http://dx.doi.org/10.5272/jimab.2015214.982

Journal of IMAB
ISSN: 1312-773X
http://www.journal-imab-bg.org

Journal of IMAB - *Annual Proceeding (Scientific Papers)* 2015, vol. 21, issue 4

PLATELET-RICH PLASMA (PRP) AND ITS APPLICATION IN THE TREATMENT OF CHRONIC AND HARD-TO-HEAL SKIN WOUNDS. A Review

Tsvetan Sokolov, Boyan Valentinov, Jordan Andonov, Sevdalin Angelov, Pencho Kosev
Department of Orthopedics and Traumatology, MHAT Ruse, Ruse, Bulgaria

ABSTRACT: In the last few years various methods are being applied in the use of platelet-rich plasma (PRP) during treatment in different orthopedic disease. They allow improvement of local biological condition and regeneration of different types of tissues. PRP is a modern treatment strategy with worldwide recognition. There is a high concentration of platelet growth factors in small amounts of plasma. PRP and its various forms have become one of the best methods to support the healing process of various tissues. PRP is used in regenerative medicine, because it provides two of three components (growth factors and scaffolds) necessary for complete tissue regeneration. The particular reason for the appearance of lesions is important in order to select an appropriate treatment method and technical application. PRP may be used for treatment of various chronic and hard-to-heal cutaneous wounds, especially when standard conventional therapy is not good enough and surgical treatment is not possible. It reduces the duration, cost of treatment and the hospital stay. There is reduction of wound pain after starting the treatment, reduced risk of blood-borne disease transmission, wound healing is restored, and local immunity is activated.

Keywords: Platelet rich plasma, platelets, platelet growth factors, chronic skin wounds

INTRODUCTION

Platelet-rich plasma (PRP) is a modern treatment strategy with worldwide recognition. PRP was introduced in the 1950s and is currently used in multiple fields of medicine. There is high concentration of platelet growth factors in small amounts of plasma. This works for offering an ''ideal environment'' for tissue regeneration and is part of the so-called biological therapy. The PRP implementation is an autologous therapy, which eliminates the risk of blood-borne infections. PRP and its various forms has become one of the best methods to support the healing process of various tissues. The term „regenerative medicine" was introduced in the 1990s. Many studies related to stem cells, growth factors, and extracellular matrix support the development of this new treatment philosophy. It differs from classic tissue engineering, where tissue regenerates ex vivo. Here, the major idea is regeneration of fully functional tissue on the spot, while damaged tissue is provided for by cellular elements and the process is managed by local factors. PRP appears to be the main source of autologous products in regenerative medicine and a true precursor and foundation of the healing process along with scaffold and stem cells [1]. PRP is used in regenerative medicine, because it provides two of the three components (growth factors and scaffolds), necessary for complete tissue regeneration. This review discusses the state-of the-art-studies on PRP action mechanisms in surgical and non-surgical implementation (treatment of tendon injuries, cartilage damage, muscle trauma, cartilage and bone pathologies, and wound healing).

Platelet Biology

Platelets are blood cells formed during hematopoiesis. They are built from cytoplasmic fragments of the long extensions of megakaryocytes and are small, discoid, and anucleate cells [2]. These extensions are interwoven through bone marrow sinusoids and are fragmented by shear forces, thus forming new platelets in the blood [3]. Their circulating lifespan is 5-9 days and their major clearance mechanism is via Kuppfer cells and hepatocytes. Platelets measure from 1 to 4 μm in diameter and apart from being enucleated, they contain different organelles. They are discoid or ellipsoid in shape and have three distinguishable zones: peripheral or outer zone, organelle zone, and cytosol zone. Peripheral zone – This is the outermost section and it contains antigens, glycoproteins and various enzymes. This zone connects the platelets with other cells and blood vessel linings [4]. Organelle zone – It is built from a variety of structures: dense granules, alpha granules, Golgi apparatus, dense tubular system and open canalicular system, lysosomes and mitochondria. The dense granules (or dense bodies) are dense structures containing 65% of the total platelet adenosine-diphosphate and adenosintriphosphate. Serotonin, pyrophosphate, antiplasmin and large amounts of calcium, necessary for platelet aggregation are also stored there [5]. Alpha granules contain various growth factors (platelet-derived growth factor— PDGF, transforming growth factor beta—TGF-b, etc.), and clotting factors. Many of the 30 bioactive proteins, playing a key role for hemostasis are contained in these granules; hemostasis is considered to be the first stage of wound healing [6, 7]. The Golgi apparatus is part of the membrane system as well [8]. Cytosol zone - It contains microtubules and forms a circumferential zone around platelets. Microtubules are connected to microfilaments. This is how the platelet cytoskeleton is formed; it directs the cell's movement, eliminates secreted products, and retracts clots. Upon platelet activation

