Treatment of Circumferential Defects with Osseoconductive Xenografts of Different Porosities: A Histological, Histometric, Resonance Frequency Analysis, and Micro-CT Study in Dogs

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ABSTRACT

Background: Finding the most effective method of minimizing the gap effect in alveolar crest remodeling constitutes a clinical challenge for immediate implant technique.

Purpose: To evaluate the effectiveness of osseoconductive xenografts with different porosities in the crestal bone region, with and without guided bone regeneration, over immediate implant installation.

Materials and Methods: Five bone defects (6 mm in diameter/4 mm depth) were prepared on one side of the mandibles of twelve dogs. Implants of 3.3 × 10 mm were installed on the mesial side of each defect, providing a 2.7-mm distal gap. Defects were randomly filled with autogenous bone, coagulum, a deproteinized bovine bone mineral (DBBM) block, a DBBM sponge, or DBBM granules. The same procedures were performed on the opposite side after 8 weeks. Collagen membranes were used to cover the defects on half of the sides. The animals were sacrificed after 8 weeks. The outcomes were evaluated by histology, histomorphometric analysis, resonance frequency analysis, and micro-CT analysis.

Results: The histomorphometry showed the DBBM sponge to provide similar bone formation to autogenous bone at 8 weeks without a membrane. The coagulum rendered better bone formation at 16 weeks (membrane) ($p < .05$). The DBBM block exhibited the poorest results between treatments (8 and 16 weeks, with or without membrane). Micro-CT analysis revealed increasing bone surface values in sites with DBBM granules, followed by the DBBM sponge (8 weeks without membrane) and autogenous bone at 8 weeks with membrane ($p < .05$). Porosity analysis of the biomaterials showed the highest number, volume, and surface area of closed pores in DBBM granules. The DBBM block presented the highest volume of open pores, open porosity, and total porosity.

Conclusions: The high-porosity block (DBBM block) failed to provide greater bone repair within the defect. Biomaterials with lower porosity (DBBM sponge and granules) showed similar or higher bone formation when compared with autogenous bone.

KEY WORDS: animal study, bone defects, guided bone regeneration, immediate implants, osseointegration

INTRODUCTION

Immediate placement of implants after tooth extraction has been established as a common procedure in dentistry. In this context, bone formation around implants has shown osseointegration outcomes similar to those obtained with conventional techniques in both human[2,3] and animal models.[1,4–6] Histomorphometric studies using animal models have shown that the installation of implants in fresh sockets results in marked dimensional changes in the ridge walls with respect to height and thickness.[7] These changes in repair pattern

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can be explained by the greater size of the socket in relation to the implant, producing a bone defect (gap) between the implant surface and the bone crest walls.[8] Finding the most effective way to minimize the gap effect in alveolar crest resorption constitutes the greatest challenge for clinical and preclinical studies that apply this technique in different experimental models.

Several materials and techniques to fill spaces surrounding the implant and facilitate the preservation of peri-implant tissues and osseointegration have been described in an attempt to increase the reliability of the treatment of post-extraction sockets.[9–11] Such methods have included guided bone regeneration (GBR) using membranes as mechanical barriers; installation of bone autografts or allografts to fill these defects; application of osseoconductive bone substitutes such as hydroxyapatite, bioglass, or deproteinized bovine bone mineral (DBBM); or various combinations of these treatment modalities.[8,9,12,13] Although most of these techniques achieve success from the clinical point of view after defect filling, providing better alveolar and soft tissue contours, they do not guarantee that implant osseointegration will occur.[10,14,15]

Among the various osseoconductive biomaterials, DBBM has been widely used in the treatment of peri-implant bone defects, maxillary sinus elevation, and alveolar socket repair for implant placement.[8,10,12,13,16–18] Some authors report similar osseointegration to that achieved with autogenous bone in defects around implants, with particle resorption and replacement by new bone,[12,13] while others report minimal resorption.[8,16] Although this biomaterial is widely used as a bone substitute in fresh sockets, experimental and clinical findings show quite contradictory results.[10,12,13,16,17] Findings that DBBM is not completely reabsorbed at the site where it is used[18] and that long-term persistence of the biomaterial particles contributes to an unstable interface between the material and the implant surface have been demonstrated in previous studies.[19]

Generally, studies using the various forms of DBBM in experimental models barely discuss the influence of the material's topography and porosity on bone formation. On analysis of the literature, a paucity of data that relate the porosity and topography of this biomaterial with bone formation is observed. It has been demonstrated that the porosity, as well as the size and interconnectivity, of osseoconductive scaffolds plays an important role in in vitro and in vivo bone formation.[20–22] When tested in vitro, lower porosity stimulated osteogenesis by suppression of cell proliferation and maturation, producing major aggregation. On the other hand, higher porosity and pore size resulted in major in vivo bone growth by facilitating capillary infiltration and deposition of bone matrix by osteoblasts. According to these studies, the architectural organization of DBBM in a porous block is maintained, while in the particulate form the interconnected macroporous structure is lost.[20,22]

The importance of porosity in bone repair was demonstrated in the study of Kuboki and colleagues.[23] Using an experimental model in rats, they performed ectopic implantation of solid or porous hydroxyapatite particles with bone morphogenic protein 2. Little or no new bone formation was observed on the surface of the solid particles, whereas direct osteogenesis occurred on the porous surfaces. Data on the porosity, pore size, and interconnectivity of biomaterials that successfully received osteoblastic cells on their surfaces can be found in the literature.[21,24,25] However, there is no consensus on the optimal porosity and pore size for bone formation. It is known that human trabecular bone has a porosity of approximately 85% and an average pore size of 850–1200 μm.[26] It is believed that the closer a biomaterial can get to these values, the greater the possibility of favorable biological response (bone formation).[27] Some studies were able to support this statement using polylactic acid/β-tricalcium phosphate-based scaffolds with porosity above 80% in an animal model. Higher tissue ingrowth and bone formation were observed in the higher-porosity areas of the biomaterial after in vivo implantation.[28,29]

Despite DBBM's known suitability for inlay and onlay grafting, there are no reports in the literature of the influence of DBBM porosity on bone formation. The hypothesis that a highly porous form of this biomaterial would provide an architecture similar to that of human trabecular bone, and would therefore be more effective in maintaining the dimensions of the alveolar crest and defect repair, remains in doubt. Additionally, the three different porosity forms of this material have not been tested and compared in the same experimental model with dental implants.

The present study aims to evaluate porous osseoconductive biomaterials in the following ways: (1) to test a DBBM block in the cervical region of the implant as a scaffold for bone regeneration; (2) to

compare a DBBM block, a DBBM sponge, DBBM granules, autogenous bone, and coagulum in an experimental model, with or without GBR; and (3) to correlate the biomaterials' porosity in vitro with bone formation in vivo.

MATERIALS AND METHODS

For this study we used 12 young dogs, weighing between 20 and 30 kg. Animals were quarantined for administration of anti-rabies vaccine, anthelmintics, and vitamins. Pre- and postoperatively, the animals were kept in kennel cages, receivimg appropriate veterinary care with free access to water and standard laboratory nutritional support throughout the trial period. This study was approved by the institutional ethics committee for animal research (# 12.1.1317.53.2).

Surgeries

The extraction of mandibular premolars and first molars was the first procedure. Animals were intravenously preanesthetized with levomepromazine (1 mg/kg; Neozine®, Cristalia, Itapira, São Paulo, Brazil) and tiletamine/zolazepam (0.1 mg/kg; Zoletil®, Virbac, São Paulo, São Paulo, Brazil) 30 minutes before surgery and anesthetized with 1% sodium thiopental (1 ml/kg – solution of 20 mg/kg diluted in 50 ml of saline; Thiopentax™, Cristalia). Throughout the surgical procedure, inhaled oxygen and intravenous saline were administered. Then, intrasulcular incision and bilateral mucoperiosteal flap reflection were performed. The four premolars and the first molars were sectioned and extracted with the use of forceps and/or elevators without damaging the alveolar walls. Tissues were repositioned and sutured with absorbable polyglactin 910 (Vicryl 4–0, Ethicon™, Johnson & Johnson, São Jose dos Campos, São Paulo, Brazil). All surgeries were performed by the same surgeon according to standards of asepsis and with abundant irrigation with 0.9% saline solution to maintain the vitality of bone.

Animals were observed throughout the postoperative period and received the analgesic tramadol hydrochloride (3 mg/kg; Anangon-Biosintetica Laboratories Ltd, São Paulo, São Paulo, Brazil) once a day for 3–4 days. Antibiotic therapy was also prescribed in the form of a suspension (Pentabiótico Veterinário™, Fort Dodge Animal Health, Campinas, São Paulo, Brazil; purchased from Agroline, Campo Grande, Mato Grosso do Sul, Brazil) at a dose of 0.5 mg/kg.

