Influence of Periodontal Biotype on Buccal Bone Remodeling after Tooth Extraction Using the Flapless Approach with a Xenograft: A Histomorphometric and Fluorescence Study in Small Dogs

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ABSTRACT

Background: Several approaches have been used to counteract alveolar bone resorption after tooth extraction.

Purpose: The aim of the present study was to evaluate the influence of gingival thickness and bone grafting on buccal bone remodeling in extraction sockets with thin buccal bone, using a flapless approach.

Materials and Methods: The gingiva of 8 dogs was thinned at one side of the mandible and mandibular premolars were extracted without flaps. The sockets were randomly assigned to the test group (thin gingiva) (TG), the test group with grafting material TG + GM, the control group (normal gingiva) (CG), or the control group with grafting material CG + GM. Ground sections were prepared from 12-week healing biopsies, and histomorphometry and fluorescence analysis were performed.

Results: In the groups with thin gingiva, numerically greater buccal bone loss was observed, while there were no differences between grafted and nongrafted sites. A numerically higher rate of mineralization was observed for the grafted sites, as compared with the nongrafted sites, at 12 weeks.

Conclusions: A thin buccal bone plate leads to higher bone loss in extraction sockets, even with flapless surgery. The gingival thickness or the use of a graft material did not prevent buccal bone resorption in a naturally thin biotype, but modified the mineralization process.

KEY WORDS: alveolar bone remodeling, animal model, extraction socket, flapless implant surgery, xenograft

During the healing process of an extraction socket a series of events occurs, including the formation and maturation of a coagulum that will be subsequently replaced by a provisional matrix and woven bone.[1–3] Further, the socket walls will be resorbed and gradually remodeled, and the distinct outline of the extraction socket will disappear.[4] When, during healing, a cortical ridge is established in the entrance of the socket, the immature woven bone is remodeled and replaced by lamellar bone and marrow.[5]

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DOI 10.1111/cid.12182

Alveolar bone resorption after tooth extraction is an inherent condition of the healing process, and the edentulous site of the alveolar process will undergo marked alterations of height and width.[4–8] The healing process following tooth removal apparently results in more pronounced resorption of the buccal aspect of the ridge than of the lingual/palatal aspect.[4,5] The higher bone density, represented by the lower number of marrow spaces, in association with the thinner aspect of the buccal bone plates, makes them more fragile to absorb compared with the lingual bone plates.[9] Further, the processes resulting in tissue reduction seem to be more pronounced during the initial phase of wound healing than during later periods following tooth removal.[4,8]

Several investigations have been performed to establish the real scale of the resorption. A recent meta-analysis on socket preservation therapies reports ridge reductions of 2.6 to 4.5 mm in width and 0.4 to 3.9 mm in height in naturally healed sockets.[10] These reductions complicate ideal implant positioning and affect the predictability of esthetic outcomes, particularly in anterior areas.[11] At the moment, the main focus of treating the extraction socket must be to preserve the tissue volume to a certain extent and improve the soft tissue conditions for delayed implant placement.

Therefore, various graft materials, including autogenous bone, bioactive glass, coralline calcium carbonate, decalcified freeze-dried bone, deproteinized bovine bone, and hydroxyapatite, have been used in attempts to preserve the alveolar ridge following tooth extraction.[12–22] One particular xenogenic graft composed of deproteinized bovine bone mineral is the material of which use in socket preservation procedures is most frequently reported in the literature.[13,16,17,19] This biomaterial acts as a scaffold for new bone formation.[23,24] Some authors have reported good osseointegration, resorption, and replacement of the graft particles with bone tissue,[24] while in some cases fibrous encapsulation of the biomaterial[25] and incorporation with minimal resorption[12,17,25] have been observed. This same deproteinized bovine bone mineral combined with collagen (Bio-Oss Collagen [BOC], Geistlich Pharma AG, Wolhusen, Switzerland) has been recently introduced in dentistry. This biomaterial seems to modify modeling and counteract marginal ridge contraction that occurs following tooth removal.[12] Despite the promising results, conflicting data exist on the outcome of placing BOC in extraction sockets, and some authors disagree with the potential of the biomaterial to limit the postoperative contour shrinkage.[6,26–29]

Ten Heggeler and colleagues[10] emphasized the scarcity of data on socket preservation therapies in humans. These authors concluded that socket preservation techniques do not prevent bone resorption but may reduce it, with consequent superior results to those recorded in cases of natural healing. Nevertheless, other aspects must be observed in an attempt to reduce the bone loss after tooth extraction, such as the initial thickness of the soft tissue and buccal bone plate and the surgical approach used.

The influence of mucosal thickness and biologic width formation on tissue health maintenance and bone wall preservation around implants has recently been discussed. The term *periodontal biotype* was introduced by Seibert and Lindhe[30] to describe the thickness of the gingiva in the buccolingual dimension, and this is an important aspect in the maintenance of gingival health. Although it seems to be an important factor for oral rehabilitation, research regarding the effects of gingival thickness on bone remodeling after tooth extraction is lacking.

The initial thickness of the buccal bone plate in the extraction socket seems to have a significant influence on the amount of horizontal and vertical crest resorption in human sockets.[31–33] Sites with thin buccal bone plate ($\leq$ 1 mm) present more height and width of bone loss after extraction of maxillary anterior teeth.[32] The literature showed that hard tissue changes during healing are consistently dependent on the baseline characteristics. Therefore, the degree of crestal resorption is dependent on the thickness of the buccal/palatal bone walls.[31,33]

The surgical approach is another important aspect that must be considered. Surgical trauma that includes the separation of the periosteum and the rupture of the connective tissue attachment at the bone surface will induce remodeling of the alveolar bone surface layer in the exposed area.[34,35] In a histological analysis of sites following tooth extraction with a full-thickness approach, osteoclasts were indeed present in the exposed area of the alveolar ridge, which exhibited signs of surface resorption.[4] This may, in part, explain the marked dimensional alterations after tooth extraction demonstrated in the above-cited study. On the other hand, leaving the periosteum in place decreases the resorption rate of the extraction socket.[36]

Based on this, the aim of the present histomorphometric and fluorescence study was to evaluate the influence of a thin periodontal biotype on buccal bone plate remodeling in extraction sockets following a flapless approach, associated or not associated with xenografts. Also, the dynamics of early bone healing were investigated.

