

Platelet-Rich Plasma: A Promising Innovation in Dentistry

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A b s t r a c t

The goal of periodontal therapy is to protect and maintain the patient's natural dentition for his or her lifetime. More specifically, after periodontal regenerative surgery, the aim is to achieve complete wound healing and regeneration of the periodontal unit. A recent innovation in dentistry is the preparation and use of platelet-rich plasma (PRP), a concentrated suspension of the growth factors found in platelets. These growth factors are involved in wound healing and are postulated as promoters of tissue regeneration. This clinical update outlines the specific effects of these growth factors, both in vitro and in vivo, on periodontal wound healing. The review focuses on current animal and human trials using PRP to promote tissue regeneration and alveolar bone repair. The article goes on to describe the clinical benefits of PRP and the step-by-step preparation of PRP in the dental office.

MeSH Key Words: blood platelets/physiology; growth substances/physiology; guided tissue regeneration/methods; wound healing

© J Can Dent Assoc 2003; 69(10):664
This article has been peer reviewed.

The goal of periodontal therapy is to protect and maintain the patient's natural dentition over his or her lifetime for optimal comfort, function and esthetic appearance.^{1,2}

After periodontal or oral surgery, healing proceeds by repair and regeneration. Repair is the healing of a wound by tissue that does not fully restore the architecture or function of the affected unit, whereas regeneration is reproduction or reconstitution of a lost or injured part.³ Open periodontal flap surgery, which provides access to the root, often results in reduction of probing depth because repair occurs with a healthy, long epithelial attachment. Occasionally, osseous surgery is needed to eliminate periodontal pockets, and gingival recession may result.^{1,4,5} Regenerative surgery, including the use of barrier membranes and graft materials, can reduce probing depths, support the formation of periodontal ligament and allow regenerative rehabilitation and functional reconstruction.^{2,6,7} The aim of regenerative periodontal procedures is to induce regeneration at the alveolar bone and cementum and to develop a new functional periodontal ligament.^{2,8,9}

The study of wound healing is a complex and growing area that deals with many cell types and growth factors.⁹⁻¹¹ After surgery, platelets begin to form a stable blood clot,

releasing a variety of growth factors that induce and support healing and tissue formation.¹²⁻¹⁴ Administration of these growth factors may be combined with tissue regeneration techniques in the repair of intrabony defects, furcations and cyst cavities. A recently developed procedure can be used to create platelet-rich plasma (PRP), a concentrated suspension of growth factors that has been demonstrated to induce healing and regeneration of tissues, including those in the periodontal area.^{8,10,15-19} This review focuses on PRP and the impact of the growth factors it contains. The aim is to inform clinicians who are interested in recent surgical techniques and to update practitioners' knowledge about the applications of PRP in dentistry.

Effects of PRP Growth Factors on Cells Involved in Periodontal Wound Healing

As in other parts of the skeleton, hormones and growth factors play important roles in the development of the maxillofacial region. Various studies have examined the effects of systemic hormones and growth factors on bone and soft-tissue metabolism.^{11,20-24} In particular, growth factors regulate cellular events in wound healing, such as proliferation, differentiation, chemotaxis and morphogenesis of tissues and organs.^{9,11} Growth factors may act in an

autocrine, paracrine or endocrine manner. They are deposited in the extracellular matrix and are then released during matrix degradation. Their interaction with surface receptors on the target cells activates an intracellular signalling pathway that induces transcription of the messenger RNA and proteins needed for the regenerative process. These growth factors, in combination with other transcription factors, then activate a set of genes. The growth factors also induce specific changes at the cellular level. All of these effects are controlled by feedback mechanisms involving binding proteins and other growth factors.^{9,11}

At a more specific level, periodontal wound healing involves gingival fibroblasts, gingival epithelial cells, periodontal ligament fibroblasts and osteoblasts, all of which are important for tissue repair and hard-tissue regeneration. A series of well-orchestrated cell-cell interactions is initiated after injury. Disruption of the vasculature as a result of injury leads to fibrin formation and platelet aggregation. Several growth factors are then released into the tissue from the platelets and from the adjacent cells after injury, including platelet-derived growth factor (PDGF), transforming growth factor-alpha, transforming growth factor-beta (TGF-b) and insulin-like growth factor I (IGF-I).²⁵⁻²⁸ Bone and cementum may also release growth factors during wound healing.⁹

Periodontal and oral surgical techniques may involve use of these factors in both soft and mineralized tissues.^{9,11,15} For example, local application of growth factors is used to promote healing, especially regeneration.^{9,11} Numerous studies, including some dental research, have shown that PDGF, TGF-b and IGF-I are found in PRP and, because of their impact on wound healing, the use of these factors has led to promising results.^{8,15-17,19,25,29-35} The next few paragraphs provide some background information about these PRP-related growth factors.

