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Review Article

Plate-Rich Plasma and its Utility in Clinical Conditions: A Systematic Review

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Abstract

Platelet-rich plasma (PRP) is an autologous product derived from whole blood through the process of density gradient centrifugation which contains a concentrated form of a large number of platelets in a small volume of plasma. PRP has a higher concentration of growth factors than whole blood. These growth factors promote natural healing. PRP is becoming more popular as a treatment option for a broad spectrum of medical disorders. PRP has been studied but has received less attention. The objective of this literature review was to focus on the utility of PRP on various medical conditions and, to consolidate the available evidence on PRP for the practicing dermatological conditions.

Keywords: *Pure Platelet-Rich Plasma (P-PRP), Leukocyte-and Platelet-Rich Plasma (L-PRP), Red - Platelet-Rich Plasma (R-PRP), Injectable Platelet Rich Fibrin (i-PRF), Platelet-rich plasma (PRP), Platelet-poor plasma (PPP), Cytokines, Growth factors.*

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INTRODUCTION

Platelet-rich plasma (PRP) can be considered as a form of autologous nonimmunogenic therapy, which contains a rich source of growth factors, cytokines, adhesion, and other molecules, which play a fundamental role in homeostasis and tissue remodeling. Human platelets release more than 300 different proteins[1]. The use of platelets as a rich source of bioactive factors was first suggested as a supplement to allogeneic fibrin glue. However, the availability of growth factors in platelet concentrates aroused great interest in this method for inducing healing and tissue regeneration, which is considered a low-cost alternative. An alternative for this platelet concentrates is platelet-rich plasma (PRP), which is a preparation from which leukocytes and erythrocytes are separated, preserving the enriched platelets. PRP has recently proved its therapeutic importance in several clinical areas, such as orthopedics, sports medicine, dentistry, gynecology, cosmetics, etc. Several trials are carried out for the clinical application of PRP with the induction of bone formation or the acceleration of wound healing in tissues, such as ligaments, muscles, or tendons[2]. Platelet-rich plasma content platelet concentration more than the normal reference value. The average normal platelet count is 200,000 platelets/µl, but after processing, a concentration of 1,000,000 platelets/µl is expected in PRP, that is, a five-fold or more enrichment[3]. PRP is obtained from autologous whole blood, so it is considered safe for clinical application, and reduce the risk of transmitting diseases. One of the issues in PRP is the presence of red blood cells and white blood cells it is considered that contaminate the PRP and leads to failure of treatment. Some studies show that the red blood cells present in intra-articular injections can lead to irritation of the synovial membrane, whereas leukocytes may be associated with both tissue protection and greater inflammation[4] and the presence of leukocytes significantly increase inflammatory cytokines, changing the regenerative potential of PRP, inducing pain and functional limitation. PRP contains several types of granules involved in coagulation, inflammation, atherosclerosis, antimicrobial host defense, and angiogenesis. Platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and Keratinocyte growth factor (KGF) are released from α-granules play a vital role in cell proliferation, migration, and differentiation. The systematic notes of platelets release growth factors are illustrated in Figure 1.