MATERIALS AND METHODS

Study Design and Randomization Procedure

This study was conducted after the approval of the Animal Experimental Ethics Committee (protocol number 11.1.138.53.6). Eight beagle dogs, around 1 year old and weighing on average 11.5 kg, were used. Such small dogs were selected to ensure that all of them would present thin buccal bone plates. All selected animals had normal mandibles, no generalized occlusal trauma, no viral or fungal mouth lesions, good overall health, and no systemic compromises attested by veterinary exams. The dogs were immunized with vaccines, received antiparasitic treatment, and were submitted to dental prophylaxis using ultrasonic points (Cavitron 3000, Dentisply York, PA, USA) for removal of dental calculus and biofilm.

A computer-generated random permuted block was used to allocate each side of each mandible to one of the four experimental groups – test group (TG; thin gingiva), test group with grafting material (TG + GM), control group (CG, normal gingiva), and control group with grafting material (CG + GM).

Presurgical Procedures

In the first stage of this study, on the test side, the buccal mucosa of the teeth scheduled for surgery was thinned using a high-speed bur (gingival peeling) (Figure 1), while the control side was left without alteration in tissue thickness. Four weeks after the gingival peeling, the gingiva of the test side had become

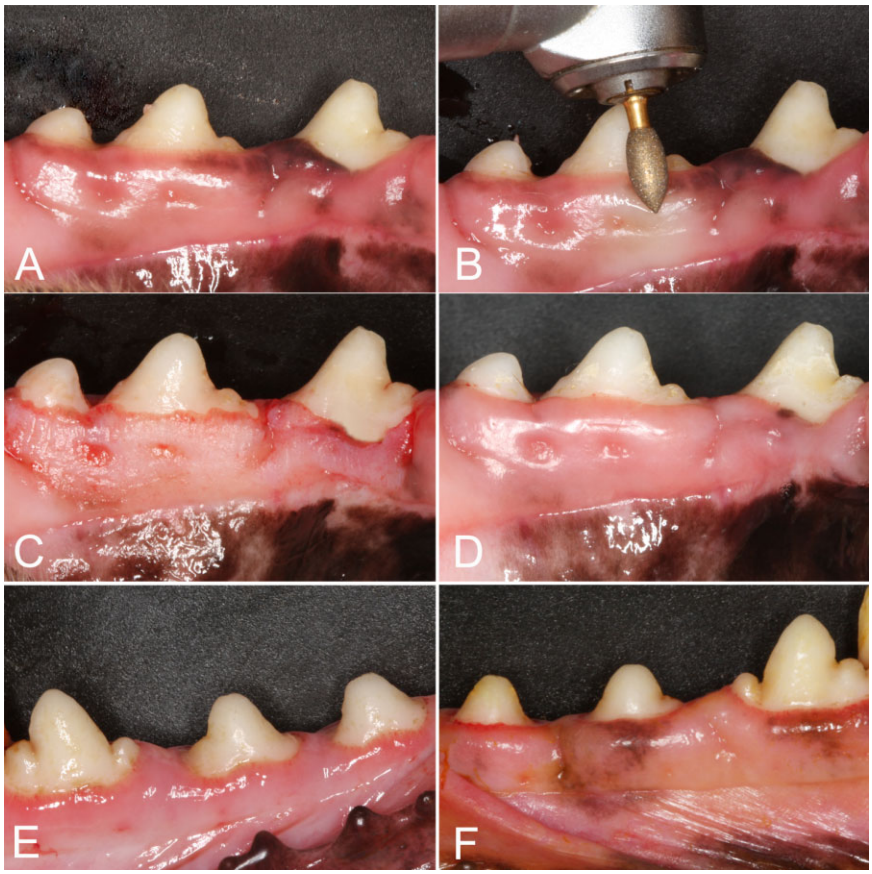

Figure 1 Gingival peeling: *A*, Initial aspect of the mucosa; *B*, The buccal mucosa of the teeth scheduled for surgery was thinned using a high-speed bur; *C*, Aspect of the mucosa immediately after the gingival peeling; *D*, Aspect of the mucosa 7 days after the gingival peeling; *E*, Gingival aspect at the moment of implant placement of the test group, showing the visible lower gingival thickness in the test group; *F*, Gingival aspect at the moment of implant placement of the control group.

similar to the control side of the mandible and another gingival peeling was carried out, followed by weekly traumatic brushing in an attempt to maintain the thinness of keratinized tissue on the test side. Eight weeks after the second gingival peeling, a statistically significant difference between test and control sides in keratinized tissue thickness was observed (see Figure 1, E and F), and the dogs were ready for the surgery procedure. During these procedures, the animals were anesthetized with intravenously administered zolazepam (0.1 mL/kg; Zoletil 50®, Verbac, São Paulo, São Paulo, Brazil) and acepromazine (2.0%; Acepran®, Univet, São Paulo, São Paulo, Brazil).

Clinical Parameters

The clinical parameters evaluated were thickness of keratinized tissue (TKT), alveolar thickness (AT), and buccal bone thickness (BBT). All clinical assessments were recorded by the same calibrated experienced periodontist (L.P.M.).

TKT and AT were assessed with the aid of an acrylic stent to determine the exact measurement sites before the first gingival peeling (T_0) and immediately before tooth extraction (T_1). The TKT was assessed using an anesthetic needle attached to a silicone disk stop. The needle was placed in an orifice in the acrylic stent, 3 mm below the gingival margin, and inserted perpendicular to the mucosa surface through the soft tissue with light pressure until a hard surface was felt. The AT was assessed using a surgical micrometer, also in the orifice of the acrylic stent. The penetration depth of both the needle and the micrometer was measured using a digital caliper with 0.05-mm resolution.

The BBT was assessed using a surgical micrometer during the surgery, after tooth extractions, and the penetration of the micrometer was measured using a digital caliper with 0.05-mm resolution.

Surgical Procedures

The surgical procedures were performed by a single experienced surgeon 12 weeks after the first gingival peeling (see "Presurgical Procedures").

The animals were kept on fast starting the night preceding the surgical procedures. For the surgery itself, the dogs were preanesthetized with 10% zolazepam (0.10 mL/kg) and acepromazine (2.0%). Anesthesia was maintained using volatile anesthetics, so the animals were submitted to tracheal intubation with a Magill probe for adaptation of the anesthetic device and for administration of oxygen-diluted volatile isoflurane (2% v/v; Isothane®, Baxter Hospitalar, São Paulo, São Paulo, Brazil). Additionally, local anesthesia was used on the premolar regions.