PDGF is a basic dimeric glycoprotein with 2 disulphide-bonded polypeptides,³⁶ referred to as A and B chains. Three isoforms of PDGF are possible: AA, BB and the heterodimeric AB.^{21,37} All isoforms of PDGF are released after adhesion of platelets to an injured site. PDGF is the most thoroughly described growth factor in terms of its effects on the periodontium *in vitro* and *in vivo*. *In vitro*, all isoforms have proliferative activity on periodontal ligament fibroblasts.³⁸⁻⁴⁰ PDGF is also chemotactic for these fibroblasts, and it promotes collagen and protein synthesis.⁴¹ Furthermore, the AA and BB isoforms enhance proliferation of bone cells,^{21,42} increasing the production of PDGF-AA in osteoblast cultures by an autocrine process.⁴³ Gamal and Mailhot⁴⁴ obtained dentin specimens from periodontally diseased and healthy teeth and cultured periodontal ligament fibroblasts over these specimens *in vitro*. Various concentrations of PDGF-BB were added, and

fibroblast adherence and cell morphology were determined after 24 hours. The optimal concentration of PDGF-BB for inducing the periodontal ligament fibroblasts to adhere to periodontitis-affected root surfaces was 50 ng/mL (similar effects were achieved at higher concentrations).⁴⁴

*In reconstructive periodontal studies in rats, in vivo application of PDGF increased bone regeneration in calvarial defects when a resorbable membrane was used as a carrier.*⁴⁵ The administration of PDGF with barrier membranes increased the gain in periodontal ligament and bone in Class III furcation defects in beagles.⁴⁶ *In periodontal lesions of monkeys, the height of alveolar bone was greater after a single dose of PDGF.*⁴⁷ PDGF also acts in combination with other growth factors, as explained below.

IGF has 2 forms, I and II, each of which has 2 single-chain peptides. IGF binds to the same receptors as insulin and is involved in the development of many tissues, including the teeth.⁴⁸⁻⁵¹ Both forms of IGF are potent factors for survival of hematopoietic cells, fibroblasts and the nervous system.⁵²⁻⁵⁴ Both forms are found in bone, and IGF-II is the most abundant growth factor in bone matrix.⁵⁵ However, in the area of periodontal regeneration, more research has been done on IGF-I. This form of IGF is chemotactic for periodontal ligament cells, and it has strong effects on periodontal ligament fibroblasts and protein synthesis.⁴¹ IGF-I stimulates bone formation by proliferation and differentiation,^{56,57} and it is synthesized and secreted by osteoblasts.⁵⁸ It also has dose-dependent chemotactic effects on osteoblasts.⁵⁹ An increase in the proliferation of human osteoblasts has been demonstrated with a combination of PDGF, IGF-I, TGF-b and epidermal growth factor.⁶⁰

*In vivo, application of IGF-I to the surface of rat molars promoted cementogenesis after reimplantation.*⁶¹ When IGF-I was given in combination with PDGF, bone formation on implant surfaces was increased.^{62,63} The combination of these factors with barrier membranes also increased the bone-implant contact rate.⁶⁴ In addition, the combination of PDGF-BB and IGF-I promoted new bone, periodontal ligament and cementum in natural disease lesions in dogs and in ligature-induced periodontal lesions in nonhuman primates.^{20,65} Human patients treated with a combination of 150 mg/mL each of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and rhIGF-I in a methylcellulose vehicle experienced 43.2% osseous defect fill, whereas the control group (vehicle only) had 18.5% osseous fill.⁶⁶

TGF-b is the name given to a group of homodimeric proteins involved in the formation and development of many tissues.⁶⁷ Once secreted, ligand binds to transmembranous heterodimeric receptors, activating a group of intracellular proteins. Then, phosphorylated intracellular

proteins start an intracellular signaling pathway which in turn activates a set of genes.^{68,69}

