Bilateral Sinus Elevation Evaluating Plasma Rich in Growth Factors Technology: A Report of Five Cases

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ABSTRACT

Purpose: The purpose of this study was to evaluate the potential effects of plasma rich in growth factors (PRGF) technology and its autologous formulations in five consecutive patients in which bilateral sinus lift augmentation was carried out.

Material and Methods: Five consecutive patients received bilateral sinus floor augmentation. All patients presented a residual bone height of class D (1–3 mm). The effects of PRGF combined with bovine anorganic bone (one side) were compared with the biomaterial alone (contralateral side). The effects of using liquid PRGF to maintain the bone window and autologous fibrin membrane to seal the defect were evaluated. A complete histological and histomorphometrical analysis was performed 5 months after surgery.

Results: One patient was excluded from the study as the Schneiderian membrane of the control side was perforated during the surgery. In two patients, the biopsies obtained from the control sides 5 months postsurgery were not acceptable for processing. PRGF technology facilitated the surgical approach of sinus floor elevation. The control area was more inflamed than the area treated with PRGF technology. Patients referred also to an increased sensation of pain in the control area. PRGF-treated samples had more new vital bone than controls. In patient number 1, image processing revealed 21.4% new vital bone in the PRGF area versus 8.4% in the control area, whereas in patient number 2, 28.4% new vital bone was quantified in the PRGF area compared with the 8.2% of the control side. The immunohistochemical processing of the biopsies revealed that the number of blood vessels per square millimeter of connective tissue was 116 vessels in the PRGF sample versus 7 in the control biopsy.

Conclusions: These preliminary results suggest that from a practical point of view, PRGF may present a role in reducing tissue inflammation after surgery, increasing new bone formation and promoting the vascularization of bone tissue.

KEY WORDS: fibrin, PRGF, sinus elevation

INTRODUCTION

The treatment of the severely reabsorbed maxilla is one of the most important challenges for the installation of endosseous dental implants. The lack of bone in this region is in general the result of the combination of alveolar bone resorption after tooth loss, pneumatization of maxillary antrum, and periodontal disease. Sinus floor elevation has become a standard procedure in the treatment of severely reabsorbed maxilla before the insertion of dental implants after its first report 20 years ago.[1,2]

Recently, we have reported a new lateral approach for sinus elevation using plasma rich in growth factors (PRGF) technology.[3] The latter enables the preparation of different formulations enriched in platelets, which after activation release multiple growth factors and bioactive proteins. Patients receiving dental implants after this sinus floor augmentation surgery with PRGF technology showed 100% survival with a mean follow-up of 33 months.[3]

PRGF technology presents many advantages that make its use in sinus lift augmentation encouraging. It provides different formulations, including liquid

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supernatant, three-dimensional PRGF scaffold, and fibrin biomaterial, which can be applied in different stages of the procedure. These formulations, once activated, secrete a large pool of proteins and factors including platelet-derived growth factor, transforming growth factor-β, vascular endothelial growth factor, insulin-like growth factor, hepatocyte growth factor, angiopoietins, platelet factor-4, and thrombospondin among others to the local milieu, driving the tissue regeneration mechanism.[4,5] Some of the distinguishing properties of PRGF include the moderated platelet concentration that has been related with optimal biological benefit[6] and the absence of leukocyte content in its formulation that avoids the pool of metalloproteases that may provoke negative tissue-destroying effects.[7]

The present paper studies the potential of PRGF technology and its autologous formulations in sinus elevation in two consecutive patients in which bilateral sinus lift augmentation was carried out. The effects of PRGF combined with bovine anorganic bone (one side) were compared with the biomaterial alone (contralateral side). Additionally, the use of liquid PRGF to maintain the bone window as well as the use of autologous fibrin membrane to seal the defect are highlighted. A complete histological and histomorphometrical analysis was performed, and the level of vascularization in the new forming bone was determined.

MATERIAL AND METHODS

The protocol was approved following the national and international (International Conference of Harmonization rules) policies on clinical studies. This study was carried out in Vitoria (Spain) and was composed of five consecutive patients (three women and two men) with a loss of height in the posterior maxilla that required application of a sinus lift technique to allow rehabilitation with dental implants. Selection of patients was carried out considering that sizes of both sinuses (lateral and contralateral) were equivalent or very similar. The exclusion criterion was the absence of any local or systemic diseases that might contraindicate the treatment and the perforation of the Schneiderian membrane. All patients included in the study presented a significant alveolar atrophy and a residual bone height of class D (1–3 mm).[8]

Elaboration of the Different PRGF Technology Preparations

Peripheral blood (70–90 mL) from each patient was taken by venipuncture before surgery and placed directly into 9 mL blood-collecting tubes® (Biotechnology Institute [BTI], Vitoria, Spain) that contain 3.8% (wt/vol) sodium citrate as anticoagulant. Liquid PRGF was prepared by centrifugation (PRGF System®, BTI, Vitoria, Spain) at 580 *g* for 8 minutes at room temperature. The 1 mL plasma fraction located just above the red cell fraction, but not including the buffy coat, was collected and deposited in a glass dish (BTI). In order to initiate clotting and the formation of a three-dimensional fibrin matrix for the continuous release of growth factors and proteins, PRGF activator® (BTI) (calcium chloride) was added to the liquid PRGF preparation (50 mL PRGF activator per milliliter of preparation). In order to prepare the autologous fibrin membrane, the 2 mL of plasma fraction located at the top of the tubes was transferred to a glass bowl (BTI). After adding PRGF activator, it was incubated at 37ºC for 40 to 45 minutes, allowing the formation of a biocompatible fibrin with excellent elastic and homeostatic properties.

