A lack of bone volume may compromise a correct three-dimensional implant placement. This study was designed to evaluate the clinical and radiographic outcomes of simultaneous horizontal guided bone regeneration (GBR) performed using autogenous bone or blood-derived products mixed with a bone xenograft. The study population consisted of patients operated on using one of two clinical protocols for GBR: group A, which used autogenous bone mixed with a bone xenograft, and group B, which used advanced platelet-rich fibrin (A-PRF) mixed with a bone xenograft. The primary outcome was the clinical gain in the peri-implant defect. The secondary outcomes included an analysis of the postoperative healing, periodontal parameters, marginal bone loss, and occurrence of adverse events. All of the surgeries were carried out successfully. One patient in each group experienced a case of early implant loss, and three patients (one in group A and two in group B) presented biologic complications. The mean peri-implant vertical defect heights at baseline in group A and group B were 3.6 ± 0.9 mm and 4 ± 1.5 mm, respectively (P = .382). No statistically significant differences in the mean residual defect heights (P = .521) or in the postoperative wound healing (P = .611) were observed. Stable peri-implant marginal bone levels were recorded after loading in both groups. The use of A-PRF combined with a particulate bone xenograft and covered with a fixed collagen membrane may provide clinical results similar to those obtained via autogenous bone mixed with bone xenograft.


Modern implantology is often subject to anatomical limitations caused by vertical and/or horizontal alveolar bone resorption.1,2 Guided bone regeneration (GBR) has been shown to be an excellent technique for alveolar ridge augmentation.3–10

Autogenous bone grafts are still considered the gold standard due to their combined osteoconductive, osteoinductive, and osteogenic properties.11 Regenerative techniques are also used in combination with particulate graft materials, such as anorganic bovine bone mineral (ABBM), freeze-dried bone allograft (FDBA), demineralized FDBA (DFDBA), β-tricalcium phosphate/hydroxyapatite, and demineralized bovine bone matrix (DBBM). Each one has different characteristics in terms of resorbability, osteoconductivity, and biologic factors, and they can also be mixed with particulate autologous bone.

Leukocyte-platelet-rich fibrin (L-PRF) was developed by Choukroun et al12 to be used in oral and maxillofacial surgery.13 It belongs to a new generation of platelet aggregates obtained from the patient’s blood. This technique requires neither anticoagulants nor thrombin of bovine origin (or any other gelling agent), giving it a comparative advantage over other derivatives, as no medical or legal problems arise regarding the manipulation of blood.

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L-PRF seems able to induce a greater proliferation of periodontal cells and a more rapid wound healing, as it is full of autologous platelets, growth factors (IGF-1, TGFβ-1, VEGF), cytokines (IL-1, IL-4, IL-6), and leukocytes. Recently, Choukroun et al. developed a new blood component, advanced platelet-rich fibrin (A-PRF), which contains a higher number of white blood cells. A-PRF is obtained via a different centrifugation protocol that reduces the centrifugal g-force, and this process leads to a greater number of leukocytes, which have a crucial role in the wound healing process, as previously mentioned.

This study was designed to evaluate the clinical and radiographic outcomes of simultaneous GBR performed using a bone xenograft mixed with either autogenous bone graft or blood-derived products on patients requiring implant placement in the maxilla or mandible followed by a horizontal bone augmentation.

Materials and Methods

This study was designed as a retrospective comparative study to evaluate the clinical and radiographic outcomes of implant placement with simultaneous GBR. The study population consisted of all patients operated on from January 1, 2016, to January 1, 2018, in the Department of Oral Surgery and Implantology of the Catholic University of the Sacred Heart for implant placement on posterior teeth using two clinical protocols for GBR: (1) autogenous bone mixed with a bone xenograft (group A), or (2) A-PRF mixed with a bone xenograft (group B). The decision to perform one of these two protocols was made following discussion with the patients. Patients who refused to undergo autogenous bone graft harvesting were treated using DBBM mixed with A-PRF and assigned to group B, whereas patients who agreed to undergo an autogenous bone graft harvesting were assigned to group A. All procedures were performed in accordance with the Declaration of Helsinki. Because of the retrospective nature of this study, it was granted an exemption in writing by the local ethics committee. Each participant who enrolled in the study received explanations about the study design and objectives, and they provided written informed consent. Participants satisfying the following entry criteria were included in the present study: (1) in need of implants in the maxilla or mandible; (2) in need of horizontal bone augmentation to cover a dehiscence defect; (3) extraction of the tooth performed at least 12 weeks prior to implant placement and bone augmentation; (4) sufficient vertical dimension; (5) primary stability of the implant; (6) full-mouth plaque and bleeding scores < 15%; and (7) age > 20 years at the time of written consent.

