Effect of Leukocyte- and Platelet-Rich Fibrin (L-PRF) on Bone Regeneration: A Study in Rabbits

Michel Knapen, BBSc, MSc;* Damien Gheldof, BBSc, MSc;† Pierre Drion, DVM, PhD;‡ Pierre Layrolle, PhD;§ Eric Rompen, DDS, MS, PhD;* France Lambert, DDS, MS, PhD*

ABSTRACT

Background: The positive effect of leukocyte- and platelet-rich fibrin (L-PRF) on osteogenesis has been widely described in vitro. However, clinical and preclinical studies are very little and controversial in demonstrating a significant beneficial effect of L-PRF in bone regeneration.

Purpose: The goal of the present study was to compare the potential effect of L-PRF in a standardized model.

Materials and Methods: A total of 72 hemispheres were implanted on the calvaria of 18 rabbits and filled with three different space fillers: L-PRF, bovine hydroxyapatite (BHA), BHA + L-PRF, and an empty hemisphere was used as control. Six rabbits were sacrificed at three distinct time points: 1 week, 5 weeks, and 12 weeks. Histological and histomorphometrical analyses were carried out.

Results: At the early phase of bone regeneration (1 week), from a descriptive analysis, a higher proportion of connective tissue colonized the regeneration chamber in the two groups containing BHA particles. Nevertheless, no statistical differences were found within the four groups in terms of bone quantity and quality at each timepoint ($p = .3623$).

Conclusions: According to the present study, L-PRF does not seem to provide any additional effect on the kinetics, quality, and quantity of bone in the present model of guided bone regeneration.

KEY WORDS: bone regeneration, histomorphometry, L-PRF, osteoconduction

INTRODUCTION

Despite the promising clinical results of short and narrow dental implants,[1,2] alveolar bone regeneration is still often required prior to or during the placement of implants to provide adequate oral rehabilitation. The use of biomaterials in alveolar bone regeneration has proven to be efficient in many clinical trials for several indications, such as localized site development, sinus lift, or socket preservation procedures.[3,4] Nevertheless, to reconstruct large defects or for vertical alveolar regeneration, autogenous bone is still needed for its osteogenic properties. In these cases, the morbidity related to the donor site remains a barrier for the patient to undergo the treatment. Moreover, bone regeneration using biomaterials alone still involves a long healing process, and there is a will to accelerate the biological process of bone healing and to provide the patient with short treatment. In this context, the use of natural or synthetic growth factors, such as bone morphogenic proteins (BMPs), platelet derived growth factor (PDGF), or transforming growth factor (TGF), combined with biomaterials, is of great interest and widely described in the literature.[5–8] Nevertheless, it is still poorly applied in a daily practice because of ethical and financial concerns.

Some authors have suggested the use of platelet concentrates to accelerate bone growth.[9–13] Indeed, platelets contain PDGF, vascular endothelial growth factor, TGF-β, and in combination with an appropriate

*Department of Periodontology and Oral Surgery, Faculty of Medicine, University of Liege, Liège, Belgium; †Hematology Laboratory-NARILIS, CHU Mont-Godinne, Department of Pharmacy, FUNDP Namur, University of Louvain, Namur, Belgium; ‡Giga-R, University of Liege, Liège, Belgium; §Inserm U957, Laboratory of Pathophysiology of Bone Resorption and Therapy of Primary of Bone Tumors, Faculty of Medicine, University of Nantes, Nantes, France

Reprint requests: Dr. France Lambert, Service de Médecine Dentaire, Domaine du Sart Tilman Bat B35, Liège B-4000, Belgium; e-mail: france.lambert@chu.ulg.ac.be

DOI 10.1111/cid.12146

scaffold, they may be able to deliver the required molecules to implement bone regeneration in situ. However, the real clinical efficacy of platelet concentrates is controversial in the available literature.[14–18] The most studied type of platelet concentrate is the platelet-rich plasma, which failed to exhibit a strong beneficial effect on bone regeneration.[14,16–18] The leukocyte- and platelet-rich fibrin (L-PRF or Choukroun's factor) is a second-generation platelet concentrate, obtained by a specific centrifugation procedure that limits the degree of blood manipulation and that does not require a platelet activator. The L-PRF has shown a satisfactory performance in soft tissue healing in periodontology,[19,20] physical medicine, or in esthetic surgery.[21]

The effect of L-PRF was studied in vitro and a promising effect on the proliferation and differentiation of osteoblast cells[22–25] through the delivery of PDG-F and TGF-β[26,27] was observed. Recent studies have also shown that L-PRF stimulated osteoblast adhesion and the upregulation of collagen protein production.[28] The fibrin architecture of L-PRF would also be an excellent scaffold for cell migration and angiogenesis, as well as a reservoir for growth factors[29] and cytokines, providing a slow release over 7 days.[26,27,30] Finally, the high content in leukocytes that are present in the thin layer near the red blood cells[31] might have a role in the regulation of inflammation and prevention of infection.[32,33]

However, there are few in vivo studies that report the positive biological effect of L-PRF in bone regeneration.[34] L-PRF has been used alone on mouse calvaria and enhanced the regeneration of the critical size bone defect.[31] Some clinical studies have described the use of L-PRF in sinus lift procedures,[35,36] a clinical model with favorable osteogenic potential due to the presence of multiple bone walls. Because of the absence of control groups and histology at different stages of the healing process, these case series do not prove the positive effect of L-PRF compared with other types of space fillers. Another clinical study showed a similar regenerated bone quantity after 6 months in sinus lifts performed using bovine hydroxyapatite alone or in combination with L-PRF.[37] However, histological samples at earlier steps in the bone regeneration process are needed to demonstrate the hypothetical beneficial effect of L-PRF on bone regeneration, but the harvesting of biopsies is ethically complicated to perform in human studies. For these reasons, it is relevant to use a standardized animal model to evaluate the performance of L-PRF in bone regeneration.

