Original Article - Prospective Study

Evaluation of inorganic bovine bone graft in periodontal defects after third molar surgery

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ABSTRACT

Aim: This study evaluated the e cacy of inorganic bovine bone graft (IBB) in periodontal defect after mandibular third molar (3M) surgery. **Methods:** The authors conducted a split-mouth, prospective, randomized, blinded, placebo-controlled clinical trial involving 20 participants with a mean age of 21.60 ± 6.5 years who had symmetrical bilateral lower 3M randomly assigned to receive IBB or left empty (blooding clot). The clinical variables studied were probing depth and clinical attachment level (CAL) at preoperative and postoperative periods of 10, 30, and 60 days. Radiographic measures included the distance from the alveolar bone crest to the cementoenamel junction and the bone density at 30 and 60 days postsurgical procedure. For statistical analysis, we used the paired t-test at a level of signi cance of 5%. **Results:** It was observed a reduction in pocket depth and CAL in both groups, but IBB did not provide better results than blooding clot ($P > 0.05$).On the other hand, IBB group showed an increased in the bone density, and a decrease in the periodontal defect on the distal surface of second molar (2M) after 30 and 60 days of surgery compared to the control group ($P < 0.05$). **Conclusion:** The use of inorganic bone graft (GenOx) did not enhance the probing depth after 3M removal. Although the radiographic ndings have showed an increase in bone density and a decrease in the periodontal defect on the distal surface of the 2M, we cannot recommend the use of IBB as a treatment for periodontal defect prevention after 3M removal.

Keywords: Attachment loss, bone density, bovine bone mineral, periodontal defect, third molar

INTRODUCTION

Several longitudinal studies have documented that third molar (3M) removal may result in bone periodontal defects on the distal surface of the adjacent second molar (2M).[1-5] Risk factors associated with bone loss following mandibular 3M removal include age, direction of the eruption, preoperative bone defects, and resorption of the 2M root surface.[5,6] Older patients are more likely to heal slowly, with reduced bone volumes, especially if other factors are present such as localized periodontitis.[2] A consideration when managing impacted teeth is the role of bone reconstruction procedure implemented at the time of 3M removal to eliminate persistent, or prevent the development of new periodontal defects on the distal surface of 2M.[7]

Autogenous bone graft are considered gold standard because of their osteogenic, osteoconductive, and osteoinductive properties,[8-10] but the search for a bone graft substitute continues

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because of some of the disadvantages associated with the use of autogenous bone grafts such as morbidity of donor sites,[11,12] longer surgical time, and higher costs.[13,14] One of the graft materials for this application is the inorganic bovine bone (IBB), a xenograft bone which presents the same chemical and physical properties as human bone.[6] In addition, to act as space filler, IBB may facilitate bone repair as stated by histological analyses, which show newly formed bone in direct contact with IBB particles.[15-17] Controversially, despite these favorable effects on bone, a delay in early bone formation has been attributed to the use of IBB in bone defects.[18,19] Supporting this, a number of *in vitro* studies have demonstrated negative effects of IBB on osteoblast cell adhesion, proliferation, gene expression, and bone matrix formation.[20,21]

To date, most of randomized clinical trials evaluating the therapeutic effect of various reconstructive techniques, such as bone substitutes as IBB mineral (Bio-Oss, Geistlich Biomaterials AG, Wolhusen, Switzerland),[6,22] guided-tissue regeneration,[22,23] soft-tissue procedures, bioactive glass,[24] and platelet rich-plasm,[25] fail to show a clinically significant benefit (improvement in attachment levels) from treatment compared with control or untreated sites.[8-12] Thus, several different treatment strategies have been proposed to decrease the risk for developing periodontal defects following 3M extraction.[1-6,22-25] The aim of this study was to evaluate a lower-cost IBB (GenOx Inorg®, Baumer S.A., Bauru, SP, Brazil) as bone graft substitute to reconstruct the 3M extraction site in order to prevent periodontal defects on the distal surface of 2M.

