Comparative Histomorphometric Analysis of Extraction Sockets Healing Implanted with Bovine Xenografts, Irradiated Cancellous Allografts, and Solvent-Dehydrated Allografts in Humans

Dong-Woon Lee, DDS, MS1/Sung-Hee Pi, DDS, PhD2/Suk-Keun Lee, DDS, PhD3/Eun-Cheol Kim, DDS, PhD4

Purpose: Bovine-derived bone xenograft and mineralized cancellous bone allograft have been successfully used as bone substitutes in dental surgery, but few clinical studies in humans have been reported. The objective of this study was to compare the osteoconductive effects of deproteinized bovine bone mineral (DBBM), irradiated cancellous allograft (ICA), and solvent-dehydrated allograft (SDA) when used to preserve extraction sockets. Materials and Methods: Twenty patients received bone grafting in extraction sockets with DBBM (n = 7), ICA (n = 8), or SDA (n = 5). Core biopsies were taken from each graft site 4 to 6 months after grafting and were evaluated histomorphometrically. One-way analysis of variance was used to compare each variable. P values less than .05 were considered significant. Results: DBBM induced more new bone deposition in the periphery of the native bone particles than ICA or SDA, whereas ICA and SDA were more frequently surrounded by fibrous tissue than DBBM. In addition, DBBM retained more residual graft bony particles than ICA or SDA. Conclusions: Based on these findings, the DBBM showed more of an osteoconductive effect than ICA or SDA, producing a more rigid bony structure. It is therefore suggested that DBBM may be more favorable for the preservation of extraction sockets than allogeneic graft materials.

Key words: bovine xenograft, histomorphometry, mineralized cancellous bone allograft, socket preservation

Although placement of dental implants with autogenous bone grafts has been performed frequently for oral rehabilitation after the loss of mandibular or maxillary bone, insufficient height or width of the alveolar bone at the implantation site still poses difficult problems. Full coverage of the implant surface with bone is required for the success of implant treatment.1 The augmentation of local defects of the alveolar ridge with bone grafts may offer a method to enable implantation in sites with insufficient bone volume. Surgical procedures to create sufficient bone volume have been developed, and an autogenous bone graft, harvested either extraorally or intraorally, appears to represent the gold standard. Xenografts, alloplastic bone grafts, and allografts have also been proposed, either alone or in combination with autogenous bone grafts, for localized alveolar ridge augmentation.2–5 Deproteinized bovine bone mineral (DBBM), irradiated cancellous allograft (ICA), and solvent-dehydrated allograft (SDA) have been used as bone grafts in dental surgery.6–9 DBBM is a natural bone substitute made from bovine bone that has been proven biocompatible and osteoconductive by many studies.10,11 However, an allograft might transmit disease, although several solutions to this problem have been introduced.12,13 Clinically, mineralized bone is more adaptable than de-mineralized bone, so mineralized bone allografts such as ICA and SDA obtained from human cadaver sources are used frequently.8,14–16 However, few clinical data are available regarding bone regeneration after the use of allografts or xenografts in human extraction sockets.

1Chairman, Department of Periodontology, Seoul Veterans Hospital, Seoul, South Korea.
2Assistant Professor, Department of Periodontology, College of Dentistry, Wonkwang University, Iksan, South Korea.
3Professor, Department of Oral Pathology, College of Dentistry, Kangnung National University, Gangneung, South Korea.
4Professor, Department of Oral and Maxillofacial Pathology, College of Dentistry, Wonkwang University, Iksan, South Korea.

Correspondence to: Dr Eun-Cheol Kim, Department of Oral and Maxillofacial Pathology, Dental College, Wonkwang University, Shinyoungdong 344-2, Iksan City, Jeonbuk, 570-749, South Korea. Fax: +82-63-850-7313. Email: eckwkop@wonkwang.ac.kr

Dong-Woon Lee and Sung-Hee Pi contributed equally to this work.

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The aim of this study was to compare the osteoconductive effects of DBBM, ICA, and SDA in conjunction with the preservation of human extraction sockets before dental implant placement.

