Dental Research Journal

Original Article

Comparison of bone regeneration in rabbit calvarial defects following the use of two different xenografts with a barrier membrane: A histological, histomorphometric, and micro-computed tomography study

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ABSTRACT

Background: This study compared bone regeneration in rabbit calvarial defects using two different xenografts with a barrier membrane by histological, histomorphometric, and micro-computed tomography (micro-CT) analyses.

Materials and Methods: This study was designed as a single-blind interventional experimental animal study that conducted on 20 male New Zealand rabbits. The rabbits were anesthetized, a full-thickness mucoperiosteal flap was elevated, and four bone defects with 8 mm diameter were created in their calvaria at the two sides of their midsagittal suture. Two defects were randomly filled with Bio-Oss and NOVA xenografts. The other two defects remained empty. Except for one control defect, the remaining three were covered with a resorbable membrane. Ten rabbits were sacrificed after 4 and the remaining 10 after 8 weeks. Histological sections underwent micro-CT and histological assessments for new bone formation, bone maturation, residual particles, degree of inflammation, foreign body reaction, and soft-tissue formation.

Results: The percentage of new bone formation increased at 8 weeks compared with 4 weeks and had a similar trend in Bio-Oss and NOVA groups ($P > 0.05$). The percentage of new bone formation was slightly higher in defects with membrane at 8 weeks ($P > 0.05$). The newly formed bone was a combination of woven and lamellar bone in all groups ($P > 0.05$). Micro-CT showed no significant difference in bone microstructure. The difference in other parameters was not significant among the groups either ($P > 0.05$).

Conclusion: Considering the similar rate of new bone formation and residual particles, both Bio-Oss and NOVA may be successfully used as scaffold with a barrier membrane for regeneration of bone defects.

Key Words: Bio-Oss, bone regeneration, heterografts, rabbits

Received: 05-Mar-2025
Revised: 06-Dec-2025
Accepted: 27-Dec-2025
Published: 07-Sep-2026

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INTRODUCTION

Dental implant treatment is the main option for replacement of the lost teeth. Several complementary

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480
DOI: 10.4103/drj.drj_120_25

How to cite this article: Tamizi M, Birang R, Abed AM, Razavi SM. Comparison of bone regeneration in rabbit calvarial defects following the use of two different xenografts with a barrier membrane: A histological, histomorphometric, and micro-computed tomography study. Dent Res J 2026;23:31.

procedures have been proposed for augmentation of atrophic alveolar bone to correct the anatomical limitations before implant placement. These procedures include autogenous bone block grafting, sinus floor augmentation, guided bone regeneration, and alveolar ridge preservation.[1] Bone grafts can be harvested from the host (autograft), another human being (allograft), or animal species (xenograft), or can be synthetic (alloplast).[1,2] Autogenous bone from intraoral or extraoral sources is the gold standard for bone grafting due to its excellent osteoconductive, osteoinductive, and osteogenic properties.[1,3,4] However, limited availability, the need for an additional surgical site, donor site morbidity, unpredictable resorption, and prolonged surgical time are among the drawbacks of autogenous bone grafting.[1,3,5] Considerable resorption of autogenous bone graft also has a negative impact on the final results.[6]

Due to the increased demand for minimally invasive implant procedures, several bone graft materials were introduced to the market for application as monotherapy or in combination with autogenous bone particles.[1] Xenografts, also known as xenogeneic or heterologous graft, are a biocompatible alternative for bone regeneration with osteoconductive properties.[1,7] Particulate bovine bone devoid of organic compounds has been extensively used in human studies for regeneration of bone defects and shown successful results. Many xenografts have the potential to be resorbed and replaced with host bone over time.[8] Inorganic bovine bone has a hydroxyapatite structure and preserves the micropores and macropores present in cancellous and cortical bone after removal of its organic content (by extraction processes using low heat and chemical agents). In the past, bovine xenografts did not yield optimal results due to graft rejection probably because of the chemical process of protein extraction with detergents, which would result in some residual protein and subsequent adverse reactions.[9] Elimination of protein components from the xenograft decreases the risk of immune reactions and disease transmission.[1]

Theoretically, xenogeneic bone graft can serve as an unlimited and available source of graft material if processed properly to ensure safe application in humans. However, xenografts, similar to allografts, lose their osteogenic and partly osteoinductive properties in their preparation process.[7] Protein-free bovine bone is commonly used for maxillary sinus and alveolar ridge augmentation. It has been claimed that it has a slow resorption rate and is suitable for use in areas where minimal resorption is required. Moreover, xenogeneic bone has excellent osteoinductive property, which results in revascularization. Given that autogenous bone is also added to xenogeneic bone, the mixture would have osteoinductive property, which would enhance osteogenesis.[10]