The goal of the present study was to assess the efficacy of L-PRF on bone healing using a guided bone regeneration model in rabbits. The benefits of L-PRF were assessed alone or in combination with bovine hydroxyapatite at several timepoints to evaluate the kinetics. Descriptive and quantitative histological analyses were carried out.

MATERIALS AND METHODS

Animals

New Zealand White rabbits were used in this study (adult males, average body weight of 3.0 kg). All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Ethics Committee of the University of Liège, Belgium. The "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, was followed carefully.

Study Design

A total of 72 hemispheres were implanted on the calvaria of 18 rabbits so that four titanium hemispheres filled with the four different studied materials were placed on each rabbit: L-PRF, bovine hydroxyapatite (BHA; Geistlich, Germany) only, BHA in combination with L-PRF, and an empty (control) hemisphere. The position of each group on the rabbit's skull was rotated. Extra rabbits were available in case of technical difficulties at the time of surgery. Six rabbits were sacrificed at three distinct time points: 1 week, 5 weeks, and 12 weeks. Therefore, each condition was repeated six times.

L-PRF Preparation

The L-PRF was produced extemporaneously according to Choukroun's protocol.[38] Four dry tubes of approximately 2 mL of blood were collected from each rabbit. They were centrifuged for 10 min at 400 *g* to obtain the L-PRF. The L-PRF clot was then dissected from the red cell clot and mixed with BHA or directly placed into the hemisphere (Figure 1, A and C).

Surgical Procedure

For analgesia, 2 hours before and for 2 days after the surgery, the rabbits received buprenorphine (0.05 mg/kg

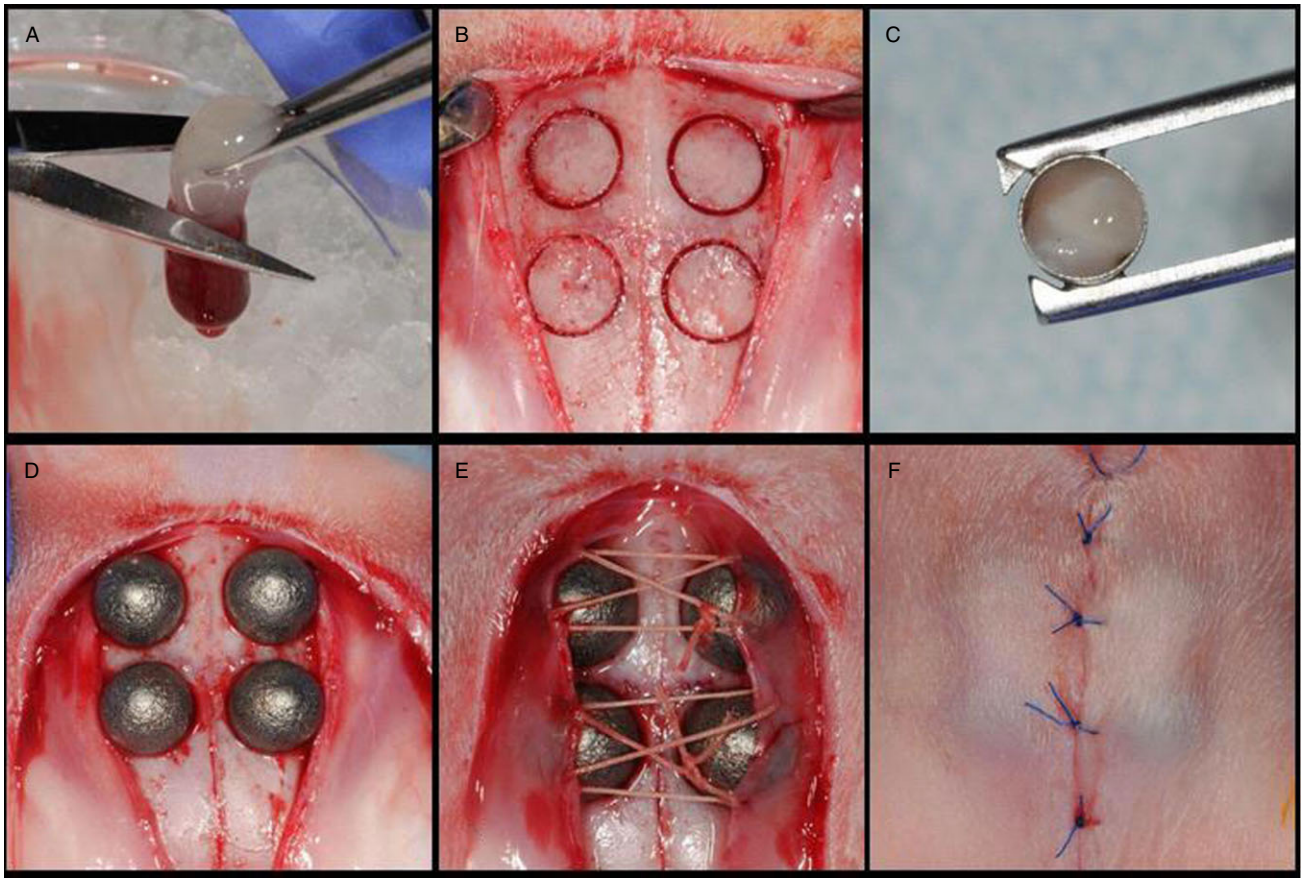

Figure 1 (A) L-PRF preparation, the red cell clot is removed; (B) the partial osteotomies; (C) L-PRF placed in the hemisphere; (D) the hemispheres inserted in the partial osteotomies; (E) periosteal closure; (F) wound closure. L-PRF = leukocyte- and platelet-rich fibrin.

