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REVIEW

Platelet preparations in dentistry: How? Why? Where? When?

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Abstract

The aim of this article is to review the outcomes of platelet preparations in dentistry. A structured electronic search discovered 348 articles, which described the use of autologous platelet concentrates with a relevance to clinical dentistry. Among these articles, 220 articles investigated platelet rich plasma, 99 investigated platelet rich fibrin, 22 investigated plasma rich in growth factors and 7 investigated the use of concentrated growth factors. Several studies reported beneficial treament outcomes in terms of enhanced bone and soft tissue regeneration.

Key words: Platelet concentrates; Platelet rich plasma; Platelet rich fibrin; Concentrated growth factors; Growth factors; Dentistry; Plasma rich in growth factors

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Core tip: Autologous platelet concentrates (platelet-rich plasma, platelet rich fibrin, plasma rich in growth factors, concentrated growth factor), are blood derivatives, prepared from patient's own blood, reach in platelets, growth factors and cytokines, which can be used to promote guided tissue regeneration in dentistry and oral surgery.

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INTRODUCTION

Bone and soft tissue regeneration is frequently required in dentistry, mainly but not exclusively for implantology and periodontology. Tissue regeneration is a complex process of healing and tissue growth, which involves different biological elements and strategies. These include the use of bone grafts[1,2], biomaterials and growth factors[3], natural or synthetic scaffolds and more recently the use of stem cells[4,5]. Nowadays, a whole range of modern surgical procedures and a variety of dental materials are available. These are performed to reconstruct bony defects of the upper and lower jaw and for augmentation of lost structures of the residual alveolar ridge. Autologous platelet concentrates are a promising and innovative therapeutic approach in various medical fields, including dentistry[6-10]. Platelets play a crucial role not only in hemostasis, but also in the

wound healing process, as they are reservoirs of growth factors and cytokines, which in turn are key promoters for bone and soft tissues regeneration. After platelets are activated, they become trapped within a fibrin matrix and release growth factors. Together the fibrin can form a scaffold and the growth factors can stimulate tissue healing and regeneration repair responses. An improved understanding of the physiologic properties of platelets in wound healing over the last two decades, has led to more successful therapeutic applications, especially in oral surgery.

Platelet concentrates

Platelet concentrates are blood derivatives[11,12], prepared from the patient's own blood and containing autologous platelets, growth factors and cytokines involved in the key processes of tissue regeneration, including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis. Platelets are packed with secretory granules, which are necessary to fulfill their functions. There are three types of secretory granules, α granules are the most abundant and have a high protein content. The granules contain cytokines and growth factors, such as vascular endothelial growth factor, epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, hepatocyte growth factor and the insulin-like growth factor as well as several others. The release of these growth factors from activated platelets can promote healing in both soft and hard tissues.

Most platelet concentrate preparations used in guided tissue regeneration surgery are termed Platelet-Rich Plasma (PRP), even if they differ slightly according to their preparation from a patients peripheral blood. These variations include differences in centrifugation speeds and times, differences in adding chemicals, and differences in the selection of supernatants and precipitates. These variations can cause differences in fibrin network structures and in platelets, leucocyte and growth factors content. Therefore the term PRP alone can be non-specific, because it does not define the actual preparation protocol. Depending on the leukocyte content and fibrin architecture, five main categories of PRPs can be defined: (1) Pure Platelet-Rich Plasma, such as cell separator PRP, Vivostat platelet rich fibrin (PRF)[13] or Anitua's PRGF[14,15]; (2) Leukocyte and Platelet-Rich Plasma; (3) Pure Plaletet-Rich Fibrin, such as Fibrinet; (4) Leukocyte- and Platelet-Rich Fibrin, such as Choukroun's PRF; and (5) Concentrated growth factors (CGF). In the following paragraphs, the use of PRP, PRF, plasma rich in growth factors (PRGF) and CGF in dentistry and oral surgery will be reviewed.