MATERIALS AND METHODS

Design

Research Ethics Committee/University of Pernambuco approved the study protocol (approval no. 036/10, CAAE 0037.0.097.000-10). We selected the participants from a pool of patients admitted to the Pernambuco School of Dentistry for regular dental treatment from January to November 2014. All participants signed an informed consent form. We conducted a prospective, randomized, blinded, placebo-controlled clinical trial and used a split-mouth design, with the right and left sides of the mouth constituting the experimental units, which we assigned randomly to two treatment groups. Each participant acted as his or her own control, which enhanced the statistical efficiency of the study.[26] We carried out the randomization process on the basis of items eight through 10 of the Consolidated Standards of Reporting Trials Statement 2001 checklist for randomized, controlled, clinical trials. Thus, a site was randomly assigned to receive the graft (IBB) in each subject while the other site received no graft (blooding clot). For each participant, the authors extracted one impacted mandibular 3M on each side of the mouth at different times

We enrolled in our study 20 healthy, nonsmoking patients (9 men and 11 women aged 18–27 years; mean age ± standard deviation [SD]; 21.60 ± 6.5 years) who were scheduled to undergo the surgical removal of bilateral and symmetrically placed impacted mandibular 3M. The participants had no known immune disorders and no contraindications for oral surgery, and they were not taking any medications. We obtained panoramic radiographs [Figure 1] to ensure the similarity of the tooth inclinations on the basis of Winter's[27] classification (mesioangular position) and Pell and Gregory's[28] classification (Class B and position 1).

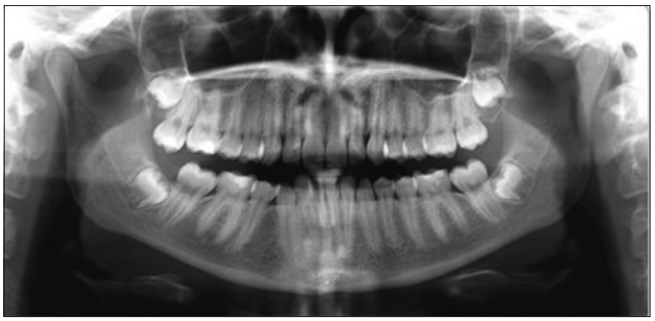

Figure 1: Panoramic radiography meeting the inclusion criteria for split-mouth study

Clinical evaluation and surgical procedures

Probing depth and clinical attachment levels (CALs) were recorded by means of a Marquis periodontal probe (Marquis, Aurora, CO, USA). All measurements were made by the same investigator (periodontist) to minimize measurement errors [Figure 2]. The same oral and maxillofacial surgeon performed all the surgical procedures using the same surgical technique on both sides of the participants' mouths to minimize discrepancies in how the oral tissues were handled. The surgeon performed extraoral antisepsis with a 2% chlorhexidine solution and intraoral antisepsis with a 0.12% chlorhexidine rinse. He administered 2% lidocaine with 1:100,000 epinephrine to anesthetize the inferior alveolar, lingual, and buccal nerves. To perform the surgical procedure, the surgeon used materials and instruments routinely required for this surgery, and he used the standardized surgical technique. An L-shaped incision was made and a mucoperiosteal flap was raised. When the surgeon performed an osteotomy and sectioned the tooth on one side, he did the same on the other side of the patient's mouth to standardize the surgical trauma. The surgeon performed all procedures using abundant irrigation with sterilized 0.9% saline solution. He used a 4-0 silk thread to close the mucoperiosteal flap. The difficulty of the removal procedure was determined based on the four grades of surgical difficulty proposed by Campbell *et al*.:[29] Simple tooth elevation (I), bone removal or tooth division (II), bone removal and tooth division (III), and very difficult bone removal and tooth division (IV). For this study, we considered grade II and III surgeries. We counted the duration of the surgery from when the surgeon made the incision until he removed the tooth. We excluded participants whose surgical procedures exceeded 30 min. When the time to perform the surgical procedure on one side exceeded that on the other side by more than 10 min, we also excluded the participant from the study. One impacted mandibular 3M was removed on the first surgical visit, and the contralateral mandibular 3M was removed on the second surgical visit, which was scheduled for 3 weeks later.

On the control side (blooding clot), using a curved curette, the distal root surface of the control tooth was debrided and the wound closed primarily. For 3M extraction sites randomized to the IBB group, following debridement of the distal aspect of the 2M with a curved curette, IBB was packed into the defect in

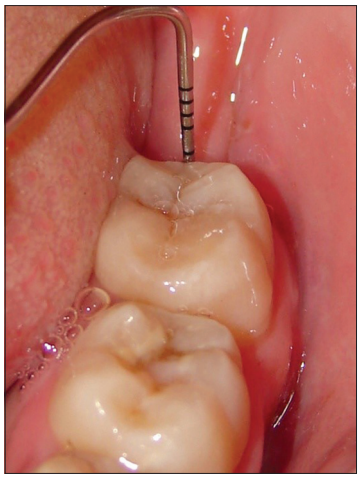

Figure 2: Probing depth with periodontal probe

a stepwise manner until the entire defect was filled to the level of the existing alveolar bone. The wound was closed primarily. Clinical measures for probing depth and CALs were collected for the distal site of each lower 2M at each of the following time points: Preoperative (D0), 10 days after surgery (D10), 1 month after surgery (D30), and 2 months after surgery (D60).

