

ORIGINAL ARTICLE OPEN ACCESS

# Bi-Layered Biphasic Calcium Phosphate Bone Substitute to Improve Bone Formation in Lateral Jaw Defects Applying the Principle of Guided Bone Regeneration (GBR)—A Pre-Clinical Randomized Controlled Study

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**Received:** 27 July 2024 | **Revised:** 6 May 2025 | **Accepted:** 28 May 2025

**Funding:** This work was supported by the Geistlich Pharma AG, Wolhusen LU, Switzerland.

**Keywords:** biphasic bone substitute | bone formation | deproteinized bovine bone mineral (DBBM) | guided bone regeneration | histomorphometry | jaw bone defects | micro-CT | regeneration | synthetic

## ABSTRACT

**Aim:** To evaluate the application of a synthetic bi-layered biphasic calcium-phosphate (BBCP) bone substitute for its capacity for new bone formation in Guided Bone Regeneration (GBR) in an acute-defect model in Beagle dogs.

**Material and Methods:** Standardized bone defects were created following the extraction of the maxillary <sup>1</sup>P<sup>1</sup>, <sup>2</sup>P<sup>2</sup>, <sup>4</sup>P<sup>4</sup> and the mesial root of <sup>3</sup>P<sup>3</sup> in six Beagle dogs. The defects were treated according to the GBR principle using the tested material, synthetic bi-layered biphasic calcium-phosphate bone substitute (Group T), deproteinized bovine bone mineral (DBBM, positive control = PC), a mixture of the test substance and DBBM in a ratio of 1:1 (Group M) and a sham-operated empty control (negative control = NC). The defects were covered with a resorbable collagen barrier membrane. Bone formation was evaluated radiologically, microtomographically, and histomorphometrically after 11 weeks of healing.

**Results:** All biomaterials resulted in increased volume of the augmented bone compared to the negative control. The augmented ridge volume developed to a greater extent in the tested area and in the combination of the tested bone substitute and the DBBM compared to the positive control alone (DBBM). A significant increment in a mineralized tissue and bone-biomaterial contact was observed between the test groups and the positive control.

**Conclusions:** The synthetic BBCP appeared to result in greater bone formation volumes than the positive control (DBBM) and resulted in less contact with soft tissue. Hence, the tested material appeared to be at least as effective as the applied standard for lateral bone augmentation (DBBM).

## 1 | Introduction

In the beginning of the 1980's, the regeneration of periodontal tissues lost as a result of periodontal infections was developed as a biologic principle, later termed as Guided Tissue

Regeneration (GTR). In a series of preclinical experiments, the tissues of the periodontium were tested for their capacity to induce cementogenesis on tooth surfaces previously deprived of the periodontal ligament (Karring et al. 1980, 1993; Nyman et al. 1980). It was conclusively demonstrated that only the

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cells of the periodontal ligament (pdl cells) have the biologic capacity to induce and promote new cementum formation, as a periodontal ligament was formed on an implant surface to replace osseointegration when the implant was in contact with an adjacent periodontal ligament (Buser et al. 1990). The GTR principle is based on the fact that a secluded space is created into which the desired cells for the regenerative process may proliferate, while the undesired cells are excluded from the area by physical barriers such as non-resorbable or resorbable barrier membranes (Gottlow 1993).

Later on, the GTR principle was adapted to the regeneration of bone in jaw bone defects with great success. Specifically, regeneration in bony defects was termed Guided Bone Regeneration (GBR) as a secluded space was created with various barriers (Dahlin et al. 1988). In addition, an attempt was made to promote bone regeneration by filling the secluded space with autologous bone as a scaffold or mixtures of autologous bone and various bone substitutes (Dahlin et al. 1990). The most established substitutes were xenografts such as deproteinized bovine bone mineral (DBBM) (Hämmerle et al. 1998). DBBM is fully biocompatible, osteoconductive, and slowly resorbable by osteoclastic activity (Jensen et al. 2014). However, DBBM is a bovine product and is limited in its osteogenic capacity.

Hence, efforts have been made to develop synthetic bone substitutes that should have similar properties as the standard of care, DBBM, namely biocompatibility, osteoconductivity, and slow or fast resorbability. The reason for choosing a synthetic bone substitute versus xenograft was the intention of reducing animal material and hereby avoiding possible transmission of prions or other infectious materials. Moreover, accessibility to the products is improved, and cost may be reduced. The implantation of  $\beta$ -Tricalciumphosphate ( $\beta$ -TCP) in intrabony defects was tested (Stavropoulos et al. 2010) and demonstrated significant Clinical Attachment Level (CAL) gains and defect fills in periodontal regeneration (Sculean et al. 2015). However, the histologic data have indicated that, following grafting with  $\beta$ -TCP, most particles were embedded in connective tissue, while the formation of a mineralized bone-like or cementum-like tissue around the particles was observed only occasionally. Similar studies with  $\alpha$ -TCP have not been done as yet.

More recently, it had been demonstrated that a newly composed synthetic, bi-layered biphasic calcium phosphate (BBCP) was a biocompatible and osteoconductive biomaterial that led to the healing of critical-size bone defects in rabbits (Saulacic et al. 2021). Furthermore, the formation of new bone was affected by the level of biomimetic nanocrystalline hydroxyapatite (HA) coating of an  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP). On the other hand, there was very little impact of the HA coating on  $\alpha$ -TCP in the bone augmentation potential and material resorption after 4 weeks when mixed with DBBM (Fujioka-Kobayashi et al. 2022). The aim of the present study was to evaluate the performance of a synthetic BBCP in view of its capacity for new bone formation in an acute-defect canine model after 11 weeks of healing. The primary outcome was the amount of newly formed bone assessed histomorphometrically.

