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# Plasma Rich in Growth Factors: Preliminary Results of Use in the Preparation of Future Sites for Implants

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This article presents preliminary clinical evidence of the beneficial effect of the use of plasma rich in growth factors of autologous origin. The plasma is obtained from the individual patient by plasma-pheresis. The macroscopic and microscopic results obtained with bone regeneration using this technique, which uses no membrane or barrier, can be observed. The incorporation of these concepts can introduce several advantages, including the enhancement and acceleration of bone regeneration and more rapid and predictable soft tissue healing.

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**Key words:** growth factors, plasma growth factors

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For the last 10 years, the dental community has been using osseointegrated implants as a routine treatment modality. Studies of the treatment of atrophic maxillae, grafting, and site preparation have been in continuous evolution. Guided tissue regeneration opened new horizons, and bone morphogenetic protein (BMP)<sup>1-3</sup> has also advanced the science. Meanwhile, graft management has been enhanced. The use of fibrin as an osseoconductive material and as a medium for compacting grafts, as well as a graft vehicle, has been reported.<sup>4-7</sup> In applying these principles, Tayapongsak et al<sup>8</sup> reported encouraging results with the use of autologous fibrin to compact grafts in patients with reconstructed maxillae. Several authors<sup>9-13</sup> underlined the importance of growth factors found in the autologous fibrin adhesive. They reported that, by means of the monoclonal antibody technique, receptors for transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), transforming growth factor- $\beta$ 2 (TGF- $\beta$ 2), and platelet-derived growth factor (PDGF) were found in medullar bone. An intense concentration of monoclonal antibodies was shown in the plasma

obtained, both in PDGF and TGF- $\beta$ , thus providing evidence of the presence of these growth factors in the original plasma used to obtain autologous fibrin.

These papers proposed a major breakthrough in their finding that the beneficial effect of fibrin is not restricted to its osseoconductive effect and the agglutination of grafts, as evidenced in several publications on orthopedics concerning the use of lyophilized fibrin.<sup>14-19</sup> In addition, autologous fibrin might have a catalyzing effect by liberating the previously described growth factors. Depending on the technique used to obtain this plasma, it is possible that additional growth factors can be obtained, thus enhancing its repair capacity. In the organizing stage of a clot, growth factors play a major role in healing and osseous regeneration phenomena.

## Nature and Actions of Growth Factors

**Platelet-Derived Growth Factor.** So named because it was first found in platelets, where it is stored in the alpha granules,<sup>20</sup> PDGF can also be found in other cells, such as macrophages,<sup>21</sup> endothelial cells,<sup>22</sup> monocytes, and fibroblasts,<sup>23</sup> as well as in bone matrix.<sup>24</sup> Platelet-derived growth factor is a polypeptide that remains stable under heat stress up to 100°C and has a cationic nature. Its isoelectric point is very basic (10.2)<sup>25</sup> and its molecular weight is 30,000 daltons.<sup>23,26-28</sup> It has a dimeric structure formed by 2 amino acid chains named A and B. These chains have a similarity in their struc-

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ture of 60%; chain A is formed of 121 amino acids, while chain B is formed of 125 amino acids.<sup>25,29,30</sup> Two genes are responsible for the encoding of PDGF: chain A of the peptide is encoded in chromosome 7, and chain B is encoded in the long branch of chromosome 22.

Platelet-derived growth factor isoforms exert their effects on target cells by binding to 2 structurally related protein tyrosine kinase receptors. The alpha receptor binds both to the A and B chains of PDGF, whereas the beta receptor binds only to the B chain. Both alpha and beta receptors induce mitogenic responses; the beta receptor, but not the alpha receptor, mediates stimulation of chemotaxis. Specific cell-directed migration, or chemotaxis of osteoblast precursors, is one of the cellular events involved in bone formation. These factors are known to favor angiogenesis through activation of macrophages that secrete factors, inducing endothelial cells to form new capillary sprouts. There is also evidence that these growth factors increase the rate of proliferation of stem cells.

**Transforming Growth Factor- $\beta$ .** This factor received its designation because it was first isolated from transformed tissues (sarcomas).<sup>31</sup> There are 2 types: alpha and beta. Transforming growth factor- $\beta$  has a dimeric structure formed by 2 subunits of 112 amino acids. It has its origin in an extracellular proteolysis of a precursor containing 391 amino acids.<sup>32,33</sup> It has a total weight of 25,000 daltons, formed by two 12,500-dalton subunits linked together by disulphur bridges.<sup>34,35</sup> A gene located in the long branch of chromosome 19 is responsible for its synthesis.