Bio-Oss is bovine bone devoid of sterilized protein, which is known as the gold standard biomaterial.[11,12] It has undergone low heat (300°C) chemical extraction for the elimination of all its organic content; however, its natural bone structure is preserved.[13] It has 75%–80% porosity, its crystals are approximately 10 nm in size and are in the form of cortical or cancellous granules. The size of its internal micropores is similar to that of natural cancellous bone, and no local inflammatory reaction by B- or T-cells has been reported following the use of this inorganic material. It appears that Bio-Oss is superior to hydroxyapatite in terms of replacement with the host bone when used for alveolar ridge augmentation. Furthermore, Bio-Oss undergoes physiological remodelling and is integrated within the host bone. It is highly biocompatible and possesses all the properties of an osteoconductive biomaterial.[11] Klinge *et al*.[14] applied Bio-Oss in artificial defects created in rabbit calvaria and showed that the size of macropores present in the morphology of this biomaterial was comparable to the size of macropores in natural cancellous bone, and it can serve as an ideal scaffold for new bone formation.

The NOVA Bone+B xenograft manufactured in Iran is a bovine bone that has undergone thermal processes at 650°C for removal of its organic content. It has 300-µm interporosities and 100–300-µm intra-porosities; 20%–30% of its particles are 250–500 µm in size, 30% are 500–700 µm in size, and 40% are 700–1000 µm in size.

Histological analyses show that xenografts have a bone bridging pattern. Residual xenograft particles are partially surrounded by the newly formed bone and adhere to the adjacent particles through this mechanism.[15] Young *et al*.[6] evaluated the efficacy of anorganic xenogeneic bone with autogenous bone particles for regeneration of maxillary and mandibular defects in rabbits. They showed that autogenous bone was actively resorbed by multinucleated cells and new bone formed close to the particles. In contrast, xenogeneic bone underwent less resorption. Newly formed bone was noted adjacent to xenogeneic bone particles and there was no evidence of resorption.[6]

Critical size defects are defined as the smallest bone defects in an animal model which would not heal spontaneously if left untreated for a certain period of time, or <10% of bone regeneration would occur in them during the animal's life span. Critical size defects are commonly used as a model for the assessment of bone regenerative potential of new biomaterials.[16] Defects are often created in the tibia, radius, mandible, or cranium of animals; among which, cranial defects can highly simulate mandibular defects in humans since they are not under tension and have limited blood supply and bone marrow.[16]

Micro-computed tomography (micro-CT) can be used for nondestructive assessment of bone trabeculae and can provide information regarding the trabecular number, thickness, and separation and the overall volume of bone and soft tissue. Its nondestructive nature and high number of cuts that yield more precise data are among the main advantages of micro-CT.[17]

Considering the recent production of NOVA xenograft in Iran, this study aimed to compare bone regeneration in rabbit calvarial defects following the use of Bio-Oss and NOVA xenografts with a barrier membrane by histological, histomorphometric, and micro-CT analyses.

MATERIALS AND METHODS

This animal study was conducted in accordance with the guidelines for the care and use of laboratory animals (IR.MUI.RESEARCH.REC.1400.511) The study was conducted on 20 skeletally mature male New Zealand rabbits weighing 2.5–3 kg. The rabbits were allowed 14 days for the purpose of acclimation. They were kept in separate cages under standard laboratory conditions in terms of lighting and humidity at 12°C–21°C temperature with *ad libitum* access to food and water.

Intervention

The rabbits were anesthetized by intramuscular injection of 45 mg/kg ketamine hydrochloride (Alfasan, Worden, Holland), 1 mg/kg acepromazine (NEUROTRANQ, Alfasan, Woerden, Holland), and 5 mg/kg xylazine (Vet-Agro, Melgiewska, Poland). The rabbits were then intubated and anesthesia was maintained by the administration of isoflurane. Local anesthesia was also induced by injection of lidocaine hydrochloride. The calvarial area was shaved and disinfected with povidone iodine. An incision was made along the midsagittal suture from the frontal to the occipital bone. A full-thickness mucoperiosteal flap was elevated to expose the calvaria. Four defects with 8-mm diameter[16] were then created at the two sides of the midsagittal suture by a trephine bur under copious saline irrigation. The defects were thoroughly rinsed to eliminate residual bone particles. The defects were then randomly assigned to four groups and coded A, B, C, and D. The first defect was filled with Bio-Oss (Geistlich Bio-Oss, spongious bone substitute, granules 0.25–1 mm, Geistlich Pharma AG, Wolhusen, Switzerland) and the second defect was filled with NOVA xenograft (NOVA Bone+B spongious, granules 0.25–1 mm, NOVA Teb Pars, Mazandaran, Iran). The third and fourth defects remained empty to fill with blood clot. The first, second, and third defects were covered with abovine-derived resorbable membrane made from Achilles tendon (NOVA Teb Pars, Mazandaran, Iran) as the control positive group. The fourth empty defect served as the control negative group and did not receive a membrane [Figure 1] as the control negative group. The flap was returned and sutured layer by layer. The periosteum was sutured with polyglycolic acid 4-0 suture (19 mm; SUPA Medical devices, Tehran, Iran) and the skin was sutured with nonabsorbable nylon suture (monofilament polyamide 5-0, 16 mm, SUPA Medical devices,

