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Biological Additives and Platelet Concentrates for Tissue Engineering on Regenerative Dentistry: Basic Science and Concise Review

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Review Article

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Abbreviations and Acronyms: PC: Platelet Concentrate; GF: Growth Factor; T-PRF: Titanium-Platelet Rich Fibrin; P-PRP: Pure-Platelet Rich Plasma; L-PRP: Leukocyte-Platelet Rich Plasma; P-PRF: Pure-Platelet Rich Fibrin; L-PRF: Leukocyte-Platelet Rich Fibrin; TGF-β1: Transforming Growth Factor β1; PDGF: Platelet Derived Growth Factors; VEGF: Vascular Endothelial Growth Factor; CTGF: Connective Tissue Growth Factor; EGF: Endothelial Growth Factor; VSMC: Vascular Smooth Muscle Cell; BV: Blood Vessel; EPC: Endothelial Progenitor Cell; BM: Bone Marrow; BCP: Biphasic Calcium Phosphate; SEM: Scanning Electron Microscope; β-TCPL: β-Tri-Calcium Phosphate; SHB: Self Hardening Biomaterials; Past: Piezotome-enhanced Sub-Periosteal; Tunnel-Technique; ITV: Insertion Torque Value; HA: Hydroxylapatite

ABSTRACT

In oral surgery, there is an increased concern for soft and hard tissue wound healing processes and the development of bioactive additives for targeted surgical sites has become an important challenge in the last three decades. Recently, platelet concentrates have been identified as satisfactory bioactive materials that increase the speed of the healing process in peri-implant surgical sites. Moreover, recent convincing results in several clinical studies and literature reviews have demonstrated the importance of these bioactive materials in the stimulation of the healing process and have provided promising results for use in the future. In order to stimulate and ensure the healing for both soft and hard tissues in the oral region, there is convincing evidence that platelet concentrates (PCs) can serve as an autologous source of growth factors (GFs) and healing cytokine biomolecules, such as platelet rich plasma (PRP), platelet poor plasma (PPP), and platelet rich fibrin (PRF) release, which plays a crucial role in promoting hemostasis and the wound healing process. In recent studies, the primary concern has been the platelet concentrates in general and particularly, platelet-rich fibrin. The following review attempts to discuss the current data for researchers and clinicians to understand the value of combining biological additives with platelet-derived products for the healing of surgical sites. This approach is of particular concern, as the critical processes and effect on the speed of action is a controversial topic for both researchers and clinicians alike.

INTRODUCTION

The modern advances in regenerative dentistry [1] have added insight from the field of molecular biology [2,3], and this novel approach can be considered a fundamental component of the therapeutic armamentarium for oral defects.

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Regenerative dentistry, especially in the field of implantology, can be defined as a category of complex biological procedures that aim to replace the destructed soft and hard tissue in the oral cavity [4,5]. In addition, obtaining harmonized functional and biological structures can be accepted as a form of regenerative therapy for dental defects in the future. The regenerative capacity for platelet concentrates (PCs) has been founded by containing various growth factors (GFs) which are considered to be a stimulant for a mitogenic response in the peri-implanted tissues regardless of whether it is soft or hard **(Table 1)**.

Table 1. Overview for the clinical application of PCs in regenerative implantology.

References	Clinical Application
Arora NS et al. [8]	Socket preservation, ridge augmentation, intra-bony defects, mandibular-maxillary reconstruction operations, enhanced peri-implant soft and hard tissue healing.
Dohan DM et al. [12]	The PRF membrane has been used for recession coverage surgeries and is considered as a healing interposition of bioactive materials.
Trombelli L et al. [9]	Maxillary sinus bone augmentation.
Choukroun J et al. [17]	Furcation-defect treatment.
Marco M et al. [10]	Guided bone regeneration (GBR).

