EXPERT OPINION

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A biological therapy to osteoarthritis treatment using platelet-rich plasma

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Introduction: Osteoarthritis (OA) is a degenerative disease affecting the synovial joint. It is caused by cells exposure to non-physiological stimuli, either mechanical or biochemical, and the loss of bone-cartilage homeostasis. Some of these changes, however, may be reversed by the use of single or combined growth factors, suggesting that the treatment of OA could be addressed using a pool of growth factors.
Areas covered: This review addresses current molecular and biological knowledge and implicates the recapitulation of some developmental processes during endochondral ossification in OA aetiology and pathogenesis. Platelets act as carriers of endogenous morphogens that may modulate cell fate and therefore affect joint tissues structure and function. We shed light on the platelet-rich plasma effects on biological level that might drive the osteoarthritic joint's improvement both in structure and function.
Expert opinion: We present the therapeutic potential of plasma rich in growth factors (PRGF-Endoret), an endogenous biological therapy that might modulate the gene expression of cells such as chondrocytes, synoviocytes, macrophages, and mesenchymal stem cells, and thereby influence an anabolic microenvironment of synovial joint which is conducive to maintaining the homeostatic state of the joint's tissues, and hence reduce pain and improve the joint motion.

Keywords: cartilage, growth factors, osteoarthritis, platelet-rich plasma

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1. Introduction

Osteoarthritis (OA) is a mechanically induced disorder that evolves as a heterogeneous, multistage, and degenerative disease provoking the synovial joint failure as an organ. OA is a relatively local disease, for the most part affecting one or two joints, typically knees or hips. The disease represents a family of synovial joint degeneration which alters every component of the tissues involved, from the molecular to the cellular and extracellular levels. Although induced by an insult, either mechanical or biochemical, OA is biochemically mediated and ultimately causes the structural and functional failure of the joint. In addition to the central role that mechanical stress and age play in the onset and progression of OA [1], we conceptualize OA as a multifaceted disease in which systemic (hormonal status, gender, and genetics) and abnormal biomechanical loading on joints (obesity, joint injury, high-intensity and prolonged sports activities) make the joint vulnerable [2]. Another additional risk factor might be mutations in genes whose products make up the extracellular matrix. Although many of the OA-associated genes are involved in the development of the joints, none of them appears to be involved in the hyaline articular cartilage degradation and loss [3] which is considered a central pathological feature of OA [4]. It is important to remember that genes only represent the cells

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Article highlights.

- Osteoarthritis is a degenerative disease that gradually affects all the joint tissues provoking pain and loss of function.
- Developmental biology has shed some light on the osteoarthritis pathogenesis by bringing growth factors into play as cell fate modulators on different joint tissues.
- Growth factors may have the capacity to establish a molecular cross-talk among joint tissues, thereby controlling the pro-inflammatory phenotype of synovial joint's cells and maintaining an anabolic microenvironment.
- Platelet-rich plasma (PRP) products deliver growth factors, cytokines and adhesive proteins as well as other plasma proteins such as fibrinogene, prothrombin, and fibronectin.
- Plasma rich in growth factors (Endoret) application to osteoarthritic joints results in reducing joint pain and improving joint function by restoring tissue homeostasis as indicated by the chondroprotective, anti-inflammatory, and cell-phenotypic modulation effect on joint tissues.
- We are only at the beginning of a new era in which we must optimize PRP procedures at the same time we continue drawing on its healing power and relief in a wide range of medical conditions.

This box summarises key points contained in the article.

potentiality for change and that is the microenvironment's signalling presence that rules cells behaviour [5].

Whereas pain represents the clinical hallmark of the disease coexisting with other clinical features such as stiffness, instability, swelling, crepitus and functional limitation [6,7], it is the degeneration of the joint's tissues and changes in the periarticular bone rearrangement and the hyaline articular cartilage breakdown in particular that constitute the major factors leading to disability and impaired quality of life [8,9]. Due to the aneural feature of cartilage, the source of nociceptive stimuli might well stem from structures which are richly innervated such as synovium, subchondral bone, periostium, and joint capsule, however this relation has yet to be established [1].

In addition to articular cartilage, mature synovial joints have classically been considered to consist of ligaments and fibrous capsules lined with a synovial membrane whose cells exude a lubricating fluid (synovial fluid). The synovium is a specialized mesenchymal soft tissue made up of a lining layer with two distinct types of cells: synoviocytes that are fibroblastic-like and secrete lubricin and hyaluronan, and macrophages, although mesenchymal stem cells (MSCs) too have been isolated both in normal and osteoarthritic human articular cartilage [10,11]. These might play an important role as chondroprogenitor cells in the reparative response to articular cartilage damage [12]. Another layer, known as subintima, includes blood and lymphatic vessels associated with nerve fibres. The multicellularity and vascularity endow the synovium with a highly reactive capacity against what their cells might interpret as an insult or stress (mechanical or biochemical). Such an insult would trigger an inflammatory defence response in order to maintain or restore joint tissue homeostasis and function [13].

