Journal of Clinical Orthopaedics and Trauma 10 (2019) 32–35

Contents lists available at ScienceDirect

Journal of Clinical Orthopaedics and Trauma

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

Improvising PRP for use in osteoarthritis knee- upcoming trends and futuristic view

Mandeep S. Dhillon [a], Sandeep Patel [a, *], Tungish Bansal [b]

[a] Department of Orthopaedics, Post Graduate Institute of Medical Education and Research, Chandigarh, 160012, India
[b] Dept of Orthopaedics, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India

ARTICLE INFO

Article history:
Received 31 August 2018
Received in revised form
8 October 2018
Accepted 11 October 2018
Available online 16 October 2018

Keywords:
PRP
Osteoarthritis knee
Scaffolds

ABSTRAC

Platelet rich plasma (PRP) has clearly emerged offering a possible solution that could modify the disease process and offer symptomatic relief in early osteoarthritis of knee. Great efforts are underway to improve PRP products for use in knee osteoarthritis (OA). Upcoming research is focused on the ideal PRP type, dose, frequency of injection and mode of injection. The combination of PRP with biocompatible carriers/scaffolds like gelatin hydrogel and chitosan appear to be promising based on early in vitro and animal studies. PRP in combination with hyaluronate has also emerged to have synergistic and additive effects. This article intends to review the recent trends and advancing ideas for improvising PRP for use in early knee OA treatment.

1. Introduction

Platelet Rich plasma is being widely used by orthopaedic surgeons for varying conditions throughout the world owing to its healing potential contained within the platelets. Positive results have been uniformly observed by various researchers for Early Knee OA in the past few years.[1,2] OA knee is a heterogenous disease wherein the dynamic equilibrium between the breakdown and repair of joint tissues becomes unbalanced. There is marked interactions between synoviocytes, cartilage matrix, Hyaluronate with changes in chemical and biological properties. PRP has clearly demonstrated its supremacy in comparison to Hyaluronic acid and placebo in various clinical trials and is undoubtedly the best option available that could modify the disease process in early Osteoarthritis of knee.[1–4]

The release of growth factors from PRP occurs immediately upon injection into an arthritic joint, and the effects can last upwards of a year.[1,2] PRP in combination with biocompatible carriers/scaffolds or with hyaluronate appear to be a way of prolonging the effects of PRP in the arthritic joint. With its safety and efficacy clearly being established, we review advances in PRP use, and new methods of harnessing the power of regenerative medicine in research, and clinical translation in the future.

1.1. Ideal PRP composition and volume

Parameters which define PRP type are the presence of leukocytes, volume, concentration, and activation. . There are various classifications[5,6] which classify PRP type based on the above factors. Different preparation methods are known to yield different PRP product in the same donor.[7] Intra-individual variation in the PRP product with the same technique at different time frames is also noted.[8] The ideal PRP needed for treatment of knee osteoarthritis is not clearly defined and requires further standardization. Additionally, all PRP preparations are not the same and the following are some of the parameters which define clinical use and our understanding.

1.1.1. Leucocyte rich versus leucocyte poor PRP

This was a hot topic of research 7–8 years back and the studies so far suggest that both are clinically effective and safe, however the minor adverse reactions like pain and effusion were less with leucocyte poor PRP and it appears to be slightly better. Recent clinical studies by Riboh et al.[9] and Filardo et al.[10] support the use of Leucocyte free PRP for knee OA.

1.1.2. Volume of PRP required for knee OA

It is suggested by some, that PRP refers to a product of at least 4 x baseline concentration of platelets ($1.5–4.5 \times 10^5$/lL) in order to label the product as PRP. What has not been studied is the ideal volume to be injected. Most of the clinical studies have used 4–5 ml

* Corresponding author.
E-mail addresses: drdhillon@gmail.com (M.S. Dhillon), sandeepdrpatelortho@gmail.com (S. Patel).