This factor has 3 different structures<sup>36-38</sup>: TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3. The  $\beta$ 1 structure is found abundantly in platelets, lymphocytes, and neutrophils, while  $\beta$ 2 is found mainly in bone extracts, platelets, lymphocytes, and neutrophils. Types  $\beta$ 1 and  $\beta$ 2 are 72% similar. Type  $\beta$ 3 is a heterodimer formed of a single chain of TGF- $\beta$ 1 and a single chain of TGF- $\beta$ 2. These factors favor bone formation by enlarging the rate of stem cell proliferation. Another suggested role is the inhibition of osteoclast formation and thus bone resorption.

Can these growth factors be obtained for use in an ambulatory patient, and is their effect really beneficial in a way that can be measured or clinically evidenced? There is little literature that describes the technique for obtaining these autologous growth factors in an ambulatory patient. Therefore, at present several protocols are under investigation for obtaining plasma rich in growth factors (PRGF) from ambulatory patients to enable its use by periodontists, oral surgeons, or general

dentists. In this report, preliminary results of the use of autologous growth factor-rich plasma obtained from an ambulatory patient are presented.

## Materials and Methods

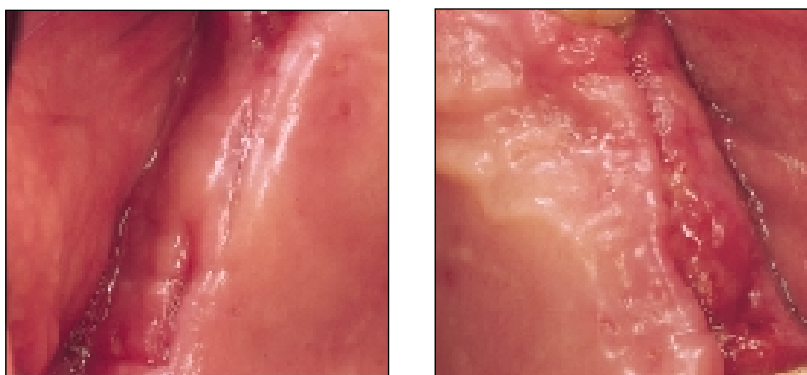
Patient selection was based on an absence of any local or systemic disease that might contraindicate the treatment. Informed consent was obtained from all patients entering the study. Twenty healthy patients, for whom an extraction was indicated because of a nontreatable tooth with vertical fractures or severe periodontal disease and who contemplated subsequent implant placement so that a biopsy of the area could be obtained without creating additional discomfort, entered the study. These patients were assigned randomly to either the PRGF group or the control group. The mean age of the PRGF group was 41 years (range 35 to 55 years); 4 patients were male and 6 were female. The mean age in the control group was 42 years (range 38 to 54 years); 4 patients were male and 6 were female.

In 3 additional patients (2 females and 1 male), multiple extractions were planned in different mouth areas. In each patient, PRGF was used in one area but not in any other, and these areas were assigned randomly. In this way it was possible to have the best control group because both treatments could be carried out in the same patient, with the same surgical procedure and identical microbiologic conditions and by the same surgeon.

**Preparation of Plasma Rich in Growth Factors.** Blood was obtained several minutes before starting surgery, prior to the administration of anesthesia. Ten to 20 mL of blood were drawn from each patient using 5 mL tubes, which contained 10% trisodium citrate solution as an anticoagulant. The tubes were centrifuged at 160 G for 6 minutes at room temperature. The blood was thus separated into its 3 basic components: red blood cells, which appeared at the bottom of the tube; PRGF in the middle of the tube; and plasma poor in growth factors (PPGF) at the top of the tube. One mL of the PPGF from each 5 mL tube was discarded. Platelets in PPGF were less than 15% of the total blood platelets, as assessed by counting ( $n = 10$ ). The remaining plasma was collected, including the upper 1 to 2 mm of the red blood phase and transferred to Eppendorf tubes, and 50  $\mu$ L of 10% calcium chloride were added to each tube containing 1.2 mL of PRGF. After 15 to 20 minutes a PRGF gel was formed. The time delay between the PRGF gel formation and the filling of the defect was standardized to 5 to 10 minutes.

**Fig 1** (Left) Fifty-four year old male treated with implants in the maxilla. One quadrant was used as a control. Four days postoperatively, epithelialization of the area treated with PRGF was almost complete. This epithelialization was considered excellent.