2. Bone-cartilage homeostasis disruption

Exposure of the joint cells to non-physiological stimuli, either mechanical or biochemical, leads to a rupture in the cartilage balance between anabolism and catabolism known as cartilage homeostasis [8,9,14]. Although the homeostatic processes within the joint occur at the cellular, tissular and organ level, the behaviour of cells such as chondrocytes, synoviocytes, macrophages and osteocytes is truly responsible for carrying them out [15]. The disarrangement of structures that make up the synovial joints such as hyaline cartilage, synovium, synovial fluid, menisci, and subchondral bone gives rise to the failure of the synovial joint, a key component in the body's motion.

Articular cartilage and the subchondral bone are endowed with different adaptive responses to mechanical load and damage, and this asymmetry might disrupt the homeostasis between them [16]. Several groups have proposed a molecular crosstalk between the bone and cartilage pointing to the subchondral bone reactions to the mechanical stress as the triggering factor in the OA [4,15-17] hence challenging the traditional view of the articular cartilage as an isolated tissue and offering a view of the possible existence of fluid, cell, and molecular communication between the cartilage and the subchondral bone (**Figure 1**) [18,19].

Tissue interactions govern most developmental processes, from the very early patterning events of cell differentiation, through a process called morphogenesis and finally growth of the many organs in the embryo. All human synovial joints share the same developmental processes. Formation of the skeleton is no exception, and most of the tissues differentiating in the newly forming limb arise from mesenchymal cells. These cells give rise to the various articular tissues, with the exception of neuronal elements and blood vessels [20,21]. The regulation of articular cartilage development and homeostatic processes throughout life is carried out under the influence of numerous growth factors and cytokines which act in concert as signalling molecular pathways [22].

3. Current cellular and molecular knowledge about the common signalling molecules and pathways underlying osteoarthritis

A variety of cells and cell signalling molecules which dynamically form the structural network of the joint tissues are extremely well communicated and may use the fluid flow to migrate and reach injured areas mainly attracted by cell signalling factors (growth factors and cytokines), biochemical gradients and matrix fragments [23-25]. Cells from different tissues of the joint but chiefly the quiescent chondrocytes undergo and sense non-physiological stimuli as an insult,