After local anesthesia, the four lower bicuspids (premolars) on both sides of the mandible were extracted without flap elevations (flapless). The teeth were buccolingually sectioned (Figure 2A), and the roots were carefully removed (see Figure 2B), avoiding damage to the alveolar bone walls. After tooth extractions, the BBT was assessed using a surgical micrometer. The mesial sockets of the second (P2) and the fourth (P4) premolars on both quadrants of the mandible were selected for the experiment. This procedure was also part of another study, and the remaining sockets were used for immediate implantation evaluation. As buccolingual sections of the middle portions of the experimental sites were evaluated in both studies, using the dogs for two experiments had no detrimental effects on the experimental model. Randomly, one socket in each hemimandible received xenografts (BOC) to fill the fresh extraction socket, while in the other socket a blood clot was allowed to form in the empty alveolus (see Figure 2C). Following this, the wounds were sutured with 5-0 nylon suture on both sides of the mandible.

For postoperative care, tramadol chlorhydrate (50 mg/mL; Tramal®, União Química Farmacêutica

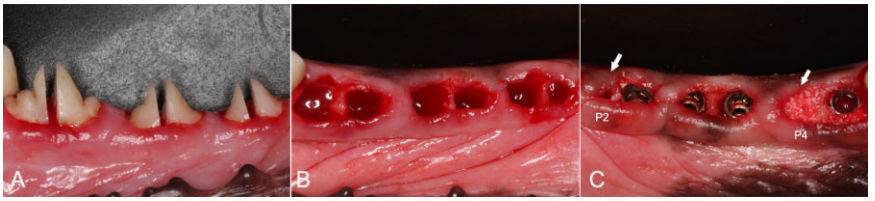

Figure 2 *A*, Hemi-section of the bicuspids. *B*, Sockets after tooth extraction. *C*, *Arrows* indicate the blood clot in the P2 mesial socket and the bone graft in the P4 mesial socket.

Nacional, Pouso Alegre, Minas Gerais, Brazil) was used at a dose of 3 mg/kg every 12 hours for 3 days as analgesic therapy, and meloxicam (2 mg/20 kg; Maxicam®, Ouro Fino Saúde Animal, Cravinhos, São Paulo, Brazil) was used for 5 days as anti-inflammatory therapy. The animals also received spiramycin (750,000 IU/10 kg) and metronidazole (125 mg/10 kg) (Stomorgyl® 10, Merial Saúde Animal, Paulínia, São Paulo, Brazil) for 10 days as antibiotic therapy. Seven days later the sutures were removed. The animals were maintained on a soft ration diet for 15 days, and healing control was performed daily with a topical application of 0.12% chlorhexidine to limit microbial biofilm adherence. The remaining teeth were cleaned monthly with ultrasonic points.

During the healing period, four different fluorescent bone markers were administered in order to observe the degree and extension of bone mineralization. One week after tooth extraction, 20 mg calcein green/kg body weight was administered i.v. in each dog; at 2 weeks, 20 mg alizarin red/kg body weight was administered i.v.; at 4 weeks, 20 mg tetracycline/kg body weight was administered i.v.; and at 12 weeks, 20 mg calcein blue/kg body weight was administered i.v. (all dyes from Sigma Chemical, St. Louis, MO, USA). All dyes were prepared immediately before use with 2% sodium bicarbonate or saline. After preparation, pH was adjusted to 7.4 and the solution was filtered through a 0.45-μm filter (Schleicher & Schuell, Dassel, Germany). Each dog received a total dose of 3 mL.

Twelve weeks after implant placement, the animals were sacrificed by induction of deep anesthesia with a subsequent intravenous sodium thiopental and potassium chloride overdose. The hemimandibles were removed, dissected, cut, and fixed in 4% phosphate-buffered formalin (pH 7) until processing. The specimens were dehydrated in increasing concentrations of alcohol up to 100%, infiltrated, and embedded in LR White resin (London Resin Company, Berkshire, UK) and hard-sectioned using the technique described by Donath and Breuner 37. From each alveolus unit, one buccolingual section representing the central area of the site was prepared. The sections were reduced to a thickness of about 25 μm by microgrinding and polishing. The sections were prepared for fluorescence analysis and then for histomorphometry after being stained with alizarin red for optic microscopic analysis.

Histomorphometric Analysis

Buccolingual longitudinal histological sections from each socket were analyzed on a Leica DM LB2 microscope outfitted with a Leica DC300F digital camera (Leica Microsystems, Wetzlar, Germany). The images were analyzed through the Leica Application Suite v. 4.1 by a single examiner.

The buccal crest level (BCL) was determined as a linear vertical measurement in two different regions: as an imaginary line from the lingual bone crest to the first point of the newly formed bone in the buccal crest (BCL-NB), and as an imaginary line from the lingual bone crest to the first point of the "old bone" in the buccal crest (BCL-OB) (Figure 3A).

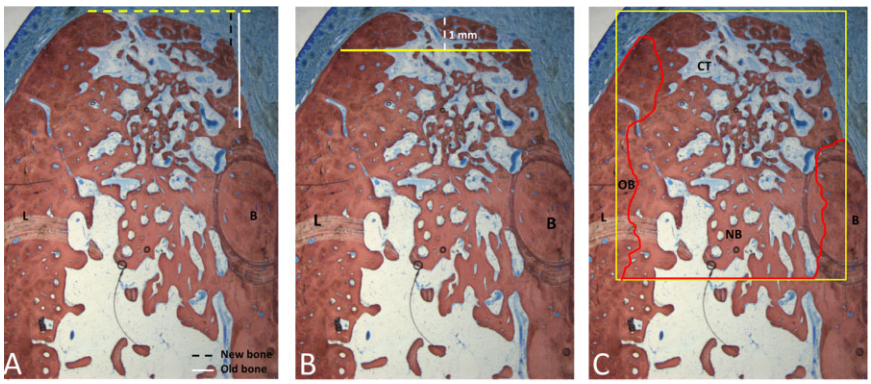

Figure 3 Histological images representing the morphometric measurements. *A*, The level of the buccal bone crest (B) in relation to the lingual bone crest (L) was determined as a linear vertical measurement in two different regions: as an imaginary line from the lingual bone crest to the first point of the newly formed buccal bone (black line), and as an imaginary line from the lingual bone crest to the first point of the "old bone" (white line). *B*, Alveolar ridge width was analyzed 1 mm below the highest point of the bone in the socket. *C*, Histological bone density was determined within a 30 mm^2 rectangle that comprised the region of the defect. The areas of connective tissue (CT), new bone (NB), and old bone (OB) were measured. (Magnification ×1.6.) Alizarin red stain.

The alveolar ridge width (ARW) was determined as a linear horizontal measurement, between both the buccal and lingual external walls, as an imaginary line 1 mm below the highest point of the bone in the socket (see Figure 3B).

The bone area was determined within a rectangle that comprised the region of the socket (30 mm^2), measured starting with the highest point of the socket. These measurements evaluated the percentage of the region occupied by mineralized bone in relation to the percentage occupied by marrow spaces. The connective tissue (CT), total bone area (TBA), and new bone area (NBA) were measured. Finally, the percentage of the sockets occupied by residual graft particles (RGP) was measured (see Figure 3C).