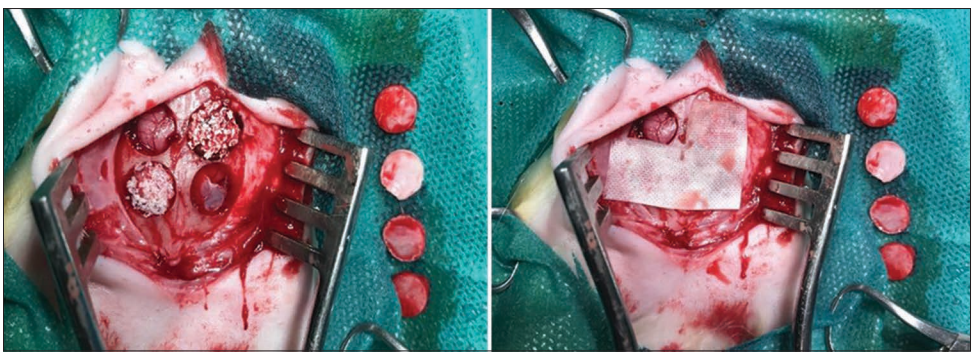

Figure 1: Application of biomaterials (left) and membrane (right).

Tehran, Iran). The surgical site was disinfected with oxytetracycline antibiotic spray (Darudarmanco, Tehran, Iran). Meloxicam injectable analgesic solution (Rooyan Daroo, Tehran, Iran) was injected subcutaneously perioperatively and for up to 5 days postoperatively at a dosage of 1 mg/kg every 24 h. Moreover, 5 mg/kg ceftriaxone (Ceftrax, Jaberebne Haian Pharmaceutical MfgCO, Tehran, Iran) was injected intramuscularly. Nylon sutures were removed after 10–14 days.

As explained above, four defects were created in each rabbit calvaria, yielding a total of 80 defects in 20 rabbits. Ten rabbits were randomly selected and sacrificed after 4 weeks and the remaining 10 after 8 weeks[1,15,16,18] by ketamine, acepromazine, and xylazine overdose followed by isoflurane overdose. Accordingly, 10 defects were evaluated in each group at each time point.

Histological, histomorphometric, and micro-computed tomography assessments

The defect site and adjacent bone were harvested *en bloc* for micro CT and histologic evaluation to assess the amount of new bone formation, residual particles, degree and type of inflammation, foreign body reaction, and the amount of newly formed soft tissue.

Micro-computed tomography

To prepare the specimens for micro-CT, 6 specimens (3 specimens from the 4-week and 3 from the 8-week group) were immersed in 10% formalin and sent to Tehran University of Medical Sciences Preclinical Core Facility. In this study, we used an *in vivo* X-ray micro-CT scanner (LOTUS inVivo, Behin Negareh Co., Tehran, Iran) at the preclinical core facility based at Tehran University of Medical Sciences. LOTUS-inVivo has a cone beam micro-focus X-ray source and a flat panel detector. In order to obtain best possible image quality, the X-ray tube voltage and its current were set to 40 kV and 150 μA, respectively, and frame exposure time set to 2 s ×2. Total scan duration was 49 min. Slice thicknesses of reconstructed images were set to 30 micrometers. All the protocol settings process was controlled by LOTUS-inVivo-ACQ software. The acquired 3D data were reconstructed using LOTUS inVivo-REC by a standard Feldkamp, Davis, Kress algorithm. Furthermore, LOTUS NDT-3D was used for rendering of reconstructed images and by adding Bone Analysis Plugin inside the software we reported bone parameters.

The following variables were assessed on micro-CT images and reported:

- The ratio of newly formed bone volume to the entire volume of the respective defect site in percentage (mm^3)
- The ratio of newly formed soft tissue to the entire volume of the respective defect site in percentage (mm^3)
- The mean trabecular number per each unit of length (1/mm)
- The mean trabecular thickness (mm)
- The mean trabecular separation (mm).

Histological and histomorphometric analyses

After removal of bone blocks from 10% formaldehyde, they were immersed in 10% formic acid for decalcification. Acid was refreshed everyday and the degree of decalcification was assessed on a daily basis. After the completion of decalcification, the specimens were sectioned into multiple slices at the largest diameter of the defect and were then immersed in alcohol for dehydration. Next, they were mounted in paraffin blocks and sectioned into 4–5-μm slices. Next, they were stained with hematoxylin and eosin.