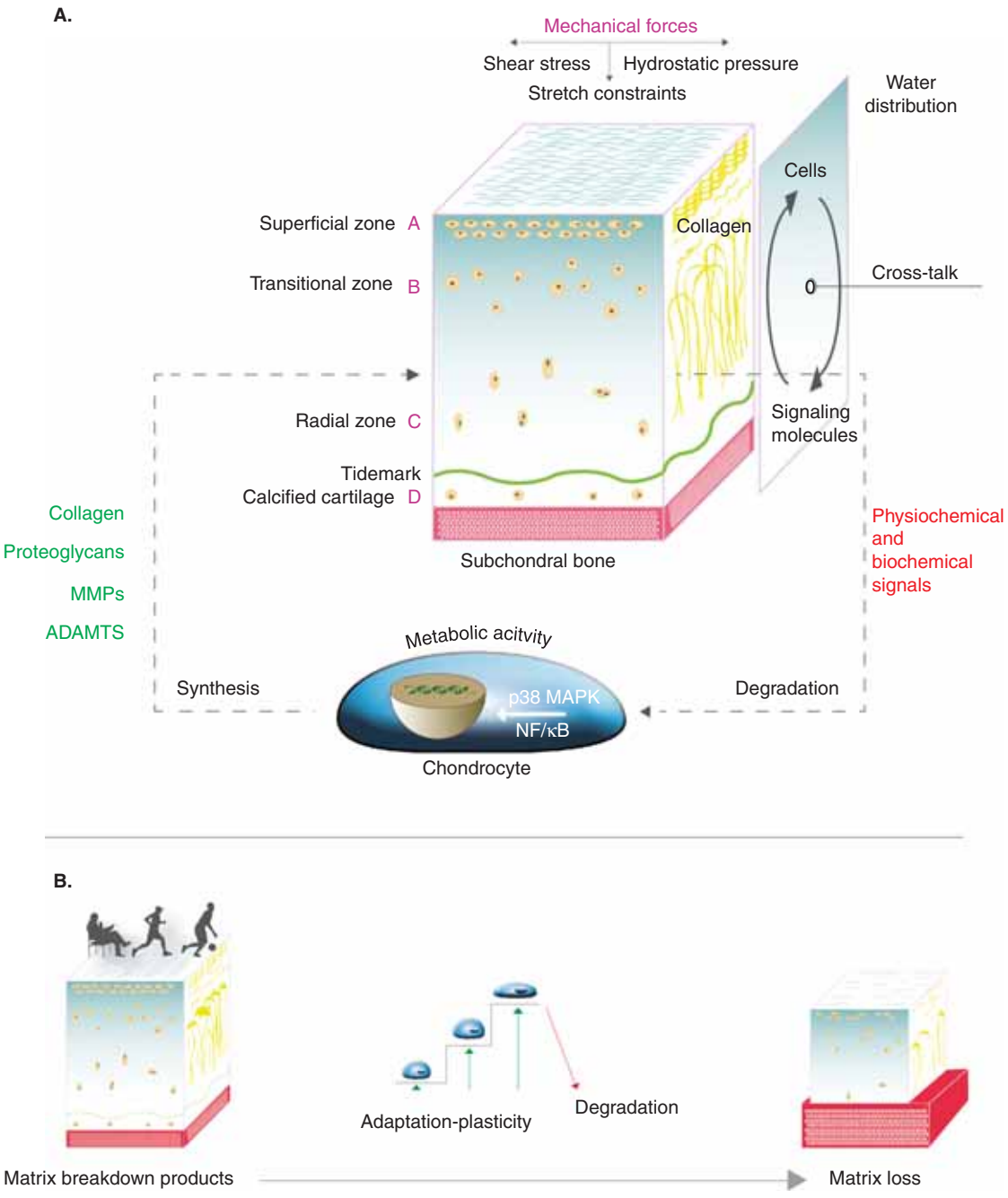

Figure 1. A. In the complex cartilage-bone-based mechanotransduction system, the mechanical energy applied to the joint is reflected on in the extracellular matrix, and subsequently in the chondrocyte nucleus. Joint cells exposure to non-physiological stimuli tips the loss of tissue balance between cartilage degradation and synthesis known as cartilage homeostasis. There appears to be a molecular, cellular, and fluid communication between the cartilage and bone. **B.** The survival/viability of the chondrocyte is affected to a large extent by the presence of a sufficient (plasticity), but not excessive (degeneration), mechanical stimulus that would inevitably lead to the disarrangement of structures such as the subchondral bone mediated mainly by deregulated chondrocytes, perpetuating a catabolic microenvironment and eventually the joint failure.

modulating and taking on a different phenotype whose gene expression products (anabolics and catabolics) orchestrate a defence-inflammatory response [26-28] in a miscued attempt to either maintain the tissue homeostasis and integrity or mimic the repair process. Nevertheless the tissue response turns out to be catabolic, thereby altering the cells' micro-environment and breaking down the extracellular matrix. The response of chondrocytes in the osteoarthritic cartilage

is heterogeneous and oriented towards hypoanabolism, which encompasses cell proliferation, apoptosis, and phenotypic alterations. Such a response results in a reactive or hypertrophic chondrocyte phenotype known as deregulated chondrocytes [26,28-30] whose catabolic gene expression causes a net loss of extracellular matrix [26]. Not only chondrocytes but also macrophages and synoviocytes influenced in a paracrine manner take on a pro-inflammatory phenotype [13,31]. The extracellular matrix which is made up mainly of water, type II collagen and aggrecans drains away and degenerates as a consequence of the action of catabolic cytokines (TNF-α and IL-Iβ), metalloproteinases (MMPs, MMP13), and aggrecanases (ADAMTS). These products are primarily released by chondrocytes, synoviocytes, and mononucleated cells, breaking the collagen and aggrecans down in a slow and relentless degenerative process [9,26,28] and thereby giving rise to articular chondrocytes expressing classic hypertrophic markers (characteristic of the growth-plate chondrocytes) and apoptosis [8,29,30]. This interpretation of biological processes strongly suggests that context matters, and that the extracellular matrix (ECM) in connective tissues, as in bone, muscle, tendon and cartilage, hosts physical–chemical processes which are key to tissue-repair processes such as cell-recruitment and differentiation, and patterning-remodelling.

There is a clear role played by tissue and cellular signalling pathways and networks in osteoarthritic aetiopathogenesis, pathways and networks which are shared in the developmental biological process [29,30,32] and which will be partially redeployed and may, in osteoarthrosis pathogenesis, adopt a role as mediator. At the molecular level the aforementioned biological processes are mediated by a group of highly conserved polypeptides known as growth factors (GFs) which are proteins specialized in signalling cellular pathways and mainly, but not solely, released by local cells such as chondrocytes, macrophages, and synoviocytes. Growth factors modulate the cell's behaviour and shape the structure of the tissues thereby determining their functions [33,34]. In addition to the inflammatory response in the osteoarthritic aetiopathogenesis, mainly led by the local cells in one reparative-reactive attempt of the cartilage, the osteoarthritic joint is the destination of several migratory cells, namely MSCs and chondroprogenic progenitor cells (CPCs) [10,12,35,36] which may come from the subchondral bone through the tidemark into the cartilage tissue. The CPC, with MSC features have a multipotent differentiation capacity towards the chondrogenic lineage [35] and may be the target of the GFs which traffic cell information through the MSCs by their trophic activity [37]. These multipotent cells might offer us the most valuable component when it comes to the repair process, namely, cells [38]. A similar process appears to be responsible for fibrocartilage repair synthesis when the Pridie drilling procedure is carried out in some reconstructive cartilage surgery. This surgical procedure has presumed that the adult marrow-derived mesenchymal stem cells (MSCs) from the subchondral bone are able to differentiate into bone, cartilage, muscle, marrow stroma, tendon-ligament, fat and other connective tissues [39]. In addition to the subchondral bone marrow, the synovium is another important source of MSCs in the joint tissues showing a high chondrogenic potential comparable to that of bone marrow-derived MSCs [12]. These observations are in accordance with insights and clinical experience, suggesting that MSCs are naturally found as perivascular cells or pericytes. Once they have migrated to the injured site, these cells behave not only as proliferative and differentiated cells but also and significantly as immunomodulatory and trophic ones [39,40].

