Review Article
Eurasian Journal of Toxicology

Platelet Rich Plasma

Mehmet Cenk Turgut [1], Sultan Tuna Akgöl Gür[2], Muhammed Furkan Erbay[2]
[1]Orthopedics and Traumatology Clinic, Palandöken State Hospital, Erzurum, Turkey
[2]Department of Emergency Medicine, School of Medicine, Atatürk University, Erzurum, Turkey

Abstract

Platelet rich plasma (PRP) is a blood product obtained by centrifuge of blood which contains more thrombocytes than normal plasma. Although this measurement may be effected by given methods, thrombocyte count is approximately 3-5 times higher in PRP than ordinary plasma. PRP is not a drug but an autologous tissue graft and it is unlikely see allergic reactions and transmitted diseases after the procedure. PRPmay be classified according to the preparation technique and the resulting product content. Aim of the PRP therapy is to administer the rich contents of alpha and dens granules contained by platelets to the relevant medium. More comprehensive studies are needed about this specific therapy in order to clear all the question marks about the topic.

Key words: Platelet rich plasma, hemostasis, plasma components

Özet

PRP (Platelet Rich Plasma) kişinin kendi kanının yüksek hızlı merkez kaç kuvveti ile bileşenlerine ayrılması sonucu oluşan kan ürünüdür. Yönteme göre değişmekle birlikte ortalama trombosit sayısı 3-5 kat artar, yani PRP sınır değerinin üzerinde trombosit içeren plazmadır. PRP bir ilaç değil bir dokudur yani otolog bir greft olması nedeniyle alerjik reaksiyonlar oluşturması veya dışarıdan bir bulaşıcı hastalık taşıyabilmesi çok zordur.PRP hazırlanış tekniği ve sonunda oluşan ürün içeriğine göre sınıflandırılabilir. PRP kullanımındaki asıl amaç, trombositlerin içerdikleri alfa ve dens granürlerinin zengin içeriklerinin ortama tedavi amaçlı salınımının sağlanmasıdır. Sonuç olarak etkileri ve tedavi süreci ile ilgili soru işareti barındıran bu tedavi yöntemi ile ilgili daha fazla çalışma yapılmasına ihtiyaç vardır.

Anahtar kelimeler: Trombositten zengin plazma,hemostaz,plazmanın hücresel komponentleri

Platelet rich plasma (PRP) is a blood product obtained by centrifuge of blood which contains more thrombocytes than normal plasma. Although this measurement may be effected by given methods, thrombocyte count is approximately 3-5 times higher in PRP than ordinary plasma. Due to high concentration of platelets, PRP contains hyper-physiological amount of growth factors[1, 2]. Henceforth PRP accelerates healing in ligament and muscle injuries and this effect has been shown in animal trials[3]. Aim of the PRP therapy is to administer the rich contents of alpha and dens granules contained by platelets to the relevant medium.

Thrombocytes are small cells without nucleus and play a major role in hemostasis. Other future of thrombocytes is to release the certain cytokines, growth factors and bioactive factors in order to give a start and to organize the wound healing[4]. Thrombocyte count reaches to peak level in the early phases of wound healing. Platelets are in discoid shape when they are inactive. When they are activated by thrombin, platelets form pseudpods. Active platelets release coagulation and growth factors in which contained dense and alpha granules in to the medium. The sources of the protein profile of the platelets are megakaryocytes and plasma[5]. Contents of the alpha and dense granules are shown in the Table 1[6]. More than 200 different proteins contained by alpha granules are defined in a study which was conducted in 2007. More than 40 different proteins contained by dense granules are defined in another study. Those proteins were shown to be crucial proteins in regeneration and glycolysis processes. Coagulation, inflammation and wound remodeling have an effect the on quantity and quality of proteins synthesized in thrombocytes[7,8].

