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Periosteal Pocket Flap technique for lateral ridge augmentation. A comparative pilot study versus guide bone regeneration

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ABSTRACT

Background: Implant rehabilitation of posterior mandibular defects is frequently associated to a horizontal bone loss. There exist several regenerative techniques to supply this bone deficiency, one of which is the Periosteal Pocket Flap Technique (PPF) proposed by Steigmann et al. to treat small horizontal bone defects. The present study proposes a modification of this technique based on the concurrent use of PPF with the use of xenogeneic and autologous bone and Plasma Rich in Growth Factors (PRGF). The aim of this study is to evaluate clinical and radiographic outcomes of the PPF with the use of xenogeneic and autologous bone and PRGF in comparison with conventional Guided Bone Regeneration (GBR) procedures.
Methods: Nine patients were enroled in the study (7 women and 2 men, mean age: 53 ± 2.74 years) and allocated to PPF or GBR. In both groups implant placement was performed simultaneously to bone regeneration. Preoperative CBCT scans were performed for each patient. Surgical time and postoperative pain were recorded, as well as tissue healing. Moreover, horizontal bone gain (mm), graft surface area (mm2) and graft volume (mm3) were evaluated.
Results: Nine surgeries were performed: 6 PPF and 3 GBR. Regarding clinical outcomes, operative time was significative greater in GBR group than in PPF group (51.67 ± 3.51 min vs. 37 ± 5.69 min; p = 0.008). Postoperative pain was higher in GBR compared to PPF (p = 0.011). Regarding radiographical results, there were not significant differences in horizontal bone gain (PPF: 9.43 ± 1.8 mm; GBR: 9.28 ± 0.42 mm), surface area (PPF: 693.33 ± 118.73 mm^2; GBR: 655.61 ± 102.43 mm^2), and volume (PPF: 394.97 ± 178.72 mm^3; GBR: 261.66 ± 118 mm^3) between groups.
Conclusions: This prospective study demonstrates that the combination of autograft/xenograft and PRGF in PPF technique is a simpler, cheaper, and faster technique than GBR technique for achieving moderate lateral bone augmentation in implant treatment. Future randomised clinical studies are needed to confirm the results.

1. Introduction

Dental implant rehabilitation of partially or fully edentulous patients is one of the most common and predictable treatments performed in daily dental practise, achieving high long-term survival rates (Chappuis et al., 2013). It has been proven that, regardless of the cause, the loss of a tooth causes significant alterations in the alveolar process, which can often compromise the placement of the implant (Balshi et al., 2015; Chappuis et al., 2013; Fischer et al., 2018; Sáez-Alcaide et al., 2021; Schropp et al., 2005). This alveolar bone resorption can be reduced with the use of different bone regeneration interventions. Among them guided bone regeneration (GBR) using a bone substitute covered by a barrier membrane is currently

Abbreviations: GBR, Guided Bone Regeneration; PRGF, Platelet Rich in Growth Factors; PPF, Periosteal Pocket Flap; ASA, American Society of Anaesthesiology; SSC, Skeletal Stem Cells; BMSC, Bone Marrow Stem Cells

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the most widely used regenerative treatment. It may or may not be performed simultaneously with implant placement (Hämmerle and Karring, 1998; Sanz-Sánchez et al., 2018; Schropp et al., 2003; Tolstunov et al., 2019).

Although non-bioresorbable membranes were considered the gold standard at the time this regenerative concept was developed (Aprile et al., 2020) with predictable results, they have also proven to have drawbacks. These include the need for a second surgical intervention to remove the membrane and fixation tacks, a higher incidence of wound dehiscence, and earlier exposure leading to easier bacterial contamination. All these sometimes jeopardise regenerative outcomes (Proussaefs and Lozada, 2003; Sheikh et al., 2017). But despite these potential problems, bioabsorbable membranes have become the state of the art in GBR procedures for lateral bone augmentation today. In fact, several authors consider horizontal bone regeneration is predictable when using resorbable membranes together with particulate grafts, which maintain the space to be regenerated and act as a scaffold for bone osteoconduction and eventually bone regeneration. In terms of graft types, autologous bone is still considered the gold standard for its unbeatable properties, although similar results have been reported with xenografts and allografts in horizontal bone augmentation procedures (Friberg, 2016; Sheikh et al., 2017). Bone grafts can also be used in combination with plasma rich in growth factors (PRGF), not only to improve their biological properties, but also to improve the immobility and graft vehiculation of the graft (Dominiak et al., 2012; Torres et al., 2009).

Steigmann et al. described in 2012 a new surgical technique for performing horizontal bone regeneration procedures under the name "periosteal pocket flap" (PPF). This technique consists of placing a slowly resorbing xenograft between the buccal portion of the mandibular bone and the overlying periosteum, which has been previously detached. In this way the periosteum acts like a biological barrier membrane, which provides great stability to the graft and avoids the use of other barrier methods. Therefore, this technique has been proposed as an alternative to horizontal GBR (Solakoglu et al., 2020; Steigmannn et al., 2012). Despite the success rate of this technique and the properties of particulate xenogeneic grafts in maintaining width, the absence of osteoinductive and osteogenic properties and insufficient stabilisation of the graft particles could compromise the results of the regenerative approach. The aim of this study is to describe for the first time a modification of the Steigmann technique using a PRGF conglomerate (combined with xenograft and autologous bone). In addition, we have performed a pilot comparative study with the standard GBR technique for horizontal bone regeneration using bioabsorbable membranes and fixation tacks associated with the xenograft to assess whether this modification PPF technique is a reliable alternative for this horizontal bone regeneration. Our hypothesis is that there is no radiographic difference between GBR and the PPF in terms of bone gain used for lateral ridge augmentation in moderate defects.