https://doi.org/10.1016/j.jcot.2018.10.005
0976-5662/

PRP.[1,3,4] Spakova et al.,[3] and Paterson et al.,[11] have used 3 ml PRP; Li et al.,[12] has used 3.5 ml. In contrast, Patel et al.,[2] and Sanchez et al.,[13] have used 8 ml PRP. Various commercial PRP kits usually focus on taking minimal patient blood ranging from 20 ml to 30 ml and the final product is usually 3–4 ml. Our experience is that for adequate treatment of osteoarthritic knees, volumes of at least 8 ml of PRP should be injected and this has been confirmed by randomized controlled studies.[2,13] The rationale is to ensure that platelets as well as plasma in the final PRP product can diffuse throughout the joint reaching all areas of the synovium. When PRP is activated, the plasma in the product forms a clot which traps the platelets thereby creating a biologic scaffold. We routinely use 8 ml PRP for knee joints and we refer to this as ***"SuperdosePRP".*** Additionally the absolute number of platelets is also more and hence more growth factors. Producing 8 ml PRP would mean drawing more blood and hence a burden for the current commercial kits available as they have to recalibrate and update the kits. This is a major clinical implication as most of the clinicians have been using less volume PRP into Knee joints. We feel the present kits which yield 3–4 ml PRP is ideal for tendinopathies where we usually need less volume.

1.1.3. Activated versus non activated PRP

The term "activation of PRP" refers to 2 key processes that are initiated during PRP preparation: Firstly, degranulation of platelets to release growth factors (GFs) from α-granules followed by fibrinogen cleavage to initiate matrix formation, a clotting process which allows the formation of a platelet gel, and therefore to confine the secretion of molecules to the chosen site. It can be done by using 10% CaCl2, 10% autologous thrombin or mixture of 10% CaCl2 +10% autologous thrombin or 10% calcium gluconate. Cavallo et al.,[14] has compared the amount of growth factor released from PRP with and without activator, and concluded that there was an increased concentration of growth factor release when PRP was used with activator, and the effects were immediate and sustained for up to 24 h. Proponents of activator believe that the main role of activator is to increase growth factor release and further augment PRP. Those who don't use activator argue that PRP once given gets activated endogenously as it comes in contact with the tissue.[3,15]

Multiple studies have been conducted investigating the clinical outcomes of use of PRP in knee OA with activation[1,2,10,12] and without activation[3,15] however, no head-to-head comparison has been published. This is a likely area for future investigation.

1.2. Platelet rich plasma and hyaluronic acid

Platelet rich plasma and hyaluronic acid are two current options that have been shown to have a disease modifying effect in early osteoarthritis of knee.[16,17] PRP and hyaluronic acid (HA) have been shown to act on the disease milieu by different mechanisms with some overlap and may have an anabolic and synergistic effect. HA acts through not only its viscoelastic and lubrication properties but also by anti-inflammatory, anti-fibrotic and chondro-protective effects.[18,19] PRP also exerts a chondro-protective effects on cartilage,[20] anti-inflammatory effect on synovium[21] and regulates the apoptotic pathway.[22] It has been shown in a few in vitro as well as clinical studies that combination of PRP with HA may exert a synergistic effect. Torrecillas et al.,[23] in their study to the effects on wound healing showed that platelet rich in growth factors PRGF with HA was better than PRGF alone in reducing ulcerated wounds in 36 days. Anitua et al., and Marmotti et al.,[24,25] have recently postulated that PRP in combination with HA may be synergistic, by enhancing the migratory potential of fibroblasts in in vitro studies. Both HA and PRP are biological approaches and their use together may be critical in the initial phases of the OA, modifying the environment through immunomodulation and improved joint hemostasis, Andia et al.,[26] confirm these findings. Chen et al.[27] have demonstrated that combination may increase cartilage growth apart from the anti inflamatory effect in their in vitro study. Inhibition of cytokines and adipokines was the postulated mechanism for their antiinflamatory effect as per another study by the same authors.[28] Better chondrocyte survival, cell count and cell proliferation were also noted in PRP encapsulated in Gelatin-HA polymer loaded in biphasic calcium phosphate scaffold by Son et al.[29] In contrast, Russo et al.[30] in their in vitro study wherein they assessed the viscoelastic and biological properties of different combinations of PRP and HA found that addition of PRP to HA exerts a dilutional effect on HA leading to poor viscoelastic and lubrication properties. They also noted higher proliferation rates of chondrocytes in cultures with PRP alone as compared to PRP + HA blend.