There is growing evidence indicating that in OA, articular chondrocytes expressing classic hypertrophic markers (known as deregulated chondrocytes) [26,28-30] with a catabolic gene expression and extracellular matrix destruction of articular cartilage, resembles that observed in the hypertrophic zone of foetal growth plate during endochondral ossification, a resemblance suggesting that developmental biology might shed some light on the OA pathogenesis [26-30]. OA is driven primarily by both mechanical stress and inflammatory signals (IL-1β and TNF-α) orchestrated by the NF-κβ signalling molecules which have been shown to mediate articular cartilage degradation by upregulation of matrix-degrading MMPs [28,41]. The activation of the NF-κβ signalling pathway can generate altered states of quiescent chondrocytes thereby pushing chondrocytes to a more differentiated, hypertrophic-like state in an attempt to maintain or restore tissue homeostasis, as well as recapitulating some developmental cell phenotypes [28,29,41]. Most of the cellular and molecular changes previously mentioned are also described in the growth plate chondrocyte and may be reversed by the use of single or combined growth factors such as TGFβ-2, FGF-2, IGF-1 or insulin [42,43] combinations that have been shown to produce synergistic effects in preserving chondrocyte homeostasis [44,45].

4. An innovative biological approach to the treatment of osteoarthritis: platelet-rich plasma

The appropriate treatment of cartilage injuries and OA remains a daunting clinical challenge despite advances in both pharmacological management of the pain and inflammation, and advances in the surgical procedures and techniques and, in extremis, OA has been considered a disease with no cure [1]. Although is not within the scope of this article to address the wide range of therapeutic strategies in the treatment of OA, we wish to remark that only a holistic approach could fulfil the goal of clinicians, namely, to control pain, to improve function and to stop the progression of disease [1]. Since the synovial joint is a complex, shock-absorbing interface in which a coordinated and sequentially ordered engagement of the joint's elements and muscles is required to

maintain the physical integrity of anatomical structures and homeostasis of the joint´s tissues, every pharmacological and surgical therapy should be assisted by mechanotherapy [46]. In this respect, and as a clinical application of cells mechanotransduction, a rehabilitation program which included the employment of PRGF in a synergistic manner would play a crucial role in both promoting the repair or remodelling of injured tissue and avoiding the degradation of cartilage and atrophy of joint's structures such as bone, periarticular muscles, tendons and ligaments with the goal of full recovery of function [46-48].

One innovative biological approach to the treatment of OA is the application of platelet-rich plasma in intraarticular injections. Although a universally accepted definition of PRPs in terms of platelet concentration and presence or absence of leucocytes is lacking, PRP products can be depicted as an autologous platelet concentrate within a plasma suspension, and whose composition is determined by the method used to obtain it. Platelet-rich plasma products include plasma and twofold or more increases in platelet concentrations above baseline levels, and the concentration of leucocytes and erythrocytes varies widely [49-51] from a complete absence of products to a high concentration of them. In particular, the PRGF is depicted as an endogenous blood-derived product which conveys growth factors, cytokines, and morphogens contained in the platelets as well as fibrinogen and other plasmatic proteins in a biologically balanced aggregate, and managed and delivered in a pharmacological manner [33,52,53]. This multifaceted, versatile, biological system is made up of an autologous, balanced blend of plasma with a moderated platelet concentration (a two- to threefold increase compared with peripheral blood) that does not contain leucocytes. The process of platelet activation and hydrolysis of prothrombin into thrombin is driven by the addition of calcium chloride, simultaneously causing the release of a plethora of growth factors and the polymerization of fibrin [33,53,54]. Once activated the liquid formulation is in the ensuing moments injected as a solution into soft tissues, and due to its local and gradual activation (*in vitro* and *in vivo*) and homogeneous distribution through and interaction with the ECM of different tissues, is converted into a matrix-like viscous and malleable structure [34].

Mammal platelets are circulating monitors, trackers and surveyors of the integrity of the vascular system and of the internal milieu as well as carriers of cytokines, chemokines and growth factors, fulfilling the function of coordinators of coagulation, inflammation and repair processes [55,56]. In addition to these bioactive mediators (α-granules: TGFB, PDGF, VEGF, FGF, EGF, IGF-1, HGF, BMPs, BDNF and dense granules: histamine, serotonin, Ca and ATP/ADP), there are other contents in the plasma of PRPs (IGF-1, HGF, fibrinogen, fibronectine and other proteins) which together with adhesive proteins expressed by activated platelets, all play a central role in the cell signalling pathways involved in both tissue injury recognition and in the repair of damaged tissues (**Table 1**) [33,56]. Platelets appear to be crucial in post-embryonic morphogenesis in identifying tissue loss or injury, factors that activate platelets thereby releasing by degranulation, growth factors and cytokines which trigger mechanisms to reconstruct structures and restore function mainly by stimulating cell migration and proliferation, regulating angiogenesis, chemoattracting circulating progenitor cells and guiding tissue remodelling [54,57-59]. Drawing on these mechanisms and observations made by Crisan and col.2008 and Caplan 2009 concerning the immunomodulatory and trophic effects of MSCs [39,40], it might be possible to suggest a synergy between platelets and MSCs.

