Is Platelet-rich Plasma the Perfect Enhancement Factor? A Current Review

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Guided bone regeneration is an accepted surgical method employed in implant dentistry to increase the quantity and quality of the host bone in areas of localized alveolar defects. The lack of predictability in osseous regenerative procedures with various grafting materials suggests that improvement in the osteoinductive properties of these materials is highly desirable. Platelet-rich plasma (PRP), a modification of fibrin glue made from autologous blood, is being used to deliver growth factors in high concentration to sites requiring osseous grafting. Growth factors released from the platelets include platelet-derived growth factor, transforming growth factor β, platelet-derived epidermal growth factor, platelet-derived angiogenesis factor, insulin-like growth factor 1, and platelet factor 4. These factors signal the local mesenchymal and epithelial cells to migrate, divide, and increase collagen and matrix synthesis. PRP has been suggested for use to increase the rate of bone deposition and quality of bone regeneration when augmenting sites prior to or in conjunction with dental implant placement. Only 6 human studies using PRP have been found in the dental implant literature and 5 were case series or reports. Thus, there is clearly a lack of scientific evidence to support the use of PRP in combination with bone grafts during augmentation procedures. This novel and potentially promising technique requires well-designed, controlled studies to provide evidence of efficacy. (INT J ORAL MAXILLOFAC IMPLANTS 2003;18:93–103)

Key words: *guided bone regeneration, growth factors, plasmapheresis, platelet gel, platelet-rich plasma*

Guided bone regeneration (GBR) is an accepted surgical procedure intended to increase the quantity and quality of host bone in localized defects of the alveolar ridge.[1] Methods described to increase the rate of bone formation and to augment the bone quantity include the utilization of autografts, allografts, xenografts, and alloplastic bone substitutes. Autogenous bone is the ideal material for increasing bone volume, but procedures to harvest the bone increase the time and cost of the surgery and require a second surgical site, thus possibly increasing postoperative discomfort. Another drawback to obtaining autogenous bone is the limited amount that can be obtained from intraoral sites such as the maxillary tuberosity, extraction sites, edentulous alveolar areas, mandibular symphysis, ramus, and retromolar areas.

The allografts most commonly used are demineralized freeze-dried allograft (DFDBA) and freeze-dried bone allograft (FDBA), and controversy exists with respect to the osteoinductive potential of these materials. It has been shown that the inductive capacity varies for DFDBA processed from different bone banks, and even different batches from the same bank respond differently. Also, the bioactivity of DFDBA seems to be dependent on the age of the donor, since the younger the donor, the more osteoinductive properties in the graft material.[2] Controversial results and patient concerns about disease transmission have encouraged the development of xenografts and alloplastic alternatives. Both

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have shown good biocompatibility and osteoconductive potential, but the clinical outcomes with these bone substitutes are unpredictable. The lack of predictability in osseous regenerative procedures using bone grafts suggests that improvement in the osteoinductive/osteoconductive properties of these materials is highly desirable.

In 1990 Gibble and Ness introduced fibrin glue, alternatively referred to as fibrin sealant or fibrin gel, a biomaterial that was developed in response to the necessity for improved hemostatic agents with adhesive properties.[3] Platelet-rich plasma gel (PRP gel) is an autologous modification of fibrin glue that has been described and used in various applications with apparent clinical success.[4] PRP obtained from autologous blood is used to deliver growth factors in high concentrations to the site of the bone defect or a region requiring augmentation.[5]

The purpose of this review of PRP was to describe the constituents participating in the healing process, discuss the different techniques and available technology for procurement and preparation, discuss risks and possible applications in implant dentistry, review human studies published to date, and provide guidance for future research.

METHODS

The literature search was conducted using the MEDLINE database, from 1960 to June 2002 in the English language. The articles were successively searched by keyword or title using the following words: PRP, platelet-rich plasma, autologous gel, and platelet gel. Articles concerned with the subject of review were included if directly related to the use of PRP in combination with bone grafts in sites intended for future dental implant placement.