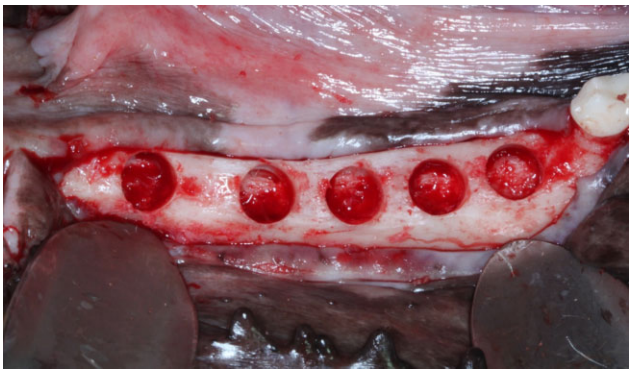

Figure 1 Surgically created bone defects.

Six weeks after dental extractions, five bone defects of 6 mm in diameter and 4 mm deep were trephined into the alveolar ridge on one side of the jaw (Figure 1). The autogenous bone was collected during drilling and stored in sterile 0.9% saline for subsequent use as particulate autogenous graft.

The defects were treated with a DBBM block (Bio-Oss Block®, Geistlich, Wolhusen, Switzerland), a DBBM sponge (Bio-Oss Collagen®, Geistlich), DBBM granules (Bio-Oss®, Geistlich; particle size between 0.25 and 1 mm), autogenous bone, or coagulum (Figure 2). At each site, one 3.3 × 10 mm implant (EasyGrip® Actives, Conexão, Arujá, São Paulo, Brazil) was installed in the mesial defect edge, leaving a 2.7-mm gap in the implant's distal aspect. Each gap was treated with the previously cited materials. All materials were inserted at defect sites after implant installation except for the DBBM block, which was trimmed beforehand with the same trephine bur used to drill the defects. The block was then adapted to the distal gap right before

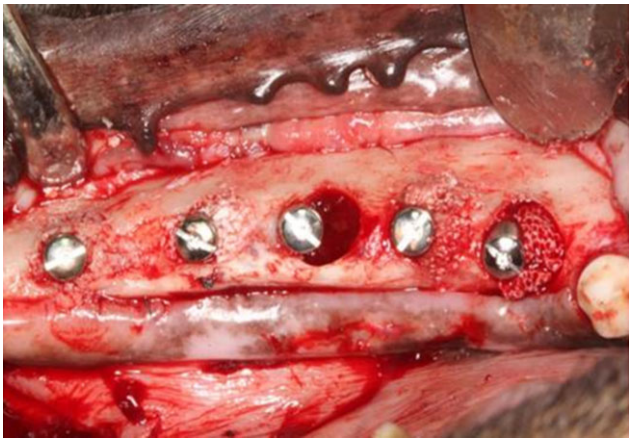

Figure 2 Bone defects treated with biomaterials.

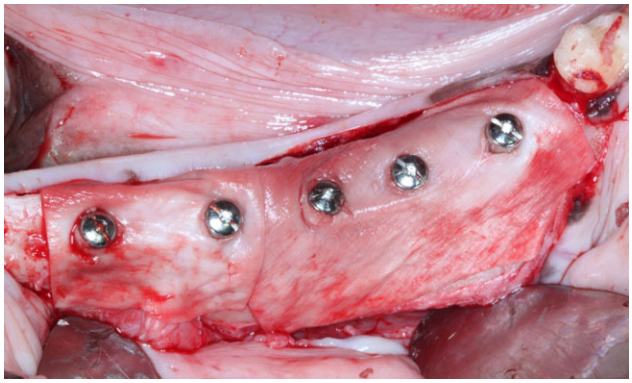

Figure 3 Collagen membrane covering the treated defects.

the installation of the implant. A 4-mm healing abutment (Conexão) was than positioned. After 8 weeks, the same surgical procedures described for the first side were performed on the opposite side. Half of the sides were covered by a membrane at both time points evaluated (Bio-Gide®, Geistlich) (Figure 3).

All implants were installed flush with the mesial-distal alveolar bone crest. The circumferential defects received different biomaterials at random, as did the sides used at each time point. All surgeries were performed by the same surgeon according to standards of asepsis and with sufficient cooling to maintain bone vitality.

During the postoperative period the animals received basic periodontal treatment (scaling, polishing, and smoothing) every 2 weeks in order to maintain oral hygiene and avoid contamination of surgical wounds. Postoperative care took place in the same environment used for tooth extraction procedures.

Implant Stability Assessment

Implants' primary stability was measured by resonance frequency analysis (RFA) of the bone/implant complex, which was done by reading the implant stability quotient (ISQ) using an Osstell Mentor™ system (Integration Diagnostics AB, Göteborg, Sweden). The evaluation was performed by the same author at the installation of implants and at the time of animal sacrifice (Figure 4).

Animal Sacrifice

The animals were sacrificed 8 weeks after the third surgical procedure by overdose of sodium thiopental (Thiopentax™).

Histological Preparation

Individual bone blocks containing the implant and surrounding bone and soft tissues were fixed in 10% formaldehyde solution, followed by dehydration with ethanol solutions of different concentrations, and finally embedded in resin (LR White™ hard grid, London Resin Co Ltd, Berkshire, UK). After polymerization, blocks were subjected to mesial-distal sections (Exakt Apparatebau, Norderstedt, Germany), stained with Stevenel's blue/alizarin red, and examined under standard light microscopy for histological and histomorphometric analysis.

Histomorphometric Analysis

The Leica DMLB-2™ microscope (Leica Microsystems GmbH, Wetzlar, Germany) was used for all measurements included in the relevant protocol. Two points, the implant shoulder (IS) and the top of the alveolar bone crest, were determined. Then, the following measurements were made using the Leica QWin™ software (Leica Microsystems GmbH):

- Vertical distance between IS and C on the distal wall;
- Bone-to-implant contact (BIC), measured as a percentage, along the whole length of the implant and inside the defects;
- Bone area (BA), measured as a percentage, formed between implant threads over the whole implant and inside the defects;
- Area of bone formation (mm),[3] analyzed inside a region of interest (ROI) of 2.7×4 mm, drawn from the implant shoulder to the defect bottom;

Micro-CT Analysis In Vivo and In Vitro

The micro-CT examinations were performed immediately after animal sacrifice, with the specimens fixed in

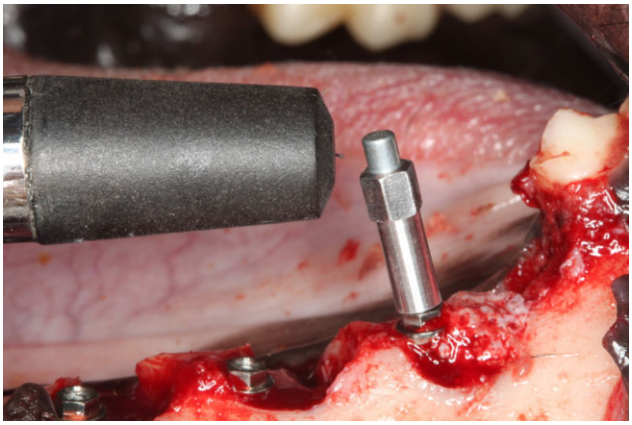

Figure 4 Stability measurement after implant installation and defect treatment.

10% formaldehyde. For image acquisition, a spatial resolution of 12 μm was adopted; images were acquired with a SkyScan® 1172 scanner (SkyScan, Antwerp, Belgium) with adjustable voltage of 50 kV.

After image acquisition and 3D reconstructions, volumes of interest (VOIs) of 4.0 × 3.0 × 6.0 mm corresponding to the defect area were established. For analysis, we adopted a grayscale threshold of 55 (maximum) and 30 (minimum) for bone tissue evaluation and 80 (maximum) and 55 (minimum) for biomaterials evaluation. The parameter values obtained for the biomaterials were subtracted from those obtained for bone tissue in order to separate the volumes of remaining biomaterials from the newly formed bone amounts. The same settings for spatial resolution, equipment adjustments, and grayscale thresholds were used for in vitro and in vivo evaluations.

The following parameters were used for the dimensional analysis of the trabecular bone microarchitecture:

- Total bone volume;
- Percent bone volume;
- Bone surface;
- Bone surface/volume ratio;
- Bone surface density;
- Trabecular thickness.

One specimen of each biomaterial used in the experiments (DBBM block, DBBM sponge, DBBM granules) was scanned separately in vitro to assess the following parameters:

- Trabecular thickness;
- Number of open and closed pores;
- Volume open and closed pores;
- Surface area of closed pores;
- Porosity of open and closed pores;
- Total porosity.

