

# Healing patterns after guided bone regeneration in human extraction sockets

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## I . Introduction

Bone resorption continues when teeth are lost. Forty to 60% of bone morphological changes occur between 6 to 24 months after the extraction<sup>1)</sup>. Implant placement in the optimal positions allows us to obtain a better load distribution, inter-arch relations and esthetics of the final prosthesis<sup>1)</sup>. Thus far, several techniques have been advocated for the generation of new bone tissue within or around a tentative implant site. For early implantation, the guided bone regeneration (GBR) procedure is performed at the extraction socket to prevent alveolar bone resorp-

tion after extraction, making implantation possible later and increasing the success rate of graft implantation. Extraction sockets have been used as a model for wound healing in many studies<sup>2-5)</sup>. GBR techniques utilize graft material alone or in combination with barrier membranes<sup>6-8)</sup>.

Human demineralized freeze-dried bone allografts(DFDBA) have been widely used in the periodontal regeneration procedure. Berglundh et al.<sup>6)</sup> reported Bio-Oss fulfills the criteria of an osteoconductive material. Wetzel et al.<sup>9)</sup> reported that Bio-Oss undergoes a slow resorption process. Although there have been studies on the use of various

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graft materials such as DFDBA and Bio-Oss during periodontal regeneration surgery, sources are lagging on histologic findings from extraction sockets after a GBR procedure in which the extraction socket is filled with the mixture of DFDBA and Bio-Oss or a GBR procedure in which GBR membrane is placed over the extraction socket after filling the socket with the mixture of DFDBA and Bio-Oss. Bio-Oss and DFDBA were mixed at a 1:1 ratio to even out osteoconduction and osteoinduction during bone formation. The combined two fillers may promote the healing after GBR in human extraction sockets. GBR membrane was used for the purpose of secure the site for retaining the graft and inducing osteoblasts.

The aim of this study is to classify the healing pattern following GBR in human extraction sockets, histologically.

## II. Materials and Methods

Twenty sites from 14 patients, (8 men and 6 women), with a mean age of 44 years (range 17 to 64) were evaluated. All the patients were medically healthy, exhibited good oral hygiene, and had no contraindications for dental treatment. The patients signed informed consent forms. Full-thickness flaps were elevated, and the sockets were thoroughly debrided with a surgical curette and a small round bur to remove the granulation tissue approximately 1 month after the extraction. The defect sites were then filled with a 1:1 mixture (vol./vol.) of DFDBA (250-500  $\mu$ m, PACIFIC COAST TISSUE BANK, U.S.A.) and Bio-Oss (0.25-1.0 mm, Geistlich, Biomaterials and Osteo-

health, Switzerland), and were covered with a resorbable collagen membrane Bio-Mesh (SAMYANG CORPORATION, Korea). The flaps were sutured using a vertical mattress suture method with 4-0 resorbable suture materials (SAMYANG CORPORATION, Korea) and 3-0 silk. Primary flap closure was performed through releasing incision. One dentist performed all surgeries. We would recommend postoperative guidelines such as the use of medications including antibiotics and mouthwash using chlorhexidine.

### 1. Specimen preparation

The specimens were retrieved with a trephine bur, a mean 6.7 months (range 4 to 13 months) after the placement of the grafts within the mandibular extraction sockets for future rehabilitation with endosseous implants. They included hard tissue from the alveolar ridge crest over the previously grafted sockets. The specimens were taken at the time of the implant placement in the regenerating bone using a trephine bur (2 $\times$ 12 mm). These biopsies were all obtained with informed consent from the patients, in accordance with the ethical standards of the Review Committee on the use of Human Subjects at Chosun University, Gwang-ju, Gwang-ju, South Korea.

### 2. Histologic and immunohistochemical staining

Each biopsy specimen from the retrieved bone cylinders was immediately fixed in a solution containing 4% paraformaldehyde

and then decalcified in a 10% EDTA (pH 7.4) solution at 4°C for 4 weeks. All the specimens were routinely embedded in paraffin, after which 5  $\mu$ m sections were cut perpendicularly to the long axis of the bone cylinders mounted on ethoxysilan-coated glass slides. The sections were stained with hematoxylin and eosin for general histological observations.