PLATELET-RICH PLASMA AND ITS CONSTITUENTS

PRP gel is formed by mixing PRP, derived from centrifugation of autologous whole blood, with thrombin and calcium chloride. PRP gel includes a high concentration of platelets and a native concentration of fibrinogen. The platelet concentrate is activated by the addition of thrombin and calcium chloride, and this results in the release of a cascade of growth factors from the platelet alpha (α) granules.[6] The growth factors are a diverse group of polypeptides that have important roles in the regulation of growth and development of a variety of tissues.[7]

Factors released from the platelets are summarized in Table 1 and include platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), platelet-derived epidermal growth factor (PDEGF), platelet-derived angiogenesis factor (PDAF), insulin-like growth factor 1 (IGF-1), and platelet factor 4 (PF-4).[8]

Platelet-derived Growth Factor

PDGF is a family of polypeptide growth factors consisting of 2-chain polypeptides linked by disulfide bonds with a molecular mass ranging from 27,000 to 30,000 daltons. Exposure to PDGF is known to stimulate DNA and protein synthesis in bone, as well as bone resorption. PDGF is stored in the alpha granules of platelets and released during the clotting cascade and is also found in monocytes, macrophages, smooth muscle cells, and endothelial cells. PDGF acts as a potent mitogen in serum for mesenchymal cells, including fibroblasts, and smooth muscle cells. The effect of PDGF is dependent upon the presence of other growth factors, and it also serves as a powerful chemoattractant for smooth muscle cells, fibroblasts, macrophages, and leukocytes. In addition to its angiogenic properties, it stimulates collagen and matrix formation in vivo.[9–11]

Transforming Growth Factor β

TGF-β is a 2-chain polypeptide that is linked together by disulfide bonds with a molecular mass of 25,000 daltons. It exists as 3 different gene products: TGF-β1, TGF-β2, and TGF-β3. TGF-β1 is found in high concentrations in bone and platelets.[9] TGF-β is a fundamental regulatory molecule that acts by both autocrine and paracrine mechanisms. It is now appreciated that TGF-β action is not limited to the local environment of the cells that produce it and that endocrine circulation of TGF-β contributes prominently to the pathogenesis of chronic fibrotic and autoimmune diseases and serves as a prognostic marker in several diseases, including carcinogenesis and atherosclerosis.[12]

When released by platelets or secreted by macrophages, TGF-β exerts its effects on adjacent cells, including fibroblasts, marrow stem cells, endothelial cells, and preosteoblasts. TGF-β stimulates angiogenesis and the production of fibronectin, glycosaminoglycans, and collagen in connective tissue.[10] One of the most important functions of TGF-β seems to be the chemotaxis and mitogenesis of osteoblast precursors. In addition, this polypeptide inhibits osteoclast formation and resorption, favoring bone formation.[13] This local connective tissue response to TGF-β in vivo is strongly anabolic and leads to fibrosis and angiogenesis.[14]

Table 1 Summary of Growth Factors Released from Platelets

Growth factor	Molecular properties	Source cells	Target	Action
PDGF	Cationic polypeptide (Mr = 30 kda)	Platelets, macrophages, monocytes, endothelial cells, smooth muscle cells	Fibroblasts, smooth muscle cells, glial cells, macrophages/ neutrophils	Stimulates chemotaxis/mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion/collagen synthesis; stimulates macrophage/neutrophil chemotaxis
TGF-β	2-chain polypeptide (Mr = 25 kda); 3 different gene products in humans: TGF-β1, TGF-β2, TGF-β3	Platelets, T-lymphocytes, macrophages/monocytes, neutrophils	Fibroblasts, marrow stem cells, endothelial cells, epithelial cells, preosteoblasts	Stimulates/inhibits endothelial, fibroblastic, and osteoblastic mitogenesis; regulates collagen synthesis/collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis
PDEGF	53-amino acid polypeptide (Mr = 6 kda)	Platelets, macrophages, monocytes	Fibroblasts, endothelial cells, epithelial cells	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis
PDAF	Acidic polypeptide (Mr = 45 kda)	Platelets, endothelial cells	Endothelial cells	Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells by direct or indirect actions; several cytokines and growth factors up-regulate PDAF, including IGF-1, TGF alpha and beta, PDGF, bFGF, PDEGF, and IL-1 beta
IGF-1	Single-chain polypeptide (Mr = 47 kda) 47% homology with insulin	Osteoblasts, macrophages, monocytes, chondrocytes	Fibroblasts, osteoblasts, chondrocytes	Stimulates cartilage growth, bone matrix formation, and replication of preosteoblasts and osteoblasts; acts as an autocrine and paracrine factor; in combination with PDGF can enhance the rate and quality of wound healing
PF-4	Homotetramer (Mr = 29 kda)	Platelets	Fibroblasts, neutrophils	Chemoattractant for neutrophils and fibroblasts; potent antiheparin agent

PDGF = platelet-derived growth factor; TGF-β = transforming growth factor beta; PDEGF = platelet-derived epidermal growth factor; PDAF = platelet-derived angiogenesis factor; IGF-1 = insulin-like growth factor 1; PF-4 = platelet factor 4; bFGF = basic fibroblast growth factor.