s.c., 12 hourly). Rabbits received premedication with thalamonal (fentanyl/droperidol) i.m. 0.22 mL/kg. Anesthesia was induced by medetomidine (Domitor, Orion Corporation, Espoo, Finland) i.m. 0.25 mg/kg and a second injection of ketamine (Imalgène, Virbac, Carros, France) i.m. 25 mg/kg. Surgical interventions were performed under strict sterile conditions. The surgical area was shaved and disinfected with iodine. A median cutaneous incision was performed on the calvaria and the soft tissues were reflected to access the left and right parietal bone. Four split thickness osteotomies were performed with an 8 mm diameter trephine under saline irrigation. To create a regeneration chamber, titanium hemispheres of the same diameter were filled with the four studied materials and anchored in the grooves that resulted from the osteotomy. When HA particles were used, they were slightly packed. The wound was sutured with 4/0 polyester thread (Permasharp, Hu-Friedy, Rotterdam, the Netherlands). Animals were sacrificed by the injection of pentobarbital i.v. 200 mg/kg after premedication with thalamonal (fentanyl/droperidol) i.m. 0.22 mL/kg. Samples were collected and soaked in fixative (10% formol).

Histological Analyses

The samples were treated for nondecalcified histology using polymethacrylate (PMMA) resin. Dehydration was performed in ascending concentrations of ethanol for 24 hours ($1 \times 70°$, $1 \times 80°$, $2 \times 95°$, $3 \times 100°$) and samples were then soaked for 24 hours in acetone. Then samples were impregnated with methyl methacrylate for 48 hours at −20°C with a refreshment of medium. Finally, they were embedded in PMMA resin in the presence of an initiator and propagator for radical polymerization at 4°C for 4 days.

The samples were cut medially in two parts: one part was used to produce 30-micron sections and descriptive analyses of the regeneration chamber; the second part underwent scanning electron microscopy and subsequent quantitative analyses of newly formed

bone and biomaterials. A diamond microtome saw (Leica SP1600, Leica, Solms, Germany) and a grinder/polisher (Metaserv 250, Grinder/Polisher, Bluewher, Dusseldorf, Germany) were used. The sections were mounted on glass plates and stained using methylene blue (1%) for 90 seconds and basic fushin (0.3%) for 25 seconds. The histological sections were acquired using a slide scanner (Nanozoomer, Hamamatsu, Hamamatsu City, Japan). Scanning electron micrographs were taken (Hitachi TM3000 Tabletop Microscope, Hitachi, Tokyo, Japan) at 50× magnification and contiguous images were assembled to assess the complete regeneration chamber. The resulting image allowed for the quantitative measurement of the mineralized bone and the quantity of biomaterial based on their gray intensity with the freeware ImageJ (ImageJ, National Institutes of Health, Bethesda, MD, USA). The following measurements were made.

Newly formed bone surface: expressed as percentages of the region of interest. Biomaterial surface: expressed as percentages of the region of interest. Noncalcified tissue surface: expressed as percentages of the region of interest.

Statistical analysis

For each variable, all sample values were expressed as the mean, standard deviation, minimum and maximum. The bone quantity was analyzed using the multifactor two-way analysis of variance (ANOVA) using time as the second variable. The statistical significance level was 5% ($p < .05$).

RESULTS

All animals recovered from surgery without complications and gained weight normally without signs of pain. From 19 rabbits, 73 hemispheres were retrieved. One extra rabbit was used at the first timepoint because of technical complications at the time of surgery. Each hemisphere was considered as a statistically independent experimental unit, and there were at least six hemispheres for each experimental condition. At euthanasia, clinical observations of the dissected samples were normal. Signs of inflammation, tissue necrosis, and infection were not observed at any timepoint and in none of the samples from the different groups.

After 1 week of implantation, none of the different groups exhibited mineralized bone in the chambers under the titanium hemispheres (Figure 2, A–D). The chambers appeared to be uniformly filled with BHA particles (Figure 2, C and D). Histology of the negative controls revealed that the majority of the left empty hemispheres appeared almost empty, not filled with tissue or a blood clot. The colonization of the hemisphere by connective tissue cells was low and was only observed in the region of the osteotomies (Figure 2E). For the L-PRF group, the L-PRF clot had substantially collapsed, and exhibited the appearance of a membrane (Figure 2F). The connective tissue cells were also observed in the region of the osteotomy but did not overrun into the L-PRF clot (Figure 2F). On the contrary, in the BHA samples, the connective tissues had colonized a larger surface of the chamber under the titanium hemisphere. Red blood cells were also observed in most of the samples (Figure 2G). In the BHA + L-PRF samples, L-PRF clots and voids were observed between the particles.

After 5 weeks, mineralized bone was observed in the chambers of all groups. The regenerated bone had already significantly progressed from the bone floor and was predominantly located along the walls of the titanium hemisphere (Figure 3, A–D). For some hemispheres, regardless of the group, the bone had only grown from one side. In the BHA and BHA+L-PRF groups, the newly formed bone was observed to be in close contact with the BHA particle (Figure 3, G and H) and the rest of the regeneration room was completely colonized with highly vascularized connective tissues. On the contrary, in the left empty and the L-PRF groups, remaining blood clots were observed (Figure 3E).