In vitro, TGF- β has been observed to promote extracellular matrix production in many cell types, such as periodontal ligament fibroblasts.^{41,70} TGF- β_1 , used alone or in combination with PDGF-BB, stimulates the proliferative activity of periodontal ligament fibroblasts.³⁸ TGF- β enhances collagen gel construction in vitro, and its effects are influenced by the combination of PDGF and IGF.⁷¹⁻⁷³ In addition, TGF- β stimulates biosynthesis of type I collagen and fibronectin and induces deposition of bone matrix.^{70,74,75}

In vivo, administration of TGF- β alone had no effect on rabbit calvarial defects; however, it increased bone regeneration when applied with gelatin scaffolds.⁷⁶ Repeated injections of TGF- β resulted in ossification by means of endochondral bone formation in the long bones.⁷⁷ When TGF- β_1 was applied with a biodegradable osteogenic material in rabbits, bone growth across calvarial defects was significantly increased.⁷⁸ In a recent study involving a canine model, the application of rhTGF- β_1 in conjunction with nonresorbable barrier membrane greatly enhanced bone regeneration in oral osseous defects (after 2 months).⁷⁹

Although basic and clinical research has focused on application of the growth factors just described, regeneration of tissues can also be achieved through gene therapy. In a recent review, Yao and Eriksson⁸⁰ reported that short shelf life and inefficient delivery to target cells are major concerns associated with local administration of recombinant human growth factors. The growth factors are expensive, and many doses may be required to achieve any therapeutic effect.⁸⁰ These authors concluded that, in light of these limitations, gene therapy may be an appropriate alternative in the future.

Another easy, cost-effective way to obtain high concentrations of growth factors for tissue healing and regeneration may be autologous platelet storage via PRP, as described below.

PRP-Related Studies

Knowledge about growth factors and wound healing has been enhanced by the development of an autologous platelet gel or concentrate, PRP, which is used in various surgical fields, including head and neck surgery, otolaryngology, cardiovascular surgery, oral and maxillofacial surgery, and periodontics, to enhance wound healing and regeneration.^{18,29} PRP is a component of blood in which the platelets are concentrated in a limited volume of plasma.^{8,10,11,15,16,29} Medical literature provides evidence that platelets contain many growth factors, including PDGF, IGF and TGF- β , that enhance wound healing and help to induce regeneration of the tissues.^{81,82} This autologous plasma is a rich source of growth factors and its application has been reported as an effective way to induce

tissue repair and regeneration.^{18,29,83,84} Once platelets have adhered to injured vessels (by collagen), they release granules containing serotonin, thromboxane and adenosine to start the clotting process, which in turn leads to the formation of fibrin.⁸⁵ From this insoluble network, the platelets then release many growth factors inside the wound, of which PDGF, IGF and TGF- β play the most important roles. For example, PDGF is known to be characteristic for monocytes and macrophages, and during wound healing it is an activator of collagenase, which promotes the strength of the healed tissue. TGF- β activates fibroblasts to form procollagen, which results in deposition of collagen within the wound.

In vitro, platelet membranes have been shown to stimulate the mitogenic activity of human trabecular bone cells, thus contributing to the regeneration of mineralized tissues.³⁴ Another study demonstrated that the proliferation rate of human osteoblast-like cells was (concentration-dependent) increased up to a certain plateau by adding thrombocytes; these in vitro results support the current assumption that the clinical use of PRP may increase bone regeneration.⁸⁶

In an in vivo study, Aghaloo and others¹⁷ grafted 8-mm rabbit calvarial defects with autogenous bone, PRP alone, or autogenous bone and PRP; the control was no treatment. The defects were evaluated by digital subtraction radiography with step wedge calibration, histologic examination and histomorphometric analysis at 1, 2 and 4 months. There was a significant increase in bone area and bone density in the defects treated with a combination of bone and PRP.¹⁷ Kim and others³¹ placed titanium dental implants in the iliac crest of dogs and used surgical methods to prepare circular defects, which were then filled with a mixture of dentin and plaster of Paris, with and without PRP. Histomorphometric analysis revealed a higher percentage of bone contact in cases where PRP was used in conjunction with the dentin-plaster of Paris mixture. The authors concluded that bone defects around the implants could be successfully treated with dentin-plaster of Paris and that the outcome of the integration could be improved by application of PRP.³¹ Another study assessed the efficacy of demineralized bone powder alone or combined with PRP in enhancing the osseointegration of dental implants in a dog model.⁸⁷ Standard histomorphometric methods at 6 and 12 weeks after surgery revealed a higher percentage of bone contact with bone powder and PRP than with bone powder alone. The authors concluded that bone defects around titanium implants could be treated successfully with bone powder and that PRP may improve bone formation.⁸⁷