A fundamental mechanism of the antiproliferative (catabolic) action of TGF-β is its ability to antagonize the mitogenic effects of other peptide growth factors such as PDEGF and PDGF.[14] Even in a single cell type, the nature of growth factor action may depend on the context set by other substances present. For example, TGF-β stimulates growth of certain fibroblasts in vitro in the presence of PDGF but inhibits their growth if epidermal growth factor is present.[15] In vivo, TGF-β enhances the proliferation and migration of macrophages, fibroblasts, and endothelial cells while inhibiting the proliferation of vascular endothelial cells in vitro.[16]

Platelet-derived Epidermal Growth Factor

PDEGF was discovered by Cohen in 1962[17] and was the first growth factor described. It stimulates epidermal regeneration, promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts, and enhances the effects and production of other growth factors.

Platelet-derived Angiogenesis Factor

PDAF has the capacity to induce vascularization in vivo. It stimulates vascular endothelial cells by direct or indirect actions, and it is involved in the process by which new blood vessels invade devascularized tissue.[18] Several cytokines and growth factors up-regulate PDAF, including IGF-1, TGF-α and β, PDGF, basic fibroblast growth factor (bFGF), PDEGF, and interleukin 1β (IL-1β). This factor is highly expressed by the induction of hypoxia.

Insulin-like Growth Factor-1

IGF-1 is a single-chain polypeptide hormone weighing 7,500 daltons. IGF-1 has 47% homology with insulin and it stimulates cartilage growth, bone matrix formation, and replication of preosteoblasts and osteoblasts. IGF-1 may directly stimulate the cells it activates (autocrine factor) and increase the alkaline phosphatase activity in osteoblastic cells. IGF-1 transcripts have been isolated from macrophages in wounds, suggesting that this growth factor may also act as a local messenger (paracrine factor).[9] IGF-1 in combination with PDGF can enhance the rate and quality of wound healing.

Platelet Factor 4

PF-4 is a chemoattractant for neutrophils also released from alpha granules, which may be partially responsible for the initial influx of neutrophils into wounds.[19] It also acts as a chemoattractant for fibroblasts and is a potent antiheparin agent.

ROLE OF PLATELET-RICH PLASMA IN THE PROCESS OF WOUND HEALING

The process of wound healing can be divided into 3 different stages: biochemical activation, cellular activation, and cellular response.[19]

Biochemical activation involves the translation of mechanical injury into biochemical signals that can be understood by the body. The trigger that starts the cascades is the Hagemann factor found in serum. When injury causes disruption of the microcirculation, plasma comes in contact with tissue proteins and the basement membrane. This activates the Hagemann factor and circulating platelets. The activated Hagemann factor in turn begins 4 cascades that amplify the initial response and result in cellular activation. The activation of the clotting cascade produces fibrin to help in hemostasis and thrombin that causes the maximal release of platelet alpha (α) granules. The complement cascade produces many biologically active molecules including C5a, a potent chemoattractant for neutrophils and monocytes that is also important in wound repair. The kinin cascade results in the production of bradykinin that causes microvascular dilatation at the wound periphery, and the activation of plasminogen produces plasmin, which degrades the fibrin. The fibrin degradation products that result from the enzymatic breakdown of fibrin are themselves biologically active molecules that can cause monocyte migration and vasodilation.

The cellular activation stage results in the influx of cells into the wound. The first cellular response involves neutrophils, monocytes, and platelets. Platelets accumulate at the wound site in response to the initial injury; in response to thrombin, platelets release their α granules that contain locally acting growth factors. These factors signal the local mesenchymal and epidermal cells to migrate, divide, and increase their collagen and glycosaminoglycan synthesis. This initial release is thought to accentuate the reparative response.

The monocytes transformed into macrophages are involved in the final cellular response. These cells assist the neutrophils in host defense and produce many of the growth factors, which direct repair until the wound is healed.