After 12 weeks, most of the samples were fully colonized with new bone tissue (Figure 4, A–D). The noncalcified tissue portions were filled with bone marrow and adipocytes in the lower parts of the chamber and with connective tissue in the upper part (Figure 4, E–H). The newly formed bone was again in close contact with the BHA particles at 3 months, while thin bone trabeculae were observed in the curvy shape of the titanium hemisphere (Figure 4, G and H). The BHA particles were still present after 12 weeks and did not seem to degrade over.

Histomorphometric analysis corroborated the qualitative results (Table 1). The bone quantity significantly increased with time in the different groups: it was 0% after 1 week and increased to approximately 5% at 5 weeks and 22% at 12 weeks. The presence of BHA particles did not significantly favor or hamper the

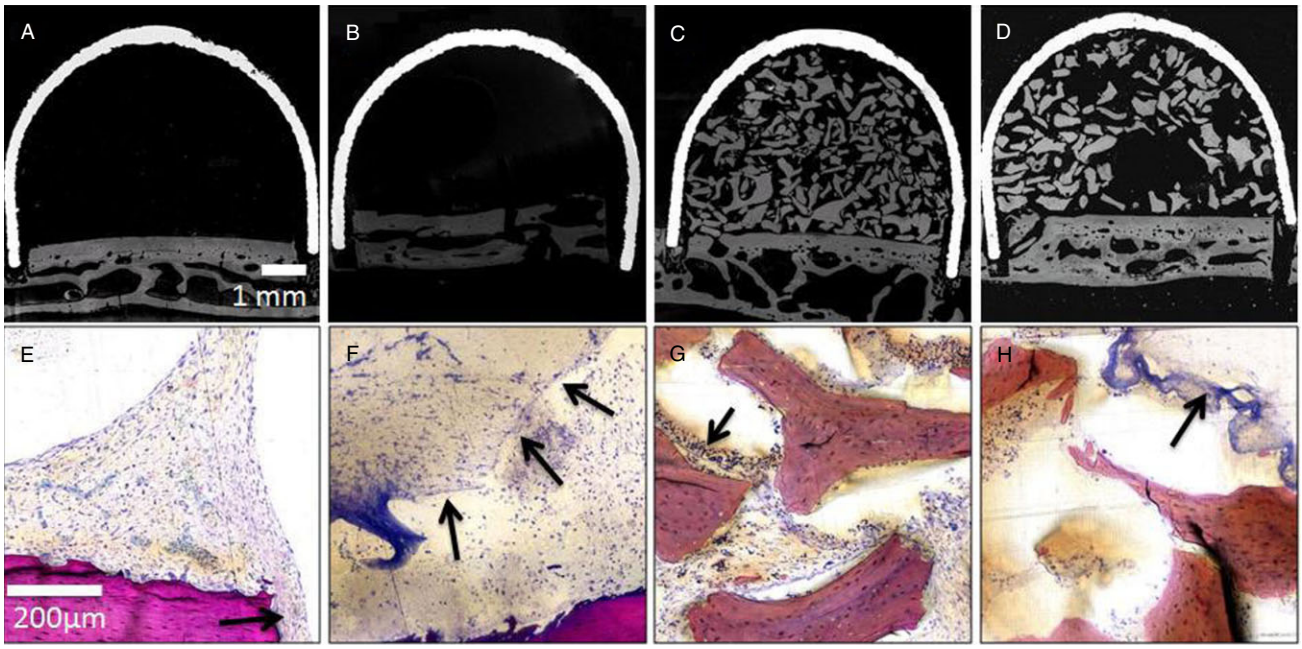

Figure 2 The same scale was used for A, B, C, D and E, F, G, H, respectively. SEM pictures at 1 week of: (A) empty hemisphere; (B) L-PRF hemisphere; (C) BHA hemisphere; (D) BHA + L-PRF hemisphere. Methylene blue/basic fushine pictures: (E) empty hemisphere, the black arrow indicates the groove of the osteotomy; (F) L-PRF hemisphere, the black arrows indicate the split between L-PRF and the connective tissues; (G) BHA hemisphere, black arrow showing red blood cells; (H) BHA + L-PRF hemisphere, the black arrow indicates the L-PRF. BHA = bovine hydroxyapatite; L-PRF = leukocyte- and platelet-rich fibrin; SEM = scanning electron microscopy.

amount of mineralized bone. The amount of BHA particles was not significantly different from 1 week to 12 weeks, remaining approximately 30%. After applying two-way ANOVA to the bone quantity data with time and conditions, no significant interaction effects between the two were found ($p = .7050$). The different conditions had no relevant effect on the quantity of bone ($p = .3623$; Figure 5), while the time effect was significant ($p < .0001$) (Table 1). The two-way ANOVA was also used to analyze the quantity of bone hydroxyapatite loaded in the BHA and BHA + LPRF groups. The time effect and the condition effect (with or without L-PRF) were significant ($p = .01837$ and $p < .0001$, respectively).

DISCUSSION

The effect of L-PRF on osteoblasts in promoting bone formation has been widely explored in vitro,[22–25] and the results are promising. However, the clinical beneficial effect of L-PRF on bone regeneration regarding the kinetics of bone regeneration has been improperly explored. A few clinical trials were carried out using L-PRF in sinus lift models,[35–37] lacking a control group and sufficient subjects/specimens. The aim of the present study was to assess the impact of L-PRF on guided bone regeneration in a standardized model alone or in combination with BHA. The main studied parameter was the bone quantity.

