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Platelet Rich Plasma: a short overview of certain bioactive components

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Abstract: Platelet rich plasma (PRP) represents a relatively new approach in regenerative medicine. It is obtained from patient's own blood and contains different growth factors and other biomolecules necessary for wound healing. Since there are various protocols for PRP preparing, it usually results with PRP generation with different amounts of bioactive substances, which finally may modulate the intensity of wound healing. The reference data about potential effect of some PRP compounds on wound healing, in different tissues, are still controversial. This review summarizes recently known facts about physiological role of certain PRP components and guidance for further research. Also, this review discusses different procedure for PRP generation and potential effect of leukocytes on wound healing.

Keywords: Platelet rich plasma, Platelets, Growth factors, Leukocytes, Wound healing

1 Introduction

Platelet rich plasma (PRP) therapy has accumulated considerable attention over the two last decades, mainly due to its potential ability in regenerative medicine, including oral and maxillofacial surgery, sports and veterinary medicine. Platelets as a main components of the PRP, contain more than 1100 different proteins, with numerous post-translational modifications, resulting in over 1500 protein-based bioactive factors [1]. These factors include immune system messengers, growth factors, enzymes and their inhibitors and other factors which can participate in tissue repair and wound healing. Another important characteristic of PRP is that represents an autologous product, which is prepared from the patient`s own blood. Therefore, the use of autologous PRP eliminates any concerns about the risk of crossed contamination, disease transmission or immune reactions [2].

The ability of PRP to provide huge amounts of growth factors and various proteins, which are able to stimulate the healing process, represents the key factor for widespread clinical use. In different tissues, including the musculoskeletal, healing process takes a long time due to limited blood supply and slow cell turnover [1]. The use of PRP speeds up the neovascularization and therefore increase the blood supply and nutrients influx necessary for cell regeneration in damaged tissue. Also, by increasing the blood supply, PRP stimulates the requirement, proliferation and differentiation of the cells, which are involved in the healing process [3].

The purpose of this article is to elucidate the PRP components, the role of some PRP growth factors in tissue repair and to discuss how certain PRP components may modulate the healing process.

2 Bioactive factors in PRP

Usually, PRP has been defined as an autologous concentration of human platelets that is 3 to 5 times greater than physiologic concentration of thrombocytes in whole blood [4]. Normal platelet count in healthy person ranges between 150000 and 350000 cell/μL of blood. These are small, discoid cells without nucleus and therefore cannot reproduce. They are formed in the bone marrow from megakaryocytes with a life span of about 7 to 10 days. Thrombocytes are usually associated with their primary function in hemostasis and coagulation. Namely, after injury with resulted bleeding, thrombocytes are activated and start

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to release their granules filled with growth factors which finally stimulate the inflammatory cascade and healing process.

Various proteins and other substances necessary for tissue repair and healing process are secreted by three types of granules (alpha, delta and lambda) located inside the platelets. α-granules are the most abundant platelet granule. They constitute approximately 10% of platelet volume and there is around 50-80 α-granules per each thrombocyte [5]. These granules contain membrane bound proteins as well as soluble proteins which are released into the extracellular space. Membrane bound proteins include integrins (αIIb, α6, β3), platelet endothelial cell adhesion molecule (PECAM), leucine-rich repeat family receptors (GPIb-IX-V complex), immunoglobulin family receptors (glycoprotein VI) and other receptors (CD36, Glut-3) [5]. On the other hand, previous studies suggested that more than 300 soluble proteins are released by α-granules [6]. These bioactive molecules are very heterogeneous with regard to function and include proteins involved in clotting, inflammation, cell growth, cell adhesion and host defense (Table 1). Delta granules (dense bodies) primarily contain molecules which stimulate clotting process, including calcium, magnesium, adenosine and bioactive amines, such as serotine and histamine [7]. Lambda granule is another type of granules in platelets and belongs to the lysosomal type organelles. Like lysosomes in other cell types, lambda granule contain enzymes necessary in protein, lipid and carbohydrate degradation process. Also, they are involved in removing the debris from damaged tissue and removing the infectious agents [1].

