

ENHANCEMENT OF OSSEOINTEGRATION BY GENERATING A DYNAMIC IMPLANT SURFACE

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KEY WORDS

Growth factors
Titanium implants
Osseointegration

Preparations of autologous plasma rich in growth factors (PRGF) are used to promote healing and tissue regeneration. We seek to determine whether covering the titanium implant surface with this preparation could enhance osseointegration. The interaction of PRGF with the surface of titanium implants was examined by environmental scanning electron microscopy (ESEM). A total of 23 implants were placed in the tibiae and radii of 3 goats; 13 implants were inserted after covering the surface and filling the alveolus with PRGF, and 10 more implants were inserted following a conventional protocol and served as controls. Histomorphometric analysis of the bone-implant interface was performed after 8 weeks. Finally, 1391 implants were placed in 295 patients after bioactivating the surface with PRGF. Stability and implant survival were evaluated. The implant surface adsorbed the protein-rich material as shown by ESEM. In the animal study, osseointegration was enhanced when the surface was covered with PRGF as shown by histomorphometry (bone-implant contact: $51.28\% \pm 4.7\%$ vs $21.89\% \pm 7.36\%$; $P < .01$). Finally, studies in patients showed that 99.6% of the implants treated with PRGF were well osseointegrated. Clinical use of this technique in oral implantology can improve the prognosis.

INTRODUCTION

In the process of tissue repair and restoration, the osseointegration of dental implants can be improved and accelerated by inducing the regenerative capacity of surrounding tissues with the appropriate stimuli. Because growth factors are expressed during different phases of tissue healing, it has been thought that they could serve as therapeutic agents to promote tissue repair.^{1,2}

Autologous plasma rich in growth factors (PRGF) has been

shown to enhance and accelerate soft tissue repair and bone regeneration in the preparation of future sites for dental implants.^{3,4} More recently, preparation with PRGF has been shown to enhance postoperative healing of ruptured Achilles tendon in professional athletes and articular cartilage repair after nontraumatic avulsion.^{5,6}

A preparation of PRGF applied to a titanium implant adheres to the metal and might create a new dynamic surface that could potentially show biological activity.⁷ This protein layer consists of

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a fibrin net embedded with growth factors that covers the whole implant surface and transforms the initial interactions of the implant surface with the surrounding tissues. It also influences cellular attachment, proliferation and differentiation, and bone matrix deposition. This coating has 2 important properties that may contribute to optimizing and accelerating the osseointegration process: the osteoconductive properties attributed to fibrin⁸ and the recognized osteoinductive activities of growth factors.⁹

Considering these findings, it was important to study the adherence of PRGF to the implant surface and its permanence after clot retraction. With this aim, environmental scanning electron microscopy (ESEM) was utilized to study the interaction between BTI implant surfaces and PRGF.

The hypothesis that PRGF-coated surfaces could accelerate implant osseointegration was tested in an animal model. In view of the good experimental results obtained, it was hypothesized that the clinical use of implant surfaces coated with PRGF would improve the outcome. To determine if clinical evidence supports our hypothesis, we prospectively studied all implant procedures performed in our office between January 2000 and June 2001.

MATERIALS AND METHODS

Preparation of PRGF

Blood was collected from human volunteers who had given their informed consent to the procedure. The blood was deposited in 3.8% w/v sodium citrate 1:9 v/v.¹⁰

Platelet-rich plasma was separated by centrifugation at 460 g for 8 minutes at room temperature (PRGF System II, BTI, Vitoria, Spain). The 0.5-mL plasma

fraction located just above the red blood cell layer was collected. Leukocytes were not included in the preparation. Fifty microliters of 10% w/v calcium chloride was added to each tube containing 1 mL of PRGF.

Interaction between plasma proteins and titanium implants

Evidence that the surface of the titanium implants directly interacted with PRGF was obtained by coating implant surfaces with PRGF in the presence of Ca²⁺ and studying the surface by ESEM (Electroskan 2020, Wilmington, NC).⁷

Studies in an animal model

Bioactive titanium dental implants were inserted in the tibiae and radii of 3 goats. The study was carried out in compliance with guidelines for the humane treatment of experimental animals in Spain (Royal Decree 2223/88). Blood was drawn from the vena cava and collected in citrated vacutainer tubes (Venject, Terumo Co, Japan), and PRGF was prepared by centrifugation at 460 g for 8 minutes.