A minimum of four sections were obtained from each specimen and were evaluated by a pathologist blinded to the group allocation of specimens using a light microscope (E400; Nikon, Japan). Photographs were obtained from each specimen at ×40 magnification and were inspected by a software program to assess the following parameters:

Type of inflammation: acute or chronic

Severity of inflammation: which was classified into four categories based on the rate of infiltration of lymphocytes:[6]

i. No inflammation: <10 inflammatory cells or <10% lymphocytic infiltration
ii. Mild inflammation: Between 11 and 25 inflammatory cells or 10%–30% lymphocytic infiltration
iii. Moderate inflammation: Between 26 and 50 inflammatory cells or 30%–50% lymphocytic infiltration
iv. Severe inflammation: More than 50 inflammatory cells or over 50% lymphocytic infiltration.

Foreign body reaction: Presence of giant cells

Amount of newly formed bone was calculated by the software and reported as percentage.

Amount of residual particles was calculated by the software and reported as percentage.

Amount of newly formed soft tissue was calculated by the software and reported as percentage.

Type of newly formed bone: Woven or lamellar

Statistical analysis

Normal distribution of data was evaluated by the Shapiro–Wilk test. The variables were compared among the groups by generalized linear model, Chi-square test, and Fisher's exact test using SPSS version 22 (IBM, SPSS Inc. Armonk, New York, USA) at 0.05 level of significance [Figures 2-5].

RESULTS

Results of histological and histomorphometric analyses

Table 1 presents the results of histological and histomorphometric analyses. As shown, no significant difference was noted among the groups in the overall percentage of new bone formation ($P > 0.05$), mean percentage of woven bone formation ($P > 0.05$), mean percentage of lamellar bone formation ($P > 0.05$), mean percentage of newly formed soft tissue ($P > 0.05$),

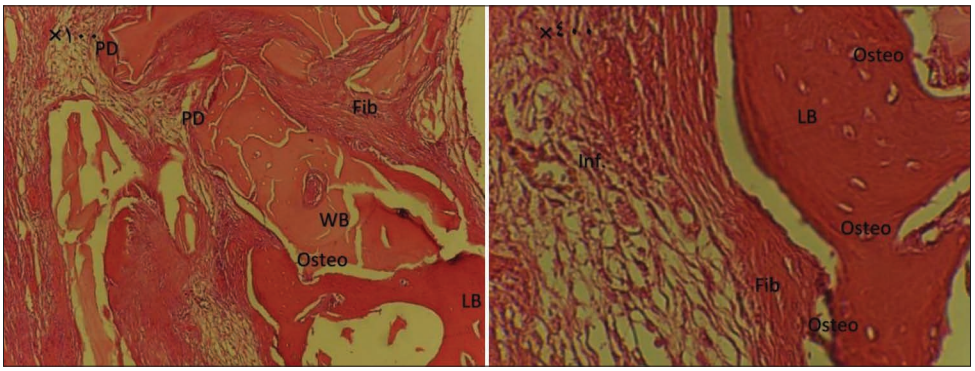

Figure 2: Photomicrograph of NOVA Bone group (H and E, ×100 and × 400).

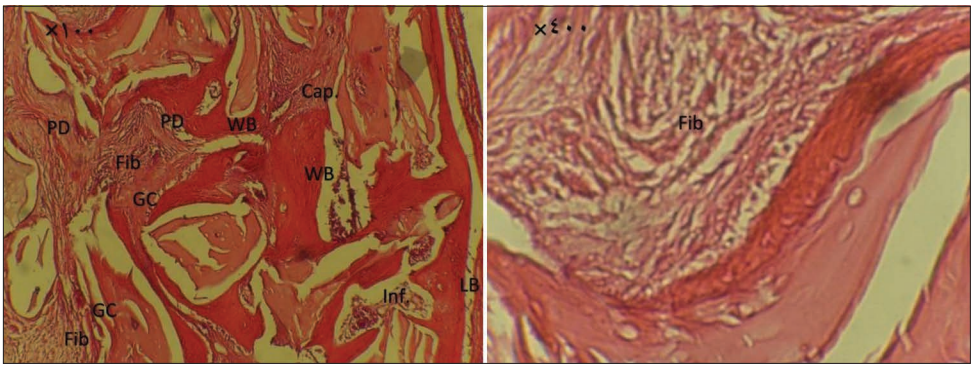

Figure 3: Photomicrograph of Bio-Oss group (H and E, ×100 and × 400).