For the immunohistochemistry, polyclonal anti-mouse ALP antibodies were purchased from ID Labs inc. (London, ON, Canada). The paraffin sections were pre-incubated with 1% BSA in 1 $\times$  PBS for 30 min, and incubated for 1 h with anti-ALP antisera diluted 1:100 in 1% BSA in 1 $\times$  PBS. After washing in 1 $\times$  PBS, the sections were incubated for 1 h at room temperature with biotinylated goat anti-mouse IgG antibodies diluted 1:200 in 1 $\times$  PBS, as the secondary antibody. The sections were then rinsed briefly with 1 $\times$  PBS, reacted with an avidine-biotin-peroxidase complex (Vector Lab., Burlingame, CA, U.S.A.) in 1 $\times$  PBS for 1 h. After color development with 0.05% DAB (diaminobenzidine tetrahydrochloride), the sections were washed and counterstained with hematoxylin.

### III. Results

Of the twenty sites, new bone formation was observed in only 9 sites without membrane exposure. F/U period in relation to healing varied according to each site and histologic healing types also varied. Table 1 shows differences according to extraction sites. Differences during the healing process of single root teeth and multiple root teeth are

listed in Table 1. Among 20 sites, there were 4 single root teeth and 16 multiple root teeth. The healing type of single root teeth was Type I in 2 sites, Type III in 2 sites. In the case of multiple root teeth, the type varied from Type II to Type V. The histological sections showed five different healing patterns according to the bone forming property, fibrous tissue proliferation and inflammation around the graft materials. Histologic differences of these 5 types are listed in Table 2. In type I, there was no new bone formation in or around the graft materials, and necrotic graft materials were surrounded by thick fibrous tissues showing mild inflammation (Figure. 1). In type II, no appreciable bone formation occurred in or around the graft materials, which were encapsulated by dense fibrous tissues (Figure. 2). In type III, the graft materials were densely packed in connective tissue that was rich in collagen fibers with only some very small, circumscribed areas of remineralization characterized by a lack of osteocyte nuclei (Figure. 3). In type IV, the newly formed bone could be distinguished from the graft materials embedded in some fibrous tissues, and the osteoblasts were exclusively located at the surfaces of newly formed bone, and the osteocytes were also laid in the lacunae of the new bone (Figure 4). In type V, the graft materials were surrounded by newly formed bone and bone deposition by osteoblast rimming was evident, which was also interconnected by newly formed bone (Figure 5). The ALP activities were not identified in the type I, II and III healing patterns, but were intensely expressed in the type IV and V healing patterns.

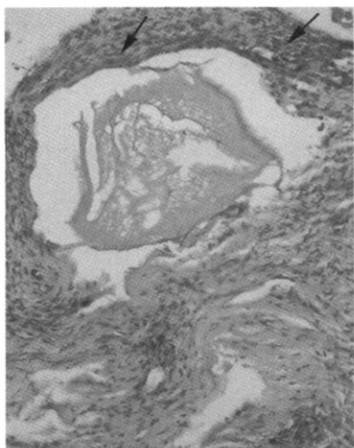


Figure 1. Histological section following augmentation with human DFDBA and Bio-Oss showing type I healing pattern. Graft materials showed necrosis and were surrounded by inflammatory connective tissues(arrows)(H-E stain, magnification  $\times 100$ ).

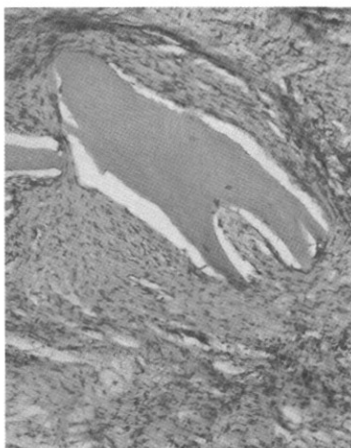


Figure 2. Histological section following augmentation with human DFDBA and Bio-Oss showing type II healing pattern. There was no new bone formation in or around the graft materials encapsulated by dense fibrous tissues(H-E stain, magnification  $\times 100$ ).