## 2 | Material and Methods

### 2.1 | Biomaterials

The test material (Group T) represents a synthetic BBCP, a composite bilayered material composed of an  $\alpha$ -TCP core epitaxially coated with a biomimetic nanocrystalline HA layer of a defined thickness. The core biomaterial is obtained by mixing powders of Ca and P in appropriate ratios, and sintering the mixture at high temperature. The coating is obtained through the transition of the surface of the sintered TCP core biomaterial into biomimetic HA. The biomimetic HA coating has a crystal size similar to that of natural bone mineral. It has a particle size of 0.25–1  $\mu$ m like the positive control DBBM. The second Group tested was a mixture of the DBBM (Bio-Oss, Geistlich Pharma AG, Wolhusen, Switzerland) and the tested BBCP bone substitute in a ratio of 1:1 (Group M). Group PC represents a positive control, namely the hitherto accepted standard of care (DBBM). As a negative control (NC), the defects were left sham operated without any GBR.

The study was designed as a randomized controlled trial with a pilot character with intra-subject controls to evaluate the capacity of the materials for GBR in a horizontal ridge augmentation model in dogs. Four treatment modalities were compared with  $n = 6$  per group.

The animal experiment was performed in the facilities of the “Centro de Biomedicina y Veterinaria in Lugo,” Spain in controlled conditions of temperatures, humidity and ventilation.

Six healthy Beagle dogs were selected and observed in a quarantine period of 21 days. They were fed a commercial granulated dog food previously wetted in water and had free access to water. The dogs were monitored twice per day during the entire experiment by the veterinarian and facility personnel trained and accredited in research animal science following the European regulations on care and use of research animals (Directive 2010/63/EU on the protection of animals used for scientific purposes). The study protocol was approved by the Ethics Committee of the Rof Codina Foundation, Lugo, Spain (05/19/AE-LU-001). In addition, the study complied with the Guidelines for Animal Research: Reporting In Vivo Experiments ARRIVE 2.0.

Following the quarantine period, a model of lateral 3-wall buccal bone defects was created to mimic the clinical situation of horizontal bone augmentation (“semi-saddle-type-defect”). The defect configuration was created similar to that described by Benic et al. (2016) but in the maxilla and not incorporating the placement of an implant. The defect represents a critical-size model with a low self-healing potential.

For the surgical procedures, the animals were sedated with a combination of medetomidine 10  $\mu$ g/kg *i.m.* (Sedodorm 1 mg/mL, Vetpharma Animal Health, Barcelona, Spain) and morphine 0.3 mg/kg *i.m.* (Morfina Braun 2%; B. Braun Medical, Barcelona, Spain). Later, general anaesthesia was induced by propofol (2 mg/kg/*i.v.*, Propofol Lipuro 10 mg/mL, B Braun VetCare SA, Barcelona, Spain) and maintained on a concentration of 1.5%–2% of isoflurane in 100% O<sub>2</sub> (Vetflurane

1000 mg/g, Virbac SA, Carros Cedex, France) by orotracheal intubation.

One dose of 0.2 mg/kg of meloxicam was administered pre-surgically for anti-inflammatory and analgesic treatment (Loxicom 5 mg/mL, Norbrook Laboratories, Monaghan, Ireland). During anesthesia, the animals were continuously monitored by a veterinarian, controlling electrocardiography, capnography, pulse oximetry, and non-invasive blood pressure, and mechanically ventilated.

The surgical sites were sanitized by chlorhexidine oral rinse 0.12% (Perio-aid, Dentaid, Barcelona, Spain) before the procedure. Local anesthesia (Artinibsa, Inibsa, Barcelona, Spain) was given by local infiltration at the soft tissues surrounding each extracted tooth. Bilateral mesial and distal premolar roots 1, 2, 4, and mesial roots of premolar 3 in the maxilla were extracted. The mandibular sites were selected for another study.

First, a flap was raised on the lingual and buccal aspects of the tooth of interest. Then, the premolars were hemisected using a tapered burr connected to a dental contra-angle handpiece. The section was performed in the buccal-lingual axis to extract each root separately. Finally, each root was carefully removed with elevators and forceps. Once the hemi-teeth were removed, sharp bony edges were reduced with a round burr, and the alveolus was rinsed with saline solution to remove potential dental debris. Once extracted, the distal root of premolar 3 was endodontically treated.

Later, in the freshly extracted sockets, areas were created with a lateral 3-wall bony defect (Figure 1). The defect dimensions were standardized and adapted to the anatomical situation with the goal of creating a critical size (i.e., low self-healing potential). The defects were 10 mm in mesio-distal, 8 mm in apico-coronal, and 6 mm in bucco-lingual dimensions. The remaining palatal bony wall was at least 1.5 mm. Measurements of the dimensions were conducted using a PCP UNC 15 periodontal probe (Hu-Friedy Co., Chicago, IL, USA) to the nearest millimeter. Sharp bony edges in contact with the mucoperiosteal flaps were flattened with a round burr.

For purposes of analysis and orientation, mini-screws were placed into the defect floor to mark the original defect bottom. The screw's position was as buccal as possible in the middle of the defects. The defects were then grafted according to an allocation table provided by a randomization protocol ([www.randomization.com](http://www.randomization.com)) to avoid bias and covered by a collagen membrane (Bio-Gide Geistlich Pharma AG, Wolhusen, Switzerland) to follow the principles of GBR. Since four treatment modalities were performed on each dog, the unit of the analysis was the animal. Potential confounders were not specifically controlled. No sample size calculation was performed due to the exploratory nature of this study. Defect filling was aimed to reach the same dimensions of height and width as encountered before the defect creation. After the treatment, the mucoperiosteal flaps were repositioned, and primary wound closure was achieved with resorbable sutures (VICRYL 5-0; Ethicon US).

The wounds were cleaned daily with gauzes soaked in chlorhexidine oral rinse (0.12%, Perio-aid, Dentaid, Barcelona, Spain) until healing was clinically complete. For infection control prior to surgery, animals received a single dose of 22 mg/kg cefazoline *i.v.* (Cefazoline Normon 1g, Laboratorios Normon SA, Madrid, Spain), and at the end of surgery, a single dose of sodic cefovecine at 8 mg/kg *s.c.* (Convenia 80 mg/mL, Zoetis Belgium SA, Louvain-la-Neuve, Belgium) was administered. Post-operative pain was controlled and managed, if necessary, by the administration of buprenorphine (0.01 mg/kg *i.m.*/8h, Bupaq, Richter Pharma AG, Wells, Austria). Inflammation was controlled by the administration of oral meloxicam 0.1 mg/kg once a day during 2 days (Loxicom 1.5 mg/mL, Norbrook Laboratories, Monaghan, Ireland).