Human studies have also shown that PRP can be advantageously and easily applied in surgery. Man and others¹⁸ used PRP in 20 patients undergoing cosmetic surgery, including face lifts, breast augmentations, breast reductions

and neck lifts. The application of PRP yielded adequate hemostasis if platelet-poor plasma (PPP) was also applied to create a seal to halt bleeding. The authors reported that bleeding capillaries were effectively sealed within 3 minutes after application of the platelet gel (PRP) and fibrin glue (PPP). They also noted the advantage of minimizing use of electrocautery so as to minimize the chance of damage to the adjacent nerves. They concluded that PRP offered significant benefits in terms of accelerated postoperative wound healing, tissue repair and regeneration if PPP was used as a hemostatic agent.¹⁸ Hiramatsu and others⁸⁸ examined the effects of reinfusion of autologous platelet concentrate after open heart surgery in patients with noncyanotic congenital heart disease. Such reinfusion of freshly prepared autologous PRP was followed by good aggregation responses and low blood loss. The authors suggested that this procedure might be useful in pediatric open heart surgery to avoid blood transfusion and minimize the need for homologous blood products.⁸⁸

The first clinical dental results with PRP were reported by Marx and others in 1998, who used PRP to improve graft incorporation in mandibular reconstructions in patients who had received cancellous bone marrow grafts after tumour removal.²⁹ Their data strongly suggested that adding PRP to bone grafts accelerated the rate and degree of bone formation. The next year, Anitua¹⁹ studied 20 healthy patients for whom an extraction was indicated because of a nontreatable tooth with vertical fractures or severe periodontal disease and who were contemplating subsequent implant placement so that biopsy samples could be obtained without additional discomfort. After the extraction, 10 of the patients received a mixture of autologous bone and PRP, whereas the control group received only autologous bone. Those who received PRP demonstrated much better epithelialization and compact mature bone with well-organized trabeculae. The author suggested that the application of PRP inside the wound improved soft-tissue repair and bone regeneration and that the augmented sites could be future candidates for dental implant placement.¹⁹ In 2000, Kassolis and others³⁰ used PRP with freeze-dried bone allograft for sinus elevation or ridge augmentation (or both) for 36 implant placements. On histological evaluation of the biopsy specimens 12 months later, numerous areas of osteoids and bone formation were observed around the freeze-dried bone allograft particles, with no evidence of inflammatory cell infiltration. The authors suggested a combination of PRP and freeze-dried bone graft as an alternative therapeutic method for implant placements.³⁰ de Obarrio and others⁸⁹ incorporated PRP into a combination technique involving bone allograft and guided tissue regeneration as periodontal therapy for intrabony defects in humans. They observed

significant gain in clinical attachment and filling of the treated defects, as revealed by 2-year follow-up.

Several methods have been demonstrated for covering gingival recession defects.⁹⁰⁻⁹³ For example, Petrungaro¹⁵ recently published a case series in which PRP, subepithelial connective tissue grafts and collagen membranes were used to cover gingival recessions. PRP was applied within the surgical area between the graft-membrane and root surface, and the site was covered with PPP as a protective layer. Although the therapy was successful in all cases, controlled trials will be needed to determine the true significance of PRP in the treatment of gingival recession defects.

