Original Article

Effect of GBR in Combination with Deproteinized Bovine Bone Mineral on the Healing of Calvarial Defects in Rabbits

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Abstract:

Statement of problem: The guided bone regeneration (GBR) technique does not always produce consistent results. Bone filling within the space provided by the membrane can be incomplete.

Purpose: The aim of this study was to evaluate the effect of a collagen membrane (Bio-Gide) in combination with or without deproteinezed bovine bone mineral (Bio-Oss) on the healing of calvarial defects in rabbits.

Materials and Methods: Twelve New Zealand white rabbits were used in this randomized single-blind experimental study. Four equal defects were created on the calvarium of all animals. Each defect in each rabbit was randomly assigned to one of the following treatment groups: Group 1(control), no treatment; Group 2, covered with Bio-Gide; Group 3, filled with Bio-Oss; Group 4, filled with Bio-Oss and Bio-Gide. The animals were sacrificed for histologic and histomorphometric analysis, 30 and 60 days after treatment.

Results: A significant difference was not observed in regenerated bone between the control and Bio-Gide groups ($P>0.05$), at 1 and 2 months. The amount of regenerated bone was significantly higher ($P<0.05$) in the Bio-Oss and Bio-Oss+Bio-Gide groups as compared to the control group. The difference in regenerated bone was not significant ($P>0.05$) between the Bio-Oss and Bio-Oss+Bio-Gide groups. Bone regeneration increased significantly in all treatment groups, between the two study periods ($P<0.05$).

Conclusion: In groups 3 and 4, the presence of a collagen membrane did not affect the amount of new bone regeneration. According to these results, use of a collagen membrane has no additional benefit in the regeneration of intrabony defects.

Key Words: Animal study; Bone graft; Deproteinized bovine bone mineral; Collagen membrane; Bio-Gide; Histomorphometry

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INTRODUCTION

Guided bone regeneration (GBR) has proven to be successful in a number of controlled animal studies and clinical trials [1,2]. The healing pattern following GBR involves all steps of de novo bone formation including blood clot development, invasion by osteoprogenitor cells, their differentiation into osteo-

blasts and apposition of an extracellular matrix. Connective tissue constitutes the main part of the extracellular matrix which finally mineralizes to form woven bone and is later remodeled into lamellar bone [3]. When membrane barriers exist between the blood-filled defects and gingival soft tissues, regenerative cells could obtain wound healing through osteogenesis [4]. However the blood clot tends to shrink during healing [5]. Therefore, bone grafts or bone substitutes are used to reduce the volume of the defect, thereby stabilizing the blood clot and impeding the tendency to shrink. Furthermore, these materials maintain space by supporting the membranes, thus preventing their collapse into a large defect [3,6,7]. Bone substitutes may be derived from natural materials of osseous or nonbony origin. They can be different in their surface characteristics and may show specific integration and degradation patterns within the augmented tissue [2,6].

Application of substitute material in combination with a membrane barrier can enhance clinical outcome [8,9]. Expanded polytetrafluoroethylene (e-PTFE) is considered a non-resorbable bio-inert membrane material of choice in bone regeneration. However, results may be unpredictable in the presence of inflammation caused by soft tissue dehiscence [4]. Utilization of biodegradable barrier membranes can result in uneventful healing of the soft tissue, making membrane retrieval unnecessary [10]. Such degradable barriers in the form of collagen membranes have been tested in animals and were found to be effective in bone regeneration in humans [9,11]. Following the use of these membranes, wound healing appeared to improve, but the risk of early degradation of the collagen still remained. It has been shown that degradation could affect the regenerating tissues and jeopardize the success of augmentation [11,12].

The resorption rate and osteoconductive properties of deproteinized bovine bone mineral (DBBM), used as a bone substitute, have not been clearly defined.

The aim of the present study was to histologically evaluate the effectiveness of a collagen barrier membrane in combination with or without DBBM on the healing of calvarial defects in rabbits.

MATERIALS AND METHODS

Surgical procedure

Twelve New Zealand white male rabbits weighing between 2.5 and 3kg were used in this randomized, single-blind experimental study. Animal selection, management, and experimental protocol were approved by the Animal Care and Use Committee of the Tehran University of Medical Sciences.

