Journal of Clinical Orthopaedics and Trauma 10 (2019) 49–52

Contents lists available at ScienceDirect

Journal of Clinical Orthopaedics and Trauma

journal homepage: www.elsevier.com/locate/jcot

Innovative regenerative medicine in the management of knee OA: The role of Autologous Protein Solution

Nicolò Danilo Vitale [a, b], Filippo Vandenbulcke [a, b, *], Emanuele Chisari [c], Francesco Iacono [a, b], Laura Lovato [a, b], Berardo Di Matteo [a, b], Elizaveta Kon [a, b]

[a] Humanitas University, Department of Biomedical Sciences, Via Rita Levi Montalcini 4, 20090 Pieve Emanuele - Milan, Italy
[b] Humanitas Clinical and Research Center, Via Manzoni 56, 20089, Rozzano, Milan, Italy
[c] Department of General Surgery and Medical Surgical Specialties, Section of Orthopaedics and Traumatology, AOU Policlinico-Vittorio Emanuele, University of Catania, 95100, Catania, Italy

ARTICLE INFO

Article history:
Received 19 July 2018
Accepted 23 August 2018
Available online 23 August 2018

Keywords:
Knee OA
Regenerative medicine
Intra articular injection
PRP
HA
APS

ABSTRACT

Osteoarthritis (OA) is one of the most common causes of chronic disability in adults due to pain and altered joint function. Although most patients report pain and functional limitation, symptoms, age of onset and disease progression are extremely variable. While inflammation could play a central role in the OA pathogenesis and progression, many underpinning mechanisms are still unclear. A number of proinflammatory mediators have been found in OA joints and could play a role, such as IL-1, IL-6, IL-7, IL-8, IL-15, IL-17, IL-18, TNF-alpha, macrophage chemotactic protein (MCP)-1, interferon-induced protein (IP)-10, monokine induced by interferon (MIG), oncostatin M (OSM), growth-related oncogene (GRO)-alpha, chemokine (C-C-motif) ligand 19 (CCL19), macrophage inflammatory protein (MIP)-1beta, and TGF-alpha. Biological approaches have recently got increasing interest due to their anti-inflammatory and immunomodulatory properties, regenerative potential, and high tolerability. The primary aim of this paper is to report the current concepts on regenerative medicine for knee OA with a particular focus on Autologous Protein solution (APS). APS is a blood derived product obtained by using a proprietary device, made of APS Separator, which isolates WBCs and platelets in a small volume of plasma, and APS Concentrator, which further concentrates platelets, WBCs and plasma proteins. The result is a peculiar formulation differing from other biologic products as it contains high levels of growth factors (EGF, IGF-1, PDGF-AB, PDGF-BB, VEGF, TGF-β1) along with high concentrations of anti-inflammatory mediators (IL-1ra, sIL-1RII, sTNF-RI, sTNF-RII) and low levels of pro-inflammatory cytokines (Il-1β and TNF-α). While emerging evidence supports the use of APS, as confirmed by in vitro studies and preliminary clinical results, the real clinical potential of APS and its benefits are still under investigation.

1. Introduction

Osteoarthritis (OA) is one of the most common causes of chronic disability in adults due to pain and impaired joint function, which are the result of deep pathologic changes in the articular tissues. Impairment in daily–life activities and working abilities is the main consequence following OA, thus justifying the relevant socio-economic burden which is associated worldwide to such disease, whose etiopathogenesis is still object of intense pre-clinical and clinical research. Age of onset and disease progression are extremely variable, and the available therapeutic strategies range from physiotherapy to pharmacological agents to surgery.[1] In the past, osteoarthritis was often misnamed as a mechanically induced degenerative joint disease. The mechanisms driving to joint tissue destruction were considered to be predominantly mechanical in a so-called “wear and tear” process. However, a variety of other key factors have been identified, such as biomechanics, inflammatory process and age-related factors. All these elements need to be further studied and they could represent a potential targeted for therapy.[2] Recently, there has been an increasing interest towards inflammatory pathways that are involved in the onset and progression of disease. The aim of this current concept paper is to discuss on the role of innovative biologic products as injective

* Corresponding author. Center for Functional and Biological reconstruction of the knee, Humanitas Clinical and Research Center, Via A. Manzoni 113, 20189, Rozzano, Milan, Italy.
E-mail address: filippo.vandenbulcke@humanitas.it (F. Vandenbulcke).

https://doi.org/10.1016/j.jcot.2018.08.019
0976-5662/

treatment of OA, with a special focus on Autologous Protein Solution, which has recently been introduced into clinical practice.