The development of bioactive additives in oral surgery was initiated by Whitman et al. [6], as they were the first researchers who explored the application of platelet rich plasma (PRP) concentrations in dental surgical operations. Moreover, they reported positive results when they found that osteoprogenitor cells were stimulated in both the host bone and grafted bone materials. However, this procedure is associated with various risks as using bovine thrombin for the PRP preparation and release antibodies can be potentially life-threatening for the patients. On the other hand, using platelet-rich fibrin (PRF) in dental surgery was first introduced by Choukrun et al. [7] and subsequently became a new generation of platelet-derived concentrates. To date, PRF consisting of fibrin enrichment provides an advantage in comparison with PRP. In the present review, we will summarize some of the advantages of PRF over PRP in a clinical context, including: 1) an Uncomplicated preparation method [8-13]; 2) The lack for the need of chemical additives during its formation, confirming that PRF is an autologous process [14]; 3) No need for any thrombin, because the formation is a natural process [12,15,16]; 4) Accelerates bone augmentation at the targeted site [12,17,18]; 5) PRF can be combined with other biological derivatives (which is what our review attempts to describe) [13,19,20], and 6) When combined with bone graft materials, it is considered to be the cheapest and fastest biological combination of materials that can be added to enhance the healing process [21].

Furthermore, some studies have mentioned several drawbacks for PRF, especially when used in clinical surgeries or laboratories; here we summarize some of the drawbacks relating to the preparation methodology: 1) After centrifugation of the sample, only a small amount is useful as it is derived from an autologous blood sample [17]; 2) it is a critical procedure that must be handled directly after collecting the blood sample and transferred within one and half minutes to the centrifuge, which is mandatory for increasing the success rate; [18] and 3)the need for special tubes to facilitate clotting polymerization, glass-coated tubes, and promising results for future PRF preparations that utilize titanium-centrifugation tubes, as well as the resultant scaffold known as T-PRF [18,22].

Among a large variety of treatment plans [23,24], only a few cases are considered to be a form of regenerative implantology, as the methodology and materials should histologically demonstrate peri-implant tissue (soft and hard) regeneration that can be formed on previously defected areas, although it can also be regarded as a regenerative modality [25-27]. Over the past two decades, different bioactive materials have been produced and examined experimentally to exhibit an obvious capability for endogenous regenerative activity; however, there were no considered definite standards [28-31].

Platelet Concentrates According to Methodology, Leukocyte, and Fibrin Networks

The various studies on platelet-derived products were simply divided into two generations [18,19,22], then according to the huge concentrations on platelet concentrates, researchers subsequently divided the products into four main families based on their endogenous fibrin and cell content [32-36]: 1) pure-platelet rich plasma (P-PRP); 2) leukocyte-platelet rich plasma (L-PRP); 3) pure-platelet rich fibrin (P-PRF); and 4) leukocyte-platelet rich fibrin (L-PRF).

Pure-platelet rich plasma (P-PRP) is leukocyte-poor platelet rich plasma, and these concentrates are produced without leukocytes. Regarding the fibrin architecture, it was found that the density of the activated fibrin network was low [37], and the product of this family can be used in the form of a liquid, solution, or in gel form [13,32]. Various methods have been introduced for producing this family of concentrates, depending on the cell pheresis (continuous flow plasmapheresis). Moreover, several authors have suggested a hematology laboratory should be used for its production, and this method has made it too difficult to be used frequently for routine clinical purposes on a daily basis [32,35,36].

Leukocyte-platelet rich plasma (L-PRP) by definition is a platelet concentrate that is prepared with a leukocyte and fibrin network as its activation is low, and P-PRP can be used as a liquid, solution, or in gel forms [32,33]. Recently, this type of platelet-rich product is prepared using special kits to reduce the amount of blood sample handling and increase the standardization of production [32,35].

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Pure-platelet rich fibrin (P-PRF), has a low concentration of leukocytes and all productions are without leukocytes and a high fibrin network density, and it can be produced in a strong gel form [32,38]. Therefore, it cannot be injected during treatment operations, and the primary drawback of this method is the production technique which is extremely costly, and the complex methodology required in comparison with other L-PRF families [32,39,40].

Leukocyte-platelet rich fibrin (L-PRF), are platelet rich products that contain leukocytes and a high density of fibrin architecture, similar to P-PRF materials. Moreover, after it is activated, it can be used as a strong gel [7,35].

In recent studies [1,4,21,41,42], many authors have concentrated on the fourth family (L-PRF), as it is the most useful PRP in comparison with the other groups, and has superior advantages regarding its preparation and application for various dental treatment methods [40]. Later in this review, we will discuss this forth platelet-rich concentrate family and its obvious effect when combined with other biological products, as well as its future potential for use by researchers and clinicians focusing on PCs [41].