The results displayed the presence of adequate osteogenesis in the four groups and no significant difference was observed at any timepoint in terms of regenerated bone quantity ($p = .3623$). These results might be surprising according to the multiple in vitro studies that show an increase in osteoblast proliferation, differentiation, and protein production[22–25] when cultured in presence of L-PRF. However, in an in vivo model, the lack of a positive effect of L-PRF on the kinetics, quality, and quantity of bone regeneration could be due to the early placement of L-PRF at the time surgery. Indeed, the load of L-PRF takes place while neither connective-vascularized tissue nor osteoblast precursors are available on site. According to the literature, L-PRF is active for 7 days[26,27,30] which does not seem to be long enough in the context of a cell-free environment like the titanium hemisphere used here. Nevertheless, if the present study does not seem to emphasize the presence of faster bone regeneration with L-PRF compared with the other groups, the limitations of the study have to be considered, because the model is not a critical size defect but a

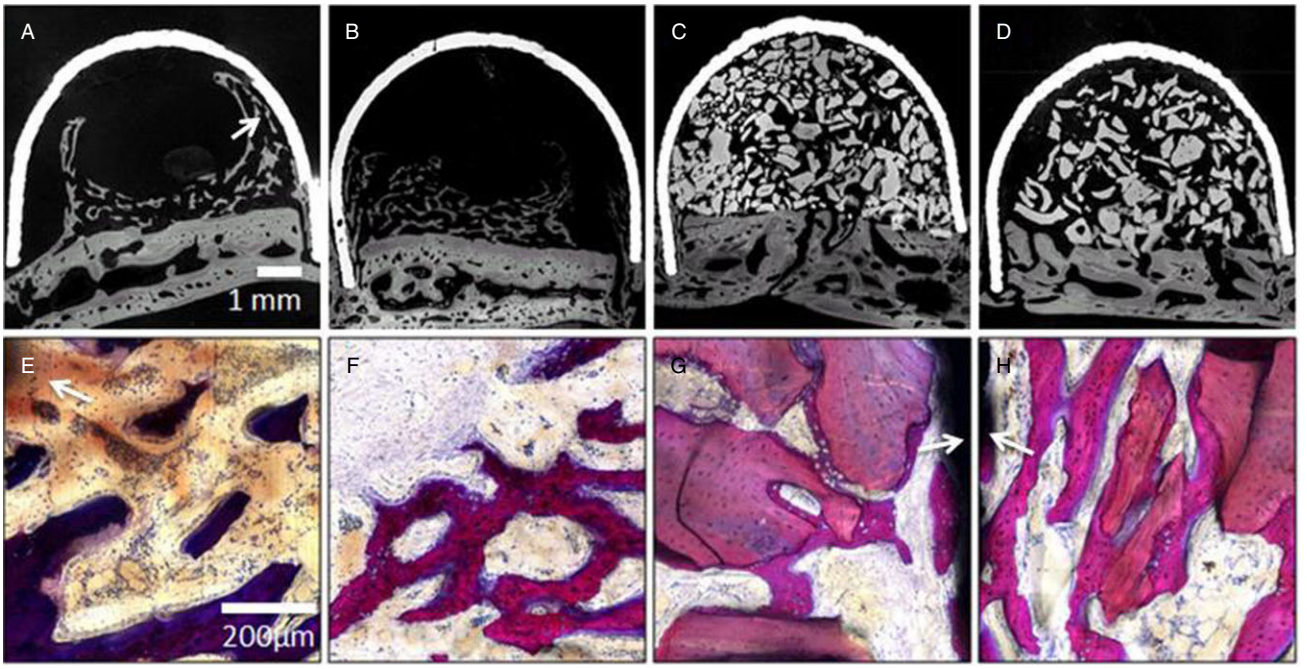

Figure 3 The same scale was used for A, B, C, D and E, F, G, H, respectively. SEM pictures at the 5-week time point: (A) empty hemisphere, the white arrow shows the bone growing against the wall; (B) L-PRF hemisphere; (C) BHA hemisphere; (D) BHA + L-PRF hemisphere. Methylene blue/basic fushine pictures: (E) empty hemisphere, the white arrow indicates a massive blood clot; (F) L-PRF hemisphere; (G) BHA hemisphere, the white arrow indicates the titan wall; (H) BHA + L-PRF hemisphere, the white arrow indicates the titan wall. BHA = bovine hydroxyapatite; L-PRF = leukocyte- and platelet-rich fibrin; SEM = scanning electron microscopy.