The exclusion criteria were as follows: (1) a general contraindication for implant placement and/or surgical treatment; (2) active periodontal disease; (3) use of any drug and medication known to affect oral status and bone turnover or contraindicate surgical treatment (immunosuppressant, corticosteroid, or bisphosphonate therapy); (4) history of malignancy, radiotherapy, or chemotherapy for malignancy; (5) smoking habit; (6) blood-related diseases; (7) limited mental capacity or language skills, or suffering from a known psychologic disorder; and (8) previously underwent a GBR procedure in the region of interest.

The peri-implant osseous defect was measured after implant placement using a periodontal probe (UNC-15), and the following parameters were recorded to classify the defects:

- Vertical defect height, measured from the implant shoulder to the first bone-to-implant contact.
- Intrabony defect height, measured from the bone crest to the first bone-to-implant contact.
- Defect width, measured from the mesial to the distal bone crests at the level of the implant shoulder.
- Horizontal defect depth, measured from the bone crest to the implant surface in a direction perpendicular to the long axis of the implant.

Outcome Variables

Clinical assessments were performed at baseline and postoperatively at weeks 1 and 3 and at months 3, 6, 9, and 24. A CBCT image was taken both prior to GBR and after 9 months of healing (before the second-stage surgery) at a
resolution of 100 mm (Orthophos XG 3D, Dentsply Sirona). The primary outcome measure was the clinical gain in the peri-implant defect at 9 months after implant placement and horizontal bone augmentation, which was calculated during the second-stage surgery with a calibrated periodontal probe. If a residual defect was observed, it was classified by analyzing its vertical height and width with the periodontal probe. The secondary outcomes included an analysis of the postoperative healing, periodontal parameters, peri-implant marginal bone loss after the prosthesis delivery, and occurrence of early and late adverse events.

Postoperative healing was assessed using the Landry, Turnbull, and Howley index every 7 days for the first 3 weeks.

The periodontal parameters measured at the two teeth adjacent to the defect at baseline and after 9 months with the periodontal probe UNC-15 were: Plaque Index (PI)\(^2\); bleeding on probing (BOP); Gingival Bleeding Index (GBI)\(^2\); probing pocket depths (PPD), which were performed at six sites on each tooth; and keratinized mucosa width.

The peri-implant marginal bone loss was assessed after prosthesis delivery and at 24 months with intraoral radiography using the long-cone parallel technique. A silicone bite was placed in the holding system, allowing for it to be repositioned precisely during each follow-up visit. Linear measurements were performed on the digital images to record the distances of the most coronal points of the bone from the implant shoulder in the mesial and distal ridge aspects.

**Surgical Procedure**

All the surgeries were performed by the same trained surgeon (P.D.A.). Prior to surgery, the patients received antibiotics (2 × 1 g amoxicillin clavulanate; Augmentin, GlaxoSmithKline) and analgesics/antiphlogistics (50 mg ketoprofen; Orudis). Surgery was performed under local anesthesia (articaine 4% with epinephrine 1:100,000). The horizontal incision was extended from the distal aspect of the mesial tooth to the mesial aspect of the distal tooth. The incision was continued intrasulcularly in both the buccal and lingual areas. Releasing incisions were performed at the buccal, mesial, and distal line angles. A mucoperiosteal flap was raised, and the bone was exposed and carefully curetted.

An osteotomic site was created following the manufacturers’ protocols, and a bone-level implant was placed. During implant placement, primary stability was assessed via insertion torque and hand testing. Defect measurements were performed. The cortical plate was perforated by means of a round bur to augment bleeding from the bone and access to the marrow cavity.

In group A, the autogenous bone chips were collected from the areas surrounding the peri-implant defect using a bone scraper, then they were placed adjacent to the implant surface and mixed with the deproteinized bovine bone mineral (particle size: 0.25 to 1 mm) using a 50:50 ratio. In group B, the peripheral blood was collected using a butterfly blood collection method and placed in a preprogrammed centrifuge (EBA 200, Hettich). Blood centrifugation was performed according to two protocols: (1) A-PRF (10 mL; 1,500 rpm for 14 minutes) and (2) injectable PRF (I-PRF; 10 mL; 700 rpm for 3 minutes). Following centrifugation, each A-PRF clot was removed from the tube and separated from the red element phase. Four A-PRF clots were squeezed between a sterile glass plate and a metal box to obtain A-PRF membranes of equal size and thickness.