The rabbits were anesthetized with intramuscular injections of 10% Ketamin (40 mg/kg) and 2% Xylazine (5mg/kg, Alfason, Woeden, Holland). All surgical procedures were carried out under sterile conditions. The periosteum was reflected laterally following a midline incision through the skin and the periosteum of the calvaria. Using a round bur, four identical full thickness bony defects (3×6mm) were created on the frontal and parietal bones of each rabbit under constant irrigation with a distance of approximately 2mm from the sagittal and coronal sutures (Fig. 1-A). The four non-critical-size defects were randomly assigned to one of the following treatment groups:

Group 1: the defect was left untreated and served as the control.

Group 2: the calvarial defect was covered by Bio-Gide® (Geistlich Biomaterials, Wolhusen, Switzerland). This membrane was a bilayered collagen barrier membrane composed of types I and III highly purified porcine collagen.

Group 3: the defect was filled with Bio-Oss® (Particle size of 0.25-1mm; Geistlich Biomaterials, Wolhusen, Switzerland).

Group 4: the calvarial defect was filled with

Bio-Oss and was covered by Bio-Gide (Fig. 1-B).

In groups 2 and 4, the membranes overlapped the margins of the defects by at least 2mm. Periosteal closure was achieved using resorbable 4/0 sutures (Vicryl Johnson & Johnson. Somerville. NJ) and the calvarial skin was closed with nonresorbable 4/0 sutures (SURGIPRO.Monofilament, polypropylene)

The animals recovered from anesthesia without complications and received postoperative narcotic pain medication (Ketoprofen 0.1mg/day) for 3 days and antibiotics (Enrofloxacin 0.6 mg/day) for one week, subcutaneously.

Sample preparation

The rabbits were sacrificed with an overdose of pentobarbital (100mg/kg) injected intravenously at 30 and 60 days after surgical procedures (six rabbits in each group).

The entire cranium was removed with a reciprocating saw, without encroaching on the grafted areas. The specimens were fixed in 10% buffered formalin solution and decalcified in 10% formic acid for two weeks. They were then dehydrated in graded alcohols and embedded in paraffin. Histologic sections were prepared with a thickness of 5µm and twenty sections were obtained from each defect (350 µm distance between two succeeding sections) and were routinely stained with hematoxylin and eosin.

Sample evaluation

Histomorphometric evaluation was performed using x40 magnification. Foreign body reaction, inflammation and the interface between bone and the biomaterial particles were assessed under light microscopy at a magnification of x400.

Trabecular bone maturation and the proportion of lamellar and woven bone in each specimen were determined by a polarized light microscope.

Photomicrographs (original magnification x40) of Bio-Oss and newly regenerated bone were evaluated using graphic software (Photoshop 8.0 CS, Adobe Photoshop CS). Areas containing newly regenerated bone were selected according to their color properties. The pixel counts of these areas were calculated and divided by the total pixel of each photomicrograph. The same procedure was completed for assessing the area of the remaining Bio-Oss particles (Bio-Oss area).

Statistical analysis

Inter-group comparison was preformed using Wilcoxon signed rank test and the Dunn procedure. Mann-Whitney test and *t*-test were used to detect statistically significant differences between treatments within the same group. Results were considered statistically significant at $P<0.05$.