PRP

PRP is blood plasma that has been enriched with platelets and it was the first generation of platelet concentrates to be used in clinical practice by Marx in 1998[16]. PRP has a platelet concentration of 1000 $\times$ 10^9/L in 5 mL of plasma, which is 3-5 times higher compared to the normal whole blood platelet count (150-400 $\times$ 10^9/L). PRP contains (and releases through platelet degranulation) several growth factors and cytokines that can stimulate bone and soft tissue healing[17-19]. PRP is prepared by drawing peripheral venous blood from a patient's arm. The fresh blood is immediately mixed with an anti-coagulant to prevent clotting and then the platelets are concentrated using a two-step gradient centrifugation method[20]. In this method, the first spin (called the hard spin) separates the red blood cells (RBCs) from the plasma containing platelets, leukocytes and clotting factors, the second spin (called the soft spin) is used to delicately separate the platelets and leukocytes, from the plasma. The soft spin produces PRP and separates it from the platelet-poor plasma (PPP), free from the interference associated with large number of red blood cells. Commonly, with commercially available systems, a one-step method is used to separate the RBCs, buffy coat and plasma into three distinct layers. The buffy coat contains platelets and leukocytes and is often collected as PRP. The top plasma layer is often called PPP, which is discarded, leaving the PRP to be injected into surgical sites to accomplish guided tissue regeneration.

PRF

PRF consists of an intimate assembly of cytokines, glycanic chains, structural glycoproteins enmeshed within a fibrin network, and is considered to be the second generation of platelet concentrates[21-23]. The cytokines, glycanic chains, structural glycoproteins can have synergetic effects on tissue healing processes. The PRF pioneers were Choukroun *et al*[24,25], who used it to promote the osseointegration of dental implants. Several studies have demonstrated the clinical effectiveness of autologous PRF to regenerate defects in hard and soft tissues. The preparation of PRF is similar to PRP and consists in collecting peripheral venous blood from the patient's arm. Except that no anti-coagulant is used during blood harvesting. After the blood is collected it is immediately centrifuged for 10 min to activate the platelets, leading to the initiation of a coagulation cascade. After centrifugation, the blood is separated into three different layers: acellular PPP (platelet poor plasma) on top, a PRF clot in the middle and RBCs at the bottom of the test tube. The PRF clot obtained after centrifugation is collected, 2 mm below the lower dividing line and the other layers are discarded. The clinical success of the PRF protocol is dependent on a quick collection of blood and its transfer to the centrifuge. Because no anti-coagulant is used, the blood sample begins to coagulate almost immediately, and a failure to accomplish the quick preparation of PRF could cause a diffuse polymerization of fibrin, which is not ideal for tissue healing.

PRGF

PRGF is prepared from peripheral venous blood drawn from a patient's arm. PRGF is prepared using

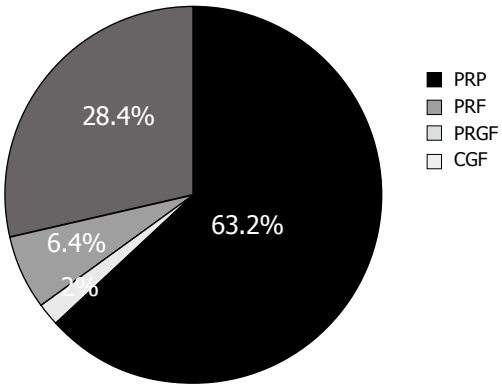

Figure 1 Number of studies with platelet rich plasma, platelet rich fibrin, plasma rich in growth factors and concentrated growth factors in dentistry up to January 2015. PRP: Platelet rich plasma; PRF: Platelet rich fibrin; PRGF: Plasma rich in growth factors; CGF: Concentrated growth factors.

