Original Article

Effects of Two Types of Anorganic Bovine Bone on Bone Regeneration: A Histological and Histomorphometric Study of Rabbit Calvaria

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Abstract

Objective: The purpose of this study was to evaluate the efficacy of two types of bone substitutes, Bio-Oss and NuOss, for repair of bone defects.

Materials and Methods: This study was performed on the calvaria of 14 New Zealand rabbits. The 6mm critical size defect (CSD) models of bone regeneration were used. Three CSDs were created in each surgical site. The first defect was filled with NuOss, the second one with Bio-Oss and the third one remained unfilled as the control. After healing periods of one and two months (seven animal for each time point), histological and histomorphometric analyses were carried out to assess the amount of new bone formation, presence of inflammation, foreign body reaction and type of new bone. Qualitative variables were analyzed by multiple comparisons, Wilcoxon, Friedman and Mann Whitney tests. Quantitative variables were analyzed using the Mann-Whitney and Wilcoxon tests. Level of statistical significance was set at 0.05.

Results: The level of inflammation was not significantly different at four and eight weeks in the Bio-Oss (P=0.944), NuOss (P=1.000) and control groups (P=0.71). At four weeks, foreign body reaction was not observed in Bio-Oss, NuOss and control groups.

There was no significant difference in the type of the newly formed bone at four and eight weeks in any group (P=0.141 for Bio-Oss, P=0.06 for NuOss and P=0.389 for the control group).

Conclusion: Deproteinized bovine bone mineral can be used as a scaffold in bone defects to induce bone regeneration.

Keywords: Bone regeneration; Deproteinized; Bovine bone; Bio-Oss; Adequate

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INTRODUCTION

Alveolar bone of adequate quality and quantity is the main prerequisite for placement of dental implants. In case of insufficient bone quantity, the bone volume can be increased by bone augmentation techniques. There are plenty of materials introduced as bone substitutes for use in bone regeneration procedures [1-3].

Autogenous bone graft is known as the gold standard of bone augmentation techniques. It is an osteoinductive substance with highly predictable results [4-6].

On the other hand, because of limitations including the donor site morbidity and limited availability, clinicians may favor other alternatives such as allografts, xenografts or a combination of both. Most of these materials introduced as alternatives to autogenous bone graft are osteoconductive and act as a scaffold for the ingrowth of osteoblasts [7-10].

One of the most commonly used biomaterials for this purpose is a type of xenograft derived from bovine bone. In fact, these materials are natural mineral matrix obtained by extracting the organic materials from bovine bone.

The purpose of this study was to evaluate the efficacy of two types of bone substitutes for repair of experimental defects on rabbit calvaria using histological and histomorphometric analyses.

MATERIALS AND METHODS

Animals:

Fourteen adult (12 months old) white male New Zealand rabbits (Oryctolagus cuniculus), weighing approximately 2.5-3 kg were used in this study. The study protocol was approved by the ethical committee of the Tehran University of Medical Sciences (TUMS) and accepted by the responsible veterinary authority.

Test materials:

In this study, two different xenografts, Bio-Oss and NuOss, were used and compared with one other.

Bio-Oss (Geistlich Biomaterials, Wolhusen, Switzerland) is the mineral part of bovine bone without any organic material, which acts as a scaffold for housing the osteoprogenitor cells. The Bio-Oss used in this study was in the form of granules measuring 0.25-1mm in size. NuOss (ACE Biomaterials, Franklin Lakes, USA), a natural porous bone mineral matrix, is produced by removing all organic components from bovine bone. It is an osteoconductive bone substitute. NuOss particles measuring 0.25-1mm in size were used in this study.

Surgical procedure:

Anesthesia was induced by intramuscular injection of 10% Ketamine (40mg/kg) and 2% Xylazine (5mg/kg) and maintained with isoflurane/02. Following shaving and aseptic preparation of the surgical site, a linear 10cm incision was made on the calvarium and full-thickness flaps were reflected. The 6mm critical size defect (CSD) models of bone regeneration were used in this study [11]. Three CSDs were created in each surgical site using a trephine bur under copious irrigation with sterile saline. In each animal, one defect was filled with NuOss, the other one with Bio-Oss and the third one remained unfilled as the control site. To prevent confusion, each site was numbered and received a code according to their distance from the transverse or sagittal sutures and recorded in a chart.