For in vitro analysis, we adopted a VOI of identical dimensions to the ROI for the analysis of specimens (4.0 × 3.0 × 6.0 mm). The software programs DataViewer® v. 1.4.1 and CTAnalyser® v. 1.11.8.0 (both from SkyScan) were used for analysis.

Statistics

A database was created for evaluation of the statistical significance with the aid of the software Statistical Package for the Social Sciences for Windows (SPSS®, version 15.0, IBM ®, Armonk, NY, USA).

The results obtained for implant stability, BIC, BA, defect area, and micro-CT analysis were subjected to ANOVA of two criteria for repeated measures for comparisons between and within groups. For the alveolar crest resorption analysis (nonparametric data), Kruskal-Wallis ANOVA and Tukey's post hoc test were applied. In all tests the considered level of significance was 5% ($p \leq .05$).

RESULTS

All animals tolerated all surgical procedures well. The three interventions were performed uneventfully. During the experimental protocol, four implants were lost: one DBBM block implant with membrane at 16 weeks, one DBBM block implant without membrane at 16 weeks, one DBBM sponge implant without membrane at 8 weeks, and one autogenous bone implant without membrane at 8 weeks. In total, the sample consisted of 116 implant sites treated with the biomaterials being tested.

Implant Stability (RFA)

The RFA measurements showed increased stability in all groups from initial to final measurements at 8 and 16 weeks. In all treatments, final ISQ scores were higher than those observed initially. Statistically significant differences were found in group comparisons for final measurements at 8 and 16 weeks ($p < .05$) (Figure 5). When a membrane was not used, final ISQ values in sites treated with the DBBM block and the DBBM sponge were similar and higher than those for DBBM granules (8 weeks). At 16 weeks, the final ISQ scores in sites treated with the DBBM sponge showed superior results to other treatments ($p < .05$). When a membrane was used, the final ISQs in sites treated with the DBBM sponge were superior to those of other treatments (8 weeks). At 16 weeks, the DBBM sponge was similar to DBBM granules and superior to other treatments.

Histological Analysis

The histological examination showed all implants were osseointegrated. In all defects, new bone formation was observed, in varying amounts according to the group evaluated (Figures 6–10). Foci of distance osteogenesis from the apical and posterior defect walls were frequently observed in all sites. Occasional regions of contact osteogenesis were also present. The regions of new bone formation displayed immature bone and

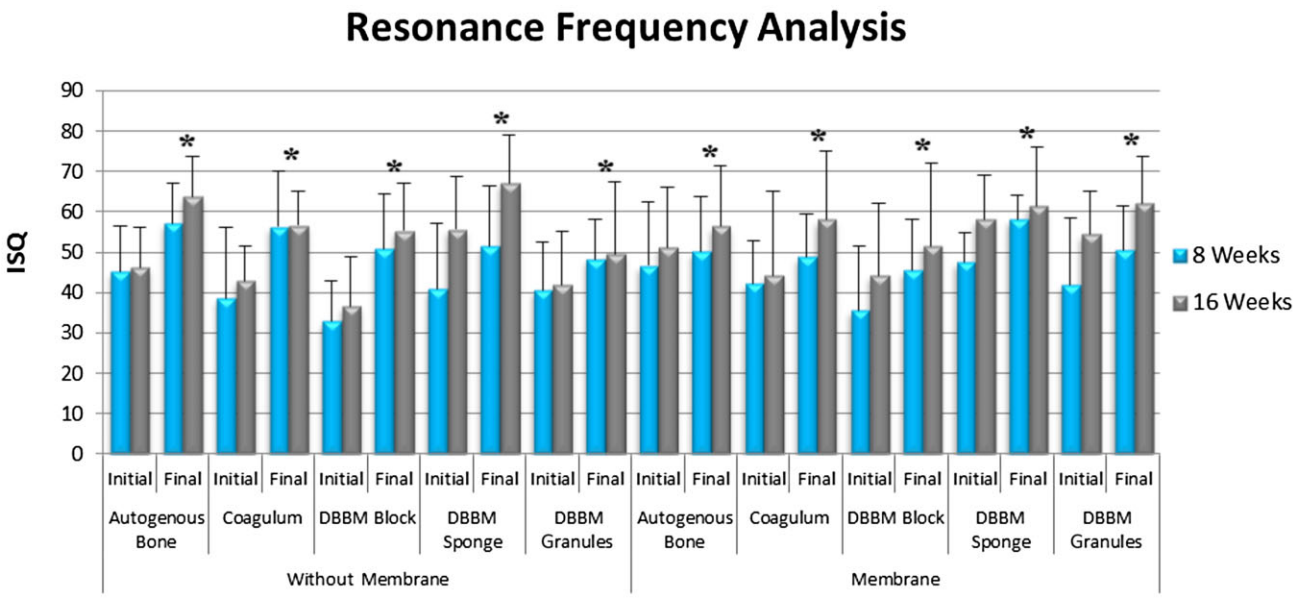

Figure 5 Means and standard deviations of initial and final implant stability quotient (ISQ) scores at 8 and 16 weeks according to group distribution. *Statistically significant difference between groups. DBBM = deproteinized bovine bone mineral.

sparse regions of lamellar bone at 8 weeks. At 16 weeks, a greater presence of lamellar bone was found, although immature bone predominated.

In sites treated with the DBBM block, most of the biomaterial scaffold was in direct contact with the connective tissue, with few foci of immature bone deposited on its surface. The presence of soft tissue within the defect increased from 8 to 16 weeks for all treatments when a membrane was not used to cover the defects. An inverse relationship was seen when defects were covered by a membrane. The amount of soft tissue decreased between the two time points in all treatments. The remaining biomaterials were observed in varying amounts at all treated sites. The DBBM block was less resorbed compared with the DBBM sponge and DBBM granules, preserving most of its micro- and macroporous trabecular structure in the defect area, with little bone filling. All biomaterials were partially

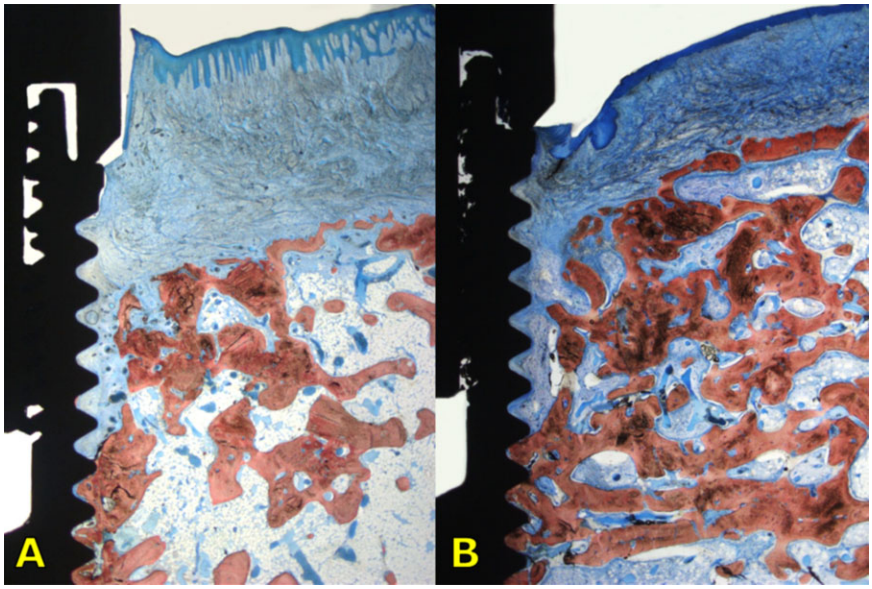

Figure 6 Mesial/distal ground sections of defects treated with autogenous bone: (A) at 8 weeks; (B) at 16 weeks. Magnification: ×1.6. Staining: Stevenel's blue + alizarin red.