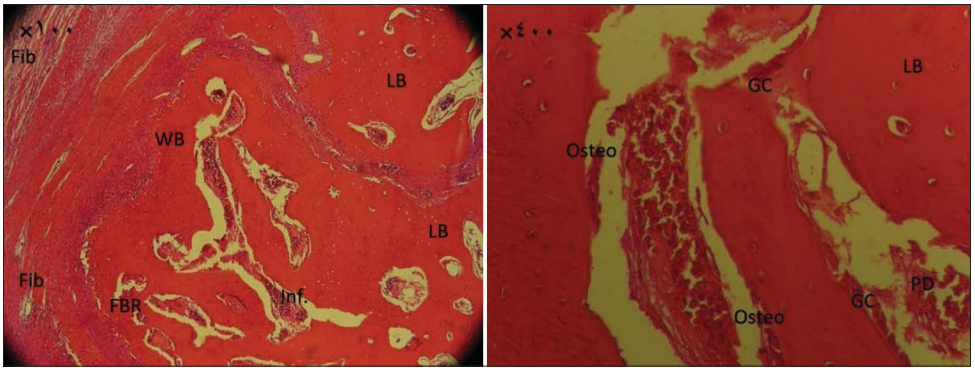

Figure 4: Photomicrograph of membrane group (H and E, ×100 and × 400).

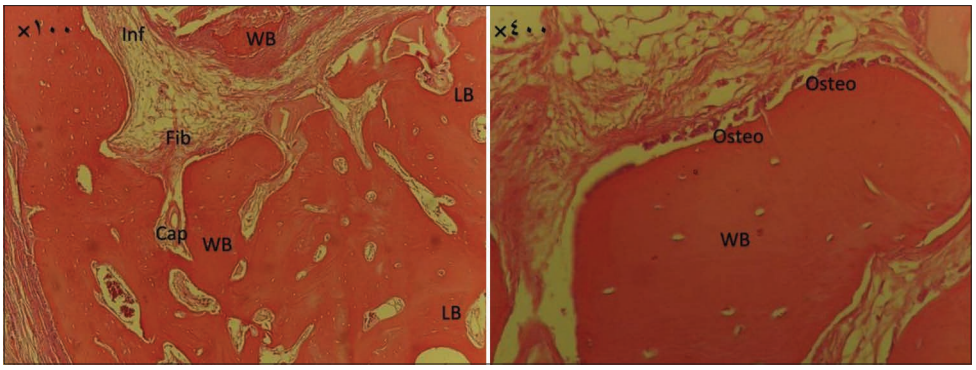

Figure 5: Photomicrograph of the control group (H and E, ×100 and × 400). WB: Woven bone, LB: Lamellar bone, Fib: Fibrous tissue), FBR: Foreign-body reaction, GC: Giant cell, Osteo: Osteoblast, Cap: Capillary, Inf: Inflammation, PD: Particle deposite.

Table 1: Results of histological and histomorphometric analyses

Time	Variable	Group				*P*
		Control	NOVA	Membrane alone	Bio-Oss	
4 weeks	Overall percentage of newly formed bone[a]	45.5±6.57	43.1±5.65	39.1±8.21	43.7±4.22	0.276
	Percentage of newly formed woven bone[b]	24.5±7.98	20.3±4.00	18.3±6.11	20.3±3.56	0.176
	Percentage of newly formed lamellar bone[c]	24.4±3.95	23±4.14	20.8±3.19	23.4±3.95	0.267
	Percentage of newly formed soft tissue[d]	26.9±4.65	24.2±6.12	26.2±3.49	26.3±4.45	0.533
	Percentage of residual particles[e]	-	31.0±6.93	29.8±6.87	29.8±7.55	0.952[#]
8 weeks	Overall percentage of newly formed bone[a]	43.7±9.82	45.1±8.01	46.8±7.24	45.2±8.81	0.401
	Percentage of newly formed woven bone[b]	22.7±5.78	24.7±7.64	25.7±6.98	23.1±7.37	0.719
	Percentage of newly formed lamellar bone[c]	21.0±6.12	22.0±4.44	24.4±3.05	22.2±3.67	0.352
	Percentage of newly formed soft tissue[d]	28.7±6.32	26.3±5.59	26.4±4.10	26.2±4.02	0.663
	Percentage of residual particles[e]	-	27.5±6.91	24.4±7.14	26.8±7.77	0.716[#]
P value for comparison of each group at 4 and 8 week		a: 0.594	a: 0.256	a: 0.002*	a: 0.626	-
		b: 0.531	b: 0.104	b: 0.007*	b: 0.307	
		c: 0.059	c: 0.578	c: 0.043*	c: 0.513	
		d: 0.065	d: 0.815	d: 0.207	d: 0.427	
		e: 0.354	e: 0.354	e: 0.025*	e: 0.289	

*Data are presented as mean±SD; **P*<0.05 was considered significant; [#]Generalized linear model with gamma distribution and logarithmic function; a: Total percentage of newly formed bone; b: Total percentage of woven bone; c: Total percentage of lamellar bone; d: Total percentage of soft tissue; e: Total percentage of particle deposites. SD: Standard deviation

or mean percentage of residual particles ($P > 0.05$) neither at 4 nor at 8 weeks. The percentage of new bone formation significantly increased at 8 weeks compared with 4 weeks in all groups ($P < 0.05$). The amount of residual particles significantly decreased at 8 weeks compared with 4 weeks in both Bio-Oss and NOVA groups ($P < 0.05$).