1.1. Inflammatory process in osteoarthritis

Inflammation play a significant role in the pathogenesis of OA, as the underpinning mechanism responsible for the production of proteolytic enzymes involved in the degradation of the extracellular matrix and, lastly, in joint tissue destruction.[3] Classically, inflammatory arthritis, such as rheumatoid arthritis (RA), has been characterized by an extensive synovial infiltrate of leukocytes with synovial fibroblast proliferation, resulting in pannus formation. Differently, in non-rheumatic osteoarthritis, the number of leukocytes in the joint fluid is low (<1000–2000 cells/ml) and synovial inflammation is best appreciated at the molecular level: it is characterized by the presence of a series of pro-inflammatory mediators, including cytokines and chemokines, that are part of the innate immune response to joint injury.[4] A number of proinflammatory mediators have been found in OA joints and could play a role, such as IL-1, IL-6, IL-7, IL-8, IL-15, IL-17, IL-18, TNF-alpha, macrophage chemotactic protein (MCP)-1, interferon-induced protein (IP)-10, monokine induced by interferon (MIG), oncostatin M (OSM), growth-related oncogene (GRO)-alpha, chemokine (C-C-motif) ligand 19 (CCL19), macrophage inflammatory protein (MIP)-1beta, and TGF-alpha[4–.6] Other inflammatory pathways include the alarmins (S100 proteins), the damage-associated molecular patterns (DAMPs) and the activation of complement.[7] Fragments released from the damaged matrix might stimulate the innate immune response and further upregulate degradative pathways through the activation of toll-like receptors and integrins.

Cartilage in OA is not just worn down mechanically: its destruction is mediated by a variety of proteases including matrix metalloproteinases (MMPs), such as ADAMTS-4, ADAMTS-5 and MMP-13. ADAMTS-4 and ADAMTS-5, also known as aggrecanases, are able to destroy the aggrecan, the large proteoglycan that provides much of the resiliency of cartilage. Collagenases, mainly MMP-13, degrade type II collagen that is the most abundant collagen in cartilage, responsible for its tensile strength. Finally, serine proteinases (HtrA1 and activated protein C) appear to be involved in the activation of MMPs by a proteolytic cleavage mechanism, whereas cysteines proteinases including cathepsin K, expressed by osteoclasts, can degrade type I-II collagen.[3]

In this complex network of different molecular pathways, numerous evidences support the role of Wnt-Beta catenin signalling network in the homeostasis of bone and cartilage, and it seems that some biological agents may modulate this pathway and therefore have an effect on OA progression, thus providing clinical benefit clinical outcome[8]

Although destruction and loss of the articular cartilage is a central component of OA, all joint tissues are affected in some way, including the subchondral bone and also soft-tissues such as the synovia, ligaments, the joint capsule, periarticular muscles and nerves,[9] so OA is “a disease of the whole joint as an organ”.[5]

2. Novel therapeutic approaches

Biological approaches for the treatment of OA, such as Platelet rich Plasma (PRP) and bone marrow concentrate have been introduced into clinical practice in the last 15 years and their therapeutic role and mechanism of action have been more and more investigated, revealing their anti-inflammatory and immunomodulatory properties besides a certain regenerative potential towards cartilage and meniscal tissue.[10] A wide spectrum of biologic substances are currently adopted by a minimally invasive procedure (i.e. intra-articular injection) with the aim of restoring joint homeostasis and providing clinical improvement and possibly a disease-modifying effect in early phases of OA.[11]

