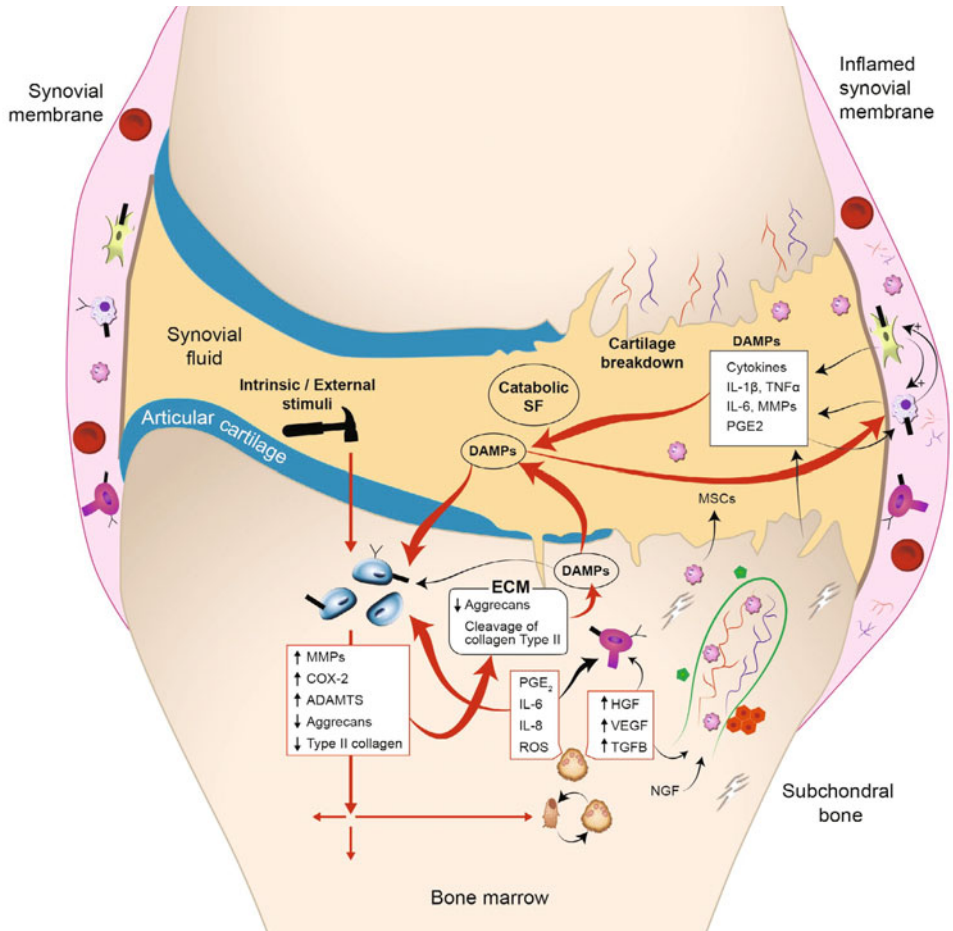

FIG. 2
Abnormal distribution of mechanical loading across joint cartilage breaks the homeostasis of articular cartilage and provokes adaptive or catabolic cell responses, which leads to an increased synthesis of matrix metalloproteinases (MMPs) and aggrecanases (ADAMTS), expression of proinflammatory 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[1,7,32,45]. 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[7], 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[7, 41, 42].
(Reprinted with permission from Sanchez, M. et al.[10])

how alterations of SB induced by mechanical or vascular stresses might be the start point in the catabolic loop of AC degradation and extend to SM (figure A)[5,35-37]. 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 as source for at least 50% of articular cartilage requirements in oxygen and glucose[37,38]. 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 SB, and then it is difficult to establish who was first[39].

2.2. Synovial membrane and subchondral bone in cartilage homeostasis and inflammation

In recent years, a great deal of evidence has been accumulating in favour of seeing as decisive the contribution of synovitis and SB on articular cartilage degradation and on the progression of KOA, where AC may after all be the victim, and not the culprit of catabolic inflammatory cytokines stemming from SM and SB, and triggered by abnormal mechanical stresses[1,7,40-42]. 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 SF[35,43].