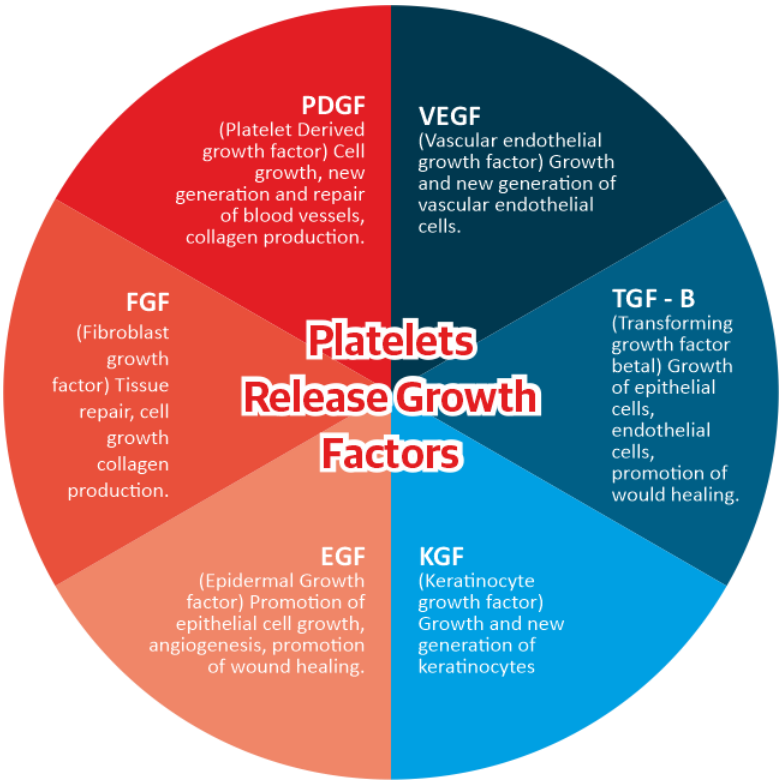

Figure 1: Platelets release growth factors

In 2009 PRP was used as an adjuvant of healing ankle injuries in sports players showed a great impact[5]. Nowadays platelet-rich plasma is used in stimulating wound healing in skin and soft tissue ulceration, accelerating wound healing in diabetic patients, and facilitating bone proliferation in orthopedic and trauma surgery. It has also applications in maxillofacial surgery, spinal surgery, plastic, and esthetic surgery, heart surgery, and burns. Many types of research proved the mechanism of action and demonstrated the efficacy in placebo trials. Plastic surgery is one of the main interest points of using PRP. Although, PRP has a wide range of therapeutic strategies in the management of injuries in the field of orthopedics and sports medicine, the approach which fulfills the objective of surgeons is to stop the progression of the disease and to improve function in the shortest period. In this respect, and as a clinical application of cell mechanotransduction, a rehabilitation program, which synergistically included the employment of PRP would play a crucial role in both promoting the repair or remodeling of injured tissue and avoiding the degradation and atrophy of structures such as the bone, peri-articular muscles, tendons and ligaments with the goal of full recovery of function.

CLASSIFICATION OF PRP

The classification of PRP is based on the preparation process. As such, based on the processing and separation methodology, PRP applications have a wide range of therapeutic applications (Figure 2). Classification of PRP is based on two main parameters: the presence of cell content (mostly leukocytes) and the fibrin architecture. This separation allowed defining four main types [6].

1. Pure Platelet-Rich Plasma (P-PRP)
2. Leukocyte-and Platelet-Rich Plasma (L-PRP)
3. Red - Platelet-Rich Plasma (R-PRP)
4. Injectable Platelet Rich Fibrin (termed i-PRF)

