

Histological Observations on Bone Healing with Bioactive Glass in Horizontal Ridge Augmentation: A Report of Four Cases

Jin-Woo Park^{1,*}, Jo-Young Suh¹

1. Department of Periodontology, School of Dentistry, Kyungpook National University

I. INTRODUCTION

The guided bone regeneration (GBR) procedure has been used successfully when there is insufficient bone volume for implant placement¹⁻³⁾. Bone graft materials are used in conjunction with barrier membranes to improve the outcomes of GBR procedures: they stabilize the blood clot, prevent membrane shrinkage and maintain the space available for new bone formation beneath the membrane⁴⁻⁶⁾. Autogenous bone is the preferred augmentation material but harvesting of autogenous bone requires surgery, which is associated with donor site morbidity, a long operation and high costs⁷⁻⁹⁾. A variety of graft materials are used as alternatives to autogenous grafts¹⁰⁻¹⁴⁾. Bioactive glass is considered an effective bone graft substitute because of its bone-binding properties^{15,16)}.

Chemical bonds form between bone tissue and a calcium phosphate layer formed by ion exchange on the surface of bioactive glass¹⁷⁻²¹⁾. Many studies have demonstrated that bioactive glass has positive effects on bone healing in human sinus floor elevation and human extraction sockets²²⁻²⁴⁾.

Despite the high efficacy of bioactive glass as a grafting material for sinus floor elevation,^{22,24-27)} histological validation of its efficacy for the treatment of horizontal ridge deficiency in conjunction with GBR is limited to a relatively short-term study (6 months)²⁸⁾. In our study, we obtained bone biopsies at various times after the operations and evaluated bone healing using histology. We evaluated the efficacy of bioactive glass particles of a narrow size range (Biogran®, 3i Implant Innovations, Palm Beach Gardens, FL, USA) for horizontal alveolar ridge augmentation in conjunction with

* Corresponding author : Jin-Woo Park, Department of Periodontology, School of Dentistry, Kyungpook National University, 188-1, Samduk 2Ga, Jung-Gu, Daegu, 702-412, Korea (E-mail: jinwoo@mail.knu.ac.kr)

the GBR procedure and titanium-reinforced expanded polytetrafluoroethylene (TR e-PTFE) membranes (Gore-Tex®, WL Gore & Associates, Flagstaff, AZ, USA) by histological examination of biopsies harvested at various periods of healing from 4 clinical cases.

II. MATERIALS AND METHODS

Patients and surgical procedure

Among the patients who received bone augmentation surgery because of inadequate alveolar ridge widths for implant placement, four systemically healthy nonsmoking patients (2 men and 2 women, 37 to 58 years old) with different healing times were admitted to the study (Table 1). The patients refused permission to harvest bone for horizontal ridge augmentation from intraoral sites. The GBR procedure and the need to harvest a core biopsy during surgical reentry for implant placement were explained to the patients, all of who gave their written consent. Patients received prophylactic antibiotics (750 mg of amoxicillin-clavulanate, Amocla®, Kuhnil Pharm Co., Seoul, Korea) 1 h preoperatively and then 375 mg of Amocla 3 times daily for 5 days postoperatively. They rinsed with 0.12% chlorhexidine gluconate for 1 min prior to surgery and twice daily for 2 weeks postoperatively. Full thickness flaps were

reflected and the cortical bone surface was perforated with a small round bur to stimulate bleeding from the marrow compartment. After placement of the Biogran® particles, a trimmed TR e-PTFE barrier membrane was applied to cover the grafts. The membrane was fixed to the bone with titanium membrane tacks (Frios®, Dentsply Friadent, Mannheim, Germany). The flap was adjusted to provide tension-free primary closure using vertical and periosteal releasing incisions. Between June 2004 and July 2005, bone biopsies were taken from the implant sites with a 2 mm diameter trephine (Dentsply Friadent, Mannheim, Germany) during the surgical reentry procedure after at least 6 months of healing.

Sample preparation and histomorphometric analysis

Bone biopsies were fixed in 4% neutral buffered formaldehyde and then decalcified in EDTA and dehydrated in ethanol before they were embedded in paraffin. Sections 5 μm thick were cut along the long axis of the core biopsy and stained with hematoxylin and eosin or Masson's trichrome stain. Histomorphometric analysis was carried out using a light microscope (BX51; Olympus, Tokyo, Japan) with an image analysis system (i-Solution, iMTechnology Inc., Daejeon, Korea) under 100 \times magnification.