Animals were anesthetized with ketamine (20 mg/kg), and anesthesia was maintained with inhaled isoflurane (2.5%). Artificial alveoli were created by making perforations (3 mm in diameter and 8.5 mm deep) in the tibiae and radii. A total of 23 implants (BTI Implant System, BTI, Vitoria-Gasteiz, Spain) were inserted in the holes in accordance with 2 protocols. Thirteen of the implants were made with a PRGF protocol (protocol A), which involved covering the implant surface with PRGF by simple adsorption and filling the alveolus with PRGF before inserting the implant. Ten of the implants were made with a non-PRGF

protocol (protocol B) consisting of control implants without added PRGF.

Biopsies were taken from all the implants with an 8-mm trephine drill after 8 weeks. Samples were numbered for blind analysis and immediately fixed in 4% formalin. Sections were stained with eosin and toluidine blue. Digitalized images were obtained with a JVC TK-C1380 color video camera (JVC, London, UK), and the central section was studied with histomorphometric techniques. Bone-implant contact (BIC), defined as the part of the implant surface in direct contact with the bone matrix, was expressed as a percentage of the total implant perimeter.

Patient selection

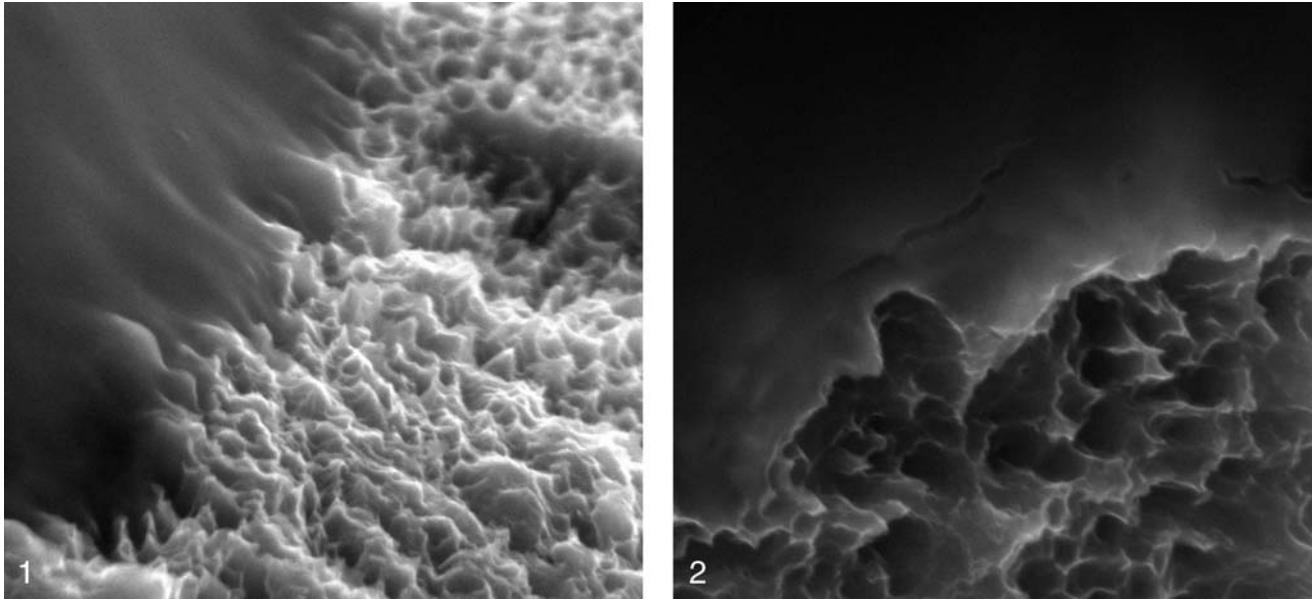
A prospective study was carried out in all patients who underwent implant rehabilitation between January 2000 and June 2001. A descriptive analysis was made and implant success was evaluated.

The patients agreed to participate in this study and gave their informed consent. All patients were healthy and had no systemic contraindications to the implantation procedure. The areas to be treated were free from infection.

Surgical procedures

A combination of panoramic and intraoral radiographs was used for preliminary evaluation of the intended implant sites. Bone quality was assessed in preoperative scans (Denta PC). Implants were placed at any site in the jaw in bone of quality I, II, III, and IV.