Surgical Protocol

Antibiotics (2 g of amoxicillin clavulanic acid) were prescribed to each patient, starting 30 minutes before surgery and during 6 days postsurgically. Midazolam (7.5 mg, one tablet) was also administered 30 minutes preoperatively. Dexamethasone (4 mg) was administered orally before the surgery and for the next 3 days with a decreasing dose (3, 2, and 1 mg, respectively). Analgesics were used pre- and postoperatively during 2 to 3 days. Patients were instructed how to maintain proper oral hygiene around implants.

Both operative areas (lateral and contralateral sinuses) were reached by means of a full-thickness flap. Access to each cavity was obtained using a periodontal ultrasonic generator (BTI Ultrasonic®) combined with an independent irrigation system with BTI sterile pyrogen-free water.[9] The osteotomy line was made by cutting and dispersing the osseous table in a controlled and progressive way. A complete osteotomy along the perimeter of the osseous window is initiated and deepened until tactile sensation of the Schneiderian membrane. Once the osseous windows were separated from both sinuses, one of them was placed in liquid PRGF

whereas the other was maintained in saline solution until they were placed again in their native anatomic locations.[3] Once the fenestrations were completed, the Schneiderian membrane in the sinus floor was carefully separated using the BTI membrane rasps to avoid any perforation of the Schneiderian membrane.

The graft material used in the present protocol to fill the cavity was a mixture of 1.5 to 2.5 g of bovine anorganic bone with a particular diameter size of 1 to 2 mm (Bio-Oss®, Geistlich Biomaterials, Wolhusen, Switzerland), with activated liquid PRGF in the case of one sinus, and saline solution in the other. Both mixtures were prepared 10 minutes before using them to fill the sinus cavity and consisted in a proportion of 0.5 g of bovine anorganic bone and 1 mL of PRGF or saline solution.

Both bone windows were placed in their original position after turning them 30°, obtaining an adequate primary stability. Afterwards, one of the windows was covered with autologous fibrin and sutured with a 5-0 monofilament suture while the other was left uncovered. Five months after sinus elevation, high-resolution scans of the mandibles were acquired with a computed tomography scanner and a bone densitometry measured using the BTI scan® program.

Sample Preparation and Histomorphometrical Analysis

Samples from both sinuses (lateral and contralateral) were obtained after a healing period of 5 months prior to implant placement using a trephine hollow drill. Processing and staining of the bone samples were carried out using a standardized protocol. Briefly, the samples were fixed in B5-fixative, decalcified with ethylenediamine tetraacetic acid (EDTA), dehydrated in graded series of alcohols, and embedded in paraffin. Then, 5 µm-thick serial sections were obtained and stained with hematoxylin and eosin. For histomorphometric analysis, histological samples were examined by conventional optical microscopy using a Leica DMLB microscopy (Leica Microsystems, Wetzlar, Germany) and photographed with a Leica DFC 300 FX digital camera (Leica Microsystems). The digitized images were analyzed using the ImageJ software (version 1.41, National Institutes of Health, Bethesda, MD, USA). For the determination of the vital bone content, a ×25 magnification was used, evaluating the complete section for each case; approximately 7.5 mm^2 were examined. The new bone, bovine hydroxyapatite particles, and soft tissue areas were measured semiautomatically and expressed as percentage of the total area. Slides stained with Alcian blue pH 2.5 (Bio-Optica, Milano, Italy) were employed to confirm the histomorphometrical analysis.

Vascular Immunohistochemistry

Immunostaining to assess the vascularity of the samples was performed with a monoclonal antibody antihuman CD34. Dako (Glostrup, Denmark) was the supplier of all reagents. The samples were rehydrated in a similar way as that for conventional histology, and the endogenous peroxidase was blocked during 10 minutes with Dako Peroxidase Blocking Reagent. After that, the slides were pretreated for antigen unmasking with FLEX Target Retrieval Solution High pH (Dako) for 20 minutes at 98°C. The sections were cooled and incubated with the primary antibody antihuman CD34 (clone QBEnd 10) during 30 minutes at room temperature. The color was developed with the Envision system (Dako), composed by two parts, a polymer conjugated with horseradish peroxidase and a substrate chromogen. The positivity was showed by the diaminobenzidine brown precipitate. Finally, the slides were counterstained with hematoxylin and mounted. The criterion for defining blood vessel was the presence of endothelial positive cells delimiting a lumen.

RESULTS

Surgical Technique and Inflammation Level

No major incidences or dental injuries were noted during the procedures. One of the patients, however, was excluded from the study as the Schneiderian membrane of the control side was perforated during the surgery (exclusion reason). Assuming that the PRGF-treated side of the excluded patient was in correct conditions, we decided to analyze and process the biopsy sample accordingly. In two other patients, the biopsies obtained from the control sides 5 months postsurgery were not minimally acceptable for processing. In fact, the control samples were inconsistent and they appear fragmented, making their complete analysis impossible. Once again, the biopsies obtained from PRGF-treated sides were more mature, which facilitated their histological and histomorphometrical processing. These determining factors conditioned the final number of patients involved in the study and clearly demonstrated that sides