Evidence in basic science, preclinical and clinical settings has been mounting for the role of synovium inflammation in the pathogenesis and progression of OA[6,7]. 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[31,42] 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 2)[7,44]. 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 (TNF-a), interferon gama (IFN-j), and nerve growth factor (NGF) among other inflammatory cytokines (figure 2)[7,41,44-46]. 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 KOA disease[26,44,47]. Another pathway involved in KOA synovitis is the activation of complement as it has been shown by Wang et al.[48] 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 remodelling such as fibromodulin, cartilage oligomeric matrix protein (COMP), and osteoadherin in SF and SM might account for this activation[7].

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[7,49]. In addition, proinflammatory cytokines may contribute to pain by stimulating hyperalgesia and sensitizing joint nociceptors to other stimuli[7,42] 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 mesenchymal stem cells (MSCs) in SF[50,51], which is commonly interpreted as a tissue response to injury[52,53], equivalent to the response of migratory chondrogenic progenitor cells from SB to injured cartilage[54,55]. Moreover, several studies have reported that the accumulation of synovial fluid MSCs increases with the severity of osteoarthritis, joint damage and the disease duration[51,56,57]. Healthy human and osteoarthritic cartilage and SF contain a population of cells with characteristics of mesenchymal progenitor cells[52,58] with migratory and chondrogenic potential[52,54]. According to these observations, endogenous mesenchymal stem cells have been postulated as a reservoir of repair cells and immunomodulatory drugstore cells to dampen inflammation[59]. Although the source of MSC increase has yet to be determined, the most likely origin may be the SM[51,52], the breakdown zone of superficial AC[58], and the SB[54,55,60,61]. 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 (figure 2)[56].

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[33] and unlike cartilage, when damaged, regenerates spontaneously due mainly to its highly elevated vascular and cellular network. Evidence is gathering 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[27,35,39,61,62].

2.4. The role of SB in the pathophysiology of osteoarthritis

Subchondral bone is the layer of bone which lies immediately below the calcified cartilage (figure 2)[63], 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[38,64]. 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[61,65]. 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[27,60,61,66].

There is now good evidence that even in a non-diseased joint, naturally occurring pores and holes enable communication between SB and AC via diffusion of small molecules[66-68]. This communication may be exacerbated by structural changes seen early in the osteochondral unit in KOA (figure 2) (Chapter 11 delves into this topic).

3. PLASMA RICH IN GROWTH FACTORS (PRGF) AS AN EFFECTIVE AND SAFE THERAPEUTIC APPROACH TO TREAT SYNOVIAL JOINT OSTEOARTHRITIS

Plasma rich in growth factors (PRGF) 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, TGF-B), cytokines (TNF-a, IL-2,3,4,5), chemokines (PF4), ECM components (fibronectin, thrombospondin, tenascin), cell adhesion (L-selectin, N-CAM), acute phase proteins, and proteins related to lipid metabolism[69,70]. By sequestering several growth factors, microparticles, and other biomolecules released from the degranulation of platelets and plasma[70-72], 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[73]. 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 (see chapter 11, figure 3) 74. When fibrinolysis begins, a gradual, sustained release of GFs and other biomolecules occurs, in contrast to a bolus delivery modality[71,75]. Such a gradual yet sustained release of GF influence on cells, mimics the biological repair process[71,72,75], which is the topic of a review published by Anitua et al. 2013[76].