THE BIOLOGIC RESPONSE TO PRP GEL

The plasmapheresis technique concentrates the platelet count in the PRP by 338% in comparison to the total blood platelet count. PRP is an autologous preparation; thus any concerns of disease transmission or immunogenic reactions that exist with allograft or xenograft preparations are eliminated.[5]

The properties of PRP are based on the production and release of multiple growth and differentiation factors upon platelet activation. These factors are critical in the regulation and stimulation of the wound healing process, and they play an important role regulating cellular processes such as mitogenesis, chemotaxis, differentiation, and metabolism.[20]

For this reason, the growth factors contained and released from PRP should enhance and accelerate soft tissue healing and the process of regenerating bone. Studies on application of single or combination growth factors using PDGF or PDGF/IGF-1 have been shown to enhance the early cascade of tissue repair processes both in vitro[21,22] and in vivo.[23–25]

Growth factors obtained from platelets offer an advantage over those used in the above-mentioned studies, which used either native or recombinant factors, because a large number of growth factors are easily available in significant amounts upon PRP activation. The actions of these growth factors are very complex, because each growth factor may have a different effect on the same tissues, as well as different responses that are dependent on specific tissues. Growth factors may also interact one with another, consequently forming a cascade of different signal proteins with multiple pathways, ultimately leading to the activation of gene expression and then protein production. This specific feature of PRP also differentiates its actions from those of recombinant growth factors that focus on a single regeneration pathway.[13]

Recent reports have suggested that more rapid epithelialization, more dense and mature bone with better organized trabeculae, and greater bone regeneration take place when PRP is added to bone autografts and allografts.[5,26–28] Most of these reports also suggest that PRP improves the handling properties of the graft material with which it is combined, facilitating graft placement and stability. PRP delivers a highly concentrated dose of autologous platelets containing a variety of biologic mediators that can be applied directly to the healing site.

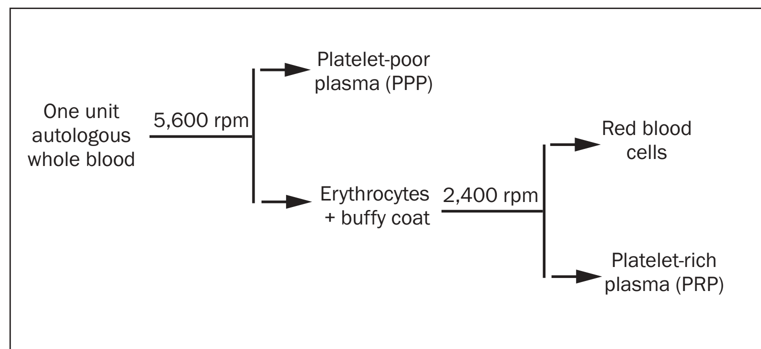

Fig 1 Preparation of PRP with general-purpose centrifuges.

PLATELET-RICH PLASMA PROCUREMENT TECHNIQUES

Use of General-Purpose Cell Separators

The clinical procedure for obtaining autologous PRP requires the use of an ultracentrifuge and gradient density cell separator, which can sequester and concentrate platelets.[27] Approximately 450 mL of whole blood is drawn from the patient directly into a standard labeled blood collection bag containing citrate-phosphate-dextrose anticoagulant.[6] The use of EDTA as an anticoagulant is not recommended during the PRP procurement because it fragments the platelets,[29] although it gives a greater yield of platelets.[30] First, the blood is centrifuged in a general-purpose cell separator, such as the ELMD-500 (Medtronic Electromedic, Autotransfusion System, Parker, CO) at 5,600 rpm to separate the platelet-poor plasma (PPP) from the red blood cells (RBC) and the PRP (also termed "buffy coat," which contains the platelets and the leukocytes). Then the centrifuge speed is slowed to 2,400 rpm to obtain further separation of approximately 30 mL of PRP from the RBC preparation into a transfer bag (Fig 1).

The procurement of PRP with this technique can be accomplished in 30 minutes, and use of the obtained PRP is recommended within 6 hours after being drawn from the patient. Platelet counts of 500,000 to 1,000,000 in the PRP are usually obtained with this plasmapheresis technique. With this processing technique, the remaining erythrocytes and PPP can be returned to the circulation or discarded.