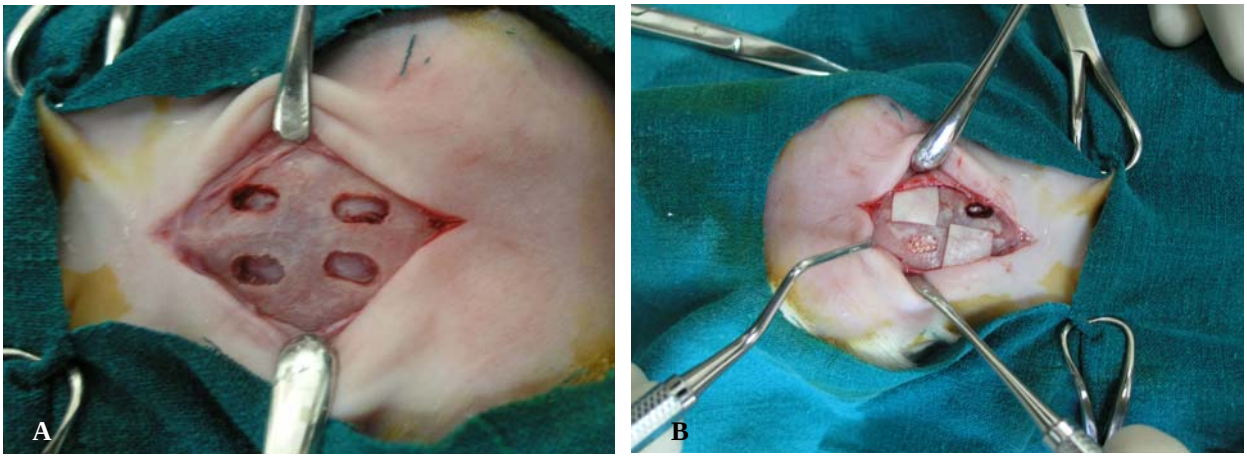

Fig.1: Surgical procedure; A: creating bony defect, and B: four different treatment group.

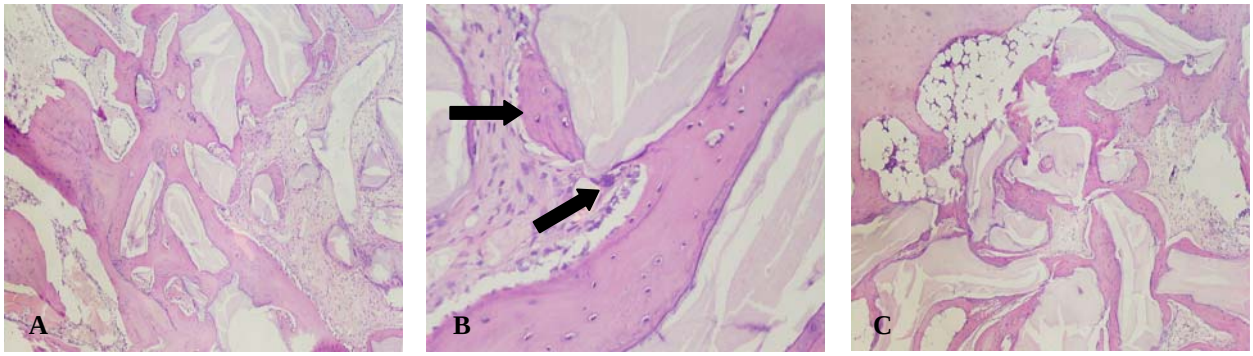

Fig. 2: Histologic results of defects repaired with Bio-Oss after one month showed newly regenerated bone in direct contact with Bio-Oss particles and multinucleated cells around the Bio-Oss particles (A: ×40 and B: ×200). C: at the end of two months (H&E, ×40).

RESULTS

Histologic results

Neither foreign body reaction nor severe inflammation was seen in the specimens. The membrane was partially degraded after 1 month and remains of the membrane were still seen after 2 months. 30 days postsurgery, newly regenerated bone was well evident in the defects filled with Bio-Oss (groups 3 and 4). The Bio-Oss particles were integrated into newly formed bone, whenever new bone formation occurred. Increased bone formation was not observed in the membrane-covered defects (Bio-Oss+Bio-Gide groups). Some of the Bio-Oss particles were surrounded by soft connective tissue mainly, in the central part of the defects. New bone formation was observed not only at the margins but also towards the center of the defects, which surrounded the Bio-Oss particles. Occasionally multinucleated cells (similar to osteoclasts) were seen adjacent to the Bio-Oss particles (Fig. 2-A, B and Fig. 3-A).

In the Bio-Gide group (non-grafted, membrane covered defects), bone regeneration was observed at the borders of the defects leaving a pronounced bony defect at the center which was replaced by newly formed connective tissue (Fig. 5-A). Bone regeneration also occurred at the periphery of the defects in the control group (Fig. 4-A).

