

# AUTOLOGOUS BLOOD PREPARATIONS RICH IN PLATELETS, FIBRIN AND GROWTH FACTORS

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## SUMMARY

**Objectives.** Bone regeneration is often needed prior to dental implant treatment due to the lack of adequate quantity and quality after infectious diseases. The greatest regenerative power was obtained with autologous tissue, primarily the bone alive, taken from the same site or adjacent sites, up to the use centrifugation of blood with the selection of the parts with the greatest potential regenerative. In fact, various techniques and technologies were chronologically successive to cope with an ever better preparation of these concentrates of blood. Our aim is to review these advances and discuss the ways in which platelet concentrates may provide such unexpected beneficial therapeutic effects.

**Methods.** The research has been carried out in the MEDLINE and Cochrane Central Register of Controlled Trials database by choosing keywords as "platelet rich plasma", "platelet rich fibrin", "platelet growth factors", and "bone regeneration" and "dentistry".

**Results.** Autologous platelet rich plasma is a safe and low cost procedure to deliver growth factors for bone and soft tissue healing.

**Conclusion.** The great heterogeneity of clinical outcomes can be explained by the different PRP products with qualitative and quantitative difference among substance.

**Key words:** platelet rich plasma, platelet rich fibrin, platelet growth factors, bone regeneration.

## Introduction

Bone deficiencies in the oral cavity differ in extension and etiology, as for example localized alveolar bone loss due to periodontal disease or extensive bone atrophy. Regeneration of the periodontal tissues following destructive episodes of inflammatory-infective periodontal diseases is an even more challenging problem, since repair processes must involve not only the affected alveolar bone, but other crucial components (the periodontal ligament and the root cementum). Bone regeneration requires the recruitment, proliferation and maturation of osteoblasts which are derived from mesenchymal stem cells (MSC). Tissues that harbor MSCs or MSC like

cells include blood, adipose tissue, skin, trabecular bone, and fetal blood, liver, and lung. Despite sharing similar characteristics, these MSCs from different sources differ in their differentiation potential and gene expression profile. Among the different types of adult stem cells, stem cells harbored in the bone marrow are considered to have the highest multilineage potential and have been studied for bone regeneration. However, long-term cultures of human MSCs have limitations because over time the cells exhibit a reduced proliferation rate attributable to telomere shortening and undergo senescence (1). The healing potential of autologous tissue is enclosed in growth factors and stem cells, and therefore the research has shifted to the blood, tissue that is most predisposed to a process for regenerating concentrate these molecules. In the

last few decades, the potential effects of growth factors in the repair and regeneration of tissues and especially of bone have been well documented. Platelet rich plasma or platelet rich fibrin are often associated with growth factors, regenerative medicine and stem cells (2, 3). It was reported that PRP accelerates the healing and regeneration of bone and soft tissue, releasing growth factors and cytokines that are associated with wound healing.

In this review, a brief description of different blood derivatives preparation for oral application is described. The literature was searched for references published up to September 2015 in MEDLINE and Cochrane Central Register of Controlled Trial databases. Three search terms – ‘platelet-rich plasma’, platelet-rich fibrin, ‘bone regeneration’ and ‘dentistry’ – were used together with their all known synonyms.

## Platelets

Platelets contain several types of secretory organelle with dense granules,  $\alpha$ -granules and lysosomes being the most important. Morphologically distinct, the granules contain storage pools of active substances that are released following adhesion to collagen or other matrix components or in a dose-dependent response to soluble agonists such as ADP or thrombin. The most abundant source of proteins is the  $\alpha$ -granule (4). The effects of platelets on bone can be viewed within the frames of bone formation and resorption. Platelets act in both processes.

Platelets contain a number of several growth factors such as platelet derived growth factor (PDGF), which exists as heterodimers of A and B chains and as homodimers of A-A and B-B chains; vascular endothelial growth factor (VEGF), insulin growth factor-1 (IGF-1), insulin growth factor-2 (IGF-2), epidermal growth factor (EGF), transforming growth factor- $\beta$  (TGF- $\beta$  and TGF- $\beta$ 2) contributing to the recruitment of osteogenic cells after the injury (5) and epithe-

lial-cell growth factor (ECGF), hepatocyte growth factor (HGF) (6).

Platelet releasate also contains other mediators, which may be involved in the bone remodeling [thromboxane A2 (TXA2) and prostaglandins (PGs)]. The essential role of platelets in wound healing was confirmed previously. These small particles release growth factors involved in clot formation and wound healing. Thus the effect of platelets on bone can be viewed in chronic (e.g. senile osteoporosis) and acute conditions (e.g. fracture) (7, 8).

Platelets enhance bone regeneration by promoting migration and proliferation of osteogenic cells (9), increasing blood vessel formation, and by inducing inflammatory reactions (10). In an *in vitro* study, Sanchez-Fernandez (11) tested the hypothesis that osteoclasts can regulate the chemotaxis of osteoblasts. The result showed that mature osteoclasts produce factors including PDGF that attract osteoblast toward the injured or inflamed sites thereby promoting new bone regeneration. Similarly the Park study (12) also reported that PDGF promotes the proliferation of osteoblasts around periodontal defects.