Fluorescence Analysis

Fluorescence microscopic images were longitudinally captured from each sample through a Leica DC300F video camera joined to a Leica DM LB2 microscope, using appropriate barrier filters. The wavelength filters used were I3 for calcein green (excitation level 450–490 nm), N2-1 for alizarin red (excitation level 515–560 nm), D for tetracycline (excitation level 355–425 nm), and A for calcein blue (excitation level 340–380 nm). All the images were adjusted and analyzed with Leica QWin software (Leica Microsystems) to determine the percentage of the alveoli occupied by marked bone. Thus, one rectangle comprising the region of the socket (30 mm^2, from the highest point of the socket) was used to evaluate the percentage of space occupied by fluorescent bone. The marked bone measurements evaluated the percentage of the total area occupied by fluorescent bone.

Statistical Analysis

Quantitative data were recorded as mean and standard deviations. The experimental unit was the dog ($n = 8$). To verify the normality of the data, the Kolmogorov-Smirnov test was used. For the parametric data (BBT, TKT, AT, and histomorphometric analysis), the one-way ANOVA was used for intragroup (T_0 vs. T_1) and intergroup (TG vs. TG + GM vs. CG vs. CG + GM) comparisons. For the nonparametric data (fluorescence analysis), the Kruskal-Wallis one-way ANOVA on ranks was applied for intergroup comparisons. A confidence interval of 95% was adopted. SigmaStat (Systat Software Inc., San Jose, CA, USA) version 3.5 was the statistical software used for the analysis.

RESULTS

Clinical Findings

The healing was uneventful, and there were no complications during the experimental period.

Clinical Analysis

Data regarding the TKT, the AT, and the BBT are summarized in Table 1. There was no statistically significant difference in mean TKT among the groups at baseline. In TG and TG + GM the mean TKT was significantly decreased from T_0 to T_1 (TG: $p < .001$; TG + GM: $p = .004$). For CG and CG + GM, the mean TKT observed at T_0 remained stable until T_1, with no statistically significant changes. This reduction in the mean TKT in TG and TG + GM resulted in statistically significant differences between these groups and the control groups (CG and CG + GM) at T_1 ($p < .001$) (see

TABLE 1 Thickness Measurements before Gingival Peeling (T_0) and at Tooth Extraction (T_1) (Mean ± SD): Intragroup and Intergroup Comparisons

	T_0	T_1	*p* Value
TKT (mm)			
TG	1.24 ± 0.13^{A}	0.73 ± 0.16^{Ba}	<0.001
TG + GM	1.19 ± 0.22^{A}	0.78 ± 0.14^{Ba}	0.004
CG	1.20 ± 0.19	1.16 ± 0.13^{b}	NS
CG + GM	1.18 ± 0.28	1.16 ± 0.14^{b}	NS
p Value	NS	<.001	
AT (mm)			
TG	3.39 ± 0.96	3.08 ± 0.46	NS
TG + GM	3.64 ± 0.86	3.44 ± 0.64	NS
CG	3.73 ± 1.00	3.15 ± 0.45	NS
CG + GM	3.39 ± 0.58	3.04 ± 0.61	NS
p Value	NS	NS	
BBT (mm)			
TG	—	0.53 ± 0.12	
TG + GM	—	0.55 ± 0.11	
CG	—	0.49 ± 0.19	
CG + GM	—	0.53 ± 0.06	
p Value		NS	

One-way ANOVA with Tukey's test. Different superscript letters represent statistically significant difference ($p < .05$).
[A,B]Intragroup comparisons.
[a,b]Intergroup comparisons.
TKT = thickness of keratinized tissue; AT = alveolar thickness; BBT = buccal bone thickness; TG = test group; CG = control group; GM = graft material; NS = not statistically significant.

Table 1). For the AT, there were no statistically significant differences among the groups at any point of the experiment (see Table 1). A low BBT was observed in all the experimental groups, with no statistically significant differences among the groups (see Table 1).

Histological Analysis

In general, the dense hard tissue walls of the alveolar process enclosed a large central area that was occupied by bone marrow and newly formed bone (Figures 4 and 5). All the groups showed the presence of parent lamellar bone, representing the "old bone", and an area of newly formed bone, characterized as parallel-fibered bone (Figures 4–7). In addition, in the grafted groups, residual graft particles were present in the socket (see Figures 5 and 7).

The newly formed bone extended from the lateral walls of the extraction site and migrated towards the middle portion, being present mostly in the center of the socket areas of all the groups. The interface between this structure and the parent lamellar bone was evident (see Figures 6 and 7). The new bone presented a lamellar pattern with parallel fibers, but in some areas, bone with interlaced fibers (woven bone) was present (see Figure 6B). In some areas, the surface of the new bone was accompanied by a layer of osteoblasts and osteoid matrix representing the remodeling process (see Figure 4B). In all areas of the socket, multinucleated cells (osteoclasts) could occasionally be observed on the surface of this new bone (see Figure 4B).

In the grafted groups, a small number of residual particles were dispersed in the middle region and surrounded by newly formed bone (see Figures 5 and 7). In some cases, the particles were lined/circumscribed by the newly formed bone, with direct contact between the structures (see Figure 5B). In other cases, the particles were surrounded by a fibrous connective tissue that differed from the native bone marrow (see Figure 5B). The marginal portion of the socket was occupied by newly formed bone in the control group and newly formed bone plus graft particles in the test groups. Both groups presented new bone in the coronal portion that "closed" the socket entrance.

Histomorphometric Results

It was observed that, in summary, all the groups presented similar results, with no statistically significant differences in any of the parameters evaluated (Tables 2 and 3).