Generally, functional properties of PRP are mainly based on the synthesis and secretion of multiple growth factors that are secreted after platelet activation. These factors are essentially stored in thrombocyte α-granules and they have key role in regulating cellular process, including chemotaxis, mitogenesis and differentiation [8]. Secreted growth factors directly stimulate local mesenchymal and epithelial cells to migrate, divide and increase the synthesis of collagen and matrix with resulting formation of fibrous connective tissue and scar formation [9]. Further, many of the growth factors released in damaged tissue express combined action and may also interact between each other, providing the activation of different intracellular signaling pathways with enhanced tissue repair [10].

The prominent growth factors of PRP, presented in Table 2, include platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF) and fibroblast growth factor (FGF) [11, 12]. PDGF was given its name after it was first found in platelets. Beside thrombocytes, PDGF is also found in other cell types, including monocytes, macrophages, fibroblasts and endothelial cells [10]. This growth factor is composed from A and/or B subunits and three isoform exist AA, BB and AB. PDGF released from platelets stimulates chemotaxis and mitosis of fibroblasts, collagen synthesis and remodeling of extracellular matrix [2]. Also, PDGF stimulates the chemotaxis of macrophages and neutrophils and enhances the secretion of TGFβ from macrophages [13].

TGF-β represents a member of TGF-β superfamily which consist of bone morphogenetic factors and three isoforms of TGF-β, TGF-β1, TGF-β2 and TGF-β3 [14]. Following the injury, active form of TGF-β1 is secreted by thrombocytes. This growth factor stimulate the production of collagen and prevent collagen breakdown. TGF-β1 pro-

Table 1: Platelet α-granule content

Type	Examples
Adhesive proteins	Von Willebrand factor, fibrinogen, trombospondi-1, trombospondin-2, laminin-8
Growth factors	Epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF), transforming growth factor β (TGF-β)
Angiogenic factors	Vascular endothelium growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF)
Chemokines	CCL5 (RANTES), CCL-3 (MIP-1α), CCL-2 (MCP-1), CCL-7 (MCP-3), CXCL8 (IL-8), CXCL2 (MIP-2), CXCL6 (LIX), CXCL-1 (GRO-α), CXCL5 (ENA-78), CXCL-12 (SDF-1α), CXCL4 (PF4)
Clotting factors and their inhibitors	Factor V, factor IX, antithrombin, factor S, protease nexin-1, protease nexin-2, tissue factor pathway inhibitor,
Integral membrane proteins	αIIb3, GPIbα-IX-V, GPVI, TLT-1, p-selectin
Immune mediators	Complement C3 precursor, complement C4 precursor, factor D, factor H, C1 inhibitor, IgG

Table 2: Growth factors in PRP and their biological functions

Name	Abbreviation	Function
Platelet derived growth factor	PDGF	Enhances collagen synthesis, proliferation of bone cells, fibroblast chemotaxis and proliferative activity, macrophage activation
Transforming growth factor β	TGF-β	Enhances synthesis of type I collagen, promotes angiogenesis, stimulates chemotaxis of immune cells, inhibits osteoclast formation and bone resorption
Vascular endothelial growth factor	VEGF	Stimulates angiogenesis, migration and mitosis of endothelial cells, increases permeability of the vessels, stimulates chemotaxis of macrophages and neutrophils
Epidermal growth factor	EGF	Stimulates cellular proliferation, differentiation of epithelial cells, promotes cytokine secretion by mesenchymal and epithelial cells
Insulin-like growth factor	IGF	Promotes cell growth, differentiation, recruitment in bone, blood vessel, skin and other tissues, stimulates collagen synthesis together with PDGF
Fibroblast growth factor	FGF	Promotes proliferation of mesenchymal cells, chondrocytes and osteoblasts, stimulates the growth and differentiation of chondrocytes and osteoblasts

motes angiogenesis, connective tissue regeneration and chemotaxis of the immune cells [15]. Further, at the site of the bone injury, TGF-β1 stimulates osteoblast proliferation and inhibits osteoclast formation, favoring bone formation over resorption [10].