Besides conveying GFs, PRGF provides the damaged tissue with a transient biological scaffold made up of fibrin which stems from the polymerization of fibrinogen, a pleiotropic blood protein that regulates coagulation, inflammation, and tissue regeneration [33,54]. The three-dimensional network, formed either "*in vitro*" as a clot or "*in situ*" as an extracellular matrix after the intraarticular infiltration over-injured areas, contains binding sites for cell adhesion as well as proteins such as thrombospondin-1 (TSP-1), alpha-1-antitrypsin fibronectin, acute phase proteins or proteins related to lipid metabolisms. Since cells that make up and populate musculoskeletal tissues, including chondrocytes are mechano-sensitive, in this varied molecular landscape, migratory cells such as MSCs and CPs might adhere and undergo physiological loading, thereby regulating their gene expression and eventually repairing the injured tissue; cells cannot express a physiological phenotype in an empty space. Therefore, after the intraarticular infiltration over the injured areas, a fibrin-scaffold formed "*in situ*" as an extracellular matrix serves as a highway for mechanical energy to transit from the environment to the cell, thereby bridging cell-to-cell tissue transition, promoting multicellular assembly and providing mechanical support as well as endowing tissues with a suitable microenvironment for biological restoration [33,34]. Since they are autologous, bio-reabsorbable, bio-compatible, and free of leucocytes and red cells, PRGF scaffolds are the best tailored among all the tissue engineering materials.

Oral and maxillofacial surgery and implantology, skin ulcers, orthopaedic surgery and bone regeneration as well as repair of injured muscle and tendon are some of the fields in which the application of platelet-rich plasma has consistently demonstrated its safety and successful outcomes in restoring tissue functions [60-64]. Therefore, the platelet-rich plasma application to osteoarthritic joints is intended to trigger and mimic the biological process of tissue healing based primarily on the synergistic influence that growth factors may exert on the joint tissues as they do in articular cartilage development and homeostasis [22], namely, by arresting type II collagen cleavage, reversing the reactive chondrocytic phenotype thereby regaining a healthier phenotype, and repairing articular cartilage [29,44,57,65,66].

Table 1. Primary platelet and plasma contents and their biological functions in tissue regeneration [28,46].

Category	Name or acronym of the molecule	Biological function
Adhesive proteins	VWF + pro-peptide, Fibrinogen (Fg), Fibronectin (Fn), Vitronectin (Vn), Thrombospondin-1,-2 (TSP-1, -2), laminin-8	Cell contact interaction, extracellular matrix composition
Proteases and anti-proteases	Tissue inhibitor of metalloprotease 1-4 (TIMPs 1-4), metalloprotease-1,-2,-4,-9 (MMP-1,-2,-4,-9), ADAMTS13, ADAMS10,17, serpin proteinase inhibitor, platelet inhibitor of FIX, C1 inhibitor, α 1-antitrypsin	Angiogenesis, vascular modelling, regulation of cellular behaviour
Growth and mitogenic factors	Platelet-derived growth factor (PDGF), Transforming growth factor β1and β2 (TGF β1, β2), Epidermal growth factor (EGF), Insulin-like growth factor 1 (IGF-1), Vascular endothelial growth factor A and C (VEGF A, C), Basic fibroblastic growth factor (FGF-2), Hepatocyte growth factor (HGF), Bone morphogenetic protein -2,-4,-6 (BMP-2,-4,-6), CTGF, SCUBE1, IGFBP3	Chemotaxis, cell proliferation and differentiation, angiogenesis
Chemokines, cytokines and others	RANTES, IL-8, MIP-α, ENA-78, MIP-2, MCP-1, MCP-3, SDF-1α, PF4, β-TG, pro-platelet basic protein (PBP), NAP-2, connective-tissue-activating peptide III T, angiopoietin-1, High mobility group box 1 (HMGB1), IL-6sR, endostatin, osteonectin, bonesialoprotein, osteoprotegerin	Regulation of angiogenesis, chemotaxis, vascular modelling, cellular interaction, bone formation
Membrane glycoproteins	alphaIIbbeta 3 (αIIbβ3), alphavbeta3 (αvβ3) PECAM-1, most plasma membrane constituent, receptors for primary agonists, CD63, CD40L, tissue factor, P-selectin, furin, GLUT3, semaphorin 4D, TLT-1, TNF-related apoptosis inducing ligand (TRAIL), syntaxin-2, SANP23	Platelet aggregation and adhesion, endocytosis of proteins, secretion, inflammation, thrombin generation, platelet-leucocyte and platelet–vascular cell interactions
Others	Content of dense granules: ATP/ADP, calcium, serotonin, histamine	Fibrin formation, capillary permeability, vascular local regulation

5. Growth factors and PRPs in cartilage repair

These biochemical modulators and regulators which are shared with developmental biological processes will be redeployed for tissue repair after injury [15,29].

