

Evaluation of Three Bone Substitute Materials in the Treatment of Experimentally Induced Defects in Rabbit Calvaria

M. Paknejad¹, AR. Rokn^{1,2}✉, B. Eslami³, R. Afzalifar⁴, A. Safiri⁵

¹Associate Professor, Department of Periodontology, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

²Associate Professor, Dental Research Center, Tehran University of Medical Sciences, Tehran, Iran

³Associate Professor, Department of Oral and Maxillofacial Pathology, Dental School of Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁴Dentist, Dental Research Center, Tehran University of Medical Sciences, Tehran, Iran

⁵Periodontist, Private Practice

Abstract:

Objective: The aim of present study was to evaluate the quality, density and thickness of newly formed bone in experimental defects treated with Combi-Pack[®], Bio-Oss[®] and Biostite[®].

Materials and Methods: Eight New Zealand white rabbits were included in this randomized, blinded study. Four equal 3×6 mm bone defects were created on the frontal and parietal bones of each animal and three were immediately grafted with Bio-Oss[®], Combi-Pack[®] and Biostite[®] while one was left untreated, serving as negative control. Histologic and histomorphometric analysis was performed four weeks after surgery.

Results: Histomorphometric bone area and trabecular maturity was significantly higher in the Bio-Oss[®] and Combi-Pack[®] samples as compared to the Biostite[®] and control cases. The amount of remaining biomaterial was almost equal in the three experimental groups at the end of the study period. Neither foreign body reaction nor severe inflammation was seen in any of the specimens except for the Biostite[®] samples.

Conclusion: It may be suggested that implantation of Bio-Oss[®] particles and Combi-Pack[®] blocks can promote bone regeneration more effectively than Biostite[®].

Key Words: Bone regeneration; Bio-Oss; Biostite

Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran (2007; Vol: 4, No.4)

✉ Corresponding author:
AR. Rokn, Department of Periodontology, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.
roknamir@sina.tums.ac.ir

Received: 20 September 2006
Accepted: 2 May 2007

INTRODUCTION

Osseous deficiency is a major issue for patients with periodontal defects and those in need of implants. Bone regeneration techniques induce restoration of lost osseous tissues and have become a necessary step in periodontal and implant treatments. Severely resorbed alveolar ridges are often encountered in individuals with periodontal problems. On the other hand, dehiscences or fenestrations may develop during the course of implant preparation in narrow ridges, which can compromise the mechanical stability of the fixture.

In order to solve this problem, autogenous/allogenic bone grafts and substitutes have been employed for reconstructive purposes resulting in alveolar ridges with sufficient bone volume [1].

Autogenous bone grafts from intra- and extra-oral donor sites are regarded as the “gold standard” in regenerative therapy of craniofacial bony defects [1]. However a disadvantage of this method is donor-site morbidity, which could be reduced by using bone replacement materials. Multiple degradable or permanent osteoconductive bone substitutes such as tri-

calcium phosphate or xenogenic hydroxyapatite ceramics are available for treatment of bone loss [1-9].

Bio-Oss[®], a deproteinized natural bovine cancellous bone mineral, is considered as one of the more popular bone substitutes. According to previous histologic reports, Bio-Oss[®] is a biocompatible osteoconductive grafting material with no signs of foreign body reaction or severe inflammation following its application [10-15]. Berglundh and Lindhe used Bio-Oss[®] for filling large self-contained defects in beagle dog mandibles. After a 3- to 7-month healing period, the graft particles were surrounded and partially replaced by newly formed bone. It was concluded that Bio-Oss[®] may be an effective osteoconductive material with osseointegration qualities [16]. In an experimental study on rabbits, Kling et al [17] found that Bio-Oss[®] particles were capable of providing a scaffold for new bone formation. This was attributed to the similarity between natural cancellous bone and the dimensions of Bio-Oss[®] macropores. Furthermore, other investigators have also shown that the crystal structure and chemical composition of Bio-Oss[®] is identical to that of normal osseous tissues [18, 19]. Histological observation of human periodontal defects demonstrated stimulation of new bone and cementum formation following grafting with Bio-Oss[®] particles [12, 20].

Biostite[®] is a recently introduced biomaterial and has been claimed to promote bone regeneration. This material is essentially a mixture of synthetic hydroxyl apatite (88%, granulometry of 160-200 micro meters, total porosity of 60%), equine type I collagen (9.5%), and chondroitin sulfate (2.5%). It seems that collagen reinforces its osteoconductive activity, prevents epithelial migration and serves as a scaffold for reformation of the periodontium [21].

