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Platelet-Rich Blood Derivatives for Alveolar Bone Regeneration

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Abstract
Platelet-rich blood derivatives have been widely used in different fields of medicine and stem cell-based tissue engineering. They represent natural cocktails of autologous growth factors, which could provide an alternative for recombinant protein-based approaches. Platelet-rich blood derivatives, such as platelet-rich plasma, have consistently shown to potentiate stem cell proliferation, migration, and differentiation. Here, we review the spectrum of platelet-rich blood derivatives, discuss their current applications in alveolar bone regeneration.
Keyword: *Blood derivatives .Platelet Rich Fibrin Platelet-Rich Plasma. Cell proliferation.Cell differentiation.*

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I. Introduction

Wound healing is initiated by clot formation, followed by proliferative stage which comprises of epithelialization, angiogenesis, granulation tissue formation, collagen deposition and finally collagen maturation and contraction.[1] This involves adherence and aggregation of platelets favoring formation of thrombin and fibrin. Platelets contain biologically active proteins, binding of these proteins within a developing fibrin mesh or to the extracellular matrix can create chemotactic gradients favoring recruitment of stem cells, stimulating cell migration, differentiation, and promoting repair. Thus, use of autologous platelet concentrates is a promising application in the field of bone regeneration and can be used in clinical situations requiring rapid healing.[2]

Tissue engineering traditionally stimulates cells using a single bioactive agent with key regenerative functions In contrast, natural tissue regeneration relies on a cocktail of signaling molecules and growth factors. During natural wound healing, activated platelets concentrate in the wound area and secrete a plethora of factors that play an instrumental role in coordinating wound healing. Using a single growth factor to steer tissue regeneration represents an oversimplified and inefficient stimulus. This is generally overcome by providing supraphysiological quantities of the growth factor of choice, which comes at the price of adverse effects [2]. In consequence, a rapidly growing number of studies have explored the efficacy of supplementing stem cell-based tissue engineering approaches with natural growth factor cocktails such as platelet concentrates. This has paved the way for improvements in (stem) cell function including cell growth, viability, proliferation, differentiation, and overall regenerative potential [3]. Platelet concentrates are therefore becoming widely used in medicine. Moreover, their translation is being fueled by their availability, cost-effectiveness, extensive range of applications, and autologous nature. Indeed, several clinical uses of platelet concentrates have been reported in the fields of dermatology, orthopedics, dentistry, and ophthalmology [4].

Platelet-Rich Blood Derivatives

Activation of platelet triggers degranulation and subsequent release of trophic factors that influence wound healing, tissue repair, angiogenesis, and stem cell behavior. There are two types of granules inside the platelets: alpha and dense granules[5].

Alpha granules affect wound healing through several typesof growth factors including the following: platelet-derivedgrowth factor (PDGF), epithelial growth factor (EGF), vascularendothelial growth factor (VEGF), endothelial cell growth factor(ECGF), fibroblast growth factor (FGF), transforminggrowth factor beta (TGFβ), and insulin-like growth factor(IGF). In general, these factors chemotactically recruit and activatestem cells as well as induce their mitogenesis and differentiation.

Dense granules promote tissue regeneration by secreting mediators such as serotonin and histamine, which increase vessels permeability and tissue perfusion [6]. Over the years, several platelet-rich blood derivative

formulations have been explored. Most notably, they have received a substantial amount of attention in tissue regeneration studies aimed at the healing of injured soft and hard tissues [7].

Platelet-Rich Plasma (First Generation Platelet Concentrate)

Platelet-rich plasma (PRP) has been introduced several decades ago [8]. PRP is prepared via a two-step centrifugation preparation of a blood sample, which was cured with an anticoagulant. During the first step of centrifugation, three layers are demarcated: plasma on top, erythrocytes on the bottom, and a middle layer that contains platelets and leukocyte. After discarding the erythrocytes, the remainder is centrifuged for a second time to ensure proper plasma separation. PRP is subsequently obtained by discarding the plasma[9] (Figure . 1 a) . This process concentrates the platelet by approximately fourfold to sevenfold as compared to untreated whole blood [11]. By varying the isolation protocol, distinct mixtures can be obtained.

In general, longer centrifugal periods slightly increased the platelet yield and decreased the concentrations of white blood cells in the upper layer. Therefore, centrifugation parameters can be used to control the amount of white blood cells in PRP [12]. Temperature proved essential to control platelet activation; low temperatures delayed platelet activation and prolonged their viability [9].