2. Material and methods

2.1. Patients

Patients with insufficient bone width (< 4 mm) and a minimum height of 8 mm were included in this study. Patients smoking more than 10 cigarettes per day and with severe systemic disease [ASA (III or IV) – American Society of Anesthesiology] were excluded. Written informed consent to participate in this study was obtained from all patients after explanation of the study objectives and protocol, as well as possible side effects. Participants were randomly assigned to PPF new technique or GBR using a random number generated by a computer programme (GraphPad Software, La Jolla, CA, USA). The flow diagram of the study has been depicted in Fig. 1.

2.2. Surgical procedure

All included patients underwent a panoramic radiograph and a cone beam computed tomography (CBCT) study to assess bone availability. The radiographic images revealed adequate bone density and favourable length; in addition, a slight deficit of bone width was observed at the implant placement site. All patients were operated by the same experienced surgeon (JT). The two different surgical procedures were:

2.2.1. Periosteal Pocket Flap technique (PPF)

Implant placement was performed simultaneously with horizontal bone augmentation using the PPF with a bone graft containing PRGF mixed with a conglomerate of xenogeneic and autologous bone particles. After local anaesthesia, a full-thickness supracrestal incision was performed, with two intrasulcular incisions in the buccal wall of adjacent teeth. A partial thickness flap was made in the buccal wall. After that, a full-thickness flap was then elevated from the lingual wall and the buccal periosteum was detached from the buccal bone, leaving three layers (mandibular bone, periosteum, and partial-thickness flap). The implants were placed following the biological drilling protocol described by Anitua et al. (Anitua et al., 2007a), obtaining autologous bone particles from the implant site.

Blood collection from patients was performed following the protocol proposed by Anitua et al., using a single-use plasma separation kit. Thirty-six mL of blood was collected in tubes with 3.8% sodium citrate (BTI Biotechnology Institute, Vitoria, Spain), to avoid blood clotting. The tubes were centrifuged at 580 g for 8 min (Centrifuge System V. BTI). 2 mL of the plasma fraction closest to the red blood cells was pipetted, avoiding aspiration of the buffy coat, deposited in a surgical sterile container, and activated with 20 µL of calcium chloride per 1 mL of plasma (Anitua et al., 2012, 2010, 2007a, 2007b; Anitua, 1999). PRGF was then mixed with xenograft bone particles (Geistlich Bio-Oss, Wolhusen, Switzerland) and autologous bone particles. This process lasted between 5 and 10 min, during which time the fibrinogen was converted to fibrin and the bone particles were integrated into the PRGF, resulting in a firm and easy-to-handle conglomerate. This graft was placed in the previously prepared pocket and the surgical wound was sutured in two planes. First with absorbable suture to join the buccal and lingual periosteum, and second with non-absorbable suture to attached buccal and lingual flaps. One week after the surgery, the sutures were removed and a clinical and radiological follow-up was performed to assess the status of the implant. The surgical procedure is described in Fig. 2.

2.2.2. Guide bone regeneration (GBR)

Implant placement was performed simultaneously with horizontal bone augmentation using an absorbable membrane (CopiOs pericardium membrane, Zimmer Biomet, Indiana, USA) fixed with pins (Master Pin Meisinger, Hager & Meisinger GmbH, Germany) with xenogeneic bone (Geistlich Bio-Oss, Wolhusen, Switzerland) as grafting biomaterial. After local anaesthesia, a full-thickness supracrestal incision was performed, with two intrasulcular incisions in the buccal wall of adjacent teeth. Two full-thickness flaps, buccal and lingual, were elevated and implants were placed following a conventional protocol.

After implant placement, the membrane was fixed to the lingual wall of the alveolar ridge, the particulate graft was placed and covered with the membrane, which is finally fixed to the buccal wall. The wound was then closed with non-absorbable suture. One week after surgery, the sutures were removed and a clinical and radiological check-up was performed to assess the implant status. The surgical procedure is described in Fig. 3.