MATERIALS AND METHODS

Study Population and Design
This study was approved by the Dental Clinic Committee of Seoul Veterans Hospital (SVH) and was conducted from May 2004 to November 2005. All included patients provided written informed consent, as requested by the Institutional Review Board of SVH before participating in the study. The patients’ medical and dental histories were reviewed. All the patients were healthy, with no underlying systemic diseases. The selected subjects were 20 patients who underwent treatment for severe chronic periodontitis. All patients had periodontally hopeless teeth without apical lesions. The exclusion criteria for socket preservation included periapical lesions, acute abscesses, and severe horizontal bone resorption. All of the subjects underwent socket preservation using bone graft materials prior to staged implant placement. After extraction, the socket depths were measured with a periodontal probe; a site was excluded if the deepest socket was 5 mm or less. The exclusion criteria also ruled out those with poor plaque control and smokers.

Surgical Procedure
The extraction sites were grafted using the socket preservation procedure. With the use of local anesthesia, minimally traumatic extraction of the tooth was performed with the use of periotomes, minimum forceps rotation, and sectioning of the tooth, if needed. Any remaining granulomatous tissue was removed carefully from the socket wall with a molar curette or similar surgical instrument. The subjects were divided into three treatment groups by two calibrated examiners, and the extraction sockets were filled with DBBM (Bio-Oss; Geistlich Pharma; n = 7), ICA (IBA; Rocky Mountain Tissue Bank; n = 8), or SDA (Puros Allograft; Zimmer Dental; n = 5) (Fig 1).
The number of patients in each treatment group differed because patient informed consent for this study was limited. Over a follow-up period of 6 months, one patient in the SDA group dropped out of the study. The three treatment modalities were not determined by randomized choice.

The grafted sockets were covered with resorbable membranes (Bio-Gide, Geistlich Pharma) and were closed using interrupted or crisscrossed sutures. Postoperatively, analgesics and antibiotics were prescribed.

### Implant Placement and Biopsies

The patients were scheduled for dental implant surgery 4 to 6 months (mean, 4.6 ± 0.7 months) after grafting (Table 1). All surgical sites healed uneventfully. The implants were placed in the center of the extraction socket using a prefabricated stent. The implant sites were prepared using a trephine bur (BonTempi) and drill. A trephine with a 2-mm inner diameter was used to harvest 2 mm of bone from the central part of the pre-existing socket. Bone cores, which did not include original host bone, were extracted from the augmented areas. Radiographs were obtained to confirm whether the area of the bone core was suitable for implant placement.

### Histologic Preparation and Analysis

The specimens were sectioned longitudinally along the major axis. The core specimens were fixed with 10% buffered formalin. Each core was decalcified with 5% formic acid and embedded in paraffin. Serial 4- to 6-µm-thick longitudinal sections were cut through the central area of the core specimens using a microtome (RM2155; Leica). Three slides were obtained for each specimen. The first slide was stained with hematoxylin and eosin (H&E), and the other two slides were stained with modified Masson trichrome for routine histology and histomorphometric examination.

A projection microscope (Olympus) with a digital image capture device was used for morphometric measurements (magnification ×40). The digital image captured from the microscope was fed into a personal computer (Sense X20; Samsung) and traced using image-analysis software (Image Pro Plus version 4.5; MediaCybernetics). These traces yielded a set of polygons, which allowed the software to calculate sets of areas. The area of each polygon was transferred to a spreadsheet program (Office Excel 2003; Microsoft) to archive the statistical analysis.
Three different areas were measured: new bone, residual graft particles, and fibrous tissue. Bone and particle fragments were distinguished by the presence of separation lines and staining characteristics. New bone was distinguished from nonvital grafted bone by the presence of cells in the lacunae of the bone samples. Each specific tissue area was divided by the total sample area to calculate the percentage of each area.

**Statistical Analysis**
Statistical significance was evaluated using one-way analysis of variance (ANOVA) using SPSS version 10.0 (SPSS). The mean and standard deviation were calculated for each variable. The Tukey multiple comparison test was evaluated at a significance level of .05 ($P < .05$).

**RESULTS**
The sample comprised 20 patients (16 men and 4 women) with ages ranging from 39 to 68 years (mean, 54.8 ± 6.8 years). At 4 to 6 months (mean, 4.6 ± 0.7 months) postoperatively, the grafted sites had healed uneventfully. None of the 20 patients experienced any complications.