Platelet-Rich Fibrin(Second Generation Platelet Concentrate)

Platelet-rich fibrin (PRF), which is a single stage centrifuged product that does not require the addition of various chemicals. In particular, blood is centrifuged immediately after drawing to prevent coagulation. Subsequently, the middle layer is separated from the two other layers[15] (Figure 1 b) . Centrifugation is usually performed at 700g for 12 min to obtain standard PRF (S-PRF) or at 200g for 14 min to obtain activated PRF (APRF).

A-PRF contains more platelets, most were found at the distal layer of PRF membrane, and S-PRF includes more neutrophils[16]. This type of white blood has the potential to enhance angiogenesis by expressing the enzyme matrix metalloproteinase [9]. Therefore, the inclusion of neutrophils in PRF could be considered if angiogenesis is of interest [17] .

PRF can release high quantities of multiple growth factors including TGFβ-1, PDGF, and VEGF [10]. The main difference between PRF and PRP resides within their respective fibrin architectures.

In PRF, this network gradually builds up during centrifugation and in the absence of anticoagulant agents. This results in a dense fibrin structure and, in PRF, acts as a network in which platelets and leukocytes are entrapped during centrifugation (Figure 1 c). This reservoir property of the fibrin network enhances the gradual release of growth factors and othermediators, resulting in prolonged maintenance and stimulation of stem cells by PRF[18] . Indeed, the release patterns of growth factor such as TGFβ and PDGF are different between PRP and PRF. In PRP,the release of TGFβ and PDGF clearly decreased after the firstday, while PRF was demonstrated to release significant amountsof TGFβ and PDGF for up to 2 weeks [19] .

Subclassification of PRP and PRF based on Leukocyte

Besides platelets, leukocytes contribute to chemical composition of PRP and PRF by secreting molecules such as interleukin 1 beta (IL1β), IL4, IL6, and tumor necrosis factor alpha (TNFα), which affect wound inflammation, vascularization, and regeneration [23] .

PRP and PRF have been classified into two main groups based on whether they contain or lack leukocytes. While leukocytes are present in traditional PRP and PRF (named L-PRP and L-PRF),

plasmapheresis of these platelet-rich blood derivatives results in leukocyte-free ‘pure’ PRP and PRF (P-PRP and P-PRF).

As inflammation is one of the fundamental stages of wound healing, leukocytes can be considered as interesting cell source for both the initiation and regulation of the tissue regeneration cascade. Furthermore, they can control excess inflammation by timely release of anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 [13] . Indeed, leukocytes provide an immune regulatory role and release of large amounts of VEGF and other cytokines. Regardless, the most commonly used platelet-rich blood derivatives rely on leukocyte limited formulations (L-PRP and L-PRF) [24].