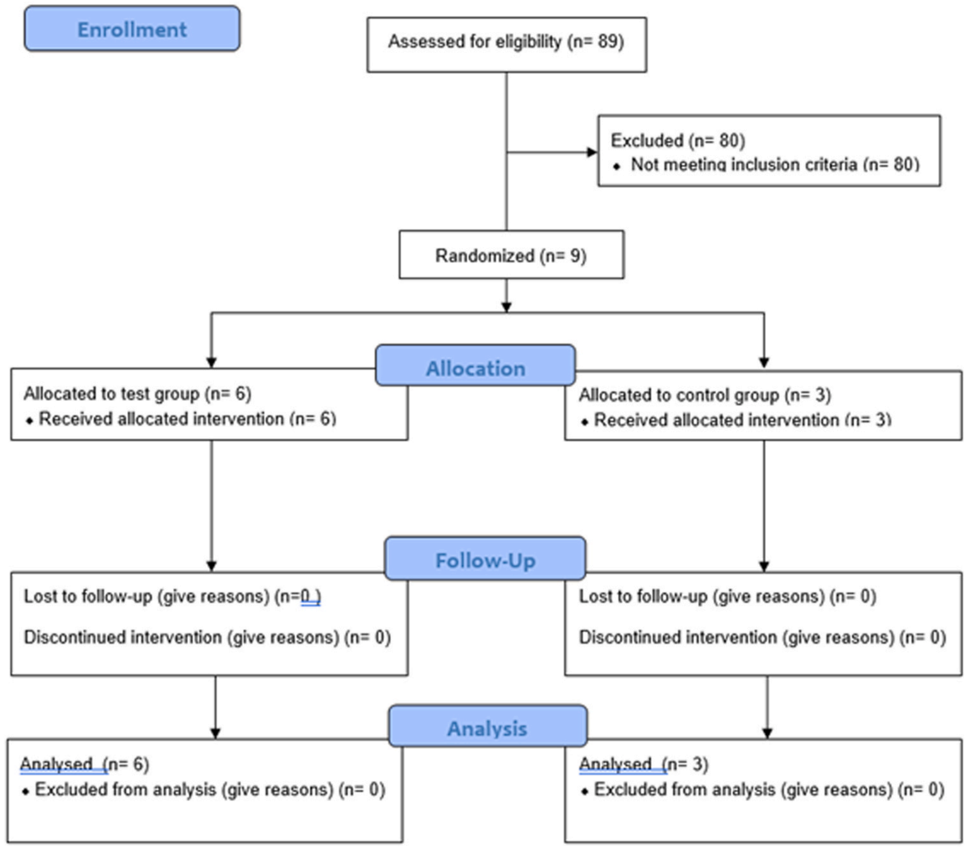

Fig. 1. Study flowchart.

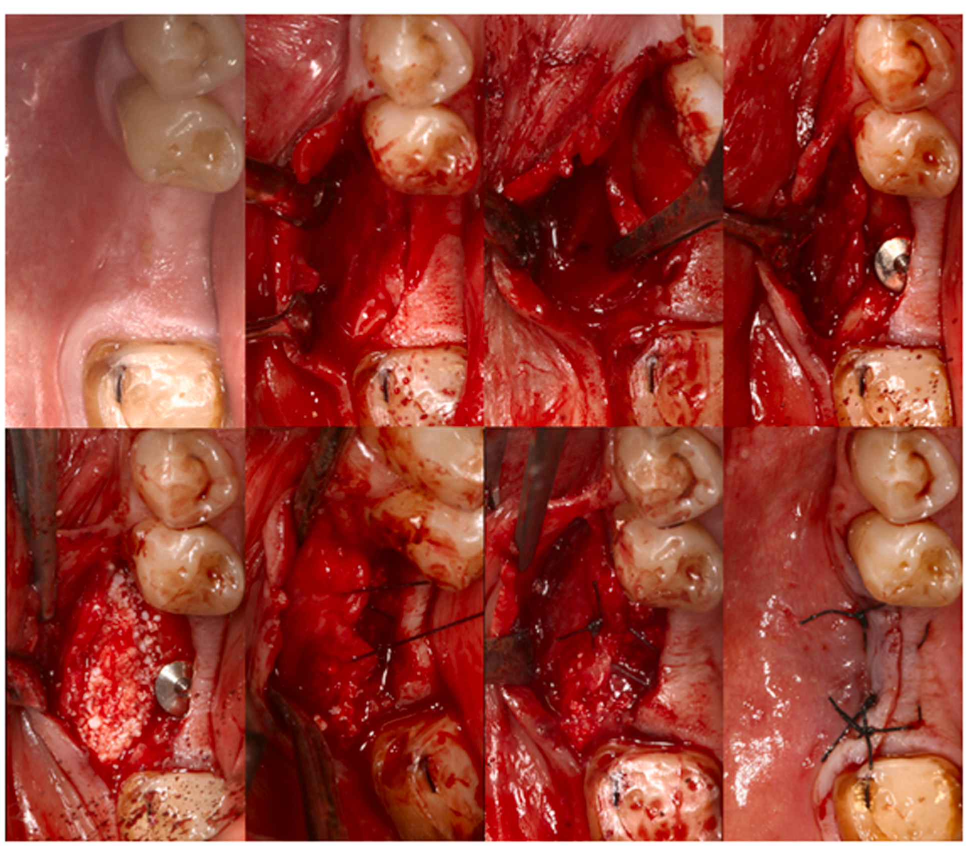

Fig. 2. Incision (A), flap preparation (B), periosteal pocket flap (C), implants placed (D), graft placed (E) and suture (F, G, H).

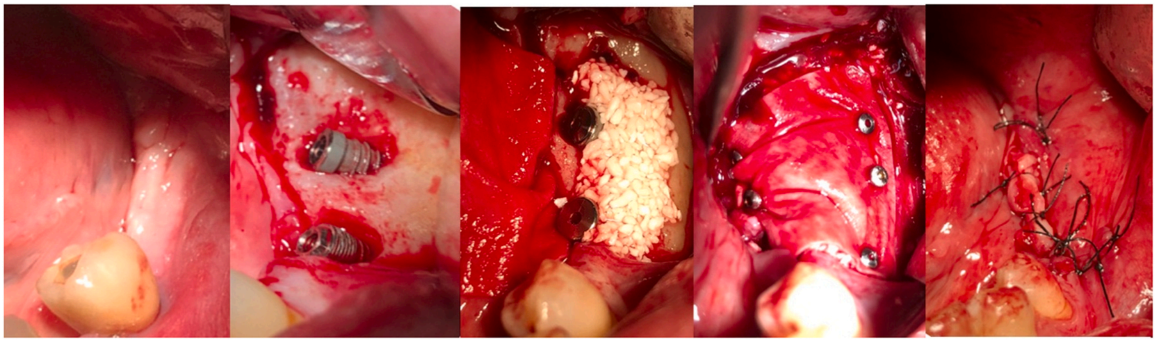

Fig. 3. Implant placement (A, B), membrane fixation to lingual wall and graft placement (C), membrane fixation to buccal wall (D) and suture (E).

2.3. Outcomes variables

2.3.1. Time consuming

In both groups, time was measured from the time immediately after exposure of the bone defect to final wound closure.

2.3.2. Post-operative pain

The degree of pain was classified into three categories with respect to analgesic drug consumption (paracetamol 1 g) during the week after surgical treatment: severe pain (when the patient took more than eight analgesics), moderate pain (when the patient took four to eight analgesics) or low pain (when the patient needed less than four analgesics).