microtubules could be seen in platelet pseudopods [4].

Functions of the Platelets

Besides participation in hemostasis, platelets also have other functions. To obtain hemostasis, the interaction of three main mechanisms is necessary: vascular response, platelet activity, and clot formation. When platelets are not activated by various stimuli, they are present in blood circulation in a state of quiescence in disc-shape form. The variety of stimuli can comprise physical or chemical ones, or a combination of both. The sub-endothelial collagen forming in the wound as a result of trauma, along with the von Willebrand factor(vWF), are the main factors that activate blood platelets in vivo, as well as thrombin, adenosine diphosphate, or a combination of them. Platelets also have non-hemostatic functions. In addition to their primary role in hemostasis, platelets participate in many non-hemostatic processes. Their secretion contains many different substances [9]. On their external layer, a number of surface receptors are arranged, including adhesion proteins, cytokines, and lipopolysaccharide [10]. Interesting is the fact that platelets also release various substances depending on the stimuli, by which they are activated [11]. Alpha granules contain many substances with directly opposing activities. This implies the existence of a mechanism for specific release of only a specific content of the granule, which is possible, but this is still not well studied [12]. Inflammation, immunity, and tissue recovery are some of the most characteristic features of platelets [13].

Platelet-rich Plasma (PRP)

The PRP as a concept can be represented as the volume fraction of blood plasma, where the platelet concentration is increased compared to the baseline serum level. In platelet rich plasma, besides platelet concentrate, small amounts of white blood cells are also present and other blood components as well. Ideal is a concentration of 1,407,640 cells in microliter (with a standard deviation of 320.100). This value corresponds to a number of platelets, approximately five times higher than the normal, number in the blood, which is usually in the range of 150,000 to 350,000 cells in micro liter (approximately an average value of about 200,000 cells in microliter) [14]. The presence of white blood cells in PRP may affect its use, independent of the concentration of the platelets [15]. The presence of the layer of leukocytes has led to the current classification, which distinguishes a clean (without leukocytes) PRP, identical to the platelet rich plasma of Anitua, and PRP with leukocytes. Also present in the classification the fibrin-rich gel known as a clean, without leukocytes, platelet-rich fibrin, and platelet-rich fibrin with leukocytes or Choukroun type [16]. PRP is an autologous therapy, which stimulates the healing of tissues and positively influences the recovery processes. It ensures a high concentration of platelets in a small volume of plasma. Once platelets are activated, platelet aggregation occurs and the contents of their solid granules and alpha granules is released [17]. In order to release the growth factors of the platelets, PRP must be activated to start the clotting cascade. The activation of platelets in vivo is done in three ways: by adenosine diphosphate, via membrane phospholipids system (arachidonic acid), and by inducing the presence of thrombin [18]. In hospital and clinical conditions, the conversion of prothrombin to thrombin takes place by means of calcium dichloride and thus platelets are activated. Another way is by means of autologous or bovine thrombin [19]. In order for the platelets and the PRP to be sufficiently functional, they should be activated as with tissue injury. As a result platelets release their contents and a cascade of events is initiated. Normal collagen is repaired. Collagen repair consists of the following phases: inflammation, proliferation, and remodelling [20]. Each of these stages is needed to restore the normal function of the tissue.