In the damaged tissue, VEGF is secreted by activated thrombocytes and macrophages. VEGF is important for stimulating new blood vessel formation and, therefore, for bringing nutrients and increased blood flow to the site of injury [16]. To stimulate angiogenesis, beside VEGF, the presence of FGF is also necessary. Earlier report demonstrated that presence of PDGF, TGF-β and EGF may highly enhance the VEGF secretion [13].

EGF stimulates chemotaxis and angiogenesis of endothelial cells and mitosis of mesenchymal cells. Different studies have shown that stimulates epithelization and markedly shortens healing process [13, 17]. Cytokine secretion by mesenchymal and epithelial cell is also increased following the EGF secretion [4].

IGF-1 is 70 amino acid polypeptide hormone which is normal component of the plasma but also can be transported into platelets by IGF binding proteins. This growth factor may be released from platelets during their activation and stimulates differentiation and mitogenesis of mesenchymal cells [2]. Further, IGF-1 promotes bone formation by proliferation and differentiation of osteoblasts [16].

FGF represents one of the most potent mitogen with multiple actions on multiple cell types. It is important mitogen for the mesenchymal cells, chondrocytes and osteoblasts [18]. Also, it stimulates the growth and differentiation of chondrocytes and osteoblasts and together with VEGF is involved in process of angiogenesis [13].

3 Production of PRP

Until today there are 40 different commercial systems able to create PRP from autologous whole blood, which are archived by means of centrifugation [19]. In majority of the available systems, two centrifugations are necessary for PRP production, with set of time and speed previously defined [2]. Primarily, PRP preparation takes advantage of different density gradients of cell components of the blood. Following the first centrifugation, the red blood cells are densest and will be separated from the plasma at the bottom of the centrifuge container. Above the erythrocytes layer, buffy coat of white blood cells is formed. Thrombocytes are at the highest concentration in plasma just above the buffy coat. Buffy coat and plasma are collected and pulled and proceeded for a second centrifugation to increase a platelet concentration [1]. Final thrombocytes concentration in PRP may vary depending the commercial system used for PRP preparation, as well as individual patient characteristics, such as age, comorbidities and circulation [20].

Moreover, PRP has been used in last few decades and there are various systems to prepare PRP, still there is no unique protocol for PRP production. Also, there is no consistent opinion about the total amount of platelets needed to be in PRP to be effective. Earlier report suggested that thrombocytes concentration from 800 to $1200x10^9$ platelets/L are needed for PRP to be effective [21]. Other researchers suggested that $1000x10^9$ platelets/L, measured in a volume of 5ml of plasma, may represent therapeutic dose of PRP [22]. Another report stated that $200x10^3$ platelets/μL is minimal thrombocytes concentration for PRP

generation [1]. On the other hand, earlier study showed that PRP should contain more than 300x10^3/μL [23].

Having this in mind various protocols are used to generate the PRP, it is easy to presume that it predominantly contains platelets, but also and other components of whole blood in varying amounts. Despite the fact that there is no universal classification of for PRP, four different categories of PRP have been proposed. Based on the total leukocyte and fibrin content inside the PRP there are: leukocyte-rich PRP (L-PRP), leukocyte reduced PRP (P-PRP; leukocyte reduced or pure PRP), leukocyte platelet-rich fibrin and pure platelet-rich fibrin [24]. By using different protocols it is possible that final PRP product contains higher amount of white blood cells. There is no consistent opinion about positive or negative role of leukocytes in L-PRP on tissue healing. Some studies proposed that leukocytes stimulate the healing process in damaged tissue and simultaneously suppress the growth of some bacteria [25, 26]. On the other hand, various reports showed positive correlation between the total number of leukocytes in PRP and increased levels of pro-inflammatory cytokines, indicating that leukocytes in PRP may inhibit the healing process [27, 28]. Also, there is ongoing debate about potential exogenous platelet activation before application to the damaged tissue. In PRP, with inactivated platelets, contact with fibrillary collagen, thrombin or basement membrane of the cells leads to thrombocytes activation, with releasing huge amounts of bioactive molecules from platelet alpha granules. However, by using certain protocols platelets may be activated before PRP application and making that great amount of growth factors may be immediately available to the target cell in the damaged tissue. Generally, total amount of growth factors is released around one hour after platelet activation, while 70% of growth factor are released 10 minutes after activation of thrombocytes [4]. Such observations may suggest that exogenous activation of platelets could lead to rapid secretion of growth factors but to decreased time that damaged tissues are exposed to the growth factors [22]. Different clinical studies are necessary to prove or not if this exogenous platelet activation represent obligatory step in PRP therapy or not.