No significant difference existed in the frequency of types of inflammation among the four groups neither at 4 ($P = 0.980$) nor at 8 ($P = 0.990$) weeks according to the Fisher's exact test. Only one rabbit in each of the four groups showed severity of inflammation of 4 at 4 weeks.

No significant difference existed in the frequency of foreign-body reaction among the four groups neither at 4 ($P = 0.280$) nor at 8 ($P = 0.990$) weeks according to the Fisher's exact test.

Results of micro-computed tomography

Table 2 presents the results of micro-CT in the four groups at 4 and 8 weeks. As shown, no significant difference was noted among the four groups in the mean volume of newly formed bone to the entire defect volume ($P > 0.05$), mean volume of the newly formed soft tissue to the entire defect volume ($P > 0.05$), mean amount of residual graft particles ($P > 0.05$), trabecular thickness ($P > 0.05$), trabecular number ($P > 0.05$), or the mean trabecular separation ($P > 0.05$) neither at 4 nor at 8 weeks [Figure 6].

Interaction effect of time and group on histological and histomorphometric results

No interaction effect was noted between the time of assessment and group ($P > 0.05$).

Table 2: Results of micro-computed tomography in the four groups at 4 and 8 weeks

Time	Variable	Group				*P*
		Control	NOVA	Membrane alone	Bio-Oss	
4 weeks	Volume of newly formed bone to the volume of entire defect[a]	76±5.28	7.1±6.68	7.8±9.56	13.0±7.14	0.400
	Volume of soft tissue to the volume of entire defect[b]	89.9±9.93	85.8±7.97	89.2±14.27	79.9±15.25	0.479[#]
	Residual graft particles[c]	-	3.3±3.32	1.7±2.91	1.3±1.69	0.832[#]
	Trabecular thickness[d]	0.15±0.04	0.24±0.05	0.21±0.04	0.26±0.06	0.211[#]
	Trabecular number[e]	0.36±0.31	0.51±0.44	0.47±0.64	0.54±0.34	0.877
	Trabecular separation[f]	4.9±4.13	4.7±6.06	2.1±8.09	2.7±2.71	0.375[#]
8 weeks	Volume of newly formed bone to the volume of entire defect[a]	9.5±4.77	13.6±5.24	13.7±2.92	13.5±1.15	0.537
	Volume of soft tissue to the volume of entire defect[b]	87.8±6.34	80.2±7.92	82.3±6.21	74.9±5.48	0.698[#]
	Residual graft particles[c]	-	1.7±1.45	1.1±1.52	1.3±1.55	0.919[#]
	Trabecular thickness[d]	0.25±0.05	0.34±0.12	0.33±0.10	0.36±0.13	0.069[#]
	Trabecular number[e]	0.26±0.12	0.52±0.26	0.44±0.16	0.57±0.36	0.610[#]
	Trabecular separation[f]	3.8±1.55	1.9±1.26	2.1±0.67	2.3±1.31	0.741[#]
P value for comparison of each group at 4 and 8 weeks		a: 0.626	a: 0.0.98	a: 0.143	a: 0.370	-
		b: 0.305	b: 0.400	b: 0.313	b: 0.450	
		c: -	c: 0.539	c: 0.518	c: 0.983	
		d: 0.027*	d: 0.068	d: 0.025*	d: 0.682	
		e: 0.710	e: 0.812	e: 0.891	e: 0.920	
		f: 0.731	f: 0.167	f: 0.085	f: 0.803	

*Data are presented as mean±SD; *P<0.05 was considered significant; [#]Generalized linear model with gamma distribution and logarithmic function; a: percentage(%); b: percentage (%); c: percentage (%); d: (mm); e: Number of trabeculae (1/mm); f: (mm). SD: Standard deviation