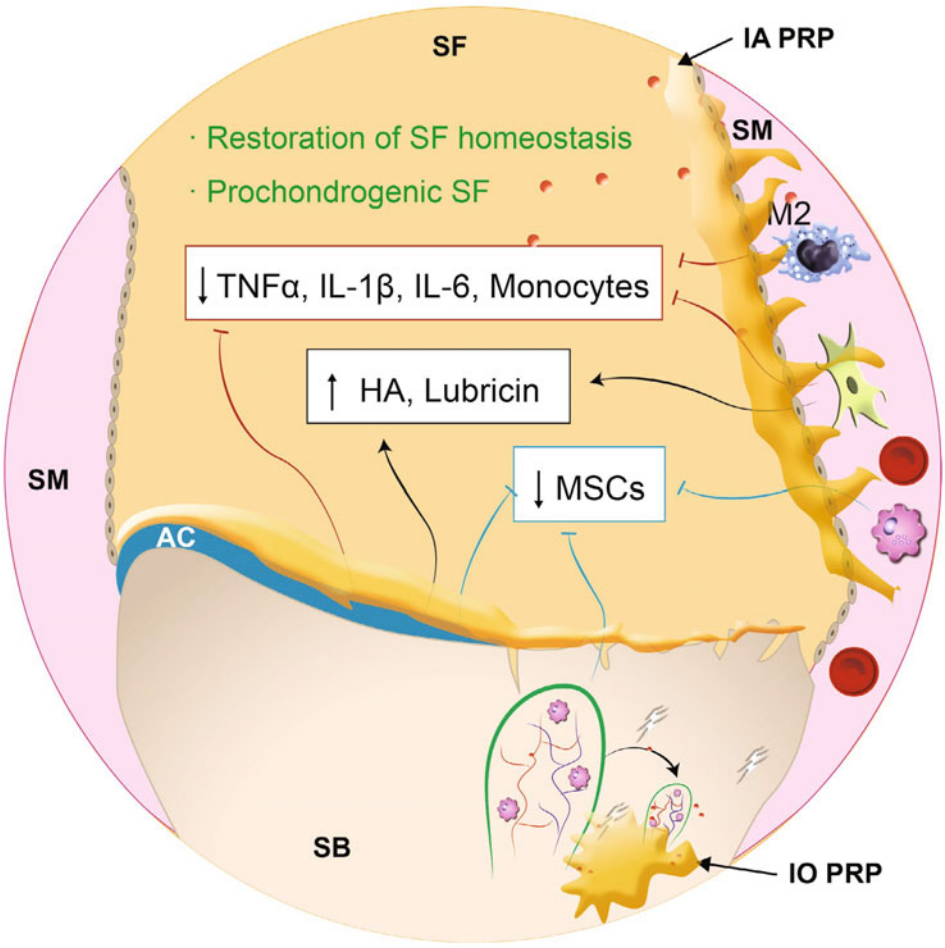

FIG. 3
Intraarticular infiltration of PRGF helps restore SF homeostasis by stimulating the synthesis of hyaluronic acid and lubricin by synoviocytes and chondrocytes respectively[16, 22, 23], dampening inflammation and suppressing the concentration chemoattractan cytokines in SF, which might contribute to the inhibition of the MSC release and migration[7, 10, 80]. PRGF might favour a homing and chondrogenic-differentiation effect on MSCs of subchondral mesenchymal progenitor cells and SF-MSCs[93-96]. (Reprinted with permission from Sanchez, M. et al.[10])

3.1. Inflammation and oxidative stress

In vitro and in vivo studies (Table I) have reported that PRGF 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[15,77,78]. IGF-I, PDGF, HG, and PRGF releasate modify the inflammatory status of chondrocytes by suppressing the NF-kβ signaling pathway[12-14], which might lead to the decreased presence of IL-β, and TNF-α and other pro-inflammatory cytokines in synovial fluid[7,79,80] (figure 3 and 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 Ikβα, 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[81]. In one recent study, Xie et al.[82] reported that PRP attenuated the multiple-cyclic tensile strain mediated MMPs, NO, and PGE2 synthesis in chondrocytes, suggesting that PRGF may protect chondrocytes from mechanically induced injury. Connective tissue factor (CTGF), one of the most abundant growth factors released by platelet activation[83] was reported to protect chondrocytes from age-related degenerative changes and from cellular stress, the latter mediated through NFkβ[84]. 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 15 and to mediate the antiinflammatory effects of PRGF on fibroblasts[53]. In a recent work, Assirelli et al.[85] observed that L-PRGF (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 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 (figs. 3 and 4)[31]. 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[29,86,87]. Osteoblasts cultured in the presence of PRP supernatant showed an up-regulation 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[17]. 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[18,19]. Hence, PRP might additionally play a role as an anti-aging factor by stabilizing AC and protecting SB against oxidative stress[17-19,84]. However, as aging is one physiological risk factor for developing OA[29,87], 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[88], that might account for some contradictory outcomes in the application of this therapy.