## PDGF (platelet-derived growth factor)

Platelet-derived growth factor (PDGF) is a potent growth factor for various connective tissue cells, many other cell types also synthesize PDGF, including macrophages, endothelial cells, fibroblasts, glial cells. The binding of PDGF to several plasma proteins and extracellular matrix facilitates local concentration of the factor. PDGF binds to specific high-affinity receptors expressed on the surfaces of various cell types and it is a powerful chemo-attractant and stimulator of cell proliferation. It has been shown that all isoforms of PDGF induce the proliferation of PDL cells *in vitro* (13). PDGF is also chemotactic for PDL cells and stimulates collagen and

hyaluronate synthesis (14). It was also reported that PDGF promotes DNA synthesis and chemotaxis in bone organ culture (15) but down-regulates alkaline phosphatase activity and osteocalcin in osteoblast-like cells *in vitro* (16). It has been shown that a single application of a combination of PDGF/IGF-I or PDGF/dexamethasone promoted periodontal tissue regeneration *in vivo* (17, 18) PDGF promote bone formation by influencing cell proliferation, chemotaxis, differentiation, and extracellular matrix synthesis (15). In addition, their protective effect on bone was shown in a few clinical trials (19, 20). Based on these studies, platelet-rich plasma (PRP) is an abundant source of growth factors implicated in bone formation. Interestingly most of these studies are dental clinical investigations or *in vitro* studies on osteoblasts suggestive of the enhancement of bone formation by PRP (21).

Studies on both animal and human cell lines illustrate controversial results about the effects of PDGF on bone formation. Some *in vitro* investigations confirmed contribution of platelets in osteoclastogenesis and bone resorption, but no exact mechanism has been proposed yet, platelets effect on osteoclasts should be also emphasized. PRP showed an inhibitory effect on the formation of osteoclast-like cells in rat bone marrow (18). Simion (22) investigated the combination of PDGF with a block of deproteinized cancellous bovine bone and in an experiment on 6 foxhound, the results are the increment of vertical periodontal bone.

Urban et al. (23), in a clinical case-report, demonstrated that use of rhPDGF in combination with bone mineral and barrier membrane enhances new bone formation around periodontal bone defect.

McAllister et al. (24), in a study on the significance of PDGF in bone regeneration around dental implants, demonstrated the 100% of osteointegration.

## VEGF (vascular endothelial growth factor)

VEGF is the most important tissue factor responsible for angioblast differentiation and tube formation. This is the most potent stimulator of endothelial cell proliferation, sprouting, migration and tube formation. VEGF and PDGF belong to the VEGF/PDGF superfamily of ligands and receptors, grouped together on the basis of their close sequence homology and evolutionary relationship. Each of the ligands contains eight conserved cysteine residues, forming a typical cystine-knot motif (25). VEGF and PDGF receptors are members of the class III receptor tyrosine kinase sub-family, which have extracellular immunoglobulin-like (Ig) domains, a transmembrane region and split intracellular kinase domains. VEGF receptors have seven Ig domains, while PDGF receptors have five extracellular Ig domains. These and other structural similarities suggest a close evolutionary relationship (25).

They are important signaling proteins involved in both vasculogenesis and angiogenesis, which paves the way for healing (26). VEGF is also a vasodilator and increases microvascular permeability.

## TGF- $\beta$ 1 (transforming growth factor)

In 1965, Urist (27) discovered the so-called bone induction principle and postulate the theory that bone matrix contained inducing agents that could help generate new bone formation when implanted into an extraskeletal site. This factor was identified as a protein that they named bone morphogenetic protein (BMPs). Currently, nearly 20 structurally related BMP's have discovered, as for example osteogenin, and these protein are known to be members of the transforming growth factor- $\beta$  (TGF- $\beta$ ) gene family (28).

TGF- $\beta$  is a multifactorial regulator of cellular growth in developing systems, and it is a prominent component of the ECM of bone. TGF- $\beta$  molecules are most abundant in the ECM of bone, and this gene family is one of the most important regulatory growth and differentiating factor superfamily (5).

TGF- $\beta$  released from platelet  $\alpha$ -granules could stimulate the proliferation of osteoblasts and beginning of bone formation. The central role of TGF- $\beta$  in controlling osteoclastogenesis by stimulating osteoprotegerin synthesis was already confirmed (29).

The effect of TGF- $\beta$  was observed to be dose-dependent on osteoclastic differentiation and system-dependent on osteoclastogenesis (30, 31).

In agreement with other studies, Graziani et al. (32) indicated that PRP induces proliferation of osteoblasts in a dose-dependent manner along with increasing production of osteocalcin and decreasing production of osteoprotegerin which are suggested to be mediated by TGF- $\beta$ .

TGF- $\beta$  produced by osteoclastic resorption is the key chemoattractant for osteoblast to navigate to sites of bone formation (33).

Recently, some clarity of the effects of TGF- $\beta$  action was shown when Tang et al. (34) demonstrated that the major action of TGF- $\beta$  was not to regulate osteoblast differentiation but to direct bone marrow stromal cells (BMSCs) to resorption sites.

Thus, the activation of TGF- $\beta$  during bone resorption represents a mechanism to link bone resorption to new bone formation, in essence serving as a local coupling factor to trigger the subsequent events involved in bone formation (33). It will induce a massive synthesis of matrix molecules, such as collagen type I and fibronectin by osteoblasts and fibroblasts.