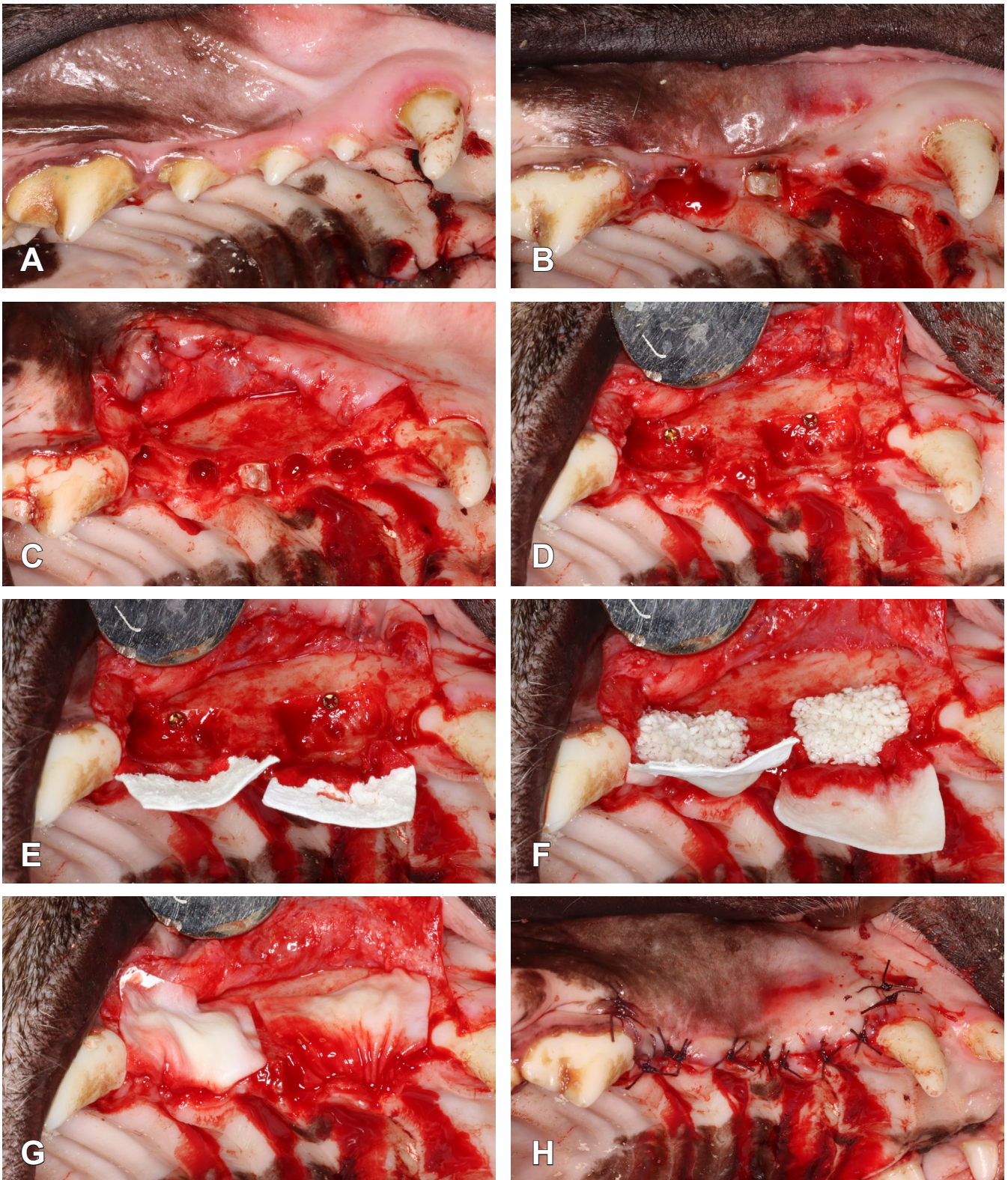
Eleven weeks after surgery, the animals were sacrificed by intravenous overdose of sodium pentobarbital (200 mg/kg *i.v.*; Dolethal 200 mg/mL; Vetoquinol Especialidades veterinarias, Madrid, Spain) under sedation with a combination of medetomidine (10 µg/kg *i.m.*) and morphine (0.3 mg/kg *i.m.*), and induced with 2 mg/kg of propofol *i.v.* Both maxillae were carefully dissected and immersed in a solution of 10% buffered formaldehyde for approximately 2 weeks.

## 2.2 | Micro-CT

After fixation, the samples were scanned using a high-resolution micro-CT (Skyscan 1172, Bruker micro-CT NV, Kontig, Belgium) freshly placed in a gauze soaked in 10% formaldehyde and surrounded by a soft plastic sheet to avoid dehydration.

The X-ray source was set at 100 kV and 100 µA with a pixel size of 13.54 µm and the use of an Aluminum/copper filter (Al/Cu) the scan was performed with a 360° rotation and images acquired every 0.4°. Both defects of each maxilla were scanned at the same time. After scanning, images were reconstructed using NRecon software (Bruker micro-CT NV, Kontig, Belgium) and based on the Feldkamp algorithm 7. The same reconstruction parameters were used for all the samples (to allow comparison) and after the correction of the possible misalignment (smoothing = 2; Beam Hardening = 40; Ring Artifact correction = 12). Each defect was reconstructed separately. Using Data viewer software (Bruker micro-CT NV, Kontig, Belgium), the three wall defects were oriented to allow the analysis (Supplementary Figure S1) placing the defect in an anatomical position and the bottom screw as vertical as possible.