Use of Platelet-concentrating Cell Separators

Recent advanced technologies permit the procurement of PRP using smaller volumes of blood, increasing the platelet concentrations and avoiding the need for RBC and PPP reinfusion.[31] Two platelet-concentrating cell separators are presently commercially available and FDA-approved for the production of PRP: the Harvest SmartPrep Platelet Concentrate System (HSPCS) (Harvest Technologies, Plymouth, MA) and the 3i Platelet Concentrate Collection System (3i PCCS) (3i/Implant Innovations, Palm Beach Gardens, FL). HSPCS and 3i PCCS use tailored centrifuge containers to manipulate the blood cells to achieve the separation and sequestration of platelets. Similar quantities of fresh whole blood are required in both units. Both systems consist of a desktop centrifuge and individual disposable kits that are designated to come into contact with blood. Each system uses centrifugal forces to separate blood cells through centrifuge cycles with short- and long-duration spins. After the first spin (short), HSPCS automatically decants the plasma into the plasma chamber, where during the second spin (long), the PPP is separated from a platelet concentrate. After the first spin in the 3i PCCS system, the operator manipulates a valve and inserts air into an air bladder, forcing the plasma into the second chamber. After the second spin, the operator forces air into a bladder that separates the PPP from the PRP. Some performance and operational data provided from manufacturers are detailed in Table 2. Recently, 2 methods of PRP procurement, the 3i PCCS system and the Curasan-type PRP kit (Curasan, Kleinostheim, Germany), were studied to compare the growth factors levels obtained with the different techniques. The authors concluded that the PRP obtained with the 3i PCCS system had both a higher platelet count and a higher content of the growth factors investigated.[32]

In summary, both platelet-concentrating cell separators are similar in performance and simplicity, and they represent a significant advance over the general-purpose cell separators. The noticeable difference

Table 2 Comparison Data of 2 Commercially Available Platelet-Concentrating Cell Separators

Performance data*	HSPCS	3i PCCS
Average % recovery of platelets	68%	65%
Average platelet count/μL in 4 mL	2,278,000	1,818,000
Average platelet count/μL in 5 mL	1,823,000	1,454,000
Average platelet count/μL in 6 mL	1,519,000	1,212,000
Operational time	15 min	20 min

*All data taken from manufacturer brochures.
HSPCS = Harvest SmartPrep Platelet Concentrate System, Harvest Technologies, Plymouth, MA; 3i PCCS = 3i Platelet Concentrate Collection System, Implant Innovations, Palm Beach Gardens, FL.

between the 2 systems is that the HSPCS requires less time to produce the PRP (15 minutes versus 20 minutes) and less operator intervention and training.

POTENTIAL RISKS WITH THE USE OF PRP

The preparation of PRP involves isolation of the PRP, after which gel formation is accelerated using calcium chloride and topical bovine thrombin (TBT).[6] The use of TBT has been reported to be associated with the development of antibodies to factors V and XI and thrombin, resulting in the risk of life-threatening coagulopathies. Landesberg and coworkers[33] reviewed reports appearing in the literature of serious coagulopathies that were difficult to treat following exposure to TBT.[34–36] TBT preparations contain factor V, which results in reaction of the immune system when challenged with a foreign protein. The factor V deficiency after thrombin exposure is thought to be caused by the cross-reactivity of anti-bovine factor V antibodies with human factor V.[34–37] To date, TBT-induced coagulopathies have been reported in 32 cases of patients undergoing cardiovascular operations, specifically in those patients with repeat exposure to TBT.[34] The severity of bleeding varies widely, from no clinical evidence to life-threatening diatheses developing 7 to 14 days after successive exposure to TBT.

However, the adverse reactions reported could depend upon an increased awareness of the coagulopathy, as well as the source and quantity of thrombin used. Differences in product purity have been documented. One brand of thrombin (Thrombin-JMI, Jones Medical Industries, St Louis, MO) applies an extra purification step to decrease the factor V concentration to less than 0.2 μg/mL.[38]

Based on the reported coagulopathies, Landesberg and coworkers[33] suggested that alternative methods of activating PRP need to be studied and made available to the dental community.

In a later study,[30] Landesberg and coworkers described a new method to activate PRP gel with the ITA gelling agent (Natrex Technologies, Greenville, NC). They stated that this method could be used more safely as an alternative to bovine thrombin for gelling the PRP; however, they did not describe the specific composition and mechanism of action of ITA. Marx has used bovine thrombin in his patients since 1996 without development of any coagulopathies; however, he indicated that the use of reagents such as recombinant human thrombin or autologous thrombin can avoid the risk or concern about the potential of bovine thrombin to cause coagulopathies.[29] In contrast, Kassolis and colleagues[27] utilized autologous thrombin to activate the PRP in their patients.