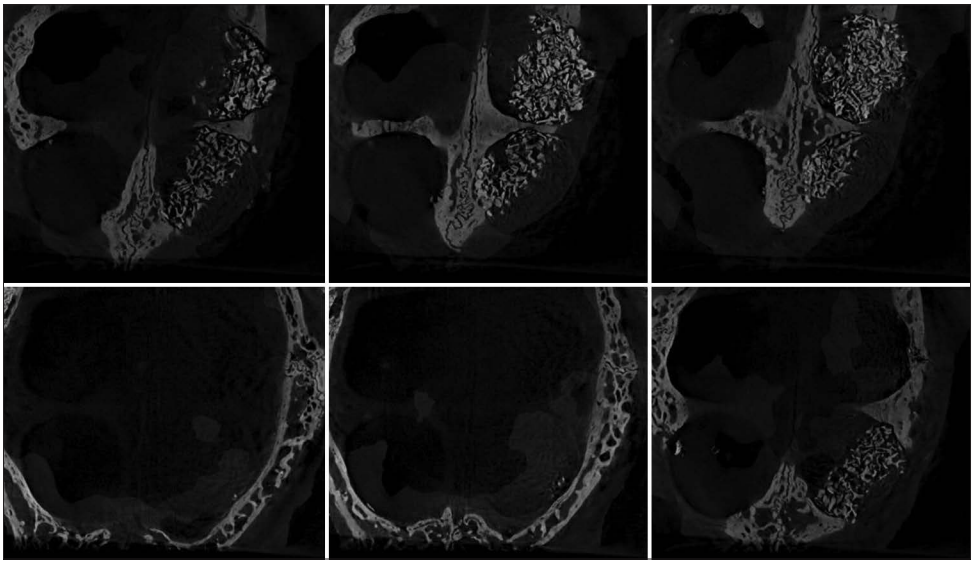

Figure 6: Axial section of micro-computed tomography images prior to reformatting in three-dimensional reconstruction software, we can see four defects. Two of them filled with bone material, one of them show the membrane remnants and the last one is the control group.

DISCUSSION

This study compared bone regeneration in rabbit calvarial defects following the use of Bio-Oss and NOVA xenografts with a barrier membrane by histological, histomorphometric, and micro-CT analyses. Selection of rabbits for this study was because of the fact that they are commonly used for animal studies due to easy handling, high rate of bone turnover, and complete maturation within 6 months.[16] Rabbit calvaria is large enough to accommodate several critical size defects for the comparison of different biomaterials. Since the embryonic process of development of both alveolar bone and calvaria vault are through intramembranous bone formation, calvaria of the rabbit was selected as the experimental site to make a more proper comparison.[19] The defects were assessed at 4 and 8 weeks in the present study because

according to Frost,[18] 8 weeks in rats equals 24 weeks in humans. Thus, assessment of histological and histomorphometric results at 8 weeks in rabbits can correspond to the time suggested for implant placement after bone augmentation procedures in humans.[18]

The present results showed that at 4 weeks (primary healing phase), the measured histological and histomorphometric parameters including the mean percentage of newly formed bone, the mean percentage of woven bone, and the mean percentage of lamellar bone were slightly, but not significantly, higher in the control group than other groups. This finding highlights the innate healing capacity of defect margins and periosteum.[16] It is possible that application of biomaterial and barrier membrane in the other three groups interfered with the innate healing potential and delayed osteogenesis. This finding was in line with the results of Jensen *et al*.[4]

At 8 weeks, the present results showed an increase in the percentage of new bone formation and woven and lamellar bone in the three groups with membrane, compared with the control group, although the difference among the four groups did not reach statistical significance in any parameter. An interesting finding was greater new bone formation in the membrane group compared with Bio-Oss and NOVA groups (although insignificant). This finding highlights the optimal efficacy of this membrane in preserving the blood clot in the defect and subsequent enhancement of osteogenesis. Also, higher new bone volume in this group can be due to the presence of residual biomaterial granules in the other two experimental groups, occupying part of the defect volume, and resulting in lower volume of newly formed bone. This finding was in line with the results of Jensen *et al*.[4] Defects grafted with bone material demonstrated significantly less new bone formation than those with membrane or control group. This is in accordance with the fact that the xenogenic bone material is stable and it is not truly resorbable. The xenogenic bone material particles due to their osteoconductive properties create a dense network for bone formation. The total fraction of mineralized tissue (graft + bone), however, was higher than the control positive and control negative groups.[4]

Comparison of NOVA and Bio-Oss groups revealed comparable rate of osteogenesis with no significant difference between them, highlighting the acceptable efficacy of NOVA for regeneration of bone defects in rabbit calvaria.

Histological assessments revealed that the newly formed bone at both 4 and 8 weeks was a combination of woven and lamellar bone and the difference in the mean percentage of each type of bone was not significant among the four groups at any time point. This result was in line with the findings of Paknejad *et al*.,[20] who compared Bio-Oss and NuOss bovine xenografts for regeneration of 6-mm bone defects in rabbit calvaria.