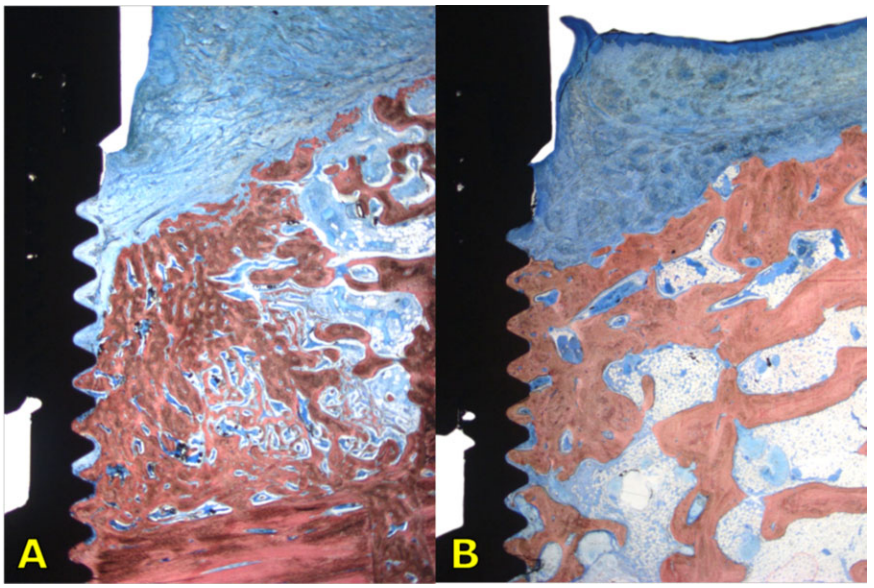

Figure 7 Mesial/distal ground sections of defects treated with coagulum: (A) at 8 weeks; (B) at 16 weeks. Magnification: ×1.6. Staining: Stevenel's blue + alizarin red.

reabsorbed from 8 to 16 weeks. Osteoclastic activity could be observed on the biomaterials' surface (Figure 11). Remnants of biomaterial particles in direct contact with the implant surface could not be found in any of the treatments or time points evaluated. Less bone deposited on the biomaterial structure was observed in the sites treated with the DBBM block as compared with the DBBM sponge and granules, independent of time point or membrane use.

Histomorphometric Analysis

Bone-to-Implant Contact. There were no statistically significant differences between or within groups ($p > .05$). When a membrane was not used, the DBBM block presented the best defect BIC at 8 weeks, and the DBBM sponge was the best at 16 weeks. With the use of a membrane, DBBM granules, autogenous bone, and coagulum showed similar defect and total BIC values at

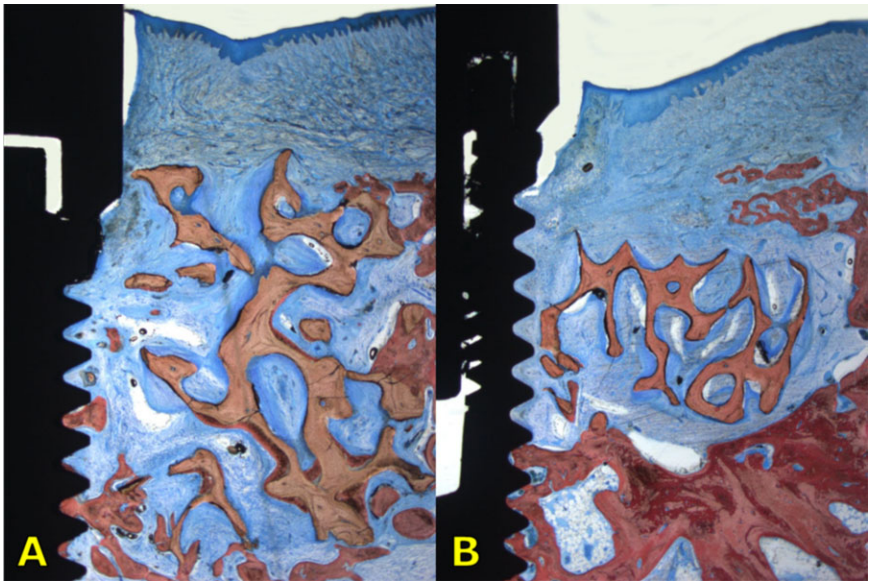

Figure 8 Mesial/distal ground sections of defects treated with deproteinized bovine bone mineral (DBBM) block: (A) at 8 weeks; (B) at 16 weeks. Magnification: ×1.6. Staining: Stevenel's blue + alizarin red.

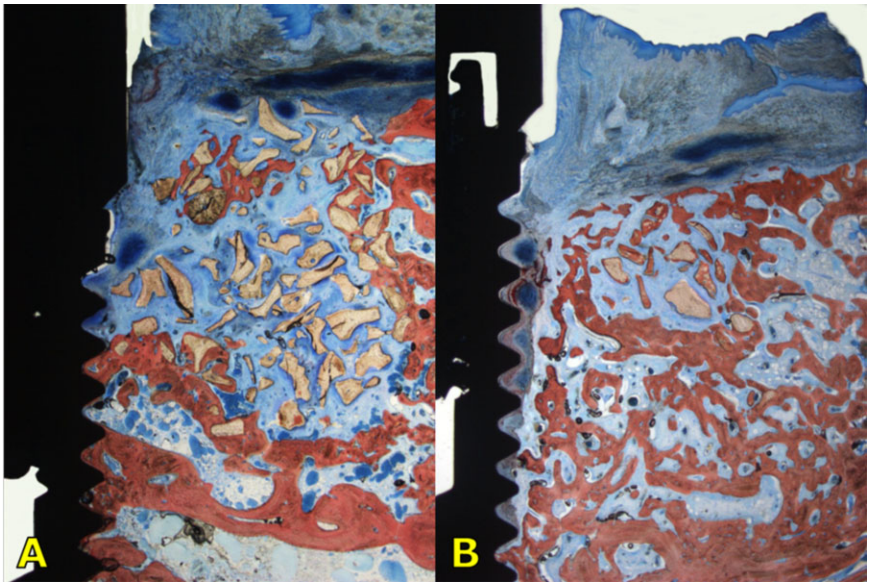

Figure 9 Mesial/distal ground sections of defects treated with deproteinized bovine bone mineral (DBBM) sponge: (A) at 8 weeks; (B) at 16 weeks. Magnification: ×1.6. Staining: Stevenel's blue + alizarin red.

8 and 16 weeks ($p > .05$). The results are displayed in Figure 12.

Bone Area within Threads (BA). There was no statistically significant difference between or within groups ($p > .05$). When a membrane was not used, the DBBM sponge and granules showed similar defect BA values to autogenous bone and coagulum at 8 weeks and values lower than those of autogenous bone at 16 weeks. The DBBM block showed the worst performance among treatments at both evaluated time points, even with the use of a membrane ($p > .05$). When a membrane was used to cover the defects, the DBBM granules and autogenous bone showed similar defect BA at 8 and 16 weeks. Values are detailed in Figure 13.

Alveolar Crest Resorption. Linear measurements (IS-C) showed less vertical height loss at sites treated with the

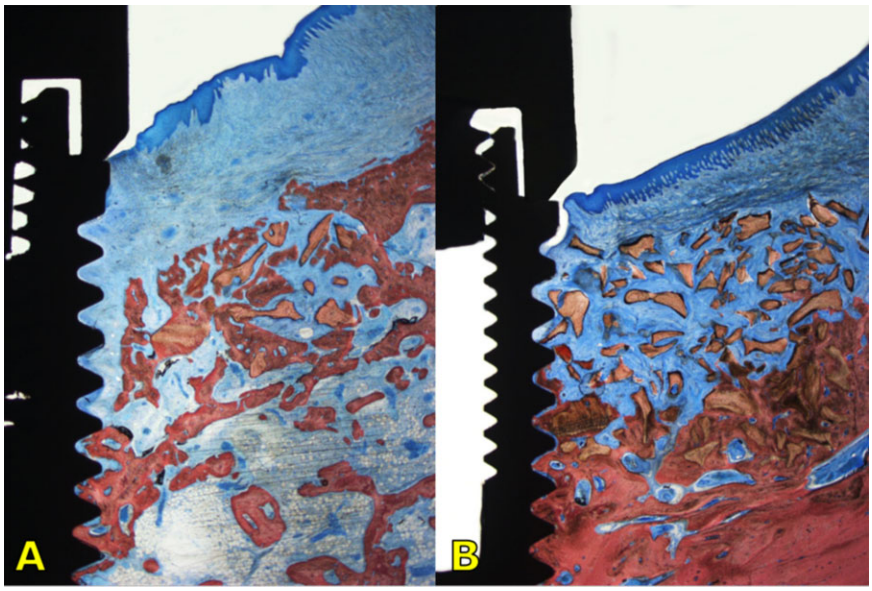

Figure 10 Mesial/distal ground sections of defects treated with deproteinized bovine bone mineral (DBBM) granules: (A) at 8 weeks; (B) at 16 weeks. Magnification: ×1.6. Staining: Stevenel's blue + alizarin red.