Using Data Viewer software, the height of the new bone regeneration was calculated inside the defect in bucco-lingual orientation as well as the original bone crest in the center of the defect and on one side, using the screw as a bottom reference. For the analysis of the samples, CTAn software (Bruker micro-CT NV, Kontig, Belgium) was used. Briefly, in the coronal view, two different Volumes of Interest (VOIs) were depicted. The height of the two VOIs was the same, taking the central slice as reference, and two different VOIs were selected; first, one VOI outlining the biomaterial (Supplementary



**FIGURE 1** | Surgical protocol. (A) Maxilla pre-surgery with teeth present. (B) Status after tooth extractions. The distal root of the  $3P^3$  was left in place. (C) Raised mucoperiosteal flap displaying the extraction sockets and the remaining  $3P^3$ . (D) Defect creation and placement of the reference screw. (E) Placement of resorbable barrier membranes. (F) Defect filling according to the treatment modalities. (G) Coverage of the augmentation area with resorbable barrier membranes. (H) Primary wound closure and suturing.

Figure S2A) was depicted by hand and later on, an automatic elliptic VOI of  $300 \times 600$  pixels ( $4064 \times 8128 \mu\text{m}$ ) in the center of the defect (Figure S2B) was selected.

The biomaterial only was delineated in the samples with material inside. For the threshold selection, the parameters 75–255 for biomaterial and 45–255 for mineralized tissue (bone plus

biomaterial) were used. Finally, the percentage of mineralized tissue, percentage of biomaterial, percentage of new bone, and percentage of soft tissue was analyzed in these two VOIs.

The last analysis performed in micro-CT scans was the volume of the augmented tissue. For this purpose, the transaxial view was stored using DataViewer software and opened in CTAn. The total volume of regeneration was selected using the mesial and distal walls of the defect as margins and manually outlining the augmented tissue using the new bone as a reference (Figure S3).

### 2.3 | Histologic Sample Preparation

After micro-CT scanning, the augmented bone defects performed in this study were carefully separated and identified. Later on, the bone blocks and the soft tissues around them were processed for undecalcified ground sectioning following the method described by Donath and Breuner (1982). In brief, the samples were dehydrated in ascending series of ethanol solutions and embedded in a light-curing resin (Technovit 7200 VLC; Heraeus-Kulzer GmbH, Werheim, Germany). From each specimen, one central buccolingual section through the augmented site was prepared using a band saw and mechanically micro-polished (Exakt Apparatebau, Norderstedt, Germany) using 1200 and 4000 grit silicon carbide papers (Struers, Copenhagen, Denmark) obtaining samples with a thickness of approximately 50  $\mu\text{m}$ . Subsequently, the samples were stained applying the Laczko and Levai (1975) staining method and digitized with bright field microscopy (DMB 6 Thunder Imager, Leica Biosystems Technology, Wetzlar, Germany).

### 2.4 | Histomorphometric Analysis

For the determination of bone formation, the digital images were painted using Photoshop software (Adobe, San Jose, CA, USA) by a masked examiner discriminating between bone, biomaterial, and bone marrow. Once painted using an automated image analysis system (CellSens, Olympus Corporation, Japan), a rectangular region of interest (ROI) of the same dimensions was created in all samples. Within the rectangle, the regenerated ROI was determined by drawing a line across the surface of the regenerated bone (Figure S4). The area and percentage of the rectangle occupied by the contour of newly formed bone were calculated. In Group M, the area and percentage of each material were determined separately.

In all samples, the distribution of tissues was analyzed as follows:

- New bone (NB) area in  $\text{mm}^2$  and percentage
- Biomaterial (BM) area in  $\text{mm}^2$  and percentage
- Mineralized tissue (MT) area in  $\text{mm}^2$  and percentage
- Soft tissue (ST) area in  $\text{mm}^2$  and percentage
- Region of Interest regenerated (ROI Reg) in  $\text{mm}^2$  and percentage
- Bone to biomaterial contact (BBC) in mm and percentage

To measure the bone to material contact, a ROI following the contour of the defect was defined. The total perimeter of all the biomaterial granules is obtained with Image Pro Premier (Media Cybernetics, Bethesda, MD, USA), and the percentage of contact between bone and biomaterial was automatically calculated. In Group M with the mixture of biomaterials, the bone to material contact was differentiated between both biomaterials histologically (different stains). One examiner assessed the parameters of  $\mu\text{CT}$  and histomorphometry, without being aware of the samples' allocation.

### 2.5 | Statistical Analysis

Results were presented as means, medians, and standard deviation (SD) values for each group. The normal distribution was checked, and normality of variances was assessed and assured using the Shapiro–Wilk test. The differences between the four groups were compared using a repeated measures ANOVA. *Post hoc* comparisons were performed using Tukey's test to identify significant differences between pairs of groups. The analysis was done using a statistical software SigmaPlot 12.5 for Windows (Systat Software Inc., San José, CA). A value of  $\alpha = 0.05$  was considered statistically significant.