2.3.3. Tissue healing

To assess soft tissue healing, patients were scheduled for checkup 7 days, 15 days, 21 days, 2 months, and 4 months after the surgical procedure and photographs were taken of surgical wound to observe any postoperative dehiscence or infection. This procedure was carried out by two independent trained observers, who evaluated the clinical images and scored them according to the healing index proposed by Landry et al. (Landry and Turnbull, 1988).

2.4. Radiographic analysis

A Carestream CS-9000-3D scanning unit (Kodak, Rochester,NY,USA) was used to obtain preoperative and postoperative images before surgery and four months after surgery, respectively. Computed tomography data of all patients were exported in Digital Imaging and Communications in Medicine (DICOM) format. DICOM data were analysed using 3DSlicer medical software (http://www.slicer.org) (Fedorov et al., 2012).

Preoperative and postoperative studies were segmented using the thresholding tool of the software and the resulting segments were superimposed. The difference between the preoperative and postoperative segment was segmented again to obtain the graft segment, which was exported to STL format. The same software was used again to analyse the graft surface and volume. This process is detailed in Figs. 4 and 5. In addition, the mean horizontal bone gain in cross-sections was assessed by following measures: (A) Distance from lingual cortical to the buccal surface of implant; (B) Distance from lingual cortical to buccal cortical; (C) Distance from lingual cortical to minimum bone width expected after regeneration; and (D) Distance from lingual cortical to the bone width achieved after regeneration. These measures are depicted in Fig. 6.

All measurements were performed in random order by two trained observers who were dentists with experience in oral radiology. Intra-observer reliability was assessed between measurements performed 2 weeks apart to eliminate recall bias and by taking three different measurements from each site which were averaged. Intra and inter-observer agreements were assessed using the intraclass correlation coefficient (ICC) for the results of continuous variables (0.91, $P < 0.001$ and 0.85, $P = 0.001$, respectively).

2.5. Statistical analysis

All variables were analysed by analysis of covariance (ANCOVA) to assess the independent effect of each variable (age, gender, smoking, surgical time consuming, post-operative pain, healing time and graft type) on the main variable (bone resorption). In addition, Chi-square and T-Student tests were used to independently assess the influence of graft type on postoperative pain and time consumption, respectively. All statistical tests were performed at 5% significance level, using the same software (IBM SPSS Statistics 22.0, SPSS, Inc., Chicago, IL).

3. Results

During the study period (May 2018 to September 2021), 89 patients were evaluated. Among these patients, 9 met the above inclusion criteria and were recruited for this prospective study. The study group consisted of 6 women and 3 men with an age range between 43 and 72 years. There was heterogeneity in the systemic diseases present in some of the selected patients such as diabetes, hypertension, and osteoporosis. However, none of these conditions are known to jeopardise the success of the implant. The characteristics of the study sample are described in Table 1.

3.1. Time consuming

The mean time elapsed from the moment immediately after exposure of the bone defect to final wound closure was 41.89 ± 9.01 min. We observed that it was significantly longer in GBR group than in PPF group (51.67 ± 3.51 min vs. 37 ± 5.69 min; $p = 0.008$) (Table 2).

3.2. Post-operative pain

Five surgical procedures were indicated as slightly painful and four moderately painful. No patient classified the surgery as very painful. In the PPF group 83.3% of the patients had mild pain and 16.7% moderate pain, while in the GBR group 100% rated postoperative pain as moderate. This difference was statistically significant ($p = 0.011$) (Table 2).

3.3. Tissue healing

Healing was uneventful in all patients and during the 5-month period following bone graft and implant placement, no graft