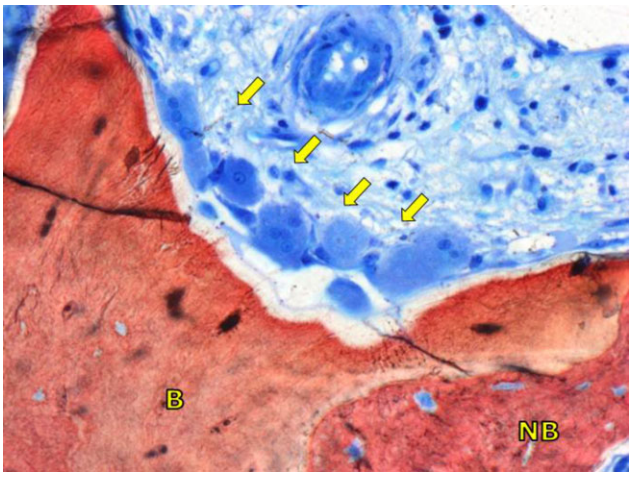

Figure 11 Ground section showing the presence of giant cells (osteoclasts) on biomaterial surface (*arrows*); "B" = biomaterial, "NB" = new bone. Magnification: ×20; Staining: Stevenel's blue + alizarin red.

DBBM block, regardless of the use of a membrane or time point evaluated ($p < .05$). When a membrane was not used, the DBBM granule's IS-C values were close to those of the DBBM block at 16 weeks ($p < .05$). With the use of a membrane, the DBBM sponge values were close to those of the DBBM block at 8 and 16 weeks ($p < .05$). All biomaterials used were superior to autogenous bone in the preservation of distal crestal bone, with or without membrane, at 8 and 16 weeks ($p < .05$). Values described are graphed in Figure 14.

Bone Formation. In the analysis of bone formation area inside the ROI, we observed statistically significant differences between treatments ($p < .05$). The DBBM block had the worst performance in bone formation (8 and 16 weeks, with and without a membrane). When a membrane was not used, the DBBM sponge showed a similar level of bone formation to autogenous bone (8 weeks), decreasing at 16 weeks. DBBM granules provided the best bone formation of the biomaterials tested (16 weeks). When a membrane covered the defects, the DBBM sponge had the best bone formation among biomaterials at 8 and 16 weeks. Membrane use improved bone formation in all treatments at 16 weeks. The values described here are graphed in Figure 15.

Micro-CT Analysis

In Vivo Analysis of Specimens. Three-dimensional reconstructions were performed (Figure 16), and no statistically significant differences were found within or between groups ($p > .05$) with regard to bone volume (Figure 17). When the defects were not covered by a membrane, DBBM granules provided the greatest bone

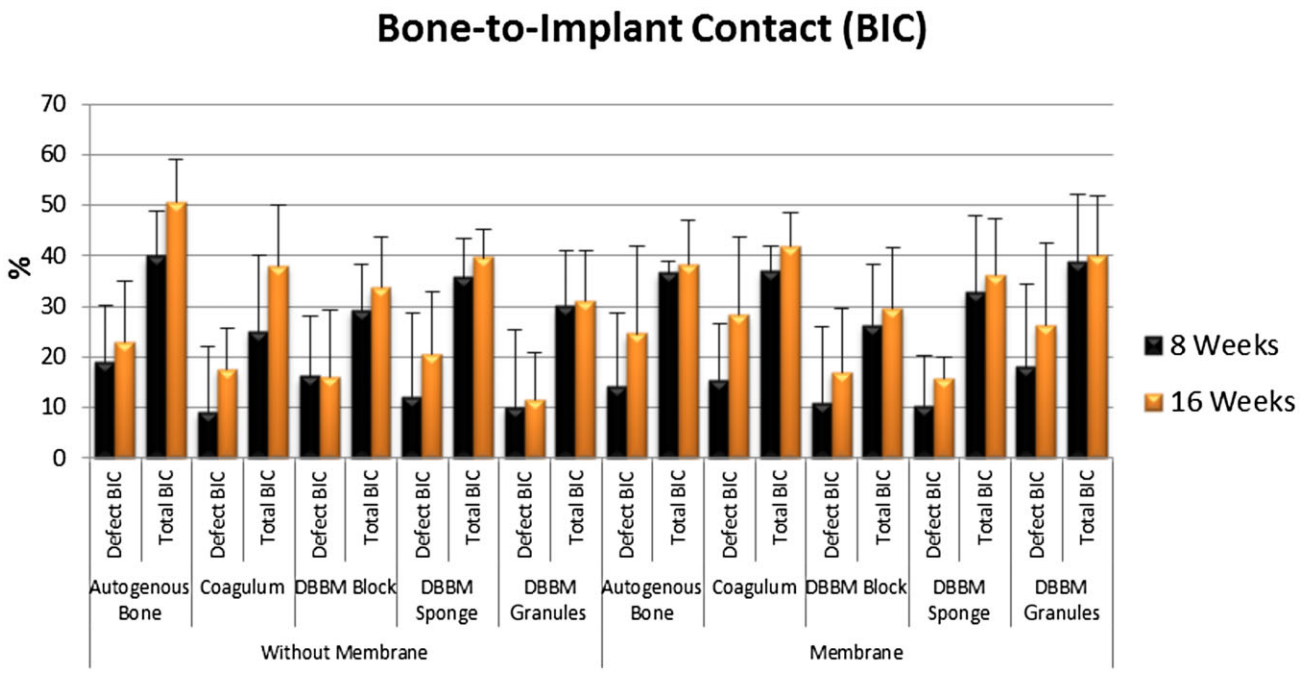

Figure 12 Means and standard deviations of bone-to-implant contact at 8 and 16 weeks according to group distribution. DBBM = deproteinized bovine bone mineral.

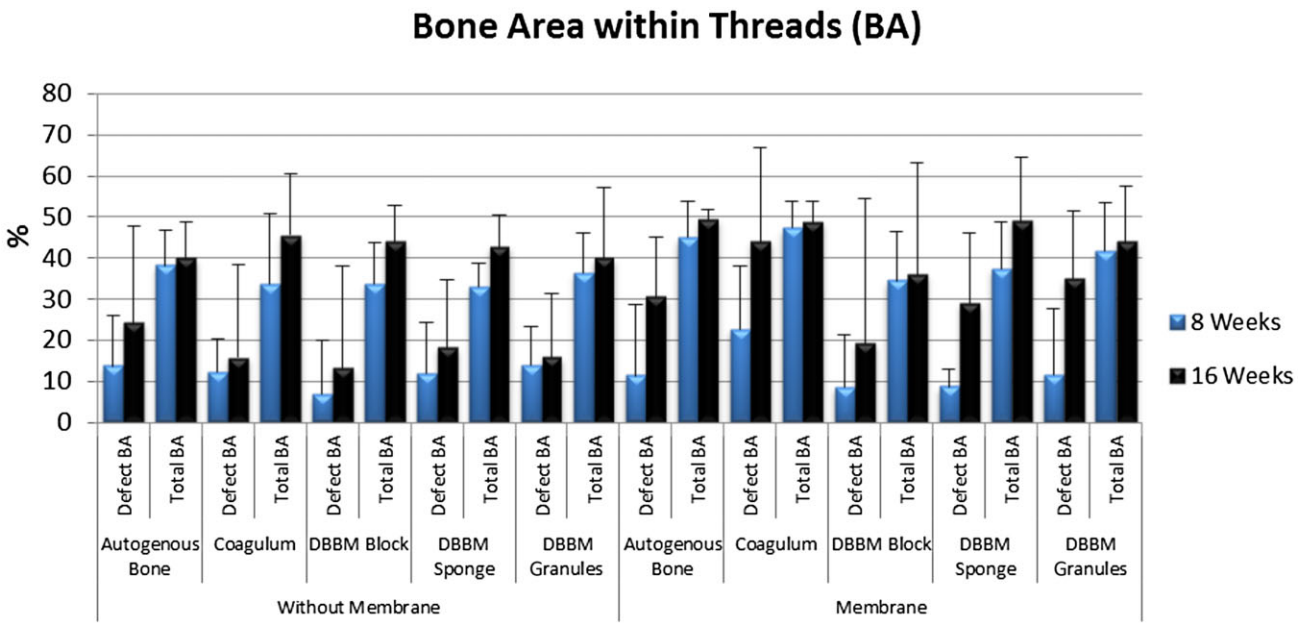

Figure 13 Means and standard deviations of bone area within threads at 8 and 16 weeks according to group distribution. DBBM = deproteinized bovine bone mineral.

volume at 8 weeks and a volume just under that of autogenous bone at 16 weeks ($p > .05$). Among the biomaterials, membrane use provided greater bone volume at 8 weeks with DBBM granules. The DBBM block had the worst performance among all treatments at 8 and 16 weeks, with or without the use of a membrane ($p > .05$).

