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Histologic and Histomorphometric Evaluation of Bone Substitutes in Experimental Defects

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Abstract: Successful aesthetic and functional outcomes of any prosthetic reconstruction require an adequate and favorable ridge contour for proper pontic design and implant placement. Often the ridge needs to be reconstructed with bone substitutes. The aim of this study was to compare the quality and quantity of newly formed bone resulting from 3 different types of commercial bone substitutes (Bio-Oss®, Bio-Gen®, PTG®). About 6 round intrabony defects were made with a trephine bur on each of ten tibias. Each defect in each tibia (except one defect in each tibia which served as control) was randomly filled with a bone substitute with or without a resorbable collagen barrier membrane. The animals were euthanized after three months and the proximal part of the tibias containing the defects were prepared for histologic and histomorphometric evaluation. The mean percentage of new bone in the three groups with grafted material was higher than those without grafted material. The mean percentage of new bone and remaining soft tissue in the PTG® group was similar to the Bio-Oss®+ collagen membrane group but the bone quality was better in the PTG® group. Application of PTG® does not interfere with the initial healing of the surgical wound.

Key words: Bone substitutes, bone grafts, titanium granules, application, grafted material, Iran

INTRODUCTION

Spontaneous healing of the edentulous alveolar ridge often leads to deformation resulting in improper sites for implant placement. Several different graft materials, e.g., autologous, allograft, alloplast or xenograft were used to preserve or reconstruct the ridge anatomy. Most of the bone substitutes used today are all more or less resorbable material. A material that is absolutely resistant to resorption and also has good clotting properties has long been a desired objective, especially for reconstructing moderately large and large defects.

Bio-Oss® is a sterilized and deproteinized bovine bone and is still considered by many dentists and dental surgeons to be the standard bone substitute. Bio-Gen® is an inorganic equine-derived bone substitute treated by an exclusive 37°C enzymatic deantigenation process. Bio-Gen® has successfully been used both for periodontal defects and in implant surgery (De Biase *et al*., 2005; Stievano *et al*., 2008).

PTG® consist of irregular and porous non-resorbable granules of commercially pure titanium. When implanted, the granules are able to interlock with each other, creating a stable porous structure and providing an environment for bone ingrowth (Turner *et al*., 2007; Wohlfahrt *et al*., 2010). PTG® have been successfully used in a series of sinus floor augmentations (Bystedt and Rasmusson, 2009). The aim of the present study is to evaluate the osteo conductivity of these bone substitutes during bone healing in experimental defects and to determine the quality and quantity of the newly formed bone.

MATERIALS AND METHODS

Surgical procedure: In this experimental animal study, 5 dogs each 1 year of age and weighing approximately 25-30 kg were used. The ethics committee of Shahid Beheshti School of dentistry and the National animal care society (Tehran, Iran) approved the protocol. Based on standards approved by the Tehran veterinary school, the animals were prepared for surgery and sedated with an intramuscular injection of xylazine hydrochloride 2% (0.1 mg kg^{-1}).

The surgical site was shaved and then scrubbed with disinfectant. An incision was made on the medial part of the tibia, penetrating the epidermis, dermis and fascias. An additional medial incision was made through the periosteum which was elevated by a self-retaining

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retractor. After irrigation with normal saline solution, 6 round intrabony defects each 6 mm in diameter and approximately 4 mm in depth were made with trephine bur. Each hole was drilled starting 3 mm anterior to the index (a titanium screw) each hole at a distance of 3-4 mm from the border of the previous hole. The 6 round defects in each tibia were treated based on a randomly-selected protocol in the following six experimental groups:

- Bio-Oss® (Geitlich Biomaterials, Wollhusen, Switzerland) + Bio-Gide® (Geistlich)
- Bio-Gen® (Bioteck, Arcugnano Vicenza, Italy) + BCG® (Bioteck)
- PTG® (Tigran Technologies AB, Malmo, Sweden)
- Bio-Gide® (Geistlich)
- BCG® (Bioteck)
- Control (blood clot)

One defect was filled with Bio-Oss® granules (0.25-1 mm) and covered with a Bio-Gide® membrane (porcine-derived collagen) another defect was filled with Bio-Gen® granules (0.5-1 mm) and covered with a BCG® membrane (equine-derived collagen); a third defect was filled with PTG® (porous titanium granules) and uncovered; a fourth defect was ungrafted but covered with a Bio-Gide® membrane; a fifth defect was ungrafted and covered with a BCG® membrane and a sixth defect was ungrafted and uncovered (control group).

The subcutaneous soft tissue was repositioned and sutured and the super-facial layers were closed with intra-cutaneous sutures. Postoperatively, antibiotics (penicillin) were administered intramuscularly once a day for 5 days. Pain and edema were controlled with mefenamic acid (250 mg) and dexamethasone, respectively.

