Histological observation of excellent bony remodeling in xenogenic bone graft for dental implant

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With the multiple practices of bone graft using different artificial bone regenerative substitutes, the bone graft procedures have been widely performed to increase the bony stabilization of dental implant. Xenogenic bone graft materials have been well developed because of their good biocompatibility and abundant source of bone materials. The present study demonstrated the histological findings from excellent bony remodeling in xenogenic bone graft biopsies compared to those findings in autogenous bone graft. For the graft bone biopsies which were usually done in 5-9 months after graft bone insertion, five types of histological grades including excellent, favorable, partial, degenerative, and poor bony remodeling could be assessed to give prognostic information for dental implant. However, recently the xenograft bone materials have been much improved and produced strong osteogenic effect. Among 239 cases of trephine bur-supported core bone biopsy the excellent bony remodeling was found in 20 cases (13.1%) out of 153 xenogenic bone grafts and in 13 cases (43.3%) out of 30 autogenous bone grafts. They produced abundant new bones on the surface of the graft bones in 5–9 months, and the graft bones were partly resorbed and also surrounded by the repetitive deposition of new bone. The osteophytic new bones showed strong birefringence under polarizing microscope, and were gradually elongated and anastomosed with each other to form trabecular bony networks which became proper stress-baring structures for dental implant. Their marrow stromal tissues were composed of loose connective tissue which was well vascularized but rarely infiltrated with inflammatory cells. The present study compared the histological features of excellent bony remodeling between xenogenic and autogenous bone grafts. Although the ratio of excellent bony remodeling in xenogenic bone graft was still low, 13.1%, the recent advance of xenogenic bone products was remarkable in biological aspect and almost comparable to the autogenous bones. Therefore, it was suggested that the xenogenic bone graft will be applicable to the bone regeneration procedures for dental implant with beneficial output in the near future.

Key words: Xenogenic bone graft, Excellent bony remodeling, Strong birefringence
I. INTRODUCTION

Xenografts have been developed extensively to use either alone or in combination with autogenous bone for the augmentation of extraction socket, lateral alveolar wall, or sinus floor elevation\(^1\)-\(^3\). However, controversy exists regarding the biocompatibility of xenograft materials. It was known that the implant survival rate was related to the quality of the reconstructed cortical plate by grafting with regenerative bony substitutes, and that the concomitant use of a collagen barrier to cover the osteotomy site seemed to improve the quality of the graft healing and survival rate of the implants loaded between 6 and 9 months after placement\(^3\).

The xenogenic materials obtained from bovine or porcine bones were similar to human cancellous bone, and showed high biocompatibility and osteoconductivity depending on the technical procedures of graft material production\(^4\)-\(^6\). In the series of our pathological observation of bone graft, the recent products of xenogenic bone graft materials have been improved and frequently achieved the excellent bony remodeling, producing good trabecular bony architecture anastomosed with each other within 5-9 months after graft bone insertion.

Although many authors insisted that the bone graft was essentially required for the socket and ridge augmentation, and sinus elevation before dental implant insertion, the bone graft still had a risk for the failure of bony remodeling\(^7\)-\(^8\). Actually some graft bones were rarely organized by new bone deposition until over 6 months after their insertion, but they remained in the tissue as dead bones and followed by inflammatory foreign body reaction\(^9\). However, little is known about the bony remodeling pattern around the inserted bone graft materials in human so far.

For the histological description of graft bone tissue, we could define five types of bony remodeling through the bone graft diagnosis for 239 trephine bur-supported core bone biopsies, i.e., excellent, favorable, partial, degenerative, and poor bony remodeling. Briefly, these histological grades of bony remodeling were made depending on the amount of new bone formation, the trabecular bony organization, and the bone graft prognosis. Therefore, the partial bony remodeling which showed weak osteogenic new bone formation was intermediate between favorable and degenerative bony remodeling in prognosis. The excellent bony remodeling indicated the complete success in bone graft, while the poor bony remodeling indicated the failure of bone graft.

However, in order to encourage to perform the xenogenic bone graft for dental implant, the present study selected the successful cases of xenogenic bone graft, which had produced the excellent bony remodeling in the histological observation. The histological characteristics of excellent bony remodeling in xenogenic bone graft were described in comparison with those of excellent bony remodeling in autogenous bone graft.