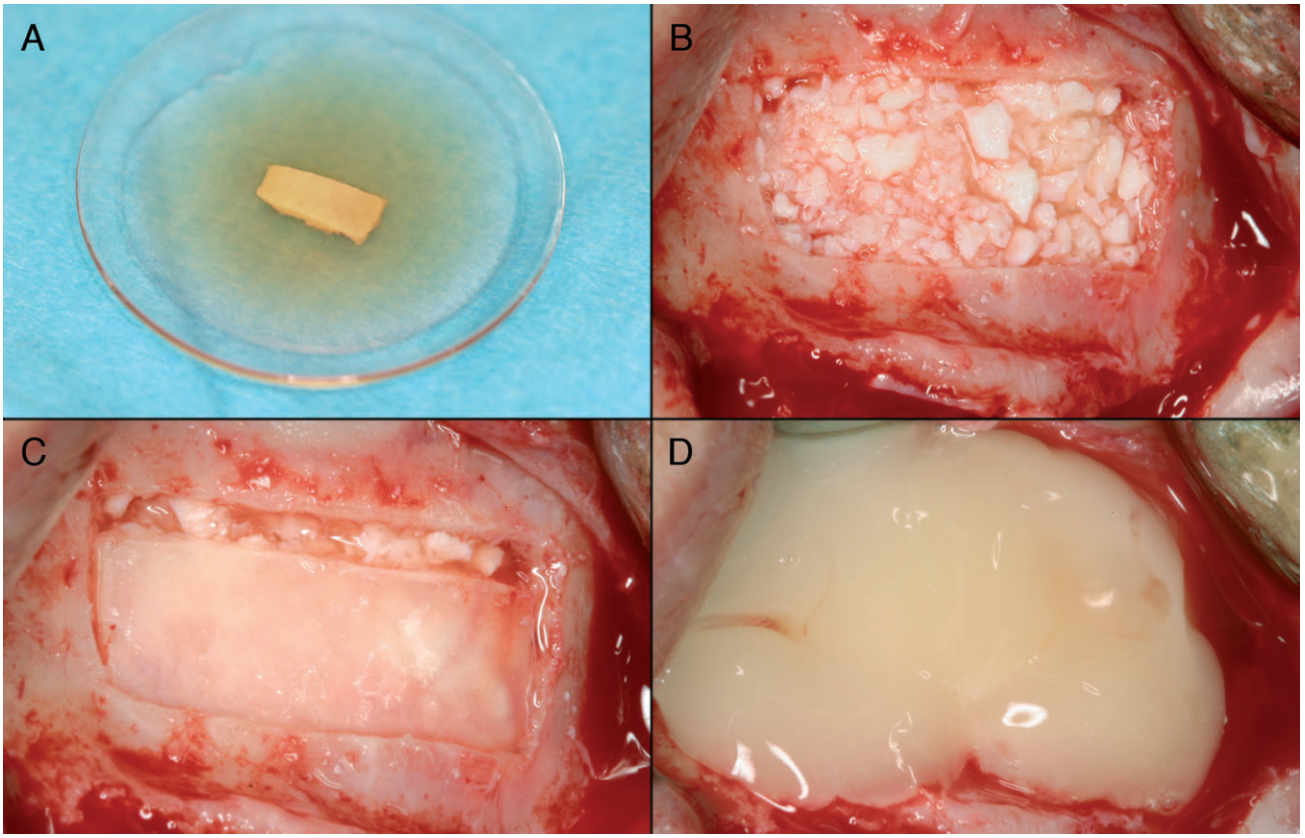

Figure 1 Plasma rich in growth factors (PRGF) technology facilitates the procedure of sinus floor elevation. *A,* The bone window from the sinus can be placed in liquid PRGF to maintain its viability and functionality. *B*, PRGF improves the manipulation and administration of anorganic bone particles into the sinus. *C*, The bone window is placed in its native position. *D*, The fibrin membrane can be used to seal the defect.

treated with PRGF from all the patients were in better conditions and showed regenerated bone compared with control sides.

From a practical point of view, the surgical procedure was clearly simplified when PRGF technology was used. As it is shown in Figure 1, the use of PRGF formulations enabled the deposition of the bone window in a growth factor-rich solution instead of saline solution. The latter may have implications for the maintenance of bone viability.[10] Additionally, the handling and administration of bovine anorganic bone were drastically improved when combined with activated PRGF, and the sealing of the defect was feasible using the retracted and hemostatic fibrin membrane.

Interestingly, the inflammation level of both sinuses was carefully evaluated after the patients referred to an increased sensation of pain and inflammation in the control area. Figure 2 illustrates the inflammation level of both hemiarcades at day 7 postsurgery. The control area was clearly more inflamed than the area treated with PRGF technology. Last but not least, patients referred also to an increased sensation of pain in the control area.

Histology, Histomorphology, and Immunohistochemical Examination

Biopsy samples, taken with a trephine hollow drill, were obtained from both hemiarcades in each of the patients before implant placement. Results showed that PRGF-treated samples were denser and had more new vital bone than the controls (Figure 3). In fact, in patient number 1, image processing revealed 21.4% new vital bone in the PRGF area versus 8.4% in the control area. In the case of patient number 2, results were similar, as 28.4% new vital bone was quantified in the PRGF area compared with the 8.2% of the control side. In the rest of the patients (the three of them were excluded from the study), the percentage of new bone ranged from 24 to 30% (data not shown).

