E-ISSN: 2964-3872

The Potential of Platelet-Rich Plasma in Promoting Axonal Regeneration: A Narrative Review

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Abstract

Platelet-rich plasma (PRP) is an autologous blood product obtained by centrifugation. PRP is characterized by a high concentration of growth factors, which are known to promote tissue healing, influence angiogenesis, and exhibit immunomodulatory effects without causing significant adverse reactions. Consequently, PRP has been used in various medical specialties, including neurology, in recent years. This review aimed to provide an overview of the current evidence on the use of PRP to promote axonal regeneration. An extensive literature search was conducted across several databases, including PubMed and Google Scholar, using keywords such as platelet-rich plasma (PRP), platelet-concentrated plasma, platelet-rich growth factors, and axonal regeneration. A detailed analysis of the relevant studies was performed, and the data were meticulously extracted and analyzed. Studies have indicated that PRP may have promising potential to facilitate nerve regeneration. Specifically, PRP has been shown to be effective in protecting nerves, preventing neuronal apoptosis, and stimulating vascular and axonal regeneration. Furthermore, PRP regulates inflammatory responses in the microenvironment, which may further contribute to its potential benefits in nerve regeneration. Understanding the biology, mechanism of action, classification of PRP, and its role in axonal regeneration is crucial for clinicians to gain a complete understanding of this therapeutic approach and appropriately interpret available data on PRP.

Keywords: Platelet-rich plasma, PRP, platelet biology, axonal regeneration.

Fundings: Universitas Malikussaleh's non-tax state revenue grant (PNBP) of 2024.

Introduction

Platelet-rich plasma (PRP) was originally introduced by Kingsley in 1954.[1] PRP is a liquid fraction that is processed from autologous peripheral blood and has a high platelet concentration, which exceeds that of normal blood plasma.[2] PRP also referred to as platelet-concentrated plasma or platelet-rich growth factors, is an autologous blood product that

contains a significantly elevated concentration of platelets suspended in a small volume of plasma, exceeding the baseline level.[3] Normal platelet levels are 150,000-350,000/ml. Concentrations of 1,000,000 / ml have been demonstrated to enhance bone and soft tissue healing. Therefore, PRP is defined as containing 5 ml of plasma with this elevated platelet concentration.[4]

The initial review of PRP by Everts et al. (2006) focused on its effects on platelet function and mechanism of action, its effect on the different stages of the healing process, and the essential role of platelet-derived growth factors in various PRP applications.[5] PRP therapy has been used to treat a variety of conditions for more than 30 years due to its potential benefits in regenerative medicine. Currently, PRP therapy is a treatment option that offers significant clinical advantages and favorable patient outcomes.[6]

Material and methods

The initial literature search was performed using Google Scholar and PubMed with the following keywords: platelet-rich plasma, 'PRP,' 'peripheral nerve injuries' AND OR combinations of these words. No time limit was applied. The extracted data were then carefully analyzed and coherently organized into a narrative structure. Whenever applicable, the data and previous studies were briefly summarized in tabular form.

Discussion

Biology of Platelets

Platelet-rich plasma (PRP) is a platelet concentrate derived from autologous blood via centrifugation. PRP was combined with a specific volume of normal saline, containing 10% calcium chloride and thrombin. The addition of thrombin and calcium chloride to PRP activated platelets, resulting in the release of numerous growth factors from the α-granules.[7,8] After platelet activation, the process of α-granules merging with the cell membrane begins, leading to degranulation and the subsequent release of growth factors.[9] Platelets, which are the key components of PRP, facilitate the release of growth factors that are critical for tissue regeneration. These growth factors have high concentrations that are essential for the recovery of tendons, cartilage injuries, and nerve tissues.[10,11] Platelet-rich plasma is characterized by a high concentration of white blood cells, including neutrophils, monocytes, and lymphocytes, which play crucial roles in pathogen and foreign substance phagocytosis. The fibrin present in PRP exhibits a three-dimensional network structure after platelet activation and aggregation and plays a crucial role in facilitating tissue regeneration.[9]