BTI implants with PRGF adsorbed onto the titanium acid-etched surface were used. Implants were inserted following a single-stage or other protocol as determined by the clinical requirements, which included



FIGURES 1 AND 2. FIGURE 1. Visualization of the interface between the plasma rich in growth factors and the titanium surface of the implant (BTI, Vitoria, Spain). The surface boundary was directly visualized with an Electroscan 2020 environmental scanning electron microscope without previous specimen preparation (magnification $\times 6000$). FIGURE 2. Visualization of the interface between the titanium surface of the implant and the developed fibrin-rich clot that remains in close contact with the microroughened surface of the BTI implant (Electroscan 2020, magnification $\times 6000$).

several surgical procedures, ridge augmentation, and sinus elevation. The same operator performed all surgical procedures.

Evaluation and criteria of success

Implants were evaluated by measuring the resonance frequencies of implants (Osstell). An implant was considered successful when (1) no sign of failure appeared in panoramic and periapical radiography, (2) no pain or symptoms of infection were present, and (3) a reverse torque force of 20 N/cm could be applied with a torque wrench (BTI) when recording impressions.

Statistical analysis

Data of BIC were obtained by IAS 2000 software (Delta Sistemi, Rome, Italy) and were expressed as mean \pm SD. The statistical significance between PRGF and non-PRGF protocols was assessed by the Student *t* test. Values of $P < .05$ were considered significant.

RESULTS

ESEM of implant surfaces coated with PRGF

The implant surface adsorbed the protein-rich material and was therefore bioactive, as is evident in Figure 1. After retraction, the clot remained perfectly adhered to the bioactive surface (Figure 2), producing a surface coated with a fibrin-rich clot. The associated growth factors remained in place to promote bone consolidation around the implant. Such a network can facilitate the adhesion and proliferation of osteoblasts at the titanium surface, a process that may be favored by chemotactic factors secreted by platelets.

Histochemical studies confirmed the tight incorporation of implants in newly formed bone tissue in a total of 23 implants in artificial alveoli in the tibia or radius of goats. Biopsies were obtained after 8 weeks, and typical histochemically stained sections of the implants are shown in

Figure 3a and b. Every biopsy of the implants made with PRGF revealed close contact between the bone and implant. In Figure 3a, newly regenerated bone fills the middle and apical thirds of the cavity. The cortical zone was indistinguishable from the medullary zone in all the samples analyzed. In the implants made according to the non-PRGF control protocol, only the middle third is surrounded by cortical bone, and osseous contact is absent in the apical third. Macroscopic images of the same biopsies are shown in Figure 3c and d. Soft tissue was probably lost during biopsy in the control implants. Mean \pm SD BIC percentages after histomorphometric analysis were $51.28\% \pm 4.7\%$ ($n = 13$) for the PRGF protocol and $21.89\% \pm 7.36\%$ ($n = 10$) for the non-PRGF control protocol. It is evident that the implants with a bioactive surface generated a significantly larger area of bone