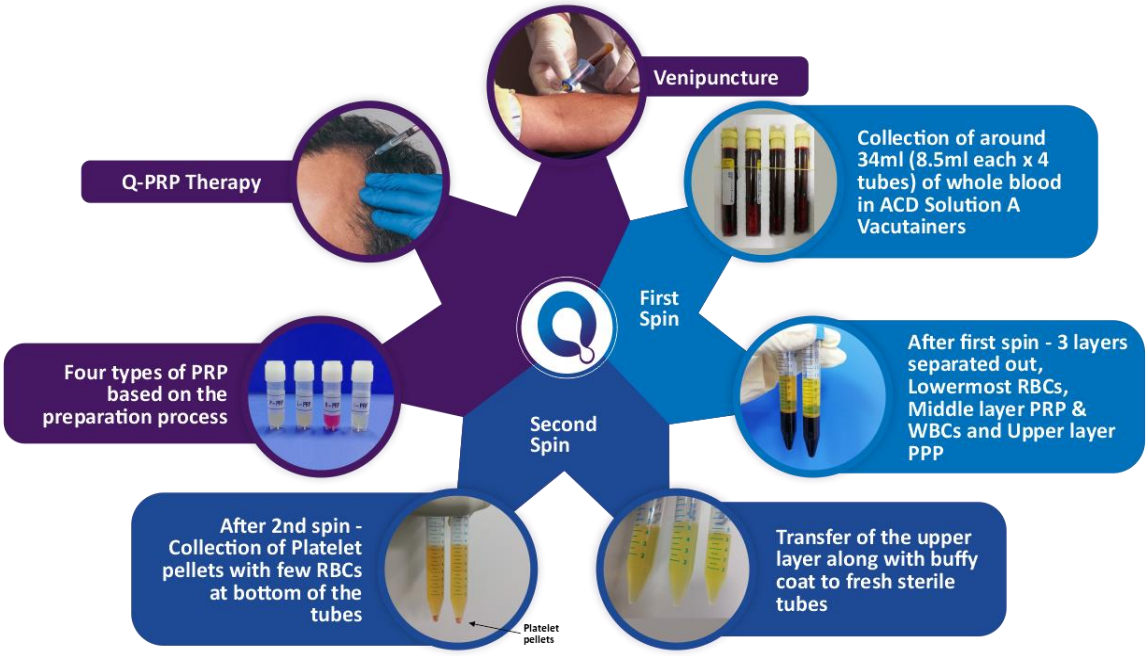

Figure 2: Summarization of the Processing, Preparation, and application of PRP for therapeutic applications.

Pure Platelet-Rich Plasma (P-PRP) – or Leukocyte Poor Platelet-Rich Plasma: P-PRP is platelet concentration with low or no leukocytes and with a low-density fibrin network after the activation process. All the types of PRP can be used in liquid form or an activated gel form. It can therefore be injected or placed during gelling on a skin wound or suture. P-PRP was widely promoted for skin ulcers. In most clinical applications this type of PRP treatment is used, P-PRP is a standard form of PRP. The process of creating the P-PRP involves the centrifugation process of the patient's whole blood to separate the plasma and concentrate the blood platelets, which are then automatically extracted and used for the PRP injection. In this type of PRP, no additives and activation are needed for preparing. P-PRP is more pure, concentrated, and customizable according to the need for treatment.

Leukocyte rich Platelet-Rich Plasma (L-PRP): L-PRP is rich in leukocytes and with a low-density fibrin network after activation. L-PRP is the largest number of commercial or experimental systems that exist with many interesting results mainly in orthopedic and sports medicine. Many automated protocols have been established, requiring the use of specific kits that allow minimum handling of the blood samples and maximum standardization of the preparations.

Leukocyte-rich platelet-rich plasma (L-PRP) is a volume of the plasma fraction of autologous blood having platelet and leukocyte concentrations above baseline. L-PRP is used in many clinical especially orthopedic conditions because of its healing properties attributed to the increased concentrations of growth factors and bioactive proteins. L-PRP is obtained when platelets are activated by thrombin, alpha granules contained in platelet release several growth factors, such as PDGF, TGF-β, insulin-like growth factor (IGF), EGF, and VEGF, this increase the potential of L-PRP and plays a prominent role in both bone and soft tissue healing processes. Besides, L-PRP contains a high concentration of leukocytes, which contribute to local debridement and exhibit bactericidal activities in acute and chronic wounds. L-PRP has proved successful in various fields including maxillofacial surgery, dentistry, neurosurgery, ophthalmology, otorhinolaryngology, wound healing, cosmetic, cardiothoracic, sports medicine, and orthopedics. L-PRP has also shown its advantages in the fields of trauma surgery for facilitating bone and soft tissue healing experimentally and clinically. In experimental studies, L-PRP has been identified to improve cellular chemotaxis, proliferation and differentiation, angiogenesis, and production of the extracellular matrix, but also responsible for stimulating defense mechanisms against infections. Clinical studies also show the beneficial effects of L-PRP, which include more rapid re-epithelialization and bone formation, reduced need for blood transfusion, reduction in postoperative swelling, bruising, and pain; shorten hospital stay, and early return to mobility.