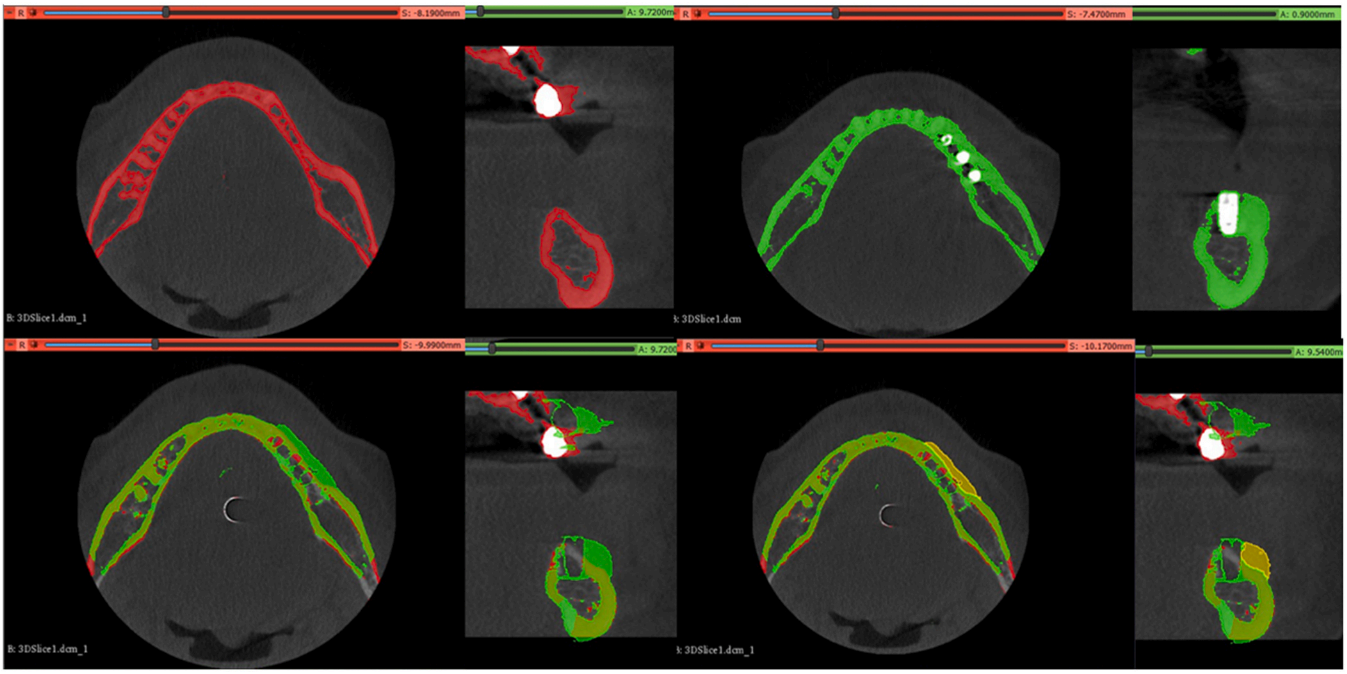

Fig. 4. Radiographic analysis of experimental group. Figure shows preoperative segmentation (A), postoperative segmentation (B), segments superposition (C) and graft segmentation (D).

exposure related to wound dehiscence was observed in any group. The mean healing period was 5 months.

3.4. Radiographic outcomes

Preoperative and postoperative CBCT analysed using DICOM 3DSlicer visualisation software (http://www.slicer.org) showed a mean surface area of 693.33 ± 118.73 mm^2 in PPF group and 655.61 ± 102.43 mm^2 in GBR group. No significant differences were observe between groups. There were also no significant differences in the mean volume achieved. The results obtained were 394.97 ± 178.72 mm^3 and 261.66 ± 118 mm^3 for MPPP and GBR groups, respectively (Table 3).

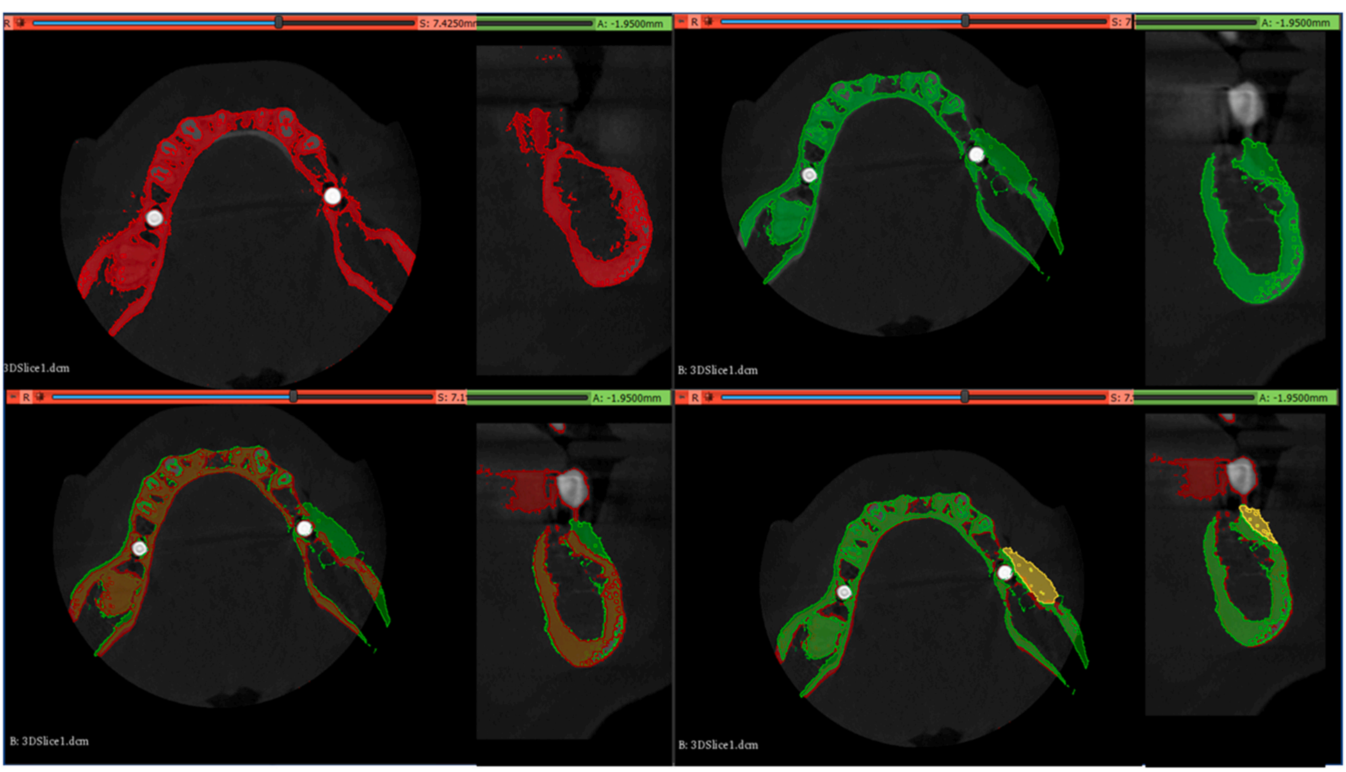

Fig. 5. Radiographic analysis of control group. Figure shows preoperative segmentation (A), postoperative segmentation (B), segments superposition (C) and graft segmentation (D).