Figure 1 Preparation protocols of PRP and PRF

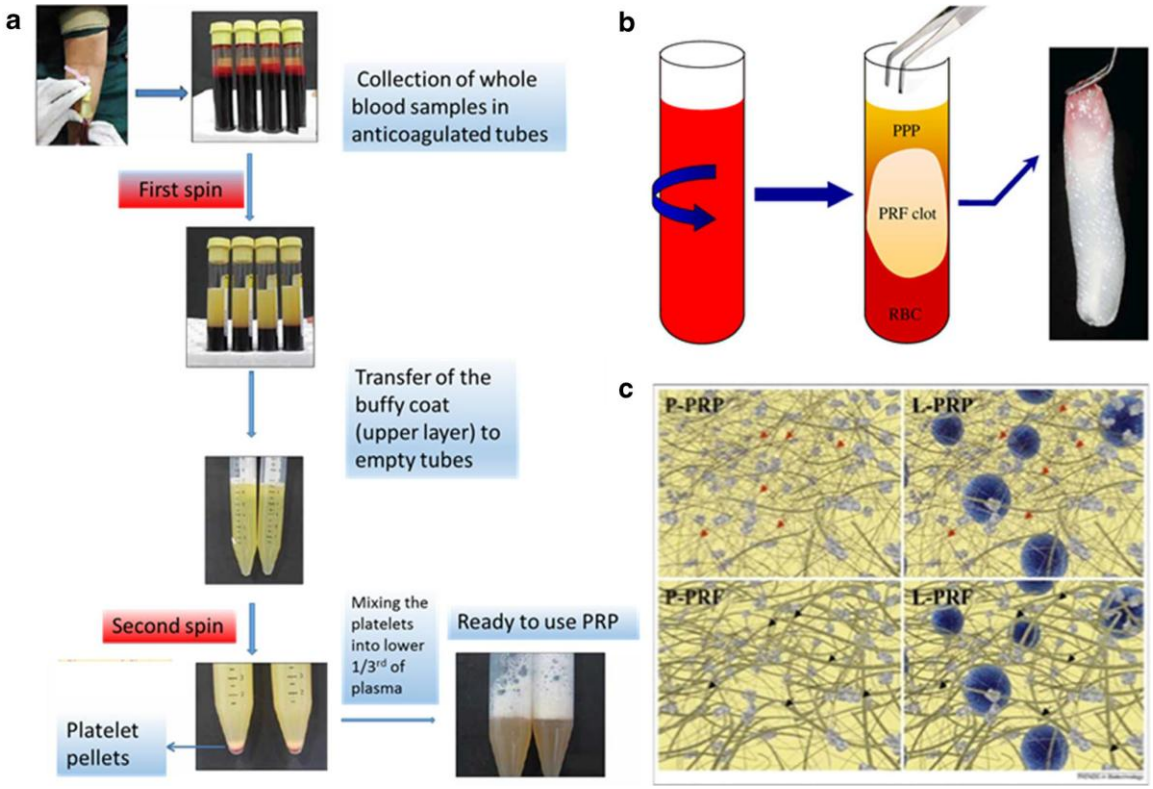
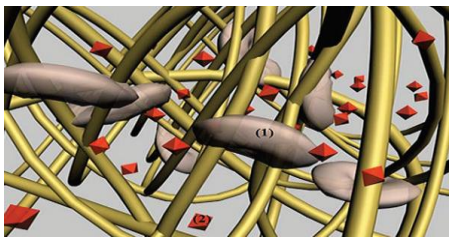
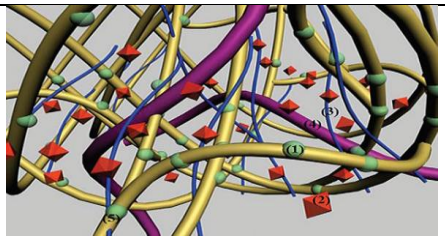


Figure 1 Preparation protocols of (a) PRP and (b) PRF. c Schematic depiction of leucocyte-free platelet-rich plasma (P-PRP) and plateletrichfibrin (P-PRF), leucocytes PRP (L-PRP), and leucocyte- and platelet-rich plasma. L-PRF leucocyte- and platelet-rich fibrin

Table 1 Difference between PRP AND PRF

		First Genration PRP	Second Genration PRF
1	BASED ON PROCESSING TECHNIQUE	Use of bovine thrombin and calcium chloride (anticoagulants) Two spin centrifugation The tube is centrifuged at 1300 rpm for 10 min (soft spin). A second centrifugation is performed at 2000 rpm for 10 min (hard spin) After blood collection, one can Wait for 10 min for centrifugation Preparation is labour intensive	No anticoagulants are used Single spin centrifugation The tube is centrifuged at 3000 rpm for 10 min It involves speedy blood collection and immediate centrifugation Simple and cost effective Based on
2	BASED ON ARCHITECTURE	Sudden fibrin polymerization‑depending on the amount of surgical additives (thrombin and calcium chloride) Bilateral junctions (condensed tetra molecular) are constituted with strong thrombin concentrations and allow the thickening of fibrin polymers leading to the constitution of a rigid network, unfavorable to cytokine enmeshment and cellular migration  Theoretical computer modeling of PRF Platelet trappedin the fibrin gel Platelet cytokine in solution	Slow natural polymerization on contact with glass particles of the test tube results in physiologic thrombin concentration Equilateral junctions (connected trimolecular) allow the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration. This 3‑dimensional organization gives great elasticity to the fibrin matrix which is observed in a flexible, elastic and very strong PRF membrane[6]

		There is Immediate release of growth factors	
3	Based on biological property	Concern over the use of bovine thrombin, bovine factor Va may be a contaminant in certain bovine thrombin commercial preparations, antibodies to bovine factor Va may cross react with human factor Va and may produce coagulopathies and rare bleeding episodes	Theoretical computer modeling of PRF Cytokine intrinsically retained within fibrin fibrillaePlatelet cytokine in solution (extrinsically associated with fibrin polymers) Fibrin‑associated glycanic chains Circulating glycoproteins (fibronectin)Fibrin fibrilla associate with glycanic chains and intrinsic cytokines
4	Based on therapeutic concern		Growth factors are released slowly over a period of 7 or more days[7] No coagulopathies and no bleeding episodes An in vitro study showed that PRF is superior to PRP, considering the expression of alkaline phosphatase and induction of mineralization, caused markedly by release of TGF‑β, and PDGF‑AB