Red - Platelet-Rich Plasma (R-PRP): Based on the color, while R-PRP further classified into three types. Red PRP is reddish so it is named red PRP, and it is often concentrated to lower levels. It's rich in white blood cells and also has some red blood cells which are the main reason for more inflammation and a stronger reaction when it is injected. Red PRP may less effective in most of the orthopedic treatments because of these numerous white blood cells. However, a few conditions may benefit with red PRP. Lower-Concentration of red PRP is Amber in color and concentrated to lower levels, or fewer platelets, Lower-Concentration red PRP is a newer type that is typically poor in white and red blood cells. It causes less tissue reaction and swelling when injected. This type is considered to be ideal, by most doctors, for injecting tendons and ligaments. Higher-Concentration amber colored PRP with more platelets, this high-concentration PRP typically cannot be created by very quick centrifugation. High-concentration PRP is ideal in joint applications and is best used to treat arthritis[7].

Injectable Platelet Rich Fibrin (termed i-PRF), is extracted from the autologous blood sample by a very specific centrifuging procedure in a completely natural process, thus refraining from any risk of immunological reaction. i-PRF is a unique rejuvenation technique that can enhance tissue regeneration, accelerate wound healing, and inducing stem cell differentiation through its growth factors

(GFs). i-PRF is a concentrated suspension of platelets (source of healing proteins and growth factors), fibrin (the wound healing matrix), and stem cells (the generators of the body's self-healing). The various cytokines that are involved in i-PRF are TGF-β, PDGF-AA, PDGF-AB, PDGF-BB, VEGF, and IGF-1.

MECHANISM OF ACTION

PRP consist of proteins such as PDGF, TGF-β, VEGF, EGF, and adhesive proteins – fibrin, fibronectin, and vitronectin is the basis of the mechanism of PRP. The list of applications of PRP mechanisms based is tabulated in Table 1.

Table 1: Applications of PRP

P-PRP	L-PRP	R-PRP	i-PRF
Intervertebral Disc Regeneration [8]	Bone Healing [12]	Tendon injection[7,13]	Rhinoplasty[14]
Stable Vitiligo [9]	Ligament injuries[12]	Ligaments injection[7,13]	Hyaluronic Acid-related Complications[15]
Bone lesion [10]	Skin Ulcers of Multifactorial Etiology [9]	Application in joints[7,13]	Regenerative Dentistry [16]
Degenerative changes in joint [10]	Chronic Diabetic Ulcer[9]	Arthritis[7,13]	Skin Plastic surgery [16]
Articular Cartilage Defect[10]	Venous Ulcers[9]		Oral and maxillofacial[16]
Osteoarthritis[10]	Leprosy Ulcers[9]		Periodontology[16]
Achilles tendons, Chronic tendinopathy[10]	Plastic and Esthetic Surgery[9]		Implant dentistry[16]
Rotator Cuff[10]	Chronic Wounds[9]		Chronic Skin ulcers[16]
Elbow, Epicodylitis[10]	Facial plastic surgery		Alopecia [17,18]
Knee Osteoarthritis[11]	Maxillofacial surgery and dentistry, bone regeneration, oral mucosa, and gingival flaps, orthopedics, neurosurgery, thoracic surgery, plastic and esthetic surgery in the skin, facelift, forehead lift, cervicofacial liposuction and rhinoplasty, traumatic wounds [16]		

CYTOKINES AND GROWTH FACTOR

The cytokines that have been investigated for their presence in PRP fall into four groups: pro-inflammatory cytokines; chemokines; interferons; and growth factors[19,20]. Some of these cytokines are leukocyte derived, whereas others are released from platelets. Because three different types of platelet products have different concentrations of leukocytes and platelets, each product type must be investigated as the cytokine level[21]. The list of cytokines and growth factors present in the PRP are tabulated in Table 2.