Cascade of Wound Healing

Wound healing is a complex process that comprises of a sequence of events, starting from the time of injury and lasting several months, which can be divided into three phases: inflammation, proliferation, and remodeling [21]. In the inflammatory phase is observed at the beginning of the cascade with the activation and aggregation of platelets and the formation of a fibrin matrix. With platelets degranulation, cytokines are released that guide the healing process. Leukocytes are attracted by the cytokines through hemostasis and migrate to the damaged area. During the proliferative phase, which spans through the next few days, monocytes migrate to the wound area under the influence of chemical signals from growth factors. The differentiating of macrophages by circulating monocytes is observed. Platelet signaling and modulation function are also performed by the macrophages. Gradually, the damaged area is impoverished of platelets. During this phase, angiogenesis also begins under the influence of growth factors and thrombin [22, 23]. At the time of the remodeling phase, contraction of the collagen and convergence of the edges of the wound is observed. Cell density and vascularization is reduced, excess matrix is removed, and the collagen fibrils are aligned along the stress lines, which increases the strength of the newly-formed tissue [21]. Accumulated granulation tissue remodels or slowly transforms into a specific tissue such as skin or bone [24].

Platelet Growth Factors

A number of growth factors are located in platelet alpha-granules. In order to be released into the injured tissue monocytes, neutrophils, fibroblasts, mesenchymal stem cells and osteoblasts need the chemotactic effect of an available growth factor. Platelet-derived growth factor (PDGF) produces such effect. This growth factor influences mitogenesis of fibroblasts and smooth muscle cells. PDGF improves the formation of fibrous tissue, assists in the three phases of the healing cascade, and also significantly affects angiogenesis and re-epithelialization. Another important growth factor is transforming growth factor beta (TGF-b). It impacts the connection between fibronectins. It influences cell migration, proliferation and replication, and is present during inflammation processes [25]. Chronic wounds' and endochondral ossification's healing processes are highly influ-

enced by vascular endothelial growth factor (VEGF). It is active in angiogenesis, too [8]. The next factor taking part in the recovery of chronic wounds is epidermal growth factor (EGF). It also influences the mitogenesis of endothelial cells, fibroblasts, and keratinocytes [26]. Hepatocite growth factor (HGF) is located in various tissues (several types of epithelium, liver, lung, kidney and tumor). It affects tissue regeneration and possesses morphogenic, mitogenic, antiapoptotic, and neurotrophic qualities [22].