The Relationship Between Growth Factors and Wound Regeneration

Platelet degranulation enhances the release of various types of soluble mediators, which are highly responsible for the initiation of wound healing; these mediators can be considered to be an initiator of the angiogenesis process [43,44]. In healthy individuals, human autologous platelet-derived fractions have been used as a tissue-repair stimulator [45]. Optimal healing depends on a cascade consisting of four phases (i.e., hemostasis of the vessels, cellular inflammation, proliferation, and tissue remodelling). The period required for these four overlapping phases to be completed depends on the vascular system [46,47].

Platelets are nucleated cell fractions that are derived from megakaryocytes in the bone marrow. These platelet fractions contain three major reservoir organelles: 1) lysosomes; 2) alpha granules; and 3) dense granules, with the most substantial part of protein storage found in the alpha granules [48]. Moreover, tissue engineering is regarded as a rapidly developing multidisciplinary field in regenerative medicine [49]. Therefore, the revascularization process enhances vascular network regenerations to obtain successful clinical results [50].

Regarding the mechanism of action for platelet concentrates in regenerative implantology, it is becoming crucial to discuss the growth factor content in the platelet-derived products (discussed above), and its release ratio **(Table 2)** in studies of platelet concentrates when evaluated by *in vitro* assays in the laboratory. Recent studies [14,39,51-55], have demonstrated the slow release of four key growth factors (i.e., transforming growth factor β1 [TGF-β1], vascular endothelial growth factor [VEGF], platelet derived growth factor [PDGF], and connective tissue growth factor [CTGF]) and three primary matrix molecules (i.e., the coagulation proteins thrombospondin1, fibronectin and vitronectin). Other studies, [56,57] revealed that platelet concentrates exist in two major forms (L-PRF and P-PRP) with a difference in the release of GFs. L-PRF has a dense architecture that exists for a longer time and shows a slow release agent lasting seven days, and does not completely dissolve when evaluated in the culture medium. P-PRP demonstrates almost all of the GFs during the first hour and is entirely dissolved only after few days [58-66]. To provide a further understanding of the role of growth factors in tissue regeneration, we summarize its functions in association with other recently related studies in **Table 3**.

Table 2. Overview of human platelet concentration growth factors.

References	Centrifugation method	Activation method	Released growth factors
Epply et al. [51]	3200 rmp for 12 mins	Thronbim and $CaCl_2$	PDGF-AB
			TGF- β1
Huang et al. [52]	Taken from a blood bank source	Thronbim and $CaCl_2$	PDGF-AB
			TGF- β1
			VEGF
Pietramaggiori et al. [53]	Purchased platelets from a blood bank	Sonication	-Fresh frozen PRP: PDGF-AB TGF- β1
			Freeze-dried PRP without additives: PDGF-AB TGF- β1
			-Freeze-dried PRP with additives: PDGF-AB TGF- β1
Roy et al. [11]	FIBRINET-PRFM system	$CaCl_2$	PDGF-BB
			TGF-β
			VEGF-A
			CTGF

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Lucarelli et al. [16]	FIBRINET	Not Mentioned	PDGF-AA
			PDGF-AB
			EGF
			VEGF
			TGF- β1
			CTGF

Table 3. Overview of growth factors and its healing functions BV, blood vessel; VSMC, vascular smooth muscle cells; EPC, endothelial progenitor cells; BM, bone marrow.

References	Growth factors	Regenerative Function
Evrard et al. [58]	Transforming Growth Factor β1(TGF-β1)	Angiogenesis enhancements of EPC.
Payakri et al. [65]	β1(TGF-β1)	Promote fibroblast contractions to enable wound closure.
Min et al .	Vascular Endothelial Growth Factor (VEGF)	Angiogenesis regulation.
Carmeliet and Jain [60]	Factor (VEGF)	Proliferation control, morphogenesis, survival of endothelial cells. Enhance the enlargement of blood vessels.
Dimmeler et al. [59]	Platelet Derived Growth Factors (PDGF)	PDGFs should be a key source of the maturation of the vessels, and employment of the EPC, which originated in the BM. Recruitment of the pericyte cells and VSMC to maintain the BV wall.
Raz et al. [61]	(PDGF)	
Herbert et al. [63]		
Hall-Gleen et al. [62]	Connective Tissue Growth Factor (CTGF)	Remodeling and regulating the BV wall by manipulating sericite cell recruitment and enhancing the effects of PDGFs on EPC.