Table 2: Cytokines and Growth Factors obtainable in PRP

Cytokines and Growth Factors			
Cytokines /Growth Factors	**Systematic Name**	**Category**	**Primary Functions**
PDGF	Platelet-Derived Growth Factor	Growth factor	Potent chemo attractant and activator of neutrophils and monocytes. Promote angiogenesis, also proliferative, migration stimulatory effects and Stimulates DNA synthesis, attracts fibroblasts to wound sites, and enhances their production of collagenase, collagen, and glycosaminoglycan. [22,23,24]
PGDF-AA	Platelet-Derived Growth Factor-AA		
PGDF-AB	Platelet-Derived Growth Factor-AB		
PGDF-BB	Platelet-Derived Growth Factor-BB		

TGF-β (TGF-β1, TGF-β2)	Transforming growth factor	Growth factor	Activin B supports wound repair and regeneration of hair follicles, promotes wound closure, Activins which are members of the TGF-β family act as enhancers for granulation tissue fibroblasts and the induction of extracellular matrix deposition and Enhances proliferation of epithelial cells, expression of antimicrobial peptides and release of chemotactic cytokines. [25,26,27]
IGF-I	Insulin-like Growth Factor	Growth factor	IGF plays a relevant role in fetal development, growth during childhood and adolescence, and adult tissue homeostasis. Besides, IGF seems to have atheroprotective actions, neural protective, and insulin-like effects (at high concentrations) and to regulate skeletal metabolism and muscle regeneration. [24,28,29]
VEGF	Vascular endothelial growth factor	Growth factor	Stimulates endothelial cell migration, proliferation, and survival. [22,24,30]
EGF	Epidermal growth factor	Growth factor	Re-epithelialization of skin wounds and promotion of wound closure. [22,24,30]
IL-1	Interleukin I	Pro-inflammatory cytokine	They influence the inflammatory phase. [27,31,32]
IL-1β	Interleukin 1 beta		They promote wound healing by controlling the proliferation. [24,31,33]
IL-6	Interleukin 6	Proliferative and Pro-inflammatory cytokines	Possess both pro-inflammatory and anti-inflammatory activities under different conditions of the wound-healing process, Promotes angiogenesis formation, Promotes epithelial cell migration, Plays an axial role in wound healing by regulating cellular responses. [27,34,35]
IL-4	Interleukin 4	Anti-inflammatory cytokines	They play a primary role in the limitation and termination of inflammatory responses. [27,36,37]
IL-13	Interleukin 13	Pro-inflammatory cytokine	IL-13 inhibits proinflammatory cytokine and chemokine production in vitro and has potent anti-inflammatory activities *in vivo*. [24,38]
IL-7	Interleukin 7	Hematopoietic Growth factor	IL-7 is a pleomorphic cytokine expressed in normal human keratinocytes, where it has been previously shown to support epidermal T-cell growth and survival. It has an important role in immunological development, including involvement in early B- and T-cell development and peripheral T-cell homeostasis. IL-7 expression was enhanced in healing chronic wounds, being expressed in all layers of the epidermis. [27,39,40]
TNF-α	Tumor necrosis alpha	Pro-inflammatory cytokine	TNF-α accelerates wound epithelialization and neovascularization in this in vivo model. TNF-α can compensate for the negative effect of macrophage reduction and seems to have a direct effect on the wound-healing. [24,41,42]
Ang-II	Angiopoietin	Vascular Growth Factor	Angiotensin II (Ang II) raises blood pressure (BP) by several actions, the most important ones being vasoconstriction, sympathetic nervous stimulation, increased aldosterone biosynthesis, and renal actions. Other Ang II actions include induction of growth, cell migration, and mitosis of vascular smooth muscle cells, increased synthesis of collagen type I and III in fibroblasts, leading to thickening of the vascular wall and myocardium, and fibrosis. [24,43]
MIP-1β	Macrophage inflammatory protein 1-alpha	Chemokines	MIP-1α and MIP-1β promote wound closure. MIP-1α and MIP-1β increase macrophage trafficking. [24,31,44]
MCP-1	Macrophage inflammatory protein 1	Chemokines	MCP-1 and its receptor (CCL2) are primarily involved in macrophage infiltration. Inflammation regulatory chemokines in the wound-healing process. [24,31,45]
RANTES	Chemokine (C-C motif) Ligand 5	Chemokines	Normal (Acute) Skin Wound Healing: Chemoattractants for monocytes/ macrophages. [24,45,46]