a modified PRP protocol developed by Anitua[26-28]. The difference between PRGF and PRP is that PRGF is optimized to deliver a more sustained release of growth factors. PRGF can create a three-dimensional fibrin scaffold which can be injected into a tissue defect, to maintain the regenerative space and can be used as a scaffold for cells to accomplish tissue regeneration. The Leukocyte content of PRGF is eliminated to prevent the pro-inflammatory effects of the proteases and acid hydrolases contained within these cells. PRGF is prepared from a small volume of patient's peripheral venous blood and is collected by a one-step centrifugation using sodium citrate as the anti-coagulant (Endoret System, Biotechnology Institut, Minano, Alava, Spain). After activation, PRGF progressively releases a pool of proteins and growth factors, which accelerate soft tissue healing as well as bone regeneration. Different formulations of PRGF with therapeutic potential can be obtained from a patient's blood depending on the degree of coagulation and activation of the samples. PRGF supernatant can be used as conventional eye-drop solution and cell culture media solution; liquid PRGF can be used to coat dental implant surfaces to promote osseointegration; the fibrillar and cellular scaffold-like PRGF can be used to fill tissue defects as part of ulcer treatment, sealing tooth sockets after tooth extraction, and promoting the epithelialization of soft tissues.

CGF

CGF, first developed by Sacco, in 2006, is an autologous fibrin network, rich in leukocytes and platelets[29,30]. CGF also contains autologous osteo-inductive growth factors derived from platelets and an osteo-inductive fibrin matrix. Similar to PRF, CGF is created using a one-step centrifugation method, but it requires a special programmed centrifuge (Medifuge MF200, Silfradent srl, Forli, Italy), which uses plastic tubes, coated with silica particles, and without the addition of exogenous substances. The final blood product is separated into three layers, two are discarded, and the CGF is collected from the buffy coat layer, which consists of a dense fibrin matrix that is rich in growth factors.

Table 1 Number of different studies published in the literature using platelet rich plasma, platelet rich fibrin, plasma rich in growth factors and concentrated growth factors in dentistry

	Platelets concentrates			
Study type	PRP	PRF	PRGF	CGF
Clinical trials	116	50	13	1
Animal studies	46	13	5	1
In vitro studies	20	17	2	1
Technical report	3	1	0	2
Case report	35	18	2	2

PRP: Platelet rich plasma; PRF: Platelet rich fibrin; PRGF: Plasma rich in growth factors; CGF: Concentrated growth factors.

STUDY STRATEGY

A structured electronic search of scientific papers up to January 2015, was conducted using two medical databases (PubMed and the Cochrane Library) and specific keywords: "platelet concentrates in dentistry", "PRF" "Platelet rich fibrin Choukroun", "platelets in dentistry and maxillofacial surgery", "PRP", "CGF", "PRGF", "periodontal regeneration". For each of these platelet concentrate categories, their therapeutic potential in dentistry was evaluated according to the following inclusion criteria: (1) clinical trials; (2) animal studies; (3) *in vitro* studies; (4) case reports; and (5) technical reports. Subsequently, the articles for each type of platelet concentrate (PRP, PRF, PRGF and CGF) were classified according to these inclusion criteria and study type.

RESULTS

A total of 563 articles were identified as meeting the inclusion criteria of investigating the clinical use of autologous platelet concentrates in dentistry. However, after all the studies not relevant to dentistry, or containing no data were excluded, 348 articles were included in this review. Of the 348 articles, 220 articles (63.2%) were about PRP, 99 articles (28.4%) investigated PRF, 22 articles (6.4%) investigated PRGF and 7 articles (2%) were about CGF (Figure 1).

The articles were classified according to the type of platelet preparations in dentistry and the type of research performed in the article, which are briefly described below and summarized in Table 1: (1) PRP: from 220 articles, 116 were human clinical trials, 46 regarded animal studies, 20 were about *in vitro* investigations, 3 were technical reports and 35 were case reports; (2) PRF: from 99 articles, 50 were human clinical trials, 13 regarded animal studies, 17 were about *in vitro* experiments, 1 was a technical report about PRF general properties and 18 were case reports; (3) PRGF: from 22 articles, 13 were human clinical trials, 5 regarded

animal studies, 2 were about *in vitro* experiments and 2 were case reports; and (4) CGF: from 7 articles, 1 was a human clinical study, 1 regarded an animal study, 1 was about an *in vitro* study, 2 were technical reports about CGF properties and its application in dental implantology and 2 were case reports.