Table 1. Clinical details of patients and biopsies.

Case	Age	Sex	Type of defect	Position of defect	Position of biopsy	Healing time (mo)	Reason for biopsy
1	54	M	Horizontal defect	24	24	6	Implant placement
2	37	M	Horizontal defect	21	21	8	Implant placement
3	49	F	Horizontal defect	35,36	36	10	Implant placement
4	58	F	Horizontal defect	16	16	18	Late implant failure

Images were captured using a digital camera (CC-12; Soft Imaging System GmbH, Munster, Germany) attached to the microscope and displayed on a computer monitor. Four evenly spaced sections were evaluated per biopsy. Histomorphometric measurement was used to quantify the relative amounts of different tissue types within the grafted area. Areas of native bone were excluded from the analyses. The following variables were measured within the boundaries of the defects: area of newly formed bone (NB%, area of newly formed mineralized bone expressed as a percentage of the total defect area) and remaining graft particle area (BG%, residual Biogran® particle area expressed as a percentage of the total defect area). Mean values for histomorphometric variables were calculated for each sample.

III. RESULTS

Clinical observations

All of the augmented sites healed uneventfully without any signs of inflammation or membrane exposure. The times at which surgical reentry procedures were performed (6, 8, 10 and 18 months postoperatively) differed between patients because of personal reasons or implant failure. In the case of patient 4, the implant was inserted at the time of the GBR procedure

and the final prosthodontic component was inserted 8 months after grafting. The implant was removed 7 months after functional loading because of mobility. The extraction site was closed without any grafting and subsequently healed. After 3 months, a biopsy was taken from the implant site, which included some of the previously augmented area. The augmented sites showed clinically significant increases in alveolar ridge width. All grafted sites exhibited resistance to drilling. After core biopsies were retrieved, all patients immediately received implants at the augmented sites. Implant stability was achieved by using long implants that engaged with the lateral or apical native bony wall.

Histological and histomorphometrical results

Histological examination revealed little new bone formation in biopsies harvested from implant sites up to 8 months after the operation (Figures 1 and 2). Cracking and fragmentation of BG particles were visible in all specimens. Most remaining BG particles were encapsulated by connective tissue (Figures 1–3). In biopsies harvested at months 6 and 8, there was no evidence of incorporation of new native bone into the graft. Histomorphometry showed that the NB% at months 6 and 8 was 2.5% and 1.9% of the total defect volumes, respectively (Table 2). The mean BG% at months 6 and 8 was

Table 2. Histomorphometry results by patients and healing time

Case	Healing time (mo)	NB (%)	BG (%)
1	6	2.5	22.3
2	8	1.9	26.5
3	10	13.2	30.7
4	18	10.7	18.9

NB = newly formed mineralized bone, BG = remaining Biogran particles

22.3% and 26.5%, respectively. A small amount of new bone ingrowth into the BG particles from native bone was observed at month 10 (Figure 3). The mean NB% was 13.2% and the mean BG% was 30.7% (Table 2). Increased new bone formation in internally excavated BG particles was observed at month 18 (Figure 4).

Newly formed bone in direct contact with residual graft particles was observed, but most BG particles were still surrounded by connective tissue and mineralized bone. In contrast, the socket left after removal of a previous implant showed more favorable bone healing (abundant and relatively