The surgical sites were rinsed and cleaned for two weeks where after the sutures were removed. After three months, the dogs were euthanized (using sodium thiopental). Immediately after euthanasia each tibia bone was removed and dissected and the proximal part of the tibia containing the defects was removed in blocks.

Histology and sample preparation: The relevant parts of the tibia were removed and fixed in a solution of 10% neutral buffered formaline. The blocks were rinsed thoroughly with water. In order to decalcify the blocks, samples were floated in a solution of 10% formic acid after which the blocks were embedded in paraffin.

Several transverse cross-sections with a diameter of 5 μm were made through the center of each defect, using a microtome device (Jung, Frankfort, Germany). However, for cutting samples that contain titanium granules, sections were ground down to a thickness of approximately 50 μm using an Accutom-50 (Struers, Denmark). The samples were then stained with hematoxilin and eosin and were examined with an optical microscope (Nikon Eclipse E400, Nikon, Sumida-KU, Japan) linked via a digital camera (Nikon Fuji HC-300 ZI, Nikon) to a personal computer.

Histomorphometric measurements were made using software. Areas of the newly formed bone, residual material and soft tissue percentages were marked manually with a mouse and calculated by a computer. To avoid possible bias, coded samples were used.

Statistical analysis: The data were analyzed using an ANOVA procedure. p-values<0.05 were considered significant. Multiple comparisons were conducted using a Tukey test. Calculations were performed using SPSS software (SPSS 17, SPSS, Inc., Chicago, IL).

RESULTS

Clinical findings: All treatment groups demonstrated an uneventful healing with completely healed wounds without infection at the time of harvest.

Histologic evaluation: Newly formed bone was well evident in all the defects but there was a clear demarcation between the old and newly regenerated bone in all sites. However, histological evaluation of the samples did not show any clear osteoclastic cells in the borderline between the materials and the new bone. Inflammatory cells were few (<10%) in all sites (Bio-Oss®, Bio-Gen®, PTG®). In the defects filled with Bio-Oss® granules (Fig. 1) or Bio-Gen® granules (Fig. 2), lamellar and a small amount of woven bone were regenerated similarily towards the center of the defect surrounding the grafted mineral granules and was not limited to the peripheral bony walls. In some of the defects filled with Bio-Oss® or Bio-Gen® granules, it was observed an abnormal appearance in the bone marrow around the granules which seemed mostly fibrovascular in nature. In the defect that filled with PTG® (Fig. 3), newly formed lamellar bone penetrated and became integrated in the macro- and micropores of the granules.

The osseous tissue was mature and well organized and the site was completely filled with bone and granules. The bone marrow formed around titanium granules appeared thoroughly normed (fatty vascular) and very similar to ungrafted sites. In the non-grafted Bio-Gide® membrane or BCG® membrane covered defects (Fig. 4), newly formed bone was noticed peripherally and

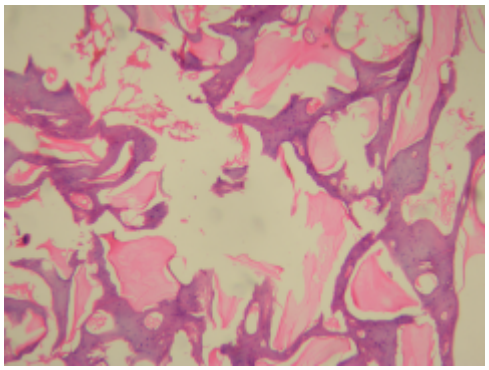

Fig. 1: Histological section (stained with hematoxilin and eosin) of a defect filled with Bio-Oss® shows a lamellar and some woven bone with a significant fibrovascular component surrounding the material granules. The bulk of granules are present, indicating that this material is only slowly resorbed. The bone does not appear to form directly in contact with the granules: however, this could be an effect of granule absorption or processing. A large part of the tissue surrounding the graft material is predominantly fibrous in nature

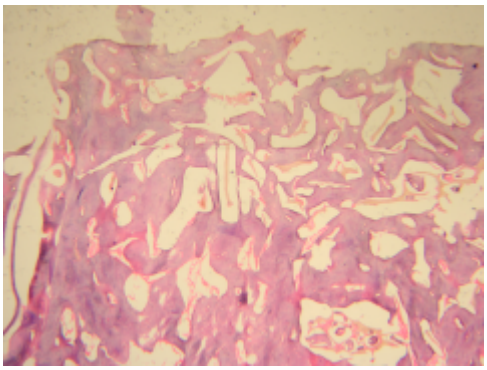

Fig. 2: Histological section (stained with hematoxilin and eosin) of a defect filled with Bio-Gen® shows lamellar and some woven bone with a significant fibrovascular component surrounding the empty areas remaining from the graft material, similar to the situation in Fig. 1. The material itself was most probably absorbed during healing, indicating that this material is highly resorbable. Alternatively, the material could be dissolved during the processing for histology

underthe membrane, tissue repair. Also in the control defects (Fig. 5), newly formed peripheral bone was

