REVIEW ARTICLE

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

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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 past three decades. Recently, platelet concentrates (PCs) 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 the use in the future. To stimulate and ensure the healing for both soft and hard tissues in the oral region, there is convincing evidence that PCs can serve as an autologous source of growth factors and healing cytokine biomolecules, such as platelet-rich plasma, platelet-poor plasma, and platelet-rich fibrin (PRF) release, which plays a crucial role in promoting hemostasis and the wound healing process. In the recent studies, the primary concern has been the PCs in general, and particularly, PRF. 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 are a controversial topic for both researchers and clinicians alike.

Key words: Bioactive materials, biological scaffold, platelet concentrates, platelet rich fibrin, regenerative dentistry

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.

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].

The development of bioactive additives in oral surgery was initiated by Whitman *et al*., 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

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

References	Year	Clinical application
Arora *et al.*[6]	2009	Socket preservation, ridge augmentation, intrabony defects, mandibular-maxillary reconstruction operations, enhanced peri-implant soft, and hard tissue healing
Dohan *et al.*[7]	2006	The PRF membrane has been used for recession coverage surgeries and is considered as a healing interposition of bioactive materials
Trombelli *et al.*[8]	1996	Maxillary sinus bone augmentation
Choukroun *et al.*[9]	2006	Furcation-defect treatment

PRF: Platelet-rich fibrin, PC: Platelet concentrate

risks; using bovine thrombin for the PRP preparation and release, antibodies can be potentially life-threatening for the patients. On the other hand, On the other hand, platelet-rich fibrin (PRF) in dental surgery was first introduced by Choukroun *et al*.,[10] 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,[7-11] (2) the lack of the need of chemical additives during its formation, confirming that PRF is an autologous process,[12] (3) no need for any thrombin because the formation is a natural process,[13] (4) accelerate the bone augmentation in the targeted site,[9] and (5) PRF can be combined with other biological derivatives (which is what our review attempts to describe).[14] 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.[15]

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;(2) it is a critical procedure that must be handled directly after collecting the blood sample and transferred within 1½ min to the centrifuge, which is mandatory for increasing the success rate;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.[16]

Among a large variety of treatment plans,[17,18] 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.[19-21] 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.[22-25]

PCS ACCORDING TO METHODOLOGY, LEUKOCYTE, AND FIBRIN NETWORKS

The various studies on platelet-derived products were simply divided into two generations, and then, according to the huge concentrations on PCs, researchers subsequently divided the products into four main families based on their endogenous fibrin and cell content:[26-29] (1) Pure PRP (P-PRP); (2) leukocyte PRP (L-PRP); (3) pure-platelet-rich fibrin (P-PRF); and (4) leukocyte-PRF (L-PRF).

1. P-PRP is a L-PRP, and these concentrates are produced without leukocytes. Regarding the fibrin architecture, it was found that the density of the activated fibrin network was low,[30] and the product of this family can be used in the form of a liquid, solution, or gel form.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 for its production, and this method has made it too difficult to be used frequently for routine clinical purposes on a daily basis.[26,29]
2. L-PRP by definition is a PC that is prepared with a leukocyte and fibrin network after its activation was low, as P-PRP can be used as a liquid, solution, or in gel forms.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.[30]
3. Pure-PRF (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.[31] 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]
4. L-PRF is 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.[33]

In recent studies, 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.[34,35] Later in this review, we will discuss this forth platelet-rich concentrate family and its obvious effect when combined with another biological products, as well as its future potential for use by researchers and clinicians focusing on PCs.