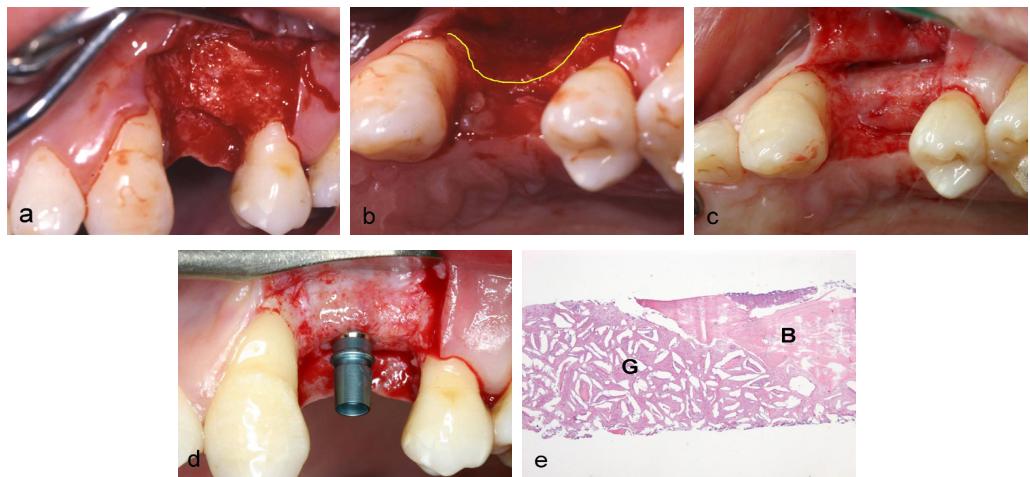


Figure 1. Case 1. (a) Buccal view of horizontal ridge deficiency of maxillary left first premolar. (b) Occlusal view of horizontal ridge deficiency. (c) Reentry surgery at 6 months showing increased alveolar ridge width after membrane removal. (d) The implant was placed in the augmented site in such a way as to confer stability. (e) Histological section of a bone core retrieved 6 months after grafting. The major part of the grafted site (G) consists of BG particles embedded in connective tissue. There is no integration between native bone (B) and the graft material (original magnification \times 40; stained with hematoxylin and eosin).

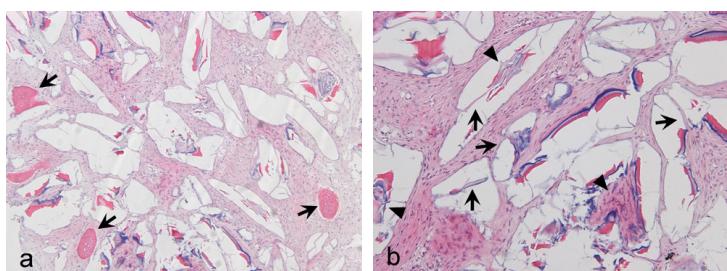


Figure 2. Histological section of a bone core retrieved 8 months after grafting (Case 2). (a) New bone formation (arrows) is limited and is not in contact with BG particles. Most graft particles are encapsulated with fibrous tissue (original magnification \times 100; stained with hematoxylin and eosin). (b) Higher magnification of Fig. 2a. Internal excavation and related filling of connective tissue (arrowheads) is evident in the centers of BG particles. Cracks and fragmentation (arrows) of BG particles are evident (original magnification \times 200; stained with hematoxylin and eosin).

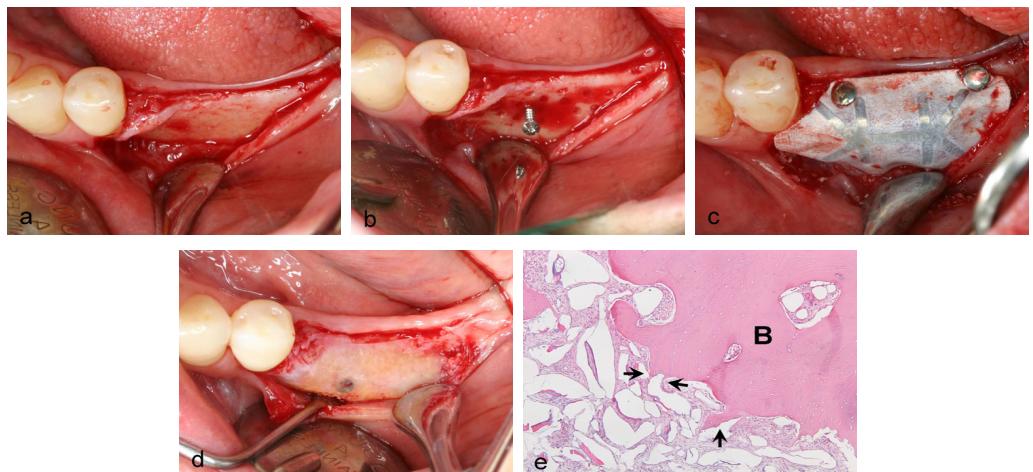


Figure 3. Case 3. **(a)** Narrow alveolar ridge in the posterior area of the left mandible. **(b)** After decortication, a titanium screw was fixed into the alveolar bone to support the barrier membrane. **(c)** After grafting, a TR e-PTFE membrane was fixed to the alveolar bone with membrane tacks. **(d)** Increased alveolar ridge width after membrane removal at surgical reentry. **(e)** Histological section of biopsy retrieved 10 months after grafting. Integration of BG particles with newly formed bone grown from native bone (**B**) is evident in some areas (arrows), but the major part consists of graft particles embedded in connective tissue (original magnification $\times 100$; stained with hematoxylin and eosin).

