CHAPTER 8

A New Approach to Treat Joint Injuries: Combination of Intra-Articular and Intraosseous Injections of Platelet Rich Plasma

AUTHORS

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SUMMARY

This chapter deals with the scientific rationale which underpins a new procedure for the treatment of severe knee osteoarthritis, namely, a combination of intra-articular and intraosseous injections of Platelet Rich Plasma. Intraarticular infiltration of platelet rich plasma is a promising treatment for knee osteoarthritis, but it still has some therapeutic limitations in severe osteoarthritis. Intraosseous infiltration delivers platelet rich plasma into the subchondral bone, acting on this tissue and consequently on cartilage-bone communication. Thus, this technique involves a new route of delivering platelet rich plasma that could be applied not only for severe osteoarthritis but also for other joint pathologies in which the subchondral bone is critical in its etiology such as osteonecrosis, osteochondral lesions, and bone marrow lesions.

This chapter explores some of the recent insights and observations concerning the involvement of subchondral bone in the pathophysiology of osteoarthritis and additionally we will describe a new technique of platelet rich plasma infiltration for the treatment of severe knee osteoarthritis.

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1. INTRODUCTION

Subchondral bone has always been present in the equation of the cartilage repair process and OA[1-3] but it has suffered neglect for decades as an important player in the etiopathogenesis of OA[3,4]. 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 (figure 1)[5-8]. As it is yet to be established precisely which of the joint tissues or structures is the primary driver of knee osteoarthritis (KOA), and therapeutic strategies targeting solely one cell or tissue target may well prove to fail[9], it is advisable that approaches to severe 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.