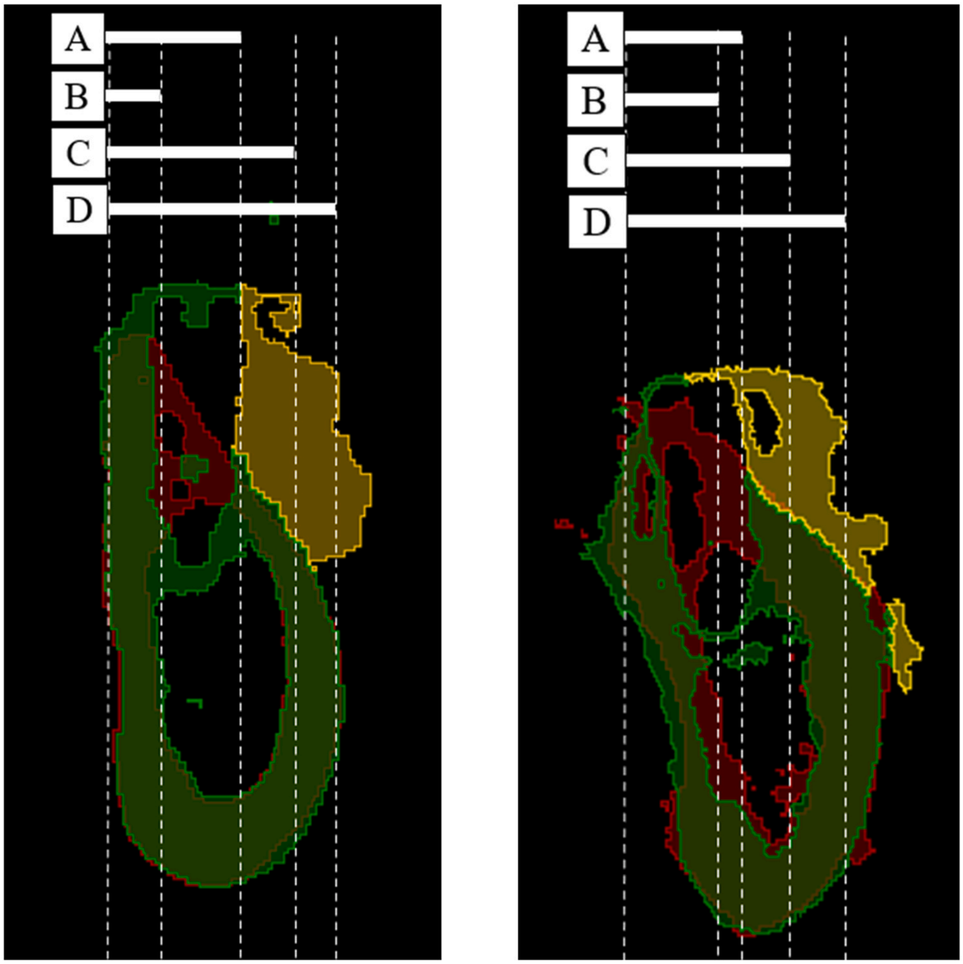

Fig. 6. Radiographic analysis of experimental and control group. Figure shows horizontal bone gain measurements. A) Distance from Lingual cortical to buccal Surface of implant; B) Distance from lingual cortical to buccal cortical; C) Distance from lingual cortical to minimal expected bone width after regeneration; D) Distance from lingual cortical to achieved bone width after regeneration.

Regarding horizontal bone augmentation, the mean horizontal bone gain was 9.43 ± 1.78 mm for PPF and 9.28 ± 0.42 mm for GBR. This difference was not statistically significant. Furthermore, in both groups, the bone gain in all cases evaluated exceeded the minimum expected bone augmentation (Table 4).

4. Discussion

To the best of our knowledge, this combination of biomaterials for PPF technique and its comparison with GBR has not been previously described for lateral ridge augmentation. Furthermore, this prospective study has shown that this technique has certain advantages over GBR exhibiting significantly shorter operative time and postoperative pain, with similar tissue healing than conventional bone regeneration. In addition, radiographic bone gain has shown similar results to conventional GBR, demonstrating similar results of success of this technique in situations requiring moderate / mild lateral bone augmentation. The results obtained are comparable to those of techniques applying the principles of bone regeneration, at least for moderate defects. Currently, the most widespread technique when horizontal bone augmentation is required is GBR, which is usually performed using absorbable collagen barrier membranes fixed with pins and particulate bone grafts. However, it is an expensive technique associated with a prolonged surgical time. Although complications are rare, some of them such as exposure of the barrier membrane, could compromise bone healing

Table 1
Sample characteristics.

Group									
PPF (N = 6)					GBR (n = 3)				
Patient	Gender (M/F)	Age (y)	Smoking (Y/N)	Area (Mx/Mb)	Patient	Gender (M/F)	Age (y)	Smoking (Y/N)	Area (Mx/Mb)
1	F	63	N	Mb	1	F	56	N	Mb
2	F	71	N	Mb	2	M	50	N	Mb
3	F	68	N	Mb	3	M	51	N	Mb
4	F	72	N	Mb					
5	F	49	N	Mb					
6	M	56	N	Mb					
Total	1 M 5 F	63.17 ± 9.11	6 N	6 Mb	Total	2 M 1 F	59 ± 14.73	3 N	3 Mb

Table 2
Main clinical outcomes.

Group	Patient	Operative Time (minutes)	Mean	SD	P value	Postoperative pain	%	P value	Dehiscences
PPF	1	35	37.00	6.23	0.008	Mild	Mild: 83,3% Moderate: 16,7% Severe: 0%	0.011	0
	2	32				Mild			
	3	41				Mild			
	4	47				Moderate			
	5	30				Mild			
	6	37				Mild			
GBR	1	48	51.67	3.51		Moderate	Mild: 0% Moderate: 100% Severe: 0%		0
	2	52				Moderate			
	3	55				Moderate			

(Fedorov et al., 2012; Hämmerle and Karring, 1998; Urban et al., 2013).

To avoid the aforementioned complications, different techniques have been developed, such as the periosteal pocket flap, proposed by Steigmann et al. in 2012, as an alternative method to resolve moderate horizontal bone defects in the posterior mandibular region (Steigmannn et al., 2012). This study describes a new PPF technique that focuses on two main points: the effect of the periosteum and the PRGF conglomerate.