Dalllari et al.[31] in their hip OA RCT compared PRP, HA and PRP + HA and concluded that PRP + HA did not offer any additional benefit over PRP alone at 2, 6 and 12 months. However, Lana et al.[32] in their RCT on early OA of knee compared PRP, HA and HA + PRP. They observed better results in combination group. So the above two clinical studies suggest conflicting evidence of efficacy of PRP and HA combination. However, it should be kept in mind that these studies may not have used the ideal combination of PRP and HA as suggested by Russo et al.[30] Loss of viscoelastic properties due to dilution or lower concentration of HA may be some contributing factors which may have given suboptimal results in the above studies. To counter this problem it has been suggested by some authors to use PRP and HA one after the other at a gap of few weeks and not simultaneously.[33] Combination of PRP and HA appear promising due to different mechanism of actions which appear to be synergistic, but further studies are needed to define the schedule, dosage and ideal concentration of PRP and HA.

1.3. Biomaterial, scaffolds and PRP

The primary effects of platelet growth factors occur during approximately 8 days (the half-life of platelets). It has led many authors to use multiple PRP injections at varying frequencies like weekly, biweekly, every-3-week, or monthly intervals to maximize effects. Due to the short term efficacy and non-sustained release of growth factors from PRP used alone, there has been increasing interest in using bio degradable materials as a carrier vehicle for PRP.

PRP has been used with many different biomaterials like gelatin hydrogels, chitosan, polylactic-co-glycolic acid (PLGA) mesh and β-tricalcium phosphate scaffolds for a prolonged and sustained release of growth factors with success. The combination of PRP with these different biomaterials, have been shown in various in vitro and in vivo studies to be significantly better than PRP alone for osteoarthritis knee.[34–36] Saito et al.,[34] in a rabbit model of knee osteoarthritis noted that PRP impregnated gelatin hydrogel resulted in sustained release of growth factors contained in PRP and had preventive effects against OA when compared to PRP alone both morphologically and histologically. It also resulted in increase in chondrocyte GAG synthesis.

Chitosan has been of particular interest and have been used widely with PRP for various applications. Various in vitro studies have shown chitosan to enhance the effect when combined with PRP. Depres-Tremblay et al.[37] noted Chitosan PRP hybrid clots to have decreased retraction, higher cumulative release of PDGF and EGF and higher cell recruitment and granulation tissue synthesis compared to PRP clots. Chitosan also helps in better platelet adhesion and aggregation. It also increases expression of glycoprotein IIIa expression and increases release of growth factors.[38]

Chitosan has some role in increasing the efficacy of PRP by providing an increased and controlled release profile of growth factors. Dwivedi et al.[39] in their study on rabbit model surgically created chronic defects in cartilage and used bone marrow stimulation (BMS) with PRP in one group and freeze dried Chitosan with PRP in the other. They noted that animals treated with BMS + Chitosan PRP had better articular scores at 8 weeks than BMS + PRP. They hypothesized that Chitosan/PRP reside for several weeks in vivo, in contrast to PRP which is quickly degraded. Segundo et al.[40] in their study on surgically created cartilage defects in rabbit knees noted that composite base of PRP, hydroxyapatite and chitosan promoted better bone healing and cartilage healing compared to control. There is encouraging in vitro and in vivo evidence that PRP can be used with biomaterials to enhance their effect, however whether this can be translated to actual clinical scenarios remains to be seen.

2. Conclusion

PRP is cementing its place in the symptomatic treatment of early knee OA. It is clear that all PRP products are not the same and hence more research into defining the ideal PRP preparation, dose, concentration, number of injections, activation status and if PRP should be ideally combined with other substances and/or engineered biomaterials. The common aim is improving the present day PRP to make it more effective, compliant and long acting for the treatment of early knee OA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcot.2018.10.005.