Application of PRP in the Treatment of Chronic Wounds

Skin wounds are defined as the absence of tissue, where epidermis and dermis are affected, while sometimes can reach the adipose tissue and muscle fascia. In this process, no natural regeneration is observed and tissue lesion is transformed into fibrotic scarring [27]. The process of healing of skin wounds is very intense and includes a number of phenomena, such as hemostasis, inflammation, formation of granulation tissue, epithelialization, neovascularization, collagen synthesis, and wound contraction. Several studies demonstrate that the growth factors are depleted in chronic wounds. Platelets have a tendency to aggregate when activated, having a leading role in skin healing. Activated platelets release growth factors, adhesion molecules, and lipids through which migration, proliferation, and the function of keratinocytes, fibroblasts, and endothelial cells are regulated [28]. Skin wounds have a frequency of 0.78% and huge funds are spent for their treatment. Annually, 2% of the budget is used for health in the European Union (EU), £ 40 million in the UK, $-1.3 billion are used for the treatment of decubitus in the US [29]. For the development of skin wounds facilitate various etiological factors such as: chronic venous disease, peripheral arterial disease, neuropathy, arterial hypertension, physical injury, hematologic disorders, skin infection, inflammatory diseases, neoplasms, iatrogenic changes, and those associated with nutrition [27]. Difficult to heal wounds are considered those that do not heal after the fourth week of their appearance after being treated with standard methods for the particular pathology. Chronic is a wound that is not healing for a period of three months. The evaluation of each wound is very important. For the purpose, a number of point scales suggested by Cancela et al. based on clinical and anatomical criteria for the size of the wound are used, while a patient variable is also taken into account. The points are randomly defined using clinical experience in wound healing. Anatomical features are noted and graded such as open bone or tendon, location of the wound, and quality of the pulse of artery dorsalis pedis and artery tibialis posterior (when related to wound location). Wounds are measured to determine their overall surface, depth, and distribution of undermining of the wound edges. The duration of the wound is determined by the history [29]. There are many studies relating to the pathophysiology of wounds. Skin wound healing is an intensive process and in it are observed a number of events such as hemostasis, inflammation, formation of granulation tissue, formation of new vessels, collagen synthesis, epithelialization, and contraction of the wound. The process of reduction of growth factors in chronic wounds is demonstrated in numerous reports as a result of their reduced production and release, sequestration, or degradation. These mechanisms can be combined [30]. Platelet aggregation has a clearly expressed effect in the process of skin wound healing. In this process, growth factors are released and, adhesion of molecules and lipids is observed, while they are responsible for the regulation of cellular migration, proliferation and function of keratinocytes, fibroblasts, and endothelial cells [6]. Platelets secrete several antimicrobial peptides when activated by thrombin. PRP has an important antimicrobial and immunoregulatory activity obtained from leukocytes [31]. A review of studies using different products rich in platelets demonstrates significant improvement in the treatment of chronic wounds. Carter et al. performed a meta-analysis of articles for chronic wounds and support the idea that the use of platelet rich plasma favors full healing compared to control groups [5]. In a study by Villela et al., they reached similar conclusions, with one of their conclusions reaching so far as to conclude that PRP is the method of choice for the treatment of wounds [29]. Anitua et al. presented an open and randomized study to evaluate the effect of PRP in chronic ulcers of 14 patients. They reported good healing response in 80% of the cases after 8 weeks of treatment, compared with 20% in the control group. Leukocytes were not detected in the analyzed products. Their explanation for the good results lies in the high concentrations of growth factors [30]. Crovetti et al. monitored the evolution of chronic skin wounds in 24 patients treated with autologous or homologous gel (depending on the case) and observed a complete healing in 9 of them after an average of 10 applications, with a reduction of pain in all cases [27]. Marté-Mestre et al. reported the recovery of vascular chronic ulcers with the use of PRP in 12 of 14 patients for an average treatment period of 2.93 months (average of 0.5 - 7 months) [29]. Margolis et al. found in a retrospective group study with neuropathic wounds in diabetic feet greater efficiency in the use of PRP against the conventional therapies, with a more obvious effect in severe wounds [29].

PRP may be used for treatment of various chronic and hard-to-heal cutaneous wounds, especially when standard conventional therapy is not good enough and surgical treatment is not possible. It reduces the duration, cost of treatment and the hospital stay. These procedures may be performed in outpatient clinics. There is reduction of wound pain after starting the treatment, reduced risk of blood-borne disease transmission, wound healing is restored, and local immunity is activated [32].

CONCLUSIONS

In the last few years various methods are applied in the use of PRP during treatment in different orthopedic disease and sports trauma. They allow improvement of local biological condition and regeneration of different types of tissues. Without doubt the use of PRP is absolutely a treatment option. According to Werner and Cramer the platelet is the most important cell for the repair processes of the body [33]. Recently, scientific research and technology have presented

a new perspective concerning the understanding of "orthobiologic" treatments and the healing process of lesions [22]. PRP treatment is widely applied in the last ten years, especially for stimulating coagulation. A number of studies show that platelets demonstrate other functions, too. They are connected to liberating bioactive proteins and growth factors. The bioactive proteins as well as the growth factors improve tissue regeneration and the process of healing. PRP represents an autologous biological material as its action is directly related to general clinical conditions of the patient.

Acknowledgments:

No institutional finding or grants as well as technical assistance or contributions of this research has been obtained by the author.

REFERENCES:

1. Cugat R. Front Matter: Foreword I. In: Lana JF, et al. (eds.), Platelet-Rich Plasma. Regenerative Medicine: Sports Medicine, Orthopedic, and Recovery of Musculoskeletal Injuries. Springer-Verlag Berlin, Heidelberg. 2014; p v-vi. [CrossRef]

2. Machlus KR, Italiano JE Jr. The incredible journey: From megakaryocyte development to platelet formation. *J Cell Biol*. 2013 Jun;201(6):785-96. [PubMed] [CrossRef].

3. Thon JN, Italiano JE. Visualization and manipulation of the platelet and megakaryocyte cytoskeleton. *Methods Mol Biol*. 2012; 788:109-25. [PubMed] [CrossRef].