BCL-NB in the control groups (normal gingiva) was numerically better as compared with the test groups (thin gingiva), but without statistical relevance. No differences between the groups that received the graft material and those that did not were observed. The

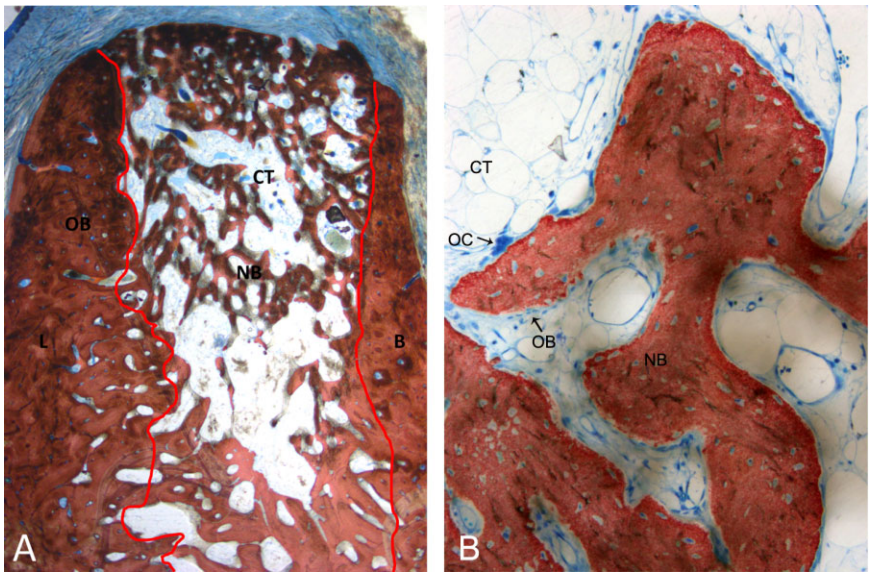

Figure 4 Histological images of the nongrafted sites. *A*, Note that the entrance of the socket was "closed" by a bridge of mineralized bone that connected the buccal (B) and lingual (L) crests. The coronal, middle, and apical areas were filled by new bone (NB) and connective tissue (CT). The red line separates the old bone (OB) from the newly formed hard tissue (NB) (magnification ×1.6). *B*, At a higher magnification (×20), the specific area of new bone shows aspects of immaturity, with the presence of osteoblasts (OB) and osteoclasts (OC), indicating ongoing bone remodeling. Alizarin red stain.

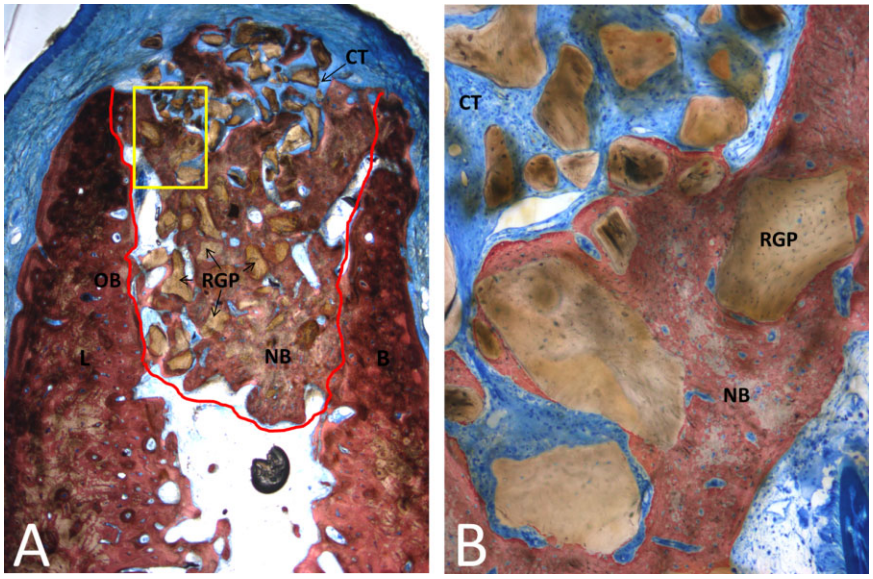

Figure 5 Histological images of the grafted sites. *A*, The fresh extraction socket was grafted with a synthetic bone graft. The residual graft particles (RGPs, brown stain) were embebbed in newly formed bone (NB) and fibrous connective tissue (CT). Note that the biomaterial occupied a large portion of the socket entrance, which was "closed" by a a bridge of mineralized bone and graft material. The red line separates the old bone (OB) from the newly formed hard tissue (NB) (Magnification ×1.6.) *B*, The circumscribed area in a higher magnification (×20), the presence of some RGPs lined/circumscribed by the newly formed bone (NB) was more evident, with direct contact between the structures, while other RGPs were surrounded by fibrous connective tissue (CT). Alizarin red stain.

BCL-OB and the ARW were very similar for the groups and without statistical relevance (see Table 2).

The total area of the frame (100%) was divided into connective tissue and bone area. All the experimental groups showed around 50% connective tissue and 50% bone area, on average, without any statistically significant difference. The new bone area represented around 35% of the total frame, also without any statistically significant difference among the experimental groups (see Table 3).

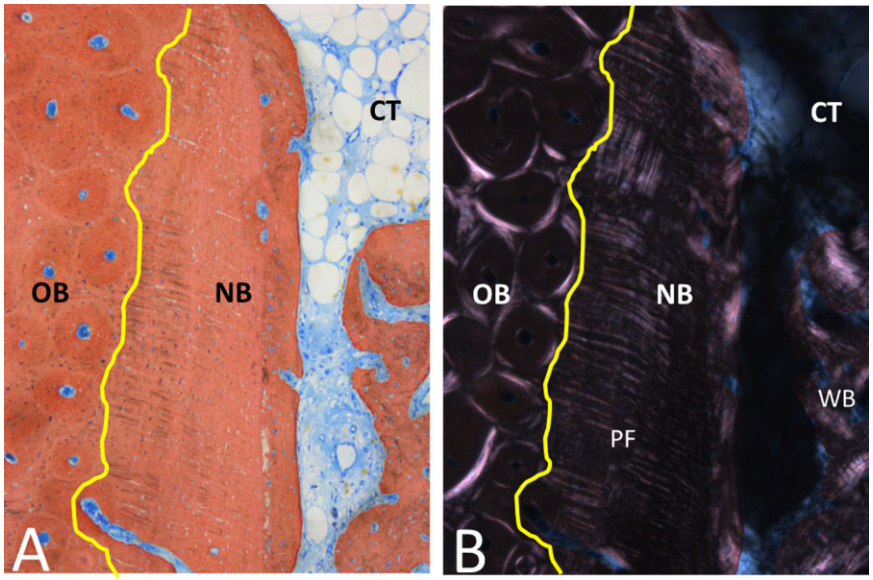

Figure 6 Histological images of the nongrafted sites. *A*, Note the difference between the new bone (NB) and the old bone (OB). The structures are connected by remodeling tissue. *B*, Under polarized light, it was possible to observe the presence of the old bone (OB) with its lamellar pattern, and the newly formed bone (NB) with areas of interlaced fibers (woven bone, WB) and parallel fibers (PF). Alizarin red stain. (Magnification ×20.)