THE RELATIONSHIP BETWEEN GFS 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.[36] In healthy individuals, human autologous platelet-derived fractions have been used as a tissue-repair stimulator.[37] Optimal healing depends on a cascade consisting of four phases (i.e., hemostasis of the vessels, cellular inflammation, proliferation, and tissue remodeling). The period required for these four overlapping phases to be completed depend on vascular system.[38,39]

Platelets are nucleated cell fractions that are derived from megakaryocytes in the bone marrow. These platelet fractions contain three major reservoirs organelles: (1) Lysosomes; (2) alpha granules; and (3) dense granules, with the most substantial part of protein storage found in the alpha granules.[40] Moreover, tissue engineering is regarded as a rapidly developing multidisciplinary field in regenerative medicine.[41] Therefore, the revascularization process enhances vascular network regenerations to obtain successful clinical results.[42]

Regarding the mechanism of action for PCs in regenerative implantology, it is becoming crucial to discuss the GF content in the platelet-derived products (discussed above) and its release ratio [Table 2] in studies of PCs when evaluated by *in vitro* assays in the laboratory. Recent studies have demonstrated the slow release of four key GFs (i.e., transforming GF β1 [TGF-β1], vascular endothelial GF [VEGF)], platelet-derived GF [PDGF], and connective tissue GF) and three primary matrix molecules (i.e., the coagulation proteins thrombospondin1, fibronectin, and vitronectin).[43,45] Other studies revealed that PCs 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 for 7 days and does not completely dissolve when evaluated in the culture medium. P-PRP demonstrates almost all of the GFs during the first hours and is entirely dissolved after only a few days.[46,47] To provide a further understanding of the role of GFs in tissue regeneration, we summarize its functions in association with other recently related studies [Table 3].

Table 2: Overview of human PC GFs

References	Year	Centrifugation method	Activation method	Released GFs
Eppley *et al.*[48]	2004	3200 rmp for 12 min	Thrombin and $CaCl_2$	PDGF-AB TGF-β1
Huang *et al.*[49]	2009	Taken from a blood bank source	Thrombin and $CaCl_2$	PDGF-AB TGF-β1 VEGF
Pietramaggiori *et al.*[50]	2006	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.*[51]	2011	FIBRINET-PRFM system	$CaCl_2$	PDGF-BB TGF-β VEGF-A CTGF
Lucarelli *et al.*[13]	2010	FIBRINET	Not mentioned	PDGF-AA PDGF-AB EGF VEGF TGF-β1 CTGF

PC: Platelet concentrate, TGF-β1: Transforming growth factor β1, PDGF: Platelet-derived growth factors, VEGF: Vascular endothelial growth factor, CTGF: Connective tissue growth factor, GF: Growth factor

Table 3: Overview of GFs and its healing functions

References	Year	GFs	Regenerative function
Evrard *et al.*[52]	2012	TGF-β1	Angiogenesis enhancements of EPC
Carmeliet and Jain[53]	2011	VEGF	Proliferation control, morphogenesis, survival of endothelial cells Enhance the enlargement of blood vessels
Dimmeler[54] Raz *et al.*[55] Herbert and Stainier[56]	2005 2014 2011	PDGFs	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
Hall-Glenn *et al.*[57]	2012	CTGF	Remodeling and regulating the BV wall by manipulating pericyte cell recruitment and enhancing the effects of PDGFs on EPC

BV: Blood vessel, VSMC: Vascular smooth muscle cells, EPC: Endothelial progenitor cells, BM: Bone marrow, GF: Growth factor, CTGF: Connective tissue growth factor, VEGF: Vascular endothelial growth factor, PDGF: Platelet-derived growth factors, TGF-β1: Transforming growth factor β1

ADDITIVES IN REGENERATIVE IMPLANTOLOGY

PCs have been investigated as potential bioactive products for enhancing bone augmentation[58] because it is easy to obtain and it contains biological proteins.[59] These proteins can enhance cellular proliferation, bone remodeling, and an intrinsic motivation for alveolar bone resorption as the GFs that are released exist in platelet-derived products seem to exhibit obvious synergetic stimulation in regenerative dentistry.[60]