Histomorphometrical analysis showed that, in the area treated with PRGF, the bovine bone was

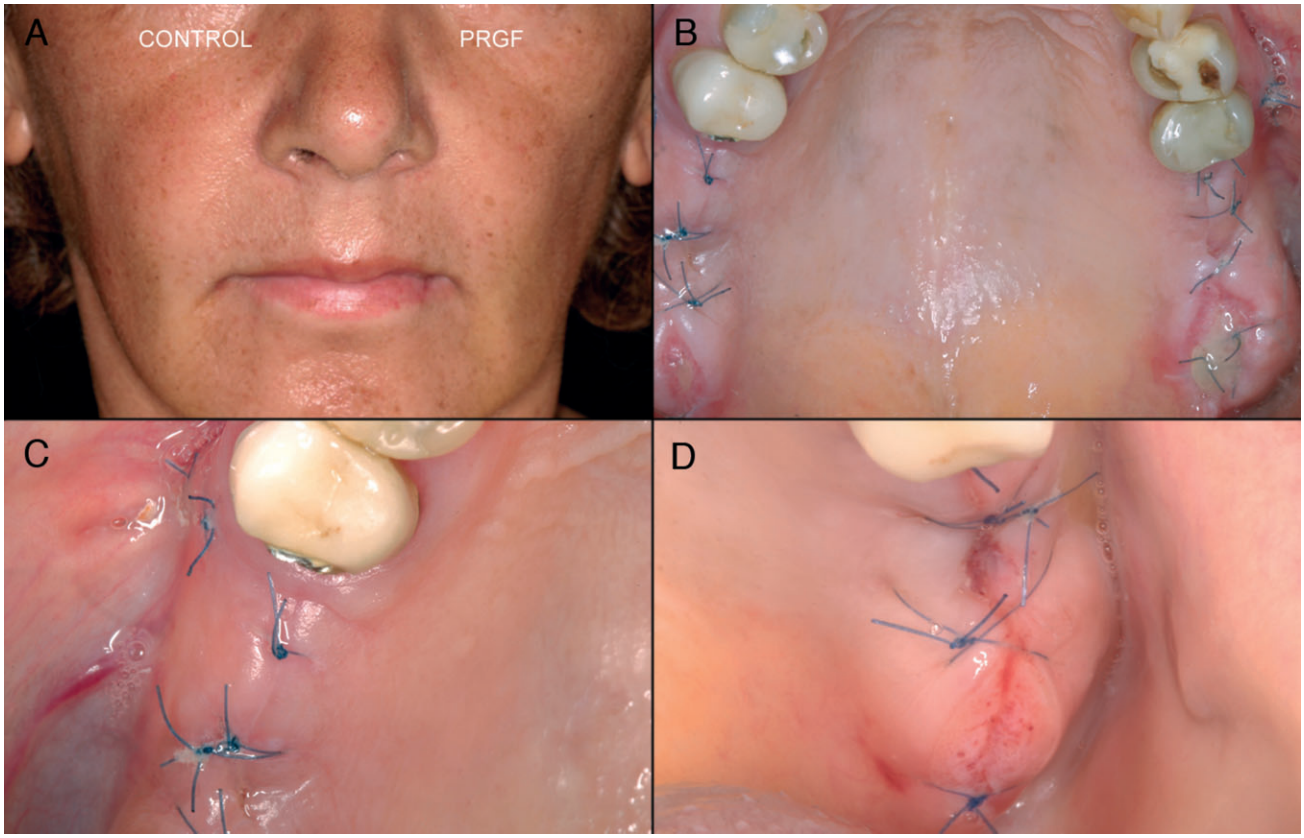

Figure 2 Plasma rich in growth factors (PRGF) reduce the inflammatory level of the treated side. (A) Illustration of one of the patients involved in the study. The inflammation is clearly reduced in the side treated with PRGF compared with the control area. (B) Soft tissue is more inflamed in the control area (*right side*) than in the PRGF-treated area (*left side*). Magnifications of the PRGF side (C) and control side (D).

incorporated into the new bone formation (Figure 4). The bone-like structure was denser and more compact than in the control area in which the process of bone regeneration was still immature.

The immunohistochemical processing of the biopsies was only successfully performed in one of the patients because of the inconsistency of the other control sample. Figure 5 compares the vascularization degree of the sample treated with PRGF and the untreated sample. The brown positivity shows the presence of endothelial cells. The quantification of the number of blood vessels per square millimeter of connective tissue resulted in 116 vessels in the PRGF sample versus 7 in the control biopsy.

Finally, Figures 6 and 7 summarize the bilateral sinus augmentation of both patients involved in the study in which PRGF technology was used in one lateral area and was not employed in the contralateral side.

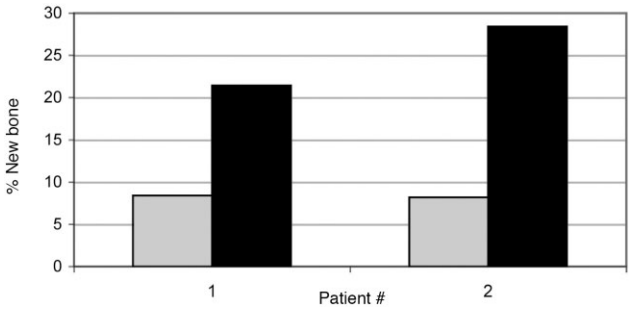

Figure 3 New bone formation in the control area (in gray) and plasma rich in growth factors-treated area (in black).