After 60 days, newly regenerated bone surrounded the Bio-Oss particles. The soft connective tissue area was diminished, but still evident. No pronounced difference was observed between the Bio-Oss and Bio-Oss+Bio-Gide groups (Fig. 2-C and Fig. 3-B, C). The Bio-Gide and control groups showed continued bone regeneration, however connective tissue still occupied large areas in the

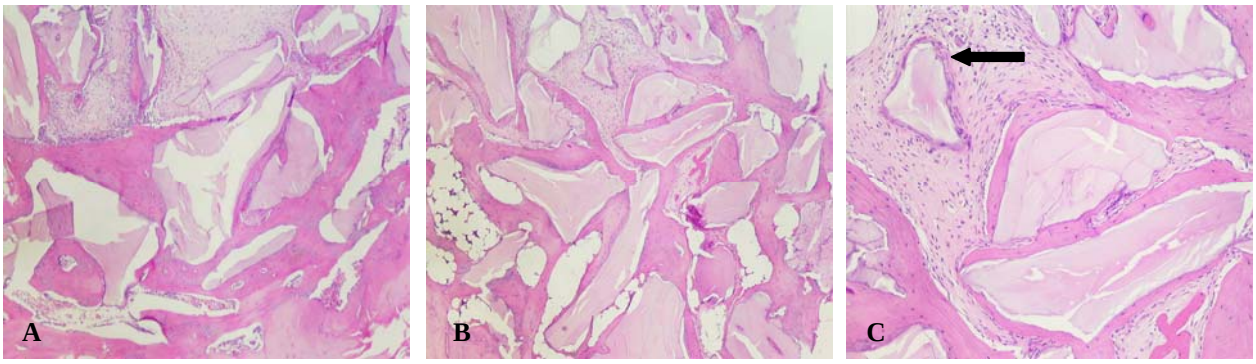

Fig. 3: Histologic results of defects repaired with Bio-Oss+ Bio-Gide after one month (A, ×40) and two months (B and C). Higher magnification (C, ×100) showed osteocytes around the Bio-Oss particle (H & E).

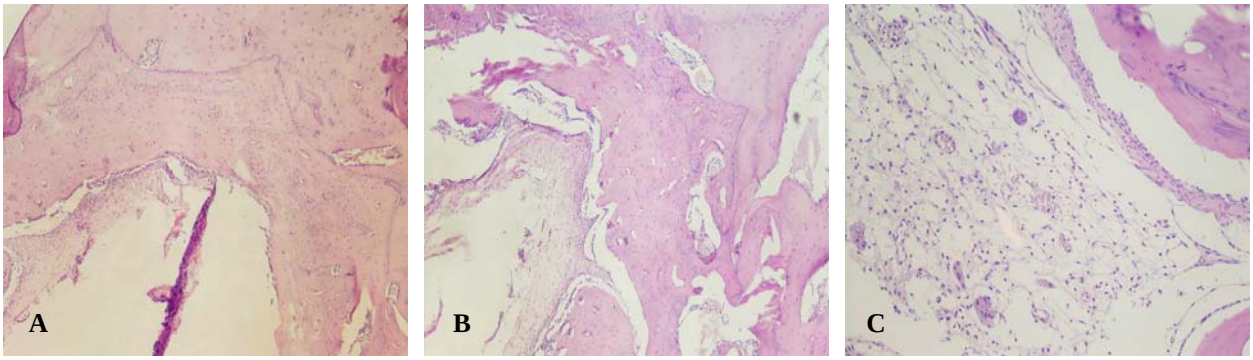

Fig. 4: Histologic results of the control group after one month (A, ×40) and two months (B and C). Higher magnification (C, ×100) showed fibro-adipose tissue in the central part of the defect (H & E).

defects. A significant difference was not observed between the control and Bio-Gide groups (Fig. 5-B and Fig. 4-B, C). There was no statistically significant difference (P>0.05) between the treatment groups regarding trabecular bone maturation between the two time periods (Table I).

Histomorphometric results

The amount of regenerated bone did not show statistically significant differences (P>0.05) between the Bio-Gide (10.37±1.02) and control (10.38± 0.72) groups, and between the Bio-Oss+Bio-Gide (17.25 ± 1.55) and Bio-Oss (17.62± 1.51) groups, at 30 days. Furthermore, no significant difference (P>0.05) was found between the Bio-Gide (16.08±1.82) and control (15.25± 0.96) groups, and between the Bio-Oss+Bio-Gide (22.41 ± 1.32) and Bio-Oss (22.95 ± 2.18) groups for bone regeneration, at 60 days. The amount of regenerated bone was significantly higher in the Bio-Oss and Bio-Oss+Bio-Gide groups as compared to the control group (P=0.028, P=0.027), at 30 and 60 days. The amount of regenerated bone was significantly higher in the Bio-Oss and Bio-Oss+Bio-Gide groups as compared to the Bio-Gide group (P=0.027, P=0.028) at 30 and 60 days (Table II).