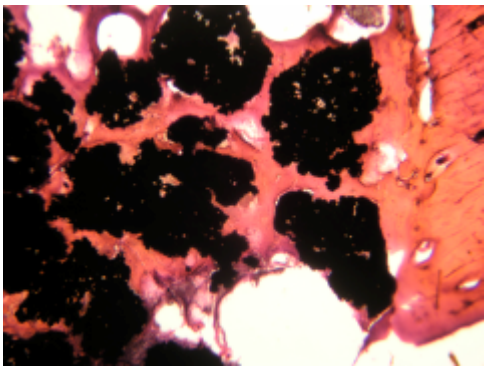

Fig. 3: Histological section (stained with hematoxilin and eosin) of a defect grafted with PTG® shows mature and well organized lamellar bone filling the intergranular space and penetrating the porosities of the granules. The mature bone completely fills the void between the granules and grows in close contact with the material that appears completely osseointegrated. Note the ultra-porosity and the irregular shape of the material allowing interlocking and securing graft stability

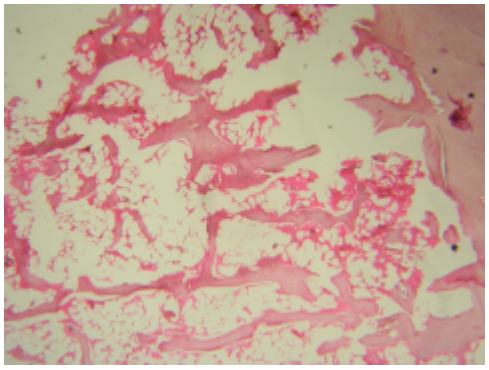

Fig. 4: Histological section (stained with hematoxilin and eosin) of a non-grafted Bio-Gide® membrane covered defect shows significant healing as a result of connective tissue repair. Only a small trabecular-like bone formation is visible

observed, leaving a significant bony defect healed by connective tissue repair.

Histomorphometric evaluation: There were significant differences in the vital bone percentage between groups ($p<0.001$) when the groups were compared using variance analysis for random blocks. The Tukey method was used for a two-by-two comparison of the groups. Three homogeneous subgroup were formed using this method (Table 1).

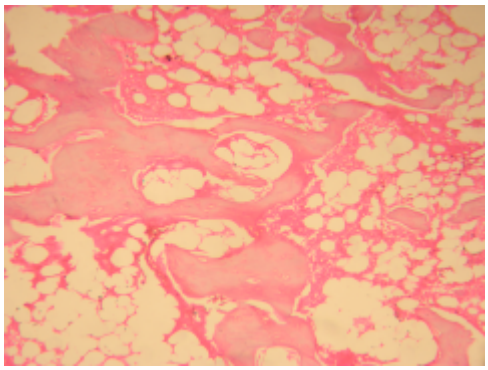

Fig. 5: Histological section (stained with hematoxilin and eosin) of a control defect shows significant healing a result of connective tissue repair. Trabecular bone formation is present, mostly growing inward from the defective edges

Table 1: Homogeneous subsets of mean (SD) vital bone in %

	Subset		
Groups	1	2	3
PTG®	67.0 (11.3)	-	-
Bio-Oss®+Bio-Gide®	65.0 (10.3)	-	-
Bio-Gen®+BCG®	-	48.8 (11.0)	-
Control (blood clot)	-	-	28.8(8.2)
BCG®	-	-	22.9(9.3)
Bio-Gide®	-	-	21.3(6.7)

DISCUSSION

This study is based on a model established for studying the performance of biomaterials in long bones. Due to the large number of comparison cases in this study, it was determined that all experimental defects needed to be managed in one place. A canine tibia provides an experimental extra-oral site which has already been used in previous studies.

The benefits of the tibia as a surgical site include the following: it is unbiased by bacterial infections and trauma from chewing. Double layer suturing (first deep soft tissue and then skin) prevents unwanted wound site exposure which accelerates the recovery process.

This procedure together with post-operative medication, most likely precluded unwanted infection thereby resulting in uneventful healing. For these reasons, the tibia was chosen for this study. On the other side, the main disadvantage of using the tibia is that there is a central predominance of soft tissue marrow space.