2.1. Biologic injective therapy

The first attempt with a regenerative blood-derived therapy for degenerative musculoskeletal diseases was made in the mid-1990s. It was observed how whole blood exposed to CrSO(4)-treated glass beads and then centrifugated leads to a serum rich in several anti-inflammatory cytokines including IL-1Ra.[12] The great immunomodulator capacity of these cytokines in vitro suggested their use as an alternative therapeutic approach in vivo. The idea was to obtain an injectable solution enriched in endogenous cytokines capable of restoring joint homeostasis preventing inflammatory diseases to degrade bone and cartilage: the “Autologous Conditioned Serum” (ACS). Baltzer et al.[13] performed a randomized, double-blinded study that showed superior improvement in symptoms and quality of life with ACS over the control groups treated by saline or hyaluronic acid. However, subsequent studies didn't confirm these encouraging results and demonstrated that in ACS not only anti-inflammatory but also pro-inflammatory cytokines (IL-1β, Il-6, TNF-α, OSM) are upregulated. Furthermore, conditioned serum showed a lack of net direct effect on cartilage metabolism.[14]More recently PRP gained increasing attention among conservative treatments for OA. PRP contains pools of growth factors, stored in platelet α-granules, that takes part in the regulation of articular homeostasis and cartilage anabolism[11,15]. Many in vitro and preclinical studies documented positive effects and showed how PRP does not act only on articular cartilage but it targets the entire joint environment, including menisci and synovia.[16] These investigations provided the rationale for several randomized clinical studies: anyway, while some of the RCTs currently available seem to support the superiority of PRP compared to HA in early knee OA,[17] there is still lack of clear evidence to endorse the use of PRP over more traditional injective approaches such as viscosupplementation. Zhang et al.[18] published a systematic review and metanalysis on the clinical efficacy of PRP compared to HA: PRP seemed to be slightly superior compared to a single injection of high molecular weight HA. However, the poor methodology of some investigations raises some relevant bias, and therefore more high quality clinical trials are required. In conclusion, the therapeutic benefits of PRP are still controversial[19] and the need of optimizing its biologic potential is well recognized. Furthermore, PRP contains sometimes white blood cells (WBCs), lacking many of plasma proteins found in other products like ACS. Debate is still open regarding whether or not WBCs should be present in a blood-derived solution and whether they could be detrimental to the clinical outcome. In fact, it has been suggested that leukocytes release reactive oxygen species, metalloproteinases and other lytic enzymes that may stimulate an early inflammatory response within the joint.[20] This hypothesis is supported essentially by in-vitro studies whereas the only in-vivo analysis of synovial fluids of patients after the injection of leukocyte-rich PRP did not reveal a significant synthesis of pro-inflammatory molecules.[21] Other authors also demonstrated more complex effects with less influences of WBCs on cartilage and synovia[22,23]. Some recent findings support a beneficial role of sub-populations of leukocytes that could release molecules positively affecting the intra-articular environment. In fact, it has been showed that leukocytes and their “secretome” can exert many different biologic actions, sometimes even opposing, based on the specific stimuli they are subject to. In light of these premises, a novel technology, called Autologous Protein Solution has been developed and transferred into clinical practice to take advantage of both platelet-derived growth factors and leukocyte-derived molecules.

2.2. The role of autologous protein solution (APS)

APS is a blood derivative technology obtained by the nSTRIDE APS Kit (Zimmer Biomet), consisting of an *APS Separator*, which isolates WBCs and platelets in a small volume of plasma, and an *APS Concentrator*, which further concentrates platelets, WBCs and plasma proteins. APS preparation is pretty simple and requires 55 ml of peripheral blood harvest, which are added to 5 ml of anticoagulant (ACD-A). Then a double centrifugation is performed: the first one, using the APS separator, lasts 15 min at 3200 rpm, whereas the second one, using the APS Concentrator, lasts 2 min at 1500 rpm. The distinctive feature of the APS preparation process is the mechanical filtration of the intermediate product thorough poly-acrylamide beads which are able to further concentrate cytokines, especially those derived from WBCs. The final output is approximately 2.5–3 ml of APS, which has a unique formulation differing from other biologic products as it contains high levels of growth factors (EGF, IGF-1, PDGF-AB, PDGF-BB, VEGF, TGF-β1) along with high concentrations of anti-inflammatory mediators (IL-1ra, sIL-1RII, sTNF-RI, sTNF-RII). On the other hand, APS provides low levels of pro-inflammatory cytokines (Il-1β and TNF-α) as the concentrate is filtrated through beads made of polyacrylamide.[24] The positive effects of APS have been demonstrated in several in-vitro studies, revealing its protective role in cartilage matrix degradation. The combined action of bioactive factors and cytokines inhibits the upregulation of inflammatory cytokines and proteases, promoting concurrent chondrocytes proliferation.[24–26]

