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Use of autologous micrografts associated with xenogeneic anorganic bone in vertical bone augmentation procedures with Barbell Technique®

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Abstract

Introduction: Bidirectional vertical ridge augmentation in the posterior maxilla is very challenging.

Purpose: To evaluate the regenerative potential of micrografts, derived from periosteum or bone tissue, added to an anorganic xenograft in vertical reconstruction of the posterior maxilla, by a prospective, controlled study.

Materials and methods: After clinical selection and the analysis of CBCT scans, 24 posterior maxillary sites, in 19 patients, were treated by using Barbell Technique®. Sites requiring both inlay and onlay reconstruction were enrolled in the study. In the Control Group (CG, $n = 8$), a xenograft was used in the inlay site and for the onlay site, a 1:1 mix of xenograft and an autograft was used. In Test Group 1 (TG1, $n = 8$), both inlay and onlay sites were grafted with the xenograft associated with the micrografts derived from periosteum. In Test Group 2 (TG2, $n = 8$), both inlay and onlay sites were grafted with the xenograft associated with the micrografts derived from bone. Six months after the procedures, CBCT scans were obtained, and bone biopsy samples were harvested during implant placement surgery. The bone specimens were analyzed histomorphometrically, by measuring the percentages of vital mineralized tissue (VMT), non vital mineralized tissue (NVMT) and non mineralized tissue (NMT). Immunohistochemically, the levels of VEGF were categorized by a score approach.

Results: Histomorphometric analysis revealed, for the inlay grafts, no significant difference among the groups for VMT, NVMT and NMT. However, for onlay grafts, CG achieved a higher amount of VMT in comparison with TG2, and the opposite occurred for NMT values. In this regard, no statistical difference was observed between CG and TG1. Concerning immunohistochemistry, the VEGF values for CG and TG1 were slightly higher than those obtained by TG2 for both inlay and onlay grafts, but without statistical significance. CBCT analysis showed a similar level of gain for all groups, for both inlay and onlay bone augmentation sites. Clinically, one implant (in CG) within a total of 50 implants installed, had early failure and was replaced after 3 months. All patients received implant supported prosthesis.

Conclusion: This study indicated that the clinical use of micrograft derived from periosteum may have some potential to increase bone formation in onlay reconstructions, unlike the micrograft derived from bone tissue.

KEYWORDS

autologous tissue, bone grafts, bone regeneration, guided tissue regeneration, sinus floor augmentation

SUMMARY BOX

What is known?

Bidirectional vertical ridge augmentation in the posterior maxilla is very challenging. So far, the procedure is typically executed using either autografts or autografts mixed with xenografts.

What this study adds?

This prospective clinical trial assessed the bone formation following bidirectional vertical ridge augmentation with Barbell Technique®, using xenografts associated with autogenous micrografts from bone or periosteum. Their performance was compared with the standard use of xenografts alone and combined with autografts.This study provides evidence that the use of xenograft associated with periosteum derived micrograft was not inferior to the xenograft mixed with the gold standard autogenous bone.

1 | INTRODUCTION

The lack of stimulus from chewing after tooth loss leads to resorption of remaining alveolar bone tissue, which many times prevents implant installation.[1] To correct this clinical issue, several bone augmentation techniques were developed, and different types of graft materials were used.[2] To this regard, the use of autogenous bone graft for dental implant installation was first described by Breine and Bränemark[3] and is currently a well-accepted and widespread procedure in maxillofacial rehabilitation planning. This graft is considered the biological gold standard, as it presents osteogenic, osteoconduction, and osteoinduction potentials.[4] Autogenous graft contains some viable cells and proteins that stimulate the mesenchymal cell differentiation in osteoprogenitor cells.[5]

Bone regeneration of resorbed jaws and pneumatized maxillary sinuses is a key factor in successful implant rehabilitation for proper implant positioning to stabilize an adequate prosthesis.[6] In the posterior maxilla with advanced resorption of the alveolar ridge, it is well known that, creating a space between the maxillary sinus floor and the Schneiderian membrane is necessary to perform a sinus floor augmentation. This procedure was first proposed by Boyne and James[7] and later successfully used for managing cases of posterior maxilla with deficient crestal bone.[8] Furthermore, some clinical situations demand an appositional bone augmentation, that is considered even more challenging. In this scope, for the sinus floor augmentation procedure the use of anorganic bovine bone alone has been shown to be adequate[9] and, for this surgical technique, no significant benefits are found with the addition of autografts.[10] However, for onlay vertical bone grafts, or large onlay horizontal bone grafts, a mix of autograft and xenograft (1:1) is desirable.[11,12] Consequently, due to the larger volume of autografts required, a higher level of morbidity is expected due to the need for a secondary donor site.[13]

Recently, a new medical device (Rigeneracons, Human Brain Wave SRL, Torino, Italy) has been introduced into clinical practice.

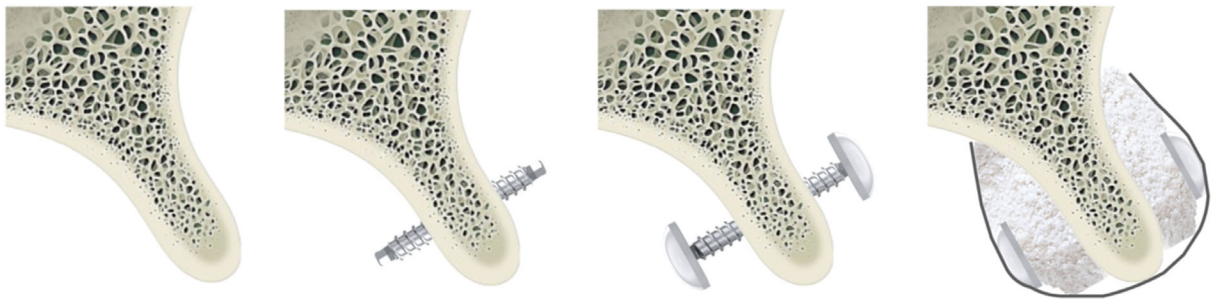

FIGURE 1 Simulation of the installation of a Barbell Technique's device for the original indication (i.e., bidirectional horizontal bone augmentation).

This device is capable of disaggregating small samples of autologous tissue. The obtained 80 μm micrograft retain high cellular viability, as well as enriched in progenitor cells and can be used immediately without any manipulation or cell culture.[14] Micrografting technology was successfully applied in oral-maxillofacial surgery, where micrografts derived by human dental pulp or periosteum were able to stimulate periodontal regeneration, atrophic maxillary bone regeneration, alveolar socket preservation and sinus floor augmentation.[15–17] This technology allows the incorporation of progenitor cells in the scaffold without the need of a large volume of autograft harvest.[14] This way, the application of Rigeneracons technology reduces the morbidity found at the donor site, making the procedure more appealing from the clinical perspective. A recent animal study[18] showed that the use of micrografts enhanced the level of bone formation when combined with an anorganic xenograft in critical sized bone defects.