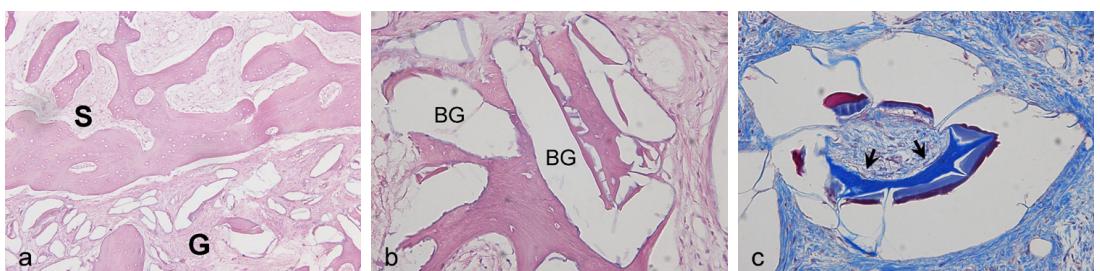


Figure 4. Histological section of bone core retrieved 18 months after grafting (Case 4). **(a)** Site of implant removal (**S**) after 3 months of healing shows favorable bone formation composed of trabecular bone that is thicker and more abundant than that of the Biogran[®] grafted site (**G**) (original magnification $\times 100$; stained with hematoxylin and eosin). **(b)** Higher magnification of Figure 4a. BG particles (**BG**) integrated into newly formed bone are evident (original magnification $\times 400$; stained with hematoxylin and eosin). **(c)** Newly formed bone (arrows) is evident within the centrally excavated BG particle (original magnification $\times 400$; stained with Masson's trichrome stain).

thick trabecular bone) than areas that had previously been augmented with Biogran[®] despite the relatively short healing period (3 months) and the absence of grafting. Histomorphometry

showed that the grafts consisted of 10.7% NB and 18.9% BG. In contrast, 41.9% of the socket left after removal of a previous implant consisted of newly formed bone.

IV. DISCUSSION

In our study, when bioactive glass was used for horizontal ridge augmentation, bone healing was poor compared to that reported in other human studies^{22–24)}. Histological analysis revealed that bioactive glass induced little formation of new bone in the first 8 months after the operation (2.5%) and a relatively low percentage of new bone in the first 18 months (10.7%), indicating that it has poor osteoconductive properties.

Many studies suggested that ionic dissolution products and internal excavation of BG particles play key roles in osteoblast differentiation and subsequent bone formation^{17,20,21,29)}. Several studies suggested that each bioactive glass particle functions as a nucleus for bone growth, thereby enhancing bone healing^{17,20)}. While many studies have demonstrated that bioactive glass has beneficial effects on bone healing *in vitro*, evidence from *in vivo* trials with humans is conflicting^{22–24,28,30)}. Tadjoedin et al^{24,31)} reported that bioactive glass particles (Biogran®, 300–355 μm) induced 36% new bone growth at month 6 of healing after sinus floor elevation. In contrast, Knapp et al²⁸⁾ demonstrated that bioactive glass had poor osteoconduction for the treatment of horizontal alveolar ridge defects in conjunction with GBR. After 6 months of healing, the grafted sites showed poor new bone formation (10% or less in 6 of 10 patients) and most residual BG particles exhibited connective tissue encapsulation, which is similar to our findings. Norton and Wilson³⁰⁾ found no evidence of new bone formation in healing extraction sockets 6 months after the operation and suggested that a longer time may be re-

quired for the graft-healing effect to become evident because a small amount of new bone was incorporated into sites with bioactive glass 7 months after the graft.

In our trial, the area occupied by residual BG particles was greater than that reported in other studies^{23,24,31)}. The residual Biogran® particles accounted for 18.9% of the defect area after 18 months, suggesting that Biogran® degrades slowly. In contrast, Tadjoedin et al^{24,31)} reported that residual BG particles accounted for 8% of the defect area at 15 months and were absent at 16 months when combined with a small amount of autogenous bone in sinus floor elevation. Froum et al²³⁾ reported that residual BG particles occupied 5.5% of the areas of extraction sockets up to month 8 of healing. Histological differences between studies may be related to differences in healing times, properties of bioactive glasses, types of defects, surgical techniques and methods of histomorphometric measurement.

