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Current concepts in intraosseous Platelet-Rich Plasma injections for knee osteoarthritis

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ABSTRACT

Knee osteoarthritis (OA) is a degenerative process that slowly destroys the joints producing pain and loss of function, and diminishes the quality of life. Current treatments alleviate this symptomatology but do not stop the disease, being total knee arthroplasty the only definitive solution. Among the emerging treatments, Platelet-Rich Plasma (PRP) has shown promising results in the treatment of OA. However, to improve its effectiveness, it is necessary to approach this pathology targeting the whole joint, not only the cartilage, but including other tissues such as subchondral bone. The pathological processes that occur in the subchondral bone have influence of the cartilage loss, aggravating the disease. The combination of intraarticular infiltrations with intraosseous infiltrations regulates the biological processes of the tissues, reducing the inflammatory environment and modulating the overexpression of biomolecules that generate an aberrant cellular behavior. Although the first clinical results using this technique are promising, further research and developing adequate protocols are necessary to achieve good clinical results.

1. Introduction

Osteoarthritis (OA) is a degenerative process that slowly destroys joints producing pain, loss of function and deformation of the affected areas. Quality of life can decrease considerably due to the pain and lack of movement, becoming incapacitating in advanced stages. As life expectancy continues to rise, and the incidence of obesity continues to increase, the prevalence of OA will also follow, leading to significant economic, social and health burden worldwide.[1] In advanced countries the estimates point to some 46 million patients with OA, more than 50% of adults over 50 years old. By the year 2030 this figure can reach 70 million.[2]

Currently no treatment is able to stop the progression of OA or reverse the damage caused, leaving total knee arthroplasty as the only real solution for these patients.[3] Conservative treatments include oral pharmacology namely, analgesics and NSAIDs, and intraarticular infiltrations such as corticosteroids and hyaluronic acid, which focused on the relief of symptoms but not resolving the disease. Research efforts employed in developing new treatments should be focused on modifying the evolution of OA.

An innovative therapy that has emerged as an alternative to current treatments is Platelet-Rich Plasma (PRP). It is a biological and autologous therapy that uses the patient's own blood in order to obtain plasma with a higher platelet concentration than blood. PRP is a source of active biomolecules as well as a transient autologous fibrin scaffold for regenerative purposes. Several of these growth factors act on the entire joint, influencing the development of OA.[4] Variables such as the number of platelets, the presence of leukocytes and type of activation condition the PRP and its consequent result. Several authors have tried to classify the different PRP to achieve standardization of protocols,[5] but there is still a great variability that sometimes causes contradictory results.[6]

The success of this treatment lies not only in the characteristics of PRP but also in its correct application. An inappropriate

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application of PRP, can lead to an ineffective biological response and unsatisfactory clinical outcomes. Intra-articular infiltrations reach the cartilage and the synovial membrane, promoting a change in the biological environment of the knee that slows the progression of OA and modulate the clinical symptoms. However, this form of infiltration does not reach the deeper layers of subchondral bone, a key element in the pathogenesis of OA.[4] This work describes the importance of subchondral bone in OA and how it can be a target for the biological action of PRP, achieving promising clinical results with suitable protocols.

2. Intraosseous PRP: targeting subchondral bone

2.1. The role of subchondral bone in osteoarthritis

The main characteristic that has always been associated with the development of OA is the loss of the articular cartilage. However, in this disease the whole joint acts as a single and complex organ, with multiple structures affected simultaneously. Other less obvious processes underlie this loss of cartilage, affecting key structures such as the synovial membrane and the subchondral bone, feeding each other and causing a total failure of the joint.[7] With this holistic approach of the pathogenesis and progression of OA, it is necessary to consider the subchondral bone as a fundamental factor in this pathology.[8]

Subchondral bone is located beneath the calcified cartilage line forming the osteochondral unit and its structure consists of a plate of cortical bone from where the bone marrow and trabecular bone areas emerges.[9] The structure of the subchondral bone along with other periarticular tissues such as muscle and tendon, lighten the load that cartilage supports, absorbing between 30% and 50% of the energy received in the joint.[10] In spite of calcified cartilage and the cortical plate being nonporous, a communication between the cartilage and the subchondral bone does exist. This cross-talk has been demonstrated in models of animal experimentation.[11] This bone-cartilage communication has been evidenced by studies showing how vessels and channels reach the cartilage from the subchondral bone, and that they are also more abundant in the cartilage of patients with OA. Channels and vessels allow the transit of molecules involved in the homeostasis of the joint as growth factors or bone morphogenetic proteins. Vessels coming from the subchondral bone provide the cartilage with an important nutritional source.[12,13] Therefore, proper communication and synergy between these tissues entails the optimal function of the joint and cartilage homeostasis maintenance.