For recovery and post-operative care, the animals were housed in Animal Experiment Unit of the Faculty of Dentistry of TUMS.

The animals were euthanized (by overdose of 3% pentobarbital sodium) after healing periods of one and two months (seven animals at each time point).

Histological analysis:

After removing the mandible from the calvarium, the samples were immersed in 10% neutral buffered formalin. After fixation, the specimens were immersed in 10% nitric acid for one week. For neutralization, the specimens were stored in 20% lithium bicarbonate for 5 minutes. Then, based on the coding on the charts, the defects were separated, placed in specific cassettes and stored in 10% formalin for 24 hours. The dehydration process was carried out by immersion in 90% ethanol for 24 hours and then the specimens were embedded in paraffin blocks.

Each paraffin block was cut into five sections (3µ-thick). The sections were stained by hematoxylin and eosin (H & E).

Quantitative evaluation (histological and histomorphometric) was performed with a light microscope (Olympus-Bx51, Olympus Co., Tokyo, Japan) equipped with a camera (Olympus- Dp12, Olympus co. Tokyo. Japan) and connected to a personal computer.

The amount of new bone formation and the remnant materials were evaluated by the Magic Wand software (Amazon co. USA, Seattle). The following parameters were assessed.

The presence of inflammation was evaluated according to the number of inflammatory cells in the high power field (400x) of light microscope. By this manner, four scores were considered for the presence and severity of inflammation: Grade one: A few scattered inflammatory cells; Grade two: Focal aggregations and five to 10 inflammatory cells in each group; Grade three: 10 to 50 inflammatory cells in each group and Grade four: More than 50 inflammatory cells in each group.

Foreign body reaction was evaluated by presence of multinucleated giant cells [12].

Based on the position and orientation of collagen fibers in the newly generated bone, the type of new bone was distinguished. In woven bone, the collagen fibers are poorly organized and are irregular in orientation. In contrast, these fibers are parallel and have concentric form in lamellar bone.

Based on the presence of each of these types of newly formed bone, three grades were considered: Grade one: more lamellar bone; Grade two: lamellar and woven bone equal in quantity; and Grade three: a greater portion of the newly formed bone is of woven type.

The presence (or absence) of the intervening connective tissue, by observation under a light microscope (40x), was used to evaluate the contact between the newly formed bone and the biomaterial. The percentage of the newly formed bone is in fact, the percentage of the defect, filled by new bone. To evaluate this parameter, digital images were obtained from the histological sections (40x magnification). Then, using the Histogram (Amazon co. USA, Seattle) and Magic Wand software, the pixels of the newly formed bone were compared with those of the defect. To evaluate the percentage of the remaining biomaterial in the defect, digital images were obtained from the histological sections stained with H & E, and Photoshop 8.0/cs software (Amazon co. USA, Seattle) and Histologic program (Amazon co. USA, Seattle) were used to determinate the percentage of the remaining biomaterial occupying the defect.

Statistical analysis:

SPSS 11.5 was used for statistical analysis. Qualitative variables (inflammation, foreign body reaction, bone formation and its type) were analyzed by multiple comparisons with Wilcoxon, Friedman and Mann-Whitney tests. Quantitative variables (number of inflammatory cells, amount of new bone) were analyzed by Mann-Whitney and Wilcoxon tests. Level of statistical significance was set at 0.05.

RESULTS

According to the Mann Whitney test, the level of inflammation was not significantly different at four and eight weeks in the Bio-Oss (P=0.944), NuOss group (P=1.000) and the control groups (P=0.71). Also, there was no significant reduction in the number of inflammatory cells at four and eight weeks in any group. It means that the severity of inflammation in all groups was similar at four and eight weeks (Table 1).

At four weeks, foreign body reaction was not observed in Bio-Oss, NuOss or the control group. At eight weeks, giant cells were observed in two cases of Bio-Oss group (28.6%) and one case in the control group (14.3%). The presence of giant cells in Bio-Oss and control groups at 8 weeks, based on the Mann-Whitney test, was not statistically significant (P>0.05)(Table 2).