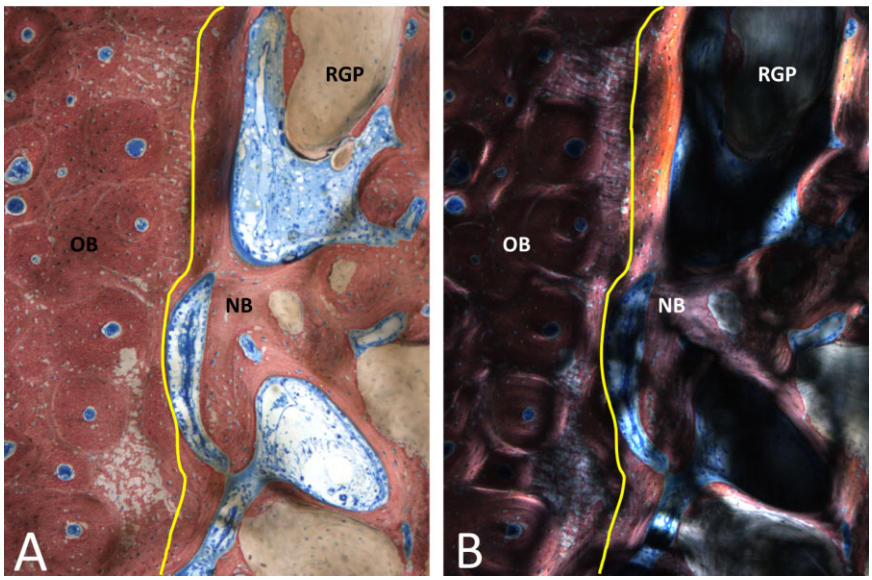

Figure 7 Histological images of the grafted sites. *A*, Note the difference between the new bone (NB) and the old bone (OB). The structures are connected by remodeling tissue. *B*, Under polarized light, it was possible to observe the presence of the old bone (OB), with its lamellar pattern, and the newly formed bone (NB). Alizarin red stain. (Magnification ×20.)

TABLE 2 Intergroup Comparisons for Linear Measurements (Mean ± SD)

	BCL-NB (mm)	BCL-OB (mm)	ARW (mm)
TG	0.73 ± 0.53	1.48 ± 0.81	3.61 ± 0.59
TG + GM	0.95 ± 0.37	1.76 ± 0.86	4.24 ± 0.53
CG	0.40 ± 0.28	1.16 ± 0.60	4.11 ± 0.58
CG + GM	0.64 ± 0.33	1.66 ± 0.53	4.01 ± 0.44
p Value	NS	NS	NS

One-way ANOVA ($p < .05$).
BCL-NB = buccal crest level of new bone; BCL-OB = buccal crest level of old bone; ARW = alveolar ridge with; TG = test group; CG = control group; GM = graft material; NS = not statistically significant.

The percentage of residual graft particles in the groups that received graft material was 2.63 ± 2.63% for TG + GM and 1.98 ± 2.48 for CG + GM, indicating that part of this biomaterial was resorbed or lost and part was present inside the socket (see Table 3).

Fluorescence Results

The analysis under fluorescent microscopy showed intense bone remodeling for all the groups evaluated. The old bone was always darker and without labeling, while alizarin red showed a red color in a smeared, diffuse pattern; calcein green showed green bands;

TABLE 3 Intergroup Comparisons for Percentage-Area Measurements (Mean ± SD)

	CT (%)	TBA (%)	NBA (%)	RGP (%)
TG	51.85 ± 9.29	55.78 ± 8.92	37.46 ± 9.28	—
TG + GM	54.50 ± 6.09	52.05 ± 4.50	35.33 ± 3.03	2.63 ± 2.63
CG	51.55 ± 7.74	53.55 ± 6.87	39.39 ± 7.24	—
CG + GM	51.52 ± 9.93	51.64 ± 8.60	35.59 ± 7.17	1.98 ± 2.48
p value	NS	NS	NS	

One way ANOVA ($p < .05$).
CT = connective tissue; TBA = total bone area; NBA = new bone area; RGP = residual graft particles; TG = test group; CG = control group; GM = graft material; NS = not statistically significant.

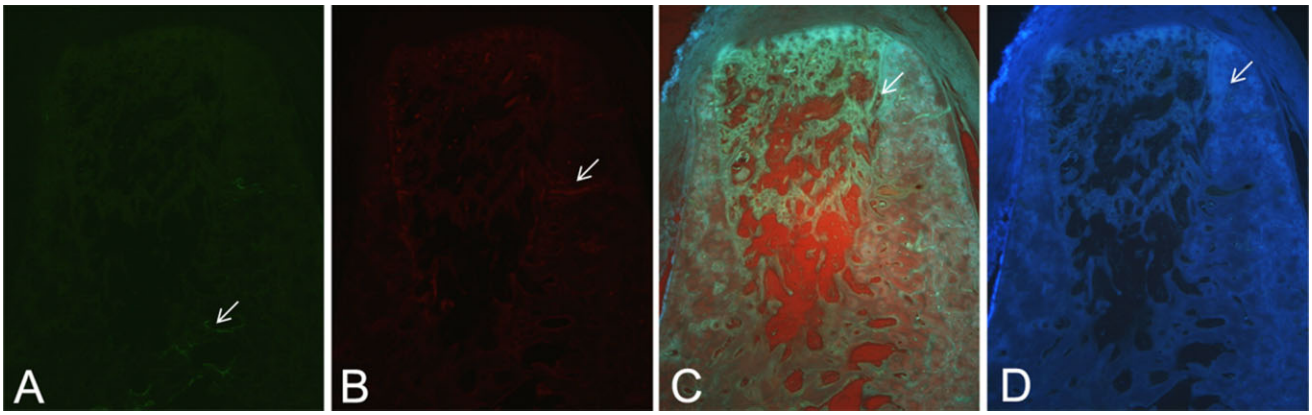

Figure 8 Histological images representing the fluorescence analysis. New bone formation was determined histomorphometrically by quantification of the bone markers. The old bone always appeared darker and without labeling. *A*, Calcein green was generally represented by clearly evident green bands; *B*, Alizarin red had a red color in a smeared diffuse pattern; *C*, Tetracycline showed thin yellow-green lines; *D*, Calcein blue was characterized by a blue color in a very diffuse pattern. (Magnification ×1.6.)

tetracycline showed thin yellow-green lines; and, finally, calcein blue was characterized by a blue color in a very diffuse pattern (Figure 8).

New bone mineralization was determined histomorphometrically by quantification of the bone markers. Sequentially, they represented the healing pattern of each different group. The percentages of space occupied by newly formed bone are described in Table 4. The mean values for the mineralization rate gradually increased over the experimental periods, with the largest increase from 2 to 4 weeks. The mineralization rate reached the highest values at week 12, except for the CG, which showed a slight decrease from 4 to 12 weeks (Figure 9). These data did not reveal any statistically significant differences among the experimental groups, but at 12 weeks the mineralization rate was numerically higher for the grafted groups (TG + GM and CG + GM) as compared with the nongrafted groups (TG and CG).