**Fig 2** (Right) The control area showed incomplete healing after 4 days and epithelialization was considered normal.



**Surgical Protocol.** Each patient received antibiotic treatment. Amoxicillin was used (1.5 g/day for 5 days). Flaps were elevated in every case so that enough visibility and first intention closing was achieved. After extraction, each site was carefully curetted. In the 10 patients who received the experimental treatment, the defects were filled with PRGF. In 5 of the 10 patients, the PRGF was mixed with autologous bone to prevent tissue collapse. In the control sites, the protocol was the same, but PRGF was not used to fill the defect. Membranes were not used in any patient; this prevented their barrier effect from interfering with the potential beneficial effects of PRGF.

**Biopsy Technique.** Wounds were biopsied between week 10 and week 16, depending on patient availability. All biopsies were made by a masked examiner who was unaware of which sites had been treated with PRGF. Biopsies were taken with trephine burs to a depth of 3 mm through what was considered to be the center of the wound. Bone biopsies were fixed in 10% buffered formalin, demineralized in 5% formic acid for 48 hours, decalcified in nitric acid, and embedded in paraffin wax. Sections 5  $\mu$ m thick were cut through each biopsy and were stained with hematoxylin and eosin. Stained sections were photographed under bright-field microscopy. All biopsies were sent to a laboratory for analysis without revealing which samples were from the control groups and which contained PRGF.

## Results

Epithelialization in all 10 patients treated with PRGF was evaluated as very good or excellent (much better than usual, and comparatively much better than the control area). The images revealed that regeneration of the treated areas was almost complete in 8 of the 10 patients. The degree of

regeneration was evaluated by means of a periodontal probe and by comparison with the previous defects, which had been photographed and radiographed. The biopsies of these areas showed compact mature bone, with well-organized trabeculae and normal morphology. The other 2 patients treated with PRGF were partially regenerated, presenting connective tissue with nonorganized trabeculae in the biopsies. Both patients, a male and a female, were smokers and both presented severe defects in 3 socket walls.

There were significant differences in the degree of trabecular organization between biopsies taken on week 10 and week 16, depending on the size and shape of the defect. In patients with severe defects that were treated with autologous grafts combined with PRGF to prevent flap collapse, a greater buccolingual/palatal width was obtained. In all 10 patients in the control group, a homogeneous situation was found at the reopening of the sites, with some variations, depending on the size and shape of the defects. All showed connective tissue filling the main part of the defect, in clear contrast with the patients treated with PRGF. All biopsies of these patients showed connective tissue and connective tissue containing osseous trabeculae. In any case, mature bone was found. The degree of epithelialization was considered normal, which was significantly different from epithelialization in the patients treated with PRGF (Figs 1 and 2).

In 3 patients, each of whom presented with 2 defects (1 treated with PRGF and the other without), epithelialization of the defects treated with PRGF was much more rapid. Biopsies of the defects treated with PRGF showed more mature bone with better organized trabeculae and greater bone regeneration.

To illustrate typical treatment and control situations, 2 patients are presented.

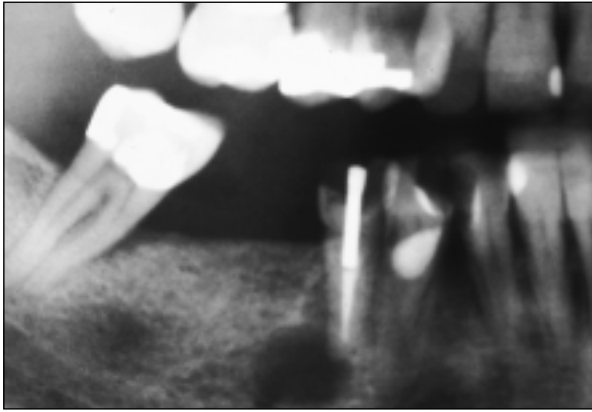


Fig 3a Radiographic image of Patient 1.

Patient 1

Forty-five year-old female with a vertical fracture in the mandibular right first premolar and a periapical granuloma, who was treated following the protocol previously explained (Fig 3).

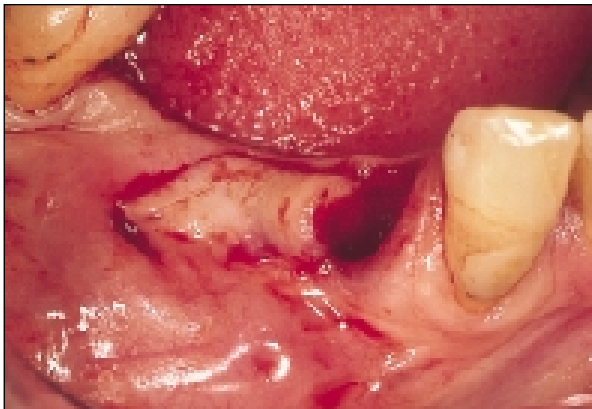


Fig 3b A flap was elevated so that the site can be sounded, the defect evaluated, and primary closure achieved after treatment.