Table 1. The percentage of inflammation in each group

Time / Group	Four weeks					Eight weeks				
	0	**I**	**II**	**III**	**IV**	**0**	**I**	**II**	**III**	**IV**
Bio-Oss	0	57.1 (4)	42.9 (3)	0	0	14.3 (1)	42.9 (3)	28.6 (2)	13.3 (1)	0
NuOss	0	57.1 (4)	42.9 (3)	0	0	0	57.1 (4)	42.9 (3)	0	0
Control	0	100 (7)	0	0	0	14.3 (1)	85.7 (6)	0	0	0

Table 2. The percentage of giant cells in each group

Time / Group	Four weeks		Eight weeks	
	0	**1**	**0**	**1**
Bio-Oss	100 (7)	0	71.4 (5)	28.6 (2)
NuOss	100 (7)	0	100 (7)	0
Control	100 (7)	0	100 (7)	0

Table 3. The percentage and type of newly formed bone in each group

Time / Group	Four weeks				Eight weeks			
	0	**I**	**II**	**III**	**0**	**I**	**II**	**III**
Bio-Oss	0	0	0	100 (7)	0	0	28.6 (2)	71.4 (5)
NuOss	0	0	0	100 (7)	0	0	42.9 (3)	57.1 (4)
Control	0	0	28.6 (2)	71.4 (5)	0	0	57.1 (4)	42.9 (3)

At four weeks, the newly formed bone in Bio-Oss and NuOss groups was type three (woven bone); and type two was observed in only one case in the control group.

At eight weeks, types two and three were both observed in all groups. According to the Friedman test, there was no significant difference among the three groups in bone formation at four weeks (P=0.194) and also at eight weeks (P=0.240).

Based on the Mann-Whitney test, there was no significant difference in the type of the newly formed bone at four and eight weeks in any group (P=0.141 in Bio-Oss, P=0.06 in NuOss and P=0.389 in the control group).

The contact between the biomaterials and the newly formed bone was perfect in both the Bio-Oss and NuOss groups. The amount of newly formed bone in Bio-Oss and NuOss groups was 16.12% and 16.17% at four weeks and 22.91% and 25.5% at eight weeks, respectively. In the control group, the amount of the newly formed bone at four and eight weeks was 13.7% and 18.7% respectively. Intra-group analysis by Mann-Whitney test revealed that in all groups, the amount of the newly formed bone was significantly different at four and eight weeks. It means that the quantity of the new bone at eight weeks was more than that at four weeks in Bio-Oss (P=0.02), NuOss

Table 4. The mean and standard deviation of the percentage of newly formed bone in each group

Time / Group	Four weeks		Eight weeks	
	Mean percentage of new bone	**Standard deviation**	**Mean percentage of new bone**	**Standard deviation**
Bio-Oss	16.1243	1.51002	22.9129	1.09354
NuOss	16.7143	1.38849	25.5171	1.68371
Control	13.2786	1.22931	18.7083	0.76883

Table 5. The mean and standard deviation of the percentage of residual biomaterial

Time / Group	Four weeks		Eight weeks	
	Mean percentage of residual biomaterial	**Standard deviation**	**Mean percentage of residual biomaterial**	**Standard deviation**
Bio-Oss	45.9886	3.76771	36.4857	3.66450
NuOss	48.4471	3.66540	41.1914	3.48467

(P=0.02) and the control groups (P=0.03).
Inter-group analysis by Friedman test revealed that the amount of new bone in Bio-Oss and NuOss groups was significantly more than in the control group at both four and eight weeks (P=0.05 and P=0.01, respectively) (Tables 3 and 4).

The amount of residual biomaterial in the Bio-Oss group was evaluated to be 45.98% at four weeks and 36.48% at eight weeks. This value in NuOss group was 48.44% at four weeks and 41.19% at eights weeks. Intra-group analysis using Mann-Whitney test revealed that the residual biomaterial decreased significantly from four to eight weeks; but this reduction was not statistically significant in the NuOss group (P=0.11). Intergroup analysis by Friedman test showed no significant difference in the amount of the residual biomaterial between the Bio-Oss and the Nu-Oss groups at both four (P=0.54) and eight weeks (P=0.368) (Table 5).