Bone regeneration increased significantly in all treatment groups, between the two study periods (P<0.05).

The remaining Bio-Oss particles amounted to 38.66± 2.53 in the Bio-Oss group and 38.95± 1.66 in the Bio-Oss+Bio-Gide group at 1 month which did not reveal a significant

Table I: Type of regenerated bone (%) in each treatment groups, at 1 and 2 month intervals.

Treatment		Woven bone	Mixed bone	Lamellar bone
1 month	Control	--	100	--
	Bio-Gide	--	100	--
	Bio-Oss	--	100	
	Bio-Oss + Bio-Gide	--	83.4	16.6
2 months	Control	--	16.6	83.4
	Bio-Gide	--	50	50
	Bio-Oss	--	33.4	66.6
	Bio-Oss + Bio-Gide	--	16.6	83.4

Table II: Descriptive statistics of newly regenerated bone (%) and remaining Bio-Oss particles (%) in each treatment groups at 1 and 2 month intervals.

Treatment		New bone Area (SD)	Bio-Oss Area (SD)
1 month	Control	10.38 (0.72)	--
	Bio-Gide	10.37 (1.02)	--
	Bio-Oss	17.62 (1.51)	38.66 (2.53)
	Bio-Oss + Bio-Gide	17.25 (1.55)	38.95 (1.66)
2 months	Control	15.25 (0.96)	--
	Bio-Gide	16.08 (1.82)	--
	Bio-Oss	22.95 (2.18)	32.23 (1.8)
	Bio-Oss + Bio-Gide	22.41 (1.32)	32.45 (1.85)

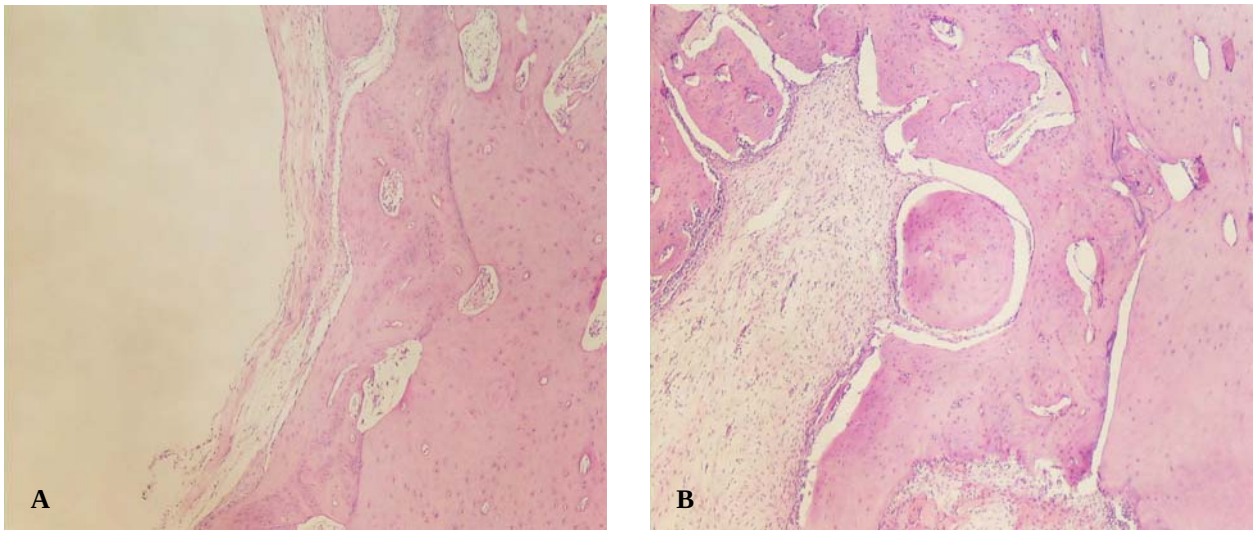

Fig. 5: Hematoxylin and Eosin stained section of the Bio-Gide group at 1 month (A) .Newly regenerated bone was noticed peripherally. After 2 months (B) newly regenerated bone was deposited towards the center of the defect however, soft connective tissue occupied large areas in the defect (×40).

difference between the two groups (P>0.05). The remaining Bio-Oss particles amounted to 32.23± 1.08 in the Bio-Oss group and 32.45± 1.85 in the Bio-Oss+Bio-Gide group, at 2 months. A significant difference was not seen (P>0.05) between the two groups (Table II).