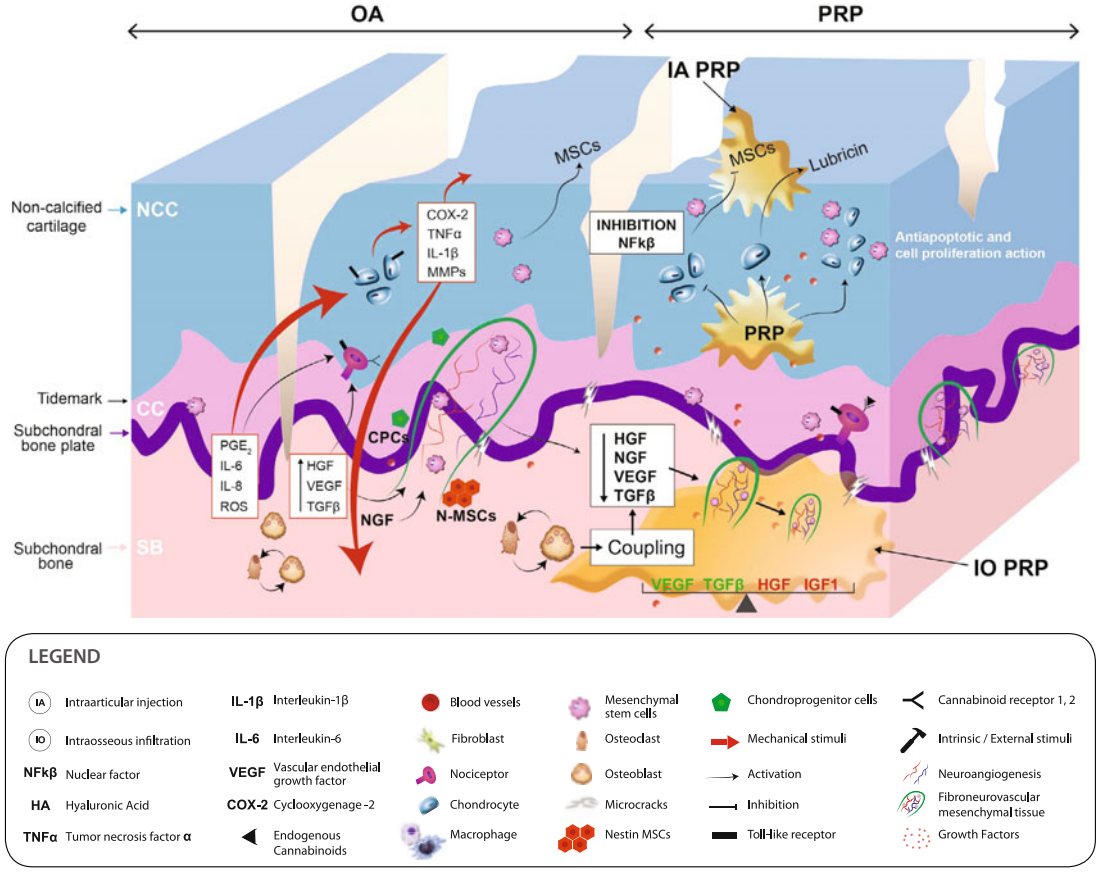

FIG. 1
Targeting the osteoarthritic subchondral bone with Intraosseous infiltration of PRP. This schematic drawing illustrates the outside-in (AC-SB) and inside-out (SB-AC) flow of mediators and cells. SB as a point of egress of morphogens and cells, through the channels and vessels breaching the osteochondral junction, partially recruited by the osteoarthritic synovial fluid 3,7. This cartilage cell invasion might be facilitated by the loss of aggrecans, collagen II cleavage, and disruption of water tissue distribution 8 of the articular cartilage as well as by the secretion by MSCs of fibrinolytic enzymes 22. The excessive presence of TGFB1 and VEGF in OA subchondral bone 3, 7 could be a driving factor for changes in osteoblast-osteoclast coupling thereby leading to a bone remodelling imbalance 28, 30, 38, NGF expression, and fibroneurovascular growth, changes that additionally might well contribute to overlying cartilage degradation, pain, and an osteoarthritic joint 25, 33, 47, 48, 49. (Reprinted with permission from Sánchez, M. et al.)[16]

In recent years, several clinical trials using intra-articular infiltrations of plasma rich in growth factors have shown promising results[10-14] ; however, there are still some concerns about whether this form of administration is able to reach the deeper layers of the cartilage and subchondral bone, thereby possibly limiting the growth factors therapeutic potential especially in severe osteoarthritis[11,15].

In light of recent studies reporting the importance of subchondral bone in the pathogenesis of osteoarthritis and cartilage-subchondral bone communication[16,17] we proposed a combination of intra-articular and intraosseous injections to treat severe osteoarthritis[18] In so doing, it is possible to expand the effective range of PRPs by not only acting on the subchondral bone and consequently on its cartilage communications, but also on mesenchymal stem cells, to modulate the affected tissue regeneration[19,20].

This chapter is based on two manuscripts published recently[16,18] an it will explore some of the recent insights and observations concerning the involvement of subchondral bone in the pathophysiology of osteoarthritis.

2. THE ROLE OF SUBCHONDRAL BONE (SB) IN PATHOPHYSIOLOGY AND CLINICAL SYMPTOMS OF OSTEOARTHRITIS

2.1. 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[2,21] 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 1)[22], 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[23,24]. Together with the articular cartilage (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[7,25]. 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[23,26]. 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[5,23,27].

2.2. 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 (figure 1)[5-7,28].

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[3,28,29]. This SB sclerosis is characterized by an increase of the osteoid volume, and a decrease of calcium bound to collagen fibre, and is associated with a gain of trabecular thickness, loss of trabecular number, and a trabecular network more separated and less interconnected[29,30]. 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[5]. Moreover, SB can undergo microdamage, such as microcracks and clefts, that modify SB stiffness and reduce the shock-absorbing capacity of SB, thereby making chronic a microdamage context and perpetuating an accelerated bone remodelling, which impairs normal mineralization of bone once it has been deposited, most likely by a modified osteoblastic phenotype[5,24,31]. 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[32], and together with bone attrition, are strong indicators of a structural deterioration in knee and hip osteoarthritis[5,33].Several studies conducted in human knee and hip OA paralleling MRI bone marrow edema lesion (BMLs) studies with histological analysis of SB retrieved at the time of joint replacement, revealed microfractures and increased bone remodelling, subchondral ingrowth of fibrovascular tissue and increased vascularity, various types of bone marrow fibrosis[32,34,35] as well as numeric and topographic alterations in native mesenchymal stem cells (MSCs)[33]. These observations were confirmed in rodent models of OA[7,36].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[25]. The neurovascular invasion of those new-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[7] and reach the noncalcified cartilage (figure 1), a finding which has been supported by animal models of OA[7].