PRP was first described by Marx et. al. but its ancestor is effects of fibrin glue in wound healing investigated by Matras. In order to accelerate the activation of fibrin glue, high amount of thrombin and thrombocyte was added in 1975-1976. Natural process of wound healing had been tried to be imitated by this method. Following this attempt, this concept was named as tissue regeneration. PRP was first used maxillofacial surgery in the early 2000. FDA approved the PRP therapy for muscle and bone injuries in 2012[9]. Later on, therapeutic area of PRP was broadened.

Corresponding Author: Sultan Tuna Akgöl Gür **e-mail:** sultantuna@hotmail.com
Received: 13.01.2020 • **Accepted:** 20.03.2020
Cite this article as: Turgut MC, Akgol Gur ST, Erbay MF. Platelet rich plasma. Eurasian J Tox. 2020;2(1):7-10.

PRP imitates natural mechanism of wound healing and growth factors, chemokines, and proteins released by alpha and dense granules in which contained platelets leads to tissue regeneration. Because of PRP contains hyper-physiological amount of growth factors, PRP improves healing in chronic wounds and accelerates the healing in acute injuries[10,11].

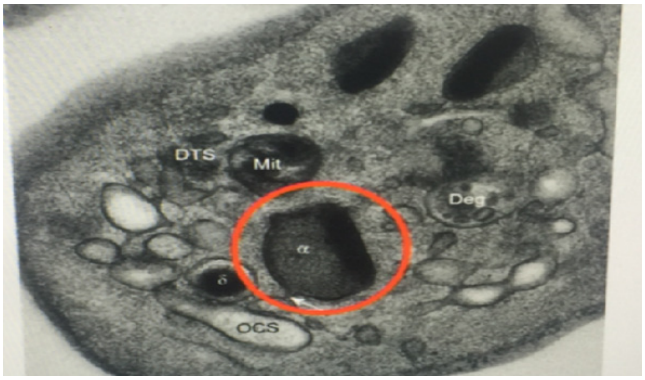

Image 1. Alpha granules under the microscope

Preparing PRP and PRP variations

Cellular components of the plasma are distributed as 93% erythrocytes, 6% platelets and 1% leukocytes. PRP contains 3-5 times higher amount of thrombocyte than complete blood. PRP is obtained by centrifuge of anticoagulated autologous complete blood. Citrate is added to complete blood before the centrifuge in order to bind the ionized calcium. So that coagulation is prevented. After the centrifuge, complete blood dissociates into 3 layers (Table 2). Upper layer contains plasma, middle layer contains thrombocytes and leukocytes which known as "buffy coat" and lower layer contains erythrocytes. It is reported that buffy coat and plasma layers may be centrifuged following the first process and PRP and platelet poor plasma may be dissociated further.

PRP may be classified as pure-platelet rich plasma (P-PRP), leukocyte and platelet rich plasma, (L-PRP), pure platelet rich fibrin (P-PRF), leucocyte and platelet rich fibrin (L-PRF) in relation to preparation method and end product. PRP must be prepared in sterile laboratory environment (Image 2). Moreover, there are many commercial PRP kits in order to obtain it.

Recently there are 4 different platelet rich plasma products[12]. Those are classified in accordance with platelet and leukocyte contents of the end product.

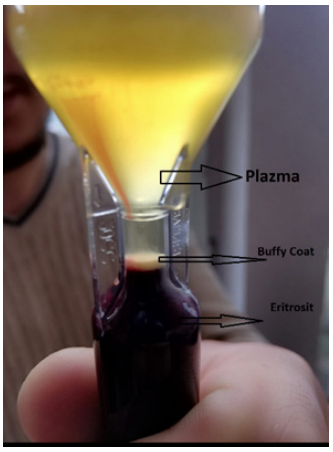

Image 2. 3 dissociated layers of complete blood after centrifuge.

P-PRP

P-PRP is dissociated from leukocytes by plasmapheresis alike methods. It is harder, longer and more expensive to obtain P-PRP than other products. There are approximately no leukocytes in it, if there is any. It may be injected in liquid form. It may be loaded to active membrane or gel.

L-PRP

Standard centrifuge methods are performed to obtain L-PRP. Obtained plasma contains thrombocytes and leukocytes. It may be used in a liquid form or activated gel form as P-PRP. Most known and most produced PRP kind is L-PRP. Aim of the kits is to make more practical to obtain L-PRP.