Platelet α-granules contain platelet growth factor (PGF) and cytokines such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), basic fibroblast growth factor (BFGF), interleukin-8 (IL-8), keratinocyte growth factor (KGF), and connective tissue growth factor (CTGF). Insulin-like growth factor-1 (IGF-1) is produced by various cell types, including Schwann cells, monocytes, skeletal muscles, and capillaries. IGF-1 receptors are found in axons, nerve terminals, Schwann cells, and cell bodies of motor neurons in peripheral nerves (Table 1).[8,12]

Preparation of PRP

Blood samples were collected from patients prior to centrifugation and platelet-rich plasma (PRP) was subsequently obtained, after which the blood components, including red blood cells, platelet-rich plasma (PRP), and platelet-poor plasma (PPP), were separated based on their distinct density gradients. In PRP, several other parameters must be considered, such as the presence or absence of leukocytes and their activation state. These factors determine the type of PRP that is used for various medical conditions.[3]

This process is typically performed in a laboratory, operating room, office, or radiology room using an appropriate centrifuge kit. The volume of blood used for the extraction process can vary, and anticoagulants can be present in the syringe or in the extraction system.[13]

Whole blood is often mixed with an anticoagulant agent, including acid-citrate-dextrose, sodium citrate, or ethylenediaminetetraacetic acid, prior to centrifugation to separate red blood cells (RBCs) from platelet-poor plasma (PPP) and "buffy coat," which contains a high concentration of platelets and leukocytes. PRP was obtained using various processing techniques and the RBC and PPP layers were discarded (Figure 2). Platelet administration to patients can be achieved by direct administration or by activating platelets using calcium chloride or thrombin. Activation causes platelets to release growth and differentiation factors during degranulation. Approximately 70% of the stored growth factors are released within the first 10 minutes of activation and almost 100% are released within an hour.[14,15]

Platelet-rich plasma can be prepared in two primary forms: plasma-based plasma and buffy coat. Both methods use whole blood, but differ in the centrifugation process, which isolates and concentrates various components of blood cell components.[1] The use of isolation techniques, such as the type of collection tube and centrifuge speed, has a direct impact on the final concentration of platelets and leukocytes in the preparation of PRP. These factors, in conjunction with the centrifugation process, contribute to the extraction of isolated cells and growth factors at higher concentrations.[10]

Plasma-Based Methods

Plasma-based techniques are designed to separate and retain only plasma and platelet components while eliminating leukocytes. Such techniques have been developed to prioritize the elimination of leukocytes, thereby preserving platelets. The primary goal of this technique is to collect platelets during centrifugation, which is why it has a slower speed and shorter duration. The outcome of this process is a platelet concentration that is typically about two to three times the baseline level (300,000 to 500,000 platelets/µL).[1]

Buffy-Coat-Based Methods

Buffy coat systems are designed to collect all available platelets during centrifugation and achieve a high leukocyte concentration, which requires high-speed and extended centrifugation. This ensures the highest possible platelet count, typically ranging from 3 to 8 times the baseline level, or 500,000 to 1,500,000 platelets per microliter.[1] PRP techniques use anticoagulants to collect blood before or during surgery, followed by immediate centrifugation to separate it into three layers. Preparing platelet concentrates usually takes up to one hour. Initially, centrifugation divides the blood into three components: red blood

cells at the bottom, acellular plasma at the top, and a buffy coat layer in between. Red blood cells and acellular plasma were removed to obtain the buffy coat layer (Figure 1). Finally, platelet concentrate is collected and administered via syringe to the injury or surgical site, with thrombin and/or calcium chloride to initiate platelet activation and fibrin polymerization.[16]

Variability in the number and speed of centrifugation steps used in PRP preparation can lead to differences in the amount of anabolic and catabolic proteins released into target tissue after injection.[1] The following factors have been identified as crucial determinants of plasma quality: an adequate number of spins (particularly with two consecutive spins), a high revolutions per minute (at least 3200 rpm), and a high g force (calculated by multiplying the rpm by the radius of the rotor in millimeters).[17] The various methods used for PRP separation typically result in similar levels of platelets (approximately 600,000 platelets/μL), erythrocytes, TGF-1, and fibrinogen. However, each system produces significantly different concentrations of PDGF, VEGF, and leukocytes (Table 1).[1]