Radiographic evaluation

The subjects were evaluated by using direct digital panoramic radiographs obtained in digital panoramic radiography apparatus CRANEX-D (Soredex, Danaher Corporation, Washington, DC, USA) with proper software acquisition and evaluation. Digital radiographic measures were collected at the following times: 1 month after surgery (D30) and 2 months after surgery (D60).

Radiographs were viewed on a computer monitor using the Digora system (Danaher Corporation, Tuusula, Finland) version 6.0, where it became possible to manipulate the images, changing the brightness and contrast, when necessary, in order to obtain a better visualization and determination points of interest [Figure 3]. Once obtained the ideal image, selected the function for linear and angular, and through the mouse, hooked up with the points of interest in a straight line. Automatically, after calibration of the image, the program provided the measurement in millimeters and tenths of millimeters. Thus, we obtained the linear measurements of left and right. The periodontal defect was established measuring the distance from the cementoenamel junction (CEJ) to the alveolar crest bone level. The density values were obtained by means of digital panoramic radiography using Adobe Photoshop software (Adobe Systems, San Jose, CA, USA).

Then, it was selected a tool for histogram density measurements, and using the mouse, we selected five areas of 441 pixels (0.20 × 0.20), using zoom 60% in order to obtain measurements of all the alveoli, the same being in such selected time intervals for evaluation. If it was necessary to compensate the distortions of panoramic radiography densities, and comparing their values, it was marked in the region of the 2M from the alveoli with 30 and 60 days, and thereafter making this change by using this value as a reference from a rule of three. On conventional radiographs, the measurement of density summed up the extent of browning at some point in the image. However,

Figure 3: Radiographs using the Digora software (Danaher Corporation)

references to time of exposure, development time, degree of deterioration of the radiographic film, processing chemicals, stable power grid, and power of the X-ray tube used, this could affect browning, which can cause measurement errors and wrong conclusions. In the direct digital system, these effects tend to be minimized, being elected easily identifiable reference points on the radiographs from the same patient taken at different times, allowing a comparative reference to their densities between images evaluated. Automatically, the program provided the measure in pixel value (bone density) ranging from 0 (black) to 255 (white). The areas studied were the same in the patient, by group, in which the points were drawn and repeated, resulting in the related measure to the density value.

Statistical analysis

For statics analysis, a normality test (Shapiro–Wilk) was used and revealed a normally distribution of the data. The comparison between groups (IBB vs. control group) on the different periods of evaluation was performed using paired *t*-test with a significance level of 5%. The software SigmaPlot 12.5 (Systat Software, Witzenhausen, Germany) analyzed all data.

RESULTS

Table 1 presents the results of the mean pocket depth and CAL according to the periods of evaluation. Although a reduction in pocket depth and CAL has been observed from D10 in both groups, IBB did not provide better results than a blooding clot.

The mean of bone density in the IBB group observed on D30 and D60 was 141.02 (SD 8.8) and 154.01 (SD 7.02), respectively. In the control group, the mean of bone density was 133.72 (SD 15.53) and 143.02 (SD 13.82) on D30 and D60, respectively. IBB showed an increased in the bone density in both periods of evaluation compared to the blooding clot [Figure 4]. Regarding the postsurgical defect, it was observed that IBB decreased the defect on the distal surface of 2M in both periods of evaluation compared to the blooding clot (D30: 2.25 ± 1.45 vs. 3.74 ± 1.86; D60: 1.51 ± 0.98 vs. 2.53 ± 1.68) [Figure 5].