DISCUSSION

The present paper has evaluated the potential of using PRGF technology in the lateral approach of sinus floor elevation. To address this issue, a number of bilateral cases have been evaluated comparing the effects of PRGF technology (one side) versus not using this biomedical approach (the contralateral side). This study included initially five consecutive patients who underwent bilateral sinus lift using the previously reported

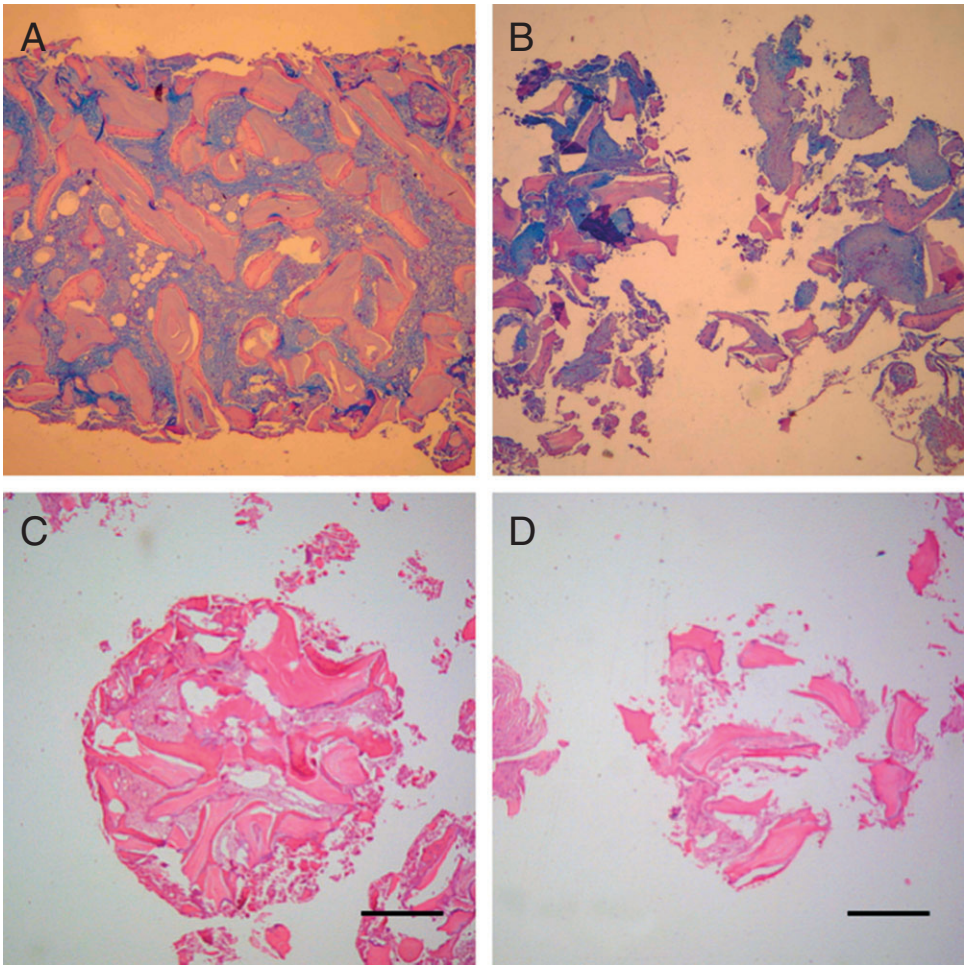

Figure 4 Histological biopsies of case one (A) for plasma rich in growth factors (PRGF) and (B) for control, and case two (C) for PRGF and (D) control. Note that the PRGF-treated biopsies have more new bone and are more compact than the controls. Biopsies are prepared using hematoxylin–eosin and Alcian blue staining. Scale bar in all cases: 500 μm.

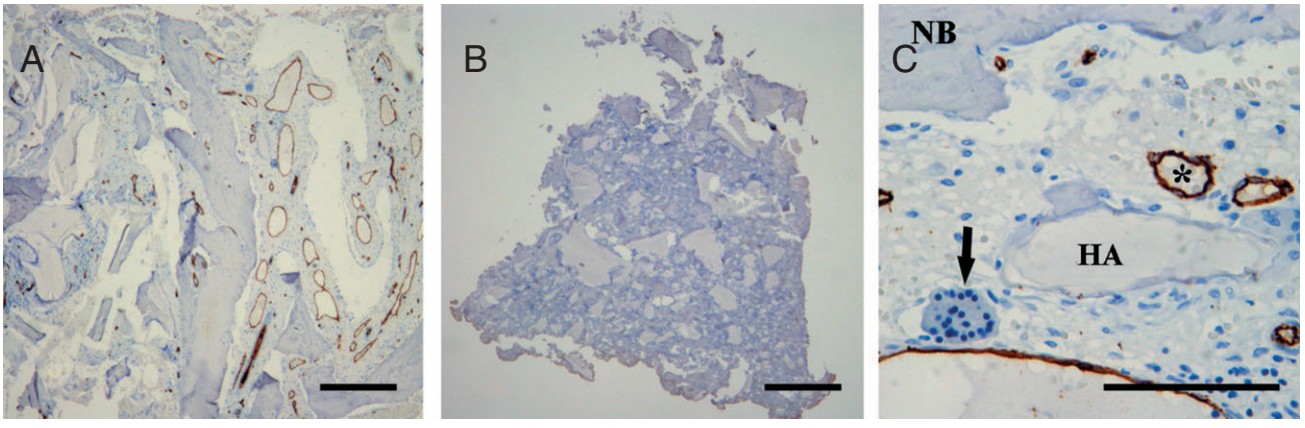

Figure 5 Immunohistochemical examination of vascular presence in case two: bilateral sample treated with plasma rich in growth factors (PRGF) (*A*) and untreated (*B*). The brown positivity shows the presence of endothelial cells. Higher magnification (*C*) showing a detailed insight of revascularization in the PRGF-treated sample. A vascular lumen is marked with an asterisk. Note the new bone (NB) and the bovine hydroxyapatite (HA) after 5 months of healing. In addition, the presence of an osteoclast (arrow) is also distinguishable. Scale bars: *A* and *B*, 250 mm; *C*, 100 mm.