2.3. 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 via diffusion of small molecules[6,37,38]. 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 28 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[7,24,39]. Reinforcing this view, Pan et al.[37] 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[6]. Therefore, the presence of these connections enables an elevated crosstalk among chondrocytes, osteoblasts, osteoclasts and MSCs through biological factors and signalling pathways (figure 1).

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 40 reported that osteoblasts in OA-affected bone exhibited a different phenotype, whose activity can degrade articular cartilage in vitro. Supporting this observation, Hilial et al.[41] reported that osteoblasts from OA subchondral bone have an abnormal metabolism with increased levels of PGE2 and TGFβ (figure 1). 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[42,43]. Moreover, osteoblasts from sclerotic subcondral bone have an elevated TGFβ expression[29] and under cyclical compression express proangiogenic factors such as VEGF,FGF, and IL-8[44]. Hepatocyte growth factor (HGF) is a pleiotropic morphogen present in articular cartilage but produced by osteoarthritic subchondral bone osteoblats, osteoclasts, and MSCs[45-47], with likely implications in both the chondrocyte

anabolic state and the proliferation of an invasive fibroneurovascular tissue in SB[5,7,47], the latter when an uncoupling osteoclast-osteoblast activity may lead to an overexpression of HGF (figure 1)[45]. The excessive presence of TGFB1 and VEGF in OA subchondral bone likely stemmed from a dysregulated osteocyte[7,48] could be a driving factor for changes in osteoblast-osteoclast coupling thereby leading to a bone remodelling imbalance[5,30], NGF expression[49], and fibroneurovascular growth changes that additionally might well contribute to overlying cartilage degradation[30,48], pain[7,24,25] and an osteoarthritic joint[30,48]. In a recent study, Zhen et al. showed that by inhibiting TGF-β signalling 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 signalling in AC[48]. Moreover, in a recent study, Campbell et al (2016) reported functional and gene expression perturbations in native MSCs which could lead to further damage escalation[33]. These findings are in accordance with previous studies that have shown that the decrease of MSCs in the synovial fluid, in low degree OA, suggests clinical improvement[50]. 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[51]. However, in vitro studies indicate that the inclusion of growth factors, as a supplementary culture medium, can be beneficial in reverting their chondrogenic activity[52].

2.4. Subchondral bone as a tissue target in OA treatment

The realization of the biological and mechanical connection between AC and SB has lead to numerous in vivo animal studies that have shown that targeting SB with some drugs can have protective structural effects on cartilage[4]. Blocking or limiting the bone remodelling with alendronate[53], zoledronic acid[54] or improving the microstructure and quality of subchondral bone in osteoarthritic and osteoporotic rabbits with parathyroid hormone[4], may prevent cartilage degradation and OA progression. Moreover, Sagar et al[55] reported a reduction in pain behaviour after a subcutaneous treatment with osteoprotegerin in a monosodium iodoacetate (MIA) rat model of OA pain, and Pelletier et al[56] demonstrated that 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[57], 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[58] reported a beneficial effect on pain and on BML evolution at 6 months. In participants from the osteoarthritis initiative, Laslett et al.[59] 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 60 and the other reducing the loss of cartilage volumes concurrent with the decrease of BMLs at 3 years of follow up[61].

Infiltrations of platelet rich plasma (PRP) into the bone marrow 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[62]. The increase of fat tissue mass in BM is correlated with decreased bone mineralization in aged SAMPS8 mice[62,63], bone demineralization that occurs in osteoarthritic subchondral bone together with cysts[24]. 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[62]. In a trial on 13 healthy volunteers, Philippart et al[64] reported fatigue on the first day as the only clini-

cal adverse effect after a self-stimulation of BM of the iliac crest by injected autologous platelet rich plasma[64]. Supporting these findings, Figure 2 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 5cc 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.