To the authors' knowledge, only 2 controlled clinical trials examining the impact of PRP on periodontal regeneration have been published. In 2002, Lekovic and others¹⁶ compared a combination of bovine porous bone mineral (BPBM), RPR and guided tissue regeneration with the combination of PRP and BPBM for the treatment of intrabony defects in humans. Patients underwent a 6-month follow-up to review defect filling. Both combinations, with or without guided tissue regeneration, were effective in patients with advanced periodontal disease.¹⁶ The same group also determined that the combination of PRP and BPBM provided additional regenerative effect in guided tissue regeneration. This regenerative potential of PRP was related to strong clinical results such as reduction of pocket depth and gain in attachment in combination with bone grafts.⁸

As outlined here, PRP offers many advantages: it decreases the frequency of intraoperative and postoperative bleeding at the donor and the recipient sites, facilitates more rapid soft-tissue healing, aids in the initial stability of the grafted tissue at the recipient sites (as a result of its cohesive and adhesive nature), may promote rapid vascularization of the healing tissue by delivering growth factors and, in combination with bone replacement materials, induces regeneration.

Preoperative PRP Preparation

PRP is prepared in a laboratory or a surgical or dental suite from blood collected in the immediate preoperative period.^{13,18,29} The use of platelet concentrates obtained from blood banks by the discontinuous plasmapheresis method is limited because of high cardiovascular stress to the recipient, known health risks and high production costs.^{94,95} However, some techniques for the preparation of small amounts of autologous PRP for dental use can be completed in minutes and involve less stress, especially for elderly patients.

Two commercial systems are available for creating PPP (fibrin glue): the SmartPreP autologous platelet concentrate system (Harvest Autologous Hemobiologics, Norwell, Massachusetts) and the Tisseel system (Baxter Heath Corp., Deerfield, Illinois). It has been speculated that the risk of disease transmission is not entirely

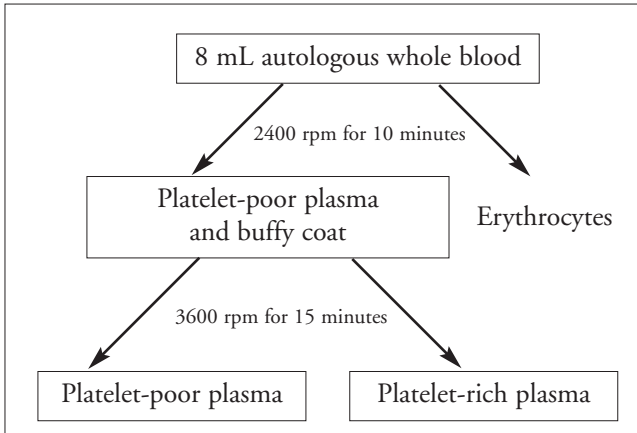


Figure 1: Steps in the preparation of platelet-rich plasma.

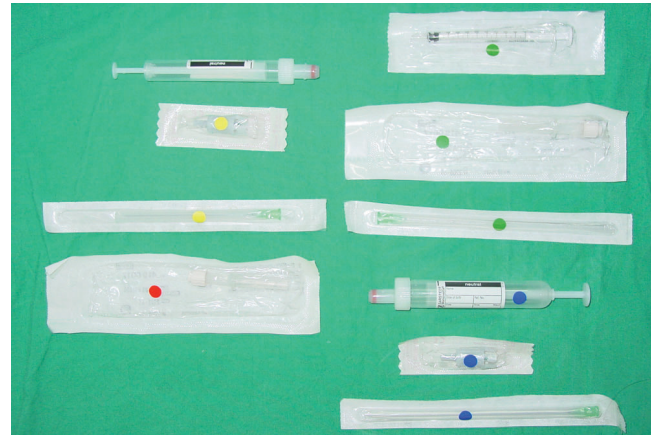


Figure 2: Colour-coded platelet-rich plasma kit (Curasan, Pharma GmbH AG, Lindigstrab, Germany).



Figure 3: The patient's blood is drawn in the dental suite.



Figure 4: The tube is centrifuged at 2400 rpm for 10 minutes. A second centrifugation is performed at 3600 rpm for 15 minutes.

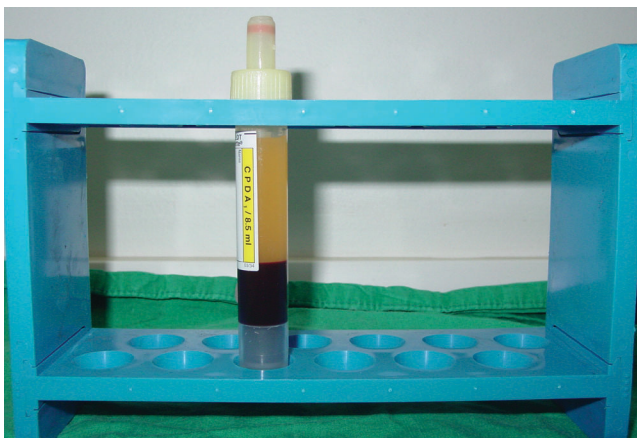


Figure 5a: After the initial centrifugation, the erythrocytes collect at the bottom of the tube.