PRP presents a significant advantage, as it facilitates the delivery of a high concentration of growth factors to areas of limited healing capacity, both in non-operative and surgical settings. This is achieved using the patient's own blood, which serves as a matrix for sustained release of growth factors that promote chemotaxis, cell proliferation, remodeling, and angiogenesis.[18,19]

Role of PRP on Axonal Regeneration

Evidence indicates that PRP holds potential for nerve regeneration by acting as a neuroprotector, preventing neuronal apoptosis, stimulating vascular and axonal regeneration, and regulating inflammatory responses in the microenvironment.[10] Schwann cells are essential for repair of damaged peripheral nerves, proliferation, and migration to form the Bünger band, which is critical for axonal regeneration. They also secrete neurotrophic factors and differentiate into myelin sheaths during nerve regeneration. Platelet-rich plasma, which is rich in growth factors, promotes Schwann cell proliferation and migration.[9] Studies on P-PRP have shown that PDGF and IGF-1 primarily influence the proliferation and migration of Schwann cells. This study found that 5% P-PRP positively affected the secretion of nerve growth factors by Schwann cells. In vitro assays assessing effect of P-PRP on NGF and GDNF production revealed significant Schwann cell proliferation, with the highest levels observed at 2.5-20% P-PRP concentration.[20]

PRP's Potential in Nerve Regeneration: Neuroprotection, Schwann Cell Activation, and Cytokine Influence

A study on the effects of P-PRP and PPP on spinal ganglion neurons indicated that P-PRP-treated neurons had higher survival rates and proliferation than the control group.[21] LL-PRF also influenced Schwann cell proliferation, neurotrophic factor secretion, and anti-inflammatory responses. Wang et al. (2020) found that L-PRF enhances Schwann cell proliferation, neurotrophic factor secretion, and the suppression of PG-LPS-induced inflammation.[22] Chondroitin sulfate (CS) improves Schwann cell proliferation and the secretion of neurotrophic factors like nerve growth factor (NGF) and glial cell line-derived

neurotrophic factor (GDNF). Additionally, CS promotes Schwann cell migration by activating integrin-beta-1-mediated focal adhesion kinase (FAK) signaling pathways.[9]

The process of axonal regeneration after peripheral nerve injury is a complex phenomenon that involves various inflammatory cells, including macrophages. Macrophages are typically classified into two major phenotypes, classically activated (M1) and alternatively activated (M2). M1 macrophages are distinguished by their pro-inflammatory response, which is largely facilitated by the expression of nitric oxide synthase (NOS), a molecule that plays a role in presenting antigens to the immune system. On the contrary, M2 macrophages mainly express arginase type 1 (Arg-1), secrete a variety of cytokines, and regulate the immune response, which is crucial for tissue regeneration. During the initial stages of nerve injury, M1 macrophages play a crucial role in phagocytosis of the myelin sheath and other cellular debris resulting from disintegration of the distal nerves. The removal of debris by macrophages significantly affects axonal regeneration. In the latter stages of the inflammatory response, M2 macrophages become the predominant phenotype and contribute to fibrosis and axonal regeneration.[9]

PRP contains growth factors that regulate the inflammatory response after injury, leading to increased macrophage aggregation, phagocytosis, and antigen presentation. Additionally, PRP promotes the transformation of macrophages into M2 macrophages, which play crucial roles in axonal repair and regeneration. Activation of the JK1/3-STAT6 pathway by PRP can also promote macrophage activation, thereby improving tissue healing. The specific mechanisms by which PRP promotes macrophage activation and tissue healing are not fully understood, but are believed to involve the regulation of growth factors and cytokines.[77] PRP has been demonstrated by Nishio et al. (2020) that have demonstrated that PRP promotes macrophage aggregation during the tissue healing process, and the effects of PRP on inflammatory cells depend on the composition and concentration of WBC, platelets, and RBC in the PRP. Leukocyte-rich PRP can lead to a more pronounced inflammatory response that promotes faster tissue repair through the accumulation and infiltration of inflammatory cells. Additionally, L-PRP can stimulate tissue cell proliferation and regeneration at a faster rate by more strongly inducing metabolism.[23]