II. Materials and methods

Total 239 cases of the trephine bur-supported core bone specimens were collected from local dental clinics, and were pathologically examined in the Department of Oral Pathology, Gangneung-Wonju National University Dental Hospital (GWNUDH). They showed slight gender difference, i.e., 138 men and 101 women, and their age range was 31 to 78 years old. The usage of biopsy specimens filed in the Department of Oral Pathology, GWNUDH was approved by our institutional review board (IRB2016-11). Because there were serious conflicts involved with commercial competition and patent registration, their commercial product names and company names were hidden in this study.
The bone graft biopsies obtained from trephine bur-supported osteotomy to prepare the insertion site of dental implant were fixed in 10% neutral formalin, decalcified in 4% nitric acid, and embedded in paraffin wax. The graft tissues were micro-sectioned in 4 µm thickness, and routinely stained with hematoxylin and eosin, and followed by histological observation under the light microscope and polarizing microscope.

In the trephine bur-supported core bone biopsy in 5-9 months after bone graft, the bony remodeling of bone graft tissue was evaluated depending on the degree of new bone formation, bony adhesion between graft bones and host new bones, and bony maturation forming anastomosing trabecular bony network. As the marrow stromal tissue was an important factor to predict the prognosis of bone graft, the stromal changes of inflammatory reaction, fibrosis, and vascular dilatation, etc., were also assessed with the histological observation. All the graft tissues could be divided into five types of bony remodeling, i.e., excellent, favorable, partial, degenerative, and poor bony remodeling, of which terms might implicate the prognosis of bone graft. Their histological characteristics were briefly described as followings.

1. **Excellent bony remodeling**

   The excellent bony remodeling showed abundant new bone formation on the surface of the graft bone in 5-9 months, and the new bones became thickened to form osteophytes by repetitive deposition of new bone. The osteophytes were gradually elongated and anastomosed with each other by additional new bone formation, and resulted in the trabecular bony networks necessary to support for dental implant. Their marrow stromal tissues were usually composed of loose connective tissue, which were well vascularized with sparse inflammatory reaction.

   Many graft bones remained were directly surrounded by new bone deposition and gradually embedded into newly formed osteophytes. The bony adherence between graft bones and new bones was almost complete, and demarcated by reversal lines. The new bones were gradually matured and mineralized, and showed strong birefringence in lamellated bone pattern under the polarizing microscope. These graft bones might play a role for osteoinductive effect to produce mature trabecular bones, and were expected to undergo with good prognosis for dental implant stabilization.

2. **Favorable bony remodeling**

   The favorable bony remodeling diffusely showed active new bone formation around/on the graft bones, producing thick osteophytes by repetitive bone deposition. But the new bone formation was usually found around/on the graft bones only, and showed focal bony anastomosis between newly formed trabecular bones. The marrow stromal tissue was usually composed of loose connective tissue with few inflammatory cell infiltration. Occasionally the marrow tissue became partly fibrosed around the graft bones remained, but these fibrous tissue was usually well vascularized and composed of many young fibroblasts with plump nuclei.

   Some graft bones remained were directly surrounded by new bone deposition and gradually embedded into newly formed osteophytes. But the bony adherence between graft bones and new bones was incomplete, thereby, easily separated during histological micro-section procedures. Many new bones were still immature, and showed weak birefringence in woven bone pattern under the polarizing microscope. These graft bones might play a role for osteoinductive effect to produce osteoid new bones, which could undergo to be mature and mineralized subsequently. These graft bones were still unorganized to support for dental implant, but they were highly expected to undergo further new bone formation later and to produce the favorable prognosis for dental implant stabilization.
3. Partial bony remodeling

The partial bony remodeling showed osteoid new bone formation around/on some graft bones limitedly, and the new bones were still rudimentary and immature. The bony elongation in trabecular pattern was partly found, but the trabecular bone anastomosis was almost incomplete. Most of graft bones were diffusely scattered and surrounded by marrow fibrous tissue. The graft bones were usually not resorbed and remained in the stromal fibrous tissue. Their marrow stromal tissues were usually composed of fibrous connective tissue filled with abundant collagen bundles, and were distributed with relatively weak vascularity. Occasionally there appeared focal inflammatory reaction around the graft bones, recruiting macrophages and small round cells. Many graft bones were not resorbed and looked rigid with angled margins, however, some osteoid new bones were partly deposited around the graft bones. The bony adherence between graft bones and new bones was poor, thereby, the graft bones were gradually separated from the new bones by the ingrowth of stromal fibrous tissue. The new bones around/on the graft bones were still immature, and showed sparse/weak birefringence in woven bone pattern under the polarizing microscope. It was usually considered that the graft bone showed sparse/weak osteoinductive effect and slight osteoconductive effect in the graft tissue, expecting that further new bone formation will be retarded and attenuated. These graft bones were still not organized for the support of dental implant, therefore, careful management should be recommended in the following dental implant treatment.