When homeostasis is altered due to biochemical and biomechanical changes, all tissues of the joint participate in restoring the biological imbalance. These efforts to recover homeostasis are translated into responses at the cellular level and the extracellular matrix in all tissues. Although the sequence and timing of steps generated in cartilage, subchondral bone and synovial membrane that trigger OA are unclear, they accelerate cartilage loss and worsen pathology (Fig. 1).[14]

Microfractures and bone edema lesions provoke an abnormal distribution of mechanical loading over the osteochondral unit, which breaks the homeostasis of the joint due to biochemical and biomechanical stimuli.[15] Products originated from extracellular matrix degradation act as toll-like receptor (TLR) ligands and damage-associated molecular patters (DAMPS) and join the TLR-2 and TLR-4 receptors of several joint cells, namely macrophages, fibroblast, chondrocytes and osteoblasts. This process triggers the intracellular signaling pathway nuclear factor kappa B (NF-kB)[16,17] that promotes a pro-inflammatory environment by means of expression of inflammatory genes and cytokines such as tumor necrosis factor alpha (TNF-α), prostaglandine E2 (PGE2) or interleukin (IL-6).[18] This abnormal biological environment promotes cartilage degradation due to overexpression of Nerve Growth Factor (NGF), Transforming Growth Factor Beta (TGF-β) and Vascular Endothelial Growth Factor (VEGF) by osteoblasts from subchondral bone that disrupts the bone remodeling and fibroneuroangiogenesis, resulting in angiogenesis and growth of sympathetic and sensory nerves.[19,20] In addition, the high levels of TGF-β in subchondral bone during OA alter Mesenchymal Stem Cells (MSCs) behavior which action is essential during bone remodeling. Several studies showed a high recruitment of MSCs in bone marrow lesions although its proliferation and mineralization is decreased, thus its repair effect is compromised.[21,22] Recent works find that the OA can be caused by senescent MSCs and those cells can be the target of future treatments, in order to improve the MSCs pool and slow down the progression of the disease.[23] Therefore, OA is the result of several pathological processes occurring in all joint tissues, with subchondral bone as a key element. Therapies targeting not only articular cartilage but also the other involved elements can potentially lead to better clinical outcomes.

2.2. Action of the PRP on the subchondral bone

Although intraarticular infiltrations of PRP to treat knee OA are showing promising results, this technique only targets articular cartilage and synovial membrane without reaching subchondral bone. Adding intraosseous injections to target subchondral bone can provide a more comprehensive treatment.

The use of drugs that act in the subchondral bone such as alendronate and zoledronic acid, have shown improvements in the quality and structure of this tissue, preventing cartilage loss.[24] It is reasonable to think that direct infiltrations of PRP into the subchondral bone can stimulate biological processes that improve the environment of this structure leading to an improvement in OA. As mentioned above, the generation of a pro-inflammatory environment is one of the most relevant factors in the pathogenesis of this disease. The anti-inflammatory effect of PRP can be one of the key elements in its therapeutic effect, achieving it through different biological pathways.