Besides the characteristics of the grafting material selected, it is by consensus agreement that the selection of an adequate surgical technique determines predictability of outcomes with bone augmentation procedures. From this perspective, one of the most challenging situations in the field of surgical implant dentistry is the bidirectional bone reconstruction. Recently, a new device called Barbell Technique®[19,20] was introduced to overcome the difficulty related to this issue. This novel technique is based on a tent pole effect inherent to the Barbell device and was presented as a reliable solution for bone regeneration in clinical situations requiring reconstruction at both buccal and palatal/lingual sides of a deficient alveolar ridge. This technique was originally designed for horizontal bidirectional bone augmentation (Figure 1) but was also used for unidirectional vertical reconstruction.[21] However, it seems plausible this technique may be used also for vertical bidirectional bone augmentation in the posterior maxilla. In many clinical cases requiring sinus floor grafting (i.e., inlay graft), there is also a deficiency requiring appositional bone augmentation of the vertical defect found with the alveolar ridge (i.e., onlay graft) concurrently.

Based on these considerations, the purpose of this clinical study was to assess the efficacy of periosteum and autologous bone micrografts (obtained by Rigeneracons technology), in conjunction with Barbell Technique®, for bidirectional bone regeneration using onlay and inlay reconstructions of the posterior maxilla.

2 | MATERIALS AND METHODS

This is a prospective, controlled study conducted to evaluate the clinical performance of periosteum and autologous bone micrografts for bone regeneration in onlay (vertical augmentation) and inlay (sinus floor grafting) reconstructions in posterior maxilla. Moreover, histomorphometric, immunohistochemical and tomographic analysis were performed.

This single-center study was conducted in the outpatient clinic of Faculdade São Leopoldo Mandic. The study design was approved by the research ethics committee (number 4.983.508, September 17, 2021) and informed consent forms for all the patients were obtained in accordance with the 1964 Declaration of Helsinki (revised in 2013). The study was conducted in compliance with the Consolidated Standards of Reporting Trials (CONSORT) statement, where applicable. Patient recruitment took place from June to October 2021.

2.1 | Patient selection

The inclusion criteria involved patients of both genders, partially edentulous for more than 1 year, with absence of at least two teeth and at most five teeth, in the posterior maxilla, and requiring implants. In the posterior maxillary quadrant, the alveolar ridge height was <4 mm, therefore requiring maxillary sinus floor augmentation combined with vertical onlay bone augmentation, either unilateral or bilateral posterior maxilla. All patients selected were between 18 and 65 years of age and maintain adequate oral health, with a bleeding score <20%. Patients were excluded if they had a history of neoplastic disease treated with radiotherapy or chemotherapy, pregnancy or breastfeeding, receiving treatment or were affected by an illness that could influence bone homeostasis, sinus pathologies, or if the patient was identified as a smoker, alcoholic, and/or have a drug addiction. Nineteen patients with 24 pneumatized sinuses were included in this study. Sample size per group was determined by background in the literature covering randomized clinical trials of sinus floor augmentation procedure.[22–24]

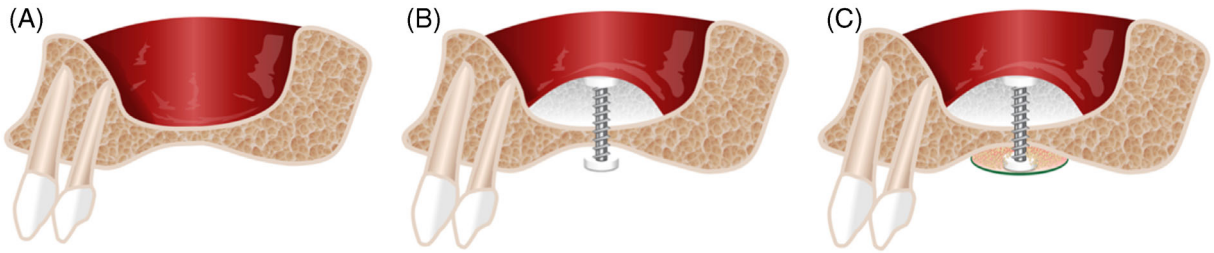

FIGURE 2 Simulation of the installation of a Barbell Technique® device for bidirectional vertical bone augmentation, allowing the replacement of the Schneiderian membrane and the space maintenance for the onlay graft. (A) Preoperative situation, showing the need of both inlay and onlay reconstruction. (B) Barbell Technique® device installed, and bone graft (white color) inserted inside the sinus. (C) Simulation of the bone graft placement (beige color) over the bone crest, covered by the barrier membrane (green color).

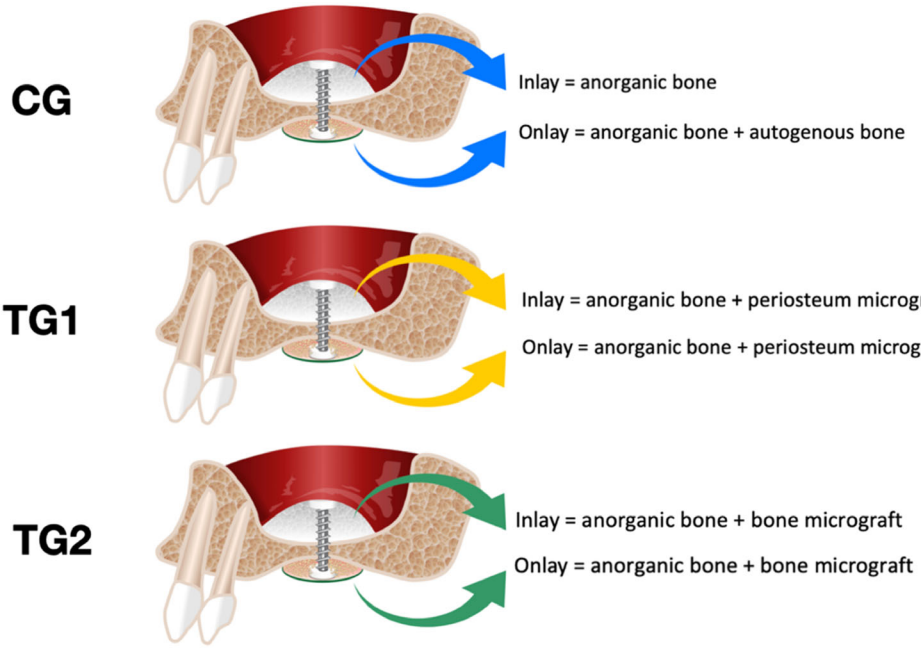

FIGURE 3 Diagram showing the experimental design of the study. CG, control group; TG1, Test Group 1; TG 2, Test Group 2.

2.2 | Study design

Periosteum or bone derived micrografts obtained by Rigeneracons were combined with an anorganic bone (Bio-Oss large granules, Geistlich Biomaterials, Wolhusen, Switzerland) for bone regeneration in onlay (vertical augmentation) and inlay (sinus floor augmentation) reconstructions in posterior maxilla. This treatment protocol was compared with standard procedures (i.e., use of anorganic bone alone for inlay/sinus grafting and a combination of anorganic bone and autograft harvested from mandibular ramus, in a 1:1 ratio, for appositional/onlay vertical augmentation). Following the principles of guided bone regeneration, as well as the principles of the Barbell Technique®, reported by Pelegrine et al.[19] and Macedo et al.,[20] titanium/PEEK devices and collagen membranes (Bio-Gide, Geistlich Biomaterials, Wolhusen, Switzerland) were used for all procedures, according to Figure 2.