On analysis of the percentage bone volume (Figure 17), DBBM granules showed higher values than other treatments at 8 weeks and were the best biomaterial at 16 weeks. With the use of a membrane, DBBM granules were the best biomaterial at 8 weeks. The DBBM block showed the worst performance, regardless of time point or membrane use. There were

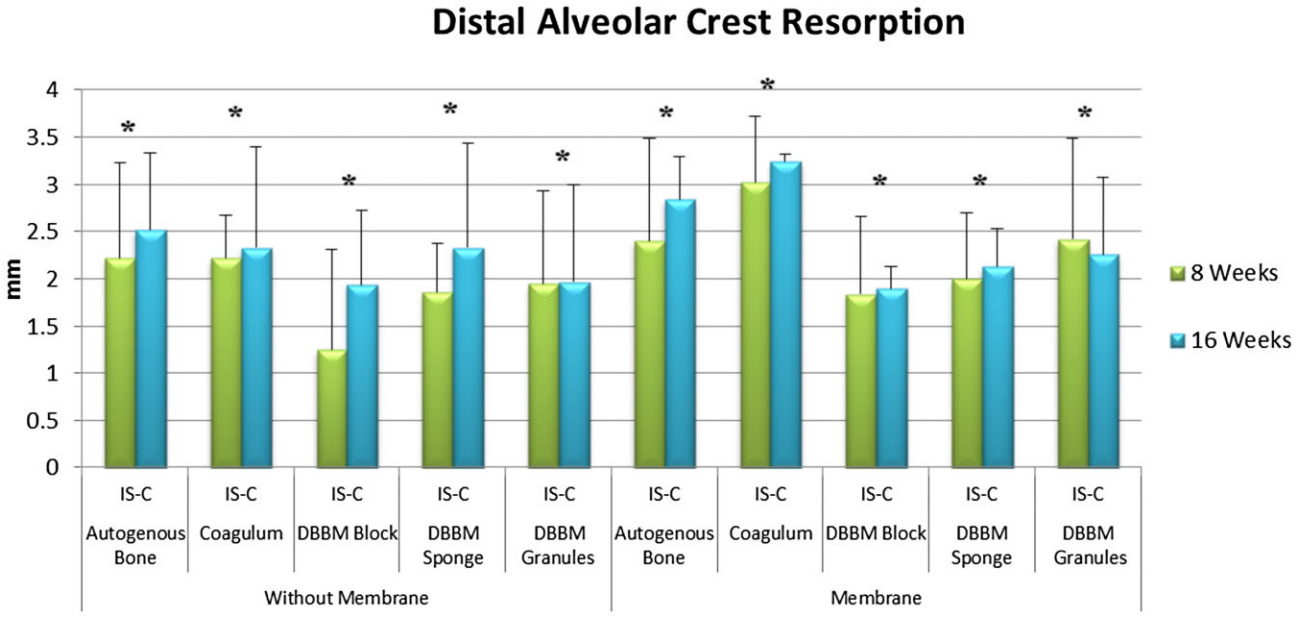

Figure 14 Means and standard deviations of distal alveolar crest resorption at 8 and 16 weeks according to group distribution. *Statistically significant differences. DBBM = deproteinized bovine bone mineral.

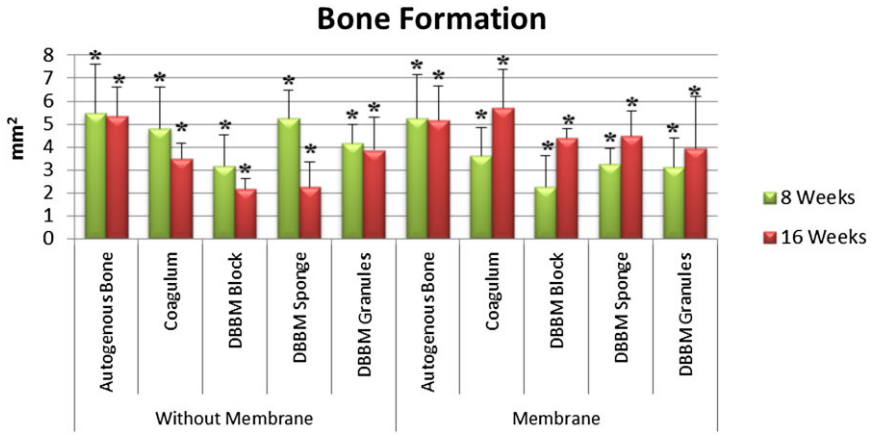

Figure 15 Means and standard deviations of bone formation area in the region of interest at 8 and 16 weeks according to group distribution. *Statistically significant difference between groups. DBBM = deproteinized bovine bone mineral.

no statistically significant differences in group comparisons ($p > .05$).

Statistically significant differences were found in group comparisons with respect to bone surface area ($p < .05$) (Figure 17). DBBM granules showed the highest bone surface area among all treatments when a membrane was not used (8 and 16 weeks) and was still the best biomaterial with membrane at 8 weeks. The DBBM sponge with membrane was the treatment with best performance at 16 weeks, followed by DBBM granules ($p < .05$).

The bone surface/volume ratio (Figure 17) showed highest values with the DBBM sponge at 8 weeks without membrane and at 8 and 16 weeks with membrane. The DBBM block showed the worst performance among biomaterials for 8 and 16 weeks without a membrane. The three biomaterials showed better results than the autogenous bone and coagulum at 8 and 16 weeks when covered by a collagen membrane. The results described above were statistically significant in group comparisons ($p < .05$).

There were no statistically significant differences in group comparisons in the analysis of the bone surface density ($p > .05$). When a membrane was not used, DBBM granules showed the highest values of bone surface density at 8 and 16 weeks, followed by the DBBM sponge (8 weeks) and the DBBM block (16 weeks). When a membrane was used, the DBBM granules rendered the highest values at 8 weeks and DBBM sponge showed the highest values within all treatments at 16 weeks ($p > .05$) (Figure 17).

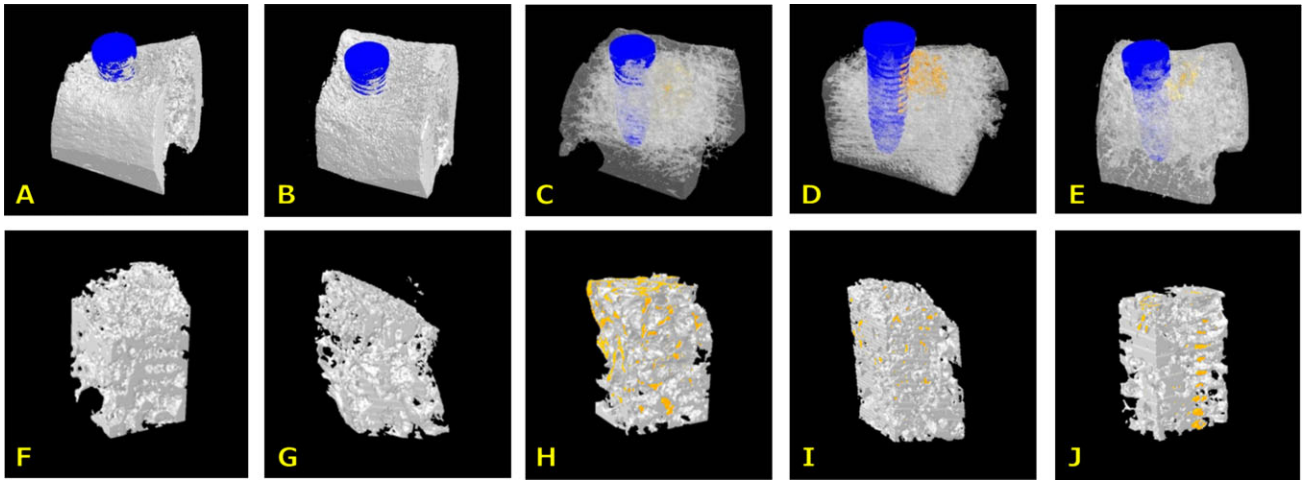

Figure 16 Three-dimensional reconstructions of, respectively, the specimens and volumes of interest of sites treated with autogenous bone (A and F), coagulum (B and G), deproteinized bovine bone mineral (DBBM) block (C and H), DBBM sponge (D and I), and DBBM granules (E and J).