4. Bittencourt CH, Bittencourt PB, Neto OAL, Arenas GCF. The Use of Platelet-Rich Plasma in Orthopaedic Injuries. In: Lana JF, et al. (eds.), Platelet-Rich Plasma. Regenerative Medicine: Sports Medicine, Orthopedic, and Recovery of Musculoskeletal Injuries. Springer-Verlag Berlin, Heidelberg. 2014; p289-313 [CrossRef]

5. Hanson SR, Harker LA . Blood coagulation and blood-materials interactions. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE (eds) Biomaterials science. An introduction to materials in medicine. Academic Press, San Diego. 1996; 193–199.

6. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost*. 2004 Jan;91(1):4–15. [PubMed] [CrossRef]

7. Harrison P, Cramer EM. Platelet alpha-granules. *Blood Rev*. 1993 Mar;7(1):52–62. [PubMed]

8. Maes C, Carmeliet P, Moermans K, Stockmans I, Smets N, Collen D, et al. Impaired angiogenesis and endochondral bone formation in mice lacking the vascular endothelial growth factor isoforms VEGF164 and VEGF188. *Mach Dev*. 2002 Feb; 111(1-2):61-73. [PubMed] [CrossRef]

9. Virchenko O, Aspenberg P. How can one platelet injection after tendon injury lead to a stronger tendon after 4 weeks? Interplay between early regeneration and mechanical stimulation. *Acta Orthop*. 2006 Oct;77(5):806–812. [PubMed] [CrossRef]

10. Clemetson KJ, Clemetson JM . Platelet receptors. In: Michelson AD (ed) Platelets, 2nd edn. Academic Press, Elsevier, Burlington. 2007;117–134.

11. Cognasse F, Hamzeh-Cognasse H, Lafarge S, Delezay O, Pozzetto B, McNicol A, et al. Toll-like receptor 4 ligand can differentially modulate the release of cytokines by human platelets. *Br J Haematol*. 2008 Apr; 141(1):84–91. [PubMed] [CrossRef].

12. Blair P, Flaumenhaft R. Platelet alpha-granules: Basic biology and clinical correlates. *Blood Rev*. 2009 Jul; 23(4):177-189. [PubMed] [CrossRef]

13. Textor J. Platelet-Rich Plasma (PRP) as a Therapeutic Agent: Platelet Biology,Growth Factors and a Review of the Literature. In: Lana JF, et al. (eds.), Platelet-Rich Plasma. Lecture Notes in Bioengineering. Springer-Verlag Berlin, Heidelberg. 2014; p 61-94. [CrossRef].

14. Dellinger RA, Britton C. Autologous platelet grafting procedure - a new approach to healing chronic wounds and comparison between current therapies. *Unpublished Monograph White Paper*. 2001;

15. Everts PA, Devilee RJ, Brown-Mahoney C, Eeftinck-Schattenkerk M, Box HA, Knape JT, et al. Platelet gel and fibrin sealant reduce allogenic blood transfusions and in total knee arthroplasty. *Acta Anaesthesiol Scand*. 2006 May;50(5):593–599. [PubMed] [CrossRef]

16. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*. 2009 Mar; 27(3):158–167 [CrossRef] [PubMed].

17. Cole BJ, Seroyer ST, Filardo G, Bajaj S, Fortier LA. Platelet-rich plasma: Where are we now and where are we going? *Sports Health*. 2010 May;2(3):203–210. [PubMed] [CrossRef]

18. Everts PA, Overdevest EP, Jakimowicz JJ, Oosterbos CJ, Schonberger JP, Knape JT, et al. The use of autologous platelet-leukocyte gels to enhance the healing process in surgery, a review. *Surg Endosc*. 2007 Nov;21(11):2063–2068. [PubMed] [CrossRef].

19. Weibrich G, Kleis WKG, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. *J Craniomaxillofac Surg*. 2002 Apr;30(2):97-102. [PubMed] [CrossRef]

20. Crane D, Oliver K. Platelet-Rich Plasma and Biocellular Grafts. In: Lana JF, et al. (eds.), Platelet-Rich Plasma. Lecture Notes in Bioengineering. Springer-Verlag Berlin, Heidelberg. 2014; p 249-264. [CrossRef].