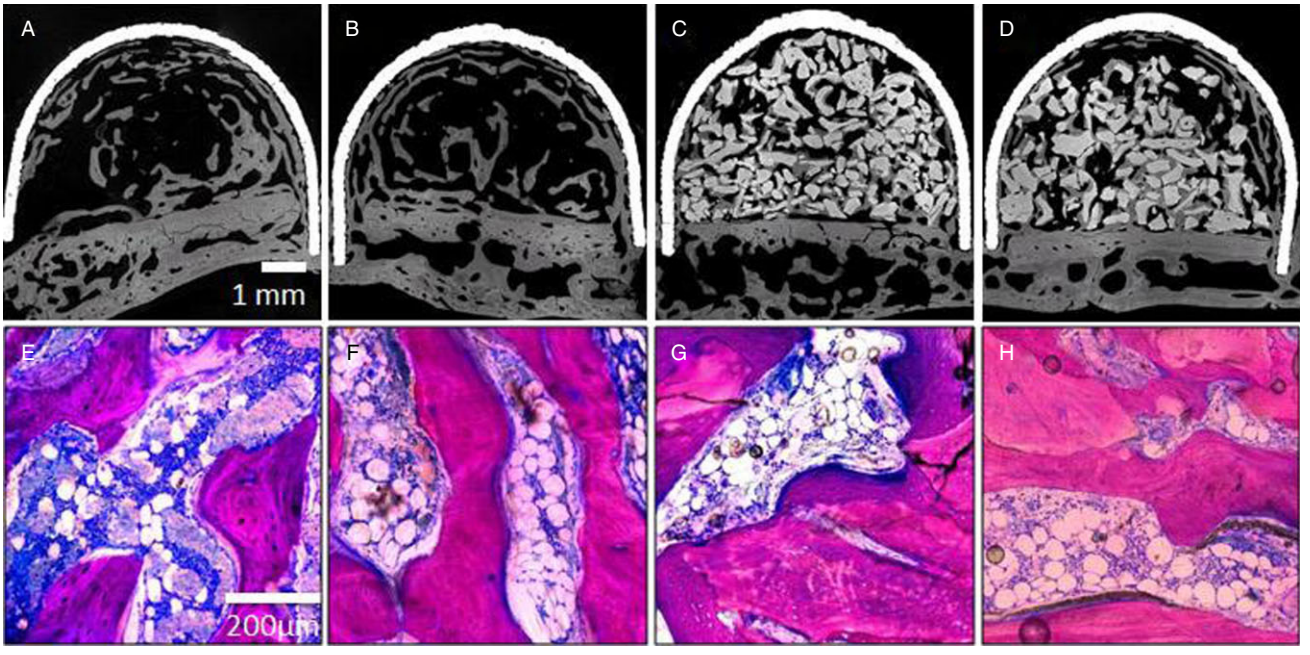

Figure 4 The same scale was used for A, B, C, D and E, F, G, H, respectively. SEM pictures at the 12-week time point of: (A) empty hemisphere; (B) L-PRF hemisphere; (C) BHA hemisphere; (D) BHA + L-PRF hemisphere. Methylene blue/basic fushine pictures: (E) empty hemisphere; (F) L-PRF hemisphere; (G) BHA hemisphere; (H) BHA + L-PRF hemisphere. BHA = bovine hydroxyapatite; L-PRF = leukocyte- and platelet-rich fibrin; SEM = scanning electron microscopy.

TABLE 1 Bone Quantity, Biomaterial Quantity, and ANOVA-2 Results

Group	Time	Bone Quantity Mean (SD) (Min-Max) (%)	BHA Quantity Mean (SD) (Min-Max) (%)	ANOVA 2 On % Bone Time Effect	*p*-Value Group Effect	Interaction
Empty	1	0.00 (0.00) (0.00–0.00)	0.00 (0.00) (0.00–0.00)	<.0001	.3623	.705
L-PRF	1	0.00 (0.00) (0.00–0,00)	0.00 (0.00) (0,00–0.00)			
Bio-Oss	1	0.00 (0.00) (0.00–0.00)	40.20 (5.34) (32.80–44.65)			
BHA + LPRF	1	0.00 (0.00) (0.00–0.00)	34.50 (3.68) (29.6–39.70)			
Empty	5	7.43 (4.41) (1.64–12.9)	0.00 (0.00) (0.00–0.00)			
L-PRF	5	11.25 (4.59) (5.91–17.07)	0.11 (0.26) (0.00–0.64)			
BHA	5	6.87 (2.73) (3.60–10.60)	39.12 (4.52) (36.00–45.73)			
BHA + LPRF	5	7.20 (4.10) (3.00–13.60)	26.25 (6.01) (21.48–36.33)			
Empty	12	25.77 (8.41) (12.80–36.43)	0.00 (0.00) (0.00–0.00)			
L-PRF	12	24.11 (6.57) (15.88–34.41)	0.00 (0.00) (0.00–0.00)			
BHA	12	21.15 (7.24) (11.00–30.00)	34.07 (4.26) (27.15–40.02)			
BHA + LPRF	12	21.42 (7.37)	29.20 (5.35)			

The effect of time was significant, but there are no significant effects of the presence of biomaterial or L-PRF.
ANOVA = analysis of variance; BHA = bovine hydroxyapatite; L-PRF = leukocyte- and platelet-rich fibrin.

bone augmentation model. A possible alternative to regenerate bone would be to load scaffolds with mesenchymal stem cells at the time of surgery. Indeed, it has been shown that human mesenschymal stem cells (hMSCs) harvested under local anesthesia from bone marrow or adipose tissue are easily isolated and amplified in culture to several hundred millions in 2 to 3 weeks. When associated with biomaterial or fibrin scaffolds at the time of surgery, hMSCs are able to regenerate bone tissue by osteoinduction.

It has been stated that L-PRF could also stimulate fibroblast migration and angiogenesis in vitro.[29] According to the present findings, L-PRF did not seem to have any positive effect on connective cell proliferation inside the regeneration room. The reason might be because of the high density of L-PRF that even seems to block cell penetration (Figure 2F). As stated before, the L-PRF would be a good support for the migration of fibroblasts[23] and angiogenesis,[29,31] because its fibrin structure can be penetrated by fibroblasts.[29,31] However, no difference in cell colonization was observed in our model in the presence of L-PRF. In clinical studies, L-PRF seems to facilitate the regeneration of soft tissues,[39–41] suggesting an effect on fibroblast migration or proliferation. Again, this effect was not observed in this study. However, it has never been clearly shown that fibroblasts are able to migrate inside the L-PRF. Instead, it might be that cells progress and develop around the L-PRF, which would effectively promote cell migration. In the present study, we did not observe any effect of L-PRF on cell colonization, although no cell counting in the chambers was performed.