The rate of biomaterial degradation was statistically significant (P<0.05) between 1 and 2 months in the Bio-Oss and Bio-Oss+Bio-Gide groups.

DISCUSSION

The principle of guided tissue regeneration (GTR) which was originally developed for the treatment of periodontal defects, has also been applied successfully in the treatment of different types of bone defects (GBR) [13,14]. The GBR technique does not always produce consistent results and bone filling within the space provided by the membrane can be incomplete [15]. This technique requires that the space for bone formation be protected against growth of fibrous connective tissue and distortion due to pressure applied by the overlying tissues [16]. Bone grafts have been placed beneath the barrier membrane to stabilize the blood clot or prevent membrane collapse and therefore improve the outcome of the GBR technique [17,18].

The present study has evaluated the effect of GBR in combination with or without a xenograft (Bio-Oss) on the healing of calvarial defects in rabbits.

Histologic evaluation in the current study, revealed Bio-Oss to be a biocompatible and osteoconductive biomaterial due to the lack of severe inflammation and foreign body reaction. Previous studies conducted by Hammerle et al [19], Slotte and Lundgren [20] and Berglundh and Lindhe [2] also reported biocompatibility and osteocunductive properties for this material. Similar results were obtained for Bio-Guide which was in accordance with studies conducted by Rothamel et al [21] and Zhao et al [22].

Maturation of the trabecular bone (from woven to lamellar) occurred with time in all treatment groups, without significant differences. This finding confirmed the results of Carmagnole et al [23] and Artzi et al [24]. According to the present study, no statistically significant difference was found between the Bio-Guide and control groups and between the Bio-Oss and Bio-Oss+Bio-Gide groups for regenerated

bone. In contrast, Mao et al [25] in an investtigation on dog mandibles, reported superior results when using collagen membranes for GTR, instead of polymer barriers and e-PTFE membranes.

Dupoirieux et al [26] stated that the use of collagen membrane in rat calvarial defects does not affect bone regeneration. The adjacent local bone is an important factor for bone regeneration in a membrane covered defect. Defects in compact bone (type I quality) have shown reduced bone fill when compared to defects in more cancellous bone types [27]. Considering that the cortical bone of rabbit calvarium could be classified as type I, this may account for the decrease in bone regeneration observed in the Bio-Gide group in the current investigation. Another reason may be the geometry of the defect, in particular, the relation between the depth and width of the defect. This relation affects the potential of the adjacent bone walls to populate the defect area with committed cells and accomplish a complete defect fill. In this respect, the shallow geometry of a calvarial defect is very demanding in the biological and geometrical aspects, because it not only provides low regenerative potential of the local bone but additionally requires superior mechaniccal properties of a barrier membrane to prevent collapse and subsequent impairment of bone regeneration in the defect [26].

In addition to initial mechanical strength, the resistance of a collagen membrane against proteolytic attack from the surrounding tissue is important for preservation of the space underneath, and protection against ingrowth of fibrous connective tissues. Resorption of commercially available collagen membranes have been reported to occur within the first 2 months [28]. Our results demonstrated partial degradation of the membrane after 1 month which may be one of the reasons for the ineffectiveness of the membrane in the present study.

In a similar investigation, Schliephake et al [28] stated that, the impairing effect of collagen membrane degradation on bone regeneration is yet unclear, but the fact that accumulation of loose membrane fragments were found in the center of the defect during early stages of healing, suggests mechanical interference with osteogenic differentiation of regenerating mesenchymal cells. This may in part explain the decreased bone regeneration seen in the Bio-Gide group, in the current investigation.

In the present study the membranes were not secured. In spite of the inherent properties of collagen to adhere and conform to underlying tissues under gentle pressure, mechanical interaction among animals or their cage surrounddings may have displaced the membrane. Therefore movement of the membrane over the healing clot may have caused a decrease in the quantity of regenerated bone in the membrane covered defects. Membrane movement often results in a layer of soft tissue between the membrane and underlying regenerated bone. When nonsecured membranes are used, their movement and resorption, combine to disrupt the surface of the clot and allow easier development of a soft tissue layer between the resorbing membrane and the disrupted clot, thus decreasing the quantity of regenerated bone [29].