1. Pure Platelet Rich Plasma (P-PRP)	→	Fibrine clot (+)
2. Leucocyte – Platelet Rich Plasma(L-PRP)	→	Fibrin clot (+)
3. Pure Platelet Rich Fibrin (P-PRF)	→	Fibrin clot (-)
4. Leucocyte – Platelet Rich Fibrin (L-PRF)	→	Fibrin clot (-)

Table 1. Contents of the granules of thrombocytes

Alpha Granules	Dense Granules
-Integral membrane proteins	-Cations
-Coagulation proteins	-Phosphates
-Adhesion proteins	-Bioactive amines
-Chemokines	-Nucleotidies
-Growth Factor	
-Pro-angiogenic and Anti-angiogenic factors	
-Microbiological proteins	

Indications of PRP
• Soft Tissue Injuries
• Bone Healing
• Cosmetic and Aesthetic Purposes
• Algology
• Chondral - Osteochondral Lesions
• Lateral epicondylitis
• Osteoarthritis
• Tendinopathies
• Acute or chronic ligament injuries
• Acute or chronic muscle injuries
• Rotator cuff rehabilitation
• ACL reconstruction
• Meniscus injuries

P-PRF

P-PRF results from two phases of centrifuge. Leukocytes are dissociated in the first phase. After aforementioned phase, added CaCl activates thrombocytes and result in platelet-fibrin clot. Because P-PRF is solid it does not fit for injection. Spesific future of this method is utilizing the seperation gel.

L-PRF

It was first described by Choukroun et. al. This method is cheap and simple. Anticoagulant substance does not added to centrifuge tube. There is no need to use activator. It can be used as an autologous biomaterial. Leukocytes and thrombocytes interacts with each other. L-PRF is also known as "the ideal blood product" because it is the most natural product among others. It does not fit for injection but may be used as a solid biomaterial.

Recently indications of PRP therapy get wider. Higher amounts of blood result in higher amount of PRP, theoretically. Small lesions like epicondylitits necessitates 3 ml of PRP however big lesions like rotatory cuff tears necessitates 5-6 ml of PRP. Local ice packs, elevation and activity modification is suggested in case patients might have local inflamatory reaction during 24-48 hours following the injection. Asetaminofens and opioids may be used for analgesic pusposes but non steroid anti inflamatory drugs (NSAIDs) are not suggested for 2-4 weeks. NSAIDs inhibts the prostoglandin pathway and may block the benefits stimulated by growth factors.

Contraindications

PRP is not a drug but an autologous tissue graft and it is unlikely see allergic reactions and transmitted diseases after the procedure. However serious allergic reactions may be encountered when cattle thrombin is used for activating thrombin, during the preparation of PRP. Local inflammation, pain and edema may occur after PRP therapy. Although it is claimed that PRP stimulates the growth factors and theoretically this condition may provoke the development neoplasms; there is no solid evidence to support this hypothesis[4]. Contraindications of PRP includes malignancy and metastasis, active infection, thrombocytopenia, anemia, pregnancy and lactation and allergy to cattle thrombin[13].

Discussion

Even though there are so many reports about the PRP in the current literature and 25% of the papers published in the last 5 years, authors do not concur the effects and protocols of the PRP. Unfortunately, there is no guideline about how to use PRP in muscle and bone diseases. However, it is reported that PRP does not cause any side effects according to AAOS guideline.

There are 3 basic debates about PRP therapy. First of them whether thrombocytes should be activated when it is injected to the relevant medium. If platelets to be activated, whether CaCl or cattle thrombin must be selected as a platelet activator. Researches showed that activated thrombocytes releases 12 different cytokines into the medium via alpha granules[14]. Especially PDGF and TGF are released in higher concentrations among other cytokines. Both PDGF and TGF binds to present clot and they are released rhythmically for a long period and attracts mesenchymal stem cells and leads to mitogenic activity[15].