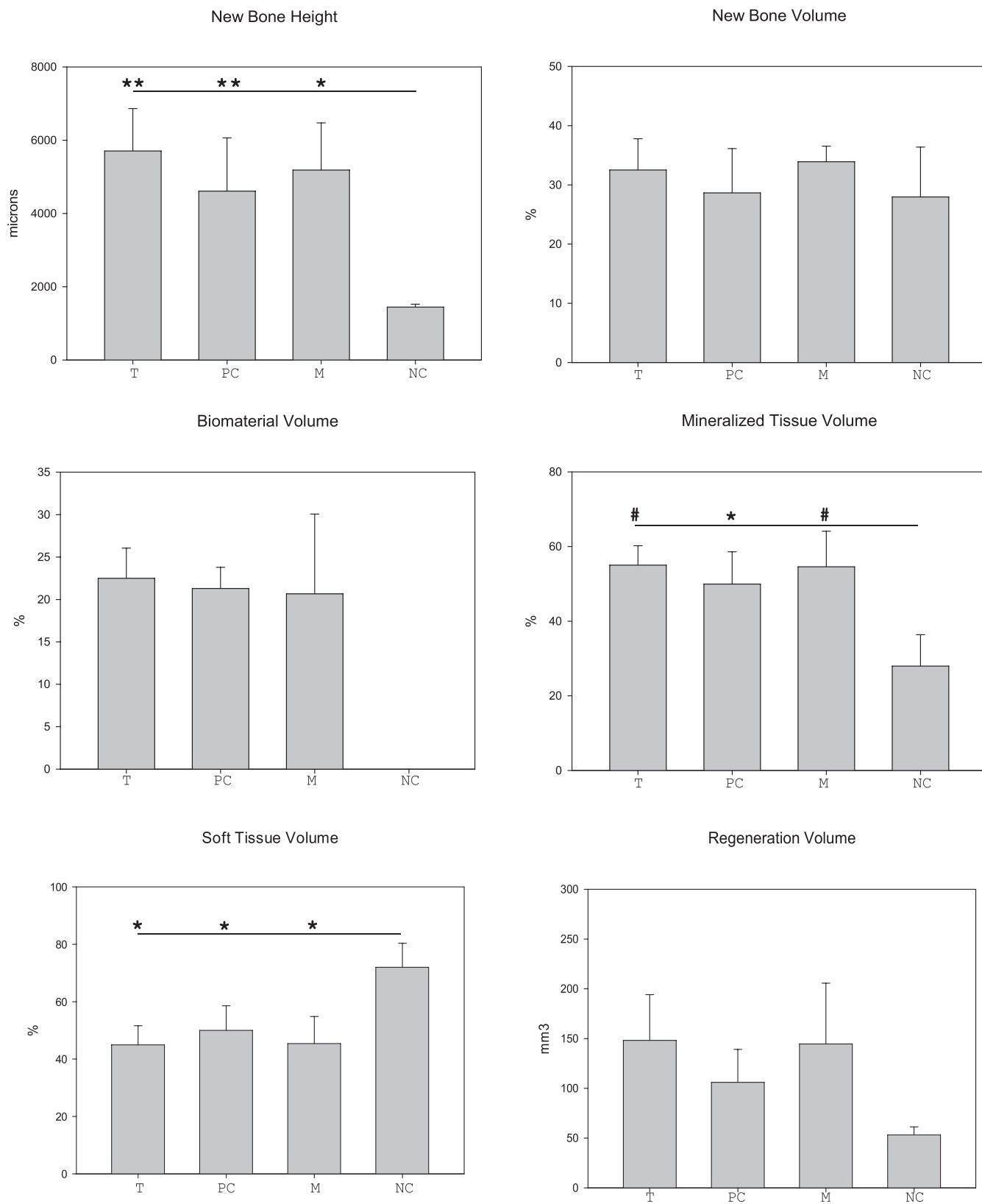
## 3 | Results

During the entire experimental period, no technical problems were observed. All animals were healthy and survived the procedures without any complications. Following necropsy, no significant alterations of the organs or the tissues were identified. All samples were included in the analysis ( $n = 6$ ).

### 3.1 | Micro-CT

Micro-CT revealed homogeneously distributed mineralized structures throughout the defect area comprising bone substitute materials and newly formed bone. New mineralized bone had formed at the borders of all defects. The regeneration heights ( $\mu\text{m}$ ) measured from the bottom of the defects to the most apical new bone for the defect heights are shown in Figure 2 (Table S1). The differences between the groups with biomaterials (T, M, PC) did not reach statistical significance, while the three groups differed statistically significantly from the NC ( $p = 0.004$ ; NC vs. T  $p = 0.003$ ; NC vs PC  $p = 0.008$ ; NC vs. M  $p = 0.021$ ).

The values for the percentages of different micro-CT parameters measured in the VOI are depicted in Figure 2 (Table S1). While there were no statistically significant differences in the percentage of new bone and percentage of biomaterial among the three groups (T, PC, M), a highly statistically significant difference was found in the VOI between the three groups (T, PC, M) and the NC for the mineralized tissue percentage ( $p < 0.001$ ; NC vs. T  $p < 0.001$ ; NC vs. PC  $p = 0.03$ ; NC vs. M  $p < 0.001$ ) and soft tissue percentage ( $p = 0.009$ ; NC vs. T  $p = 0.011$ ; NC vs. PC  $p = 0.03$ ; NC vs. M  $p = 0.011$ ).



**FIGURE 2** | Micro-CT analysis. Representative bar charts Box Plot for the comparison of the 4 groups for new bone crest height, relative values (%) of new bone, biomaterial, mineralized tissue and soft tissue and regeneration volume (\* $<0.05$ , \*\* $<0.01$ , and # $<0.001$ ).

The regenerated volume was higher in all the augmented groups (T, PC, M) compared to the NC, but the difference did not reach statistical significance ( $p=0.087$ ). There was

a tendency to greater volume in the groups treated with the test and with the combination products (T, M) compared to the PC.