Growth factors as well as platelet microplates within PRP increase the presence of M2 macrophages phenotype, which is related to reparatory functions instead of inflammatory response.[25] Several studies have demonstrated the balanced action of growth factors in PRP such as Hepatocyte Growth Factor (HGF) and (Insulin-like Growth Factor-1 (IGF-1), inhibiting the NF-kB signaling pathway in synovial fibroblast, chondrocytes and osteoblast, reducing the synthesis of TNF-α and IL-1β and interrupting the inflammatory process.[26] Finally, PRP acts on the mechanism of oxidative stress, which influences the catabolic state of subchondral bone.[27] PRP activates the antioxidant response element (ARE) in osteoblast cultures, protecting cells from reactive oxygen species (ROS) and oxidative stress.[28] Thanks to these processes, restoring a favorable biological environment has a positive impact on the bone remodeling and fibroneurovascular growths of subchondral bone during OA. Avoiding or reducing uncontrolled tissue fibrosis or angiogenesis can be decisive in stopping or slowing the progression of pathology. Although PRP contains proangiogenic and profibrotic factors, no aberrant growth has been reported during PRP treatments for knee pathologies.[29]

Restoring joint homeostasis also influences the behavior of MSCs that coordinate bone remodeling of subchondral bone. The modulating action of PRP could reduce overexpression of TGF-β responsible for aberrant MSCs during OA. Zhen et al. achieved attenuation of articular cartilage degeneration by inhibiting TGF-β signaling in nestin positive-MSCs present at subchondral bone.[20] Moreover, in vivo studies showed that intraosseous infiltrations

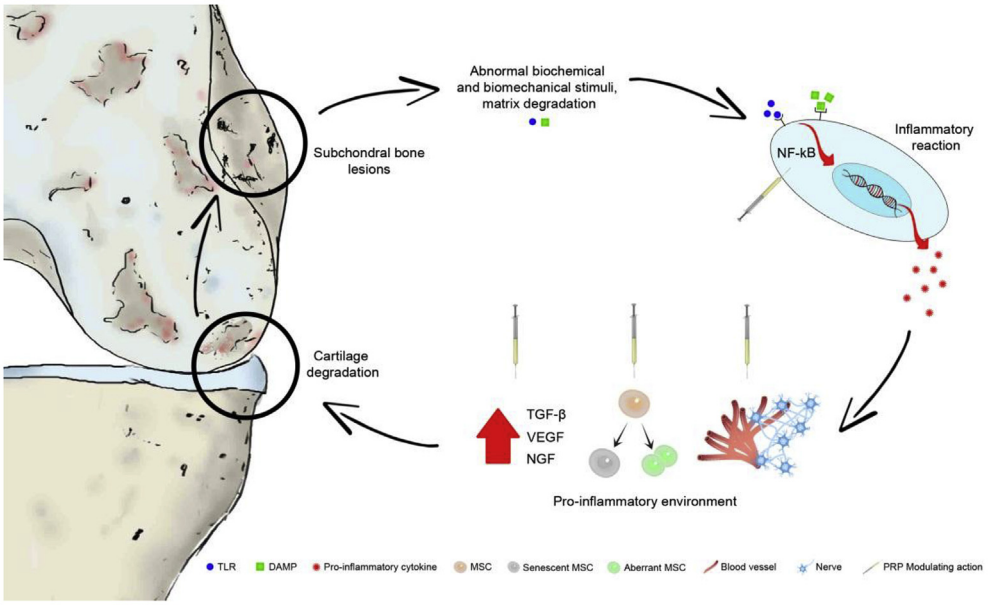

Fig. 1. Osteoarthritis pathogenesis and PRP biological effects.
Subchondral bone lesions such as edemas or microfractures result in a load decompensation that produces biochemical and biomechanical stimuli in part because of degradation of the extracellular matrix. This activates the NF-kB intracellular pathway that generates an inflammatory environment due to the production of cytokines. In this environment there is an imbalance at the molecular, cellular and tissue level generating cartilage degeneration and restarting the process again. The modulating action of PRP acts on the inflammatory response, the overexpression of biomolecules, the MSCs alteration and the growth of neurovascular tissue. TLR: toll-like receptor; DAMPS: damage-associated molecular patters; MSC: Mesenchymal Stem Cell; PRP: Platelet-Rich Plasma; TGF-β: Transforming Growth Factor Beta; VEGF: Vascular Endothelial Growth Factor; NGF: Nerve Growth Factor.

of PRP rescued MSCs from senescence and consequently recovered cell potential, enhanced their osteogenesis and prevented from oxidative stress.[30] Therefore, the direct action of PRP on subchondral bone positively stimulates subchondral bone cells. This therapeutic effect influences articular cartilage, because of communication and cross-talk between both tissues which is more pronounced during OA.