Additives in Regenerative Implantology

Platelet concentrates have been investigated as potential bioactive products for enhancing bone augmentation [66], because it is easy to obtain and it contains biological proteins [67]. These proteins can enhance cellular proliferation, bone remodelling and an intrinsic motivation for alveolar bone resorption as the growth factors that are released exist in platelet-derived products seem to exhibit obvious synergetic stimulation in regenerative dentistry [68,69].

In vitro PRP demonstrates cellular proliferation and osteogenic evidence in human osteoblast cells [70]; however, *in vivo* studies on the effects of PRP on bone remodelling is contradictory [71,72] and there is no enhanced bone regeneration when the PRP gel has been used. This is beside the short period of the PRP effect, due to the fast fading rate of bioactive proteins, and new research has concentrated on the prolonged effect of platelet concentrates [73,74].

Recently, the second generation of platelet concentrates consisting of autologous platelets enriched with leukocytes and fibrin was discovered by Dohan et al. and was subsequently termed Choukroun's Platelet Rich Fibrin. Fresh blood without anticoagulants or thrombin should be immediately centrifuged within in two minutes (3000 rpm for 10 mins) and the resultant bioactive material with natural leukocytes and fibrin matrix, demonstrates a slow GF release system in comparison with other PCs [15,32].

To date, various studies discuss the effect of platelet-derived materials on regenerative dentistry, particularly the implantology field, as well as the biological additives in combination with PCs and the synergistically enhanced healing process [75,76].

Current Directions Regarding the Additive Effects

Despite the obvious concern regarding the effect of platelet-derived products on clinical dentistry, researchers have started to insert biological additives in their studies, and also focused on PRF as it is supposedly superior among the various types of platelet concentrates.

Bölükbaşı et al. [77], aimed to evaluate the efficacy of PRF in combination with biphasic calcium phosphate (BCP) on bone formation. In their study, they created 5 mm surgical bone defects, of which the defects were left without additives or filled by PRF, BCP, or PRF+BCP together focused on PRF as it is considered to be superior. Bölükbaşı et al. [77] revealed histomorphometric results revealed no signs of necrosis in all groups and there was an increase in bone formation when PRF and BCP were used together [77].

In addition to the broad uses of PCs during clinical operations, Pallotta et al. [78] focused on the limitations of platelet concentrates and its fibrin network due the capacity of PDGF to enhance direct wound healing. These limitations include poor mechanical form, rapid degradation speed, and the lack of the control of GF release in the targeted sites [78]. The activity and modification of the released GFs were manipulated by the charge of the silk protein, and the silk-platelet gel augmented both the mechanical and rheological properties of the platelet gel. When rVEGF was added to a 2% w/v silk solution, the concentration (by ELISA) was constant over 16 days. In contrast, rVEGF diluted in PBS, was approximately degraded during 2 days, as expected. According to this evidence, the ability of silk to stabilize the growth factors released by PG-Silk was documented. Moreover, versus

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the platelet derived gels, silk-platelet gel applications show cellular infiltration and blood vessel production, which represent a serious step towards new gel formation for future clinical and laboratory studies.

Tunalı et al. [23] developed a new type of PRF known as titanium platelet rich fibrin (T-PRF), with a novel hypothesis based on using titanium tubes instead of glass tubes that are used for Choukroun's produced leukocyte-platelet rich fibrin 1. The aim of the study was to focus on the comparison between titanium and silica in activating PRF using blood samples from ten healthy volunteers have been collected for their study, and divided into two groups based on the production of T-PRF and L-PRF. After centrifugation, they were divided into two halves again, with one-half of each clot processed under a scanning electron microscope (SEM), and the other half examined using fluorescent and light microscopy. The titanium-platelet rich fibrin exhibited organized and high integration architecture in comparison with leukocyte-platelet rich fibrin, and the histomorphometric results showed a thicker and larger area of fibrin networks in T-PRF than in L-PRF.

Although their case report was focused on periodontal furcation recession, Sambhav et al. [79] described promising future perspectives for regenerative peri-implants for the pupose of soft and hard tissue defects. Their case showed that the treatment of an advanced (grade II) buccal furcation area with the use of PRF in combination with β- tricalcium phosphate (β-TCP), and a simple pedicle flap can enhance root coverage [79]. They claimed that the aim of their study was to treat the fraction area with combined therapy (PRF and β-TCP), and demonstrated promising results [79].