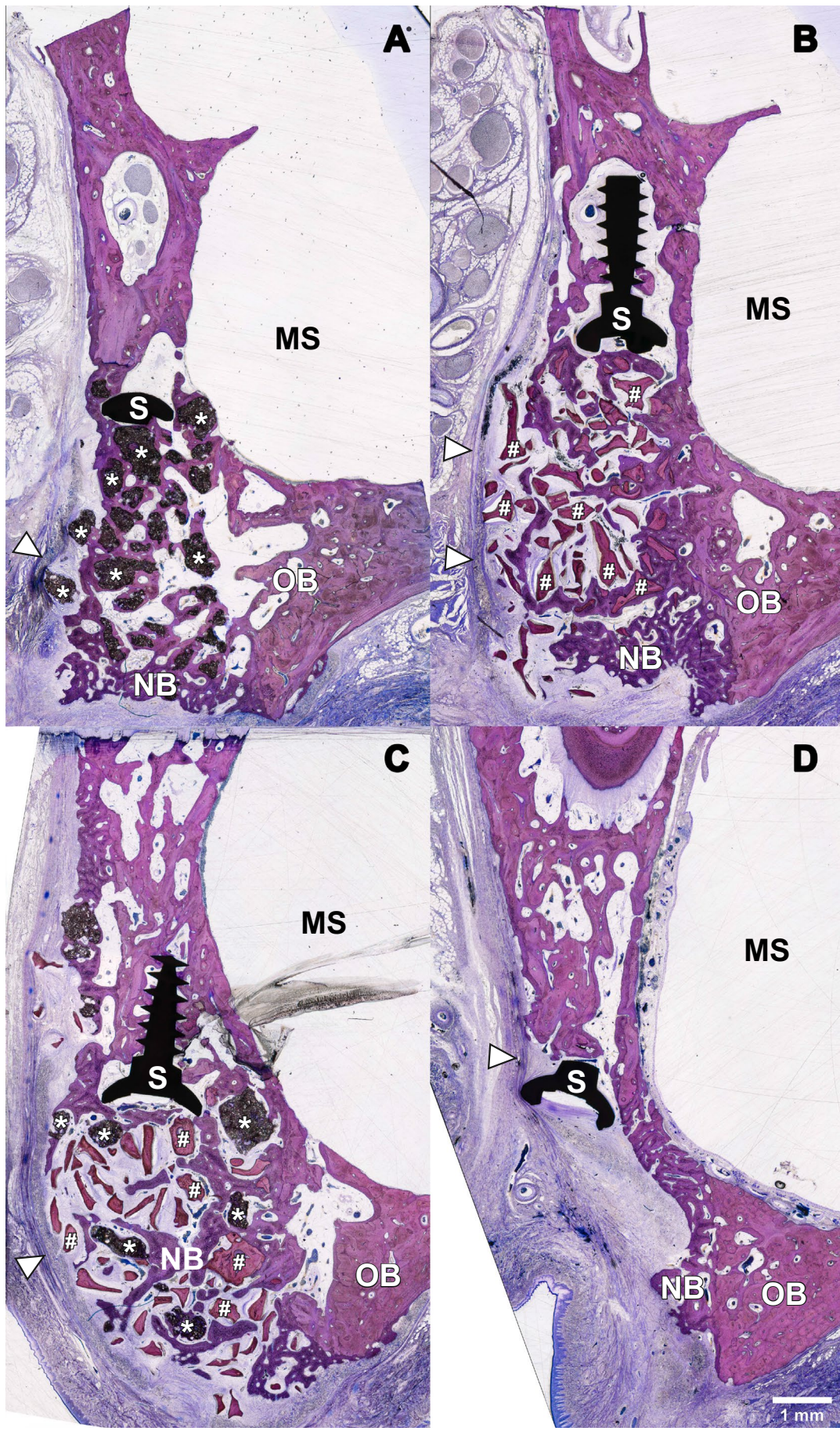
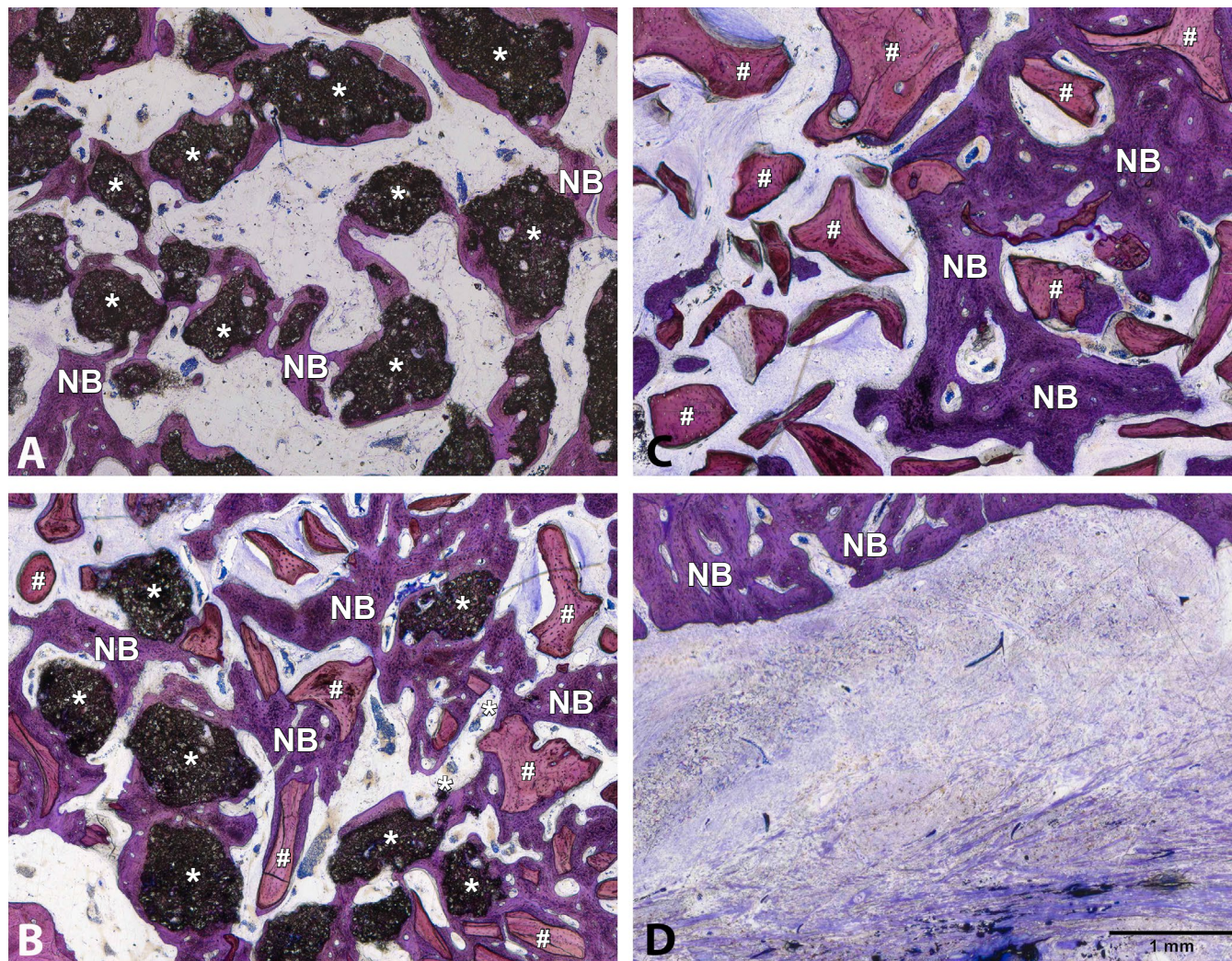


FIGURE 3 | Legend on next page.

**FIGURE 3** | Examples of histologic sections of each group (A: T; B: PC; C: M; D: PC). Magnification 4X. OB: old bone; NB: new bone; \*: BBCCP; #: DBBM; arrowhead: collagen membrane; S: screw; MS: maxillary sinus.