3. Clinical outcomes of intraosseous injections

The current body of clinical research for intraosseous application of PRP in treating OA is in its infancy. The technique was largely absent from research until its introduction by Sánchez et al., in 2014.[31] However, the evolution up to this point is strongly correlated with our expanding knowledge of the role of subchondral bone in OA development in conjunction with preclinical studies investigating the role of PRP on the subchondral environment. A strong influence can also be found from other intraosseous treatments that have been used to treat bony pathology, namely the Subchondroplasty (Zimmer Biomet, Warsaw, IN) for treating bone marrow lesions and intraosseous injections for osteonecrosis.

The importance of subchondral bone and its role in OA has become more apparent as we gain understanding of the communication between subchondral bone and articular cartilage, a connection referred to as the osteochondral functional unit. Clinically, bone marrow lesions (BMLs) have been shown to be strongly associated with disease worsening in OA, as well as increased pain in knee OA.[32,33] In addition, the presence of BMLs has been attributed to more rapid progression of joint degradation and increased risk of total knee arthroplasty (TKA).[34] Significant effort is being devoted to understanding the true cellular mechanisms for BML's association with pain and how they contribute to disease progression. A recent study utilized microarray analysis to look at the histological characteristics and genetic expression within BMLs. The study not only found pain to be linearly correlated with OA progression, but also with changes in the microenvironment of subchondral BMLs. Analysis of the BMLs showed reduced bone marrow volume replaced by dense fibrous connective tissue, new blood vessels, hyaline cartilage and fibrocartilage. Furthermore, BML's were characterized as regions of high metabolic activity with gene expression involved in pain, neuronal development, ECM turnover, cartilage and bone formation and angiogenesis.[35]

Various intraosseous injection techniques have been utilized in the past for other bony pathology, such as the Subchondroplasty (Zimmer Biomet, Warsaw, IN) for bone marrow edema. The procedure involves fluoroscopic and arthroscopic injection of Calcium Phosphate into subchondral bone lesions.[36] A retrospective case review in 2017 of 133 knee subchondroplasties showed the procedure to be an effective and well received treatment for patients with knee OA and bone marrow edema. The study showed that only 25% of patients, who failed conservative measures and were considering TKA, actually converted to TKA after receiving a subchondroplasty at 2.5 year follow up.[37] Another study from 2018, examining 164 patients with bone marrow lesions who were treated with Subchondroplasty (Zimmer Biomet, Warsaw, IN) showed significant functional improvement and pain reduction after subchondral calcium phosphate treatment, as well as a 70% reduction in TKA conversion, from patients who had a previous indication for surgery.[38]

Intraosseous biologics have also been used for treatment of osteonecrosis. The technique was first introduced by Hernigou and Beaujean in 1993. They injected 189 hips (116 patients) with MSCs, derived from a patient's bone marrow, through a core decompression tract into the area of necrosis. Patients with early disease showed positive results at 5–10 years follow-up, with only nine of 145 hips requiring total hip arthroplasty.[39] Since this preliminary research, there have been many other studies throughout the procedure's evolution, involving various techniques and preparations for utilizing intraosseous MSCs[40] as well as PRP[41] for osteonecrosis. A systematic review published in 2017 found that of the 10

studies with level III evidence, patient-reported outcomes showed improvements in the cell-therapy groups compared with the control group. Overall, 24.5% (93/380 hips) that received cell-therapy showed radiographic disease progression compared with 40% (98/245 hips) in the control group. Nine of 10 studies that reported failure rates showed a lower total hip arthroplasty conversion rate in the cell-therapy group 16% (62/380 hips) compared with the control group 21% (52/252 hips).[42] Although the most of aforementioned studies were carried out with cell-therapy products, the intraosseous infiltration of PRP can achieve a biological effect by stimulating MSCs of the subcondral bone niches. A study by Kruger et al. suggested that PRP may enhance the migration and stimulate the chondrogenic differentiation of human subchondral progenitor cells.[43] In addition, Muinos-Lopez et al. showed that subchondral infiltration combined with intraarticular application of PRP reduced the number of MSCs in synovial fluid of OA knees, where intraarticular application alone did not cause changes in the synovial fluid MSC population, illustrating the potential role of subchondral PRP injections in modulating the intraarticular environment.[44] It is worth noting that high levels of MSCs in synovial fluid are associated with more severe joint OA, and that most MSCs in degenerated joints are thought to be diseased, dysfunctional or senescent. This decrease in MSC concentration in synovial fluid after PRP infiltration is thought to return MSC level to a healthy concentration.[45] In addition, PRP meets advantages that justify its choice as a therapeutic tool such as being a less invasive technique and a composition in which the amount of white blood cells and proinflammatory factors is lower than in that of the bone marrow concentrates.[46]