Growth Factor Release of PRP and PRF

In addition to the composition of PRP and PRF, their delivery method plays a pivotal role on their ability to affect (stem) cells as well as on their clinical outcome. A hydrogel system utilizing alginate carriers for PRP growth factor release revealed that beads or capsules had different release profiles of PDGF-AB, TGFβ-1, and IGF-1 [25]. Alginate beads demonstrated higher release of TGFβ-1, whereas capsules favored PDGF-AB release; alginate capsules showed higher levels of SaOS-2 cell proliferation as compared to beads and controls.. Currently, there are over a dozen ongoing large clinical trials studying the regenerative potential of PRP, which would help elucidate the potential benefits and pitfalls of this blood derivative [4].

Cell Proliferation

PRP has consistently been shown to increase cell proliferation in all explored cell types. This includes differentiated cells such as osteoblast-like cells and chondrocytes [25 26 28 34], periodontal ligament cells [31 35 36] tendon cells[37 38], preadipocytes, and endothelial cells[33 39] as well as multipotent cells such as mesenchymal stem cells [40 – 43] and adipose-derived stem cells [5 45 - 47]. PRP mediates this increase in proliferation in a dosedependent manner [29 - - 38]. Osteoblasts were shown to increase proliferation with PRP [33], although one study showed platelet poor plasma (50 %) to have a greater enhancement of cell proliferation [31]. In alveolar bone cells, higher concentrations of PRP suppressed proliferation, whereas low concentrations (1–5 %) stimulated proliferation [30] .Regardless, PRP exerts a stimulatory effect on the proliferationof stem cells such as MSCs (Fig. 1 c), exhibiting also dose dependency [40 – 45]. PRP can also indirectly stimulateproliferation by enhancing (stem) cell adhesion, highlightingthe use of PRP for increased (stem) cell adherence in biomaterials[45] . Besides proliferation, PRP can contribute to an increase in cell expansion rates by reducing cell death. In particular, PRP supplementation reduced Bcl-2 expression and apoptosis [46].

Different animal models have corroborated the capabilities and limitations of PRP usage for cell proliferation. In a diabetic rat femur fracture model (noncritical), PRP supplementation at the fracture site increased cell proliferation, torque to failure, and torsional rigidity [49]. In another study, PRP releasedfrom gelatin gels implanted in a bone defect promoted bone regeneration (Fig. 3). However, in a long bone defect model, PRP combined with a collagen scaffold did not exert differential effects in bone volume, mineral density, and mechanical rigidity [50]. These potentially conflicting reports might point toward the dire consequences of different growth factor compositions between distinct patients, thus emphasizing the irregular nature of PRP composition and its clinicalimplications[51].

Cell Differentiation

PRP can have a potent effect on the differentiation of a variety of stem cells. PRP releasate has shown a particular affinity for stimulating the differentiation of stem cells into skeletal cell types such as cartilage, bone, blood vessel, and tendon [47, 52 - 54]. Periodontal ligament cells as well as osteoblast-like cell lines SaOS-2 and

HOS showed increase in alkaline phosphatase (ALP) activity and increased the differentiation into mineralized tissue formation cells upon exposure to PRP [25 3436] . Moreover, these cell lines rapidly increased the expression of the osteogenicdifferentiation markers such as osteopontin (OPN), osteoprotegerin, and runt-related transcription factor 2 (RUNX2) [34]. In adipose-derived stem cells, PRP was found, at later stages (21 days), to promote osteogenic differentiation by increasing ALP, OPN, osteocalcin (OCN), and RUNX2 in a dose-dependent manner [47]. Interestingly, PRP exposure not only increased the osteogeneic potential of stem cells but also reduced their adipogenic differentiation efficacy (Fig. 3) . PRP is hypothesized to exert its osteogenicstimulation through synergistic effects with BMP2, BMP4, BMP6, and BMP7 [55]. Indeed, BMPs have a stronger effect on osteoblast differentiation than PRP but demonstrated augmented effects when Stem Cell Rep supplemented with PRP [29]. This makes PRP a suitable synergistic agent to steer stem cell osteogenesis for bone tissue engineering applications. Besides osteogenesis, PRP's effect on chondrogenesis of stem cells has been well-characterized [32,56] .