The aim of the clinical study by Angelo et al. [80] was to examine the biomechanical stability of dental implants in the augmented sites using different biomaterials and PRF, as well as investigate the augmented sites in the maxillary bone using self-hardening calcium phosphate biomaterials (SHB), with and without PRF, using a piezotome-enhanced subperiosteal tunnel-technique (PeSPTT). In the methodology of this study, patients with anterior maxillary bone ridge defects were selected and treated with PeSPTT, using the application of biphasic or monophasic SHB, with or without PRF. After the implant insertion in the targeted sites, the insertion torque value (ITV) was measured as clinical evidence for biomechanical stability, and favourable results were documented when PRF and SHB were combined; this shows another promising future result for combining therapy in the future.

Kumar et al. [81] studied extended pulp necrosis in the surrounding periapical region and periodontal tissues, which leads to a periapical lesion that causes bone recession. The purpose of this recent clinical study was to evaluate the attempt of healing and bone regeneration when PRF is used in combination with hydroxyapatite bone crystals (HA), on the basis of their clinical outcomes, and two years after the radiography follow up. The authors concluded that the use of PRF in combination with HA seems to enhance the bone regeneration [81].

Future Perspectives

In summary, different methodologies for the assessment of the function of platelet concentrates are available [82,83] and activated biological materials including platelet-derived products play a crucial role in bone remodelling [84]. However *in vitro* studies have raised some concerns regarding osteogenic differentiation or the trans-differentiation of platelet concentrates in regenerative medicine,[85] which will be an obvious perspective for regenerative dentistry and implantology in future studies [86]. Furthermore, various bioactive materials are a component of bone augmentation that is capable of enhancing healing processes. It was also found that combining them with platelet concentrates will lead to the acceleration of the recommended process, because of the well-directed release of growth factors on the targeted sites [14,72]. More detailed studies should be conducted to examine the addition of a biological scaffold in enhancing the release of growth factors and its effects on the entire process.

PRF is the second generation PRP and seems to be more useful than PRP, due to the slow and an elongated period of growth factors that are released regarding the presence of a fibrin matrix [87-89]. For this purpose, to obtain promising results from PRP in comparison with PRF, combination therapy should be used [90]. Hence, strategies to improve the slow release of growth factor mechanisms, such as the new concentration of scaffold preparations and related studies provide promising perspectives for future exploration [3,90-92].

Finally, there is obvious lack of controlled clinical studies, especially related to regenerative dentistry, and available clinical research consists primarily of case-control studies. Double-blinded control studies are strongly recommended to provide obvious evidence and a supportive perspective for future regenerative dentistry, particularly the field of implantology [3,85,93,94].

CONCLUSION

Most of the clinical studies and research outcomes appear to demonstrate the significant increase of growth factor release [95,96] when platelet-derived products were used. In contrast, there is an increase in the maintenance of bone augmentation with its use. PRF with its slow GF release appears to have better effects on regenerative dentistry compared to PRP. Moreover, despite the variability of study designs, methodologies, and evaluations, standard platelet-derived product (the four families) preparations and activation methods should be established for bone grafting goals. The constraints of the platelet numbers and growth factor concentrations can be recorded in every study, and despite the various methodologies, the actual effect of the platelet concentrates supposed be recognized.

With this recent knowledge, it can be affirmed that platelet concentrates; especially L-PRF is a useful therapeutic biomaterial. However, despite the unobvious regenerative outcomes from platelet-derived biomaterials, and the role of growth factor releases on the healing process, substantiation of its clinical application when other bioactive materials are combined remains limited.

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Recently, a formulation of new biological materials based on combining preparations (platelet concentrates and bioactive materials) was demonstrated, which revealed promising results that seem to enable the control of growth factors to be released. The mechanical feature of the resultant materials, which shows steady contributions of both biological components, also appears to increase its portentous architecture. In addition, the increased stability of the resultant combined components can prolong the time scale for bone remodelling and the bioactivity of these new materials.

Finally, it can be concluded that combined methodologies, based on the PCs in other bioactive materials, represent an obvious step towards the development of scaffold preparations and its effect on regenerative dentistry. Additional randomized controlled clinical experiments are required to defend against the long-term advantages and ultimate outcomes associated with biological scaffolds.

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CONFLICT OF INTEREST

Regarding the content of this review, the authors have no conflicts of interest.

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