**FIGURE 4** | Representative histological details of the three augmented and the negative control sites. A: T, B: M, C: PC, D: NC; NB: new bone; \*: BBCCP; #: DBBM.

### 3.2 | Qualitative Histology

A representative sample of the four groups analyzed is depicted in Figure 3A–D. The new bone in the peripheral area was mainly lamellar and in the central region composed of both woven and lamellar bone. New bony tissue was found on the surfaces of both types of granules (Figure 4). All the particles of the augmented region are surrounded by new bone formed in contact with the test product (Figure 4A), whereas some of the DBBM particles are located within the connective tissue (Figure 4B). In the region of the sham operated NC, minor signs of new bone formation were visible, and the region was mainly occupied by connective tissue (Figure 4D).

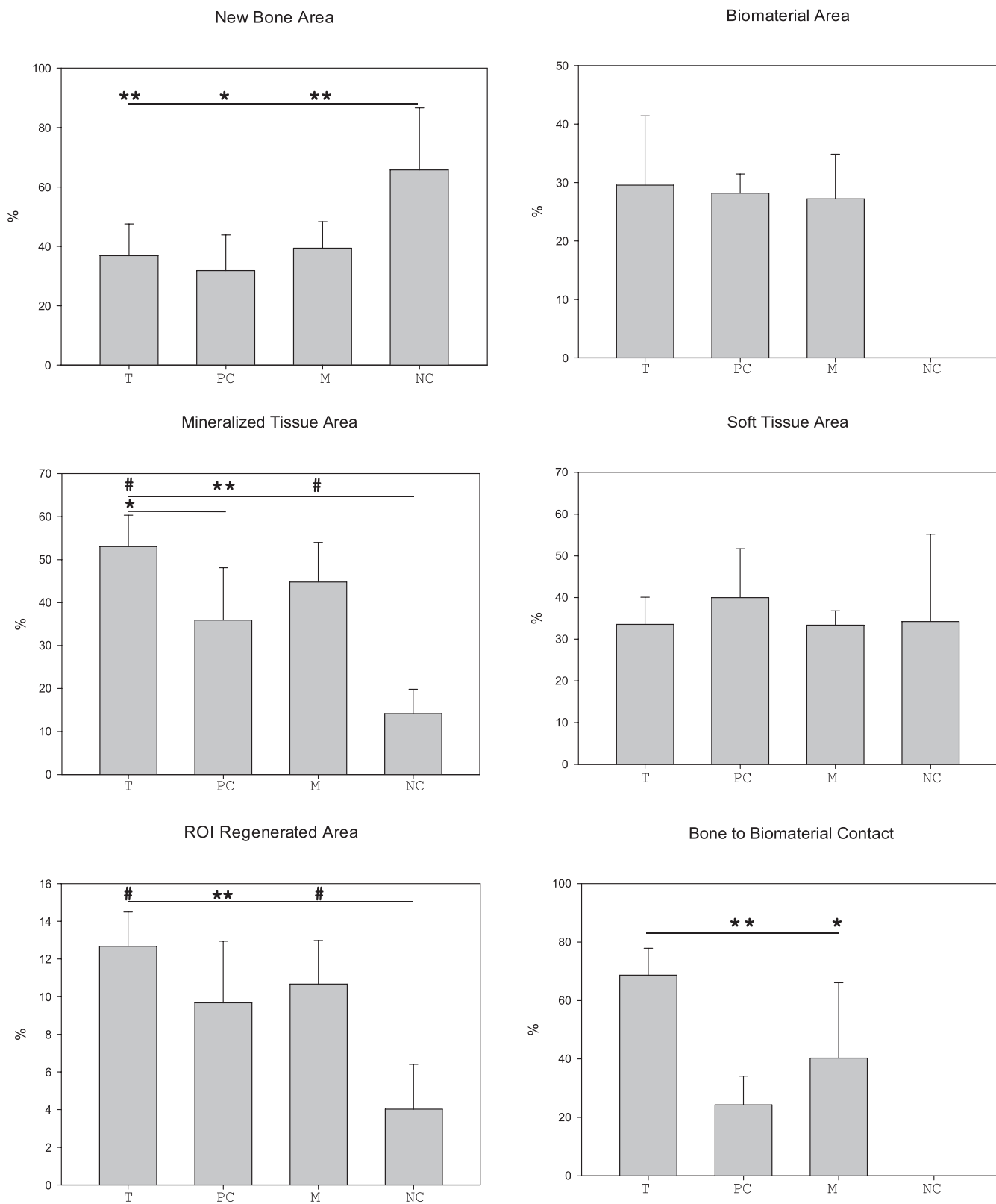
### 3.3 | Histomorphometry

The results of the proportions of new bone formation, biomaterial, mineralized tissue, and soft tissue are represented in Figure 5

(Supplementary Table 2). The NC showed the highest new bone area, being significantly higher than the other three groups ( $p=0.002$ ; NC vs. T  $p=0.009$ ; NC vs. PC  $p=0.015$ ; NC vs. M  $p=0.003$ ). Three groups with biomaterials showed higher values of mineralized tissue areas as compared to the NC ( $p<0.001$ ; NC vs. T  $p<0.001$ ; NC vs. PC  $p=0.002$ ; NC vs. M  $p<0.001$ ), while the T group also reached significance in comparison to the PC ( $p=0.01$ ).

When the percentage of regenerated areas was compared, the difference was also highly significant ( $p<0.001$ ). Nonetheless, only NC showed significantly lower values as compared to the other three groups (NC vs. T  $p<0.001$ ; NC vs. PC  $p=0.004$ ; NC vs. M  $p<0.001$ ).

There were statistically significant differences among three groups for bone to biomaterial contact ( $p=0.001$ ). The *post hoc* comparison showed that the difference between Groups T and PC, and between Groups T and M, reached  $p$ -values of 0.001 and 0.02, respectively.



**FIGURE 5** | Histomorphometrical analysis. Representative bar charts for relative area values (%) of new bone, biomaterial, mineralized tissue, soft tissue, regenerated area, and bone to biomaterial contact (\* $<0.05$ , \*\* $<0.01$ , # $<0.001$ ).