Angiogenesis

PRP is also known to stimulate angiogenesis, which is an essential part of tissue regeneration and stem cell recruitment. It increases migration and tube formation in HUVECs [39]. In a coculture of endothelial progenitor cells (EPCs) and dental pulp stem cells, PRP was shown to increase VEGF and PDGF secretion thereby promotingvasculogenesis and stimulating EPCs to form vessel-like structures [59]. PRP contains high levels of angiopoietin 1 (Ang1), which mediates angiogenesis. Inhibition of Ang1-Tie2 signaling suppressed the proangiogenic effect of PRP [60]. Interestingly, PRP preventedendotoxin-induced pulmonary edema through the same pathway [61], which might be due to the PRP's potential to stabilize vascular integrity and permeability. PRP diminishedthe disruption of cell-cell junctional integrityinduced by inflammatory cytokines [61]. Indeed, PRPsupplementation has consistently been associated with improved angiogenesis in a variety of models and methodsofapplication (Fig. 4)[60, 62 64]. Therefore, blood derivatives such as PRP could be explored as an autologous competitor for the traditionally used recombinant protein VEGF to induce blood vessel formation within implanted bioengineered constructs.

Chemotaxis and Inflammation

In addition to PRP's and PRF's direct effects on proliferation, differentiation, and angiogenesis, they also affect wound healing indirectly via the chemotactic recruitment of cells and local control of the inflammatory environment. Indeed, PRP has been reported to chemotactically attract human MSCs [54]. Another promising chemotactic effect was also observed in a rat tendon healing model, where PRP was able .

Activated PRP demonstrated high levels of hepatocyte growth factor (HGF), IL-4, and TNFα[67]. In chondrocytes, high levels of PRP-derived HGF and TNF-α decreased the trans-activating activity of NF-κB by acting as an anti-inflammatory trigger [67] .

Additionally, in osteoarthritic chondrocytes challenged with IL1B, PRP reduced the levels of NF-κB activation and had multiple anti-inflammatory effects [68]. In another osteoarthritic model utilizing osteoarthritic cartilage and synovium from patients, PRP, with or without leukocytes, had similar anti-inflammatory effects [69]. Moreover, in tendon cells treated with IL-1B, PRP induced the expression of VEGF, RANTES, and HGF and decreased pro-inflammatory cytokines IL-6, IL-8, and MCP-1 [70]. Taken together, the set of reports mentioned above highlight a role of PRP in growth factor release and its importance for chemotaxis and inflammation.

Not surprisingly, PRP has been considered for the management of inflammation and pain for specific diseases such as osteoarthritis [71].

Neuroprotection and Other Effects

In organ cocultures of brain cortex and spinal cord, PRP was also found to promote axon growth, mediated by IGF-1 and VEGF, but not by PDGF-AB [72]. Furthermore, inhibition of TGFβ-1 results in higher axon growth, suggesting that TGFβ-1 inhibits or impairs axon growth. Besides axon growth, PRP has neuroprotective properties. In a rat brain focal ischemic stroke model, PRP lysate administered systemically or locally provided neuroprotective effects [73].

However, the effects in reducing infarct volume were higher with local intra-arterial infusion of PRP lysate compared to systemic administration. Additionally, PRP minimized neurological deficits in the same model. PRP also showed antimicrobial activity by inhibiting bacterial growth. Agar plates with PRP inhibited initial bacterial growth of Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus faecalis[74]]. This inhibition was mediated by the release of anti-bacterial chemokine ligand 1, 3, and 5. Interestingly, PRP was shown to upregulate the pluripotency markers and decrease lineage-specific marker expression in human MSCs, human muscle-derived progenitor cells, and adipose-derived stem cells [43, 75].

Moreover, PRP has been demonstrated to protect cell viability during cryopreservation [42]. Thus, PRP holds promise to augment stem cell expansion while minimizing loss of stemness.