Following nerve injury, axonal regeneration is accompanied by the regeneration and reorganization of blood vessels. In the initial stage, which typically occurs within the first week, temporary dilation of the blood vessels occurs; however, the number of vessels does not increase. This leads to the recruitment of macrophages, which subsequently phagocytose cell debris. In the subsequent stage, the increase in the number of blood vessels facilitates axonal elongation, cell proliferation, and myelinization.[24]

Role of VEGF and Neurotrophic Factors in PRP Therapy for Axonal Regeneration and Nerve Repair

PRP contains vascular endothelial growth factor (VEGF), a potent angiogenic factor that has been found to have chemotactic effects and plays a critical role in vasculogenesis, which is the formation of neovascularization. This process leads to increased angiogenesis and vascular permeability and stimulates the proliferation of myoblasts and endothelial cells. VEGF is a crucial modulator of angiogenesis in embryonic development and peripheral

nerve regeneration. Furthermore, VEGF promotes neuronal survival and axonal regeneration, making it a valuable component in PRP therapy. Vascular endothelial growth factor has shown neuroprotective properties, which can enhance the survival of motor neurons and reduce their susceptibility to external factors. Furthermore, this growth factor promotes blood vessel formation and nerve regeneration. These findings suggest that VEGF can facilitate and promote nerve regeneration by using its angiogenic, neurotrophic, and neuroprotective capabilities.[9]

Vascular endothelial growth factor, released by Schwann cells and activated macrophages, significantly contributes to neovessel formation, supplying oxygen and nutrients for Bünger's band development after Wallerian degeneration. Schwann cells also secrete NGF and GDNF, which promote their proliferation and Bünger band formation. They migrate along the blood vessels, thereby aiding axon regeneration. Platelet-rich plasma, which is rich in various growth factors, effectively enhances Schwann cell activities, such as migration, proliferation, and NGF secretion. Additionally, the high VEGF concentration in PRP promotes revascularization (Figure 3).[9]

Several studies have indicated positive effects of neurotrophic factors, including BDNF, NGF, FGF, and GDNF, on the growth and regeneration of axons. A study by Lu et al. (2019) revealed that BDNF promotes neuronal differentiation and axonal remyelination and enhances the survival of endothelial cells, thus maintaining the stability of blood vessels.[25] Cho et al. (2010) observed increased expression of neurotrophic factors such as BDNF, NGF, FGF, and GDNF after injection of PRP in guinea pigs after fascial nerve injury, suggesting that PRP and mesenchymal stem cells (MSC) act as a source of neurotrophic factors. The study also demonstrated an increase in the number of axons and myelinization in the PRP-administered group.[26]

Experimental Evidence of PRP and PRF Efficacy in Rat Models for Peripheral Nerve Injury and Regeneration

Rats are commonly used to study peripheral nerve injury because they provide an opportunity to observe the axonal regeneration process in a dynamic manner. In the study conducted by Firat et al. (2016), the impact of PRP and hyaluronic acid on the regeneration of the peripheral nerves of rats after injury was examined, and the results indicated that the rats administered PRP demonstrated superior axonal regeneration and improvements in electromyographic and gait compared to the control group.[27] Wu et al. (2012) reported that local injection of 200µl PRP into the corpus cavernosum was shown to increase the number of myelinated axons and facilitate recovery of erectile function in rats with bilateral cavernous nerve injury.[28]

According to a recent study, PRP administered intracavernously to injured rats in a dose of 400µL with 1,500,000/µL platelets showed a substantial improvement in myelinated axon regeneration, a decrease in apoptosis, and an increase in the proliferation of smooth muscle cells in the corpus cavernosum at the early stage.[29] Another study reported that PRP administered locally at a dose of 400µl after bilateral cavernous nerve injury in rats has a

positive effect on cavernous nerve regeneration and functional improvement, which can increase the number of myelinated axons and improve erectile function.[30]

Histological analysis was conducted to investigate nerve regeneration, revealing a significant increase in the number of inflammatory cells and endoneurium vacuoles in the PRP group, which was administered 3 days post-injury.[31] Furthermore, PRP demonstrated a substantial impact in improving nerve conduction and increasing the number of axons in the sciatic nerve of rats after injury compared to the control group.[32]