4. Degenerative bony remodeling

The degenerative bony remodeling showed only focal immature new bone formation around/on the graft bones, but generally the graft bone were rarely resorbed and densely surrounded by collagenous fibrous tissue. The graft bones were almost not resorbed and looked rigid with acute angled margins, and they were also separated from each other likely floating rocks in the fibrous stromal tissue. The marrow stromal tissue was usually composed of dense fibrous tissue filled with thick collagen bundles, and diffusely infiltrated with macrophages and small round cells even in the absence of bacterial infection.

The bony adherence between graft bones and new bones was almost not observed, and resulted in the sequestration of graft bones in the stromal fibrous tissue. The focal new bones around/on the graft bones were found but almost immature and showed sparse birefringence under the polarizing microscope. It was usually considered that the graft bone showed no osteoinductive effect and rare osteoconductive effect in the graft tissue, expecting no further new bone formation later. These graft tissue may give sparse/weak support of dental implant and be easily affected by periodontal inflammation, therefore, active management should be recommended in the following dental implant treatment.

5. Poor bony remodeling

The poor bony remodeling hardly showed new bone formation from graft bones, which were almost sequestered likely bony foreign bodies. The graft bones became sclerosed and basophilic by staining with hematoxylin, and were not attached by the stromal fibrous tissue. Their marrow stromal tissues were also sclerosed with thick fibrous collagen bundles, and were distributed with only tiny vascular channels. There appeared diffuse inflammatory cell infiltration throughout the graft tissue, accompanied with focal bony resorption by osteoclasts.

The focal osteoid new bones around the graft bones could be found, but they were usually formed by the reparative reaction of host bony tissue. It was usually considered that the graft bone showed no osteoinductive and osteoconductive effect in the graft tissue, expecting no further new bone formation later. These graft tissue may give no support for
dental implant, but can deteriorate the periodontal environment leading to granulomatous inflammation. The aggressive management to get rid of the hazard of graft bones should be recommended in the following dental implant treatment.

With the above findings of bone graft, the present study was aimed to define the histological findings of excellent bony remodeling, which was considered as a complete success in bone graft for dental implant, particularly focused on the xenogenic bone graft which was frequently used for the preparation of dental implant. The histological findings of excellent bony remodeling in xenogenic bone graft were compared with those of excellent bony remodeling in autogenous bone graft. And in order to know their bony maturation, the decalcified sections of graft bony tissues were observed under the polarizing microscope. Their microscopic images were captured by a digital camera (DP-70®, Olympus Co., Japan).

Among 239 cases of trephine bur-supported core bone biopsies, 153 cases of xenogenic bone graft and 30 cases of autogenous bone graft were analyzed for their clinical and histopathological features. 20 cases out of 153 xenogenic bone grafts and 13 cases out of 30 autogenous bone grafts showed excellent bony remodeling. Among the 20 xenogenic bone grafts exhibiting excellent bony remodeling, 9 cases were from bovine bones, and 11 cases were from porcine bones. Both the xenogenic and autogenous bone grafts exhibiting excellent bony remodeling showed similar gender distribution and graft insertion duration, therefore, it was considered that both groups were comparable with each other to evaluate their biocompatibility (Table 1).

### III. Results

Total 239 cases of graft bone biopsies were examined through histological observation, and among them 33 cases (14.1%) showed the features of excellent bony remodeling, expecting to be complete success in bone graft for dental implant. Among 33 cases of excellent bony remodeling, 20 cases (13.1%) was from xenogenic bone grafts (153 cases), and 13 cases (43.3%) was from autogenous bone grafts (30 cases). The xenogenic bone graft was more frequently done for ridge augmentation (9 cases) and sinus elevation (7 cases) than socket augmentation (4 cases), while the autogenous bone graft was more frequently done for socket augmentation (9 cases) than ridge augmentation (3 cases) and sinus elevation (1 case).