We observed that BHA particles mixed with L-PRF were less packed than the BHA alone. This explains

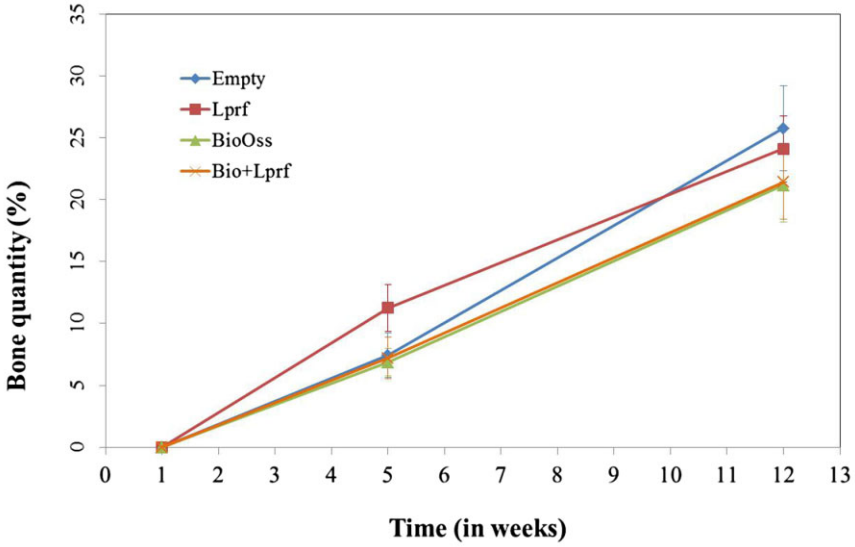

Figure 5 Distribution of the bone quantity over time in the different groups. There is no significant difference between the four groups.

the significant difference in terms of the BHA particle surface in the two conditions, and this difference should not be interpreted as biomaterial resorption in the presence of L-PRF. Nevertheless, the quantity of bone was not significantly different between the two groups, even though the BHA particles were less packed in the L-PRF + BHA group.

Osteoconduction was clearly observed along the titanium walls, and the shape of bone trabeculae appeared to be driven by the hemisphere architecture (Figure 3, G and H). This observation is in accordance with the results of other studies that used a similar model[42–44] and could be explained by the fact that the titanium surface was not completely smooth.[36,45,46] Moreover, in many samples at 5 and 12 weeks, the regenerated bone was found only on one side of the hemisphere. The hypothetical explanation of this observation might be related to the lack of basal bone perforation[47] and thereby an unhomogenous blood supply comes mainly from the osteotomy.

The L-PRF might also have an antibacterial effect related to the presence of leukocytes. However, this hypothesis was not explored in the present study, and further investigation of that matter may be of interest.

CONCLUSION

According to the present study, L-PRF does not seem to provide any additional effect on the kinetics, quality, and quantity of guided bone regeneration in vivo. Further investigations, including critical size defect models, are needed to confirm these findings. Nevertheless, no negative effect was observed, and its clinical use in oral surgery might be relevant for soft tissue healing.

ACKNOWLEDGMENTS

The authors wish to express thanks to Jerome Amiaud and Luc Duwez for their technical expertise and support in histological procedures and animal care, respectively. The authors declare that there is no conflict of interest in this study. The study was self-funded by the authors and their institution.

REFERENCES

1. Esposito M, Maghaireh H, Grusovin MG, Ziounas I, Worthington HV. Interventions for replacing missing teeth: management of soft tissues for dental implants. Cochrane Database Syst Rev 2012; (2)CD006697.
2. Renouard F, Nisand D. Impact of implant length and diameter on survival rates. Clin Oral Implants Res 2006; 17(Suppl 2):35–51.
3. Pjetursson BE, Tan WC, Zwahlen M, Lang NP. A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. J Clin Periodontol 2008; 35:216–240.
4. Lambert F, Vincent K, Vanhoutte V, Seidel L, Lecloux G, Rompen E. A methodological approach to assessing alveolar ridge preservation procedures in humans: hard tissue profile. J Clin Periodontol 2012; 39:887–894.
5. Kaigler D, Avila G, Wisner-Lynch L, et al. Platelet-derived growth factor applications in periodontal and peri-implant