Huang et al. (2020) conducted a study to investigate the potential effects of platelet-rich fibrin (PRF) administration on the nerve conduit (PRF-NGC) for peripheral nerve regeneration. This study compared the effects of PRF-NGC with those of autologous nerve grafts (ANG) and polyurethane (PUR) by evaluating the histological results after 12 weeks. The results demonstrated that the PRF and ANG groups exhibited significantly thicker myelin sheaths, larger fiber diameters and more regenerated axons than the PUR group.[33] [84] According to Ikumi et al. (2018), the activation level of Schwann cells, the count and the diameter of axons in rats given PRF after sciatic nerve injury were significantly higher than in the control group.[34]

Conclusion

Platelet-rich plasma (PRP) has emerged as a novel therapeutic option for pathologies related to nerve damage, and studies have indicated its potential for nerve regeneration. PRP serves as a neuroprotective agent, prevents neuronal apoptosis, and stimulates vascular and axonal regeneration. PRP supports nerve repair by preserving neuronal integrity and function. It enhances vascular regeneration, supplies essential nutrients and oxygen to healing tissues, and promotes axon regrowth via growth factors that reduce inflammation. PRP offers a comprehensive approach to nerve regeneration, necessitating further research and clinical trials to optimize its therapeutic use for various neurological conditions.

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Table 1 Growth factor and cytokine components in PRP.[6,8,13]

PGF and Cytokines	Sources	Function
PDGF	Platelets, macrophages, endothelial cells, smooth muscle cells	Cell replication; angiogenesis; mitogen of various cells (fibroblasts, mesenchymal cells, osteoblasts, glia cells, smooth muscle cells); regulates collagen secretion and synthesis; macrophage activation and neutrophil chemotaxis
IGF-1	Thrombus, plasma, epithelial cells, endothelial cells, fibroblasts, osteoblasts, bone matrix	Regulation of tissue growth and differentiation in various organs; chemotaxis of fibroblasts and stimulation of protein synthesis; promote osteoblast proliferation and differentiation.
TGF	Macrophages, T lymphocytes, keratinocytes	Stimulates the proliferation of mesenchymal cells; regulates the mitogenesis of fibroblasts, osteoblasts, and endothelial cells; regulates the secretion and synthesis of collagen; regulates the mitogenic effect of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits the proliferation of macrophages and lymphocytes.
VEGF	Platelets, macrophages, neutrophils, keratinocytes, endothelial cells	Vasculogenesis; increases angiogenesis and vascular permeability; stimulates proliferation of myoblasts and endothelial cells
EGF	Platelets, macrophages, and monocytes	Proliferation of mesenchymal cells, epithelial cells, keratinocytes, and fibroblasts; stimulates endothelial cell mitogenesis
KGF	Fibroblast, mesenchymal cell	Proliferative effects on epithelial cells and mediates keratinocyte migration
CTGF	Platelets, fibroblasts	Angiogenesis; cartilage regeneration, fibrosis, and platelet adhesion
FGF	Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts	Stimulates the growth and proliferation of chondrocytes and osteoblasts; mitogen of mesenchymal cells, chondrocytes, and osteoblasts
TNF	Macrophages, mast cells, T lymphocytes	Regulates monocyte migration and fibroblast proliferation; macrophage activation; angiogenesis

Abbreviations: PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; KGF, keratinocyte growth factor; CTCG, connective tissue growth factor; FGF, fibroblast growth factor; TNF, tumor necrosis factor.