Table 2 MERITS and DEMERITS of PRF

MERITS OF PRF	DEMERITS OF PRF
.Ease of preparation/application .Lack of biochemical modification .Simplified and cost effective process .Long-term effect .Able to support cytokines enmeshment and cellular migration .Increased incorporation of the circulating cytokines in the fibrin meshes (intrinsic cytokines) .It is an immune organizing node .It supports and accelerates the healing process due to slow polymerization .Helps in hemostasis .Three-dimensional structure giveselasticity and flexibility to PRF membrane	.Low quantity of PRF is obtained,because of autologous blood so application in general surgery is limited .The clinical benefit of PRF depends on time interval between speed of handling between blood collection and centrifugation as PRF is prepared without any addition anticoagulants .The fibrin matrix contains the circulating immune cells and all the highly antigenic plasmatic molecules, that is why PRF istotally specific to the donor] .PRF membrane should be used immediately after preparation as it will shrink resulting in dehydration altering the structural integrity of PRF and leukocyte viability will be adversely affected altering its biologic properties .PRF when stored in refrigerator can result in risk of bacterial contamination

Table 3: Merits and demerits of Platelet Rich Plasma

MERITS OF PRP	DEMERITS OF PRP
.Soft autologous preparation, . Free from concerns over transmissible disease such as HIV, hepatitis etc. .Convenient for patien . Blood is collected in the immediate preoperative period .Presence of platelets brings cytokines and growth factors to the site of surgery which helps in rapid regeneration in a manner that would not occur with fibrin glue	.Concern over the use of bovine Thrombin the fact that bovine thrombin has been associated with development of antibodies to clotting factors V, XI and thrombin, which had occasionally lead to life threatening coagulopathies .Lack of uniformity in PRP preparation protocol as different platelet concentration has different storage time

Clinical Translation

PRP and PRF have many clinical applications in regenerativemedicine. Numerous in vitro and animal studies consistently reported that these blood-derived products can promote stem cell differentiation to some cell lineages. Currently, stem cell laden PRP scaffolds are clinically explored. In particular, they are investigated in maxillofacial procedures, orthopedic surgeries, and wound or burn therapies. PRP has been reported to accelerate chronic skin ulcer repair as well as improve fat graft outcomes. For instance, patients with lower extremity ulcers were treated with PRP mixed by fat tissue base or with a control treatment consisting of collagen mixed with hyaluronic acid. After 7 weeks and 10 weeks, the wounds treated with PRP were reepithelializedcompletely in respectively 61.1 and 88.9 %, compared to 40 and 60 % of control group. A similar study was held for to explore the effect of PRP on the treatment of different maxillofacial defects. Patients received either treatment with PRP and fat grafting or fat graft only. One year post surgery, graft survival, and maintenance of three-dimensional tissue volume were 70 % in PRP-treated group, as compared to 31 % in the fat graft only control group [76]. In the orthopedic field PRP is regularly included as a bioactive component.

In dentistry field, PRP was explored to treat a Black Triangle, which is the interproximal distance between the teeth caused by receded papilla height.

II. Summary

The early clinical results indicated that platelet based blood derivatives such as PRP and potentially PRF are promising supplements for current stem cell-based therapies. In particular, they have consistently been shown to represent a safe and readily available source of growth factors.It is a long time that soft tissue maintenance, wound healing and protecting tissue from bacterial infection is the clinician's primary intention. PRF belongs to a new generation of platelet concentrate, it represents a new step in the platelet gel therapeutic concepts with simplified processing with no artificial biochemical modification. PRF includes ancytokines, glycanic chains, and structural glycoproteins enmeshed within a slowly polymerized autologous fibrin network. PRF releases high quantities of three main growth factors TGF-β1, PDGF-AB, vascular endothelialgrowth factor, and an important coagulation matricellularglycoprotein (TSP-1) during 7 days. Apart from this PRF secrete EGF, FGF, and three important proinflammatorycytokines - interleukin (IL)-1b, IL-6, and TNF-α. The easily applied fibrin acts much as a fibrin bandage with biochemical components that already have well known synergistic effects on healing processes. The presence of fibrin network composed up of leukocytes and cytokines play a significant role in self-regulation of the inflammatory and infectious phenomenon within the

grafted material. Apart from its application in various disciplines of dentistry PRF is also been used all over the world in a various medical field that too includes orthopedic and plastic surgery. Although many merits and demerits of PRF is in front of us still numerous prospective of this new generation platelet concentrate, have to be obtained and searched for. The results obtained from PRF for various treatments are quite encouraging but still further studies are necessary to support its common use in day today practice with its clinical efficacy and long‑term stability. Most importantly, establishing a scientifically sound, evidence‑based rationale is critical to have the ultimate success of PRF.

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