Second debate topic is the comparison of the effectivity of L-PRP and L-PRF. L-PRF, firstly developed by Chokuron et. al. in 2001, is thought to be safer and more practical because thrombin and CaCl are not needed in the preparation period of L-PRF and there is a one certain protocol to administer it[14,16]. Disadvantageous sides of the L-PRF are limited obtainable amount of the L-PRF and specificity of donor[17].

Third debate topic is the leukocyte content of PRP. Nowadays since leukocytes may increase the local inflammation, leukocyte poor substances are believed to be superior to leukocyte rich ones[18].

It is believed that PRP is beneficial since it increases cell proliferation and matrix synthesis however the reasons of the different effects of it on the individual basis is still unknown. Also the is no concurrence about the optimum PRP formula, duration and doses of administration and rehabilitation after PRP proper protocol. More comprehensive studies are needed about this specific therapy in order to clear all the question marks about the topic.

References

1. Nguyen RT, Borg-Stein J, McInnis K. Applications of platelet-rich plasma in musculoskeletal and sports medicine: an evidence-based approach. PM&R. 2011;3(3):226-50.
2. Paoloni J, De Vos RJ, Hamilton B, Murrell GA, Orchard J. Platelet-rich plasma treatment for ligament and tendon injuries. Clinical Journal of Sport Medicine. 2011;21(1):37-45.
3. Rees JD, Maffulli N, Cook J. Management of tendinopathy. The American journal of sports medicine. 2009;37(9):1855-67.
4. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. The American journal of sports medicine. 2009;37(11):2259-72.
5. Park JE, Barbul A. Understanding the role of immune regulation in wound healing. The American Journal of Surgery. 2004;187(5):S11-S6.
6. Siegel A, Lüscher E. Non-identity of the α-granules of human blood platelets with typical lysosomes. Nature. 1967;215(5102):745.
7. Maynard D, Heijnen H, Horne M, White J, Gahl W. Proteomic analysis of platelet α-granules using mass spectrometry. Journal of Thrombosis and Haemostasis. 2007;5(9):1945-55.
8. Rendu F, Brohard-Bohn B. The platelet release reaction: granules' constituents, secretion and functions. Platelets. 2001;12(5):261-73.
9. Sonnleitner D, Huemer P, Sullivan DY. A simplified technique for producing platelet-rich plasma and platelet concentrate for intraoral bone grafting techniques: a technical note. International Journal of Oral & Maxillofacial Implants. 2000;15(6).
10. Khan KM, Cook JL, Bonar F, Harcourt P, Åstrom M. Histopathology of common tendinopathies. Sports Medicine. 1999;27(6):393-408.
11. de Mos M, van der Windt AE, Jahr H, van Schie HT, Weinans H, Verhaar JA, et al. Can platelet-rich plasma enhance tendon repair? A cell culture study. The American journal of sports medicine. 2008;36(6):1171-8.
12. Yıldız C, Özgürtaş T. Trombositten zengin plazma. TOTBİD Dergisi, 2017, 16(3): 247-58
13. Sampson S, Gerhardt M, Mandelbaum B. Platelet rich plasma injection grafts for musculoskeletal injuries: a review. Current reviews in musculoskeletal medicine. 2008;1(3-4):165-74.
14. Breen A, O'Brien T, Pandit A. Fibrin as a delivery system for therapeutic drugs and biomolecules. Tissue Engineering Part B: Reviews. 2009;15(2):201-14.
15. Catelas I, Dwyer JF, Helgerson S. Controlled release of bioactive transforming growth factor beta-1 from fibrin gels in vitro. Tissue Engineering Part C: Methods. 2008;14(2):119-28.
16. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunité en paro-implantologie: le PRF. Implantodontie. 2001;42(55):e62.
17. Prakash S, Thakur A. Platelet concentrates: past, present and future. Journal of maxillofacial and oral surgery. 2011;10(1):45-9.
18. Jain A, Bedi RK, Mittal K. Platelet-rich plasma therapy: a novel application in regenerative medicine. Asian journal of transfusion science. 2015;9(2):113.