4 Leukocytes in PRP

Since the PRP contain all components of the whole blood, depending on the used protocol leukocytes may be found in PRP in different amounts. Neutrophils represent first type of leukocytes that migrate to the damaged tissue. Their primary goal, at the wound site, is to induce phagocytosis of debris, necrotic tissue and microbes. Monocytes are second type which arrive at the damaged tissue following neutrophils. Circulating monocytes induce extracellular matrix breakdown by releasing the MMP-2 (matrix metalloproteinase), MMP-9, and MMP-13 [1]. By destroying extracellular matrix, they allow cellular migration through tissue, making the healing more efficient [29]. Shortly after arriving into the damaged tissue, monocytes transform themselves into macrophages. Macrophages continue process of phagocytosis and remove the rest of cellular debris and neutrophils. Beside the named functions of macrophages, they are able to secrete growth factors important for healing, such as TGF-β1, PDGF, VEGF, IGF-1, EGF and others [30]. By secreting the bioactive molecules, macrophages are essential for neo-angiogenesis. By forming the new blood vessels, delivering the nutrients, oxygen and other inflammation cells is increased, together with forming the granulation tissue and removing necrotic tissue. On the other hand, poor angiogenesis results with slow wound healing and ulcer formation [31].

Leukocytes are able to secrete many proteinases, including metalloproteinases and serine which have important role in process of wound healing [32]. Even proteinases have ability to induce lymphocyte and platelet activation, activation of cytokines and formation of fibrin-platelet plug [30], they are able to control the intensity of inflammatory process by deactivating the inflammatory cells [32]. Also, proteinases secreted by leukocytes are able to control the activity of secreted growth factors. TGF-β1 is released in inactive form, but it is easily converted in active form by proteinases [30]. Further, TGF-β is stored bound to the extracellular matrix. Activation of proteinases secreted from leukocytes are able to degrade the matrix and release the growth factor which may take place in wound healing process [32]. Earlier report documented that wound healing process may be enhanced by application of L-PRP in damaged oral mucosa [33]. In line with those results, another report demonstrated that reduced levels of macrophages at the fracture site resulted with reduced blood vessel density and delayed bone formation. Simultaneously, it has been shown that PDGF and TGF-β1 released from leukocytes have key roles in fracture repair [34].

On the other hand, some studies have suggested that PRP rich in leukocytes may inhibit the wound healing process mainly by massive release of reactive oxygen species (ROS) by neutrophils in damaged tissue [23]. As neutrophils are first cell which arrive to the wound site, they release ROS and nitric oxide to eliminate potential microbes and debris in wounded tissue [35]. A higher con-

centration of leukocytes may be responsible for delayed wound healing and, therefore, the influx of neutrophils must be controlled [1]. Also, macrophages induce neutrophil apoptosis and may prevent potential negative effect of huge amounts of neutrophils in damaged tissue. Lack of adequate studies of potential positive or negative effect of leukocytes in process of wound healing, definitive conclusion still remains to be clarified. However, positive or negative effects of leukocytes in PRP may not be generalized to all tissues, since PRP rich in white blood cells have positive effects in some conditions [4].

5 Conclusion

PRP represents important therapy in regenerative medicine. By using different methods it is possible to get various PRP types, regarding the content of bioactive molecules. Beside platelets, as a dominant PRP factor, other bioactive factors may be involved in modulation of immune response. PRP rich in leukocytes is able to enhance the healing process, by removing the potential microbes and stimulating growth factor release. However, large concentration of white blood cell in PRP may have inhibitory effect. The optimal concentration of platelets, leukocytes and other plasma components remain to be clarified and researcher should be aware that PRP effect is not only based on thrombocytes concentration.

Conflict of interest: Authors state no conflict of interest.

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