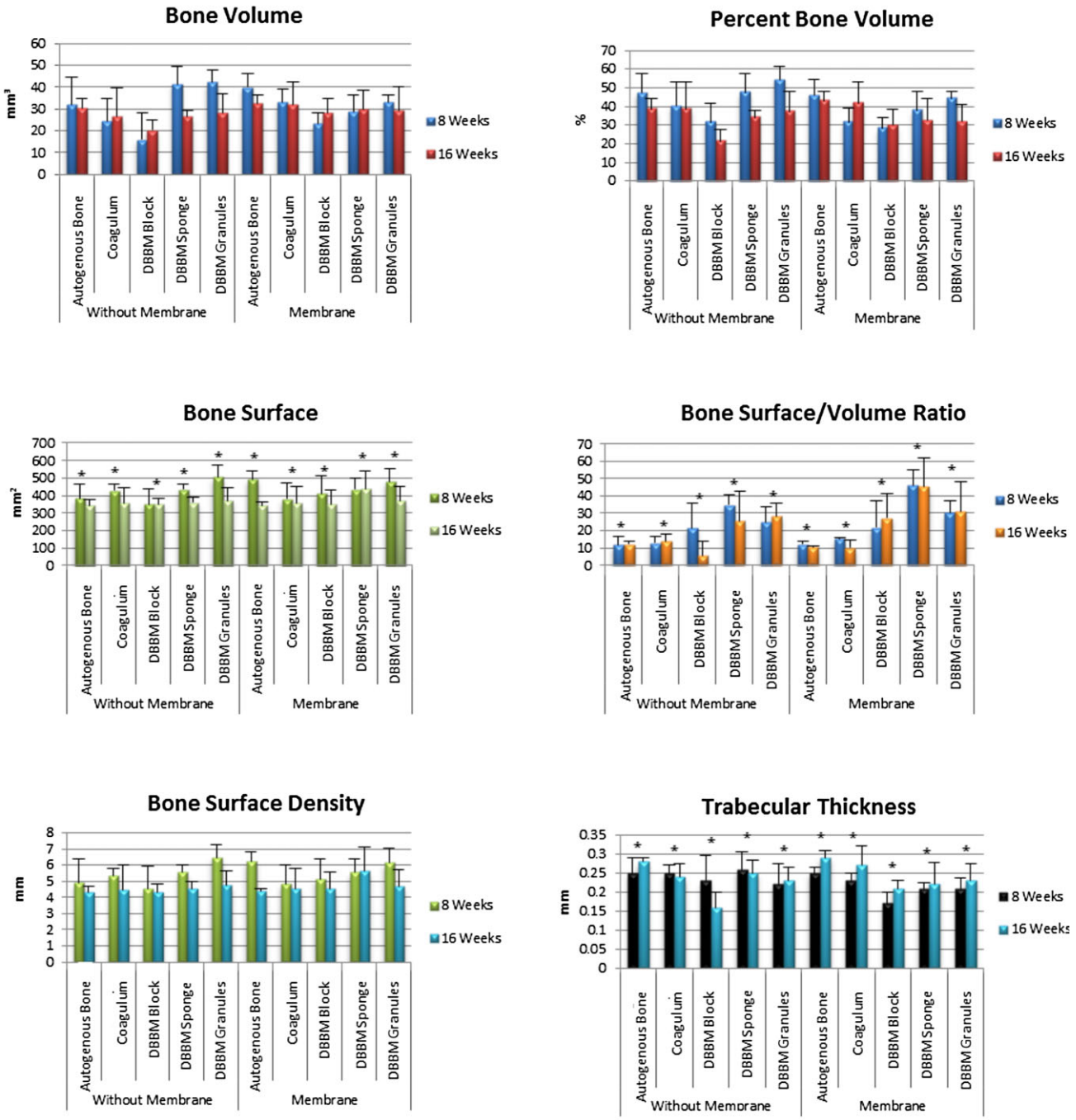

Figure 17 Means and standard deviations of micro-CT parameters: bone volume, percent bone volume, bone surface, bone surface/volume ratio, bone surface density, and trabecular thickness at 8 and 16 weeks according to group distribution. *Statistically significant difference between groups. DBBM = deproteinized bovine bone mineral.

The trabecular thickness analysis demonstrated a statistically significant difference in group comparisons ($p < .05$). When a membrane was not used, the highest trabecular thickness was shown by the DBBM sponge (8 weeks). Among biomaterials, DBBM sponge and DBBM granules were similar at 8 weeks, and DBBM granules showed greater values at 16 weeks (Figure 17).

In Vitro Analysis of Biomaterials. In vitro analysis of biomaterials (Figure 18) was carried out, adopting identical VOI dimensions to those used for the specimens. The DBBM block presented the highest trabecular thickness, volume and open pore porosity, and total porosity. DBBM granules showed the greatest number, volume, and surface area of closed pores. The DBBM

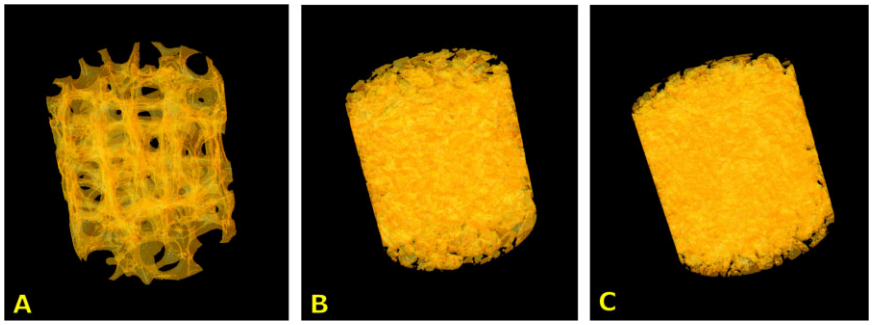

Figure 18 Three-dimensional reconstruction of biomaterials samples in a lateral view: (A) deproteinized bovine bone mineral (DBBM) block, (B) DBBM sponge, (C) DBBM granules.

sponge had intermediate values compared with the others in all parameters evaluated (Table 1).

DISCUSSION

This study compared the outcomes of the use of osseoconductive biomaterials of different porosities in the treatment of 2.7-mm cervical bone defects around implants and evaluated the influence of GBR at two time points (8 and 16 weeks). Histology, histomorphometry, resonance frequency analysis, and micro-CT analysis were applied in order to investigate the influence of different in vitro porosities and in vivo biological response. There are no previous reports in the literature of the use of porous block biomaterials in experimental models of circumferential defects around dental implants that consider both the variety of in vivo parameters that can be analyzed and the physical properties of biomaterials.

The implant stability analysis revealed statistically significant differences between groups in final ISQ value ($p < .05$). Nevertheless, significant differences within and between groups in initial ISQ values were not observed ($p > .05$). It has been reported that implants with low initial ISQ values provide major increases in values over time compared with implants that have high ISQ at the time of installation,[30–32] demonstrating the method's sensitivity to changes in the bone-implant interface during the osseointegration process.[33–38] In all tested treatments in the present study, the ISQ values increased from the initial to the final measurements and from 8 to 16 weeks, regardless of the use of a membrane covering the defects. Implants placed in defects treated with DBBM sponge showed higher final ISQs than other treatments at 16 weeks without a membrane and at 8 and 16 weeks with a membrane ($p < .05$). With regard to the ROI, the DBBM sponge presented a good pattern of bone formation, which would explain the higher implant stability in these sites.

TABLE 1 Assessment of Biomaterial Porosity In Vitro

Parameter	DBBM Block	DBBM Sponge	DBBM Granules
Trabecular thickness (mm)	0.12	0.05	0.03
Closed pores			
Number	5.8490	45.1807	73.1256
Volume (mm^3)	0.02	0.22	0.52
Surface area (mm^2)	14.67	144.02	312.64
Porosity (%)	0.17	0.98	2.17
Open pores			
Volume (mm^3)	37.05	27.06	25.66
Porosity (%)	74.19	54.18	51.38
Total porosity (%)	74.24	54.63	52.44

DBBM = deproteinized bovine bone material.

The histological analysis showed biomaterial particles surrounded by lamellar and/or immature bone and some embedded in connective tissue. No biomaterial was seen in direct contact with the implant surface at either of the two experimental time points. The absence of biomaterial contact with the implant has also been reported in several animal studies.[12,14,39,40] An important finding regarding active participation of the three forms of DBBM was the presence of giant cells and osteoclasts on their surfaces, suggesting remodeling activity and its gradual elimination. This finding concurs with what has been found in other studies.[39,41–44] In defects treated with the DBBM block, most of the scaffold was in direct contact with connective tissue, but with few foci of new bone contacting its surface. Similar reports have been made in studies using this biomaterial in different experimental models.[45,46] It has been described in the literature that bone formation pattern within the defects

occurs predominantly by distance osteogenesis, starting in greater proportion at the apical region and then the bone walls.[44,47,48] This regeneration pattern resembles the analysis of the present study.