The excellent bony remodeling in xenogenic bone graft tissue usually showed abundant new bone deposition on the surface of graft bones. The primary linear new bones became elongated and thickened, and underwent to form the anastomosing trabecular bony network (Fig. 1 A1-C1). Under the polarizing microscope the graft bones themselves showed no birefringence, but the new bones induced by the graft bones became organized/mineralized and showed conspicuous birefringence in woven bone pattern (Fig. 1 A2-C2). Some xenogenic bones were almost surrounded by new bones, and embedded into the new bones. The adherence between the embedded graft bone and the new

### Table 1. Excellent bony remodeling of xenogenic and autogenous bone grafts found in 239 cases of graft bone biopsies

<table>
<thead>
<tr>
<th>Types</th>
<th>case number (total)</th>
<th>female/age (cases/years)</th>
<th>male/age (cases/years)</th>
<th>socket augmentation</th>
<th>ridge augmentation</th>
<th>sinus elevation</th>
<th>duration (months)</th>
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</thead>
<tbody>
<tr>
<td>xenogenic bone graft</td>
<td>20(153)</td>
<td>7/59.6</td>
<td>13/61.3</td>
<td>4</td>
<td>9</td>
<td>7</td>
<td>6.9</td>
</tr>
<tr>
<td>autogenous bone graft</td>
<td>13(30)</td>
<td>8/55.3</td>
<td>5/63.5</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>5.2</td>
</tr>
</tbody>
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Fig. 1. Photomicrographs of excellent bony remodeling in xenogenic bone graft tissue, stained with hematoxylin and eosin, A2, B2, and C2: Observation under the polarizing microscope in the same areas of A1, B1, and C1, GB: graft bone, NB: new bone, A1: Abundant new bone deposition on the surface of graft bones, forming anastomosing trabecular bony network, A2: Under the polarizing microscope the graft bones (GBs) showed no birefringence, B1: Osteoinductive potential of GB, producing new bones (NBs) in woven bone birefringence (B2), C1: Some xenogenic bones (arrows) were almost surrounded by NBs, The embedded GB (arrows) showed rare birefringence (C2), Also noted the marrow spaces filled with relatively loose connective tissue.
Fig. 2. Photomicrographs of excellent bony remodeling in xenogenic bone graft tissue (A and B) and autogenous bone graft (C), stained with hematoxylin and eosin, A2, B2, and C2: Observation under the polarizing microscope in the same areas of A1, B1, and C1, GB: graft bone, NB: new bone, A1: Active new bone deposition on the surface of graft bones (GBs), producing thick trabecular bones, A2: Under the polarizing microscope the GBs showed no birefringence, B1: Some xenogenic GB was separated from the NBs, but continuously osteoconductive to form mature NB exhibiting strong birefringence (B2), C1: Autogenous GBs produced abundant NBs. They were rapidly surrounded by the NBs, The embedded GB still showed marked birefringence (C2).
bone was almost perfect, so that the embedded graft bones were found in the central part of the osteophytic bones. And the marrow stromal tissue was usually composed of loose connective tissue (Fig. 1 B1 and C1).

In the comparison between the xenogenic and autogenous graft bones the xenogenic graft bones showed the active new bone deposition on the surface of graft bones, producing thick trabecular bones, similar to the autogenous graft bones (Fig. 2 A1-C1). But under the polarizing microscope the xenogenic graft bones showed no birefringence due to their intensive purification procedures, but the autogenous graft bones showed strong birefringence in lamellated bone pattern (Fig. 2 A2-C3). However, the new bones induced by the xenogenic graft bones rapidly matured and showed strong birefringence similar to the new bones induced by the autogenous graft bones (Fig. 2 B2 and C2).

IV. Discussion

Xenogenic bones obtained from vertebrate animals may be composed of the same calcium hydroxide apatite with human bones, but they may have different organic matrices which are strongly antigenic to human immune system. The multitude animals familiar to human being, i.e., bovine, porcine, equine, and gout, etc., are available for the xenogenic bony substitutes so far. The present study demonstrated the xenogenic graft bones from bovine and porcine for their excellent bony remodeling in the dento-alveolar tissue. Among 239 cases of trephine bur-supported core bone biopsy, only 20 cases of excellent bony remodeling were found in 153 xenogenic bone grafts, whereas 13 cases of excellent bony remodeling were found in 30 autogenous bone grafts. As the excellent bony remodeling has an implication that their bony healing and organization are almost complete to support the associated dental implant, the 20 cases of excellent bony remodeling (13.1%) out of 153 xenogenic bone graft are statistically significant enough to indicate the possibility of perfect bone graft success in the near future, if the xenogenic bone graft will be improved properly.