Patients were randomly divided into three groups according to the material used: Control Group (CG) (6 patients, $n = 8$ pneumatized sinuses), following the standard approach, with using Bio-Oss alone inside the sinus (inlay) and a mix of Bio-Oss and autograft (1:1) for the appositional graft (onlay); Test Group 1 (TG1) (7 patients, $n = 8$ pneumatized sinuses) with Bio-Oss combined with micrograft obtained from the periosteum at used in both sites, that is, inside the sinus (inlay) and extra-sinus, along the alveolar ridge for the appositional graft (onlay); and Test Group 2 (TG2), (6 patients, $n = 8$ pneumatized sinuses) with Bio-Oss combined with micrograft obtained from the maxillary tuberosity bone used in both sites, that is, inside the sinus (inlay) and for the appositional graft (onlay) (Figure 3 and Table 1).

All patients were prosthetically rehabilitated using osseointegrated implants and fixed prostheses at the end of the study.

2.3 | Randomization and blinding

The patients receiving the different treatments were assigned to groups randomly using the website Randomization.com (http://www.randomization.com), and the random assignment was placed in a sealed opaque envelope. Allocations of the procedure was revealed to

investigator (Luiz Antonio Mazzucchelli Cosmo) according to the sequence of the enrollment. The patients were blinded to the planned treatment and all the analyses were performed by a blind operator. All the analyses were performed by a blind operator.

2.4 | Recipient bed surgical protocol

At the preoperative stage, cone-beam computed tomography scans (CBCT) of the posterior maxillary region were obtained to measure the height of the posterior maxillary alveolar ridge and the size of the alveolar crest defect and pneumatized maxillary sinus (for details see "Tomographic Analysis" section).

After local anesthesia, a crestal incision followed by two releasing incisions were used and, after flap reflection, a lateral window was prepared using number 3 PM spherical diamond bur (Medical Burs Ind. E Com. de Pontas e Brocas Cirúrgicas Ltda. Cotia, Brazil) on the buccal aspect of the maxillary sinus. The Schneiderian membrane was carefully released using specific curettes (Neodent, Curitiba, Brazil) (Figure 4).

The sinus membrane was maintained in an apical position by using Barbell Technique® devices (DSP Biomedical, Campo Largo, Brazil), with screws 14 mm in length, that were also used to maintain the space for the vertical onlay graft, thus allowing standardization of vertical bone augmentation among groups. After bone graft placement, a collagen membrane of porcine origin (Bio-Gide, Geistlich Biomaterials, Wolhusen, Switzerland) was used to cover the graft for all groups, thus excluding soft tissue from the graft region (Figure 5). The bone grafts placed inside the sinus (inlay grafts) and over the bone crest (onlay grafts) did not have contact with each other, as the native bone was able to maintain separation of the two different compartments for all patients.

After 6 months, new CBCTs were taken, Barbell devices removed (except the PEEK capsule positioned inside the sinus) and bone biopsies (two per grafted site—one from the inlay and the other from the onlay grafts) were harvested using a 2 mm trephine bur (Figure 6). Immediately after that, the titanium implants (IntraOss, Itaquaquecetuba, Brazil) were placed. Horizontal mattress sutures followed by multiple single interrupted sutures, with ETHILON-Nylon 4-0 (Ethicon, MA), were used to achieve primary closure. All patients were instructed to not wear any removable prosthesis during the healing time and were medicated with amoxicillin (500 mg; Amoxil; EMS, Hortolândia, Brazil) three times a day for 7 days, as well as Nimesulid (100 mg; Scaflam, Eurofarma, São Paulo, Brazil) twice a day for 5 days. Patients were also instructed to rinse twice a day with a 0.12%

TABLE 1 Baseline subject characteristics.

Patient	Gender	Age (years)	Group	Side	BI (%)
1	M	54	TG1	LEFT	11
2	F	67	TG2	LEFT	13
3	F	63	CG	BOTH	19
4	M	42	TG1	LEFT	18
5	F	46	TG1	LEFT	10
6	M	44	CG	RIGHT	19
7	F	63	TG2	BOTH	16
8	F	33	TG2	LEFT	11
9	M	42	CG	BOTH	17
10	F	50	TG1	BOTH	15
11	F	49	CG	RIGHT	16
12	F	61	TG1	LEFT	8
13	M	72	TG2	RIGHT	19
14	F	64	TG1	RIGHT	16
15	F	57	CG	RIGHT	8
16	F	69	TG2	BOTH	11
17	F	30	TG1	RIGHT	13
18	M	59	TG2	LEFT	15
19	M	58	CG	LEFT	16

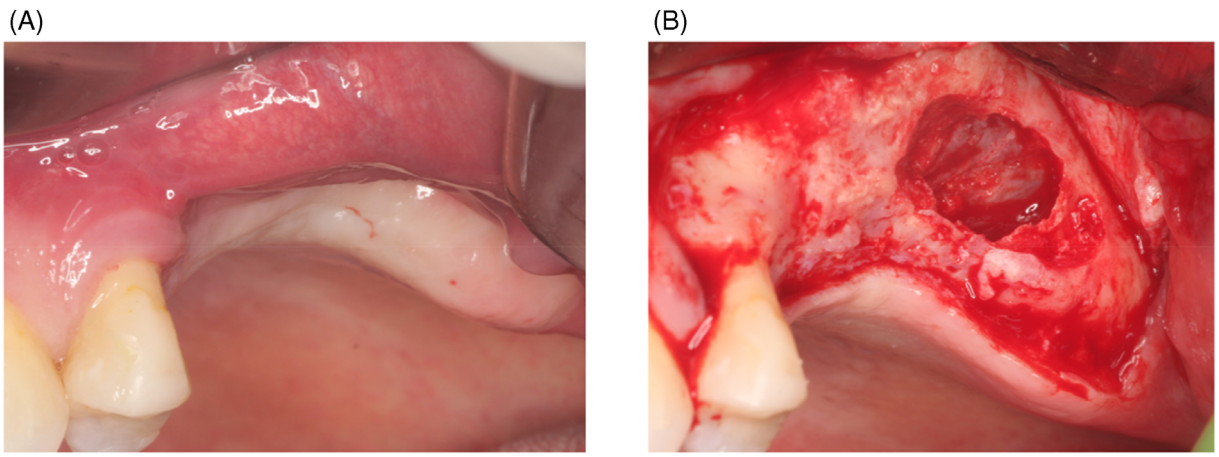

FIGURE 4 Clinical case requiring major bone augmentation. (A) Preoperative view of an edentulous posterior maxilla requiring bone reconstruction and (B) Elevation of a full-thickness buccal flap (note the Schneiderian membrane seen through the lateral window in the maxillary sinus).