The distal alveolar crest resorption analysis revealed statistically significant differences between groups in value of IS-C ($p < .05$). The DBBM block was the treatment that provided the least vertical bone loss in the distal crest at the two evaluated time points (8 and 16 weeks), regardless of the use of a membrane to cover the defects ($p < .05$). This finding was also made in the work of Faria and colleagues,[49] who used a porous block of titanium-based material in surgically created bone defects. In contrast, De Santis and colleagues,[46] in a study that compared implants placed in sites previously augmented with a DBBM block or autogenous bone blocks, reported extensive height resorption in bony ridges grafted with a DBBM block. Some studies emphasize the benefits brought by the use of a DBBM sponge or DBBM granules in minimizing remodeling effects in the bone crest,[44,50] while others report limited influence on this process.[51–54] The three biomaterials used in our study were superior to autogenous bone in the vertical preservation of the distal bone crest at 8 and 16 weeks with and without the use of a membrane ($p < .05$).

The defect area analysis quantified bone formation area within the region of interest (ROI). Statistically significant differences among groups were found ($p < .05$). Despite the morphological changes of the post-extraction socket, it undergoes a phase of accelerated bone formation within the first 30 days of repair and subsequent resorption of approximately 15% of newly formed bone, with gradual replacement by bone marrow.[55]

In the present experimental model – with surgically created defects – a decrease in bone formation was observed from 8 to 16 weeks in all treatments when the membrane coating was applied. Generally, the DBBM block showed the lowest bone formation area among all treated sites in both periods of evaluation, regardless of whether a membrane was used ($p < .05$). Other experimental studies that used block biomaterials and evaluated bone formation corroborate our findings. In the applied models, little bone formation was seen, but with good biocompatibility, biological interaction with the recipient bone, and final volume maintenance.[22,46,49] The DBBM sponge showed the best performance on bone formation among biomaterials at 8 weeks (without membrane) and at 8 and 16 weeks (membrane), just below autogenous bone and coagulum ($p < .05$). These findings are supported by studies that also emphasize its efficacy.[56,57] The use of a collagen membrane to cover defects allows clot maintenance and migration and settlement of bone-forming cells with no competitive migration from adjacent tissues.[58] A beneficial biological effect of its use can be observed in the present study. Defects coated by a membrane were observed to have more bone formation in all treated sites compared with those without covering ($p < .05$).

Based on micro-CT analysis, this study assessed and quantified bone formation at peri-implant defects subjected to different treatment types. Statistically significant differences between groups for the parameters bone surface, bone surface/volume ratio, and trabecular thickness were observed ($p < .05$). The defect treated with DBBM granules showed greater bone surface than others at 8 and 16 weeks without a membrane and a surface similar to that of autogenous bone at 8 weeks with a membrane. Chackartchi and colleagues[59] compared amounts of newly formed bone after sinus floor elevation procedures by histomorphometry and micro-CT with two different sizes of DBBM granules. After data analysis, they observed that both particle sizes showed similar bone surface area and bone formation. In our study, the DBBM sponge provided the greatest bone surface area at 16 weeks with the use of a membrane ($p < .05$). On evaluation of the bone surface/volume ratio, it was found that use of the DBBM sponge resulted in the highest values at 8 weeks without a membrane and at 16 weeks with and without a membrane. Nevins and colleagues[60] used the aforementioned biomaterial to repair defects in teeth affected by severe vertical bone loss in patients with advanced periodontal disease. After a period of 9 months, biopsies were performed and submitted to micro-CT and histological analysis. They emphasized the good ability of DBBM sponge to provide bone formation in periodontal defects. Furthermore, the three biomaterials used in this study showed superior results to autogenous bone and coagulum, regardless of the time point or membrane use ($p < .05$). The trabecular thickness also showed higher values in sites treated with autogenous bone at 8 and 16 weeks with and without the use of membrane ($p < .05$). Using autogenous bone as a standard control of natural and physiological bone formation, the DBBM sponge was the biomaterial that provided the closest values to

those of autogenous bone at all tested time points ($p < .05$).

On comparing the data obtained in the micro-CT and histomorphometric analyses, several divergences could be observed. Some studies have assessed the degree of correlation between these two methods. While some have found strong correlations,[61–63] another reported no correlation between the analyses.[64] In the present study, the treatment that performed the best was divergent in most cases when the data of the two analyses were compared. This mismatch between the absolute values can be explained by the procedure of defining the grayscale threshold for the micro-CT data evaluation. Besides the lack of standardized scales across the various studies, it is known that the accuracy of the analysis is based on the human ability to define correct thresholds, separating the levels of gray of the image. After the described setup, the software quantifies the images and provides values according to the parameters. In some situations, it is difficult to determine the boundary between newly formed bone and remaining biomaterial, and there may be a small influence from an artifact area formed by the titanium part of the implant.[60,64] In our study, the VOIs were defined outside of the implant artifact area. In addition, standard values for grayscale thresholds were defined for the bone tissue and biomaterials, so that after the two measurements were taken separately, it was possible to subtract values in order to obtain the actual value of newly formed bone within the defect. Another factor that could explain this divergence between the values of the two analyses is the 3D assessment provided by the micro-CT. While a histological ground section is a single cut through the center of the defect of approximately 90 μm in thickness, the micro-CT analysis scanned the information of 217 sections of 12 μm each, covering the entire defect area in three dimensions.

Porosity can be defined as the percentage of empty space in a solid, being considered a morphological feature of the biomaterial concerned.[65] It is known that the presence of pores is necessary for bone tissue formation, allowing space for the migration of osteoblasts and mesenchymal cells and the process of vascularization.[23] In addition, a porous surface increases the mechanical interaction between the biomaterial and the surrounding bone in a critical interface.[66,67] In our study, biomaterials' in vitro porosity was assessed by micro-CT. Measurements of trabecular thickness; number, volume, surface area, and porosity of closed pores; volume and porosity of open pores; and total porosity were taken. There are no reports in the literature of data on the topography and porosity of the biomaterials used in our study. The DBBM block presented the highest trabecular thickness, volume and porosity of open pores, and total porosity. DBBM granules showed the highest number, volume, and surface area of closed pores. The DBBM sponge was in an intermediate position. When comparing the results observed in this analysis with the findings in histomorphometric and micro-CT specimens (in vivo), an inverse relationship could be observed between porosity and bone formation. The DBBM block was seen on in vitro analysis to be the most porous material tested. However, it presented the worst performance in bone formation in vivo, contradicting the theory that a large surface area of pores and microporosity contribute to greater protein adsorption from blood and interstitial fluid and greater ion exchange, enabling close interaction between the biomaterial and the environment and more favorable conditions for bone formation.[66] Conversely, the DBBM granules, which on in vitro analysis were shown to be the least porous biomaterial, showed the best results in vivo. Despite the proven benefits of porous biomaterials, it appears that neither porosity nor pore size nor volume alone can be the key to obtaining favorable results with bone formation, due to the high complexity of the biological processes occurring during bone metabolism. The difficulty in reproducing the same results obtained in vitro in an in vivo experimental model illustrates such limitations.[68]

In the present study, the hypothesis that the use of a block biomaterial with a micro- and macroporous scaffold similar to human bone architecture to reconstruct the defect would provide more effective bone formation was not confirmed. It is suggested that the sparse but well-distributed bone formation on the DBBM block surface could advance to a more satisfactory level over longer periods than that evaluated in the present study (16 weeks). The slow pattern of xenogenic bone resorption would provide long-term stability to the newly formed bone within the defect. Furthermore, the technical difficulty of introducing a protocol closer to clinical situations (stabilizing the block via the buccal aspect) means the results related to crestal bone resorption are to be extrapolated with caution. The other porous materials used (DBBM sponge and DBBM granules) demonstrated a significant effect on bone formation,

with equal or superior performance to autogenous bone in specific situations.

Based on the results obtained in this study, the most effective treatment to minimize the effects of the intense remodeling process of alveolar bone walls after tooth extraction and complete defect repair remains uncertain. Future research into the molecular biology of bone metabolism could explain this process. The development of tissue engineering materials with ideal biocompatibility, biomechanical properties, and porosity manufactured for patients' specific needs, combined with therapy using stem cells and growth factors, seems to be the way to reach success, solve problems, and achieve successful treatment outcomes.

CONCLUSIONS

Within the limitations of this study, it can be concluded that the highest-porosity block (DBBM block) used in the cervical region of the implant was the worst biomaterial for bone repair within the defect, and it was the treatment that most reduced distal alveolar bone crest resorption. Biomaterials with lower porosity (DBBM sponge and DBBM granules) showed similar or higher bone formation and implant stability compared with autogenous bone. The GBR technique resulted in greater bone formation in all treatments and at both evaluation time points. An inverse relationship between porosity of biomaterials in vitro and in vivo bone formation was observed in this experimental model.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Distribution of best treatment results for each parameter by time point and membrane use.