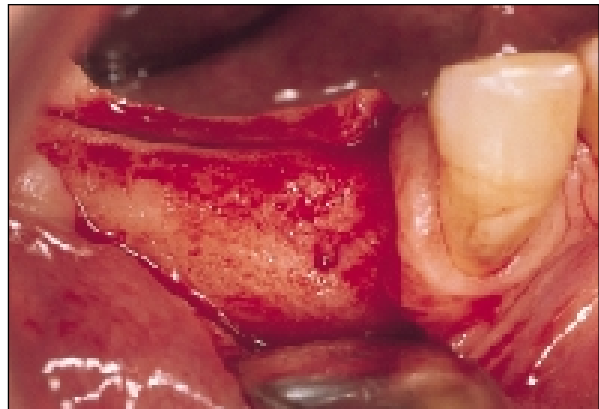


Fig 3c After 14 weeks, the site was reopened and an implant was placed. Dense bone can be observed almost completely filling the former defect.

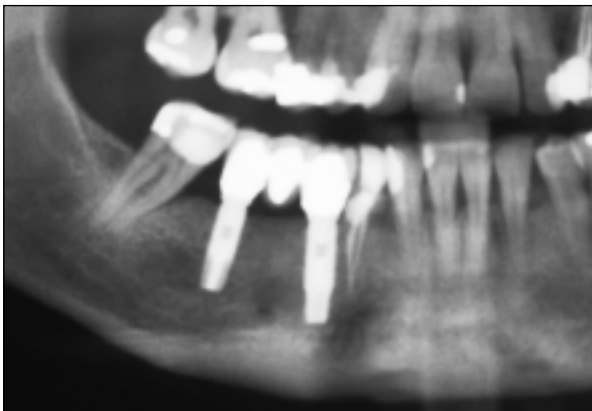


Fig 3d Radiographic image of Patient 1, 1 year postoperatively.

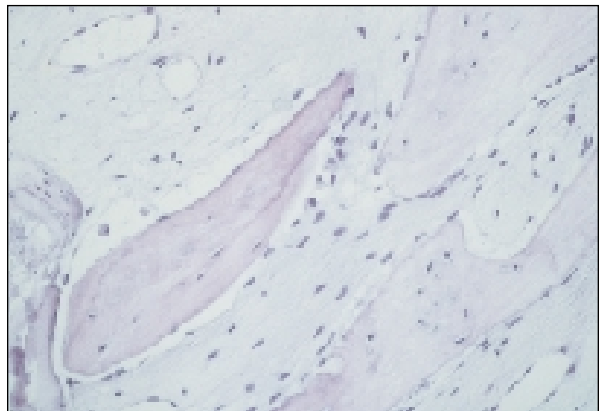
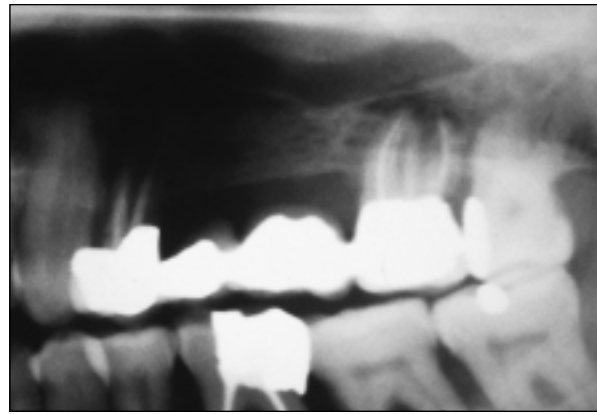


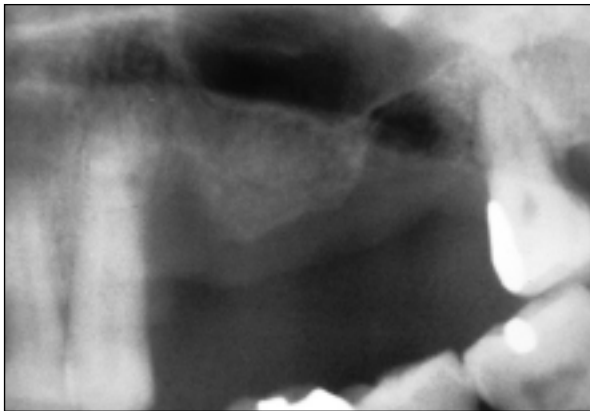
Fig 3e Image of the biopsied fragment from area seen in Fig 3c. Parallel digitiform trabeculae with typical cell morphology were observed. Osteocytes with normal morphology can be seen inside the trabeculae.