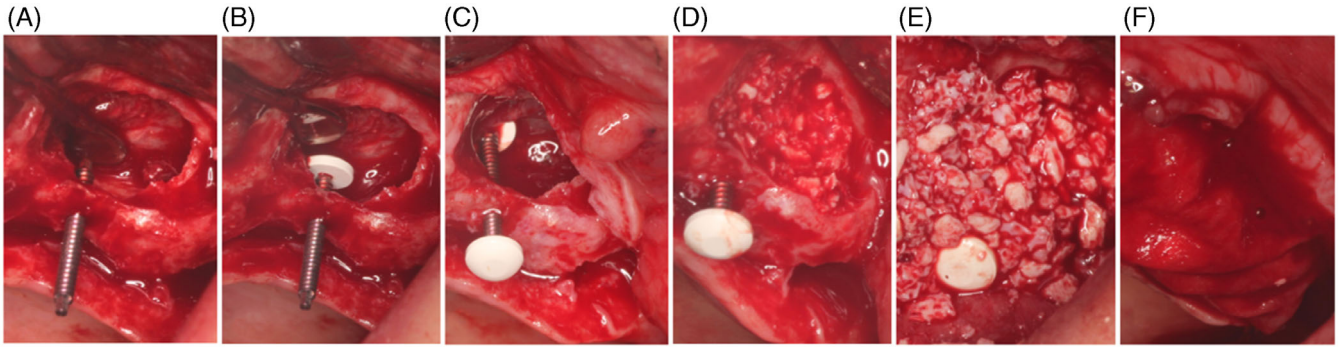

FIGURE 5 Barbell Technique® device installation and grafting. (A) Barbell screw being inserted; (B) First PEEK capsule fixed in the apical tip of the screw; (C) Second PEEK capsule fixed after final positioning of the Barbell screw; (D) Inlay graft in position; (E) Onlay graft in position; and (F) Collagen membrane covering the whole grafted area.

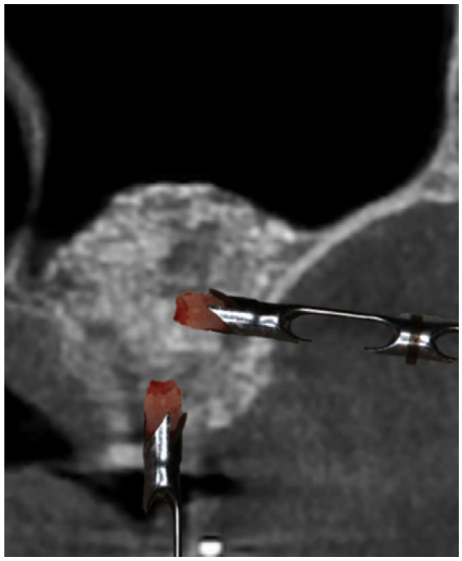

FIGURE 6 Simulation of the two areas where bone biopsies were harvested after healing, on a CBCT scan image. Note that the upper trephine bur is oriented horizontally for collection of a sample from the inlay grafted site, and the lower trephine oriented vertically for collection of a sample from the onlay grafted site.

chlorhexidine solution (Periogard; Colgate, São Bernardo do Campo, Brazil). Sutures were removed after 10 days. All patients were monitored monthly, and included in a hygiene program, until the end of the study.

2.5 | Grafts preparation

In CG, for the inlay graft, an anorganic bone from bovine hydroxyapatite (Bio-Oss large granules, Geistlich Biomaterials, Wolhusen, Switzerland) was used and hydrated with saline solution. In this group, for the onlay vertical graft, the same anorganic bone from bovine hydroxyapatite was mixed in a 1:1 proportion in volume with an autograft harvested from the mandibular ramus using a bone scraper (IM3, São Paulo, Brazil).

To obtain the micrografts in both TG1 and TG2, the micrografting disposable device Rigeneracons coupled to the Rigenera machine (Human Brain Wave SRL, Turin, Italy) was used, following the manufacturer's instructions. The samples harvested to use as the micrografts for TG1 and TG2 were harvested from the same surgical site, near the recipient bed. The periosteum samples were harvested from the inner layer of the flap elevated for access to the surgical site (3 mm periosteal tissue), with the aid of a 15C scalpel—TG1. The bone samples were harvested from the tuberosity, with the aid of a with a 2 mm trephine drill—TG2. Both tissues were disaggregated with the addition of 1.5 ml of saline solution in the device. This tool filters and select cells measuring 50–80 μm in size to create autologous micrografts that can be used without extensive manipulation.[14,25,26] The mechanical disaggregation was then activated, inserting the device in the Rigenera machine (70 r/min and 15 Ncm) and after 2 min, the micrograft suspension was collected with a syringe coupled to the device. The micrograft suspension was used to embed the anorganic bovine bone for 10 min (Figure 7).

The quantity of solution used to hydrate the bovine hydroxyapatite was calculated by using the following proportion: 1.5 ml to hydrate 1.0 g of bovine hydroxyapatite.

2.6 | Specimen preparation and histologic/immunohistochemical processing

The bone biopsies were fixed in 4% formaldehyde solution (Merck, Darmstadt, Germany). The biopsies were demineralized in 20% formic acid, dehydrated and embedded in histological paraffin in order to perform 4 μm thick cuts in the central region of the samples. For histology, the samples were stained with hematoxylin–eosin, and then mounted on resin slides.

In this study, immunohistochemistry was performed to verify vascularization by using a previously published method for VEGF immunostaining.[27] Images were captured on a computerized imaging

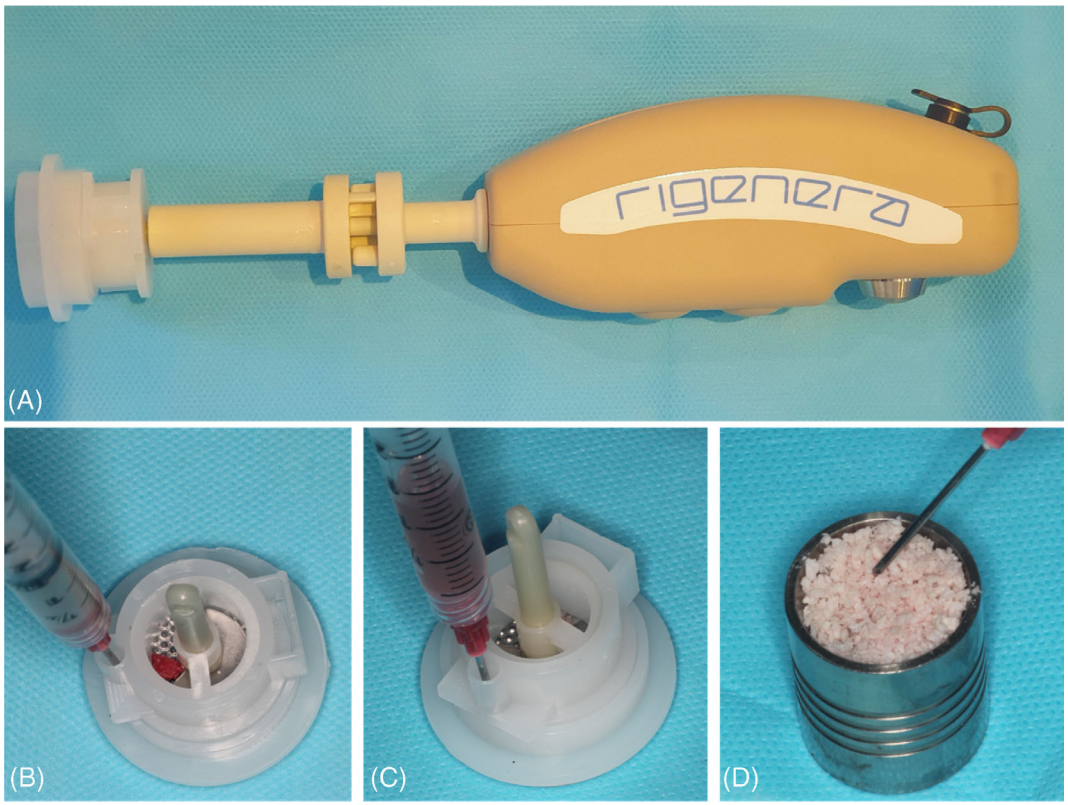

FIGURE 7 Micrografting obtained after sample collection. (A) Rigeneracons coupled to the Rigenera machine; (B) Tissue sample positioned inside Rigeneracons disposable device and insertion of 1.5 ml saline solution; (C) After spinning (70 r/min and 15 Ncm) for 2 min, the micrograft suspension was collected with a syringe using the dedicated access hole; and (D) Micrograft suspension mixed with the anorganic bovine biomaterial.

system (AxioVision rel 4.8, Carl Zeiss, Oberkochen, Germany) coupled to the Axioskop 2 Plus light microscope (Carl Zeiss, Oberkochen, Germany).