Although the volume of bone involved in lateral ridge augmentation is small compared with that involved in sinus floor elevation, most of the area to be augmented receives its blood supply from the marrow compartment of bone through cortical perforations. The cancellous portion of the alveolar ridge is reduced as atrophy of the bone progresses and the number and diameter of vessels decreases with time³²⁾. In most cases of GBR of severely atrophic alveolar ridges, blood supply to the grafted sites is limited. The grafted sites are supplied with blood through intramarrow perforations. Moreover, blood flow from the periosteum to the grafting materials is blocked by the barrier membrane. This differs from self-contained

defects created by surrounding bony walls in sinus floor elevation. These differences may explain the delayed resorption of BG particles and poor bone formation in our study relative to that reported in other studies because enhanced angiogenesis emanating from the surrounding native bone and increased numbers of circulating stem cells contribute to graft healing^{33–36}.

In our study, newly formed bone occupied only 10.7% of the total defect area at 18 months, while 41.9% of the area of the socket from which an implant was removed consisted of trabecular bone at 3 months, which is similar to the normal trabecular bone content of the maxilla²⁴. These findings are in agreement with the results of recently published studies in which the authors showed that Biogran® may interfere with new bone formation in animals when used with or without GBR^{37,38}. Stavropoulos et al³⁷ examined the long-term influence of bone substitutes combined with GBR on bone formation and demonstrated that newly formed bone occupied 12.6% of the area of Biogran® grafted defects and 88.2% of the area of non-grafted control defects after 1 year. However, it should be noted that conflicting histological results have also been reported for similar types of defects such as human extraction sockets^{23,30} and periodontal osseous defects^{39–42}.

With GBR, other bone substitutes, such as deproteinized bovine bone, induced greater new bone formation than the bioactive glass used in our study. Studies of human alveolar ridge augmentation showed that deproteinized bovine bone induces 17%–27% new bone formation with a considerable degree of direct contact between newly formed bone and the residual graft after

6 months of healing^{11,14,43}. These differences in bone healing indicate that more human histological studies are needed to confirm the effectiveness of BG in the treatment of osseous defects.

In our study, the increase in the width of the alveolar ridge induced by bioactive glass was sufficient for placement of an implant combined with a titanium reinforced e-PTFE barrier membrane but histological evaluation revealed poor bone healing, even after 18 months. The limited information obtained from this case series suggests that bioactive glass particles are not suitable for bone regeneration with GBR for treatment of horizontal ridge defects.

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수평적 치조제증대술에 사용된 Bioactive glass의 골재생에 관한 조직학적 관찰: 증례보고

박진우, 서조영

경북대학교 치과대학 치주과학교실

임프란트 식립을 필요로 하는 환자의 수평적 치조제 결손의 증대를 위해 골유도재생술과 병용한 bioactive glass (BG) (Biogran[®]) 이식의 골재생 양상을 각기 다른 치유기간을 부여한 4명의 환자에서 평가하였다. 6, 8, 10, 18개월의 치유기간 후 임프란트 식립부위에서 조직절편을 채득하여 골재생을 조직계측학적으로 평가하였다. 임프란트 식립을 위한 surgical reentry시 모든 이식부위는 임상적으로 명확한 수평적 치조제 폭경의 증가를 관찰할 수 있었다. 하지만 조직학적 분석결과 BG는 불량한 골전도성을 나타내었다. 6, 8개월의 치유기간후, 이식부위에서 신생골이 거의 관찰되지 않았으며(2.5%이하), 이식부와 기존 골의 경계부위에서 BG particle에 대한 신생골 성장과 결합양상 또한 관찰할 수 없었다. 10개월의 치유기간후 기존 골조직으로부터 성장한 신생골의 BG particle과의 직접적인 접촉양상을 일부 관찰할 수 있었다. 이식부는 13.2%의 광물화된 신생골조직을 보였고, 대부분의 BG particle은 결체조직으로 둘러싸여 있었다. 18개월의 치유기간이 부여된 환자의 조직절편에서 신생골은 이식부의 10.7%를 차지하여 비교적 낮은 신생골 형성양을 나타내었고, 이식부에 존재하는 잔존 BG particle은 대부분은 결체조직으로, 일부분에서 광물화된 골조직으로 둘러싸여 있었다. 6, 8, 10, 18개월에서 잔존 BG particle양은 전체 이식부 면적에 대해서 각기 22.3%, 26.5%, 30.7%, 18.7%로 나타났다. 본 증례보고는 비록 한정적인 4명의 환자에서의 조직계측학적 평가결과이지만, 수평적 치조제 결손의 증대를 위해 골유도재생술과 병용한 bioactive glass이식은 불량한 골전도성으로 인해 효과적인 골재생을 위한 이식재로서는 적절하지 않을 수 있음을 나타낸다.