In this study the xenogenic bone graft was more frequently done for ridge augmentation (9 cases) and sinus elevation (7 cases) than socket augmentation (4 cases) compared to the autogenous bone graft, which was usually done for socket augmentation (9 cases out of 13 cases). These differences were closely related to the fact that the xenogenic bone was more available to use large amount of graft bone materials for the ridge augmentation and sinus elevation procedures compared to the autogenous bone.

The allogenic bone obtained from human bone is actually limited in usage due to the ethical and legal problems, and possible transmission of hidden diseases, i.e., prion diseases, viral diseases, etc. Thus, it is thought that the xenogenic bones obtained from fresh animal bones fed in less polluted environment would be the best choice for regenerative bony substitutes, which are relatively free from ethical and legal problem.

Although the xenogenic graft bones are obtained from different animals including, bovine, equine, porcine, etc., their basic structures of bony matrix are almost similar. So that it is thought that regardless the bony sources the success of xenogenic bone graft is usually depending on the purification procedures of bone matrix to remove the foreign organic materials from xenogenic bone. However, in this study the xenogenic bones found in the cases of excellent bony remodeling showed almost no birefringence under the polarizing microscope contrast to the autogenous bones, which continuously revealed the strong birefringence similar to the newly formed trabecular bones. These findings may indicate that the xenogenic bones were highly purified by different methods of
deproteination and demineralization\textsuperscript{17,18}).

The cancellous bony remodeling is also achieved through the wound healing procedures accompanied with \textit{de novo} angiogenesis and osteogenesis in the marrow stromal tissue. And the loads of foreign materials, the xenogenic bones, should be resorbed and organized by the cellular reaction from the marrow stromal tissue\textsuperscript{19-21}). In the histological observation the regenerating features of the marrow stromal tissue are mostly important to induce the osteogenic cell differentiation for \textit{de novo} osteogenesis. The marrow stromal tissue is usually composed of well vascularized loose connective tissue in the normal bony tissue\textsuperscript{20}). Therefore, in this study the status of marrow stromal tissue was primarily assessed for the excellent bony remodeling of xenogenic bones, and followed by the histological features of new bone production and bony adhesion between the xenogenic bones and the newly formed bones.

In the present study the excellent bony remodeling of xenogenic bones showed remarkable histological features of abundant new bone formation, organization of newly formed trabecular bones to produce the anastomosing bony network, and well vascularized marrow stromal tissue. Particularly, the marrow stromal tissue was composed of loose connective tissue with many young fibroblasts and minimum fibrous collagen bundles. Because every cellular reactions for the \textit{de novo} angiogenesis, osteogenesis, and inflammation may occur in the marrow stromal tissue, the histological findings of marrow stromal tissue in the graft bone are important criteria to predict the following progresses of bony remodeling\textsuperscript{19}). The excellent bony remodeling of both xenogenic and autogenous bones clearly revealed the well vascularized marrow stromal tissue composed of relatively loose connective tissue with few inflammatory cell infiltration.

The present study is a preliminary report from the accumulated data of trephine bur-supported core bone biopsies in order to announce the successful progresses of xenogenic bone grafts performed recently. Although many failures and complications were reported in the bone graft treatment using different kinds of xenogenic bony materials\textsuperscript{22)}, the number of successful xenogenic bone graft has been gradually increased in the pathological diagnosis of trephine bur-supported core bone biopsies. In this study five histological types of graft bone remodeling progresses were tentatively described, and defined the histological features of excellent bony remodeling in the xenogenic and autogenous bone grafts. It was assumed that the excellent bony remodeling in the xenogenic bone grafts showed almost perfect bony production and organization similar to those of autogenous bone grafts, and that the excellent bony remodeling might imply the good prognosis for the dental implant success. The statistical ratio of excellent bony remodeling in xenogenic bone grafts is still 13.1\% which is much less than 43.3\% in autogenous bone grafts. However, the successful value, 13.1\%, is more than the scientific negative value, 5\%, thus it is significant enough to encourage the investigation of xenograft bony materials to produce the appropriate regenerative bony substitutes for the beneficial output of dental implant in the future.

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\textbf{REFERENCES}