2.7 | Histomorphometric and Immunohistochemical analysis

The entire area of the trephine biopsy above and below the native bone of the sinus were defined as region of interest. The software ImageJ Pro Plus 4.5 for Windows software (National Institute of Health, Bethesda, MD) was used for all measurements.

For histomorphometry, hematoxylin–eosin stained samples were evaluated on photomicrographs taken at ×100 magnification. The following parameters were considered: (1) nonvital mineralized tissue (NVMT), (2) vital mineralized tissue (VMT), and (3) nonmineralized tissue (NMT).[27] For analysis, four different areas of each fragment were evaluated, which were then averaged out. All results were noted in square micrometers and, subsequently, stated as a percentage of the total area.

For immunohistochemistry, the expression of VEGF was analyzed. To verify, a semiquantitative approach was used to define the percentage of cell positivity, which was graded 0 to 3, where 0 corresponded to <10% positive cells, grade 1 ranged from 10% to 25% positive cells, grade 2 ranged from 25% to 50% positive cells, and grade 3 contained >50% positive cells.[28]

2.8 | Tomographic analysis

Cone beam computed tomography (CBCT) scans were obtained using the i-Cat apparatus (Imaging Sciences International, Hatfield, PA) set to operate at 120 kVp, 36 mA, with field of view of 13 cm and an exposure time of 40 s. Images obtained in DICOM format with 96 dpi resolution, 14-bit gray scale and 0.25 mm voxel size were converted into BSB format using Blue Sky Plan software (Blue Sky Bio, Libertyville). The software was downloaded and all analysis performed in a MacBookPro 13 inches, M2, 2022 (Apple Inc., Cupertino). The scans were taken at 2 time points: baseline, before the bone augmentation surgery and 6 months after surgery, before surgical reopening for implant placement.

The measurements performed on CBCT images were done to establish the amount of pristine bone and the total amount of vertical bone gain inside (i.e., inlay) and outside (i.e., onlay) the sinus. All

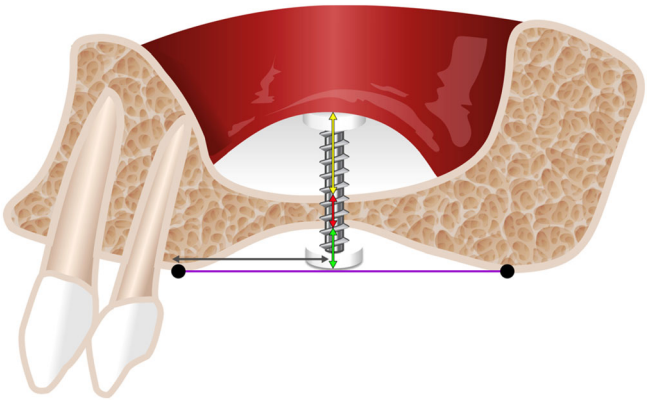

FIGURE 8 Diagram showing the references and measurements performed in the study. Note the two reference points (black dots) and the reference line (purple line). The dark gray arrow represents the distance between the mesial reference point and the Barbell screw after 6 months, which was transferred to the baseline scans for comparison at the same location. The yellow arrow represents the inlay gain, the red arrow represents the pristine bone and the green arrow represents the onlay gain (in this scheme simulating a maximum gain).

measurements were taken by using the Panoramic and Cross-Sections view of Blue Sky Bio Planning software (Blue Sky Bio, Libertyville). To measure the bone volume gained, CBCT scans were taken after 6 months of healing to compared with the initial, baseline CBCT scan. For standardization of the measurement points (Figure 8), in baseline CBCT scans, two reference points at the most coronal aspect of the edentulous posterior ridge were considered, being one in the mesial and the other in the distal. After, a reference line (purple, Figure 8) was traced between the two reference points.

At 6 months post-surgery CBCT scans, the distance between the Barbell screw to the mesial reference point was measured (dark gray arrow, Figure 8), and transferred to the baseline CBCT to identify the same measurement location in both pre-surgery and post healing scans. This standardization allowed the measurement of the height of pristine bone (red line, Figure 8) and inlay (yellow line, Figure 8) and onlay (green line, Figure 8) bone gain.

For the onlay bone gain, the percentage was calculated by measuring the obtained gain, in millimeters, divided by the maximum vertical appositional bone gain that could be achieved, using the space present between the reference line and the pristine bone.

2.9 | Statistical analysis

For histomorphometric and tomographic analysis, a Wilcoxon–Mann–Whitney *U* test were used for comparison among the groups. These statistical analyses were based on patient means and one summary value was calculated per trial participant (i.e., one value per trial participant was analyzed—19 patients). The level of significance assumed for deciding on all statistical tests performed were set at 5%.

3 | RESULTS

Fifty implants were installed in the grafted sites, for the 19 patients. There was no drop-out during the follow-up. During the healing period after implant placement, only one implant failed to osseointegrate (in CG). The percentage of pre-loading implant failure in this study was 2% (i.e., one implant lost from 50 installed implants). This single implant loss was managed by placement of a new implant, 3 months after the removal of the failed implant. All patients received fixed prosthesis reconstruction after osseointegration was achieved and their oral function re-established.

The results of the histological and histomorphometric analysis for the three groups, at the two locations (inlay and onlay) are depicted in Figure 9.

For the inlay grafts, there was no significant difference among the groups for VMT, NVMT and NMT (Table 2). However, for onlay grafts, a higher amount of VMT was observed for CG in comparison with TG2, and the opposite occurred for NMT values. Moreover, no statistical difference was observed between CG and TG1 and between TG1 and TG2, for all parameters (Table 3).

Concerning the immunohistochemical analysis, the distribution of sites by group and score can be seen in Table 4. The immunohistochemical results for VEGF are illustrated in Figure 10 and Table 5.

The CBCT analysis showed that in all groups either in onlay and inlay defects, it was observed the same bone gains (Figures 11 and 12), with no significant difference among groups. Moreover, the onlay defects at baseline were similar for all the three groups. All CBCT results are shown in Table 6.