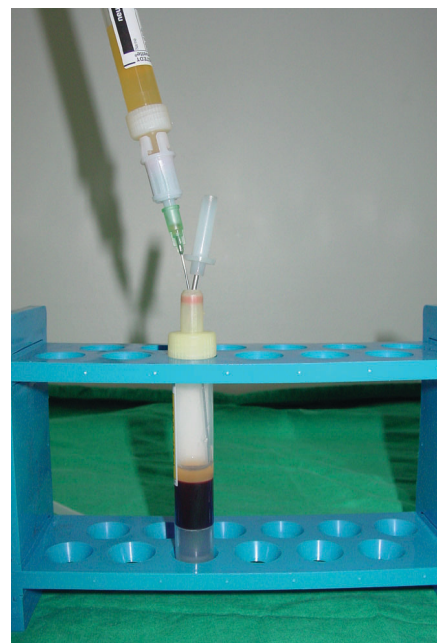


Figure 5b: The supernatant is removed by means of a long cannula.

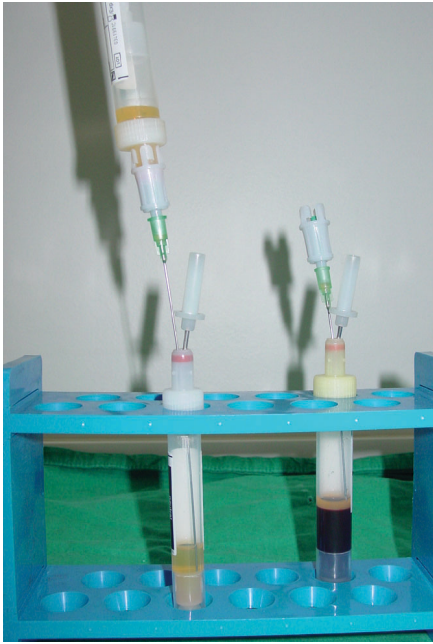


Figure 6: After the second centrifugation, the platelet-rich plasma is removed by a long cannula.

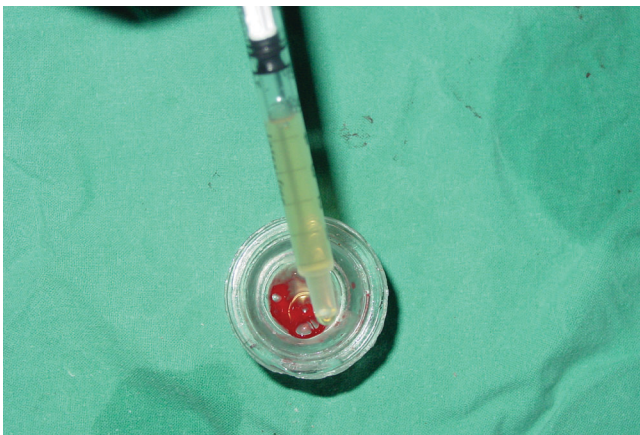


Figure 8: Platelet-rich plasma is mixed with calcium chloride and thrombin to start the clotting cascade, and this mixture is then transferred into the defect.

eliminated with the allogeneic Tisseel system, whereas the SmartPreP system is an autologous system and hence there is no risk of disease transmission.¹⁸ In addition, the SmartPreP system produces PRP gel as well as fibrin glue; the PRP could be the more important product because of its platelet-associated growth factors. Furthermore, the SmartPreP system has larger blood containers for centrifugation. This is important because it is advisable to obtain 90 to 180 mL of whole blood, as this amount of blood will yield sufficient PRP for maxillofacial or plastic and reconstructive surgical procedures.¹⁸

Two additional systems are now available commercially for office use by dental practitioners:^{33,35,96} the



Figure 7: Platelet-rich plasma (in the syringe) and platelet-poor plasma (in the centrifugation tube). The latter can be used to cover the surgical area.