3.2. 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[11] in patients with KOA treated by 3 weekly infiltrations of PRP[8,9,11,89]. The

mechanism/s causing osteoarthritis pain remain yet to be fully identified[49] 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[7,42]. The second is the reported significant amount of endogenous cannabinoids within PRP 20 that might act as ligands for cannabinoid receptor 1 (CB1) and 2 (CB2) of chondrocytes, synovium cells, and bone cells 90 of OA patients, thereby supporting both a pain and inflammation reduction by targeting the endogenous cannabinoid systems (Figure 3 and 4)[20,90].

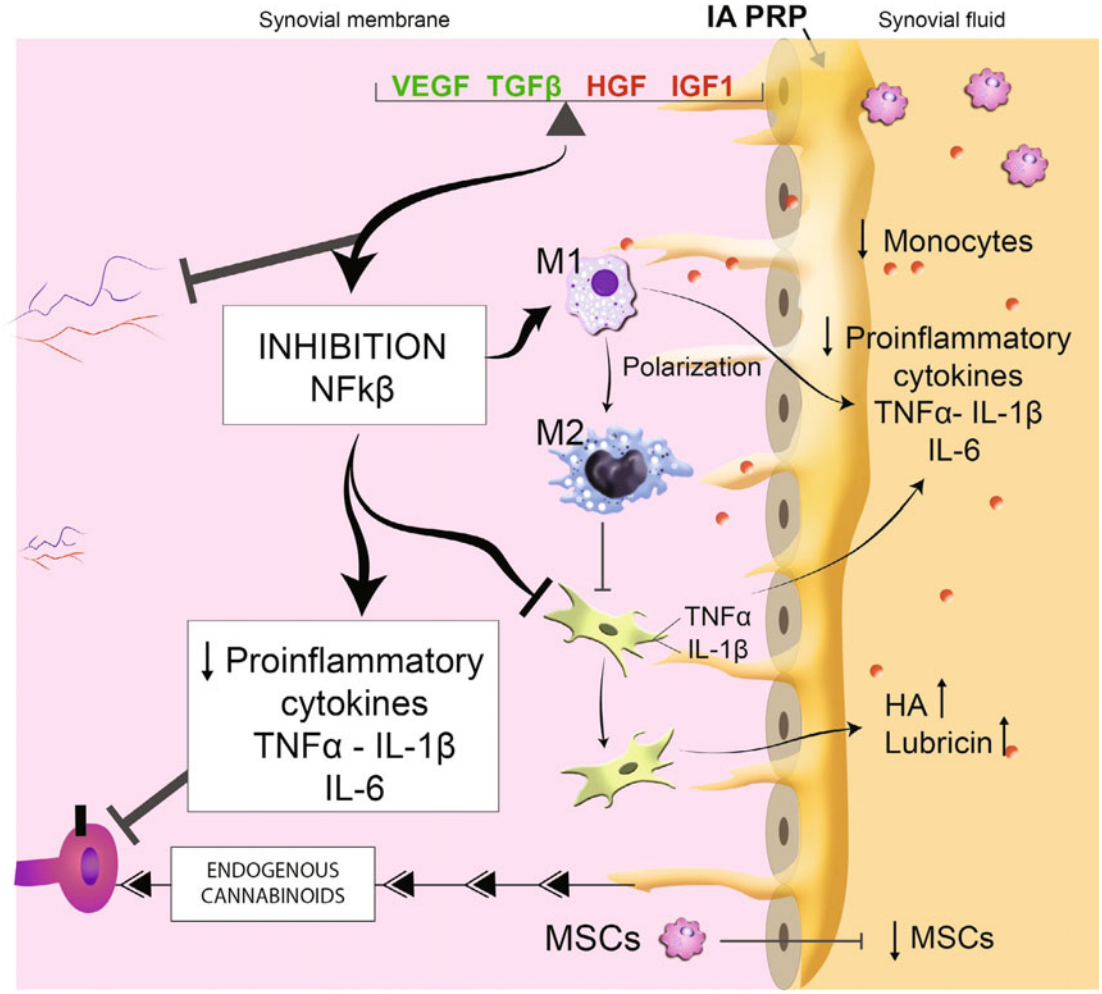

FIG. 4
This repertoire of antiinflammatory responses induced by PRP may break the catabolic loop, and dampen inflammatory response in SM and AC when these cells are exposed to proinflammatory cytokines and to abnormal mechanical stress and DAMPS, which is the significant OA context[5-7]. This sterile disruption of ECM homeostasis in osteoarthritic joint and an early inflammatory response has been suggested to resemble a chronic injury[7]. (Reprinted with permission from Sanchez, M. et al.[10])

Cell type/Animal model	Intervention	Outcome	Reference
Immortalized human chondrocytes	PRP releseate after thrombin and CaCl2 activation and single centrifugation	Reduction of transactivating activity of NFκB, decreased COX-2 and CXCR4 expression	Bendinelli P. 2010
Human monocytic tumor cell line	PRP releseate after thrombin and CaCl2 activation and single centrifugation	Decreased chemotaxis	Bendinelli P. 2010
Human osteoarthritic chondrocytes	10% PRP releseate after CaCl2 activation	Decreased IL-1β-related inflammation, inhibition of NFκB activation	Van Buul GM. 2011
Primary canine chondrocytes	Medium supplemented with HGF and IGF-1	Inhibition of IL-1β-mediated activation of NFκB, decreased apoptosis in chondrocytes	Montaseri A. 2011