To prepare the bone graft, the A-PRF membranes were cut into small pieces using scissors, then they were mixed with deproteinized bovine bone mineral at a ratio of 2 membranes per 0.5 g biomaterial (which provides, approximately, a 50:50 ratio). Subsequently, the mixture was hydrated using the A-PRF exudate from the preparation box to create a homogeneous graft, which was stirred gently. The graft was then shaped into the desired form, and the I-PRF was injected on the graft. The horizontal bone ridge augmentation procedure was performed using a resorbable membrane fixed on the lingual/palatal side with two or three fixation pins. The graft was then placed carefully to overfill the defect area. The residual A-PRF membranes were placed over the graft, and the collagen membrane was closed and fixed on the buccal side using two or three fixation pins. Periosteal releasing incisions
were used to release the mucoperiosteal flaps. The crestal incision was sutured with 5-0 PTFE mattress sutures; finally, single sutures were placed on the vertical incisions and between the mattress sutures.

Figures 1 and 2 show the surgical procedures for groups B and A, respectively.

The patients were instructed to rinse twice daily with a 0.2% chlorhexidine mouthrinse (Dentosan, Recordati) and to continue the antibiotic regimen for 5 days. In addition, analgesics (50 mg Orudis) were prescribed for the next 3 days according to individual needs. Patients were also instructed to refrain from mechanical plaque removal in the area of implantation for 2 weeks. The sutures were removed 21 days after surgery.

Statistical Analysis

The descriptive statistics used for the continuous factors included the mean, standard deviation (SD), median, and interquartile range (IQR); for categorical factors, absolute and relative frequencies (in percentages) were utilized. Two-sample Wilcoxon rank-sum (Mann-Whitney) test was adopted to make between-group comparisons for the continuous variables, and Pearson chi-square and Fisher exact tests were used in the case of categorical or binary factors. Multivariate logistic regression modeling was adopted to test the association between vertical gain (≥ 5 vs < 5) and treatment group while adjusting for all other factors and confounding variables. A stepwise backward selection algorithm was used to obtain the final model, using a significance level of 0.2. All analyses were conducted using Stata software (version 14.2, StataCorp). Two-tailed probabilities were reported, and an observed significance level of .05 was used to define nominal statistical significance.

Results

In total, 37 patients were included in this study. Group A consisted of 17 patients (mean age: 58.5 ± 9.7 years; 7 women and 10 men), while group B consisted of 20 patients (mean age: 59.6 ± 9.8 years; 11 women and 9 men).

All of the surgeries were carried out successfully, and no intraoperative complications were recorded. All implants obtained an adequate primary stability. Implant lengths ranged from 8 to 12 mm, and implant diameters ranged from 4.1 to 4.8 mm. One patient in each group experienced a case of early implant loss, and three patients (one in group A and 2 in group B) presented biologic complications during the first 3 weeks after surgery. All complications were cases of wound dehiscence, which were treated with local disinfectant (rinsing with 0.2% chlorhexidine mouthrinse and applying 1% chlorhexidine gel), and all affected patients recovered completely after 2 to 3 weeks.

The baseline clinical parameters were comparable among both groups, with no significant differences (P > .05). The mean vertical defect heights at baseline in group A and group B were 3.6 ± 0.9 mm and 4 ± 1.5 mm, respectively (P = .382). After 9 months, statistically significant reductions in BOP and PI were recorded in both groups compared to baseline findings (P < .05). The statistical comparisons of clinical parameters revealed no significant differences between the groups (P > .05 in all cases). In group A, three cases had a residual defect height, while there were two cases in group B. The mean residual defect widths of the two groups also showed no statistically significant difference (P = .53). No statistically significant difference between the postoperative keratinized mucosa widths was observed between the groups (P = .273). Finally, in both groups, a significant reduction in the keratinized mucosa width was observed from baseline (P < .05 in both cases). Statistically significant differences in postoperative wound healing were observed for the first week (P = .022), with a trend toward a greater degree of healing in group B. No statistically significant differences in postoperative wound healing were observed at 3 weeks (P = .611). Stable peri-implant marginal bone levels were recorded after loading in both groups, and no implant showed a marginal bone loss > 1.5 mm. No statistically significant difference in marginal bone loss was found between the two groups (P > .05). All the
prosthetic restorations were monolithic screw- and cement-retained, and at the 24-month follow-up, 86% of patients were free from biologic or prosthetic complications.