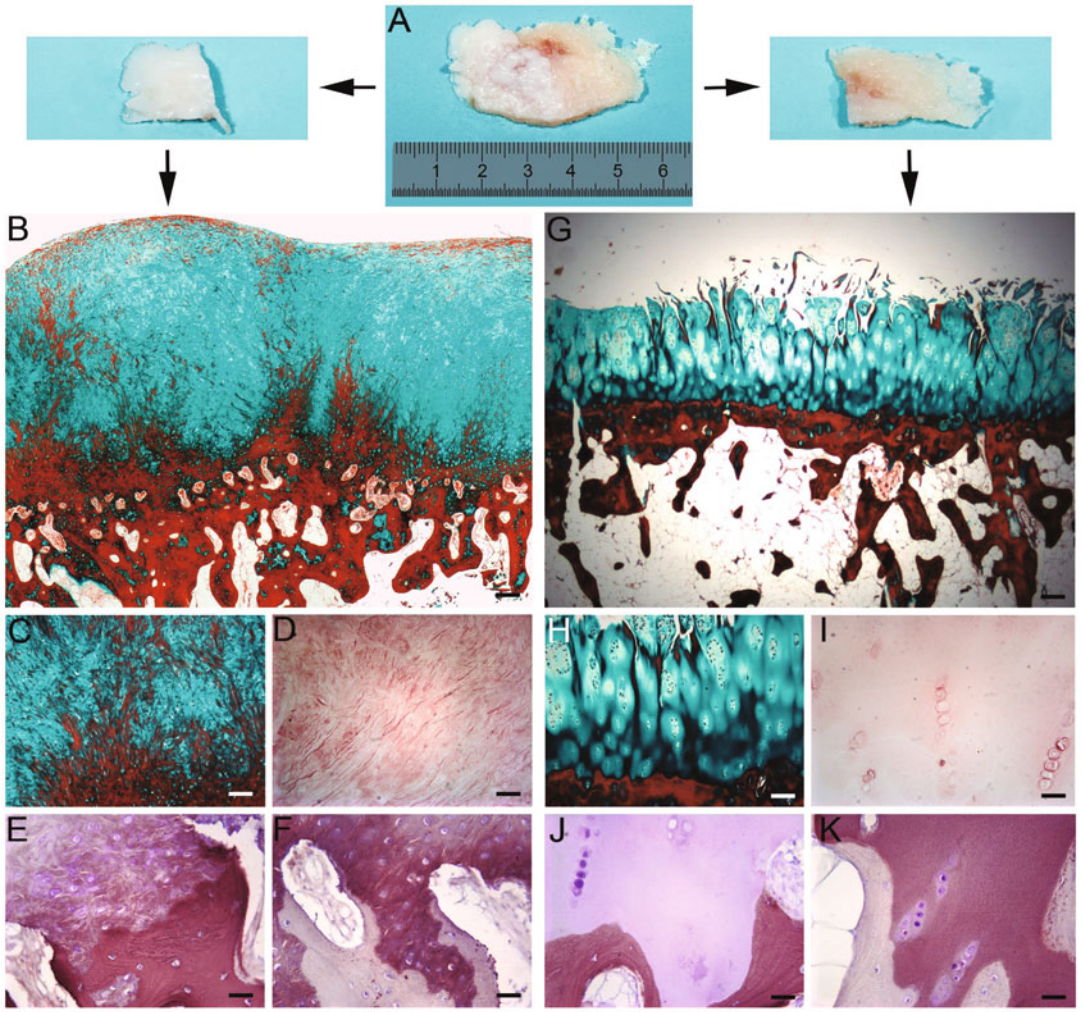

FIG. 2
Fibrocartilage repair tissue after intraosseus PRGF infiltrations in the treatment of human knee osteoarthritis: a histological study. (A) Macroscopic morphology of the sample. The sample was divided into two pieces. The fragment on the left corresponds to fibrocartilage repair tissue (B to F) 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 fibres is demonstrated by Orcein staining (D and I). These fibres 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. (Reprinted with permission from Sánchez, M. et al.)[16]

3. INTRAOSSEOUS INFILTRATIONS OF PLASMA RICH IN GROWTH FACTORS

In light of the aforementioned research and others not mentioned here due to space limitation, and the significant clinical improvement obtained in some but not all patients with KOA treated with intraarticular infiltrations of PRP[11,13,14,65] our group arrived at the strategy of combining another drug delivery route, namely, the intraosseous infiltrations combined with intraarticular infiltrations of PRP[18-20,66,67]. (Figure 3)

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 2mL of 2% mepivacaine. Intraosseous infiltrations are performed with a 13G trocar used