PRP studies in dentistry

PRP was used to treat periodontal intrabony defects in fifteen studies[31-45]. Nine studies described the use of PRP in cyst enucleations/periapical surgeries[46-54]. Forty-eight studies investigated PRP in sinus floor elevation treatments[55-101]. Twenty-two studies reported the use of PRP for the treatment of periodontal and periimplant defects[102-123]. Four studies used PRP for covering the roots of teeth[124-127]. Six studies investigated the efficacy of PRP for the treatment of gingival recession[128-133]. Four studies evaluated the benefits of using PRP to repair furcation defects[134-137]. Twenty-five studies investigated PRP for the repair of mandible/maxilla fractures[138-160]. Thirty-one studies investigated the use of PRP in endodontic surgery[161-188]. Eighteen studies investigated the use of PRP for dental extraction socket preservation before implant placement[189-206]. Twenty-two studies investigated the stimulating effect of PRP on alveolar bone regeneration and reconstruction[207-224]. Eight studies investigated the use of PRP to improve the healing and regeneration of soft tissues[225-231], mostly for periodontal ligament repair, and reducing the incidence of complications. Eight studies investigated PRP using *in vitro* protocols to enhance the migration and proliferation of human dental stem cells and gingival fibroblasts[232-237] (Figure 2).

PRF studies in dentistry

PRF was used in six studies to treat periodontal intrabony defects[238-243]. Four studies used PRF to regenerate tissue following cyst enucleations, and periapical surgeries[244-246]. Eleven studies investigated the ability of PRF to regenerate tissues following sinus floor elevation[247-256]. Eight studies investigated the use of PRF to treat periodontal and periimplant defects[257-263]. One study tested PRF as a potential root coverage repair treatment[264]. Two studies investigated the efficacy of PRF in gingival recession treatment[265,266]. Four studies investigated PRF to treat furcation defects[267-269]. Eight studies applied PRF to heal mandible or maxilla fractures[270-276]. Twenty one studies investigated the usefulness of PRF as part of endodontic surgery to repair periapical tissues[277-293] . Eleven studies investigated the ability of PRF to preserve tooth sockets after tooth extraction in preparation for dental implant placement[294-302]. Nine studies investigated the ability of PRF to stimulate alveolar bone regeneration and reconstruction[303-309]. Ten studies investigated the ability of PRF to improve the healing and regeneration of soft tissues, especially periodontal ligament, reducing complications[310-319]. Four studies investigated the *in vitro* effects of PRF to enhance the migration and proliferation of human dental stem cells and gingival fibroblasts[320-323] (Figure 2).

PRGF studies in dentistry

PRGF was investigated in two studies to treat periodontal bone defects[324,325]. PRGF was investigated in two studies to regenerate tissues following cyst enucleations and periapical surgeries[326,327]. The potential of PRGF to heal tissues following sinus floor elevation treatment[328,329] was reported in two studies. Two studies reported that PRGF had a positive effect on the healing of periodontal and periimplant defects[330,331]. One study investigated the use of PRF to cover the roots of teeth[332]. One study investigated the efficacy of PRGF to heal tissues following gingival recession treatment[333]. Two studies investigated the benefits of PRGF for the treatment of furcation defects[334,335]. One study investigated the effectiveness of PRGF to heal mandible/maxilla fractures[336]. One study investigated the effectiveness of PRGF to heal periapical soft tissues following endodontic surgery[337]. Four studies investigated the clinical potential of PRGF to preserve tissue in tooth extraction sockets prior to dental implant placement[338-340]. One study investigated the stimulating effect of PRGF on alveolar bone regeneration and reconstruction[341]. One study investigated the ability of PRGF to improve the healing and regeneration of soft tissues, especially the periodontal ligament[342]. Two studies investigated the *in vitro* effect of PRGF to enhance the migration and proliferation of human dental stem cells and gingival fibroblasts[343,344] (Figure 2).