Various cytokines are observed in all types of PRP mainly pro- and an anti-inflammatory biomolecule, interleukin (IL)-1b, IL-4, IL-6, IL-10, IL-17a and IL-22; macrophage inflammatory protein- 1a (MIP-1a/CCL-3), regulated upon activation, normal T-cell expressed, and secreted (RANTES/CCL-5), monocyte chemoattractant protein-3 (MCP-3/CCL-7), growth-regulated oncogene-a (Gro-a/CXCL-1), platelet factor 4 (PF-4/CXCL-4), epithelial neutrophil-activating peptide- 78 (ENA-78/CXCL-5), neutrophil-activating peptide-2 (NAP-2/ CXCL-7), IL-8/CXCL-8, fractalkine/CX3CL-1 and soluble CD40 ligand (s-CD40L) are observed in P-PRP, L-PRP, and PPP. As a proof of evidences, the values of growth factors and cytokines present in PRP evidenced by researchers are listed in the Table 3.

Table 3: Values of Growth factors and Cytokines present in PRP

PRP Cytokines and Growth Assessment Chart			
Evidences	**Cytokines & GF studied**	**Observed Values**	**Supporting Studies**
1	PDGF-BB	17±8 ng/ml	Growth Factor Content in PRP and their applicability in medicine.[47]
	TGF-β	120±42 ng/ml	
	VEGF	955±1030 pg/ml	
	EGF	470±317 pg/ml	
	PDGF-AB	117.57 ng/ml	
2	TGF-β1	300 ng/ml	Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). [48]
	PDGF-BB	15 ng/ml	
	VEGF	800 pg/ml	
	IL-1β	14 pg/ml	
	IL-6	10 pg/ml	
3	PDGF-AB/BB	>10,000 pg/ml	Characterization of the cytokine profile of platelet-rich plasma (PRP) and PRP-induced cell proliferation and migration: Up regulation of matrix metalloproteinase-1 and -9 in HaCaT cells. [49]
	PDGF-AA	>10,000 pg/ml	
	bFGF	370 pg/ml	
	RANTES	3,228 pg/ml	
	GRO	3895 pg/ml	
	SCD40L	3418 pg/ml	
	TGF-β1	2435 pg/ml	
	TGF-β2	103.5 pg/ml	
4	PDGF-AB	47±0.94 ng/ml	Platelet-Rich Plasma: Quantitative Assessment of Growth Factor Levels and Comparative Analysis of Activated and Inactivated Groups. [50]
	PDGF-BB	37.15±1.62 ng/ml	
	TGF-β	118.7±1.84 ng/ml	
5	PDGF-AA	7.9±5.6 pg/ml	
	PDGF-AB	37.6±14.9 pg/ml	
	PDGF-BB	20.1±10.0 pg/ml	Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. [24]
	TGF-β1	318.6±118.4 pg/ml	
	TGF-β2	365.2±389.5 pg/ml	
	EGF	438.5±195.3 pg/ml	

The concentration of cytokines released from PRP varied over time and was influenced by various activation protocols (Table 4). The effect of the activation was shown to be dependent on the preparation method as well as on the type of cytokine and, accordingly, proper PRP components with activation methods should be selected by considering their biomolecular characteristics and patient indications. Cytokines such as PDGF-BB, TGF-β1, VEGF, and FGF concentrations plays important role in cell proliferation, chemotaxis, cell differentiation, and angiogenesis. PDGF is a powerful mitogen for fibroblasts and smooth muscle cells. TGF-β helps in stimulating the proliferation of

undifferentiated mesenchymal stem cells and the chemotaxis of endothelial cells and angiogenesis. VEGF stimulates endothelial cell mitogenesis and cell migration, and FGF proliferates and differentiates a wide variety of cells and tissues. Conversely, IL-1β, and MMP-9 are catabolic cytokines that are known for inflammation or matrix degradation. Interleukin-1β is a primary cytokine during inflammation and matrix degradation, and it is a common target to reduce inflammation by manipulating IL-1ra. MMP-9 is known to degrade collagen and other extracellular matrix molecules and has been implicated as a predictor of poor healing[51]. EGF is a growth factor that stimulates cell growth, proliferation, and differentiation by binding to its receptor EGFR [18].