## IGF-I (insuline-like growth factor)

The insulin-like growth factor, also known as IGF or somatomedins, are a group of peptide

hormones by the anabolic properties, produced by the liver under the stimulation of growth hormone (GH) produced by the pituitary gland. Two different IGFs (IGF-I and IGF-II) have been described (35). Both were isolated initially as serum factors with insulin-like activities that could not be inhibited by anti-insulin antibodies (36). The first isoform (IGF-I) is always present in the life, decreasing with the age. The second form (IGF-II) is present only during the fetal age (37). The structure of both IGFs is homologous to human pro-insulin. However, IGFs do not cross-react immunologically with each other. IGF is constitutively produced in many tissues, including liver, kidney, heart, lung, fat tissues, and various glandular tissues. IGF-I is also produced by chondroblasts, fibroblasts, and osteoblasts. IGF-I is chemotactic for fibroblast and stimulates protein synthesis (38). IGF-I is a single chain protein that binds to a specific cell surface receptor (IGF-I-R) and directly stimulates type I collagen biosynthesis, and bone formation by inducing cellular proliferation and differentiation, IGF-I stimulates bone matrix synthesis in cultured rat calvaria, an effect that is partly dependent on its stimulatory effect on DNA synthesis (37, 38). In periodontal research, it was shown that IGF-I is chemotactic and mitogenic for PDL cells (39). Although a single application of IGF-I only slightly induces periodontal tissue regeneration, several lines of evidence suggest that IGF-I combined with other growth factors-such as bFGF, PDGF, and TGF- $\beta$ -may augment the osseous wound-healing process (35).

It was shown that IGF-I increases bone turnover in patients with low bone mineral density (36, 40).

## CTGF (connective tissue growth factor)

This new growth factor was described by Kubota et al. (41). Platelets adhere to CTGF at injured tissue wound sites, where it is overexpressed



along with the platelet coagulation process. In their experiments they showed that non-activated platelets contain considerable amounts of CTGF and that is released by activated PRP and that CTGF endorses angiogenetic activity, cartilage regeneration and fibrosis. Cicha et al. (42) showed that CTGF is expressed in bone marrow cells, but not by platelet producing megakaryocytes, suggesting that the total amount of CTGF in platelets is the result of endocytosis from the extracellular environment in bone marrow.

## EGF (epidermal growth factor)

Acts by binding with high affinity to the epidermal growth factor receptor (EGFR) on the cell surface, triggering an increase in the expression of certain genes that ultimately lead to DNA synthesis and cell proliferation (43).

Stimulates epidermal regeneration, promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts, and enhances the production end effects of other growth factors (44).

## PF-4 (platelet factor)

It is also released from the a granules of platelets and may be partially responsible for the initial influx of neutrophils into wounds. It also acts as a chemoattractant for fibroblasts and promotes blood coagulation by moderating the effects of heparin-like molecules (45).



## Technologies

The aim of processing whole blood sample is to separate the blood components in order to discard element considered not usable (red blood

cells) and concentrate the therapeutic parts (platelets, fibrin, leukocytes and growth factors (46, 47). Many names were attributed to platelet concentrates but many Authors prefer the term concentrated platelet rich plasma (cPRP) (48, 49). We will describe the different systems in according to the classification hold as guidelines for all publications on the topic (50, 51).

## P-PRP (pure platelet-rich plasma) and L-PRP (leukocyte and platelet-rich plasma)

The platelet-rich plasma was first introduced in oral surgery by Whitman et al. in 1997 and is defined as the portion of autologous plasma having a platelet concentration greater than 8 times the baseline value (150,000-400,000/ $\mu$ l) (52-54). Within PRP, the increased number of platelets delivers an increased number of growth factors to the surgical area, it is an autologous source of PDGF and TGF- $\beta$  that is obtained by sequestering and concentrating platelets by gradient density centrifugation. There are some advantages of using PRP. First of all, it is easy to obtain PRP from patient's own blood (55). Secondly, by regulating the processing technique and activation protocol, it is possible to control the dose of growth factors released on activation (55). PRP is the combination of seven native growth factors within a normal clot as the carrier. The clot is composed of fibrin, fibronectin, and vitronectin, which are cell adhesion molecules required for cell migration as for example in osteoconduction, wound epithelialization, and osseointegration.

Various studies document a regenerative potential marrow capable of increasing the quantity and quality of the bone. In addition, the mucosal wound healing appears to be much faster than normal (20, 56, 57).

The platelet rich plasma is a concentrate of autologous growth factors separated with a double

centrifugation (56).

Different methods of locally usable platelet rich plasma (PRP), components have been described (58). In implant dentistry, the most obvious application of PRP would be to accelerate autogenous grafts used for site preparations, sinus lifts, osseointegrations, ridge augmentations, etc. To date, no positive clinical benefits have been documented, nor can be expected, with the use of PRP with non-vital bone substitutes. The target of PRP remains viable osteoprogenitor cells and stem cells.

However, an enhanced bone regeneration can be expected when PRP is used with mixtures of autogenous bone and bone substitutes and with recombinant human growth factors such as recombinant BMP (56). Zimmerman et al. (58) have reported that PDGF and TGF content of concentrated platelet samples is not correlated to the platelet concentration, but a remarkable inter-individual variability in growth factor levels. There are two other factors influencing the release of growth factors from locally usable platelet components: platelet activation and WBE contaminations (59, 60).