The aggregate of research exploring the role of the osteochondral functional unit in OA, in combination with preclinical studies, as well as other intraosseous techniques have led to the development of preliminary human trials for intraosseous PRP in the treatment of OA. Sanchez et al. published the preliminary results of a pilot study involving 14 patients with severe knee OA in 2016.[47] The patients received an intraarticular injection on 8 ml of leukocyte poor PRP, as well as two subchondral intraosseous injections containing 5 ml of PRP into the medial tibial plateau and the medial femoral condyle with fluoroscopic guidance. They received 2 more intraarticular PRP injections at 7 and 14 days after the initial procedure. At 6 months follow up, patients showed a statistically significant improvement in KOOS pain score from 61.55 ± 14.11 at baseline to 74.60 ± 19.19 after treatment (?? = 0.008), as well as all other areas of the KOOS scale. In 2018, Sánchez et al. performed an observational study (n = 60) comparing intraarticular PRP (IA) alone versus intraosseous + intraarticular PRP (IO + IA) for severe knee OA.[48] At 2, 6 and 12 months after treatment, the IO + IA group had a significant improvement in all KOOS and WOMAC subscales ($P < 0.05$), while the IA group did not improve in any of the scores. Sixteen out of 30 IO + IA group showed minimal clinically important improvement (MCII) compared to 8 out of 30 in the IA group at 6 months ($p < 0.05$). At 12 months, 14 patients of IO group and 5 patients of the IA group showed MCII ($p < 0.05$). The most recent study by Su et al., in 2018 further examined intraarticular (IA) and intraosseous (IO) applications of PRP for 86 patients with knee OA. Patients were randomly assigned to 1 of 3 groups: IA + IO PRP (group A), IA PRP (group B), or IA HA (group C). Patients in group A received IA + IO PRP (administered twice, 2 weeks apart). Patients in group B received IA injection of PRP every 14 days. Patients in group C received a series of five IA injections of hyaluronic acid every 7 days. The combination of IO + IA PRP resulted in significant clinical outcomes, with sustained lower VAS and WOMAC scores and improvement in quality of life at 18 months follow up ($p < 0.05$, n = 82).[49] Finally, intraosseous PRP has also been applied for treatment of hip OA. In 2017, Fiz et al. presented a similar technique involving intraarticular and intraosseous PRP for hip OA.[50] The technique combined a conventional 8 ml intraarticular leukocyte poor PRP injection with two 5 ml fluoroscopic guided subchondral intraosseous injections of PRP into the acetabulum and femoral head. Patients also received repeat ultrasound guided intraarticular PRP injections at 7 and 14 days after the initial treatment.

4. Intraosseous infiltration of PRP for KOA: a technical note

In order to reach all the key tissues in more advanced KOA, it is necessary to combine intraarticular with intraosseous infiltrations of PRP.[31] Once the blood is extracted to prepare PRP, the patient is sedated and positioned supine on an operating room table. Preparation of the sterile field is required to maintain aseptic conditions throughout the treatment. In general, conscious sedation is administered prior to the procedure.

1. The first step is to perform intra-articular infiltration. The joint is penetrated through the external patellar wing with a 21G or 22G needle. Once it is placed into the joint space, synovial fluid arthrocentesis is conducted if required, and without removing the needle, 8 ml of PRP (2–3x platelet concentration, no leukocytes) is infiltrated into the mind-point area of the femoropatellar region using a lateral infrapatellar approach. The injection into the synovial membrane is avoided because it may cause pain for the patient.
2. Next, intraosseous injections are performed on the tibial plateau and the femoral condyle using either an 11, 13, or 15 gauge trocar for both cases:
   a Infiltration into the medial tibial plateau is conducted intto the middle area of this structure. The trocar is placed 1 cm close to the tibial plateau surface using an inclination of 45° (Fig. 2A).
   b Concerning intraosseous femoral condyle infiltration, the trocar is applied to the thickness of the medial femoral condyle, as far as the middle area of it. An inclination of 45° from cranial to caudal is used, placing the trocar 1 cm close to the subchondral bone (Fig. 2B).