Based on these data, apparently other methods should be considered in the preparation of PRP gel. Safer methods to consider could include the utilization of recombinant human thrombin, autologous thrombin, or perhaps extra-purified thrombin.

PRP PREPARATION TECHNIQUE

The PRP application described by most authors requires initiating the coagulation process with a mixture of 1,000 US units of TBT powder suspended in 10 mL of sterile saline with 10% calcium chloride. The protocol for PRP activation requires the use of an individual 10-mL syringe for each mix. Each mix draws 6 mL of PRP, 1 mL of the 10% calcium chloride, 1 mL of thrombin, and 1 mL of air to act as a mixing bubble. The syringe is agitated for a few seconds to initiate clotting and is then ready to be applied to the bone grafts. Once added to the grafts, the fibrin, fibronectin, and other cell adhesion molecules establish a network that may serve as osteoconductors in bone growth.[39]

THERAPEUTIC POTENTIAL OF PRP GEL IN IMPLANT DENTISTRY

PRP has been recommended for use in increasing the rate of bone deposition and quality of bone regeneration when augmenting edentulous sites for future implant placement.[20,26,27,31] Most reports on PRP in the literature have proposed its utilization as a bone graft enhancement material rich in growth mediators.[5,6,13,30] In this context, PRP may be valuable for

Table 3 Summary of Human Studies Involving the Use of PRP in Combination with Bone Grafts

Authors	Study type	Patients	Groups	Control	Random-ization	Length	Defect type	Graft	Biopsy
Marx et al[5]	Prospective	88	44 PRP+graft/ 44 graft	Yes	Questionable	4 months	Mandibular discontinuity	Autologous	Yes
Anitua[26]	Case series	20	10 PRP, 10 control	Yes	Questionable	6 months	Postextraction	None/ Autologous*	Yes
		3	3 split-mouth	Yes	No	Same	Same	None	Yes
Kassolis et al[27]	Case series	15		No	No	12 months	Ridge and SA	FDBA	Yes
Rosenberg and Torosian[28]	Case report	1		No	No	9 months	SA	Alloplastic	No
Shanamen et al[45]	Case reports	3		No	No	No	RA	1 DFDBA, 1 alloplastic/ DFDBA/ autograft, 1 DFDBA	Yes
Froum et al[46]	Case reports	3	Split mouth	Yes	Questionable	7–9 months	SA	ABB	Yes

*In 5 patients in the PRP group.
PRP = platelet-rich plasma; SA = sinus augmentation; RA = ridge augmentation; DFDBA = demineralized freeze-dried bone allograft; FDBA = freeze-dried bone allograft; ABB = anorganic bovine bone.

use in conjunction with bone autografts, allografts, xenografts, or alloplasts in sinus lifting and in alveolar ridge augmentation procedures prior to or in conjunction with dental implant placement. PRP has also been recommended for use alone or in combination with bone grafts and barrier membranes in the treatment of peri-implant defects created as a consequence of immediate implant placement or as a result of peri-implantitis.

PRP is thought to accelerate soft tissue healing by promoting a more rapid revascularization and reepithelialization of flaps and cell proliferation. Therefore, there may be merit in applying it to the flap margins and to the underlying tissues. Garg and coworkers[40] proposed that resorbable barrier membrane materials be infused with PRP. They have proposed that this PRP-based membrane could serve as a short-acting biologic barrier, since all platelets contained in PRP will degranulate within 3 to 5 days, and their initial growth activity expires within 10 days.

Nine articles were found documenting the clinical use of PRP for humans, but 3 were excluded; 2 of these involved a case report and a clinical reentry study on the use of PRP in intraosseous periodontal defects,[41,42] and the other study was a case report on a new technique to expand the alveolar ridge using a piezoelectric scalpel.[43] In the latter case, the author did use PRP, but without any comment on the PRP performance, and focused primarily on the performance of the new device.[43] Of the 6 articles reviewed, only 1 had a prospective design, and of the 5 remaining, 2 were case series and 3 were case reports (Table 3). Only 3 included controls and randomization, but the experimental design was inadequate.