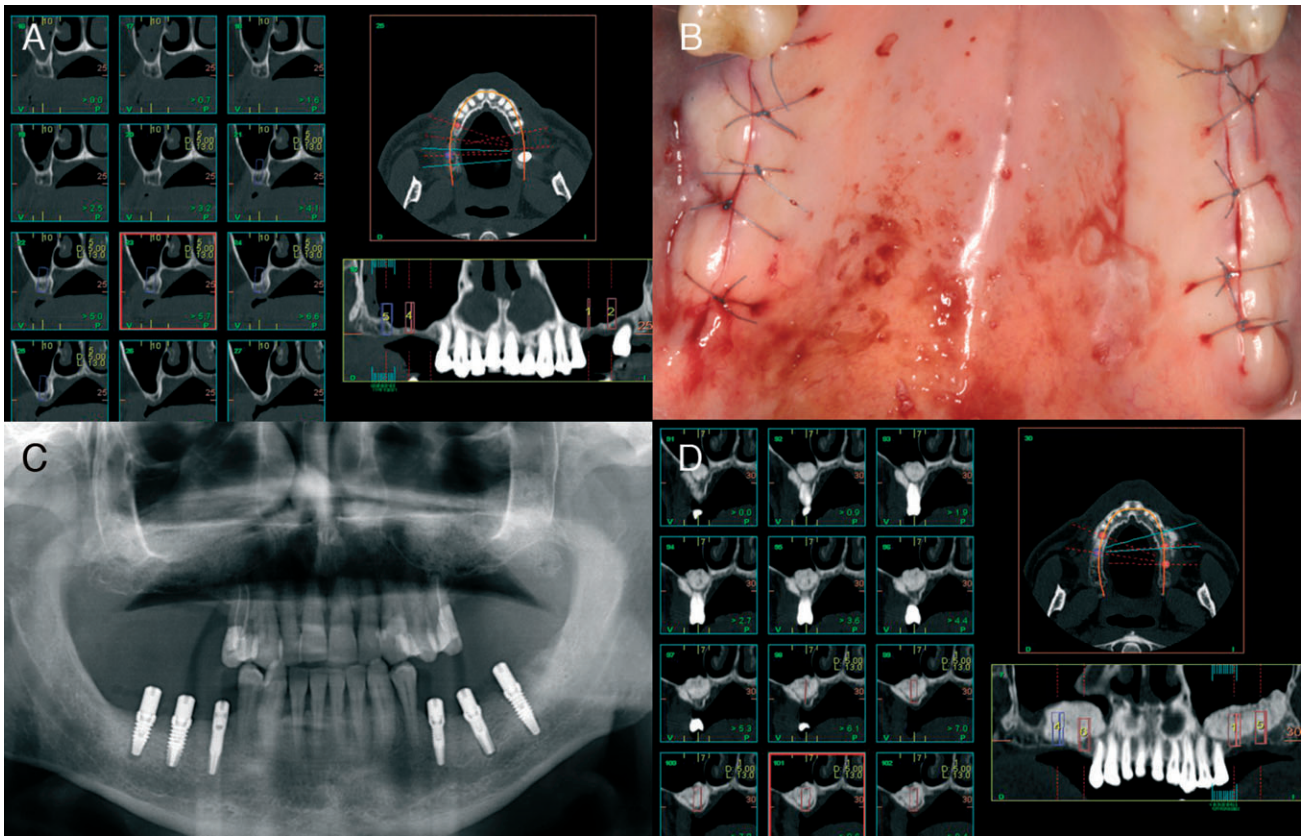

Figure 6 Summary of the first patient involved in the study. *A*, Pretreatment radiographs. *B*, Level of inflammation in both sides. *C* and *D*, Final radiographs.

approach.[3,9] One of the patients was excluded from the study as the Schneiderian membrane of the control side was perforated during the surgery. Unfortunately, when the histological and histomorphometrical evaluation of the biopsies was carried out, only in two of the cases the control samples were acceptable for processing. In general, the control biopsies were inconsistent and they appear fragmented, making their complete analysis impossible. Therefore, one important consideration of this work is that PRGF provided a more mature bone in only 5 months as all the samples obtained from the five patients in the PRGF side were successfully processed and evaluated.

The preliminary data presented here indicate some potential advantages of PRGF technology that should be more completely demonstrated in future studies involving more patients. One of the first advantages of using this platelet-based preparation is that it facilitates the protocol and improves the biosafety of the approach. The former is because of the adhesive properties of the PRGF scaffold that improve handling and administration of the bone substitute while avoiding the uncontrolled displacement of particular graft materials into the sinus cavity, especially in the case of Schneiderian membrane perforation. The latter is mainly because of the biocompatible and hemostatic properties of the fibrin membrane.[11] This biomaterial can be used as an autologous sealant agent not only to cover the window but also in the case of Schneiderian membrane perforation. It has been reported that perforations of the Schneiderian membrane represent the major intraoperative complication of the lateral approach in sinus floor augmentation, with an incidence ranging from 20 to 25%.[12–14] The use of a patient's own fibrin membrane is an excellent option to seal the membrane perforations, especially when these are small or medium sized.