Micro-CT assessment was also performed in the present study since a combination of micro-CT and histological observations can provide comprehensive information about the regenerated bone and enables better assessment of the outcome at the defect site.[21] Micro-CT findings in the present study revealed superior results in the three experimental groups, compared with the control group. The amount of newly formed bone significantly increased at 8 weeks compared with 4 weeks in the three experimental groups. Also, the mean trabecular number and thickness at both 4 and 8 weeks were higher in Bio-Oss followed by NOVA group while trabecular separation was higher in the control group. Although none of these differences were statistically significant, they indicate that both Bio-Oss and NOVA served as osteoconductive scaffolds in bone defects, enabling greater bone formation, and higher trabecular number and thickness three-dimensionally, compared with the control group. Slightly superior performance of Bio-Oss to NOVA in osteogenesis can be attributed to their different structure, which is influenced by the manufacturing process.[22]

Four important structural parameters that affect osteoconduction, healing process, and bone formation include pore size of xenograft particles, grain size, surface morphology, and crystallinity of xenograft material. All these parameters are affected by the processing method, and the applied heat. Differences in these parameters can probably explain the relative superiority of Bio-Oss to NOVA in the present study.[23]

In the current study, the mean residual particles in Bio-Oss and NOVA groups significantly decreased at 8 weeks compared with 4 weeks. The amount of residual granules in NOVA group was slightly higher than that in Bio-Oss group; although this difference did not reach statistical significance. This

finding indicates slower resorption rate of NOVA. It is believed that resorption or biodegradability of a xenograft is correlated with its calcium phosphate structure. If crystallinity and density of the processed xenograft increase by the processing method, its resorption decelerates. The degree of heat applied in the manufacturing process of xenografts determines their crystallinity. Higher temperatures increase crystallinity of xenografts, and decelerate their resorption.[22] Ramírez Fernández *et al*.[24] demonstrated that a xenograft treated at lower temperatures had a faster resorption rate while a xenograft treated at a higher temperature had a slower resorption rate due to having higher crystallinity and density. Comparison of the synthesis of Bio-Oss and NOVA revealed that Bio-Oss is manufactured at a lower temperature (300°C) while NOVA is fabricated at a higher temperature (650°C). Thus, NOVA probably has higher crystallinity and density, which explains its slower resorption rate compared with Bio-Oss. Also, in the membrane group, parts of the membrane were observed on histological sections at both 4 and 8 weeks, indicating its slow resorption. Slow resorption is an important characteristic for optimal efficacy of a barrier membrane.[25]

Several investigations have addressed the resorption time of Bio-Oss and evidence shows its slow resorption. Some studies have even reported that it remains at the site for several years.[6,11,26] Piattelli *et al*.[11] evaluated the resorption rate of Bio-Oss used for sinus floor augmentation during a follow-up period of 4 months to 6 years. Osteoclasts attempting to degrade the Bio-Oss particles were noted at 18 months and 4 years. Duda and Pajak[26] evaluated the bio-resorption of Bio-Oss in bone defects. Biopsy samples taken after 30 months revealed new bone formation; however, Bio-Oss residues were still present. Thus, according to the present results, it may be predicted that NOVA particles, similar to Bio-Oss, remain for long periods of time in the reconstructed defects. However, what is important is that NOVA can serve as a scaffold, gradually undergo resorption, and enhance new bone formation at the defect site.

The results regarding the severity of inflammation and foreign body reaction in the present study were in agreement with the results of some previous studies.[20,26] All specimens showed grade 1 (no inflammation) or grade 2 (mild inflammation) inflammation, and only one rabbit in all four groups showed severe inflammation (grade 4) at 4 weeks. Foreign body reaction was noted in 13 out of 80 specimens.

The reason behind the selection of anorganic xenogeneic bovine bone as a bone substitute by most clinicians and researchers is that it brings about the most favorable clinical, histological, and histomorphometric results.[27-31] The anorganic xenogeneic bovine bone particles are gradually resorbed and replaced with new bone with a structure highly similar to that of human bone.[11,12,32] Moreover, Taylor *et al*.[33] reported release of type I collagen in the process of resorption of Bio-Oss, enabling greater accumulation of hydroxyapatite crystals at the defect site. This phenomenon probably plays a role in enhancement of osteogenesis. Thus, the presence of residual xenogeneic granules and their gradual degradation over time cannot be considered as a drawback.

Further studies with a larger sample size and smaller number of larger defects are required. Furthermore, randomized clinical trials are required to assess the efficacy of NOVA for regeneration of human bone defects. Furthermore, molecular studies are recommended on bone growth factors and angiogenic factors.

CONCLUSION

Considering the absence of a significant difference in new bone formation between the Bio-Oss, NOVA, and collagen membrane groups, both Bio-Oss and NOVA can be successfully used as a scaffold with a barrier membrane for regeneration of bone defects.

Financial support and sponsorship
Nil.

Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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