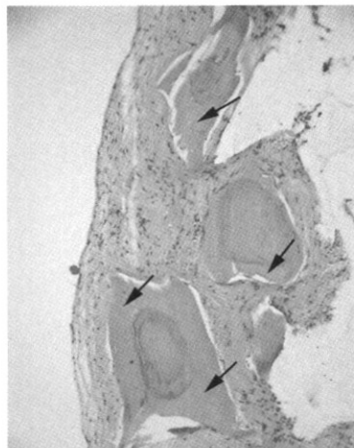


Figure 3. Histological section following augmentation with human DFDBA and Bio-Oss showing a type III healing pattern. Graft materials were surrounded by new bone(arrows) characterized by a lack of the osteocyte nuclei and connective tissues(H-E stain, magnification  $\times 100$ ).

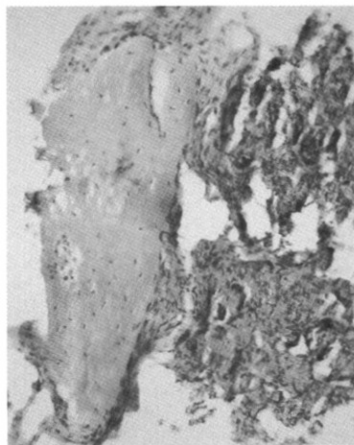


Figure 4. Histological section following augmentation with human DFDBA and Bio-Oss showing type IV healing pattern. Dense fibrous tissues were in contact with some osteoblasts (H-E stain, magnification  $\times 100$ ).

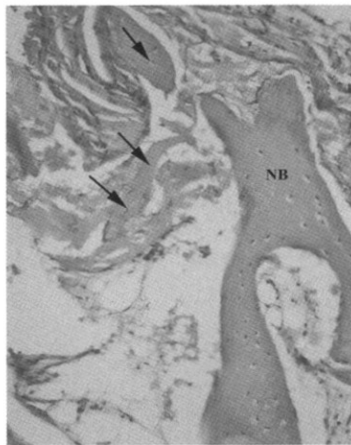


Figure 5. Histological section following augmentation with human DFDBA and Bio-Oss showing type V healing pattern with bone dust. The newly formed bone(NB) could be unambiguously distinguished from the graft materials(arrows)(H-E stain, magnification  $\times 100$ ).

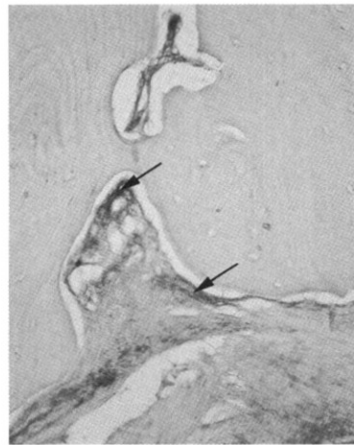


Figure 6. Immunohistochemical localization of ALP in the sections showing a type V healing pattern. ALP localization denoting the active new bone formation(arrows)( $\times 100$ ).

Table 1. Healing patterns around the graft materials

Patient	Sex	Site	Healing time (month/day)	Membrane exposure	Healing pattern
1	M	#37, #38	13/16	No	Type V
2	M	#36	7/25	No	Type V
3	M	#46	6	No	Type V
4	F	#36	4/7	No	Type V
5	M	#36	4/15	No	Type V
6	F	#46, #47	4/12	No	Type IV
7	F	#46	4/10	No	Type V
8	M	#25, #26	13	Yes	Type I & II
9	M	#36	4/15	Yes	Type III
10	M	#36	4/5	Yes	Type III
11	M	#11, #21	7	Yes	Type III
12	F	#45, #46	4/12	Yes	Type II
13	F	#46, #47	4/6	Yes	Type II
14	F	#25	6/3	Yes	Type I

Table 2. Interpretive standard for typing of healing patterns around the graft materials

Healing patterns	New bone formation	Connective tissue proliferation	Inflammation/ necrosis	ALP activities
Type I	-	+	+	-
Type II	-	+	-	-
Type III	±	+	-	-
Type IV	+	±	-	+
Type V	+	-	-	+

ALP: alkaline phosphatase, -: absent, +: present, ±: partially present

## IV. Discussion

Tooth extraction as a result of advanced periodontitis often leads to osseous deformities of the alveolar ridge. These deformities can present significant complications performing implant surgery. Periodontal and alveolar ridge defects resulting from several factors often require a surgical correction prior to a prosthodontic reconstruction<sup>10-13</sup>. It has been suggested that extraction sockets are ideal defects for evaluating bone healing.