4 | DISCUSSION

In this study, we showed for the first time the concomitant use of micro-grafts and Barbell Technique® for bidirectional vertical bone augmentation. The autologous micro-grafts were obtained by a CE Class I medical devices called Rigeneracons, which can increase the cell viability in the recipient surgical site.[29,30] We elected to verify the performance of Rigeneracons by using two tissue sources commonly used for cell therapy related with bone tissue engineering purposes: the periosteum and autogenous bone tissue. These two micrografts were mixed with a commonly used bovine anorganic biomaterial. To allow the comparison of this technology with the standard procedures, the Control Group received bovine anorganic biomaterial alone in the inlay (i.e., sinus floor grafting) procedure and a mix of the bovine anorganic biomaterial with autograft (in a 1:1 ratio) for the onlay (i.e., appositional) reconstruction. This approach is supported by the literature for vertical bone augmentation.[9–12]

Posterior maxilla vertical bone reconstruction can be obtained by sinus augmentation, onlay bone grafting or combination of both approaches. As Schmitt et al.[31] showed that a higher vertical height can be achieved by application of both methods together, a combined vertical bidirectional bone augmentation procedure was selected for

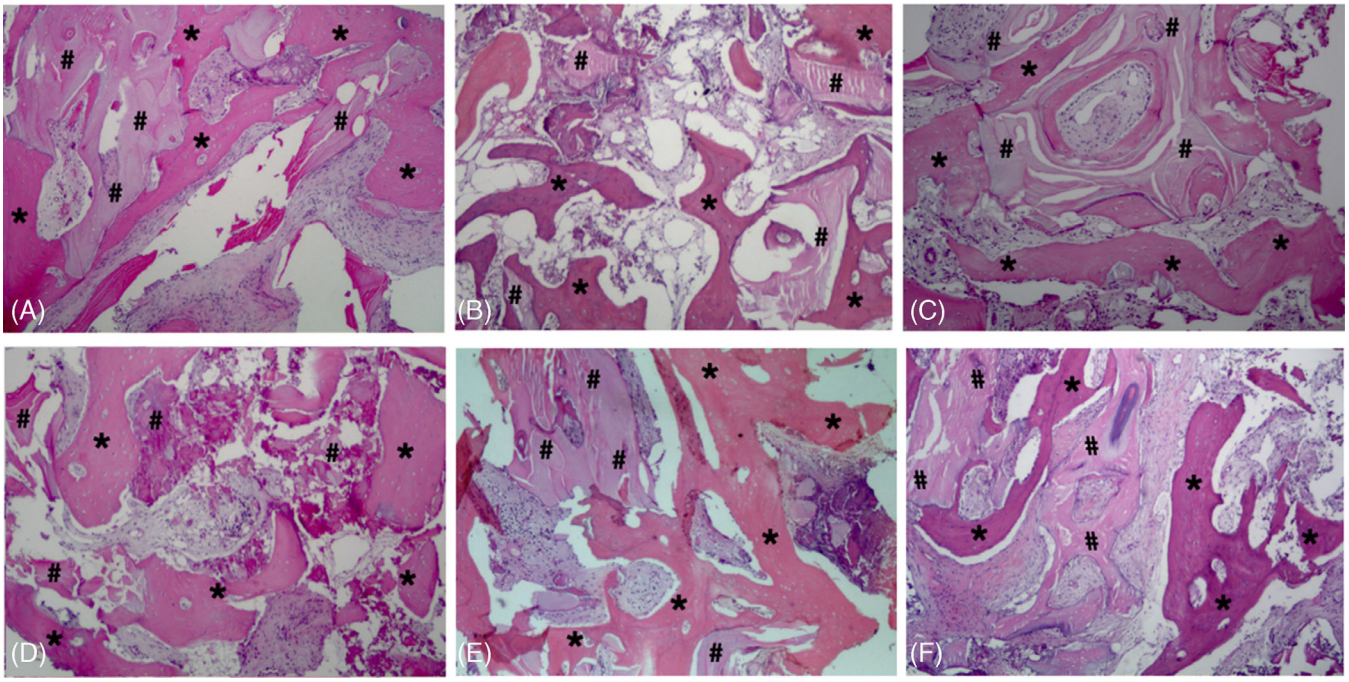

FIGURE 9 Photomicrographs of histologic slides showing remaining hydroxyapatite biomaterial (#) and newly formed bone (VMT, *). (A) CG, inlay; (B) TG1, inlay; (C) TG2, inlay; (D) CG, onlay; (E) TG1, onlay; and (F) TG2, onlay (×100 magnification).

TABLE 2 Histomorphometric evaluation results for the inlay sites.

Group	VMT (%)	NVMT (%)	NMT (%)
CG	31.13 ± 8.13 A	18.00 ± 11.95 A	50.87 ± 13.79 A
TG1	31.75 ± 13.13 A	16.35 ± 11.56 A	51.91 ± 14.89 A
TG2	31.71 ± 16.77 A	12.77 ± 6.13 A	55.52 ± 18.08 A
p value CG vs. TG1	0.6744	0.7527	0.6744
p value CG vs. TG2	0.7527	0.4622	0.5286
p value TG1 vs. TG2	0.7527	0.5286	0.6744

Note: Different letters in the same column indicate a significant difference (Wilcoxon–Mann–Whitney *U* test, $p < 0.05$).
Abbreviations: CG, control group; NMT, non-mineralized tissue; NVMT, non-vital mineralized tissue; TG1, Test Group 1; TG2, Test Group 2; VMT, vital mineralized tissue.

TABLE 3 Histomorphometric evaluation results for the onlay sites.

Group	VMT (%)	NVMT (%)	NMT (%)
CG	46.19 ± 10.42 A	7.85 ± 4.24 A	45.96 ± 9.82 B
TG1	43.26 ± 14.03 AB	11.38 ± 10.62 A	54.64 ± 14.34 BA
TG2	32.74 ± 6.45 B	8.68 ± 7.85 A	58.59 ± 11.26 A
p value CG vs. TG1	0.6363	0.5606	0.7527
p value CG vs. TG2	0.0157	0.7927	0.0274
p value TG1 vs. TG2	0.1152	0.6340	0.0929

Note: Different letters in the same column indicate a significant difference (Wilcoxon–Mann–Whitney *U* test, $p < 0.05$).
Abbreviations: CG, control group; NMT, non-mineralized tissue; NVMT, non-vital mineralized tissue; TG1, Test Group 1; TG2, Test Group 2; VMT, vital mineralized tissue.

this study. For this purpose, the Barbell Technique was the surgical technique of choice. This surgical technique permitted a tent pole effect to prevent invagination of soft tissue and maintain space necessary for new tissue formation. The tenting screw also served as a reference point for measurements at baseline and 6 months postoperative. This technique was originally designed for clinical situations requiring bidirectional horizontal reconstruction (i.e., at buccal and palatal/lingual sides), such as knife edge ridges.[19,20] The authors of this

study decided to use this technique for bidirectional vertical reconstruction, as the clinical cases selected in the present study required bidirectional grafting, a sinus floor augmentation (i.e., inlay graft) and a vertical appositional bone augmentation (i.e., onlay graft). The Barbell device was changed from a horizontal position (used in the original technique) to a vertical one (used in the present study) maintaining the available space for new bone formation.