Another potential advantage of using the plasma-derived and platelet-derived bioactive molecules present in PRGF is the capacity to reduce tissue inflammation after surgery. As it has been observed in both cases, but also in other patients not included in this study, the inflammation degree was clearly reduced in the PRGF-treated area compared with the control side. It has been observed that platelet products suppress monocyte

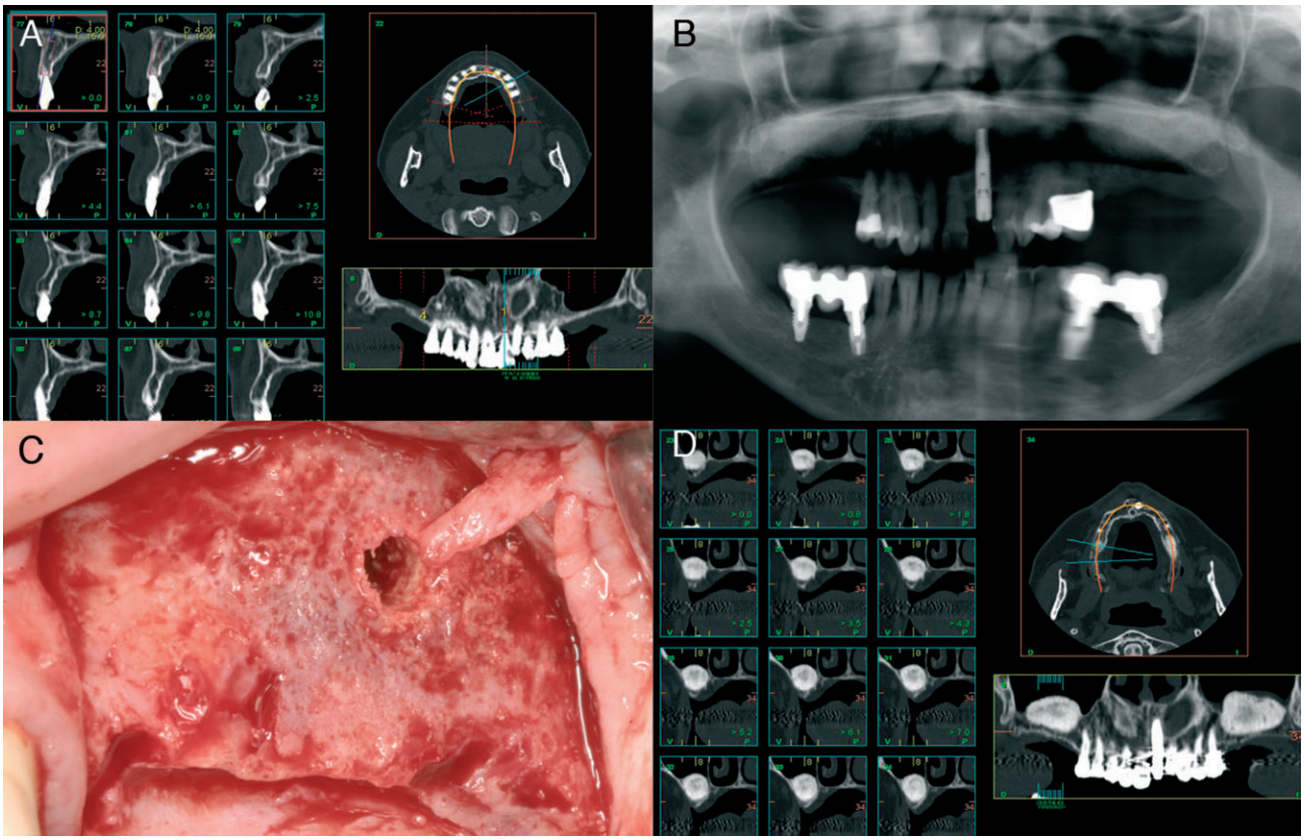

Figure 7 Summary of the second patient involved in the study. *A* and *B*, Pretreatment radiographs. *C*, Reopening 5 months postsurgery. *D*, Final radiograph.

cytokine release and limit inflammation.[15] Furthermore, recent results suggest that platelets cause an initial suppression of interleukin-1 (IL-1) release from activated macrophages. The initial suppression of the inflammatory response could have broad implications in the explanation of a mechanism by which platelet-rich products may act as an anti-inflammatory agent.[16] In addition to this activity, some reports also indicate that platelet-based products can exert potent antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*.[17]

The complete histological and histomorphometrical evaluation of the biopsies carried out 5 months after surgery revealed that there was significantly more new vital bone in the PRGF-treated area than in the control area. These data suggest a role of PRGF in promoting and stimulating bone formation. PRGF preparations contain two to three times the concentration of platelets compared with peripheral blood. Platelets can be considered as a potential source of multiple autologous growth factors and proteins, some of which are involved in bone tissue regeneration.[18] For example, platelet-derived growth factor is a powerful mitogen for connective tissue cells,[19] transforming growth factor-β stimulates osteoprogenitor cells to proliferate but also blocks in later stages cell differentiation and mineralization,[20] insulin-like growth factor might promote the late-stage differentiation and activity of osteoblasts, and vascular endothelial growth factor induces endothelial cell proliferation and migration, thus initiating the angiogenic response[21] and even acts directly on osteoblasts.[22]

To test this hypothesis, we performed an immunohistochemical evaluation of the samples to quantify the presence of newly formed vessels in each biopsy. Results indicated that PRGF-treated areas were more vascularized than control sides. This may suggest that PRGF-secreted bioactive molecules may initiate the sprouting of blood vessels that will contribute to accelerate the regeneration of bone tissue. In fact, actually, it is totally accepted that there exists an intimate connection, both physically and biochemically, between bone and blood vessels.[22] Inadequate or inappropriate bone vascularity is also associated with decreased bone formation and bone mass.[23]