The present animal study was designed to comparatively evaluate the histologic and histomorphometric pattern of healing in experi-

mentally induced cranial defects treated with Bio-Oss[®], Bio-Oss[®]COLLAGEN (Combi-Pack[®]) and Biostite[®] grafting materials.

MATERIALS AND METHODS

The current study was performed according to the methods described by Soleymani Shayesteh et al [22].

Animal Surgical Procedure

Eight New Zealand white male rabbits weighing between 2.5 and 3 kg were included in this randomized, blinded prospective investigation. The study protocol was approved by the ethics committee of Tehran University of Medical Sciences. Each animal was anaesthetized with 10% ketamine (40 mg/kg) and 2% xylazine (5 mg/kg; Alfason, Woerden, Holland), administered intra-muscularly. The cranium was shaved and, scrubbed with 7.5% povidine iodine and draped in a sterile fashion. A coronal-sagittal approach was used for the surgical procedure. The periosteum was dissected and four identical full thickness bony defects (3×6 mm), terminating over the dura mater, were created with a round bur on the frontal and parietal bones. In each rabbit, one defect was left untreated (negative control) and the other three were randomly filled with Bio-Oss[®] (Bio-Oss[®], Geistlich and Sons, Wolhusen, Switzerland), Combi-Pack[®] (Combi-Pack[®], Geistlich and Sons, Wolhusen, Switzerland), and Biostite[®] (Biostite[®], vebas S.R.L, S. Giuliano M, Milan, Italy). The periosteal and calvarial skin were closed with resorbable 4/0 (Vicryl, Johnson & Johnson, Somerville, NJ, USA) and non-resorbable 4/0 (SURGIPRO[™], Richmond, VA, USA) sutures, respectively. All rabbits recovered from anesthesia without complications. A postoperative narcotic (ketoprofen, 0.1 mg/day for 3 days) along with an antibiotic (Enrofloxacin, 0.6 mg/day for 1 week) was injected subcutaneously for each animal.

Sample Preparation

The rabbits were sacrificed one month after

surgery by intravenous administration of pentobarbital (100 mg/kg). Care was taken not to damage the grafted areas while removing the cranium. The specimens were decalcified in a 20% solution of formic acid for three days, dehydrated in graded alcohol and embedded in paraffin. Five-micrometer sections were prepared and stained with haematoxylin and eosin.

Sample Evaluation

Foreign body reaction was assessed through observation of giant cells and a concomitant granulomatous reaction under a light microscope (Olympus BX 51, Olympus Co., Tokyo, Japan) at a magnification of $\times 400$. The interface between bone and biomaterial particles was also evaluated with the same magnification. The proportion of lamellar and woven bone was estimated under polarized light. Collagen bundles in lamellar bone are concentrically aligned, while woven bone contains irregularly oriented fibers. Magnified photomicrographs ($\times 40$) were used for assessment of bone and biomaterial areas using graphics software (Photoshop 8 CS, Adobe Photoshop CS). Evaluators were blinded to the type of biomaterials implanted in the calvarial defects. Statistical evaluation

Statistical analysis was performed by Friedman test and Dunn procedure for pairwise comparisons using SPSS software (SPSS for Windows version 11.5).

RESULTS

Histological Evaluation

Foreign body reaction and severe inflammation were absent in all studied specimens except for two of the Biostite[®] samples. Half of the control cases did not have any inflammation and the remaining 50% showed a mild inflammatory response. Accordingly, a significant difference in the severity of inflammation was found between the case and control groups ($P < 0.05$).

In experimental cases that demonstrated new

bone formation, osseous tissues were observed in direct contact with the biomaterials without intervening connective tissue. A mixture of woven and lamellar bone was observed in all case and control specimens with bone regeneration.

Histomorphometric Evaluation

The amount of newly formed bone was 11.0% (SD=0.7%), 14.5% (SD=1.8%), 16.3% (SD=1.4%) and 17.3% (SD=0.8%) in the control, Biostite[®], Bio-Oss[®] and Combi-Pack[®] samples, respectively. The difference between the four groups was significant ($P = 0.001$).

Pairwise comparisons revealed a significantly lower amount of bone regeneration in the control defects as compared to the Biostite[®]-treated samples ($P < 0.05$). Also, the amount of new bone formation was found to be higher in the Bio-Oss[®] and Combi-Pack[®] groups in comparison to the Biostite[®] specimens ($P < 0.05$). However the difference between the former groups was not statistically significant. The percentages of remaining biomaterial in the Biostite[®]-, Bio-Oss[®]- and Combi-Pack[®]-treated defects were 23.3% (SD=1.6%), 23.7% (SD=1.6%) and 23.5% (SD=1.6%), respectively. A significant difference was not observed between these samples ($P = 0.854$).