Studies in the literature described the PRP as a concentration of PDGF obtained from the destruction of platelets. Potentially offers a significant osteogenic impact but clinical studies attest the bone growth to 10% of the volume applied. Marx (20) described the importance of PRP on the stimulation of fibroblasts.

## Platelet apheresis (AP)

A blood cell separator is used with the dual-needle WBC-reduction system, with isotonic saline solution used for priming and ACD-A as the anticoagulant.

Platelet suspensions obtained by apheresis are centrifuged at 3000 g for 15 minutes, the supernatants are discarded and the platelet pellets resuspended in 1 ml of plasma.

## Buffy coat-derived platelets (BCP)

Buffy coat is prepared by the top-and-bottom method after centrifugation of whole blood (450 ml), at 3000 g for 13 minutes, the PRP is prepared after centrifugation of the resuspended buffy coat for 5 minutes at 400 x g.

## Tube method (TP)

Whole blood (50 ml) is centrifuged at 1650 x g for 10 minutes, the collected supernatant is centrifuged at 730 g for 15 minutes; platelet pellet is resuspended in about 1,5 ml of plasma.

## PRP-techniques

In all available PRP techniques, venous blood is collected with an anticoagulant to avoid platelet activation and degranulation (EDTA-ethylenediaminetetraacetic acid or CTAD-citrate, theophylline, adenosine, and dipyridamole) and then it is immediately processed by centrifugation. It should be stressed that the production of PRP has been developed according to various protocols by many companies, especially diversifying the method of sampling of separation stages.

Established PRP production methods concentrate platelets and leukocytes to different extents. The time for platelet concentrate preparation is about an hour. The first centrifugation step (soft spin) is designed to separate the blood into three layers: red blood cells (RBCs) are located at the bottom and constitute 55% of total volume, acellular plasma or platelet-poor plasma (PPP) is at the top of the tube and it is rich in fibrinogen, and a "buffy coat" layer appears between the 2 fractions, in which platelets are concentrated. The first spin will separate the red blood cells from the plasma, which contains the platelets, the white blood cells, and the clotting

factors. The goal of the subsequent steps is to discard both the RBC layer and the PPP to collect only the buffy coat layer (47). Using a sterile syringe the practitioner aspirates PPP, PRP and some red corpuscles and transfer them to another tube.

The second centrifugation (called the hard spin) finely separates the platelets and white blood cells together with a few red blood cells from the plasma. This soft spin produces the PRP and separates it from the platelet poor plasma (PPP) free from the obstruction provided by a large number of red blood cells.

The result is a separation between platelet-poor plasma (PPP) in the upper part of the tube and platelet rich plasma (PRP) in the lower part. The latter is taken and combined to bovine thrombin and calcium chloride which it activates the gelling of platelet and fibrinogen concentrates (46-49). L-PRP products are preparations with leukocytes and with a low density fibrin network after activation (51).

One method of P-PRP is known as PRGF (platelets rich growth factors); it has been defined by Anitua et al. (61-64), by which it is possible to obtain different autologous preparations rich in growth factors from the same patients blood depending on the coagulation and activation degree of the samples. One of these preparations is the scaffold-like PRGF composed of fibrillar and cellular components, which may be used to induce bone regeneration in post-extraction sockets. Another interesting formulation is the liquid PRGF, which may be used to humidify and bioactivate dental implant (66) surfaces to improve their osteointegration.

The centrifugation is carried out on venous blood collected in 5 ml tubes containing sodium citrate (10%) used as an anticoagulant. The duration is 6 minutes and the rotational force is 160 G. The separation produces 3 phases. One on the bottom of the tube containing RBCs (Red phase), a central where it accumulates the fibrinogen and growth factors (PRGF) and at the top the plasma (PPGF). Subsequently, through a pipette laboratory, it picks up the PDGF and part

of PPGF. To this is added the calcium chloride to activate the coagulation, which after 10-15 minutes produces the gel of PDGF.

## PRF (platelet-rich fibrin) and L-PRF (leukocyte-and platelet-rich fibrin)

Fibrinogen is a soluble fibrillary molecule that plays a determining role in platelet aggregation during hemostasis, it is transformed into a insoluble fibrin by thrombin while the polymerized fibrin gel is the first cicatricial matrix of the wounded site (47, 48, 65). This protocol was developed by Choukroun et al. in 2001 for specific use in oral and maxillofacial surgery and represents a step forward in ease of production of the fibrin gel. The collection of venipuncture occurs in the tubes coated with glass and immediately place in the rotor, where after 10-12 minutes of centrifugation at 2700-3000 rpm, it completes the formation of the gel.

The coagulation occurs without addition of gelling agent but to interaction with the silicon dioxide present in the glass coating of the tube. The absence of anticoagulant determines the activation of most platelets in contact with the tube walls and the release of coagulation cascades. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom of the tube and acellular plasma supernatant at the top. Platelets accumulate in the lower part of the fibrin clot, mainly at the junction between the red corpuscles and the PRF clot. A fibrin clot charged with serum and platelets is obtained and by driving out the fluids trapped in the fibrin matrix, practitioners will obtain very resistant autologous fibrin membranes (48). The fibrin network presents a particularly homogeneous 3-dimensional organization, a progressive polymerization increases incorporation of the circulating cytokines in the fibrin meshes (47). The PRF red extremity would be of interest for clinical use and even more effective than the higher

part of the fibrin clot (65). PRF inlike the other platelet concentrates, would be able to progressively release cytokines during fibrin matrix remodeling (47, 48). The technique is very quick, inexpensive and allows to produce large quantities of fibrin clots and membranes in a very short time (50, 66, 67). When performing ridge augmentation, PRF membranes are used to protect and stabilize the graft materials and act as fibrin bandages, accelerating the healing of the soft tissues, facilitating the rapid closure of the incision. This is currently the main technique in oral and maxillofacial surgery, particularly because the L-PRF membranes and clots are very simple to use. The presence of leukocytes has probably a great impact on the biology of these products, not only because of their immune and antibacterial properties, but also because they are actors of the wound healing process and the local factor regulation. But it depends which leukocytes, in which quantity and in which state the centrifugation process can softly activate the white cells (45, 50).