#### 4 | Discussion

The present study was designed to evaluate the capacity for new bone formation of a synthetic bi-layered biphasic

calcium-phosphate (BCCP) bone substitute for lateral bone augmentation procedures. As a preclinical *in vivo* model, critical size defects were prepared in dogs. Such animal models have been widely used in preclinical research and have achieved

objective and repeatable results in terms of the outcome of regenerative procedures. It has to be realized that the model applied in the present study is an acute-defect model and hence may provide treatment outcomes that are not completely identical to similar procedures in chronic defects (Schenk 1992). Whether or not such differences are indeed observed will have to be determined in additional preclinical studies. Nonetheless, an acute bone defect advantages a reproducible, standardized defect dimensions and histomorphometrical analysis. Results of the present study confirmed that critical size lesions would not heal to a restitution as the spontaneous healing capacity would not allow filling the defects with bone within the given observation period. Hence, it is imperative to present outcomes of a sham-operated negative control against which the presumptive materials were tested.

In the present study, a positive control, namely the application of a DBBM has been selected to compare outcomes of the tested materials with the generally propagated standard for bone substitute in GBR (Mizraji et al. 2023). For this reason, the BBCP was tested as a scaffold for bone regeneration as well as in combination with a 1:1 mixture with DBBM. If the BBCP was to result in similar outcomes as the positive control, it may, in the future, be considered as an alternative to the DBBM. Both the histomorphometric and the micro-CT analyses for the test product and the combination of the test product with DBBM yielded very similar outcomes with no statistically significant differences.

In terms of bone formation, all the augmenting materials including the positive control reached more bone formation and higher augmentation of the maxillary bone volume compared to the negative control. Likewise, the improved proportion of mineralized tissue of the augmented sites when compared to the negative control was documented by both micro-CT analysis as well as by histomorphometric analysis. Unexpected was the finding that the test product, the synthetic bi-layered biphasic Calcium Phosphate bone substitute as well as the mixture of this with DBBM displayed better outcomes in terms of bone-to-biomaterial contact. This, in turn, allows the assumption that the osteoconductivity of BBCP appears to be superior compared to the positive control DBBM. Likewise, the area of mineralized tissue in conjunction with the test product or Group M showed significantly better outcomes. The reason for this apparent superiority of Groups T and M in the present study is only speculative and may be related to small sample size and only one observation period. It may be anticipated that additional research may show more clarity and hence, it is recommended to initiate further preclinical studies with more than one healing time and a bigger sample size.

Despite the differences in degradation kinetics, the BBCPs volume maintenance of the HA/ $\alpha$ -TCP (Schaller et al. 2020; Saulacic et al. 2021) or HA/ $\beta$ -TCP (Helder et al. 2018; Yip et al. 2015) types was found to be similar. In its present form, the BBCP appears to have potential as an alternative to other alloplastic materials. Prior to this study, two other reports have presented in vivo data for the synthetic material alone or in combination with DBBM (Saulacic et al. 2021; Fujioka-Kobayashi et al. 2022). The results of the present study have confirmed the results from

the rabbit calvaria defect study (Saulacic et al. 2021). Moreover, the addition of collagen to BBCP has addressed the closure of calvaria defects at 3 months of healing in a recent study (Schaller et al. 2020). It had been demonstrated that the addition of collagen to biphasic calcium phosphate resulted in no significant improvement in terms of bone formation (Schaller et al. 2020). If synthetic biomaterials such as BCP or TCP were added to DBBM, new bone formation was evident in all defects augmented with the biomaterials, providing the evidence for osteoconductivity of the materials (Fujioka-Kobayashi et al. 2022). Hence, the degradation kinetics of bone substitute materials are essential for their osteoconductive properties (Bouler et al. 2017). Occasionally, single particles of BBCP were noted isolated from adjacent bone and surrounded by soft tissue (Supplementary Figure S5). These particles were almost exclusively seen outside the defect volume, but not within the confined defects. Whether or not multinucleated cells are involved in the degradation process remains to be demonstrated.

In another study (Saulacic et al. 2021) the influence of the intensity of the biomimetic HA coating of  $\alpha$ TCP on biomaterial degradation and bone formation was explored. The study indicated the highest degradation rate in the case of BBCP concomitant with the highest rate of bone formation. In the present study, the application of BBCP bone substitute yielded higher potential for bone regeneration than that known for the standard of care, DBBM. It may therefore be concluded that BBCP provides a biomaterial with characteristics that are at least as favorable as the hitherto used positive control, DBBM alone.

It may be speculated that on the basis of the availability of the product and the cost, the tested BBCP may be applied as an alternative to the bovine products routinely used. Further studies are indicated to explore in more detail the degradation rate and the augmentation potential in chronic lesions.

In conclusion, the present preclinical animal study applying an acute-defect canine model demonstrated that a synthetic bi-layered biphasic calcium phosphate may provide equal properties to the hitherto recommended bovine bone mineral in providing a scaffold for bone formation in GBR.

#### Author Contributions

**Kiri N. Lang:** data curation, investigation, formal analysis, visualization, writing – original draft. **Niklaus P. Lang:** conceptualization, data curation, validation, writing – review and editing. **Fernando M. Muños Guzon:** methodology, visualization, writing – original draft, formal analysis, data curation. **Nikola Saulacic:** conceptualization, investigation, funding acquisition, project administration, writing – review and editing, validation.

#### Acknowledgments

The contributions of Dr. Jürg Zumbrunn, Geistlich Dental Regeneration, Wolhusen LU, Switzerland are acknowledged. The competent co-operation and excellent animal care rendered in the Centro de Biomedicina y Veterinaria (CEBIOVET) in Lugo, Spain are highly appreciated. The material support by Geistlich Pharma AG, Wolhusen LU, Switzerland and the partial financial support for laboratory work are appreciated. Open access publishing facilitated by

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### Ethics Statement

The current study was approved by the Ethics Committee of the Rof Codina Foundation, Lugo, Spain (05/19/AE-LU-001).

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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### Supporting Information

Additional supporting information can be found online in the Supporting Information section.