4.1. Periosteum effect

Periosteum is a thin sheet of specialised connective tissue composed of a highly vascularises and innervated fibrous membrane that covers most of the bones. This tissue has a structure composed of an outer “fibrous” layer and an inner “cambium” layer. The outer “fibrous” layer contains elastic fibres, fibroblasts, and blood vessels. And the inner “cambium” layer contains blood vessels, nerves, preosteoblasts/bone lining cells, osteoblasts, and undifferentiated mesenchymal stromal/stem cells. Moreover, the periosteum has two essential properties that enhance the success of this technique: the regenerative capacity mainly due to the inner layer and the mechanical properties due to the outer layer (Alexander et al., 2017; Evans et al., 2013; Malizos and Papatheodorou, 2005). (Fig. 7).

4.1.1. Regenerative capacity (inner layer)

The regenerative potential of periosteum is well described in scientific literature. Several studies have pointed out that the periosteum is an important source of Skeletal Stem Cells (SSC) for bone repair. SSC are activated in the early steps of bone regeneration and are the basis of this extraordinary bone regenerative capacity. Many studies have focused on the characterisation of Bone Marrow Stem Cells (BMSCs), which are currently used in cell-based therapy approaches in orthopaedics, however recent studies have identified that periosteal cells have a higher capacity for cell growth and clonogenicity, as well as superior regenerative capabilities compared to BMSCs / SSC (McBride et al., 2011). Immediately following a fracture or injury, separation of the periosteum from the bone occurs, triggering an inflammatory response that, joint to platelet released factors, activates cells in the inner and outer layer, stimulating the processes of osteogenesis, chondrogenesis and angiogenesis (Evans et al., 2013).

4.1.2. Mechanical properties (outer layer)

The outer layer consists of mainly of collagen, aligned with the longitudinal axis, and elastin, and is believed to have a structural (mechanical) function due to its barrier properties (Colnot et al., 2012; McBride et al., 2011). Initially, under small strains, the periosteum is very flexible and at the transition point, reported to be slightly longer than the in situ length, the periosteum becomes significantly stiffer (Bertram et al., 1998). Also, several previous studies have demonstrated the non-linear behaviour, i.e., transition from a flexible elasticity at low strains to stiffer elasticity at high strains, of periosteal tissue (Bertram et al., 1998; Uchiyama and Yamakoshi, 1998). Tension-free primary closure is considered one of the essential requirements to achieve successful bone regeneration. Our proposed technique, like the original by Steigmann et al., allows mobilisation of soft tissues over horizontally augmented alveolar ridges. Bioabsorbable barrier membranes of native porcine collagen are widely documented in the scientific literature for their favourable handling properties and histological tissue integration, although they have limited rigidity and a rapid degradation rate (between 8 and 24 weeks) (Rothamel et al., 2005). Unlike these barrier membranes, periosteum has no reabsorption, so the graft's containment effect is longer than that of any membrane, even those with long-term reabsorption. The results observed in this study could also be explained by adequate stiffness and flexibility of the periosteum, which may increase the space maintenance effect. For this reason, PPF avoids the use of any type of barrier membrane because the periosteum acts like a biological membrane. Consequently, the absence of barriers and fixing pins, makes it a cheaper and simpler technique than conventional ones. Also, but not least, it decreases the surgical time.

4.2. PRGF conglomerate

This modification focuses on two main points. On the one hand, the combination of xenograft with autologous bone obtained from a biological drilling technique at the implant site (Steigmannn et al., 2012) allows collect live autologous bone particles helping regenerative processes due to their osteoinductive, osteogenic and

Table 3
Main radiographical surface area (mm^2) and volume (mm^3) reached by each technique.

Group	Patient	Surface area (mm2)	Mean	SD	P value	Volume (mm3)	Mean	SD	P value
PPF	1	784.07	693.33	118.73	0.655	327.78	394.97	178.72	0.253
	2	825.04				620.19			
	3	732.31				550.24			
	4	647.81				454.46			
	5	490.49				269.09			
	6	680.24				148.06			
GBR	1	772.23	655.61	102.43		280.73	261.66	16.66	
	2	614.38				254.34			
	3	580.23				249.91			

Table 4
Main radiographical outcomes related to horizontal bone gain. A) Distance from Lingual cortical to buccal Surface of implant; B) Distance from lingual cortical to buccal cortical; C) Distance from lingual cortical to minimal expected bone width after regeneration; D) Distance from lingual cortical to achieved bone width after regeneration.

Group	Patient	A	Mean	SD	P value	B	Mean	SD	P value	C	Mean	SD	P value	D	Mean	SD	P value
PPF	1	6.1	5.95	1.25	0.503	6.4	4.70	1.84	0.585	8.2	7.82	1.27	0.538	9.61	9.43	1.78	0.895
	2	5.6				3.3				7.4				8.79			
	3	7.5				5.4				9.3				11.7			
	4	5.1				2.3				6.9				8.18			
	5	4.3				3.9				6.1				7.08			
	6	7.3				7				9.1				11.2			
GBR	1	7.6	6.60	1.41		4.9	4.07	0.74		9.4	8.40	1.41		9.52	9.28	0.42	
	2	5				3.7				6.8				8.79			
	3	7.3				3.6				9.1				9.53			

osteoconductive properties. On the other hand, bone graft particles mixed with PRGF result in the formation of three-dimensional fibrin matrix, which retains the grown factors and releases them slowly, improving wound healing and decreasing postsurgical pain and inflammation (Anitua et al., 2012, 2010, 2007a, 2007b; Anitua, 1999; Fuerst et al., 2009; Fernández-Tresguerres Hernández-Gil et al., 2004; Tresguerres et al., 2020). But even more important in this technique are the physical characteristics of the mixture that prevents the mobility of the graft, creating a sticky bone complex (Fig. 8) with great stability, allowing its perfect placing in the graft site. In addition, the graft remains immobile thanks to the periosteum layer, which behaves as a biological barrier membrane, as mentioned above. (Hur et al., 2010; Lin et al., 2017).