## In vitro studies

PRP modulates cell proliferation in a cell type-specific manner similar to what has been observed with TGF-beta1, PRP stimulated DNA synthesis in gingival fibroblasts and periodontal ligament cells (68). The effect of platelet-rich plasma (PRP) on the proliferation of osteoblast-like cells SaOS-2 *in vitro* was investigated by Ogino et al. (29). Cells were cultured in the presence of platelet-poor plasma (PPP), whole blood, or PRP and the cell number was counted after 36 and 72 hours. The mean platelet count of PRP was  $1546.36 \pm 382.25 \times 10(3)/\text{microL}$ , and the mean levels of PDGF-AB, TGF-beta1 and IGF-I were  $0.271 \pm 0.043$ ,  $0.190 \pm 0.039$ , and  $0.110 \pm 0.039 \text{ ng}/1500 \times 10(3) \text{ platelets}$ , respectively. Cell proliferation was enhanced in all PRP groups in a dose-dependent manner. The presence of neutralizing antibodies of PDGF and TGF-beta1, significantly suppressed prolifera-

tion. These results show the beneficial abilities of PRP in the proliferation of osteoblast-like cells from the standpoint of growth factors, including the contribution of each factor.

A comparative study of PRF and PRP effect on proliferation and differentiation of rat calvaria osteoblasts *in vitro* was obtained by He et al. (69). The exudates of PRP and PRF were collected at the time points of 1, 7, 14, 21, and 28 days. PRP released the highest amounts of TGF-beta1 and PDGF-AB at the first day, followed by significantly decreased release at later time points. PRF released the highest amount of TGF-beta1 at day 14 and the highest amount of PDGF-AB at day 7. Cells treated with exudates of PRF collected at day 14 reached peak mineralization significantly more than negative and positive control groups.

PRF released autologous growth factors gradually and was superior to PRP, from the aspects of expression of ALP and induction of mineralization.

The aim of the study of Giovanini et al. (70) was to evaluate the effect of PRP in the presence of levels of TGF- $\beta$  on PRP samples, as well as in the presence of collagen III and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), by means of a histomorphometric analysis of the bone matrix and fibrous deposition on the bone repair. Four bone defects were created on the calvarium of 21 rabbits. The surgical defects were treated with either particulate autograft, particulate autograft mixed with PRP and PRP alone. The histomorphometric results demonstrated intensive deposition of fibrous tissue while hinder bone deposition occurred in PRP groups. These results coincided with higher values of the TGF- $\beta$  on the PRP sample, also larger occurrence of diffuse collagen III deposition and higher presence of  $\alpha$ -SMA positive cells spread among the fibrous tissue. It suggests that higher levels of TGF- $\beta$  on PRP samples may produce a fibroproliferative event and consequently hinder the bone matrix deposition.



## Animal studies

Anitua and Andia (71) showed that adding PRP-clot around roughened titanium implants at the moment of their implantation in goats improved both the extent and the quality of bone regeneration around the implant.

Two studies evaluated the use of PRP with Bio-Oss in a rabbit calvarial bone defect model.

Kim et al. (72) created 2 cranial defects in each of 20 rabbits. The defects were grafted with Bio-Oss with or without PRP. Digitized plain films and computed tomography scans both showed significantly greater bone density with the use of PRP at both 1 and 2 months. Aghaloo et al. (73) created 4 rabbit cranial defects in each of 15 rabbits. Three were grafted with either Bio-Oss, Bio-Oss with PRP, or autogenous bone. The fourth received no graft as a control. Histomorphometric evaluation showed that the addition of PRP significantly increased the percentage of bone formation over that of Bio-Oss alone at all 3 time periods (1, 2, and 4 months). However, in this study, autogenous bone was still significantly better than either Bio-Oss or Bio-Oss with PRP. Kim et al. (74) in a bone defect in the iliac crest of dogs demonstrated that PRP combined with demineralised bone powder enhanced bone formation around titanium implants.

It is possible that a higher concentration of platelets may aid in human wound healing, whereas other animal species may heal extremely well even without additional platelets, making it more difficult to show a benefit of PRP in these models (75). The potential effect of the platelet cytokines, massively released during platelet activation and fibrin gelling, looks to be extremely limited in time because are released too quickly during the fibrin matrix polymerization (47, 48, 76, 77).