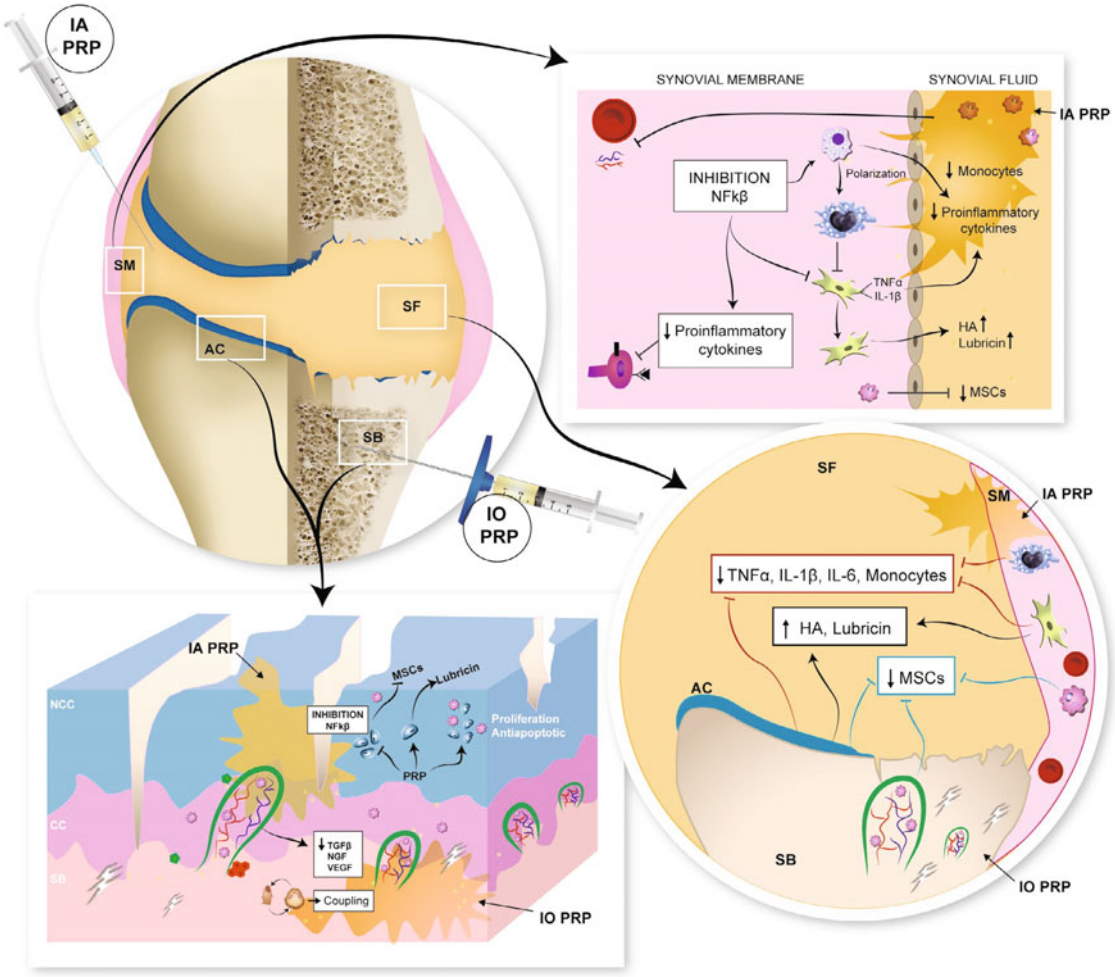

FIG. 3
Depiction of a new strategy to treat severe knee OA by targeting different knee joint structures such as synovial membrane (SM), synovial fluid (SF), articular cartilage (AC), noncalcified cartilage (NCC) and calcified cartilage (CC), and subchondral bone (SB) with intra-articular injections (IA) and intraosseous infiltrations (IO) of platelet rich plasma (PRP). This procedure reduces pain and mesenchymal stem cells (MSC) in SF, besides significantly improving knee joint function of patients with severe OA (Reprinted with permission from Sánchez, M. et al.)[19]

for bone biopsy, and the control of trocar placement is facilitated using a fluoroscope (Figure 4, 5, and 6)[18]. The first treatment includes one PRP intraarticular infiltration and two PRP intraosseous infiltrations (in femoral condyle and tibial plateau).Two more weekly intraarticular infiltrations are performed. The group of Sanchez et al. have found after a 6 month follow-up, a significant pain reduction and decrease of MSC and CFU-F in synovial fluid with no adverse effects[19,20,66]. 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[68].