The CBCT analysis showed no significant difference between groups for both inlay and onlay augmentation. This may be related with the bovine hydroxyapatite's slow resorption rate used in the present study, as Chávarri-Prado et al.[32] showed just a slight height reduction (5.36 ± 2.41%) 6 months after sinus lift procedures with the bovine xenograft. However, the histomorphometric analysis showed a higher level of vital mineralized bone in the onlay reconstruction sites where the standard autograft (CG) was used, in comparison with the bone micrografting group (TG2). However, CG was not different from TG1, where the periosteum derived micro-graft was used. Accordingly, despite the lack of volumetric difference between groups, the bone healing process were different and unfavorable for the group where bone derived micro-graft was used. Therefore, it can be stated in this experimental model that the periosteum derived micro-graft performed as well as the biological gold standard, autograft, for the primary outcome (newly formed bone) at the more challenging onlay sites. For the inlay sites, all groups performed similarly. One possible explanation for this finding is the significant anatomic difference between the deficient sites requiring an inlay or onlay graft. In the inlay graft, the graft is positioned inside the antrum of the sinus, which

TABLE 4 Distribution of sites by group and score.

SCORE	CG	TG1	TG2
Onlay			
0	1	3	4
1	5	3	2
2	1	1	2
3	1	1	0
Total	8	8	8
Inlay			
0	0	0	1
1	3	3	3
2	4	4	3
3	1	1	1
Total	8	8	8

Abbreviations: CG, control group; TG1, Test Group 1; TG2, Test Group 2.

TABLE 5 Immunohistochemical results (average score [minimum score/maximum score]).

CG	TG1	TG2
Inlay		
2.0 [1/3]	2.0 [1/3]	1.5 [0/3]
Onlay		
1.0 [0/3]	1.0 [0/3]	0.5 [0/2]

Abbreviations: CG, control group; TG1, Test Group 1; TG2, Test Group 2.

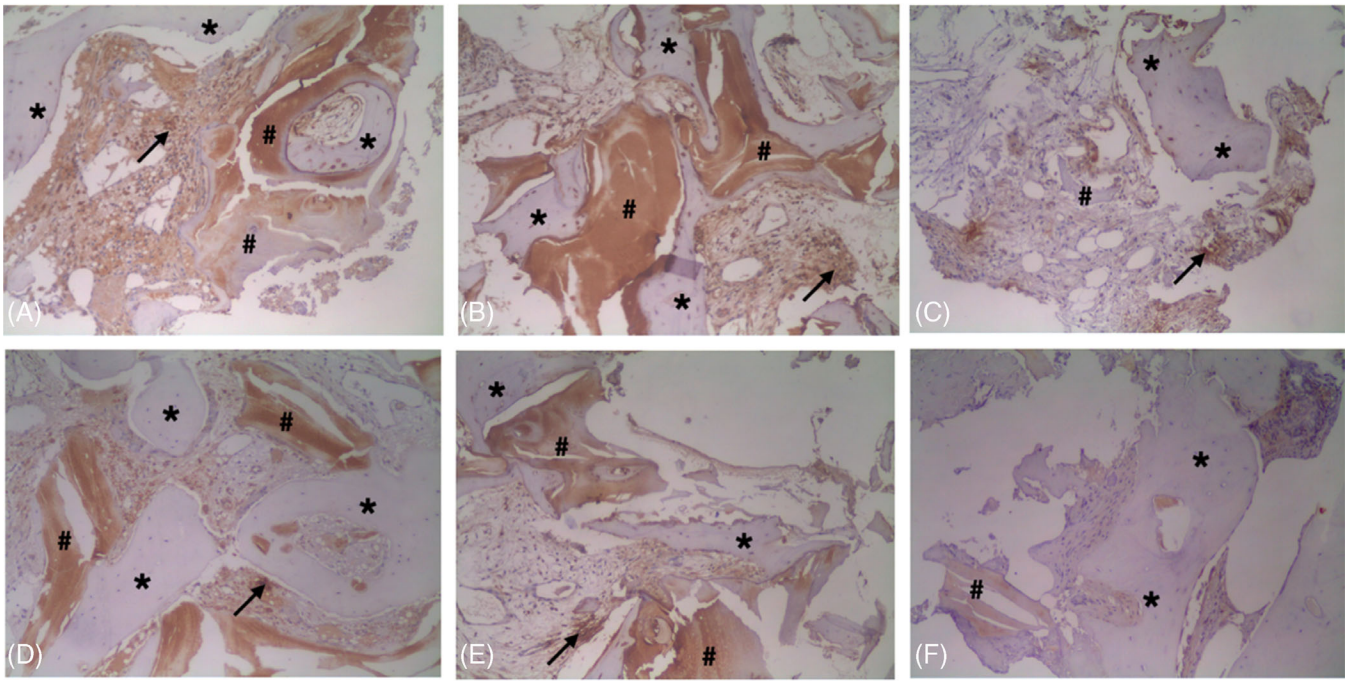

FIGURE 10 Photomicrographs of the immunohistochemical reactions for VEGF, showing remaining hydroxyapatite biomaterial (NVMT, #), newly formed bone (VMT, *) and VEGF positive cells (arrows). (A) CG, inlay; (B) TG1, inlay; (C) TG2, inlay; (D) CG, onlay; (E) TG1, onlay; and (F) TG2, onlay (×100 magnification).

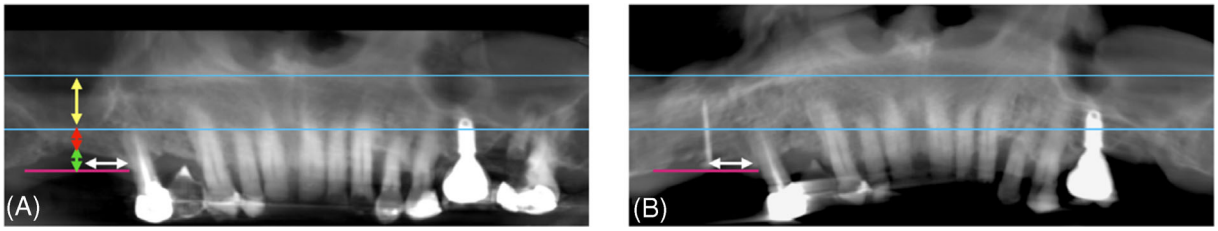

FIGURE 11 CBCT panoramic view at (A) T1 and (B) T2. The white arrow represents the distance between the mesial reference point and the Barbell screw at T2, which was transferred to T1 scans for comparisons at the same site. The yellow arrow represents the inlay gain, the red arrow represents the pristine bone and the green arrow represents the onlay gain.

FIGURE 12 Cross-Sections of the same site at T1 (A) and T2 (B). The yellow arrow represents the inlay gain, the red arrow represents the pristine bone and the green arrow represents the onlay gain.

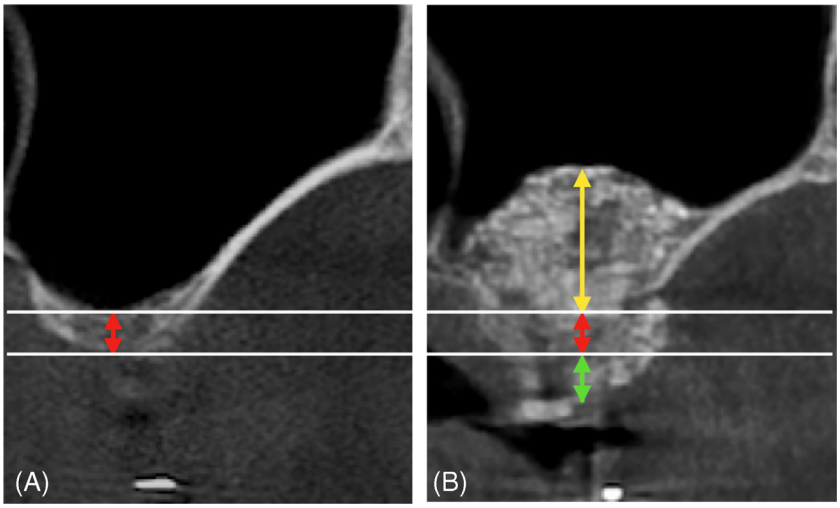

TABLE 6 Tomographic evaluation results.