Five mL of PRP is infiltrated both into the tibial plateau and into the femoral condyle. After infiltration is completed, the site undergoes cryotherapy as needed. In the days following surgery, the patient can bear weight as tolerated and take analgesics (acetaminophen) for pain Image guidance is necessary to perform this procedure correctly.

The use of fluoroscopy facilitates the placement of the trocar, achieving precision during infiltration. However, radiation can be a limitation in the use of this technique. In order to overcome this drawback, the injection can be guided by ultrasound instead of x-ray. In this case, the meniscus is used as a reference to localize the articular line. Thus, after locating the meniscal wall by ultrasound, a 25G needle is placed into it to have the reference of articular line. For the tibial injection, the trocar is introduced 2 cm distally from the articular line with an inclination of 45° and a depth into the bone of 1.5 cm. In the case of the femoral injection, trocar is placed 2 cm proximally from the articular line using an inclination of 30° and with the same depth as in the tibial injection.

5. Conclusion

Despite advances in our understanding of OA, no treatment is definitive apart from total knee arthroplasty. However, arthroplasty is a less desirable option for younger, active patients because of associated surgical risks and complications. PRP is a promising, minimally invasive therapeutic tool, however both the cellular

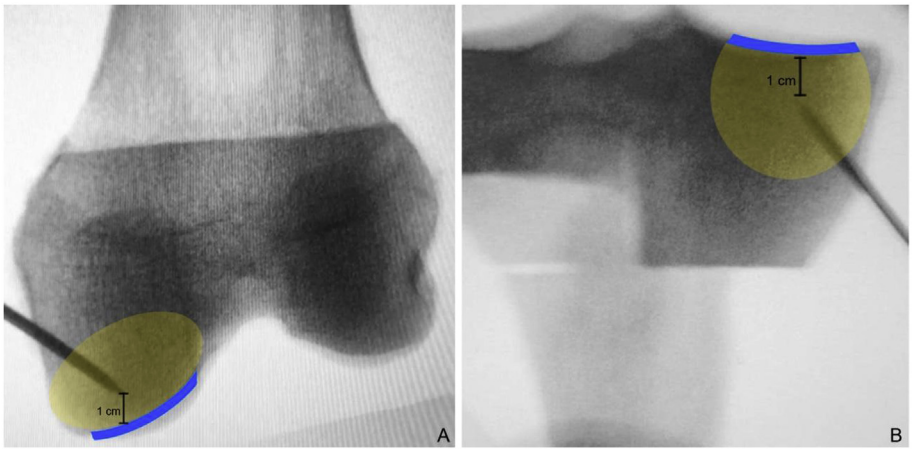

Fig. 2. Fluoroscope image of intraosseous infiltration of PRP.
During intraosseous infiltration into femoral condyle, the trocar is applied to the middle area of medial femoral condyle, placing the trocar 1 cm close to the subchondral bone (A). Tibial infiltration is conducted into the middle area of the medial tibial plateau, just to of this structure. The trocar is placed 1 cm close to the tibial plateau (B).

composition and route of administration are important in its clinical efficacy. The combination of intraarticular application with intraosseous infiltration targets cartilage, the synovial membrane as well as subchondral bone, all key tissues in the development of osteoarthritis. Acting on the biological processes of these structures could delay or even stop disease progression. The clinical research for intraosseous application of PRP for joint OA is currently in the early stages. The rationale for its progression is largely based in our expanding knowledge of the role of the osteochondral functional unit in the development of joint OA, as well as increased preclinical studies, and other intraosseous techniques for other bone pathology. Further research is needed in this area to better understand the cellular processes behind its potential mechanism of action, and future directions for intraosseous injections.

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