Another reason for the lack of a significant difference in the amount of regenerated bone between the Bio-Oss and Bio-Oss+Bio-Gide groups may be the osteoconductivity of Bio-Oss that can cause acceleration of new bone growth. Furthermore, particle aggregation by itself serves as a type of physical barrier that may inhibit soft tissue cell migration into the defect [24]. On the contrary, Donos et al [30] demonstrated that the predictability of bone formation in critical-size defects depended mainly on the presence or absence of a barrier membrane. They stated that the combined use of GBR with deproteinized bovine bone

mineral did not significantly enhance the potential for complete healing.

In guided bone regeneration the osteogenic potential of progenitor bone cells, periosteum or periodontal ligaments are used to create new bone growth in a variety of osseous defects. Thus in the current study, placement of a barrier membrane and separating the periosteum from the calvarial defects (type I bone) may be one of the reasons for the decreased bone quantity encountered in the membrane-covered defects of the calvarium.

Due to the small size of the rabbit cranium, it was not possible to create four critical-size defects on the calvaria, therefore in the present study, non-critical-size defects were used to evaluate the healing process. This size difference may be responsible for the similar results that were observed in the defects with and without membrane-coverage.

According to our results, xenogenic biomaterial (Bio-Oss) may have the ability to induce physiologic bone remodeling as manifested by the significant reduction in the area of the defects filled with Bio-Oss, between 1 to 2 months.

CONCLUSION

In the current study, the presence of a membrane in the Bio-Gide and Bio-Oss+Bio-Gide groups did not positively affect the amount of new bone regeneration.

Therefore, within the limits of this study it was concluded that utilization of a collagen membrane has no additional benefit in the regeneration of non-critical-size calvarial defects in rabbits. Further investigations are warranted to determine the effectiveness of a collagen membrane in the healing of bone defects.

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REFERENCES

1- Buser D, Dula K, Hirt HP, Schenk RK. Lateral ridge augmentation using autografts and barrier membranes: a clinical study with 40 partially edentulous patients. J Oral Maxillofac Surg 1996 Apr;54(4):420-32; discussion 432-3.

2- Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with Bio-Oss. An experimental study in the dog. Clin Oral Implants Res 1997 Apr;8(2):117-24.

3- Hammerle CH, Chiantella GC, Karring T, Lang NP. The effect of a deproteinized bovine bone mineral on bone regeneration around titanium dental implants. Clin Oral Implants Res 1998 Jun;9(3):151-62.

4- Lang NP, Hammerle CH, Bragger U, Lehmann B, Nyman SR. Guided tissue regeneration in jawbone defects prior to implant placement. Clin Oral Implants Res 1994 Jun;5(2):92-7.

5- Hammerle CH, Schmid J, Olah AJ, Lang NP. A novel model system for the study of experimental guided bone formation in humans. Clin Oral Implants Res 1996 Mar;7(1):38-47.

6- Buser D, Hoffmann B, Bernard JP, Lussi A, Mettler D, Schenk RK. Evaluation of filling materials in membrane--protected bone defects. A comparative histomorphometric study in the mandible of miniature pigs. Clin Oral Implants Res 1998 Jun;9(3):137-50.

7- Kohal RJ, Trejo PM, Wirsching C, Hurzeler MB, Caffesse RG. Comparison of bioabsorbable and bioinert membranes for guided bone regeneration around non-submerged implants. An experimental study in the mongrel dog. Clin Oral Implants Res 1999 Jun;10(3):226-37.

8- Kohal RJ, Mellas P, Hurzeler MB, Trejo PM, Morrison E, Caffesse RG. The effects of guided bone regeneration and grafting on implants placed into immediate extraction sockets. An experimental study in dogs. J Periodontol 1998 Aug; 69(8):927-37.