Mouse bone marrow derived macrophages	Medium supplemented with HGF	Decreased IL-6 production, increased IL-10 production, reduction of transactivating activity of NFκB	Coudriet GM. 2010
Human osteoarthritic synoviocytes	Autologous conditioned plasma	Decreased TNF-α concentration, decreased MMP-13 expression, increased HAS-2 expression	Sundman EA. 2014
Human osteoarthritic chondrocytes	Autologous conditioned plasma	Decreases TNF-α concentration, increased cartilage synthetic activity	Sundman EA. 2014
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	Tohidnezhad M. 2014
Human adipose-derived stromal cells	PRP releseate after thrombin activation	Increased cell proliferation, ALP activity and mineralization	Liu HY. 2011
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	Liu HY. 2014
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	Liu HY. 2014
Human keratinocyte cell line	PRP releseate after freeze-thaw cycle activation and single centrifugation	Increased endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) production	Descalzi F. 2013
Mouse model of acute inflammatory pain induced	PRP releseate after freeze-thaw cycle activation and single centrifugation	Reduced nociceptive behavior	Descalzi F. 2013
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	Wu CC. 2011
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	Spreafico A. 2009
Human osteoarthritic synoviocytes	20% PRP and 20% PRP releseate after CaCl2 activation	Increased hyaluronic secretion and HGF production	Anitua E. 2007 (Rheumatology)
Human synoviocytes, chondrocytes and anterior cruciate ligament-derived cells	Autologous conditioned plasma	Increased cell proliferation and superficial zone protein production	Sakata R. 2015
Mouse macrophages cell line	Different formulations of human and mouse PRP	Decreased nitric oxide, TNF-α and inducible NO synthase	Renn TY. 2015
Human acute monocytic leukemia THP-1 cells	Platelet-derived micropartilces	Promoted monocytes towards a resident phagocytic phenotype	Vasina EM. 2011