21. Pietrzak WS, Eppley BL. Platelet rich plasma: Biology and new technology. *J Craniofac Surg*. 2005 Nov; 16(6):1043–1054. [PubMed] [CrossRef]

22. Lana JF, Weglein A, Vicente E,

Perez AG, Rodrigues A, Luzo AC, et al. Platelet Rich Plasma and Its Growth Factors: The State of the Art. In: Lana JF, et al. (eds.), Platelet-Rich Plasma. Lecture Notes in Bioengineering. Springer-Verlag Berlin, Heidelberg. 2014; p1-59. [CrossRef].

23. Lorenz HP, Longaker MT. Wounds: Biology, pathology, and management. In: Norton JA, Bollinger RR, Chang AE, et al. (eds.) Surgery: basic science and clinical evidence, Springer, New York. 2001;77–88.

24. Clark RAF. Overview and general considerations of wound repair. In: Clark RAF (eds.) The molecular and cellular biology of wound repair. Plenum Press, New York. 1996;3–50.

25. Molloy T, Wang Y, Murrell G. The roles of growth factors in tendon and ligament healing. *Sports Med*. 2003;33(5):381–394. [PubMed]

26. Bennett SP, Griffiths GD, Schor AM, Leese GP, Schor SL. Growth factors in the treatment of diabetic foot ulcers. *Br J Surg*. 2003 Feb;90(2):133–146. [PubMed] [CrossRef]

27. Crovetti G, Martinelli G, Issi M, Barone M, Guizzardi M, Campanati B, et al. Platelet gel for healing cutaneous chronic wounds. *Transfus Apheres Sci*. 2004 Apr;30(2):145–151. [PubMed] [CrossRef]

28. Yuan T, Zhang CQ Tang MJ, Guo SC, Zeng BF. Autologous platelet-rich plasma enhances healing of chronic wounds. *Wounds*. 2009 Oct;21(10):138-141. [PubMed]

29. Cancela AM, Lana JF, Annichino-Bizzachi JM, Belangero WD, Luzo ACM. Use of Platelet-Rich Plasma (PRP) in Treating Chronic Wounds. In: Lana JF, et al. (eds.), Platelet-Rich Plasma. Lecture Notes in Bioengineering. Springer-Verlag Berlin, Heidelberg. 2014; p 281-288. [CrossRef].

30. Anitua E, Aguirre JJ, Algorta J, Ayerdi E, Cabezas AI, Orive G, et al. Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. *J Biomed Mater Res B Appl Biomater.* 2008 Feb;84(2):415-421. [PubMed]

31. El-Sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M, et al. Platelet-rich plasma: Growth factors and pro- and anti-inflammatory properties. *J Periodontol*. 2007 Apr; 78(4):661-669. [PubMed] [CrossRef]

32. Greenlagh DG. The role of growth factors in wound healing. *J Trauma*. 1996 Jul;41(1):159-167. [PubMed]

33. Da Silva RT, Heidrich F. Therapy with Use of Platelet-Rich Plasma in Orthopedics and Sports Traumatology: Literature Review, Evidence and Personal Experience. In: Lana JF, et al. (eds.), Platelet-Rich Plasma. Lecture Notes in Bioengineering. Springer-Verlag Berlin, Heidelberg. 2014; p153-170. [CrossRef].

Please cite this article as: Sokolov T, Valentinov B, Andonov J, Angelov S, Kosev P. Platelet-rich Plasma (PRP) and its application in the treatment of chronic and hard-to-heal skin wounds. A Review. *J of IMAB.* 2015 Oct-Dec;21(4):982-986. DOI: http://dx.doi.org/10.5272/jimab.2015214.982

Received: 17/09/2015; Published online: 09/12/2015

Address for correspondence:
D-r Tsvetan Sokolov
Department of Orthopedics and Traumatology, MHAT Ruse, Ruse, Bulgaria
2, Nezavisimost str., 7002 Ruse, Bulgaria.
e-mail: sokolovi2001@abv.bg