Platelet Concentrate Collection System [PCCS] (3i Implant Innovations, Palm Beach Gardens, Florida) and the Curasan PRP kit (Curasan, Pharma GmbH AG, Lindigstrab, Germany). Published reports^{33,35,96} indicate that these systems have greater ease of handling and shorter preparation times than the SmartPreP and Tisseel systems. The PCCS and Curasan systems use different protocols, but the end product is suitable for the same oral surgical applications.

In a comparison of the PCCS and Curasan systems, whole blood was drawn from healthy donors, and PRP was prepared with each system. Higher platelet counts were achieved with the PCCS system.⁹⁶ The concentrations of TGF- β_1 and IGF-I were significantly higher and that of PDGF-AB was lower with the PCCS than with the Curasan system. The authors concluded that the PCCS end product had a higher platelet count and a higher total content of growth factors.⁹⁶ The same comparison was performed by Appel and others.³⁵ Whole blood was drawn from healthy individuals and processed with the PCCS and Curasan systems, as well as a procedure used in transfusion medicine. The absolute gain in platelets was higher with the PCCS system, but the highest concentration of platelets per microlitre was obtained with the Curasan system. The procedure used in transfusion medicine may offer an alternative if a commercial preoperative system is not available. The authors recommended that future studies should assess the ideal concentration of the various growth factors, characterize other physiochemical factors that may be present in the platelet concentrate and explain the beneficial effects of PRP treatment in bone regeneration.³⁵

Use of the colour-coded Curasan kit is described here to demonstrate the ease of preparing a small amount of PRP for use in reconstruction of periodontal and osseous defects, augmentation of extraction sockets, and connective tissue grafting procedures (Fig. 1 and Fig. 2).³³

Recent publications have indicated that PRP prepared from 8 to 10 mL of whole blood is sufficient for periodontal regenerative therapies.^{8,16,25} However, in oral and maxillofacial reconstruction, 8 to 500 mL of whole blood should be drawn, so as to obtain the greater amounts of PRP needed for larger surgical defects.^{13,18,29}

The blood sample is drawn into a citrated tube (Fig. 3). If more than 8 mL is needed (e.g., for larger defects), more than one tube of blood should be drawn. The sample tube is then spun in a standard centrifuge for 10 minutes at 2400 rpm (Fig. 4) to produce PPP. The PPP is taken up into a syringe with a long cannula and an additional air-intake cannula (Figs. 5a and 5b). A second centrifugation (15 minutes at 3600 rpm) is performed to concentrate the platelets. The second supernatant is also taken up by a long cannula and an air-intake cannula (Fig. 6). For each 8 mL of blood, the volume of supernatant is about 0.6–0.7 mL (Fig. 7); this is the PRP, to be used for the surgical procedure (Fig. 8). At the time of the application, the PRP is combined with an equal volume of a sterile saline solution containing 10% calcium chloride (a citrate inhibitor that allows the plasma to coagulate) and 100 U/mL of sterile bovine thrombin (an activator that allows polymerization of the fibrin into an insoluble gel, which causes the platelets to degranulate and release the indicated mediators and cytokines); the result should be a sticky gel that will be relatively easy to apply to the surgical defects.^{8,16,18} The PPP can be stored for use as a protective barrier over the wound (Fig. 7).

Conclusions

PRP is a new application of tissue engineering and a developing area for clinicians and researchers. It is a storage vehicle for growth factors, especially PDGF and TGF- β , both of which influence bone regeneration. Although the growth factors and the mechanisms involved are still poorly understood, the ease of applying PRP in the dental clinic and its beneficial outcomes, including reduction of bleeding and rapid healing, hold promise for further procedures. Most important, this autologous product eliminates concerns about immunogenic reactions and disease transmission. Animal studies and recently published human trials have demonstrated successful results. More well-designed and properly controlled studies are needed to provide solid evidence of PRP's capacity for and impact on wound healing, soft-tissue reconstruction and (in combination with bone grafts) augmentation procedures, especially in oral and periodontal therapy. ♦

Acknowledgement: The authors would like to acknowledge TÜB İTAK (Scientific and Technical Research Council of Turkey).

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The authors have no declared financial interests in any company manufacturing the types of products mentioned in this article.

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