Table 2 Comparison of growth factor levels and cell counts in peripheral blood and PRP.[35]

Components	Peripheral Blood	PRP
PDGF (10-50 pg/ml)	45 pg/ml	360 pg/ml
TGF (10-70 pg/ml)	35 pg/ml	320 pg/ml
VEGF (15-85 pg/ml)	55 pg/ml	560 pg/ml
IGF-1 (0,5-19,5 pg/ml)	13 pg/ml	175 pg/ml
Platelets (150.000-350.000/mm^3)	265.000/mm^3	1.250.000/mm^3
Leukocytes (3.200-9.000/mm^3)	5.600/mm^3	20.000/mm^3
Granulocytes	60% (3.300/mm^3)	24% (480/mm^3)
Mononuclear	35% (1.960/mm^3)	70% (14.000/mm^3)
$CD34^+$	0,5/mm^3	175/mm^3

Figure 1 (A) After the second spin cycle, platelet-rich plasma (PRP) is obtained under sterile conditions. (B) Then, it was introduced into two syringes for later administration, and the PRP was ready for injection into sterile bags. (C) Scheme showing how to obtain the PRP from platelet-poor plasma (PPP) after 2 spin cycles.[17]

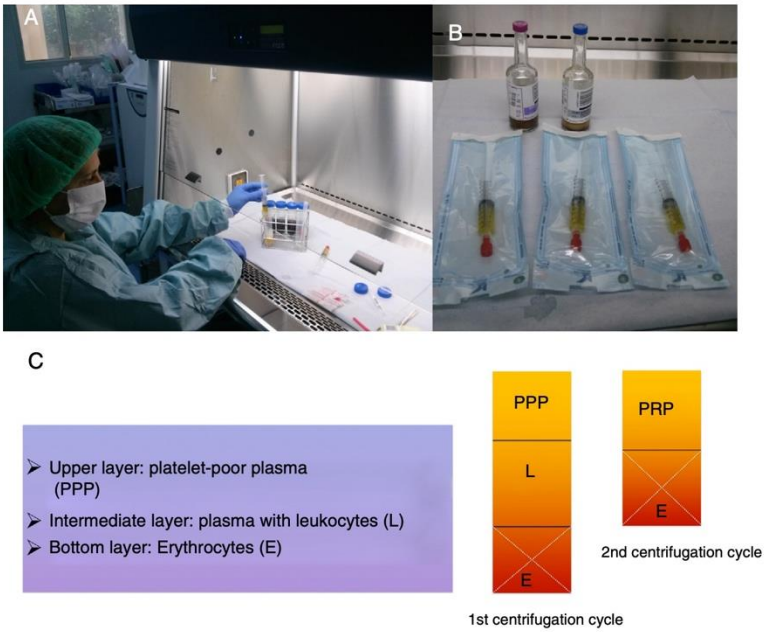

Figure 2 Classical technique of separation of PRP by two-stage centrifugation; PPP, platelet-poor plasma; BC, buffy coat; RBC, red blood cell; P-PRP, pure PRP; L-PRP, leukocyte-rich PRP.[16]

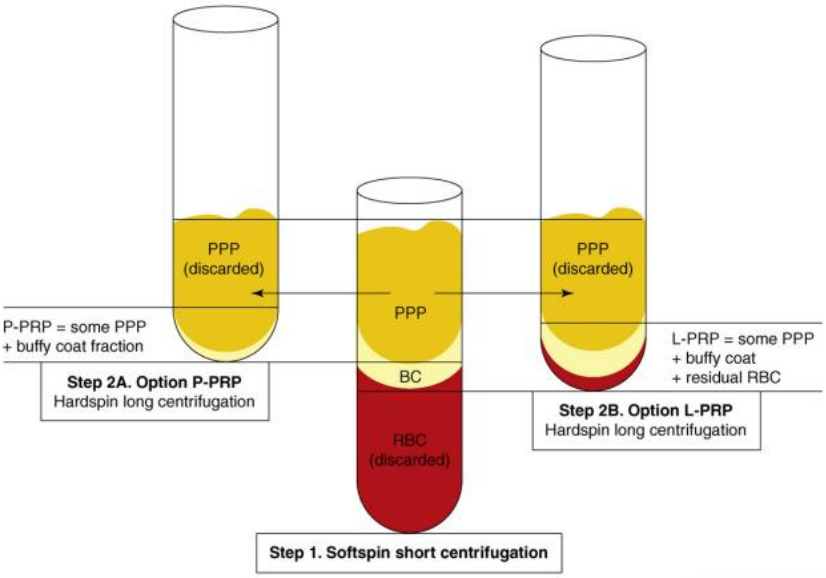

Figure 3 The role of PRP in axonal regeneration after peripheral nerve injury.[9]