Anitua et al. studied its potential bone regeneration effects of the scaffold-like PRGF from goats' blood, on empty artificial defects made in the tibiae of goats, which simulate post-extractive defects. The PRGF elaborated resulted in a significant enrichment in platelet number, 3.17-

fold increase comparing with peripheral blood. On the contrary, leukocyte content was below the detection limit of the coulter, confirming the absence of leukocytes in the PRGF preparations, which will improve the homogeneity of the product and reduce donor to-donor variability (62-64). None of the goats showed signs of weakness after retrieval of blood before surgery. At sacrifice, no clinical signs of inflammation or adverse tissue reactions were observed. Histological analysis of all artificial defects was performed 8-weeks post-surgery. The damage caused by the drilling procedure appeared to be limit and no signs of immune response were observed around the defects. Histological and histomorphometric results demonstrated that application of liquid PRGF increased the percentage of bone-implant contact in 84.7%. You et al. (78) have compared the effect of platelet-enriched fibrin glue and PRP on bone defect repair adjacent to 6 titanium dental implants (80, 81) in the dog tibia to simulate immediate implantation into fresh tooth extraction sockets in the presence of large gaps (>2mm). Bone-implant contact achieved with PRP was lower than with platelet-enriched fibrin glue. Even the study of Jeong et al. (79) confirmed this result: the Authors evaluated the effect of tooth ash and platelet-rich plasma (PRP), and platelet-rich fibrin (PRF) grafts into bone defects around implants on bone formation on six adult dogs. Histomorphometric examination revealed that the rate of new bone formation in group PRF after 4-weeks was significantly higher than that in the control group. In this study, a bone graft method using a mixture of tooth ash and PRF was found to increase new bone formation compared to the method using PRP.

## Clinical studies

The PubMed and Cochrane search resulted in 442 papers, from assessing the titles and abstracts, 31 randomized clinical trials were selected but only the 20 most relevant and with a fol-

low-up  $\geq 4$  months were been reported and explained in Table 1. In implant dentistry, the most obvious application of PRP would be to accelerate autogenous grafts used for site preparations, sinus lifts, osteointegrations, ridge augmentations, etc. In the first formulation, conceived by Marx et al. in 1998 (20), the gel obtained (Metronics) was inserted in association with autologous bone to compensate for mandibular bone defects.

88 patients with mandibular continuity defects were randomized to be reconstructed with autogenous cancellous bone grafts with or without the addition of PRP. Subjectively evaluated radiographs determined that the degree of maturation of the grafts was significantly greater with the addition of PRP (1.6 to 2.2 times faster). This first study found a greater and more rapid densification of bone repair. Histomorphometric evaluation of core samples taken at 6 months revealed a significantly greater percentage of trabecular bone with the addition of PRP (74% with PRP compared with 55% without PRP).

Anitua (61) first studied the deposition of a PRP-clot with or without autogenous bone in a series of 20 patients undergoing tooth extraction due to vertical fractures or severe periodontal disease. Subsequent implant placement was another precondition. In some patients with multiple extractions, PRP-clot was deposited into the socket on one side of the mouth while the other served as the control. Bone biopsies were taken at extraction sites between 10 and 16 weeks. In most of the patients with PRP-clot, bone regeneration was extensive and bone tissue was compact with well-organized trabeculae. In contrast, in the control group the cavity was mainly filled with connective tissue. The results document a complete recovery in 8 of 10 sites. The 2 patients with partial healing had severe bone defects and were smokers. PRP and bovine porous bone mineral provided an added regenerative effect to GTR in promoting the clinical resolution of intrabony defects on 18 patients with severe periodontitis (80).

Treatment with PRP to support the osseointegration of endosseous dental implants has also been

described (81, 82), suggesting that PRP may be useful in accelerating the osseointegration of titanium implants (74).

Hanna et al. (83) have enrolled 13 patients in a randomized, split mouth, double-masked clinical trial. Bilateral defects were matched according to their intrasurgical measurements. Qualifying defects had loss of attachment of  $>$  or  $= 6$  mm, a radiographically detectable defect of  $>$  or  $= 4$  mm, at least two remaining osseous walls, and not primarily related to a furcation involvement. During open flap debridement, the defects were randomly assigned to receive either bovine derived graft mixed with PRP (Smart PreP) or graft alone. The mean baseline and 6-month PD, CAL, and REC of the deepest buccal and lingual measurements related to the defect for each group were computed. At 6 months, comparisons within groups showed statistically significant benefits with both treatment modalities and a significant differences between treatments for PD and CAL. The addition of a high concentration of autologous platelets to a bovine derived xenograft to treat infrabony defects significantly improved their clinical periodontal response.

Wiltfang et al. (84) analysed 35 patients that underwent floor augmentation of the maxillary sinus and were randomly assigned to  $\beta$ -TCP ( $\beta$ -tricalcium phosphate) and PRP (Curasan kit) or b-TCP alone. Hystomorphometric outcome showed a positive effect of 9%.

Choukroun et al. (77) evaluated the potential of PRF in combination with freeze-dried bone allograft (FDBA) to enhance bone regeneration in lateral sinus floor elevation. Nine sinus floor augmentations were performed with 6 sinuses receiving PRF + FDBA particles (test group) and 3 sinuses receiving FDBA without PRF (control group). Histologic evaluations showed the presence of residual bone particles surrounded by newly formed bone and connective tissue. The use of PRF in combination with FDBA to perform sinus floor augmentation seemed to accelerate bone regeneration.