Transforming growth factor-β superfamily (TGFβ) has been shown to play an anabolic role in cartilage repair. In particular, TGFβ1 the major growth factor within PRPs and one of the most important in cartilage regeneration, stimulates both chondrogenesis of synovial lining and bone marrow-derived MSC [67,68] and chondrocyte synthetic activity with matrix deposition [69]. Moreover, TGFβ1 counteracts the catabolic activity of IL-β1 including the degradation of type II collagen and proteoglycan produced by chondrocytes [70,71] and increases chondrocyte phenotype expression [72]. Insulin-like growth factor (IGF-1) is another component of PRPs with a potent anabolic effect on articular cartilage metabolism and its presence is required to maintain the integrity of articular cartilage [73]. In addition to positive influence of IGF-1 on the repair of extensive areas of damaged cartilage and protection of the synovial membrane from chronic inflammation [57], IGF-1 is, together with PDGF, a potent chemotactic factor for chondrocytes, which stimulates synthesis of extracellular matrix in human OA but does not avoid the matrix catabolism [74]. Moreover, its presence in cartilage enhances the effect of other growth factors present in articular cartilage [75].

PRPs application to cartilage repair is underpinned by a substantial body of evidence in basic science, as well as in preclinical and clinical levels of practice. *In vitro*, treatment of mature porcine chondrocytes with L-PRP releasate stimulates cell proliferation, and glycosaminoglycan and collagen synthesis [58]. The presence of PRGF releasate without leucocytes on human osteoarthritic synoviocyte cultures enhances the synthesis of hyaluronic acid (HA) and HGF compared to synoviocytes cultured on a platelet-poor medium. Moreover, the enhanced secretion of HA and HGF by PRGF was maintained despite the fact that synoviocytes were treated with interleukin-1β [59,76]. In one proteomic study conducted on human osteoarthritic chondrocytes cultured with different mediums, the PRP-enriched medium showed to be more efficient than other mediums at increasing cell proliferation and reverting and restoring the pattern of gene expression determined in a normal chondrocyte phenotype without undergoing hypertrophy [77,78]. Bendinelli *et al.* have reported an important HGF-mediated anti-inflammatory and anabolic effect of platelet-rich plasma on immortalized chondrocytes lineage by attenuating or reducing the transactivating activity

of NF-κB [78], a proposal that has been reinforced by the results obtained in osteoarthritic chondrocytes by Van Buul *et al.* [79]. In addition, PRP decreased the expression of COX2 and CXCR4 target genes, whose products might be involved in controlling chemotaxis of inflammatory cells such as monocytes thereby reducing local inflammation [78]. Wu *et al.* [80] have shown, using a 3D *in vitro* model, that the combination of PRP with a collagen matrix (with immortalized human chondrocytes) recovered type II collagen and proteoglycan synthesis which had been inhibited by 3 days of treatment with IL-1β+TNF-α, thereby illustrating the protective efficacy of PRP on chondrogenic-specific gene expression such as Col lI and AGN [80]. In another recent study, Anitua *et al.* determined that synovial fibroblast culture incubated with plasma rich in growth factors (Endoret) + HA induced a greater increment in synovial cell migration compared with the response to HA alone [81].

Furthermore, drawing on the aforementioned evidence, some *in vivo* studies have used PRP in an attempt to restore local hyaline cartilage injuries. When PRP liquid was loaded in microporous poly-lactic-glycolic acid scaffolds and applied on large osteochondral defects in a rabbit model, the neochondrogenesis induced showed chondrocyte-like cell and a high ECM synthesis and the defects were totally filled with a repair tissue similar to hyaline cartilage, compared with the control that showed a fibrous tissue repair [82]. The preventive effect of PRP infiltrations delivered in gelatin hydrogel microspheres in a rabbit model has been reported, showing a suppression of histomorphological signs of the OA progression compared with microspheres containing PPP [83]. Therefore, it has been suggested that the treatment of OA might be carried out using a combination of growth factors [29,57,65] in an attempt to redress the extracellular matrix through the cells behaviour.

6. Conclusions

There is increasing recognition and evidence of a molecular crosstalk between cartilage and subchondral bone which might be harnessed by growth factors delivered from PRPs, thereby counteracting the influence of catabolic gene expression of immature or deregulated chondrocytes on the extracellular matrix, triggered and maintained by mechanical stress. PRGF-Endoret might influence an anabolic microenvironment, containing the right combination of chemical cues, which is conducive to maintaining the homeostatic state of the joints tissues, reducing pain and improving the joint motion, structure, and function.

7. Expert opinion

The potential of endogenous regenerative technology (Endoret) for *in situ* regenerative medicine has yielded positive and promising clinical–surgical outcomes in musculoskeletal system pathologies [34,61,84]. This autologous and biological therapy to cartilage repair is underpinned by a substantial body of evidence in basic science [58,76,80] as well as in preclinical [82,83] and clinical levels of practice [66,85-91].