Marx and coworkers[5] performed the first and most compelling study available on the use of PRP in combination with bone grafts. They evaluated the effect of PRP on bone graft reconstructions of mandibular continuity defects 5 cm or greater arising from tumor extirpations. One group received cancellous cellular marrow grafts with the addition of PRP, and the other group received a cancellous cellular marrow graft alone, serving as the positive control. In this study, a complete description of the technique for procurement and application of PRP was given. The grafts were allowed to consolidate and mature for 6 months, and panoramic radiographs were obtained at 2, 4, and 6 months. These radiographs were used to evaluate the age (maturity) of the graft at each interval. From this subjective radiographic assessment, the authors calculated a graft maturity index but they did not provide an explanation of this index. From the results of this questionable index, the authors claimed that the bone grafts combined with PRP showed a maturity index more than twice and slightly less than twice the actual maturity at 2 and 4 months, respectively.

Marx and coworkers[5] also performed a monoclonal antibody study on the platelets sequestered by the centrifugation process and on the harvested bone graft material. The data indicated that PDGF and TGF-β from the platelets had been absorbed by the graft, and receptors for PDGF and TGF-β were present within the autogenous grafts. Similar

analyses of core bone specimens of each graft at 6 months indicated that TGF-β was still present, but PDGF was not identified. Marx and coworkers also compared the baseline mean platelet counts with PRP mean platelet counts and found an increase of 338% in the platelet count because of the sequestration process. The authors also performed a histomorphometry graft assessment at 6 months. A core bone biopsy was taken from each grafted site (bone graft and bone graft with PRP). These bone specimens from grafted sites were compared to 10 resection specimens retrieved from the midbody of the mandible (native bone). The trabecular bone area (TBA) was measured in the 3 groups; a TBA of 38.9 ± 6% was seen in the native bone group, 55.1 ± 8% in the bone graft group, and 74.0 ± 11% in the bone graft/PRP group. They concluded that the addition of PRP accelerated the rate and degree of bone formation and that bone autografts contained platelets and thus were positive for receptors for the growth factors. Furthermore, it was noted that the PRP was an autologous preparation obtained at the time of the surgery, thus eliminating concerns about disease transmission, immunogenic reactions, and mislabeling of the sample. It was reported that the subjects were randomized into 2 groups; however, no explanation of the randomization procedure was given and therefore it is questionable. Current standards of experimental design expect that the method used for randomization be reported.[44]

Anitua[26] reported the results of the use of PRP in a series of patients who underwent tooth extraction because of root fracture or periodontitis. Ten patients were treated with PRP. In 5 of these patients, autogenous bone was used in combination with PRP to prevent soft tissue collapse. The remaining 10 patients served as controls; the extraction sockets were left to heal without application of PRP. In the same study, another 3 patients, who needed multiple extractions on both sides of the mouth, were assigned in a split-mouth fashion to PRP and control treatment modalities alternatively. Biopsies of the sites in all 23 patients were taken between 10 and 16 weeks. Anitua reported total regeneration of bone in 8 of the 10 patients based on periodontal probing, and biopsies of these sites showed compact mature bone with well-organized trabeculae and normal morphology. In 2 patients in the PRP group, partially regenerated bone was found with connective tissue and non-organized trabeculae. The 10 patients in the control group showed connective tissue filling the main part of the defect, and no mature bone was found. In the 3 patients assigned to the split-mouth design, the sites treated with PRP demonstrated more mature bone, with better organized trabeculae and greater bone regeneration. In all patients treated with PRP, the epithelialization was described based on subjective observations as very good to excellent.

Kassolis and colleagues[27] presented case reports of 15 patients undergoing ridge and sinus floor augmentation treated with PRP and FDBA. Seventeen areas, including 14 sinuses and 3 maxillary ridges, were treated and 36 endosseous implants were placed. Twenty-nine implants were placed at the time of the GBR procedure. Thirty-two of the 36 implants (89%) were successful and 4 implants were determined to have failed at the time of uncovering. Histologic study of bone retrieved from 2 patients confirmed the presence of vital bone formation in apposition to residual FDBA particles. The authors suggested that the use of PRP may allow for earlier implant placement and loading, but in the absence of a control group and an adequate study design this conclusion is unacceptable.

Rosenberg and Torosian[28] presented a case report concerned with the use of PRP in combination with an alloplastic graft in a sinus floor augmentation procedure. A 70-year-old male patient underwent a sinus grafting procedure using PRP in addition to an alloplastic bone particulate graft. In this case, the patient was scheduled at 3 months for implant placement. One month after implant placement, a 4-unit provisional restoration was placed and 5 months later the definitive prosthesis was connected. Based on this case alone, the authors erroneously concluded that the duration of the treatment was reduced by one half with the use of PRP. It was not appropriate to draw conclusions regarding a novel treatment based on the clinical results of the treatment of a single patient because of the high variability among patients and lack of appropriate study design.