When the PRP is activated with the external dose such as thrombin, calcium chloride was added exogenously to the PRP; a low level of thrombin formed endogenously and allowed a slower GF release over a longer period than exogenous thrombin. Along with this, thrombin caused a rapid aggregation of platelets and an excessive condensing of the fibrin matrix with rapid activation of the platelets. A low dose of thrombin has been shown to increase the migration and the number of mesenchymal progenitor cells derived from bone marrow whereas high concentrations have been demonstrated to have limited effects on the proliferation of osteoblasts and alveolar bone cell, suggesting that the thrombin dose plays a role in the GF-release kinetics of the PRP preparations.

Table 4: List of PRP Activators

Name of the activator	Research Studies	Activator
Calcium Chloride	Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors	Platelet-rich plasma was activated using 20 mM $CaCl_2$. [52]
Calcium chloride (CaCl2) + thrombin	Platelet-rich plasma: the choice of activation method affects the release of bioactive molecules	10% of a mixture of $CaCl_2$ + thrombin. [53]
Collagen type I	Platelet-rich plasma: the choice of activation method affects the release of bioactive molecules	10% of collagen type I (final concentration 4 µg). [53]
Freeze-Thaw	Activation of equine platelet-rich plasma: comparison of methods and characterization of equine autologous thrombin	Freeze Thaw. [54]
Hyaluronic Acid	Hyaluronic acid induces the release of growth factors from platelet-rich plasma	1 mL of Platelet-rich plasma and 0.6 mL of Hyaluronic acid. [55]
PRP activation using a bead mill homogenizer	A method for the activation of platelet-rich plasma via bead mill Homogenizer for Mesenchymal stem cell culture	Bead mill homogenizers. [56]
Thrombin	Platelet-Rich Plasma: The Choice of activation method affects the release of bioactive molecules	10% of autologous thrombin. [53]
Vitamin C	The combined use of platelet-rich plasma and vitamin C positively affects differentiation in vitro to the mesodermal lineage of adult adipose equine mesenchymal stem cells.	Combining vitamin-C and plasma-rich-platelet positively affected the ability of MSC to differentiate in vitro into mesodermal lineages. [57]

The effect of the activation depended on both the preparation method and the type of cytokine assessed. Ca-only activation had a significant effect on the double spin PRP preparation (VEGF, FGF, and IL-1β concentrations) whereas Ca/thrombin activation had significant effects on both single spin and double spin PRP preparations (PDGF-BB and VEGF concentrations sustainably and TGF and FGF concentrations shortly). These interpretations may be due to the biological activity of the platelets is sensitive to any kind of process-related stress and that more platelets are activated during the process with the double spin method. These results are also consistent with the previous findings, it was testified that the individual dynamics of the growth factors release depend exclusively on the type of growth factors rather than on the preparation method. In overall, as per the scientific evidences, TGF-β1 and bFGF are promptly released within 24 hours of exogenous activation whereas the growth factors release of the PDGF-BB and the VEGF is more dependent on the technique that is used.

CONCLUSION

Platelet-rich plasma works by delivering a supra-physiologic amount of growth factors and cytokines contained within platelets that influence the healing of tendon, ligament, muscle, and bone. PRP proves to be a promising treatment modality with clear evidence of safety. The efficacy of PRP has been based on mixed and highly dependent on composition and the specific indication. This article reviewed the basic science of PRP, and it describes the current clinical applications.

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