DISCUSSION

Different approaches have been advocated to preserve or improve the dimension and contour of the ridge following tooth extraction. The present study evaluated the influence of the periodontal biotype on buccal bone remodeling in extraction sockets following a flapless approach. This process was also compared between sites that were or were not grafted with xenografts.

It is important to note that this study was conducted in small beagle dogs with a thin periodontal biotype. All the dogs used in the study presented a thin buccal bone plate at the moment of tooth extractions, measuring about 0.5 mm on average. The presurgical procedures succeeded in reducing the gingiva of the test groups to a critical thickness, from 1.2 mm to 0.8 mm on average ($p < .05$). Despite the significant reduction in gingival thickness, there were no statistically significant changes in AT.

TABLE 4 Fluorescence Analysis: Intergroup Comparisons of the Percentages of the Alveoli Occupied by Newly Formed Bone over the Period of Evaluation (Mean ± SD)

	Rate of Mineralization (%)			
	1 Week	2 Weeks	4 Weeks	12 Weeks
TG	0.41 ± 0.21	0.87 ± 0.46	4.65 ± 3.44	6.37 ± 2.43
TG + GM	0.62 ± 0.37	1.06 ± 0.69	4.06 ± 1.99	7.65 ± 4.34
CG	0.81 ± 0.47	1.07 ± 1.32	5.72 ± 4.07	5.52 ± 2.32
CG + GM	0.82 ± 0.56	3.6 ± 4.43	4.04 ± 1.42	8.39 ± 4.89
p Value	NS	NS	NS	NS

Kruskal-Wallis one-way ANOVA on ranks ($p < .05$).
TG = test group; CG = control group; GM = graft material; NS = not statistically significant.

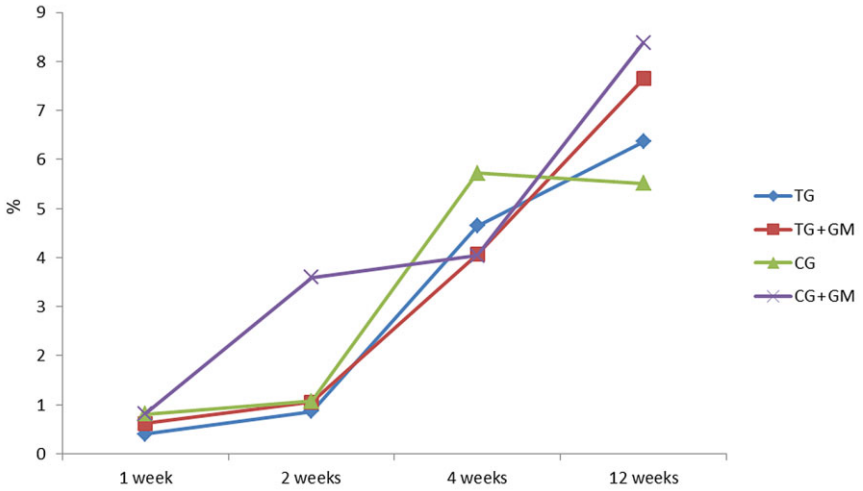

Figure 9 Percentages of the sockets occupied by newly formed bone for the experimental groups over the period of evaluation.

The buccal crest level in all specimens was slightly apical to the corresponding lingual bone crest. All the groups showed new bone formation above the old bone crest level, reducing the differences between the buccal and lingual crests during the healing period. No statistically significant difference was observed between the test groups (thin gingiva, TG and TG + GM) and the control groups (normal gingiva, CG and CG + GM), but the distance between buccal and lingual crests was numerically superior for the test groups, mainly in relation to the new bone. This difference, although it is small, and despite this being an animal study and therefore using a small sample size, may have clinical implications. Both animal[38] and human trials[39–41] that evaluated the influence of thin ridge mucosal tissues on crestal bone stability around dental implants reported more pronounced crestal bone loss in cases with insufficient gingiva. The results of the current experiment suggest that the influence of gingival thickness on bone healing occurs after tooth extraction, independently of implant placement.

No difference between grafted (CG + GM: 0.40 ± 0.28 mm) and nongrafted (CG: 0.64 ± 0.33 mm) sites was observed in our study regarding the distance between the buccal and lingual crests. Similar results have been reported in the literature.[22,42,43] Bashara and colleagues[42] studied the effect of different biomaterials on hard tissue remodeling following their placement into fresh extraction sockets in dogs after 6 months of healing, and reported a similar amount of vertical buccal bone loss in sites treated with bovine bone (Bio-Oss) (0.65 ± 0.40 mm) and sites that healed with a blood clot (0.70 ± 0.35 mm). Rothamel and colleagues,[43] evaluating the alterations in ridge dimensions following application of a nanocrystalline hydroxyapatite paste in fresh extraction sockets in dogs, observed a difference in bone height between buccal and lingual bone of 0.54 ± 0.62 mm in grafted sites and 0.85 ± 0.17 mm in nongrafted sites at 3 months of healing. Suaid and colleagues[22] also reported low bone loss in extraction sockets grafted with biphasic calcium phosphate. The authors reported a buccal bone level of 0.26 ± 0.67 mm apical to the lingual bone in grafted sites and 0.80 ± 1.14 mm apical in the nongrafted sites. On the other hand, Araújo and colleagues,[26] who assessed the effect on bone remodeling following the placement of the same xenograft in fresh extraction sockets in dogs, found a mean vertical distance between the buccal and lingual bone crests of 2.1 ± 0.5 mm at nongrafted sites and 1.9 ± 0.6 mm at grafted sites. These values, higher compared with ours, could be attributed to the flap elevation performed in that study.

The advantageous effects of tooth extraction without the elevation of a mucoperiosteal flap on bone remodeling of extraction sockets were analyzed by Fickl and colleagues[28] in an animal model. The authors demonstrated significantly lower resorption rates in the flapless group. Also, Araújo and colleagues[12] compared hard tissue healing following tooth extraction with and without prior elevation of mucosal full-thickness flaps and found better results for the flapless approach regarding the distance between the cementum/enamel junction of the adjacent tooth and the bone crest (flap: 1 ± 0.1 mm; flapless: 0.7 ± 0.2 mm).