On 16 patients underwent bilateral sinus floor augmentation after 4 months the PRP group showed higher bone activities but a progressive

**Table 1** - Published results on PRP and PRF clinical application. OMR (oral and maxillofacial reconstruction), (PISP) Pre-implant Socket preservation, (SERA) sinus elevation and/or ridge augmentation, (IBD) interproximal bony defects, (FD) buccal degree II furcation defects, (GTR) guided tissue regeneration, (BAOSFE osteotome sinus floor elevation), (SRP) scaling root planning, (OFD) open flap debridement, Hys (Hystomorphometry), RMI (radiographic graft maturity index), radiographic bone density (RBD), residual bone height (RBH), RBL (radiographic bone loss (RBL), probing pocket depth recovery (PPDR), clinical attachment level gain (CALG), ABG (autologous bone graft), freeze-dried bone allograft (FDBA), bovine derived xenograft (BDX),  $\beta$ -tricalciumphosphate ( $\beta$ -TCP) hydroxyapatite (HA), distal second molar defect (SMD).

Year	First Author	Participants	Methods	Treatment	Measure	Months	Results	Author's Conclusions
1998	Marx <sup>(20)</sup>	88	Metronics+CaCl+ Bovine Thrombine	OMR	Hys	6	ABG+ PRP>ABG ≈19% ABG+ PRP>ABG ≈0.56	Bone grafts with growth factors added by means of PRP demonstrated greater trabecular bone density than did bone grafts without PRP
1999	Anitua <sup>(61)</sup>	20	160G + CaCl	PISP	Hys	4	ABG+PRGF>ABG No quantitative analysis	The epithelization has been complete and significantly better than in areas not treated with PRGF. Better bone regeneration.
2000	Kassolis <sup>(61)</sup>	15	Autologous thrombine	SERA + 36 implants	Hys	12	FDBA+PRP No quantitative analysis	Numerous areas of osteoid and bone formation around FDBA particles, with no evidence of inflammatory cell infiltrate. 4 implants were removed-no control
2002	Camargo <sup>(60)</sup>	18	5600 r.p.m.	IBD	PPDR	6	BDX+GTR+PRP>GTR ≈ 1.36-1.39mm	PRP and BDX provide an added regenerative effect to GTR in promoting the clinical resolution of intrabony defects on patients with severe periodontitis
2003	Wiltfang <sup>(64)</sup>	35	Curasan kit	SERA	CALG	6	BDX+GTR+PRP>GTR ≈1.75-1.84mm $\beta$ -TCP+PRP> $\beta$ -TCP ≈9%	when PRP was added to $\beta$ -TCP, bone regeneration was supported to a small extent but the degradation of $\beta$ -TCP was not accelerated
2004	Oyama <sup>(60)</sup>	12	160G+400G+huma n fibrin glue	OMR	RBD	5-6	ABG+PRP> ABG ≈17%	PRP could enhance the osteogenesis of alveolar bone grafting in cleft lip and palate
2004	Hanna <sup>(63)</sup>	13	SmartPRP +CaCl+ Bovine Thrombine	IBD	PPDR	6	BDX+PRP>BDX ≈ 1.01mm BDX+PRP>BDX ≈ 0.84 mm	The addition of PRP to BDX for the treatment of intrabony defects demonstrated good clinical result respect to the use of graft alone
2005	Okuda <sup>(61)</sup>	70	2400rpm+3600 rpm + sodium alginate	IBD	PPDR	12	HA+PRP>HA ≈ 1.2 mm HA+PRP>HA ≈ 1.4 mm	The added benefits of PRP combined with HA are of clinical but not radiographycal significance
2005	Sammartino <sup>(92)</sup>	18	1200rpm + batroxobine+ calcium gluconate	SMD	PPDR	3-4	SRP+ PRP> SRP ≈ 3.5 mm SRP+ PRP> SRP ≈ 3.4 mm	PRP method is valid in inducing and accelerating bone regeneration of periodontal distal defects of the mandibular second molar after surgical extraction of deeply impacted third molars.
2005	Thor <sup>(63)</sup>	19	Metronics+CaCl+ Bovine Thrombine	OMR	RBL	12	ABG+IMPLANT+PRP±A BG+IMPLANT	no positive effects of PRP
					RBD		ABG+PRP±ABG	