References

1. Filardo G, Kon E, Buda R, et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. *Knee Surg Sports Traumatol Arthrosc*. 2011;19:528–535.
2. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med*. 2013 Feb;41(2):356–364.
3. Spaková T, Rosocha J, Lacko M, Harvanová D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. *Am J Phys Med Rehabil*. 2012 May;91(5):411–417.
4. Cerza F, Carnì S, Carcangiu A, et al. Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. *Am J Sports Med*. 2012 Dec;40(12):2822–2827.
5. Mishra A, Harmon K, Woodall J, et al. Sports medicine applications of platelet rich plasma. *Curr Pharmaceut Biotechnol*. 2012;13:1185–1195.
6. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. *J Arthrosc Relat Surg*. 2012;28:998–1009.
7. Magalon J, Bausset O, Serratrice N, et al. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. *Arthroscopy*. 2014;30(5):629–638.
8. Mazzocca AD, McCarthy MBR, Chowaniec DM, et al. Platelet-rich plasma differs according to preparation method and human variability. *J Bone Joint Surg*. 2012;94(4):308–316.
9. Riboh JC, Saltzman BM, Yanke AB, et al. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. *Am J Sports Med*. 2016;44(3):792–800.
10. Filardo G, Kon E, Pereira Ruiz MT, et al. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: single- versus double- spinning approach. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(10):2082–2091.
11. Paterson KL, Nicholls M, Bennell KL, Bates D. Intra-articular injection of photoactivated platelet-rich plasma in patients with knee osteoarthritis: a double-blind, randomized controlled pilot study. *BMC Muscoskel Disord*. 2016 Feb 9;17:67.
12. Li M, Zhang C, Ai Z, Yuan T, Feng Y, Jia W. [Therapeutic effectiveness of intra-knee-articular injection of platelet-rich plasma on knee articular cartilage degeneration]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi Zhongguo Xiufu Chongjian Waike Zazhi Chin J Reparative Reconstr Surg*. 2011 Oct;25(10):1192–1196.
13. Sanchez M, Anitua E, Azofra J, et al. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. *Clin Exp Rheumatol*. 2008;26:910–913.
14. Cavallo C, Roffi A, Grigolo B, et al. Platelet-rich plasma: the choice of activation method affects the release of bioactive molecules. *BioMed Res Int*. 2016, 6591717.
15. Duymus TM, Mutlu S, Dernek B, Komur B, Aydogmus S, Kesiktas FN. Choice of intra-articular injection in treatment of knee osteoarthritis: platelet-rich plasma, hyaluronic acid or ozone options. *Knee Surg Sports Traumatol Arthrosc Off J ESSKA*. 2017 Feb;25(2):485–492.
16. Di Y, Han C, Zhao L, Ren Y. Is local platelet-rich plasma injection clinically superior to hyaluronic acid for treatment of knee osteoarthritis? A systematic review of randomized controlled trials. *Arthritis Res Ther*. 2018 Dec;20(1):128.
17. Zhang HF, Wang CG, Li H, Huang YT, Li ZJ. Intra-articular platelet-rich plasma versus hyaluronic acid in the treatment of knee osteoarthritis: a meta-analysis. *Drug Des Dev Ther*. 2018;12:445.
18. Musumeci G, Loreto C, Carnazza ML, et al. Acute injury affects lubricin expression in knee menisci: an immunohistochemical study. *Ann Anat*. 2013;195(2):151–158.
19. Abate M, Pulcini D, Di Iorio A, Schiavone C. Viscosupplementation with intra-articular hyaluronic acid for treatment of osteoarthritis in the elderly. *Curr Pharmaceut Des*. 2010;16(6):631–640.
20. Sundman EA, Cole BJ, Karas V, et al. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. *Am J Sports Med*. 2014;42:35–41.
21. Anitua E, Sánchez M, Nurden AT, et al. Platelet-released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. *Rheumatology*. 2007;46(12):1769–1772.
22. Bendinelli P, Matteucci E, Dogliotti G, 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*. 2010;225(3):757–766.
23. Ramos-Torrecillas J, García-Martínez O, De Luna-Bertos E, Ocaña-Peinado FM, Ruiz C. Effectiveness of platelet-rich plasma and hyaluronic acid for the treatment and care of pressure ulcers. *Biol Res Nurs*. 2014 May 20, 1099800414535840.