DISCUSSION

One of the major goals in periodontal therapy is to regain the lost periodontium including osseous tissues. This has been achieved through application of biomaterials using different techniques. Ongoing investigations seek to identify better bone regeneration substitutes and superior grafting methods. Growth factors can play an important role in improving and accelerating wound healing of both soft and bony tissues. According to the results obtained in the present study, bone regeneration was more efficient in cases filled with Bio-Oss[®] and Combi-Pack[®] than in Biostite[®]-treated defects. Additionally better results were observed in all experimental groups as compared to con-

tol cases at the end of the study period.

Regeneration of osseous tissues in our samples was significantly higher in the Biostite[®] specimens in comparison to the Bio-Oss[®] and Combi-Pack[®] cases. These results are in agreement with those reported by Piattelli et al [6] who found physiologic bone remodeling to be better in Bio-Oss[®] than in hydroxyapatite. Previous investigations on Bio-Oss[®] and Biostite[®] have shown a greater amount of bone regeneration in Bio-Oss[®] and a slower pace of hydroxyapatite replacement for Biostite[®]. A number of studies have also suggested that Bio-Oss[®] is well tolerated and more biocompatible than Biostite[®] [6].

Artzi et al [10] investigated the effect of Bio-Oss on the microscopic appearance of newly formed bone during healing of extraction sockets. The average amount of osseous tissue adhered to Bio-Oss[®] particles was 46.3% after 9 months. The current study employed a 4-week period for evaluation of bone regeneration; therefore our findings do not provide information on the rate of new bone formation in different intervals. Bone substitutes are alloplastic or xenogenic materials capable of conducting bone regeneration. Considering their eventual resorption and replacement by osseous tissues; it is conceivable that with time, histomorphometric measurements would show an increase in the quantity of newly formed bone.

Controversial data exist regarding the amount and mechanism of graft material resorption. According to several investigators, bone substitutes do not have osteoinductive or osteoproliferative properties and resorb very slowly, thus cannot completely replace autogenous bone [11-14]. However, others have shown biodegradation of bone graft materials, following physiologic normal bone remodeling or normal osteoclastic resorption [14,17-20].

Despite the fact that new bone formation was found in all our specimens and has been reported in numerous other studies, the resorp-

tive capability and turnover rate of grafted materials remains unclear. Therefore, differences in resorption rate, may affect the amount of bone replacement, and future studies are required to clarify the behavior of grafts in relation to newly formed osseous tissues over time.

An important finding in the present investigation is the lack of severe inflammation and foreign body reaction, which demonstrates physical and chemical similarities between Bio-Oss[®] and the recipient bone. The osteoconductive property of this biomaterial was apparent based on the observation of increased osteogenesis without intervening fibrous tissue. The newly formed bone encountered within the Bio-Oss[®] particles was vital. This indicates the ability of the grafted material to induce revascularization and revitalization of the bony tissue which may occur because of the porous architecture of Bio-Oss.

It can be suggested that porosity in bone substitute materials, similar to autogenous bone, is necessary for vascularization and osteogenesis. The interconnecting pore system of Bio-Oss[®] has been shown to promote angiogenesis and facilitate the migration of osteoblasts. [23]. Furthermore it is reasonable to assume that the biocompatibility of Bio-Oss[®] is greater than Biostite[®].

CONCLUSION

According to the results obtained in the current investigation, it may be proposed that implantation of Bio-Oss[®] particles and Combi-Pack[®] blocks can accelerate bone regeneration more effectively than Biostite[®]. In addition it was shown that filling bony defects with each of the studied materials would be better than leaving them unfilled.

ACKNOWLEDGMENT

This study was supported by the Research Council of Tehran University of Medical Sciences grant no. 132/4401.