The efficacy of guided bone regeneration (GBR) therapy for rebuilding of atrophic alveolar bone prior to implant placement has been well-established in the literature. The technique of guided bone regeneration using barrier membranes are frequently combined with the application of autogenous bone or bone substitutes<sup>14</sup>. Cancellous autogenous bone is an ideal graft material for use in surgical procedures aimed at enhancing a deficient alveolar ridge particularly for vertical ridge augmentation<sup>14-15</sup>. However, patients frequently do not accept autogenous bone transplants because it requires 2 surgical sites, and may require general anesthesia. For these reasons, many authors have suggested the use of a variety of materials for grafting<sup>16-21</sup>.

DFDBA appears to affect bone formation because of the presence of bone morphogenic proteins<sup>22</sup>. Piattelli et al.<sup>23</sup> reported that significant new vital bone formation was observed 6 months after the placement of a DFDBA. DFDBA is used in the preservation of extraction sockets and in the repair of re-

sorbed alveolar ridges to provide a sufficient quantity of bone for the placement of endosseous implants<sup>24</sup>. Bio-Oss is a natural bovine bone mineral, which has the osteoconductive properties<sup>25-27</sup>. Valentini et al.<sup>28</sup> reported the use of Bio-Oss as a grafting material to augment the sinus floor. Berglundh et al.<sup>6</sup> examined the healing process after 3 and 7 months of bone defects filled with natural cancellous bovine bone mineral. They reported that with time Bio-Oss, as an osteoconductive material, becomes integrated and subsequently replaced by newly formed bone. Wetzel et al.<sup>9</sup> reported that Bio-Oss undergoes a slow resorption process in the sinus area. Valentini et al.<sup>28</sup> suggested use of the composite grafts, DFDBA and Bio-Oss, as an alternative to autografts in the sinus floor lifting procedure, and natural bovine bone mineral matrix could be indicated alone in the case of sufficient remaining bone around the sinus.

We believed that the mixture of Bio-Oss, for the purpose of securing the space by retaining a graft for a limited period of time, and DFDBA, for the purpose of inducing osteoblasts in the surrounding autogenous bone, would be effective in maximizing the effect of GBR procedures. In this study, Bio-Oss and DFDBA were osteoconduction and osteoinduction, respectively, during bone formation. Therefore, the combined two fillers may promote the healing after GBR in human extraction sockets.

It was reported that histologic healing state after GBR procedures is analyzed at extraction sockets but the histologic healing type wasn't classified. Therefore, we classi-

fied the histologic healing types from Type I to Type V in hope of aiding future studies on GBR. F/U period in relation to healing varied according to each site and histologic healing types also varied in this study. Differences between Type I and Type V are listed in Table 2. GBR results are based upon space maintenance, clot protection, clot isolation, maintenance of primary soft tissue closure, and management of overlying postoperative forces<sup>29-33)</sup>. The early exposure of the barrier membrane was considered to be an actual complication that can hinder the effectiveness of the GBR for future implant placements. Retention of the connective tissue or necrotic and inflamed tissue in the grafted sites may weaken the bone at the sites. It is likely that the results from a variety of techniques may differ. Within limited studies, suggests that membrane exposure in GBR is related to a negative effect in new bone formation following grafting in the extraction sockets<sup>34)</sup>. In this study, the healing type of membrane non-exposure group was Type V in 7 sites, Type IV in 2 sites. In the case of membrane exposure, the type varied from Type I to Type III. The specimens with a type I, II and III healing pattern might not show any evidence of new bone formation and the ALP activities because of membrane exposure. Type V showed new bone formation and positive ALP but did not show the proliferation of connective tissue, inflammation and necrosis. The proliferation of connective tissue differed between Type IV and Type V. In other word, the histology was that of normal bone in Type V compare with Type