As regards pre-clinical studies, APS has been first studied as treatment for horse OA in a prospective RCT with saline as control.[27] Horses treated with APS had significantly better improvements in lameness grade, asymmetry indices of vertical peak force and range of joint motion 14 days after injection. Horses originally treated by saline were administered APS at the end of the study, and the owners independently assessed lameness and comfort as improved at 12 and 52 weeks after injection. Another feature of APS is that it is possible to achieve a high concentrations of growth factors and anti-inflammatory molecules regardless of the degree of articular cartilage degradation and age of the patient, as demonstrated by O'Shaughnessey et al.[28] They showed, in a pool of 82 patients, that no single patient parameter correlated ($R^2 > .7$) with specific key cytokine concentrations in APS. Therefore, APS can be prepared from a broad range of OA patients, with different disease phases, while still obtaining a high-quality product with a strong presence of anti-inflammatory cytokines over the pro-inflammatory one.

In addition to APS unique cytokine, platelets and growth factors pattern, it has been studied the white blood cells (WBC) relationship with the overall outcome of the therapy in OA patients.[20] While other studies involving similar biologics reported an enhancement of the inflammation after administration of leucocyte rich solutions[22,29], what emerged from preliminary APS laboratory tests is that the presence of leucocytes and their molecular activity could be one of the essential components of APS efficacy.[20] In particular, King et al.[20] designed a clinical study to clear their role in APS therapy outcome. While the patients sample was limited to eleven subjected with knee OA, the results were encouraging: the WBC concentration in APS was significantly ($p < 0.05$) and strongly ($R^2 > 0.7$) correlated with IL-1ra in APS but not significantly correlated with IL-1β. The ratio of IL-1ra to IL-1β in APS was significantly correlated with improved WOMAC pain scores one week and six months post-injection, thus supporting a beneficial role coming from the presence of WBC. 85.7% of subjects whose APS had an IL-1ra:IL-1β ratio greater than 1000 or a WBC count greater than 30 k/μl, were OMERACT-OARSI responders six months post-injection. One potential mechanism by which WBCs may promote the positive outcome is through the action of microparticles derived from platelets and stimulating monocytes to become M2 pro-healing cells in the joint.[30] Other autologous solution containing WBCs have been shown to down-regulate NFκβ expression through both an inhibition of cyclooxygenase 2 (COX2) expression and a higher production of NFκβ inhibitor α (Iκβα) by chondrocytes.[31]

Looking at clinical data, a preliminary evaluation including 11 patients with knee osteoarthritis treated by a single intra-articular injection of APS confirmed the safety of APS and the benefit in terms of improvement in WOMAC pain scores, paving the way for further clinical studies.[20]

More recently a pilot multicenter, double-blind, randomized, saline-controlled trial was conducted to assess clinical outcomes of APS at 1 year follow-up.[32] Forty-six patients were randomized in a 2 APS:1 saline distribution. In each group, a single injection of either APS or saline was performed under ultra-sonographic guidance. Concentrations of IL-1ra, sIL-1RII, IL-1β, sTNF-RII and TNFα were measured using ELISA kits (R&D Systems), confirming the elevated quantity of anti-inflammatory cytokines and the low amount of pro-inflammatory ones. All patients were evaluated before the injection and at 2 weeks, 1, 3, 6 and 12 months through WOMAC LK 3.1, Knee injury and Osteoarthritis Outcome Score (KOOS) VAS for pain score, SF-36 The Outcome Measures in Rheumatology-Osteoarthritis Research Society International (OMERACT-OARSI) responder rate was also measured, and responders were defined as subjects who achieved a high degree of improvement in two of the three outcomes (pain, function, global assessment), according to defined criteria.[33] No significant difference in improvement in WOMAC and VAS pain scores in comparison to control group was found at 2 weeks, 1 month, 3 months and 6 months. However at 12 months APS group reported a mean 65% improvement in WOMAC and 49% in VAS, in both cases significantly higher compared to them mean 41% improvement in WOMAC and 13% in VAS reported in the saline group. The OMERACT-OARSI responder rate showed that at 12 months responder in the APS were 65.5%, compared to 50.0% of the control group. A radiological evaluation was also performed, recording changes on X-ray and MRI from baseline to 3 and 12 months after treatment. Possible changes in MRI Osteoarthritis Knee Score (MOAKS) and in joint space narrowing on radiographs from pre-treatment screening to 3 and 12 months follow-up were also evaluated. The MRI analysis showed significant differences between APS group and control in bone marrow lesion (BML) dimensions and osteophytes comparing baseline to 12 months follow-up: in particular, both osteophytes and BML in the lateral femoral condyle remained unchanged over time in the group of patients treated with APS, whereas their size increased in the saline group. No other significant differences were observed. In conclusion this pilot study confirmed safety and efficacy of a single injection of APS up to one year of follow-up, with overall superior outcome compared to saline Laboratory analysis also confirmed its peculiar concentrations of both growth factors, anti-inflammatory cytokines and activators of inflammatory processes. However, the aforementioned pilot RCT included just a small number of patients evaluated at short-term follow-up: therefore, the clinical in-vivo effects of APS and its benefits have to be better investigated.