REFERENCES

- 1-Nkenke E, Schultze-Mosgau S, Radespiel-Troger M, Kloss F, Neukam FW. Morbidity of harvesting of chin grafts: a prospective study. *Clin Oral Implants Res* 2001 Oct;12(5):495-502.
- 2-Aybar B, Bilir A, Akcakaya H, Ceyhan T. Effects of tricalcium phosphate bone graft materials on primary cultures of osteoblast cells in vitro. *Clin Oral Implants Res* 2004 Feb;15(1):119-25.
- 3-Babbush CA. The use of a new allograft material for osseous reconstruction associated with dental implants. *Implant Dent* 1998;7(3):205-12.
- 4-Carmagnola D, Berglundh T, Araujo M, Albrektsson T, Lindhe J. Bone healing around implants placed in a jaw defect augmented with Bio-Oss. An experimental study in dogs. *J Clin Periodontol* 2000 Nov;27(11):799-805.
- 5-Dong J, Uemura T, Shirasaki Y, Tateishi T. Promotion of bone formation using highly pure porous beta-TCP combined with bone marrow-derived osteoprogenitor cells. *Biomaterials* 2002 Dec;23(23):4493-502.
- 6-Piattelli M, Favero GA, Scarano A, Orsini G, Piattelli A. Bone reactions to anorganic bovine bone (Bio-Oss) used in sinus augmentation procedures: a histologic long-term report of 20 cases in humans. *Int J Oral Maxillofac Implants* 1999 Nov-Dec;14(6):835-40.
- 7-Nkenke E, Fenner M. Indications for immediate loading of implants and implant success. *Clin Oral Implants Res* 2006 Oct;17 Suppl 2:19-34.
- 8-Lynch SE, Genco RJ, Marx RE. Tissue engineering, applications in maxillofacial surgery and periodontics. 1st ed. Chicago: Quintessence Publishing; 1999.
- 9-Hatcher J, Lemon A. Laboratory Animal studies of Apatite and collagen Biomaterials. *Journal of Dental Research* 1986;65(Special Issue):1604.
- 10-Artzi Z, Tal H, Dayan D. Porous bovine bone mineral in healing of human extraction sockets. Part 1: histomorphometric evaluations at 9 months. *J Periodontol* 2000 Jun;71(6):1015-23.
- 11-Skoglund A, Hising P, Young C. A clinical and histologic examination in humans of the osseous response to implanted natural bone mineral. *Int J Oral Maxillofac Implants* 1997 Mar-Apr;12(2):194-9.
- 12-Schlegel AK. Bio-Oss bone replacement material. Long-term results with Bio-Oss bone replacement material. *Schweiz Monatsschr Zahnmed* 1996;106(2):141-9.
- 13-Maiorana C, Redemagni M, Rabagliati M, Salina S. Treatment of maxillary ridge resorption by sinus augmentation with iliac cancellous bone, anorganic bovine bone, and endosseous implants: a clinical and histologic report. *Int J Oral Maxillofac Implants* 2000 Nov-Dec;15(6):873-8.
- 14-Spector M. Anorganic bovine bone and ceramic analogs of bone mineral as implants to facilitate bone regeneration. *Clin Plast Surg* 1994 Jul;21(3):437-44.
- 15-Schlikewei W, Paul C. Experimentelle untersuchung zum knochenersatz mit bevinem Apatit. In: Huggler A, Kunner E, eds. A ktuller stand beim knochenersatz; Hefte zur unfallheilkunde. Berlin: Springer; 1991. p. 59-63.
- 16-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.
- 17-Klinge B, Alberius P, Isaksson S, Jonsson J. Osseous response to implanted natural bone mineral and synthetic hydroxylapatite ceramic in the repair of experimental skull bone defects. *J Oral Maxillofac Surg* 1992 Mar;50(3):241-9.
- 18-Wetzel AC, Stich H, Caffesse RG. Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. *Clin Oral Implants Res* 1995 Sep;6(3):155-63.
- 19-Merkx MA, Maltha JC, Freihofer HP. Incorporation of composite bone implants in the facial skeleton. *Clin Oral Implants Res* 2000 Oct;11(5):422-9.
- 20-Aaboe M, Schou S, Hjorting-Hansen E, Helbo M, Vikjaer D. Osseointegration of subperiosteal implants using bovine bone substitute and various membranes. *Clin Oral Implants Res* 2000 Feb;11(1):51-8.
- 21-Sakalliglu U, Acikgoz G, Ayas B, Kirtiloglu

T, Sakallioğlu E. Healing of periodontal defects treated with enamel matrix proteins and root surface conditioning--an experimental study in dogs. *Biomaterials* 2004 May;25(10):1831-40.

22-Soleymani Shayesteh Y, Khorsand A, Motahhary P, Dehghan M, Shafiee Ardestani MA. Evaluation of platelet-rich plasma in combination with deproteinized bovine bone mineral in the tab-

bit cranium: a pilot study. *J Dent TUMS* 2005; 2(4):127-34.

23-Orsini G, Traini T, Scarano A, Degidi M, Perrotti V, Piccirilli M, et al. Maxillary sinus augmentation with Bio-Oss particles: a light, scanning, and transmission electron microscopy study in man. *J Biomed Mater Res B Appl Biomater* 2005 Jul; 74(1):448-57.