9- Hockers T, Abensur D, Valentini P, Legrand R, Hammerle CH. The combined use of bioresorbable membranes and xenografts or autografts in the treatment of bone defects around implants. A study

in beagle dogs. Clin Oral Implants Res 1999 Dec;10(6):487-98.
10- Simion M, Misitano U, Gionso L, Salvato A. Treatment of dehiscences and fenestrations around dental implants using resorbable and nonresorbable membranes associated with bone autografts: a comparative clinical study. Int J Oral Maxillofac Implants 1997 Mar-Apr;12(2):159-67.
11- Zitzmann NU, Naef R, Scharer P. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. Int J Oral Maxillofac Implants 1997;12(6): 844-52.
12- Aaboe M, Pinholt EM, Schou S, Hjorting-Hansen E. Incomplete bone regeneration of rabbit calvarial defects using different membranes Clin Oral Implants Res 1998 Oct;9(5):313-20
13- Karring T, Nyman S, Gottlow J, Laurell L. Development of the biological concept of guided tissue regeneration animal and human studies. Periodontol 2000. 1993 Feb;1:26-35.
14- Nyman R, Magnusson M, Sennerby L, Nyman S, Lundgren D. Membrane-guided bone regeneration. Segmental radius defects studied in the rabbit. Acta Orthop Scand 1995 Apr;66(2):169-73.
15- Schenk RK, Buser D, Hardwick WR, Dahlin C. Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible. Int J Oral Maxillofac Implants 1994 Jan-Feb;9(1):13-29.
16- Buser D, Bragger U, Lang NP, Nyman S. Regeneration and enlargement of jaw bone using guided tissue regeneration. Clin Oral Implants Res 1990 Dec;1(1):22-32.
17- Simion M, Trisi P, Piattelli A. Vertical ridge augmentation using a membrane technique associated with osseointegrated implants. Int J Periodontics Restorative Dent 1994 Dec;14(6):496-511.
18- Buser D, Dula K, Belser UC, Hirt HP, Berthold H. Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. Int J Periodontics Restorative Dent 1995 Feb;15(1):10-29.
19- Hammerle CH, Olah AJ, Schmid J, Fluckiger L, Gogolewski S, Winkler JR, Lang NP. The biological effect of natural bone mineral on bone neoformation on the rabbit skull. Clin Oral Implants Res 1997 Jun;8(3):198-207.
20- Slotte C, Lundgren D. Augmentation of calvarial tissue using non-permeable silicone domes and bovine bone mineral. An experimental study in the rat. Clin Oral Implants Res 1999 Dec;10(6):468-76.
21- Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. Clin Oral Implants Res 2005 Jun;16(3):369-78.
22- Zhao S, Pinholt EM, Madsen JE, Donath K. Histological evaluation of different biodegradable and non-biodegradable membranes implanted subcutaneously in rats. J Craniomaxillofac Surg 2000 Apr;28(2):116-22.
23- Carmagnola D, Adriaens P, Berglundh T. Healing of human extraction sockets filled with Bio-Oss. Clin Oral Implants Res 2003 Apr;14(2): 137-43.
24- Artzi Z, Givol N, Rohrer MD, Nemcovsky CE, Prasad HS, Tal H. Qualitative and quantitative expression of bovine bone mineral in experimental bone defects. Part 1: Description of a dog model and histological observations. J Periodontol 2003 Aug;74(8):1143-52.
25- Mao C, Sato J, Matsuura M, Seto K. Guided tissue regeneration around dental implants in immediate extraction sockets: comparison of resorbable and nonresorbable membranes. Chin Med Sci J 1997 Sep;12(3):170-4.
26- Dupoirieux L, Pourquier D, Picot MC, Neves M. Comparative study of three different membranes for guided bone regeneration of rat cranial defectsInt J Oral Maxillofac Surg 2001 Feb;30(1): 58-62.
27- Zitzmann NU, Scharer P, Marinello CP. Factors influencing the success of GBR. Smoking, timing of implant placement, implant location, bone quality and provisional restoration. J Clin Periodontol 1999 Oct;26(10):673-82.
28- Schliephake H, Tavassol F, Gelinsky M, Dard M, Sewing A, Pompe W. Use of a mineralized collagen membrane to enhance repair of calvarial

defects in rats. Clin Oral Implants Res 2004 Feb;15(1):112-8.
29- Fugazzotto PA. GBR using bovine bone matrix and resorbable and nonresorbable membranes. Part 1: histologic results. Int J Periodontics Restorative Dent 2003 Aug;23(4):361-9
30- Donos N, Lang NP, Karoussis IK, Bosshardt D, Tonetti M, Kostopoulos L. Effect of GBR in combination with deproteinized bovine bone mineral and/or enamel matrix proteins on the healing of critical-size defects. Clin Oral Implants Res 2004 Feb;15(1):101-11.