IV according to the pattern of bone healing. Many studies reported that early exposure of the membranes during healing hinders the effectiveness of the GTR in the peri-implant tissues. It was reported that membrane exposure is more prevalent in smokers but all of the subjects in this study were non-smokers. In this study, most extraction sockets resulted from tooth extraction due to periodontitis. In some cases, endodontic treatment was done in the tooth adjacent to the extraction socket. In this study, the healing type of single root teeth was Type I in 2 sites, Type III in 2 sites. In the case of multiple root teeth, the type varied from Type II to Type V. Single root teeth were either extracted due to chronic periodontitis or endodontic treatment was done in the adjacent teeth. We believe that the tooth being extracted and the status of adjacent teeth would affect histologic healing types when comparing the histologic healing types of single root teeth and multiple root teeth.

## V. Conclusions

In this study, we classified the histologic healing types after GBR in human extraction sockets. And we compared the histologic healing pattern in two groups, i.e., the membrane exposure and membrane non-exposure group. The histologic healing types were classified from Type I to Type V. The healing type of membrane non-exposure group was Type V in 7 sites, Type IV in 2 sites. In the case of membrane exposure, the type varied from Type I to Type III. New bone formation was showed only Type IV and

Type V without membrane exposure. Within the limitation of this study, these results demonstrated that membrane exposure is related to a negative effect in new bone formation following grafting in the extraction sockets. Our results suggest that the classification of healing pattern may be useful in the evaluation of outcome of the GBR.

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## 인간의 발치와 내에서 골유도재생술 후의 치유양상

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이 연구는 임플란트를 식립하기를 원하는 전신건강상태가 양호하며 구강위생상태가 좋은 14명 환자(남자:8명, 여자:6명, 평균나이 : 44세)의 20개의 발치와 내에 흡수성 차폐막(BioMesh, Sam Yang Corporation, Korea)과 함께 탈회냉동 건조동종 골(dem-ineralized freeze-dried bone allografts, 250-500 $\mu$ m, Pacific Coast Tissue Bank, U.S.A.)과 이종골(Bovine-Bone, Bio-Oss 0.25-1.0 mm, Geistlich, Biomaterials and Osteohealth, Switzerland)을 1:1(부피)로 혼합하여 이식한 후 그 치유양상을 관찰하고자 조직학적 및 면역조직화학적으로 평가하였다. 이식재가 탈락되는 것을 방지하기 위하여 발치한 후 1개월이 경과된 후에 이식재와 차폐막을 위치시켰다. 표본제작을 위하여 이식술을 시행한 지 약 6개월 후에 임플란트를 식립하기 직전 식립부위에서 trephine bur로 골을 채취하였는데, 20증례 중 7증례에서 임플란트를 식립하기 전에 차폐막이 노출되었다. 차폐막이 노출되지 않은 것을 대조군으로, 노출된 것을 실험군으로 설정하였다. 조직학적인 관찰을 위하여 통상적인 방법에 따라 탈회 표본을 제작하였고, alkaline phosphatase(ALP)를 이용하여 면역조직화학적 염색을 시행한 후 골 형성 상태를 평가하여 다음과 같은 결과를 얻었다. 본 연구에서는 발치와내에서 골유도재생술 후 나타나는 치유 형태를 5가지 형태로 분류할 수 있었다. Type I, II와 III는 새로운 골 형성을 나타내지 않았고, 면역조직화학적 검사 시 ALP 음성 소견을 나타내었다. Type V는 새로운 골 형성과 ALP 양성 소견을 나타내었으나 염증, 괴사, 결합조직의 증식 등은 없었다. Type IV와 Type V의 차이는 결합조직의 증식여부로 구분되었다. 막이 노출되지 않은 증례들 중 7 증례에서는 Type V의 치유 형태를, 2증례에서는 Type IV의 치유 형태를 나타내었다. 막이 노출되었던 증례에서는 Type I, II, III의 다양한 치유 형태를 나타내었다.

본 연구결과, 발치와 내에 골유도재생술을 시행한 후 차폐막의 노출 여부가 신생골 형성에 중요한 영향을 미칠 것으로 사료되며, 본 연구에서 분류한 치유 형태가 향후 골유도재생술 후의 결과 분석에 활용될 수 있을 것으로 사료된다.