CGF studies in dentistry

Compared to the other platelet articles, only a few had investigated the use of CGFs as part of dental treatment. A reason for the lack of CGF articles may be because it is newest of the platelet protocols and there has not been enough time for many articles to be published. Three studies were found which investigated CGF for tissue regeneration following sinus floor elevation[345-347]. One study investigated the *in vitro* effectiveness of CGF to enhance the migration and proliferation of human dental stem cells and gingival fibroblasts[348]. One study investigated the healing effects of CGF for tissue repair following endodontic surgery[349]. Two studies investigated soft tissue and periodontal ligament healing after using CGF to accomplish guided tissue regeneration[350,351] (Figure 2).

DISCUSSION

Dentists have different types of biomimetic biomaterials to help guided bone and soft tissue regeneration. All these biomaterials have advantages and limitations and no single type of biomaterial has all the properties needed to be the universal dental regeneration biomaterial. A natural scaffold regeneration material is the blood clot, and several protocols have been

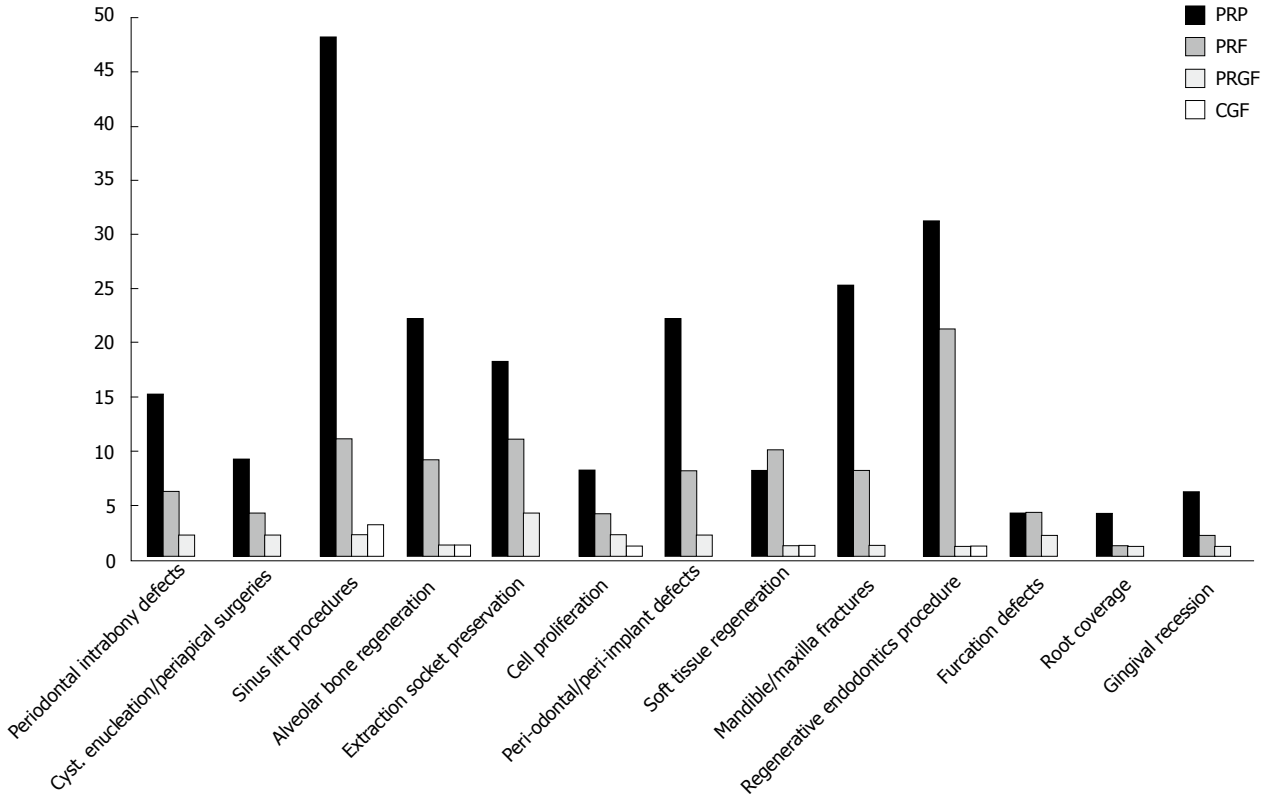

Figure 2 Platelet rich plasma, platelet rich fibrin, plasma rich in growth factors and concentrated growth factors application in dentistry up to January 2015. PRP: Platelet rich plasma; PRF: Platelet rich fibrin; PRGF: Plasma rich in growth factors; CGF: Concentrated growth factors.

developed to improve the scaffold and growth factor properties of the blood clot (PRP, PRF, PRGF and CGF). These platelet rich preparations have been shown to improve healing, quicken tissue regeneration, improve the quality of tissues that are regenerated, and to reduce the incidence of complications. Alternatively, there are also many studies, which have shown that platelet rich preparations had little or no effect on tissue healing in comparison to biomimetic scaffolds. This explains the need to carefully investigate the uses of platelet concentrates as part of dental treatments.

After the careful analysis of the literature, the follow questions could be asked: (1) How is platelet rich fractions of blood prepared? (2) Why use platelet concentrates in dentistry? (3) Where to use platelet concentrates in dentistry? and (4) When to use platelet concentrates in dentistry?

The answer to the first question about how platelet rich fractions of blood are prepared, was answered in the previous paragraphs. All the techniques involve the centrifugation of the patient's peripheral venous blood and the use of fractions containing fibrin, platelets, leukocytes and growth factors. Red blood cells are discarded.