The successive intraarticular injections of platelet-rich plasma in the knee joint of osteoarthritic patients have shown significantly higher reductions in knee pain and stiffness and improvement in physical function, even compared with hyaluronic acid (HA) [64,82,87] although this product has not yet been proven to really modify the overall histology or molecular composition of OA cartilage. In these clinical trials, the surrogate marker for OA amelioration was the pain. The trials did not evaluate the influence of Endoret on histological and molecular make-up of osteoarthritic cartilage. Although PRPs open a new disease-modifying OA therapy, we acknowledge that this biological approach may only play a part, albeit, a key part in solving this condition. We must not lose sight of the fact that physical rehabilitation as well as other systemic factors such as nutritional deficiencies can affect the joint vulnerability [1]. Healing does not mean "regenerating", and repairing does not mean "recovering the function".

These clinical outcomes have demonstrated that Endoret use is safe as well as efficacious. Taking into account the overall results in basic science, in preclinical and in osteoarthritic patients, we are led to suggest four synergetic effects on the osteoarthritic context (**Figure 2**). First, there is a chondroprotective effect of the synovial joint due to both the hyaluronic acid secretion by synoviocytes [76] and the arresting of type II collagen cleavage by the combination of TGFβ and FGF [29] which contribute to the homeostasis of the articular cartilage. Second, we see an anti-inflammatory effect on human chondrocytes on the basis of the HGF effect both present in PRP and secreted by the synoviocytes [64] inhibiting the intracellular signalling regulator of the inflammatory and stress-induced response [41] pathway NF-κβ [78,79]. Moreover, PRP up-regulates chondrogenic-specific genes and down-regulates the expression of inflammatory molecules on immortalized human articular chondrocyte cell hPi [80]. Third, there is a cell-phenotypic modulation of both chondrocytes which prevent hypertrophic differentiation and maintain them in an arrested state [28-30] and of MSCs and CPCs which promote chondrogenic differentiation once they have migrated from vascular areas (synovium and subchondral bone) [12,17,35,36] towards injured areas under the action of PRP [80], GFs such as TGF-β and IGFs [71,92,93] or FGF-2 [94]. Fourth, by attenuating and reducing the joint's pain [64,86-88,90,95] the physical activity level might improve and increase the physiological load tolerable for the joints. The increased tolerable physical load might entail a chondroprotective effect since it has been proved that moderate mechanical loading [14,48] has an anticatabolic effect on the articular cartilage through either the action of CITED2 [96] or by suppressing NF-κβ activation and, in this manner, it may mediate the anti-inflammatory effect of moderate joint motion [14,48,97]. But not all PRPs are the same, and in a clinical trial conducted by Filardo *et al.* [98] which compared the efficacy and safety of intraarticular injections of Endoret