DISCUSSION

In the current study, the inflammatory response in the two test groups was more than in the control group. Also, in all groups the inflammatory reaction at four weeks was greater than at eight weeks. In the control group, one case showed no inflammation. In the NuOss and Bio-Oss groups, most cases showed grade one or two (mild to moderate) inflammatory response. Artzi et al. [14] used Bio-Oss in extraction sites and found some lymphocytes in the sockets. This finding is in line with our study. In another study, by Terries et al, [15] a critical size defect on rabbit calvaria was filled with Bio-Oss and after eight weeks the level of inflammatory reaction was the same as in our study. It seems that two weeks after the surgical trauma to the rabbit calvaria, the acute phase of the inflammatory response subsides and the mononuclear inflammatory cells (chronic ones) migrate to the surgical site; gradually, the inflammatory cells decrease in number.

In Contrast, Piatteli et al, [16] Molly et al, [17] and Degidi et al. [18] did not report the presence of inflammatory cells following the use of Bio-Oss as graft material in their studies. This controversy may be due to the differences in the methodology of studies or samples and also application of different surgical techniques. In our study, after four weeks giant cells were not observed in any case in the three groups. After eight weeks, the giant cells were observed in only two cases of Bio-Oss group. In most previous studies, the foreign body reaction was not reported following the use of Bio-Oss as the graft biomaterial.

Tapety et al. [19] reported the presence of osteoclasts on the Bio-Oss, which was used as the bone substitute in critical size defects in rabbit femur after 14 days. This finding is in line with our study. In contrast, in a study by Noumbissi et al, [20] no foreign body reaction against Bio-Oss was observed in rats after 10 months.

According to kling et al, [21] Bio-Oss can accelerate bone formation and is easily and rapidly replaced with the host bone.

Based on a few other studies, the resorption process of Bio-Oss is very slow and it is never resorbed in some cases [22-24]. Piatelli et al. [25] reported that Bio-Oss can be resorbed by osteoclasts. According to a study by Commack et al, [26] presence of giant cells in the histological sections reveals foreign body reaction against Bio-Oss. On the other hand, Slotte and Lundgren [27] reported that no giant cells were observed in the defects filled with Bio-Oss. It seems that these controversies may be due to the different definitions of the foreign body reactions and different indexes used for this purpose.

In the current study, no foreign body reaction was observed in the NuOss group, which may indicate the biocompatibility of this biomaterial. In our study, there was no significant difference in the type of the newly formed bone between groups after four weeks and in all cases, the type of the newly formed bone was

grade three and entirely woven bone, except for one case in the control group which showed grade two bone. After eight weeks in the Bio-Oss group, 71.4% of cases were grade three and 28.6% were grade two; but in NuOss group, 57.1% of cases were grade three and 42.9% were grade two.

Oh et al. [28] reported that the type of the newly formed bone in an experimental defect around an implant in dog, using Bio-guide membrane, was woven after four weeks and lamellar after eight weeks.

In another study by Cormagnola et al. [29] it was reported that after four months the type of the new bone in the extraction site, following the use of Bio-Oss and membrane was grade two. The type and quality of the newly formed bone, significantly depends on the duration of the healing process. It means that in studies with longer durations, more lamellar bone may be observed in histological sections [20]. The current study showed that there was a direct contact between the biomaterial and the new bone in 100% of the cases of Bio-Oss and NuOss groups. In our study, there was no significant difference in the quantity of the newly formed bone between the Bio-Oss and NuOss groups. This finding shows that the conductivity potential of these two biomaterials is the same and this is in line with other studies [30-34]. In the current study, the amount of residual bone graft material at four and eight weeks was 45.98% and 36.98% in Bio-Oss group, respectively. These values were 48.44% and 41.19% in NuOss group, respectively. In another study by Terrace [35], the same results were obtained. Lindhe et al. [36] reported that Bio-Oss particles resorbed and decreased over time.

CONCLUSION

Our study showed that deproteinized bovine bone mineral can be used as a scaffold in bone defects to induce bone regeneration.

The NuOss and Bio-Oss show similar physicochemical characteristics.

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