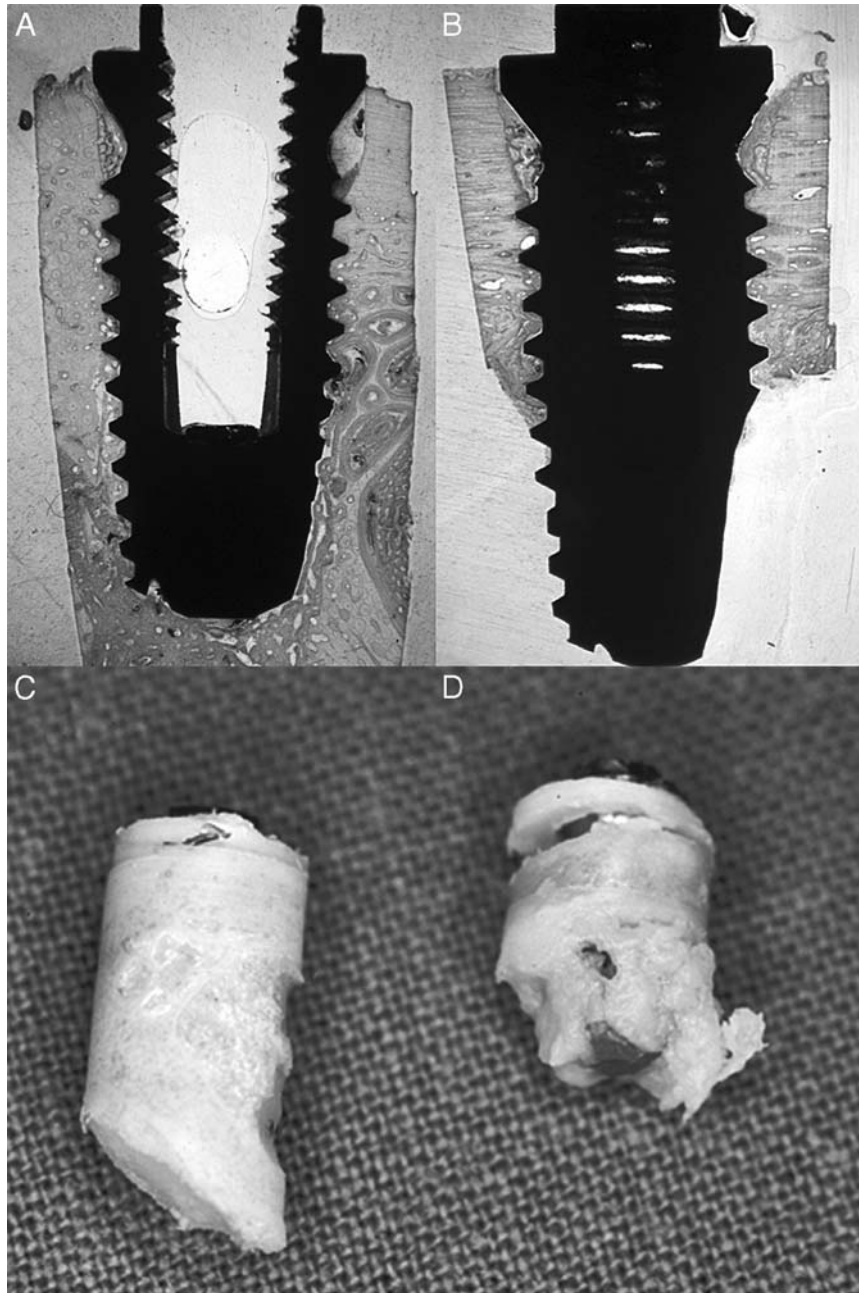


FIGURE 3. Analysis of the effect of the plasma rich in growth factors on bone regeneration around a titanium implant in an animal model. Bone structure was examined histologically after 8 weeks. (A) A stained section shows the compact cortical structure of the bone surrounding the entire implant. (B) A typical section from a control implant where soft tissue has been lost from the apical portion during the biopsy. (C and D) Macroscopic views of similar treated and control implants, respectively.

contact at 2 months than did the control group ($P < .01$).

Implantation success in patients treated with bioactive surface implants

A total of 1391 implants were placed in 265 patients, including

193 women (65.5%) and 72 smokers (24.5%). Mean patient age was 55 years (range: 19–84). Of 1391 implants, 992 (71.3%) were placed in a 1-stage protocol, 111 (8%) were placed in a 2-stage protocol, and 288 (20.7%) were immediately loaded. Seven hundred

eighty-two implants (56%) were placed in the maxilla and 609 (44%) in the mandible. In the maxilla, most of the implants ($n = 518$) were placed in posterior positions. In the mandible, posterior positions were also dominant ($n = 520$). Osseointegration failed

in 5 implants of 5 patients. All 5 implants were located in the maxilla: 3 in type IV bone, 1 in an alveolar ridge expansion procedure, and 1 in a totally edentulous patient.

DISCUSSION

In light of available knowledge about the effect of growth factors on bone healing, new treatment protocols have been developed. The gold standard implant treatment protocol¹¹ evolved as a consequence of the introduction of new biological concepts in daily surgical practice. Our new understanding of the healing process has led to the routine use of growth factors in oral clinical practice. Some of the many uses of PRGF that have been devised include filling the alveolus after dental extraction,⁴ compacting bone grafts,¹² or using them as biological membranes. We developed a new application for PRGF in which it is used to create a protein layer covering the implant surface. The rationale is that such surfaces may stimulate mechanisms of bone formation at the implant-bone interface, thus modulating the healing process. Other authors¹³ have reported stimulation of bone response adjacent to implants coated with bovine-purified bone morphogenetic protein.