It is important to highlight that although many studies evaluating the effect of the use of a biomaterial graft after tooth extraction on bone remodeling did not show statistically significant differences between grafted and nongrafted sites, most of them reported better numerical values for the grafted groups.[22,26,42,43] Also, recently published systematic reviews of the literature have concluded that socket preservation may aid in reducing the changes in bone dimensions following tooth extraction, although it does not prevent bone resorption, as a loss in width and height will probably occur.[10,44] In the current study this was not observed, probably due to the thin aspect of the buccal bone.

The ARW, measured 1 mm below the highest point of the bone in the socket, was very similar among the groups, approximately 4 mm. Suaid and colleagues,[22] with a similar methodology, reported 5.2 mm and 5.0 mm for grafted and nongrafted sites, respectively. These values, higher than those presented in this study, make it clear that our dogs were smaller and, consequently, with thinner oral tissues.

In a clinical study, Spinato and colleagues[32] observed significant reductions in both crestal height and ridge width after extraction of maxillary anterior teeth with the use of a cancellous mineralized human bone allograft. However, in the group that did not receive the graft material, the sites with thin buccal bone plate (≤ 1 mm) presented more loss in height as well as in width. Additionally, in nongrafted sockets, the amount of bone in the "thick" group was statistically greater than that in the "thin" group, indicating that the thickness of the buccal bone plate markedly influenced new bone regeneration in human sockets 4 months after extraction. Tomasi and colleagues[33] also stated that the thickness of the buccal bone wall influences hard tissue changes during healing.

A comparison of the variously treated sockets with respect to tissue composition (see Table 3) indicated that the process of tissue remodeling progressed according to the same pattern in all the experimental groups. This observation is in agreement with studies made previously with the same biomaterial.[12,13] The similarity in the quantity of mineralized tissue between the groups with and without graft material indicates that at 3 months of healing BOC did not enhance healing and/or stimulate hard tissue formation in the socket, but served as a scaffold for tissue formation during healing. Regarding the new bone area, in our study around 30% of the total bone area consisted of new bone in all experimental sites. Using a similar methodology, Suaid and colleagues[22] observed 15.62% new bone to be in the grafted sites and 22.24% in the nongrafted sites. In this study, higher levels of new bone formation were obtained, which may be due to the thickness of the lingual and buccal bone plates and perhaps to the biomaterial that was used.

Furthermore, in this study, the histological examination revealed the presence of biomaterial, mainly in the coronal portion of the extraction sockets. Araújo and colleagues,[25] evaluating the dynamics of BOC incorporation in fresh extraction wounds, stated that it involves a series of different processes that eventually result in *de novo* bone formation and hard tissue integration of the biomaterial. Bashara and colleagues[42] reported the presence of newly formed bone around particles of an inorganic bovine graft material at 6 months of healing, with little evidence of the inorganic biomaterial being replaced by bone. The same was observed by Araújo and Lindhe,[12] who also observed the presence of graft material in locations outside the bone tissue, such as in the oral mucosa. The authors suggested that during the modeling process of the outer portion of the crestal region, the particles may be dislodged from the newly formed bone. In the current experiment the residual particles occupied 2.63 ± 2.63% (TG + GM) and 1.98 ± 2.48 (CG + GM) of the tissue volume, which is a small amount compared with previous studies. Araújo and colleagues[26] and Araújo and Lindhe[13] utilized the same grafting material and the same healing time and found 12.2% and 8.6% residual graft particles, respectively. It is conceivable that these particles would not have been resorbed in such a short period of time, and the hypothesis of the dislodgment of the particles could be a good explanation for the data observed. Also, it is important to emphasize that the use of a flapless approach could lead to a more pronounced exfoliation of the biomaterial, especially if the sockets were not covered with membranes or connective tissue, as in the present study. The graft material was in contact with the oral environment, and part of it could be lost during the healing process. Another important point to be discussed is that although the biomaterial has enhanced handling characteristics, it is composed of only 10% highly purified porcine collagen; the other 90% consists of bovine cancellous bone granules, which makes its compaction at the bottom of the socket hard to achieve.

Further, the study aimed to use fluorescence analysis to investigate the dynamics of early bone healing in fresh extraction wounds in thin buccal bone filled with BOC. The application of bone markers at different time points permits evaluation of bone formation and remodeling throughout the different stages of healing. Alizarin, calcein green, tetracycline, and calcein blue fluorochromes present different colors and supply sequential information when applied intercalated. The bone markers used in the present study can be compared because they bind to calcium ions by chelation,[45] correctly indicating the areas of active mineralization. The fluorochrome incorporation followed a pattern among the different groups over the period of evaluation from 7 days to 12 weeks. Similarly to the histomorphometric findings, comparison among the groups did not show statistically significant differences in the fluorescence analysis results. Generally, it was observed for all the experimental groups that the area of marked bone was slight in the first week and gradually increased at 2 and 4 weeks, reaching the highest values at the 12-week evaluation. According to Araújo and colleagues,[25] the first steps of bone remodeling in extraction sockets filled with BOC involved the presence of a clot containing large numbers of mesenchymal cells, leukocytes, and vascular structures, followed by the formation of woven bone. In the interval between 2 and 4 weeks, the rate of new bone formation is pronounced. These observations support the results found in the present study that showed an increase of almost four times in the mineralization rate from 2 to 4 weeks, except for the CG + GM. In the sequence of this ongoing process, at 4 weeks the woven bone in such locations is in the process of remodeling, indicating that the mineralization rate could continue to increase in the subsequent weeks. This continuous dynamic is also observed in naturally healed tooth extraction sockets, where there is a pronounced alteration of the tissue within the extraction socket between 4 and 8 weeks.[4] At 8 weeks a cortical ridge is formed, sealing the entrance of the extraction site, and the woven bone is to a large extent replaced by lamellar bone and marrow.[4] The results of the present study corroborate the findings in the literature, showing an increase in the mineralization rate between 4 and 12 weeks.

Also, although no statistically significant difference was observed among the groups regarding the mineralization rate, at 12 weeks it was numerically superior for the grafted groups. The results support the histomorphometric findings of previous studies,[26,46] which stated that the placement of this biomaterial in an extraction socket may modify the modeling process that occurs following tooth removal.

CONCLUSION

Based on these findings, it can be speculated that a naturally thin buccal bone plate leads to higher bone loss in extraction sockets, even with flapless surgery. Reduction in gingival thickness did not result in a significant contribution to bone loss. The use of graft material did not prevent buccal bone resorption in a thin biotype, but modified the mineralization process that occurs following tooth removal.

ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Graduated Personnel (CAPES) for the scholarship that supported Ms. Maia, the State of São Paulo Research Foundation for the financial support of this study (process number 11/00674-6) and Geistlich Pharma AG (Wolhusen, Switzerland) for the donation of the graft material used in this study.

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