**Discussion**

The results of the present study reveal that the parameters of the peri-implant defects improved significantly after the regeneration procedure and the healing period. No statistically significant differences could be observed between the two groups in terms of bone regeneration. Fig 1 Case 1 (group B). (a) A CBCT scan was taken prior to GBR. (b) A-PRF membranes after centrifugation. (c) Osteotomy sites were created following the manufacturer’s protocol. (d) Implants were placed in the osteotomy sites. (e) A collagen membrane was placed and fixed using fixation pins. (f) Clinical view of the final suturing. (g) Postoperative CBCT scans of the mesial implant and (h) the distal implant, both taken prior to surgical reentry. (i) Intraoral radiographic view after implant placement. (j) Lateral view at prosthesis delivery.
regeneration, peri-implant defect closure, periodontal parameters, and postoperative wound healing.

In a systematic review by Thoma et al, the most commonly used treatment in the case of peri-implant bony dehiscence was the combination of a native collagen membrane and a xenogeneic particulate graft. According to that review, there is no evidence that a specific treatment modality is required, as the heterogeneity between the studies was large and several barrier membranes and grafting materials were used. The positive results in terms of defect resolution found in the present study may be related to the favorable tridimensional architecture of the peri-implant defects, which provided significant regeneration potential. Another explanation may be the effect of the graft stabilization achieved in both groups. Group B took advantage of the stabilization effect of platelet concentrates in addition to the fixation pins. It has been reported in the literature that fixing membranes with pins or osteosynthesis screws increases their stability and leads to more predictable and stable results over time.

Concerning the type of graft, the combination of autogenous bone chips with a DBBM is a well-documented and studied approach. In this approach, the accelerated bone formation may be related to the release of growth factors, which can facilitate the
osseointegration of implants and earlier graft consolidation. However, the use of PRF has several advantages: (1) simple preparation protocol; (2) significant angiogenic potential due to several angiogenic factors, such as basic fibroblast growth factor, vascular endothelial growth factor, and platelet-derived growth factor; and (3) it also participates in the osteogenic process due to the presence of the bone morphogenetic proteins in the fibrin matrix.\textsuperscript{12,25} Choukroun et al\textsuperscript{16} showed that A-PRF stimulates a greater amount of growth factor release when compared to the standard PRF; moreover, the distribution of neutrophilic granulocytes in the A-PRF clot is likely responsible for the improved functionality of the monocytes/macrophages and lymphocytes and their actions in tissue regeneration. In addition, A-PRF can promote the colonization, adhesion, and proliferation of cells due to the presence of several types of growth factors that chemotactically activate the stem cells and induce mitogenesis and differentiation.\textsuperscript{26}

There are two main methods for combining PRF with GBR procedures. The first method involves flattening the PRF to produce a barrier membrane that can be placed below the resorbable membrane, appearing to have a resorption time of 10 to 14 days. The second method is to cut PRF membranes into small fragments, mix them with grafting materials, and then combine the mixture with I-PRF. The reason that PRF is combined with a bone xenograft is that it adds a bone-inducing agent that could function as a biologic matrix, promoting the migration of osteoprogenitor cells and inducing neoangiogenesis due to the release of bioactive molecules and growth factors.\textsuperscript{11,22} This process could hypothetically lead to faster integration of the graft, although histologic comparisons should be made to confirm this hypothesis.\textsuperscript{22} Furthermore, mixing the cut PRF with graft materials also improves the handling properties of the graft by making it stickier and more stable because the activation of the coagulation cascade traps the biomaterial, giving it greater consistency and elasticity.\textsuperscript{11} In a study by Cortellini et al,\textsuperscript{22} the combined use of a particulate xenograft and L-PRF without autologous bone resulted in a safe and effective procedure for horizontal bone augmentation, with a mean horizontal bone gain of $4.7 \pm 2$ mm.

Even though 86% of patients in the present study were free of biologic and technical complications, autogenous bone remains the gold standard and it should nonetheless be remembered that this procedure may cause serious complications if accurate case selections and surgical procedures are not performed. Caution is required because the lack of autogenous bone may compromise the quality and vitality of the regenerated bone. Furthermore, randomized long-term studies with comparative histologic analyses should be performed to clarify the advantages of A-PRF use in GBR procedures.

## Conclusions

Within the limitations of this clinical retrospective study, A-PRF combined with a particulate bone xenograft in GBR is a predictable procedure for closing favorable three-dimensional peri-implant defects. Further randomized and long-term studies are required to determine the clinical and histologic outcomes of GBR performed using A-PRF mixed with a bone xenograft.

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## References