This characteristic limited the depth of defects to no >4 mm to prevent interference with the central bone marrow space. Creating a defect of critical size is crucial when testing the osteoconductivity of materials (Schmitt *et al*., 1997; Schmitz and Hollinger, 1986). There is still no agreement regarding the critical size for defects in the canine tibia but since, the healing of control defects (ungrafted, uncovered) was incomplete and inferior to the defects treated with bone substitute (Bio-Oss®, Bio-Gen®, PTG®), the size (4 mm deep, 6 mm in diameter) was considered suitable.

In all 3 bone substitute groups, the slow resorbable (Bio-Oss®, Bio-Gen®) and the inresorbable (PTG®) highly mineralized tissue formed directly in the granules indicate that these materials are indeed osteoconductive. Although, the remodeling process had started after 3 months all grafted material was still present and had not started to degrade. Therefore, the 3 months were sufficient for comparing these 3 groups. This conclusion concurs with previous studies using Bio-Oss® (Artzi *et al*., 2003; Gholami *et al*., 2009).

No significant difference in bone formation percentage between the samples with membrane only (without bone substitute) and with the control group is a result that opposes findings in several previous studies (Imbronito *et al*., 2002; Piattelli *et al*., 1996). This may be the result of collapsed overlaying soft tissue due to lack of grafted material inside the defect. It is possible that if the membrane had been fixed with screws, the results might have been different.

CONCLUSION

Within the limitations of this study, It is observed that the application of PTG® does not interfere with the initial healing of the surgical wound. Bone marrow around PTG® was fatty vascular but in some locations where grafted materials (Bio-Oss® and Bio-Gen®) were used, the bone marrow was fibrovascular which provides evidence that the bone formation remodeling process in the samples containing PTG® was developing correctly. The PTG® group bone quality was better than that of the Bio-Oss® group. The density of the bone trabecular around the PTG® and the penetration of bone in between the granules indicate that PTG® help in the formation of cortical bone. PTG® are considered an osteoconductive graft material suitable for bone regeneration.

RECOMMENDATIONS

It is recommend that in the future, histologic and histomorphometric sample evaluation be done in a specific sequence and at specific intervals to allow better comparison of the results.

REFERENCES

Artzi, Z., N. Givol, M.D. Rohrer, C.E. Nemcovsky, H.S. Prasad and H. Tal, 2003. Qualitative and quantitative expression of bovine bone mineral in experimental bone defects. Part 1: Description of a dog model and histological observations. J. Periodontol., 74: 1143-1152.

Bystedt, H. and L. Rasmusson, 2009. Porous titanium granules used as osteoconductive material for sinus floor augmentation: A clinical pilot study. Clin. Implant Dent. Relat. Res., 11: 101-105.

De Biase, A., F. Guerra, L. Cipriano, L. Lamazza and E. Tucci, 2005. Subantral filling by deantigenated heterologous bone and immediate fixture placement. Minerva Stomatol., 54: 99-108.

Gholami, G.A., A. Ghassemi, H. Gholami, G.A. Rad and G. Ansari, 2009. Assessing periodontal status of patients with active caries or faulty restorations. J. Med. Sci., 9: 276-279.

Imbronito, A.V., J.H. Todescan, C.V. Carvalho and V.E. Arana-Chavez, 2002. Healing of alveolar bone in n resorbable and on-resorbable membrane-protected defects: A histologic pilot study in dogs. Biomaterials, 23: 4079-4086.

Piattelli, A., A. Scarano, P. Russo and S. Matarasso, 1996. Evaluation of guided bone regeneration in rabbit tibia using bioresorbable and non-resorbable membranes. Biomaterials, 17: 791-796.

Schmitt, J.M., D.C. Buck, S.P. Joh, S.E. Lynch and J.O. Hollinger, 1997. Comparison of porous bone mineral and biologically active glass in critical-sized defects. J. Periodontol., 68: 1043-1053.

Schmitz, J.P. and J.O. Hollinger, 1986. The critical size defect as an experimental model for craniomandibulofacial nonunions. Clin. Orthop. Relat. Res., 205: 299-308.

Stievano, D., A. Di Stefano, M. Ludovichetti, S. Pagnutti, F. Gazzola, C. Boato and E. Stellini, 2008. Maxillary sinus lift through heterologous bone grafts and simultaneous acid-etched implants placement. Five year follow-up. Minerva Chir., 63: 79-91.

Turner, T.M., R.M. Urban, D.J. Hall and G.B. Andersson, 2007. Bone ingrowth through porous titanium granulate around a femoral stem: Histological assessment in a six-month canine hemiarthroplasty model. Ups J. Med. Sci., 112: 191-197.

Wohlfahrt, J.C., M. Monjo, H.J. Ronold, A.M. Aass, J.E. Ellingsen and S.P. Lyngstadaas, 2010. Porous titanium granules promote bone healing and growth in rabbit tibia peri-implant osseous defects. Clin. Oral Implants Res., 21: 165-173.