### Patient 2

Fifty-two year-old male with a vertical fracture in the maxillary left first premolar and severe periodontal disease in the second molar of the same quadrant. The molar area was treated with PRGF, and the premolar area was considered the control area (Fig 4).

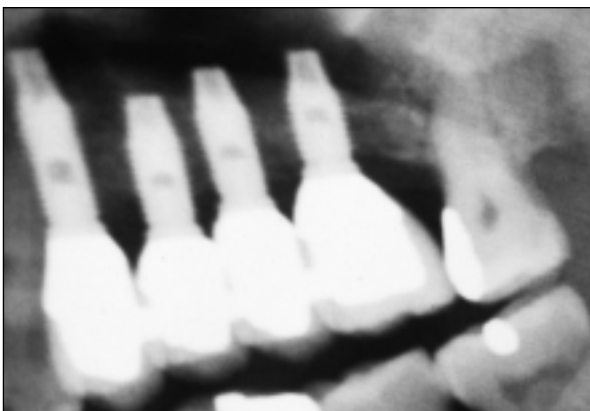


**Fig 4a** Radiographic image of maxillary left posterior quadrant in Patient 2.

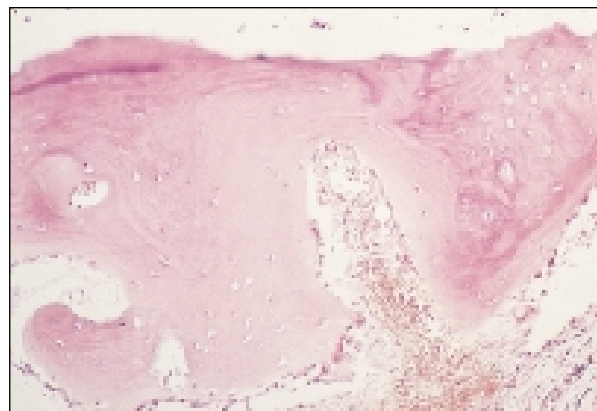


**Fig 4b** Postextraction radiographic image. Plasma rich in growth factors was placed in the molar area. In the premolar area, the site was not filled.

**Fig 4c** (Right) At the 10th week the area was reopened. Hard consistent bone that fills the molar area can be observed. A biopsy was taken and implants were placed. In the premolar area, a softer tissue was observed and biopsied.



**Fig 4d** Radiographic image of the implants placed, 15 weeks after completion of treatment.



**Fig 4e** Biopsy of the molar region in Patient 2. Mature trabeculae with continuous osteoblastic ribbon, osteocytes, and concentric calcification lines were observed. In the biopsy of the premolar area (control area), connective tissue was found.

## Discussion

Platelets were identified in previous studies as a rich source of PDGF and TGF- $\beta$ . In this study, a rapid and convenient method for obtaining autologous PRGF in ambulatory patients is presented. The use of PRGF provides conditions for obtaining more rapid and effective bone regeneration. This PRGF gel (which is a coagulated mass) is easy to manipulate, but it must be applied without delay to preserve growth factor activity. In addition to these growth factors, other proteins carried within platelets<sup>39</sup> may act in concert with other cytokines released from other cellular sources, modulating hemostasis. These results suggest that reinforcing growth factor concentration through the application of PRGF in the wound improves soft tissue repair and bone regeneration. The results are in agreement with several preclinical experimental studies in animals showing a high degree of consistency in the bone regeneration effect of PDGF.<sup>40,41</sup> Recently, recombinant PDGF-BB and IGF-I have been tested in patients with periodontal disease, and a significant improvement in bone growth and filling of periodontal defects has been found.<sup>42</sup> Optimal dosage is yet to be determined.

The use of this technique does not introduce any risk for the patient whose blood is used in a very short period of time after the extraction (30 minutes), and it is not mixed with any other component of animal or human origin. At the present time, some 250 patients have been treated with apparently good clinical results. Future studies are needed to assess the ideal concentration of the different growth factors and to characterize other physiochemical factors that may be present in PRGF that might further explain the beneficial effect of PRGF treatment in wound healing and bone formation.

## Conclusions

No negative effect has been found in the use of PRGF. The epithelialization, in 100% of the cases, has been complete and significantly better than in areas not treated with PRGF. Osseous regeneration of mature bone has been found in a larger quantity and quality than in control areas. The incorporation of this technique can bring patient benefit without risk of infection or disease transmission.

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