Groups	Inlay Gain (mm)	Onlay Gain (mm)	Onlay Defect (mm)	Onlay Gain (%)
CG	8.95 ± 1.79 A	1.72 ± 1.56 A	2.56 ± 1.17 B	58.07 ± 31.68 A
TG1	8.80 ± 2.48 A	1.79 ± 2.14 A	2.57 ± 1.83 B	61.15 ± 44.95 A
TG2	8.65 ± 2.62 A	1.52 ± 1.50 A	2.48 ± 1.28 B	51.23 ± 34.24 A

Note: Different letters in the same column indicate a significant difference (Wilcoxon–Mann–Whitney *U* test, $p < 0.05$).
Abbreviations: CG, control group; TG1, Test Group 1; TG2, Test Group 2.

is a self-contained defect with surrounding walls. The alveolar defect requiring an onlay graft is much more challenging from a biological point of view, as it demands the positioning of the graft over just one wall. This one-wall defect is non-self-containing and does not have as much vascularity or cellularity from the pristine bone recipient bed. Therefore, for inlay reconstructions there is not a high demand for osteoinduction and osteogenesis properties from the graft, as is necessary for onlay augmentations. The self-containing three wall defect of the maxillary sinus allow the clinicians to more predictable outcomes just using an osteoconductive anorganic bovine bone alone for augmenting the sinus floor.[9]

The immunohistochemical analysis indicated a slightly higher expression of VEGF values for CG and TG1 than those obtained by TG2, for both inlay and onlay grafts, but without statistical significance. In this sense it's important to state that, for statistical comparison between groups, a Proportion test (Chi-Square) was used because the analysis involved score counts per group. So, as a semiquantitative approach was used to evaluate the immunohistochemical results, the median was not the main measure to test VEGF results between groups. In this study, the expression of the VEGF was selected as it is an important stimulator of angiogenesis and bone formation.[33] The slightly higher score for VEGF expression in TG1 than in TG2 might be attributed to the presence of progenitor cells and factors in periosteum micrograft that may regulate vascularization and bone formation. Trovato et al.[14] showed that micro-grafts obtained by periosteum samples are enriched of progenitor cells, which can maintain its capacity to differentiate. Periosteal cells have the ability to self-commitment toward osteogenic lineage,[34,35] which may had contributed for the results observed in TG1. In addition, the abundance of pericytes related to vascularization[36] as well the presence of growth factors and cytokines[37] derived from progenitor cells presented in the periosteum tissue may contribute to a better

performance in bone tissue repair and regeneration when the periosteum micrografts were used compared to bone micrografts.

Another intriguing factor is the difference in hardness properties between the two tissues, which may play a role in changes in micrograft processing. Because of its hardness, mechanical disaggregation of bone tissue employing Rigeneracons may fail to properly disaggregate the tissue, inhibiting the release of cytokines and growth factors necessary for bone formation. Furthermore, during mechanical disaggregation, osteocytes, the primary cellular component of skeletally mature adult bone tissue,[38] may undergo apoptosis, resulting in the generation of RANKL, the cytokine essential for osteoclast activation.[39] This might explain the slight difference concerning VEGF expression, for inlay augmentations, between the sites where just the anorganic bone was used (i.e., CG) and the sites where the anorganic bone was associated with the bone micrografts (i.e., TG2). In this regard, despite an in vivo study showed good performance in bone regeneration when using micrografts derived from bone tissue, it is important to highlight that in that study the entire amount of bone removed from a 5 mm size rat calvaria defect[18] was used, instead 2 mm size samples were used in the present study, which may explain the different results between the animal and human study.

The clinical outcomes of the present study showed a single early implant failure out of 50 installed implants. This level is consistent with the current literature.[40] This single implant failure occurred in CG and was managed successfully by placing a new implant 3 months after the failed implant removal. The authors of the present study did not correlate this failure with the maintenance of the PEEK capsule in an apical position, as PEEK is biocompatible, increasing adhesion and viability and permitting proliferation of osteoblasts and gingival fibroblasts compared with titanium implant material.[41] Moreover, we achieved only one loss within 50 implants installed in the same way. However, as the device's PEEK capsule positioned into the sinus cavity is not removed and will not resorb, future longitudinal research should be designed to verify the long-term outcomes concerning eventual biological complications. Nonetheless, in the authors' opinion, a possible explanation for the one implant failure may be associated with the lack of the patient compliance. The patient used a removable prosthesis during the osseointegration phase. Inadequate prosthodontic management has been correlated to causing early failure of dental implants.[42] In this regard, as all patients enrolled in this study had posterior missing teeth, the decision of avoiding the use of a removable prosthesis during osseointegration was adopted as a standard protocol. This single patient failed to follow this protocol and wore a removable prosthesis immediately following the implant placement surgery. All patients received fixed prosthesis reconstruction after osseointegration was achieved and their oral function re-established.

The results of the present study reinforced that micrografts derived from periosteum may be an alternative approach for cell therapy in attempting to achieve bone regeneration, especially in clinical situations that require appositional bone augmentation. Considering the morbidity associated with harvesting large volume of autograft from a secondary donor site, this medical device with its ease of handling may provide a less traumatic alternative for harvesting donor tissue. Studies comparing the presence of viable cells as well cytokines and growth factors present in different tissue sources for micrografting should be performed in future investigations.

5 | CONCLUSION

This study indicates that clinical use of micrograft derived from periosteum may have potential to increase bone formation in onlay reconstructions in the posterior maxilla when used together with Barbell Technique®, unlike the micrograft derived from bone tissue.

AUTHOR CONTRIBUTIONS

André Antonio Pelegrine: concept/design, data analysis/interpretation, drafting article; Luiz Antonio Mazzucchelli Cosmo: data analysis/interpretation, drafting article, data collection; Luís Guilherme Scavone de Macedo: data analysis/interpretation; Reginaldo Machado Coutinho: drafting article; Antonio Carlos Aloise: data analysis/interpretation; Sérgio Jorge Jayme: critical revision of article; Elizabeth Ferreira Martinez: critical revision of article; Peter Karyen Moy: critical revision of article, approval of article; Antonio Graziano: approval of article; João Pedro Grandini Zeferino: data collection.

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CONFLICT OF INTEREST STATEMENT

Antonio Graziano is the CEO of Rigenera HBW (the producer of the micrograft device). André Antonio Pelegrine and Luís Guilherme Scavone de Macedo are the inventors of the Barbell Technique.

DATA AVAILABILITY STATEMENT

The data that support the findings will be available in Barbell (Vertical) Research at https://dspbiomedical.com.br following an embargo from the date of publication to allow for commercialization of research findings.

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