3. Conclusion

Knee OA is a multifactorial pathology characterized by inflammation and immune response in the joint. Several innovative products have been proposed as injective treatment for OA. In this context APS is one of the most promising biologic treatment currently under investigation. Its peculiar feature lays in the concentration of WBCs cytokines through mechanical filtration in

order to further increase the therapeutic potential. While emerging evidence supports the use of APS, as confirmed by in vitro studies and preliminary clinical results, its real clinical potential has yet to be clarified. Anyway, based on the available data, the role of leukocytes in biologic injectable products should be reconsidered and better elucidated both at the in-vitro and in-vivo level. To this regard, two large pivotal RCTs comparing APS to saline and to Hyaluronic Acid are running in US and in Europe, with the aim of providing sounding data to support the use of APS as a first-line therapy for knee OA.

References

1. Lane NE, et al. OARSI-FDA initiative: defining the disease state of osteoarthritis. *Osteoarthritis Cartilage*. May 2011;19(5):478–482.
2. Loeser RF, Collins JA, Diekman BO. Ageing and the pathogenesis of osteoarthritis. *Nat Rev Rheumatol*. 2016;12(7):412–420. Nature Publishing Group.
3. Troeberg L, Nagase H. Proteases involved in cartilage matrix degradation in osteoarthritis. *Biochim Biophys Acta Protein Proteonomics*. 2012;1824(no. 1): 133–145. Elsevier B.V.
4. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. *Nat Rev Rheumatol*. 2015;11(1):35–44. Nature Publishing Group.
5. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum*. 2012;64(6):1697–1707.
6. Sohn DH, et al. Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4. *Arthritis Res Ther*. 2012;14(1):1–13.
7. Wang Q, et al. Identification of a central role for complement in osteoarthritis. *Nat Med*. 2011;17(12):1674–1679.
8. M. De Santis, B. Di Matteo, et al , "The Wnt pathway as a potential therapeutic target in osteoarthritis :," BioMed Res Int.
9. Roos EM, Herzog W, Block JA, Bennell KL. Muscle weakness, afferent sensory dysfunction and exercise in knee osteoarthritis. *Nat Rev Rheumatol*. 2011;7(1): 57–63.
10. de Girolamo L, et al. Regenerative approaches for the treatment of early OA. *Knee Surg Sports Traumatol Arthrosc*. 2016;24(6):1826–1835. Springer Berlin Heidelberg.
11. Filardo G, et al. Non-surgical treatments for the management of early osteoarthritis. *Knee Surg Sports Traumatol Arthrosc*. 04-Jun-2016;24(6):1775–1785.
12. Meijer H, Reinecke J, Becker C, Tholen G, Wehling P. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. *Inflamm Res*. Sep. 2003;52(10):404–407.
13. Baltzer AWA, Moser C, Jansen SA, Krauspe R. Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis. *Osteoarthritis Cartilage*. Feb. 2009;17(2):152–160.
14. Rutgers M, Saris DB, Dhert WJ, Creemers LB. Cytokine profile of autologous conditioned serum for treatment of osteoarthritis, in vitro effects on cartilage metabolism and intra-articular levels after injection. *Arthritis Res Ther*. 2010;12(3). R114.
15. Olivotto E, et al. Cultures of a human synovial cell line to evaluate platelet-rich plasma and hyaluronic acid effects. *J. Tissue Eng. Regen. Med.* Jun. 2018;12(8).
16. Filardo G, Kon E, Roffi A, Di Matteo B, Merli ML, Marcacci M. Platelet-rich plasma: why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. *Knee Surg Sports Traumatol Arthrosc*. Sep. 2015;23(9):2459–2474.