Furthermore, regarding clinical outcomes related to postoperative pain, the reduction in the operative time associated to the PPF may be the reason why patients in the study group suffered less postoperative pain.

Finally, despite the limitations of the present study, such as the small sample size and short follow-up time, the results of the present pilot study suggest that this modification of the original

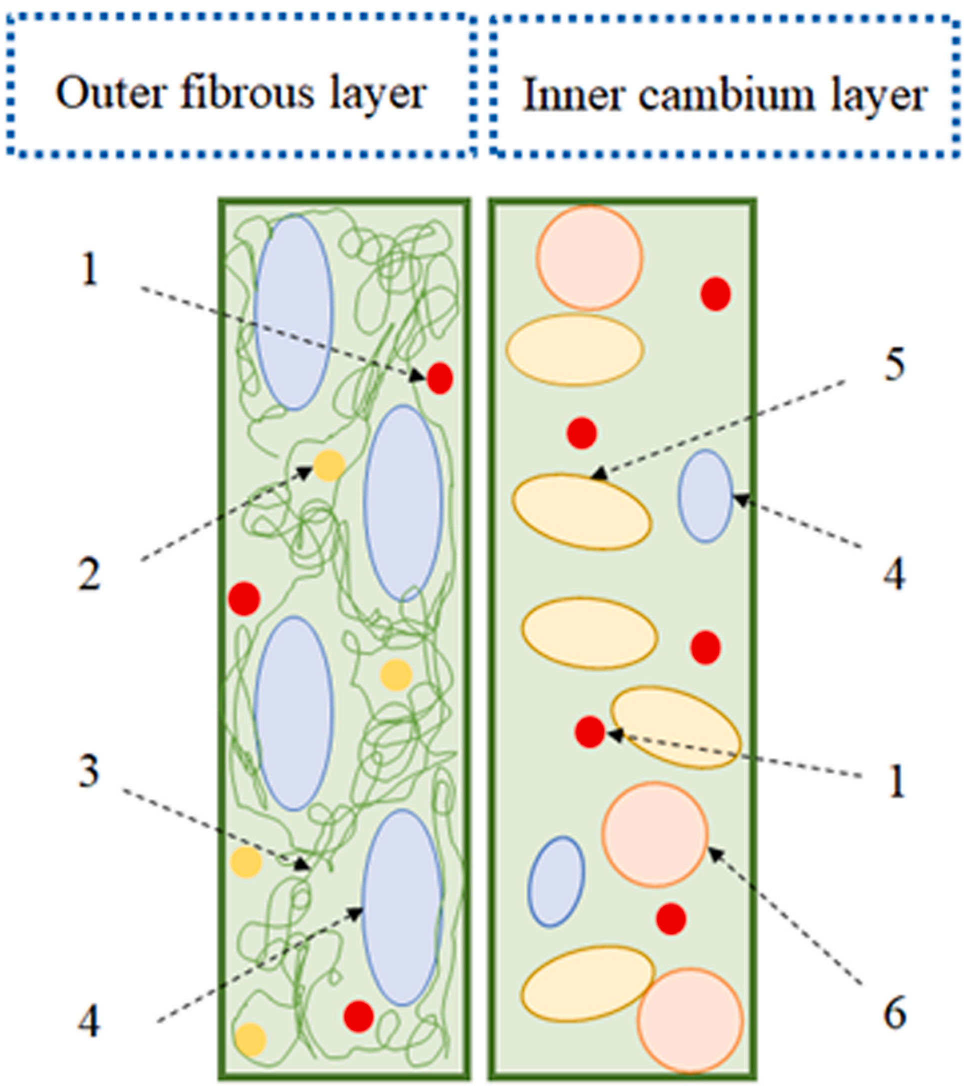

Fig. 7. Description of the structure of periosteum. Outer “fibrous” layer contains blood vessels (1), nerves (2), collagen fibres (3) and fibroblasts (4); and inner “cambium” layer contains blood vessels (1), fibroblasts (4), osteoblasts and preosteoblasts (5) and mesenchymal stem cells (6).

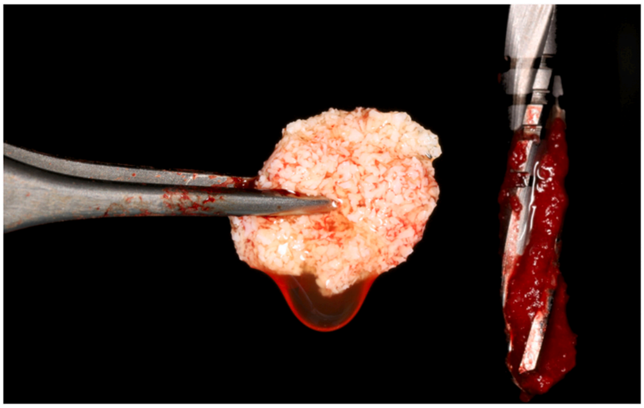

Fig. 8. Sticky bone complex composed by PRGF, and autologous bone obtained from implant site through biological drilling technique.

technique makes it possible to achieve moderate lateral bone augmentation using an easier, cheaper and faster technique.

5. Conclusion

In conclusion, this prospective study demonstrates that the combination of autograft/xenograft and PRGF in PPF technique is a simpler, cheaper and faster technique than the GBR technique to achieve moderate lateral bone augmentation in implant treatment. Future randomised clinical studies are needed to confirm these results.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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