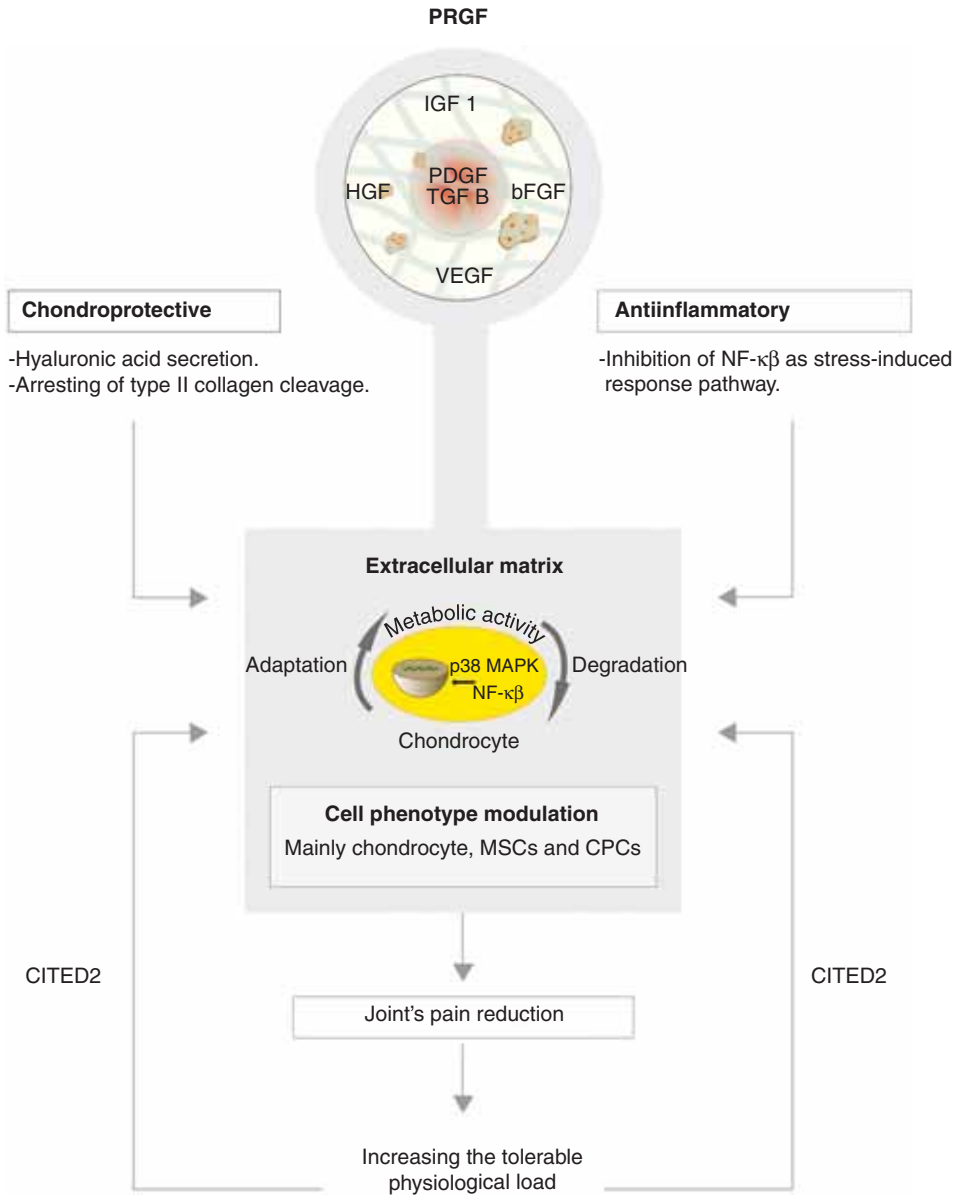

Figure 2. The overall outcomes in basic science, preclinical, and clinical studies suggest four synergetic effects of PRP application on the osteoarthritic joint. By modulating gene expression and gene products, PRP may well influence cells behaviour which are conducive to maintaining the homeostatic state of the joint's tissues thereby reducing pain and improving joint function and motion.

against a leucocyte-PRP in the treatment of OA, patients treated with Endoret had fewer side effects than those treated with leucocyte-PRP whose patients presented more pain and swelling events.

The aforementioned observations emphasize the important role that both growth factors and autologous platelets and plasma products play by providing a storm of signalling factors which are biologically active soluble metabolites, and by regulating a vast range of cellular behaviours both in osteoarthritis and in the articular cartilage repair process. By modulating gene expression and gene products of cells such as chondrocytes, synoviocytes, macrophages, mesenchymal stem cells as well as their cell cycles through epigenetic mechanisms [12,28,80] PRP might influence an anabolic microenvironment, containing the right combination of physical as well as chemical cues, which is conducive to maintaining the homeostatic state of the joint tissues, reducing pain and improving the joint motion, structure, and function.

Platelet-rich plasma therapy draws on the autologous biological system of growth factors and fibrin whose effects on different joint cells and their microenvironments are promising. The biological approach with the application of Endoret

on osteoarthritic joints results in reducing joint pain and improving joint function by restoring tissue homeostasis as indicated by the chondroprotective, anti-inflammatory, and cell-phenotypic modulation effect on joint tissues.

However, there remain some mechanistic and dosage aspects that must be elucidated in order to determine, harness, and optimize the therapeutic potential of platelet-rich plasma products. Although somewhat controversial, one differential element among the various biological compositions of PRPs that might clearly alter its healing potentiality is the leucocyte concentration. Several unanswered questions remain, such as how many infiltrations would be ideal in a first approach, the interval between them, and whether there should be a 1-year anniversary repetition of infiltrations. Due to the heterogeneous composition of PRPs, stemmed from the myriad of methods to obtain them as well as from individual variability, it is difficult to ascertain general guidelines in order to optimize them. We have to acknowledge that we are only in the dawn of biological therapies and PRP products are just in their infancy. The fact that such endogenous PRP therapy acts on a variety of tissues which can be seen as biological systems or networks themselves should not be seen as an absence of accuracy, like a scatter shot, simply because most of the proteins in platelet-rich plasma exert a broad regulatory and pleiotropic function. Indeed, it is only in rather exceptional cases that a specific physiological function can unconditionally and unambiguously be assigned to a given protein as a discrete entity [99]. There seems to be no specific biological factor for each specific cellular function. There are simply biological factors which, in a particular tissue environment, and acting together, induce the expression of cell phenotypes with different cell behaviours [100]. In recognition of the emerging current view of the bone-cartilage as a biological unit [16,17,19,35] mentioned previously in this paper, the articular cartilage should be regarded as only part of the target in the OA treatment. Therefore, in the coming years attempts to harness subchondral bone's source of migratory cell such as MSCs and CPCs [10,12,35,36] and of signalling factors (growth factors and cytokines) must include the subchondral bone as an additional target in the OA treatment with Endoret.

In the light of basic science and clinical studies, we may state that the application of Endoret, in addition to being safe, has been shown to be clinically efficacious in OA treatment although many interesting challenges remain. As knowledge about the regenerative effect of growth factors is growing, their application is being extended, and new challenges arise. We are only at the beginning of a new era in which we must optimize this procedure at the same time we continue drawing on its healing power.

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G Orive and S Padilla designed the proposal. S.P. drafted the first version of the article. E Anitua, M Sánchez and G Orive revised and edited the final version. All authors have reviewed and approved the manuscript.

Declaration of interest

E Anitua, G Orive and S Padilla are scientists at BTI Biotechnology Institute, a biotech company that has developed the technology of plasma rich in growth factors. The remaining authors have no competing interests to declare. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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