Recently Shanaman and coworkers[45] published 3 case reports. Patient 1 underwent GBR prior to implant placement using e-PTFE plus DFDBA/PRP. In patient 2, GBR was accomplished using e-PTFE plus alloplastic/DFDBA/autogenous bone in combination with PRP. Patient 3 underwent GBR using bioabsorbable membrane plus a PRP/DFDBA combination. The histologic observations demonstrated new bone formation, and the authors stated that the PRP did not appear to enhance the quality of newly formed bone. The authors concluded from the results that PRP used in conjunction with various derivatives/substitutes and a barrier membrane can support new bone formation in localized ridge augmentation. However, they indicated that the results in these 3 patients appeared comparable to other GBR studies without the use of PRP, but this

conclusion is also null because of the inappropriate experimental design.

More recently, Froum and associates[46] presented reports of 3 patients undergoing sinus floor augmentation treated with PRP + anorganic bovine bone (ABB). On the day of surgery, a flip of a coin, an unacceptable method for randomization, determined the experimental side (PRP+ABB) and the control side (ABB). In patient 3, miniature test implants, 2.0 mm in diameter and 10 mm in length, were placed through the crestal bone into the sinus grafts. Placement of the permanent implants was performed at 7 months, 7.5 months, and 11 months, respectively, for patients 1, 2, and 3. At the time of implant placement, trephine cores 3 mm in diameter and 10 mm in length were harvested from the former site of the lateral window. In addition, the 3 test implants were removed with a trephine and the cores were sent for histologic and histomorphometric evaluation. The histomorphometric analysis of vital bone in the grafted sinuses demonstrated similar values in the percentages of vital bone between the experimental and control groups. The 2 test implants placed in the experimental group sinuses showed slightly higher percentages of bone-implant contact (37.6% and 38.8%) than the test implant placed in the contralateral control sinus (33.8%). Therefore, it was concluded from these case reports that PRP did not make a significant difference in the production of vital bone or in the bone contact at the implant interface. These conclusions are also apparently invalid because of the lack of appropriate study design.

All the available literature reviewed on PRP consisted of case series and individual reports, which in some instances involved a quasi-experimental design. In some instances, the authors used subjective indices to measure the ability of PRP to increase the bone density[5] or reached conclusions based on subjective observations.[26–28,44] Because of the absence of adequate controls and because the subjects were not selected randomly, the experimental designs were flawed.

CONCLUSIONS

Several conclusions can be drawn from this review.

- The platelet sequestration process results in a high platelet concentrate that upon activation releases a cascade of growth factors contained in the alpha granules.
- Growth factors released from platelets seem to signal the local mesenchymal cells and epithelial cells to migrate, divide, and increase collagen and matrix formation.
- PRP is an autologous preparation; thus it eliminates concerns about disease transmission or immunogenic reaction.
- PRP gel seems to improve the handling characteristics of grafts.
- Some of the limited number of studies in the dental literature suggest some benefit when PRP is combined with autogenous bone. Specifically, PRP seems to improve the rate of bone formation and the quality of bone formed.
- To avoid possible development of coagulopathies associated with bovine thrombin, the use of an alternative method for PRP activation, other than TBT, is advisable.
- Recent advanced technologies permit the procurement of PRP using smaller volumes of blood, increasing the platelet concentrations and avoiding the need for RBC and PPP reinfusion.
- Longitudinal studies are needed to determine whether the addition of PRP to bone substitutes would allow earlier implant placement and loading and increase the predictability of regenerative procedures.

SUMMARY

This literature review demonstrates a lack of scientific evidence to support the current use of PRP in combination with bone grafts during augmentation procedures. Most of the evidence on the clinical potential of PRP comes from case series and case reports. While it has been recognized that these types of studies represent the starting point, they are not definitive. It has been suggested that this treatment-observation-description strategy is at the lowest level in the hierarchy of evidence. The need for evidence-based clinical decisions regarding treatment recommendations and alternatives is quite apparent. PRP may have strong clinical potential associated with the growth factors it contains, but there is a need for well-controlled randomized clinical studies to assess the ideal concentration of the different growth factors and to determine whether PRP application to different bone graft substitutes and different barrier membranes provides a beneficial effect. Well-designed controlled studies are necessary to determine the efficacy of the technique.

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