DISCUSSION

Removal of mandibular 3M may cause periodontal defects on the distal surface of adjacent 2M, especially in patients with

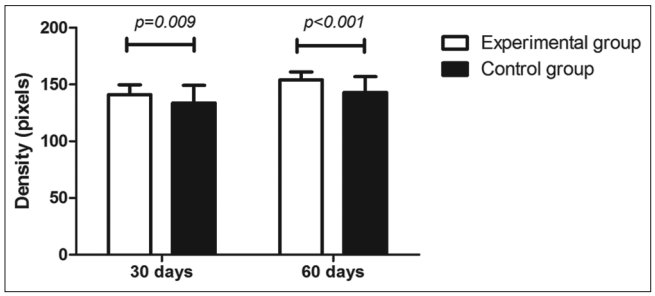

Figure 4: Bone density evaluation between groups at D30 and D60

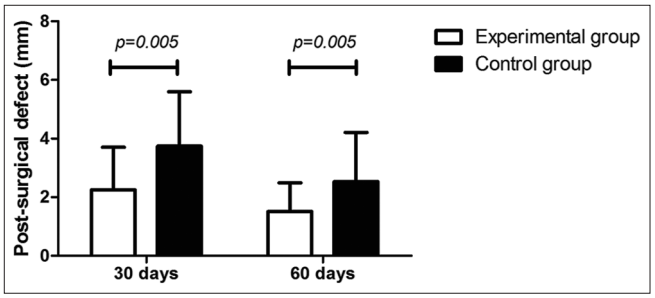

Figure 5: Periodontal bone defect evaluation between groups at D30 and D60

Table 1: Statistics of probing depth and clinical attachment level according to the periods of evaluation and study groups

Variable	Evaluation	Mean±SD (median)		*P*
		Experimental group	Control group	
PD	Baseline	3.49±0.93 (3.33)	3.58±0.85 (3.50)	0.717
	10 days	5.05±1.44 (5.00)	5.53±2.13 (5.83)	0.153
	30 days	3.33±0.77 (3.33)	3.27±0.83 (3.00)	0.746
	60 days	3.08±0.59 (3.00)	3.10±0.65 (2.83)	0.930
CAL	Baseline	1.20±0.68 (1.33)	1.21±0.67 (1.33)	0.966
	10 days	2.60±1.26 (2.33)	3.05±1.80 (3.00)	0.147
	30 days	1.47±0.62 (1.33)	1.47±0.75 (1.33)	1.000
	60 days	1.25±0.64 (1.33)	1.35±0.61 (1.33)	0.532

SD=Standard deviation; PD=Probing depth; CAL=Clinical attachment level

preexisting periodontal disease.[30] In the last years, some studies have been conducted in order to evaluate the efficacy of bone grafts after 3M removal.[1-6,22-25] However, this study is the first trial using the IBB (GenOx) for periodontal defect prevention after 3M removal.

Our results showed that probing depth and CAL decreased from D10 to D60 either using IBB or nothing (blooding clot), but there were no differences between groups. In a study performed by Kugelberg[3] and Kugelberg *et al*.,[5] it was also observed an improvement in probing depth on the distal surface of 2M after 2 and 4 years of follow-up without bone graft. However, in this study, it is possible that the decrease in probing depth can be explained by the reduction of inflammatory edema, resulting in less tissue penetration by the probe, and it does not represent a real improvement of insertion from D10 to D60.

Although we have not found periodontal clinical differences between groups, the radiographic analysis showed that postsurgical defect, measured from alveolar bone crest to CEJ, was reduced using IBB compared to blooding clot in the periods of evaluation D30 and D60. In addition, bone density was also improved in the IBB group similar to that found in previous studies.[22,25,31,32] However, we cannot confirm that exists a more calcified tissue into the 3M defect due to the presence of the bone substitute that was not resorbed in a 1 year follow-up.[20] To verify the quantity and quality of new bone formation, it would require a histological evaluation of the graft, however, according to Sammartino *et al*.,[25] bone biopsy in this region should not be done by the bioethics principles since that a second surgical procedure on the graft area is unnecessary.

In a previous study,[17] we used IBB (Bio-Oss) to coat allograft blocks and proximal areas since it is known that the IBB is a biomaterial slowly resorbed as it is in contact with newly formed[33] and can remain *in situ* for extended periods.[34] Moreover, others[35] demonstrated a reduction of resorption of autogenous grafts by means of Bio-Oss coverage and Morad and Khojasteh[36] showed the effectiveness of IBB in reducing the percentage of absorption of autograft bone augmentation techniques. With this knowledge, we decide to use a lower-cost IBB (GenOx). To date, this is the first report covering the 3M defect with GenOx, which resulted in similar clinical results.

The use of inorganic bone graft (GenOx) did not enhance the probing depth after 3M removal. Although the radiographic findings have showed an increase in bone density and a decrease in the periodontal defect on the distal surface of the 2M, we cannot recommend the use of IBB as a treatment for periodontal defect prevention after 3M removal. In addition, the lack of differences in clinical attachment may be attributed to a lengthened junctional epithelium in the control group.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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