4. DISCUSSION AND FUTURE PERSPECTIVES

Intraarticular delivery is an alternative modality to convey PRP in patients with KOA and it has been shown to be safe and efficacious in improving clinical symptoms[11,13,65]. This route of drug delivery reaches the synovial membrane (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[69,70]. 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, since the lymphatic drainage clears proteins in a few hours. This is not the case of PRGF, since it acts as a dynamic liquid scaffold with a fibrin network from where GFs are gradually released into the tissue[71,72]. Moreover, the increasingly recognized role of SB in the pathophysiology of OA[3,7,24,28] might make the intraarticular route insufficient to tackle all the joint tissues involved in KOA.

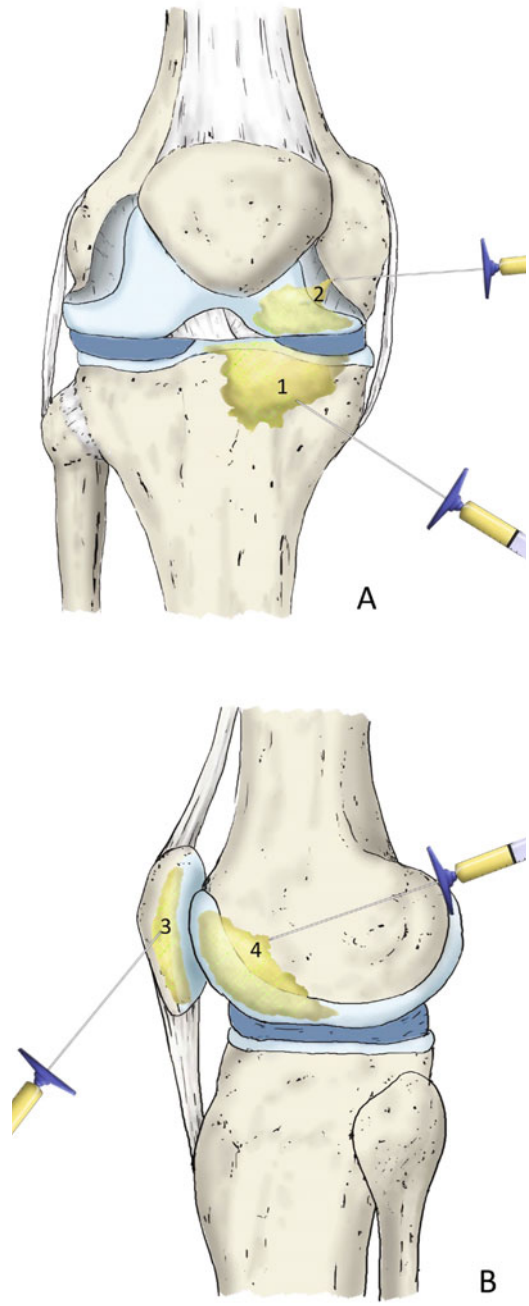

FIG. 4
(A) The platelet-rich plasma (PRP) intraosseous infiltration of a knee with severe femorotibial osteoarthritis is performed into the medial tibial plateau (1) and medial femoral condyle (2). (B) If the patient presents with femoropatellar osteoarthritis, the approach is external and the patella (3) and trochlea (4) are infiltrated. Before these intraosseous injections are performed, conventional knee intraarticular infiltration of PRP is conducted. (Reprinted with permission from Sánchez, M. et al.)[18]