24. Anitua E, Sanchez M, De la Fuente M, et al. Plasma rich in growth factors (PRGF-Endoret) stimulates tendon and synovial fibroblasts migration and improves the biological properties of hyaluronic acid. *Knee Surg Sports Traumatol Arthrosc*. 2012;20:1657–1665.
25. Marmotti A, Bruzzone M, Bonasia DE, et al. One-step osteochondral repair with cartilage fragments in a composite scaffold. *Knee Surg Sports Traumatol Arthrosc*. 2012;20:2590–2601.
26. Andia I, Abate M. Knee osteoarthritis: hyaluronic acid, platelet-rich plasma or both in association? *Expert OpinBiolTher*. 2014 May;14:635–649.
27. Chen WH, Lo WC, Hsu WC, et al. Synergistic anabolic actions of hyaluronic acid and platelet-rich plasma on cartilage regeneration in osteoarthritis therapy. *Biomaterials*. 2014 Dec 31;35(36):9599–9607.
28. Chen WH, Lin CM, Huang CF, et al. Functional recovery in osteoarthritic chondrocytes through hyaluronic acid and platelet-rich plasma–inhibited infrapatellar fat pad adipocytes. *Am J Sports Med*. 2016 Oct;44(10):2696–2705.
29. Son SR, Sarkar SK, Linh NT, et al. Platelet-rich plasma encapsulation in hyaluronic acid/gelatin-BCP hydrogel for growth factor delivery in BCP sponge scaffold for bone regeneration. *J Biomater Appl*. 2015 Feb 1;29(7):988–1002.
30. Russo F, D'Este M, Vadalà G, et al. Platelet rich plasma and hyaluronic acid blend for the treatment of osteoarthritis: rheological and biological evaluation. *PloS One*. 2016 Jun 16;11(6), e0157048.
31. Dallari D, Stagni C, Rani N, et al. Ultrasound-guided injection of platelet-rich plasma and hyaluronic acid, separately and in combination, for hip osteoarthritis a randomized controlled study. *Am J Sports Med*. 2016 Mar 1;44(3):664–671.
32. Lana JF, Weglein A, Sampson SE, et al. Randomized controlled trial comparing hyaluronic acid, platelet-rich plasma and the combination of both in the treatment of mild and moderate osteoarthritis of the knee. *J Stem Cells Regen Med*. 2016;12(2):69.
33. Patel S, Dhillon MS, Bansal T. Randomized controlled trial comparing hyaluronic acid, platelet-rich plasma and the combination of both in the treatment of mild and moderate osteoarthritis of the knee-Letter to the Editor & Author Response. *J Stem Cells Regen Med*. 2017;13(2):80.
34. Saito M, Takahashi KA, Arai Y, et al. Intra-articular administration of platelet-rich plasma with biodegradable gelatin hydrogel microspheres prevents osteoarthritis progression in the rabbit knee. *Clin Exp Rheumatol*. 2009;27:201–207.
35. Busilacchi A, Gigante A, Mattioli-Belmonte M, Manzotti S, Muzzarelli RA. Chitosan stabilizes platelet growth factors and modulates stem cell differentiation toward tissue regeneration. *Carbohydr Polym*. 2013;98(1):665–676.
36. Kurita J, Miyamoto M, Ishii Y, et al. Enhanced vascularization by controlled release of platelet-rich plasma impregnated in biodegradable gelatin hydrogel. *Ann Thorac Surg*. 2011;92(3):837–844.
37. Deprés-Tremblay G, Chevrier A, Tran-Khanh N, Nelea M, Buschsaitomann M. Chitosan inhibits platelet-mediated clot retraction, increases platelet-derived growth factor release, and increases residence time and bioactivity of platelet-rich plasma in vivo. *Biomed Mater*. 2017;13(1), 015005.
38. Shen E, Chou TC, Gau CH, Tu HP, Chen YT, Fu E. Releasing growth factors from activated human platelets after Chitosan stimulation: a possible biomaterial for platelet rich plasma preparation. *Clin Oral Implants Res*. 2006;17(5):572–578.

39. Dwivedi G, Chevrier A, Hoemann CD, Buschmann MD. *Freeze Dried Chitosan/platelet-rich-plasma Implants Improve Marrow Stimulated Cartilage Repair in Rabbit Chronic Defect Model*. San Diego, CA: Poster Presentation at Orthopaedic Research Society; 2017.

40. Segundo FA, Costa EI, Azevedo AS, et al. Platelet-rich plasma, hydroxyapatite, and chitosan in the bone and cartilaginous regeneration of femoral trochlea in rabbits: clinical, radiographic, and histomorphometric evaluations. *Journal of healthcare engineering*. 2018;2018.