The answer to the second question about why platelet concentrates are used in dentistry is because they are cheap natural scaffolds and source of growth factors to stimulate tissue regeneration. Platelet concentrates are biocompatible and can sometimes offer potential benefits including rapid wound healing and bone regeneration. A controversial advantage is a reduction of postoperative pain and an unequivocal advantage is the lack of risk of infectious disease transmission. Sometimes platelet concentrates cannot be used where a patient does not want to donate their own blood, or when a special-needs patient or child refuses to cooperate with the collection of their blood.

The answer to the third question about why autologous platelet concentrates are used in oral and maxillofacial surgery and periodontal regenerative therapy is because of some promising results for tissue regeneration following sinus floor elevation (especially with PRP and CGF)[55-101,345-347], bone filling of periodontal intrabony defects[102-123,238-243,324,325], regeneration of alveolar ridges[207-224,303-311,343], dental extraction socket preservation[189-206,294-302,338-340], gingival recession treatment[128-133,265,266], mandibular and maxilla fractures[138-160,270-276,333]. Platelet concentrates have been used to manage bisphosphonate-related osteonecrosis of the jaw to enhance wound healing and bone maturation[271,272,352].

The answer to the fourth question about when to use platelet concentrates is the most difficult to reach for most dentists. A general rule of guidance is to use platelet concentrates, scaffolds, or biomaterials, in surgical situations where the prognosis for tissue repair is poor in the absence of a tissue regeneration scaffold and addition of growth factors[225-231,310-319].

CONCLUSION

In conclusion, platelets concentrates represents innovative tools in dentistry. The results, demonstrate that these concentrates are effective at improving bone and soft tissues healing. Moreover, well-enhanced bone regeneration can be obtained when PRP, PRF and CGF are used together with autogenous bone, with recombinant human growth factors such as recombinant BMP and also with other biomaterials (as for example Bio-Oss- *Geistlich-Switzerland* and Hydroxyapatite)[95-96]. However, the definition and validation of accurate protocols is a key issue for the long-term development of these techniques. So for further research is required to establish a standardized protocol for the use of these concentrates in the treatment of tissue regeneration.

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