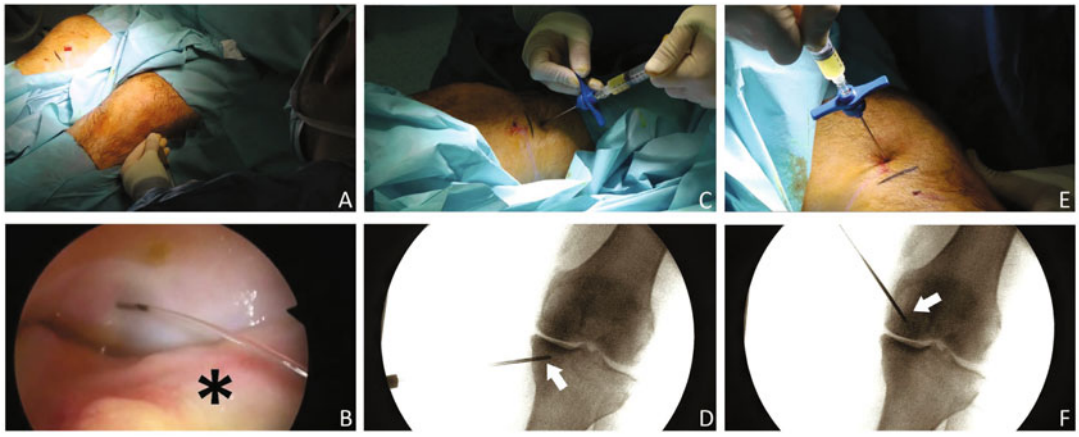

FIG. 5
After the patient is positioned supine on the operating room table, (A) intra-articular infiltration is performed into the joint through the external patellar wing, centred in the central region of the patella in the craniocaudal plane; (B) the infiltration is directed into the midpoint area of the femoropatellar region using an external approach and preventing infiltration into the synovial membrane, (asterisk). (C, D) Intraosseous tibial plateau infiltration is conducted into the medial tibial plateau, just to its middle area. The arrow indicates the trocar. (E, F). With respect to intraosseous femoral condyle infiltration, a trocar (arrow) is applied to the thickness of the medial femoral condyle, as far as the middle area of the medial condyle. (Reprinted with permission from Sánchez, M. et al.) [18]

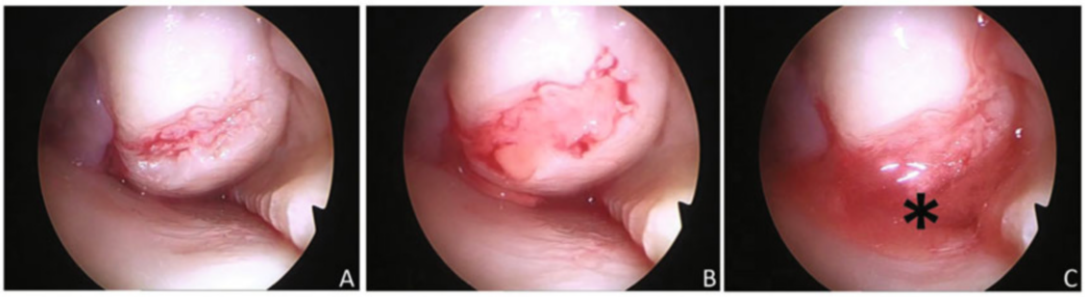

FIG. 6
(A) Communications between cartilage and subchondral bone are more pronounced in degenerated cartilage. (B) The platelet-rich plasma infiltrated into subchondral bone flows through the degenerated zones, and because of its viscous consistency, (C) it remains in the area, creating a matrix (asterisk). (Reprinted with permission from Sánchez, M. et al.) [18]

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[73,74]. 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 effect reported[75]. There is good in vitro and in vivo evidence that events in the subchondral bone concur with and have a direct effect on the overlying articular cartilage[4,27,29,76]. Moreover, although the titles and much of the text of Liu et al[63] and Philippart et al[64] papers are not focused on osteoarthitis, these studies shed important light on the role that intraosseous infiltrations of PRGF might play in subchondral bone homeostasis by targeting both osteoblast-osteoclast coupling and mesenchymal stem cell 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 PRGF intraosseous infiltrations (figure 3)[18-20].These PRGF infiltrations convey a mimetic biomaterial embedded with a pool of growth factors acting as a smart scaffold[77] 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[78-81], all conveyed by PRGF 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 PRGF 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 PRP on several cell phenotypes[79-81]. This new reestablished homeostatic balance between TGFB1 and HGF[39,48] 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[82].

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 due to the presence/absence of leukocytes, the platelet concentration, the donors age, and the manner in which PRPs are applied to the damaged tissues[83]. Moreover, we need to delve into the particular effect of PRP on MSCs in addition to the systemic effect that this procedure might entail since few human studies have been carried out regarding PRP treatments and systemic effects[84,85].

5. CONFLICT OF INTERESTS

The authors declare the following competing financial interest(s): One author is the Scientific Director of and two authors are scientists at BTI Biotechnology Institute, a dental implant company that investigates in the fields of oral implantology and PRGF-Endoret technology.

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