17. Görmeli G, Görmeli CA, Ataoglu B, Çolak C, Aslantürk O, Ertem K. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial. *Knee Surg Sports Traumatol Arthrosc*. Mar. 2017;25(3):958–965.
18. Zhang H, Wang C, Li H, Huang Y, Li Z. Intra-articular platelet-rich plasma versus hyaluronic acid in the treatment of knee osteoarthritis: a meta-analysis. *Drug Des Dev Ther*. Mar. 2018;12:445–453.
19. Filardo G, et al. Platelet-rich plasma intra-articular knee injections show no superiority versus viscosupplementation: a randomized controlled trial. *Am J Sports Med*. Jul. 2015;43(7):1575–1582.
20. King W, van der Weegen W, Van Drumpt R, Soons H, Toler K, Woodell-May J. White blood cell concentration correlates with increased concentrations of IL-1ra and improvement in WOMAC pain scores in an open-label safety study of autologous protein solution. *J. Exp. Orthop.* Dec. 2016;3(1):9.
21. Mariani E, et al. Leukocyte-rich Platelet-rich Plasma injections do not up-modulate intra- articular pro-inflammatory cytokines in the osteoarthritic knee. *PLoS One*. Jun. 2016;11(6). e0156137.
22. Assirelli E, et al. "Effect of two different preparations of platelet-rich plasma on synoviocytes," *Knee Surgery. Sport. Traumatol. Arthrosc.* Sep. 2015;23(9): 2690–2703.
23. Cavallo C, et al. Comparison of platelet-rich plasma formulations for cartilage healing: an in vitro study. *J. Bone Jt. Surg. - Ser. A*. Mar. 2014;96(5):423–429.
24. O'Shaughnessey KM, Panitch A, Woodell-May JE. Blood-derived anti-inflammatory protein solution blocks the effect of IL-1β on human macrophages in vitro. *Inflamm Res*. Oct. 2011;60(10):929–936.
25. Matuska A, O'Shaughnessey K, King W, Woodell-May J. Autologous solution protects bovine cartilage explants from IL-1??- and TNF??-induced cartilage degradation. *J Orthop Res*. Dec. 2013;31(12):1929–1935.
26. Woodell-May J, Matuska A, Oyster M, Welch Z, O'Shaughnessey K, Hoeppner J. Autologous protein solution inhibits MMP-13 production by IL-1β and TNFα-stimulated human articular chondrocytes. *J Orthop Res*. Sep. 2011;29(9): 1320–1326.
27. Bertone AL, et al. Evaluation of a single intra-articular injection of autologous protein solution for treatment of osteoarthritis in horses. *Am J Vet Res*. Feb. 2014;75(2):141–151.
28. O'Shaughnessey K, et al. Autologous protein solution prepared from the blood of osteoarthritic patients contains an enhanced profile of anti-inflammatory cytokines and anabolic growth factors. *J Orthop Res*. Oct. 2014;32(10): 1349–1355.
29. Braun HJ, Kim HJ, Chu CR, Dragoo JL. The effect of platelet-rich plasma formulations and blood products on human synoviocytes: implications for intra-articular injury and therapy. *Am J Sports Med*. May 2014;42(5):1204–1210.
30. Vasina EM, Cauwenberghs S, Feijge MAH, Heemskerk JWM, Weber C, Koenen RR. Microparticles from apoptotic platelets promote resident macrophage differentiation. *Cell Death Dis*. Sep. 2011;2(9):e211.
31. Bendinelli P, et al. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: mechanisms of NF-κB inhibition via HGF. *J Cell Physiol*. Dec. 2010;225(3):757–766.
32. Kon E, Engebretsen L, Verdonk P, Nehrer S, Filardo G. Clinical outcomes of knee osteoarthritis treated with an autologous protein solution injection: a 1-year pilot double-blinded randomized controlled trial. *Am J Sports Med*. Jan. 2018;46(1):171–180.
33. Pham T, et al. OMERACT-OARSI Initiative: osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. *Osteoarthritis Cartilage*. May 2004;12(5):389–399.