to be continued

continued from Table 1

2006	Choukroun <sup>(77)</sup>	9	2500rpm	SERA	Hys	4	FDBA+PRF±	In the control group, bone sampling after a healing time of 4 months was not possible, underestimation of PRGF effect
2007	Consolo <sup>(85)</sup>	16	1200rpm+4400rpm + autologous thrombine	SERA	RBD	8	FDBA	
						4	ABG+PRP>ABG ≈71%	A progressive extinguishment of the PRP effect is recorded after an interval longer than 6-7 months
2008	Schaaf <sup>(86)</sup>	53	1000g+2900g+10% calcium gluconate	SERA	Hys	7	ABG+PRP±ABG	
						4	ABG+PRP±ABG	No enhancement in bone volume after sinus floor elevation with PRP.
2008	Diss <sup>(87)</sup>	20	3000rpm	BAOSFE + 35 implants	RBH	12	PRF-T12>PRF-T0 ≈1.7-4.9mm	Healing period of 2-3 months was found to be sufficient to resist a torque of 25 N.cm applied during abutment tightening. No control
2011	Marukawa <sup>(88)</sup>	20	800g+1500g-calcium chloride	OMR	RBL	12	ABG>ABG+PRP ≈9%	PRP may preserve the width and height of the graft better than control, making ABG with PRP useful for alveolar bone grafting in cleft patients
2012	Anitua <sup>(94)</sup>	5	PRGF System®+ PRGF activator® (calcium chloride)	SERA	Hys	5	BDX+PRGF>BDX ≈9% new bone	PRGF may present a role in reducing tissue inflammation after surgery, increasing new bone formation and promoting the vascularization of bone tissue
2012	Lekovic <sup>(95)</sup>	17	1000g	IBD	PPDR	6	Bio-Oss+PRF>PRF ≈0.96-1.22	Bio-Oss has the ability to augment the effects of PRF in reducing pocket depth, improving clinical attachment levels and promoting defect fill
					CALG		Bio-Oss+PRF>PRF ≈1.42-1.63	
2013	Bajaj <sup>(96)</sup>	37 (72sites)	PRF (500g) PRP (400g+autologous thrombin)	FD	PPDR	9	PRF>PRP≈1mm PRF>OFD≈2.71	The use of autologous PRF or PRP, were effective in the treatment of furcation defects with uneventful healing of sites
					CALG		PRF+PRP>OFD≈1.5	
2013	Kumar <sup>(89)</sup>	20	5600rpm+2400rpm +autogenous thrombin+al2	OMR	RBD	6	ABG+PRP>ABG≈28%	A combination of autologous bone graft and PRP can increase the bone formation
2015	Anitua <sup>(97)</sup>	26 (41 short implant)	PRGF-Endoret	SERA	RBH	35	PRF-T12>PRF-T0 ≈ 2.2-6.2mm	Transalveolar sinus floor augmentation in association with plasma rich in growth factors and short implants have resulted in a stable bone height gain of 4.2 1 2.0 mm in 3 years' follow-up



reduction of the effect was recorded after a time of 6-7 months (85).

Schaaf et al. (86) showed no significant differences in bone volume and implant failure using autologous bone graft alone or in combination with PRP in 34 sinus floor augmentation (92).

The aim of this prospective study was to document, radiographically, changes in the apical bone levels on microthreaded implants placed in subsinus residual bone height, according to a bone-added osteotome sinus floor elevation technique with platelet-rich fibrin (PRF) as grafting material.

Diss et al. (87) placed implants on 20 patients using PRF as grafting material in the bone-added osteotome sinus floor elevation technique. The survival rate at abutment tightening (6 to 12 weeks of healing) and at 1 year was calculated. At 1 year, all implants were clinically stable and the definitive prostheses were in function, resulting in a survival rate of 97.1%, a healing period of 2-3 months was found to be sufficient to resist a torque of 25 N.cm applied during abutment tightening. At 1 year, formation of a new recognizable bone structure delimiting the sinus floor was identified radiologically and led to a predictable implant function.

In 2012 Marukawa et al. (88) have evaluated the effectiveness of platelet-rich plasma (PRP) on the regeneration of autogenous cancellous bone and marrow grafted in the alveolar cleft on 14 patients. The bone graft mixed with PRP was packed into the alveolar cleft and postoperative bone density was assessed in a qualitative analysis. Quantitative evaluation of regenerated bone was made with computed tomography and panoramic radiographs at 1 month, 6 months and 1 year after surgery. Satisfactory bone bridging formation was observed in all patients without any complications. The added PRP to autogenous cancellous bone grafting reduced the resorption of regenerated bone postoperatively.

More matured bone was formed when PRP was used with autogenous bone graft on ten patients who need reconstruction in the oral and maxillofacial region, the regenerated bone showed an opacity close to native bone and the compact

bone was clearly differentiated from cancellous bone after six months. The bone density was measured in terms of Hounsfield Units (89). Other publications in the oral and maxillofacial surgery confirm the ability of PRP to promote bone healing (90-97).

## Conclusions

The totality of the above studies emphasizes the importance of growth factors contained in the various extracts of blood centrifuged. In fact, various experiments have researched the amount of growth factors most representative in the various separation products (78, 98).

Autologous platelet rich plasma is a safe and low cost procedure to deliver growth factors for bone and soft tissue healing. The guiding concept is the assumption that greatly increasing the concentration of growth factors in a wound the bone regenerative response will be better and faster (99). Another feature of the PRP-enhanced graft is its high content of fibrinogen (fibrin glue) that enhanced wound stability and promotes a favorable scaffold for cellular migration (53).

An important role is played by the type of polymerization of fibrinogen into fibrin, dictated by the concentration of thrombin. The fibrin can have two different geometries of polymerization: a tetramolecular given by high concentrations of thrombin, and another trimolecular triggered by minimum quota of thrombin. The first form is presented as a network dense and thickened, with higher mechanical properties, this represents an impediment to the migration of cytokines. In fact in the second mode of polymerization, the network is more flexible and the mobility of cytokines results to be higher.

The great heterogeneity of clinical outcomes can be explained by the different PRP products with qualitative and quantitative difference among substance. PRP activation is another source of variability, some Authors not activate platelets, and others use autologous thrombin and calcium chloride and other methods (100). If platelets become

activated by the preparation before a final centrifugation step, growth factors may be lost with the discarded supernatant. Knowledge of growth factors levels in PRP or PRF samples is necessary to ensure reliability and reproducibility to the use of PRP in oral surgery, since the regenerative potential depends on growth factor levels (90).

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