In vitro PRP demonstrates cellular proliferation and osteogenic evidence in human osteoblast cells;[61] however, *in vivo* studies on the effects of PRP on bone remodeling are contradictory,[62,63] and there is no enhanced bone regeneration when the PRP gel has been used. This is beside the short period of PRP effect, due to the fast fading rate of bioactive proteins, and new research has concentrated on the prolonged effect of PCs.[64]

Recently, the second generation of PCs consisting of autologous platelets enriched with leukocytes and fibrin was discovered by Dohan *et al*., and subsequently, termed Choukroun's PRF. Fresh blood without anticoagulants or thrombin should be immediately centrifuged within 2 min (3000 rpm for 10 min), and the resultant bioactive material with natural leukocytes and fibrin matrix demonstrates a slow GF release system in comparison with other PCs.

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.[65,66]

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 among the various types of PCs.

Bölükbasi *et al*. 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. The 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.[67]

In addition to the broad uses of PCs during clinical operations, Pallotta *et al*. focused on the limitations of PCs and its fibrin network due to 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. 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 enzyme-linked immunosorbent assay) was constant over 16 days. In contrast, rVEGF diluted in phosphate-buffered saline was approximately degraded during 2 days, as expected. According to this evidence, the ability of silk to stabilize the GFs released by PG-Silk was documented. Moreover, versus the platelet-derived gels, silk-platelet gel applications show cellular infiltration and blood vessel production, which represent a serious step toward new gel formation for the future clinical and laboratory studies.[68]

Tunali *et al*. developed a new type of PRF known as titanium PRF (T-PRF), with a novel hypothesis based on using titanium tubes instead of glass tubes that are used for Choukroun's produced L-PRF 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, and another half examined using fluorescent and light microscopy. The T-PRF exhibited an organized and high integration architecture in comparison with L-PRF, and the histomorphometric results showed a thicker and larger area of fibrin networks in T-PRF than in L-PRF.[69]

Although their case report was focused on periodontal furcation recession, Sambhav *et al*. described promising future perspectives for regenerative peri-implants for the purpose 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. 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.[70]

The aim of the clinical study by Angelo *et al*. was to examine the biomechanical stability of dental implants in the augmented sites using different biomaterials and PRF, as well as to 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 was measured as clinical evidence for biomechanical stability, and favorable results were documented when PRF and SHB were combined; this shows another promising future result for combining therapy in the future.

Kumar *et al*. studied extended pulp necrosis in the surrounding periapical region and periodontal tissues, which leads to a periapical lesion that causes a 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 2 years after the radiographically follow-up. The authors concluded that the use of PRF in combination with HA seems to enhance the bone regeneration.[71]

FUTURE PERSPECTIVES

In summary, different methodologies for the assessment of the function of PCs are available[72,73] and activated biological materials including platelet-derived products play a crucial role in bone remodeling.[74] However, *in vitro* studies have raised some concerns regarding osteogenic differentiation or the transdifferentiation of PCs in regenerative medicine,[75] which will be an obvious perspective for regenerative dentistry and implantology in the future studies.[76] 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 PCs will lead to the acceleration of the recommended process, due to the well-directed release of GFs on the targeted sites. More detailed studies should be conducted to examine the addition of a biological scaffold in enhancing the release of GFs and its effects on the entire process.

PRF is the second-generation PRP and seems to be more useful than PRP, as the slow and an elongated period of GFs are released regarding the presence of fibrin matrix.[77-79] For this purpose, to obtain promising results from PRP in comparison with PRF, combination therapy should be used. Hence, strategies to improve the slow release of GF mechanisms, such as the new concentration of scaffold preparations and related studies, provide promising perspectives for future exploration.[80-82]

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.[83-84]

CONCLUSION

Most of the clinical studies and research outcomes appear to demonstrate the increase of GF release[85,86] 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 GF concentrations can be recorded in every study, and despite the various methodologies, the actual effect of the PCs should be recognized.

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

Recently, a formulation of new biological materials based on combining preparations (PCs and bioactive materials) was demonstrated, which revealed promising results that seem to enable the control of GFs 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 remodeling 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 toward 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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