Platelets are activated and release a number of stimulatory factors (eg, growth factors and other metabolites) that can promote the formation of bone, epithelium, and blood vessels. Fibrin in conjunction with fibronectin acts as a provisional matrix¹⁴ for the influx of local cells. These migrating cells use integrin receptors that recognize fibrin,

fibronectin, and vitronectin to interact with the implant surface. Extracellular matrix molecules can provide signals for gene expression through integrin receptors, so the interaction of local cells with the matrix could be expected to alter cell function.¹⁵ Studies in a goat model confirmed that the PRGF layer enhanced ossification around implants. Our findings confirm and build on the results of other authors. Platelet concentrates have been found to promote bone healing after dental implants in a pig model,¹⁶ as well as total tissue ingrowth into porous hydroxyapatite in a rat model¹⁷ or in skull defects in rabbits.¹⁸

CONCLUSION

Osseointegration was enhanced by covering the implant surface with PRGF before insertion into the alveolus. The clinical use of this biologically active surface in oral implantology might improve the prognosis. Long-term clinical studies are needed to validate the findings of this study.

REFERENCES

1. Bostrom MPG, Yanf X, Koutras I. Biologics in bone healing. *Curr Opin Orthop*. 2000;11:403-412.
2. Lieberman JR, Daluiski A, Einhorn Th A. The role of growth factors in the repair of bone: biology and clinical applications. *J Bone Joint Surg Am*. 2002; 84-A:1032-1044.
3. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants*. 1999;14: 529-535.
4. Anitua E. The use of plasma-rich in growth factors (PRGF) in oral surgery. *Pract Proced Aesthet Dent*. 2001;13:487-493.
5. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous plate-

lets as a source of proteins for healing and tissue regeneration. *Thromb Haemost*. 2004;91:4-15.

6. Sanchez M, Azofra J, Santisteban J, et al. Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. *Med Sci Sports Exer*. 2003;35: 1648-1653.

7. Aparicio C, Gil FJ, Planell JA, Engel E. Human-osteoblasts proliferation and differentiation on grit-blasted and bioactive titanium for dental applications. *J Mater Sci Mater Med*. 2002;13:1105-1111.

8. Clark RAF. Fibrin and wound healing. *Ann N Y Acad Sci*. 2001;936:355-367.

9. Anitua E, Andía I. *Un Nuevo Enfoque en la Regeneración Ósea*. Anitua E, ed. Vitoria, Spain: Puesta al Día Publicaciones; 2000.

10. Nurden P, Savi P, Heilmann E, et al. An inherited bleeding disorder linked to a defective interaction between ADP and its receptor on platelets. *J Clin Invest*. 1995;95:1612-1622.

11. Branemark P-I, Zarb GA, Albrektsson T, eds. *Tissue-Integrated Prostheses: Osseointegration in Clinical Dentistry*. Chicago, Ill: Quintessence; 1985.

12. Anitua E. Gel di plasma rico di piastrine. *Implantol Orale*. 2001;3:9-24.

13. Bessho K, Carnes DL, Cavin R, Chen H-Y, Ong JL. BMP stimulation of bone response adjacent to titanium implants in vivo. *Clin Oral Implants Res*. 1999;10:212-218.

14. Yamada KM, Clark RAF. Provisional matrix. In: Clark RAF, ed. *Molecular and Cellular Biology of Wound Repair*. New York NY: Plenum; 1996:51-93.

15. Davis JR, Lofthouse RA, Frondoza CG, Jinnah RH, Hungerford JD. Does mechanical stimulation alter integrin expression by human osteoblasts? *J Bone Joint Surg Br*. 1999;81B:338.

16. Zechner W, Tangi S, Tepper G, et al. Influence of platelet-rich plasma on osseous healing of dental implants: a histologic and histomorphometry study in minipigs. *Int J Oral Maxillofac Implants*. 2003;18:15-22.

17. Siebrecht MA, De Rooij PP, Arm DM, Olsson ML, Aspenberg P. Platelet concentrate increases bone ingrowth into porous hydroxyapatite. *Orthopedics*. 2002;25:169-172.

18. Kim ES, Choung PH. Platelet concentration and its effect on bone formation in calvarial defects: an experimental study in rabbits. *J Prosthet Dent*. 2001;86:428-433.