TABLE 1 (I)
Summary of in vitro and in vivo effects of Platelet-Rich Plasma and growth factors. (Reprinted with permission from Sanchez et al.[10])

Cell type/Animal model	Intervention	Outcome	Reference
Primary human gingival fibroblast and primary human alveolar fibroblast	Leukocyte-rich PRP	Increased NFκB activation, decreased cell proliferation, increased pro-inflammatory cytokines production	Anitua E. 2015
Bovine chondrocytes	PRP releseate after CaCl2 activation and single centrifugation	Increased type II collagen and aggrecan messenger RNA expression, decreased cyclic tensile strain-mediated catabolic and inflammatory response	Xie X. 2015
Huma osteoarthritic synovial fibroblasts	Leukocyte-rich PRP	Increased FGF-2, IL-1β and IL-8 production, decreased HGF and TIMP-4 production	Assirelli E. 2014
Human nasoseptal chondrogenic cells and human bone marrow mesenchymal stromal cells	PRP releseate after CaCl2 activation and single centrifugation	Promoted chondrogenic differentiation and their recommitment	do Amaral RJ. 107
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	Kruger JP. 2012
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	Kruger JP. 2014
Human subchondral mesenchymal progenitor cells	Different PRP formulations	Modulated chondrogenic differentiation by PRP formulation	Kreuz PC. 2015
Human tenocytes	Different PRP releseate after CaCl2 activation supplemented with PDGF and TGF-β1	Modulated cell proliferation and collagen type I, HGF and VEGF production by TGF-β1 addition	Anitua E. 2007 (Plast Reconstr Surg)
Primary human keratocytes and conjunctival fibroblasts	PRP releseate after CaCl2 activation and single centrifugation	Stimulated cell proliferation and migration, inhibited TGF-β1-induced myofibroblast differentiation	
Anitua E. 2011			
Human tenocytes	PRP releseate after CaCl2 activation and single centrifugation	Stimulated cell proliferation and HGF and VEGF production	Anitua E. 2005
Human tenocytes and synoviocytes	PRP releseate after CaCl2 activation and single centrifugation	Stimulated cell migration	Anitua E. 2012
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	Braun HJ. 2014
Human osteoarthritic chondrocytes	Different PRP formulations	Stimulated cell proliferation and chondrocyte anabolism by PRP, stimulated catabolic pathway by leukocyte-rich PRP	Cavallo C. 2014
Rabbit chondrocytes	Pool of rabbit PRP loaded in hydrogel scaffold	Increased cell viability and cannabinoid receptor CB1 and CB2 expression	Lee H-R. 2012
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	Lee H-R. 2012

TABLE 1 (II)
(Reprinted with permission from Sanchez et al.[10])

3.3. 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[22,24,91] and on rabbit chondrocyte when GFs are delivered in a sustained manner through the upregulation of CB1 and CB2 receptors[92]. 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[21] or decreasing catabolism by reducing MMP-13 expression and TNF-α concentration in synoviocyte and cartilage co-cultured systems with PRP media 16. 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[16,23,24], which help restore the SF homeostasis and function (figure 3), the latter preventing chondrocyte apoptosis, synovial cell overgrowth, cartilage breakdown, and inhibition of the MSC release and migration[24,80,93]. 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[93-95]. 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[93,96]. Last but not least, dysregulated angiogenesis and fibroneurovascular tissue proliferation are two histological features of osteoarthritic SM and SB (figure 4). 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[97] nor were tissular fibrosis or an aberrant angiogenesis induced[97-100].

4. CONCLUSIONS

Intraarticular delivery is the conventional modality to deliver PRP in patients with KOA and it has been shown to be safe and efficacious in improving clinical symptoms. 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 via capillaries of the subsynovium. However this route does not target subchondral bone, and some mechanistic and dosage aspects remain to be elucidated in order to determine, harness, and optimize the therapeutic potential of platelet-rich plasma products. Some of these issues will be tackled in the ensuing chapters.

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