bone regeneration. Expert Opin Biol Ther 2011; 11:375–385.
6. Gutwald R, Haberstroh J, Stricker A, et al. Influence of rhBMP-2 on bone formation and osseointegration in different implant systems after sinus-floor elevation. An in vivo study on sheep. J Craniomaxillofac Surg 2010; 38: 571–579.
7. Triplett RG, Nevins M, Marx RE, et al. Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. J Oral Maxillofac Surg 2009; 67:1947–1960.
8. Boyne PJ, Lilly LC, Marx RE, et al. De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. J Oral Maxillofac Surg 2005; 63:1693–1707.
9. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85:638–646.
10. Gruber R, Varga F, Fischer MB, Watzek G. Platelets stimulate proliferation of bone cells: involvement of platelet-derived growth factor, microparticles and membranes. Clin Oral Implants Res 2002; 13:529–535.
11. Carlson NE, Roach JRB. Platelet-rich plasma: clinical applications in dentistry. J Am Dent Assoc 2002; 133:1383–1386.
12. Sánchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. Int J Oral Maxillofac Implants 2003; 18:93–103.
13. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost 2004; 91:4–15.
14. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009; 27:158–167.
15. Dohan Ehrenfest DM, Bielecki T, Mishra A, et al. In search of a consensus terminology in the field of platelet concentrates for surgical use: Platelet-rich plasma (PRP), platelet-rich fibrin (PRF), fibrin gel polymerization and leukocytes. Curr Pharm Biotechnol 2012; 13:1131–1137.
16. Del Fabbro M, Bortolin M, Taschieri S. Is autologous platelet concentrate beneficial for post-extraction socket healing? A systematic review. Int J Oral Maxillofac Surg 2011; 40:891–900.
17. Esposito M, Grusovin MG, Rees J, et al. Effectiveness of sinus lift procedures for dental implant rehabilitation: a Cochrane systematic review. Eur J Oral Implantol 2010; 3:7–26.
18. Simonpieri A, Del Corso M, Vervelle A, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery. Part 2: bone graft, implant and reconstructive surgery. Curr Pharm Biotechnol 2012; 13:1231–1256.
19. Chang YC, Zhao JH. Effects of platelet-rich fibrin on human periodontal ligament fibroblasts and application for periodontal infrabony defects. Aust Dent J 2011; 56:365–371.
20. Del Corso M, Vervelle A, Simonpieri A, et al. current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery. Part 1: periodontal and dentoalveolar surgery. Curr Pharm Biotechnol 2012; 13:1207–1230.
21. Cieslik-Bielecka A, Choukroun J, Odin G, Dohan Ehrenfest DM. L-PRP/L-PRF in esthetic plastic surgery, regenerative medicine of the skin and chronic wounds. Curr Pharm Biotechnol 2012; 13:1266–1277.
22. Clipet F, Tricot S, Alno N, et al. In vitro effects of Choukroun's platelet-rich fibrin conditioned medium on 3 different cell lines implicated in dental implantology. Implant Dent 2012; 21:51–56.
23. Dohan Ehrenfest DM, Diss A, Odin G, Doglioli P, Hippolyte M-P, Charrier J-B. In vitro effects of Choukroun's PRF (platelet-rich fibrin) on human gingival fibroblasts, dermal prekeratinocytes, preadipocytes, and maxillofacial osteoblasts in primary cultures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108:341–352.
24. Dohan Ehrenfest DM, Doglioli P, de Peppo GM, Del Corso M, Charrier J-B. Choukroun's platelet-rich fibrin (PRF) stimulates in vitro proliferation and differentiation of human oral bone mesenchymal stem cell in a dose-dependent way. Arch Oral Biol 2010; 55:185–194.
25. Chang IC, Tsai C-H, Chang Y-C. Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts. J Biomed Mater Res A 2010; 95:327–332.
26. Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): a gold standard to achieve for all surgical platelet concentrates technologies. Growth Factors 2009; 27:63–69.
27. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101:e45–e50.
28. Wu CL, Lee SS, Tsai CH, Lu KH, Zhao JH, Chang YC. Platelet-rich fibrin increases cell attachment, proliferation and collagen-related protein expression of human osteoblasts. Aust Dent J 2012; 57:207–212.
29. Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101:e56–e60.
30. Dohan Ehrenfest DM, Bielecki T, Jimbo R, et al. Do the fibrin architecture and leukocyte content influence the growth factor release of platelet concentrates? An evidence-based answer comparing a pure platelet-rich plasma

(P-PRP) gel and a leukocyte- and platelet-rich fibrin (L-PRF). Curr Pharm Biotechnol 2012; 13:1145–1152.
31. Kang Y-H, Jeon SH, Park J-Y, et al. Platelet-rich fibrin is a bioscaffold and reservoir of growth factors for tissue regeneration. Tissue Eng Part A 2011; 17:349–359.
32. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101:e51–e55.
33. Bielecki T, Dohan Ehrenfest DM, Everts PA, Wiczkowski A. The role of leukocytes from L-PRP/L-PRF in wound healing and immune defense: new perspectives. Curr Pharm Biotechnol 2012; 13:1153–1162.
34. Simonpieri A, Del Corso M, Vervelle A, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 2: Bone graft, implant and reconstructive surgery. Curr Pharm Biotechnol 2012; 13:1231–1256.
35. Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101:299–303.
36. Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM. Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: a radiologic and histologic study at 6 months. J Periodontol 2009; 80:2056–2064.
37. Zhang Y, Tangl S, Huber CD, Lin Y, Qiu L, Rausch-Fan X. Effects of Choukroun's platelet-rich fibrin on bone regeneration in combination with deproteinized bovine bone mineral in maxillary sinus augmentation: a histological and histomorphometric study. J Craniomaxillofac Surg 2012; 40:321–328.
38. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101:e37–e44.
39. Gorlero F, Glorio M, Lorenzi P, Bruno-Franco M, Mazzei C. New approach in vaginal prolapse repair: mini-invasive surgery associated with application of platelet-rich fibrin. Int Urogynecol J 2012; 23:715–722.
40. Del Corso M, Sammartino G, Dohan Ehrenfest DM. Re: "Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study". J Periodontol 2009; 80:1694–1697. author reply 1697-1699.
41. Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. J Periodontol 2009; 80:244–252.
42. Tamura T, Fukase Y, Goke E, et al. Three-dimensional evaluation for augmented bone using guided bone regeneration. J Periodontal Res 2005; 40:269–276.
43. Yamada Y, Nanba K, Ito K. Effects of occlusiveness of a titanium cap on bone generation beyond the skeletal envelope in the rabbit calvarium. Clin Oral Implants Res 2003; 14:455–463.
44. Lundgren D, Lundgren AK, Sennerby L, Nyman S. Augmentation of intramembraneous bone beyond the skeletal envelope using an occlusive titanium barrier. An experimental study in the rabbit. Clin Oral Implants Res 1995; 6:67–72.
45. Bacakova L, Filova E, Parizek M, Ruml T, Svorcik V. Modulation of cell adhesion, proliferation and differentiation on materials designed for body implants. Biotechnol Adv 2011; 29:739–767.
46. Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Implants Res 2009; 20(Suppl 4):172–184.
47. Rompen EH, Biewer R, Vanheusden A, Zahedi S, Nusgens B. The influence of cortical perforations and of space filling with peripheral blood on the kinetics of guided bone generation. A comparative histometric study in the rat. Clin Oral Implants Res 1999; 10:85–94.