In the last decade, several methods to prepare platelet-rich products have been published. This is a particularly important issue as the different available techniques yield to substantially different amounts of platelets, growth factors, and proteins, leading to different and sometimes conflicting biological effects. In fact, in one intraindividual comparison of patients with bilateral sinus floor augmentation, higher bone regeneration was observed in patients treated with platelet-rich plasma (PRP).[24] Similarly, the results of a clinical trial with 16 patients who underwent bilateral sinus lift seem to indicate a certain regenerative potential of PRP when used with autologous bone.[18] However, a recent study showed no positive effects of PRP on bone density in sinus floor augmentation.[25]

The technique presented herein is the pioneering and unique procedure to prepare different 100% autologous platelet-based formulations with therapeutic potential.[4,5,26] Previous studies have suggested that this new approach for sinus elevation and implant installation using PRGF technology can be considered safe, simple, effective, and predictable.[3,9] Although data presented in this case report should only be considered as preliminary information, results suggest that from a practical point of view and in the short-term evaluation, PRGF may present a role in reducing tissue inflammation after surgery, increasing new bone formation and promoting the vascularization of bone tissue.

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REFERENCES

1. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg 1980; 38:613–616.
2. Tatum H. Maxillary and sinus implant reconstructions. Dent Clin North Am 1986; 30:1207–1229.
3. Anitua E, Prado R, Orive G. A lateral approach for sinus elevation using PRGF technology. Clin Implant Dent Relat Res 2009. (In press).
4. Anitua E, Sánchez M, Orive G, Andía I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials 2007; 28:4551–4560.
5. Anitua E, Sánchez M, Orive G, Andía I. Delivering growth factors for therapeutics. Trends Pharmacol Sci 2008; 29:37–41.
6. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone 2004; 34:665–671.
7. Schnabel LV, Mohammed HO, Miller BJ, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. J Orthop Res 2007; 25:230–240.
8. Jensen OT, Shulman LB, Block MS, Iacone VJ. Report of the Sinus Consensus Conference of 1996. Int J Oral Maxillofac Implants 1998; 13:11–32.
9. Torrella F, Pitarch J, Cabanes G, Anitua E. Ultrasonic osteotomy for the surgical approach of the maxillary sinus: a technical note. Int J Oral Maxillofac Implants 1998; 13:697–700.
10. Anitua E, Carda C, Andia I. A novel drilling procedure and subsequent bone autograft preparation: a technical note. Int J Oral Maxillofac Implants 2007; 22:138–145.
11. Anitua E, Sánchez M, Nurden AT, Nurden P, Orive G, Andía I. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol 2006; 5:227–234.
12. Khoury F. Augmentation of the sinus floor with mandibular bone block and simultaneous implantation: a 6-year clinical investigation. Int J Oral Maxillofac Implants 1999; 14:557–564.
13. van den Bergh JP, ten Bruggenkate CM, Krekeler G, Tuinzing DB. Maxillary sinus floor elevation and grafting with human demineralized freeze dried bone. Clin Oral Implants Res 2000; 11:487–493.
14. Becker ST, Terheyden H, Behrens E, Springer I, Wiltfang J. Prospective observation of 41 perforations of the Schneiderian membrane during sinus floor elevation. Clin Oral Implants Res 2008; 19:1285–1289.
15. El-Sharkawy H, Kantarci A, Deady J, et al. Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. J Periodontol 2007; 78:661–669.
16. Woodall J Jr, Tucci M, Mishra A, Asfour A, Benghuzzi H. Cellular effects of platelet rich plasmainterleukin1 release from prp treated macrophages. Biomed Sci Instrum 2008; 44:489–494.
17. Bielecki TM, Gazdzik TS, Arendt J, Szczepanski T, Krol W, Wielkoszynski T. Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances. J Bone Joint Surg Br 2007; 89:417–420.
18. Consolo U, Zaffé D, Bertoldi C, Ceccherelli G. Platelet-rich plasma activity on maxillary sinus floor augmentation by autologous bone. Clin Oral Implants Res 2007; 18:252–262.

19. Graves DT, Valentin-Opran A, Delgado R, Valente AJ, Mundy G, Piche J. The potential role of platelet derived growth factor as an autocrine or paracrine factor for human bone cells. Connect Tissue Res 1989; 23:209–218.
20. Maeda S, Hayasi M, Komiya S, Imamura T, Miyazono K. Endogenous TGF-beta signalling suppresses maturation of osteoblastic mesenchymal cells. EMBO J 2004; 23:552–563.
21. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003; 9:669–676.
22. Carano RAD, Filvaroff EH. Angiogenesis and bone repair. Drug Discov Today 2003; 8:980–989.
23. Glowacki J. Angiogenesis in fracture repair. Clin Orthop Relat Res 1998; Oct.(355 Suppl):S82–S89.
24. Bauer FJM. Der einfluss von plattchenreichem plasma (PRP) auf sinusbodeaugmentate-eine intraindividual vergleichsstudie. Medical dissertation, University of Munich, Munich, Germany, 2004.
25. Schaaf H, Streckbein P, Lendeckel S, et al. Sinus lift augmentation using autogenous bone grafts and platelet rich plasma: radiographic results. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106:673–678.
26. Anitua E. Plasma rich in growth factors: preliminary results of the use in the preparation of future sites for implants. Int J Oral Maxillofasc Implants 1999; 14:529–535.