Histologically, new bone was deposited in the periphery of the DBBM particles, with increased osteoblastic activity (Figs 2a and 2b). Inflammatory cell infiltration was rare in the regenerated graft tissue. Eventually, most of the DBBM particles were incorporated with thin cortical bone that was newly deposited, forming osteophytes, while only a few DBBM particles were directly connected with the adjacent stromal connective tissue.

The ICA was gradually resorbed and remained as basophilic coagula containing many dead bone chips, on which new bone was irregularly deposited, producing thick bony trabeculae that were partly anastomosed with each other (Figs 3a and 3b). Inflammatory cell infiltration was focally observed in the stromal connective tissue, where ICA particles were resorbed by osteoclasts. Consequently, the implanted ICA was partly incorporated with new bone, irregularly forming osteophytes, whereas many ICA particles still remained in the fibrous stroma of the graft site.

The SDA was actively resorbed and remained as bony spicules partially incorporated with new bone (Figs 4a and 4b). The inflammatory cell infiltration was rare. The resorbed SDA particles actively induced new bones covered with a thick osteoid rim and conspicuous osteoblast layer. The new bone formation on the surface of SDA particles was usually irregular and separated, so that the bony trabeculae were thick and rarely anastomosed.
Although the DBBM, ICA, and SDA particles all participated in new bone formation, the DBBM particles had resorbed less than the ICA and SDA particles during the 4 to 6 months (mean 4.6 ± 0.7 months) after grafting. The DBBM showed mostly osteoconductive new bone formation, whereas the ICA and SDA usually showed osteoinductive new bone formation. The DBBM produced more intimate contact by “bridging” new bone between DBBM particles (and new bone) than the other graft materials. In contrast, the ICA and SDA were gradually surrounded by more abundant fibrous tissue than the DBBM.

The average areas of new bone, fibrous tissue, and remaining graft particles are summarized in Fig 5. Based on the one-way ANOVA and Tukey test, DBBM had a larger area of new bone (23.6%) and a lower area of fibrous tissue (34.1%) than ICA (45.9%) or SDA (46.3%). In addition, DBBM had significantly more residual bony particles than ICA or SDA (Fig 5).

DISCUSSION

Because it is a deproteinized bone substitute, DBBM contains only a calcium phosphate medium without bone matrix proteins. It has been used to augment the resorbed alveolar ridges of adults10 and has been proven biocompatible and osteoconductive in several studies.10,17 In human studies using DBBM as a bone substitute, the reported area of new bone formation was 16% to 46% and that of residual graft particles was 16% to 30%.17–20 In this study, areas grafted with DBBM showed mean areas of new bone and residual graft particles of 23.6% and 25.4%, respectively, similar to previously reported values.18–21 The minor differences in values are likely related to differences in study design, operative procedures, and healing period.

ICA is an allograft material that was irradiated with 0.00025 to 0.00038 Gy to remove its antigenicity and then frozen at –75°C. Although SDA is another allograft material in which antigenicity and virus transmission are reduced using a five-step process,15,16 it should be resorbed by osteoclasts in the graft site, and then may produce intense osteoinductive effects for new bone formation. Some studies have found that ICA reacts similarly to autologous bone graft and is replaced by new bone consistently and predictably at low cost with few complications.22,23 Most studies using ICA have been preliminary, and few histologic or histomorphometric findings have been reported. In this study, ICA resulted in a mean new bone area of 17.2%, which is a better result than in other studies.24,25

SDA has demonstrated partial removal of its matrix proteins by different solvent solutions, has
been grafted in extraction sites and human maxillary sinuses, and examined histologically in case reports. From et al found that the results with SDA (25.2% vital bone, 58% marrow tissue, 16.8% remaining particles) 9 months after sinus grafting were favorable compared to other bone substitutes. 26 Nounibisi et al reported 40.33% new bone and 4.67% remaining graft particles, implying a difference in graft turnover caused by structural changes during the preparation of bone. 27 With SDA, the present authors obtained less new bone (12.0% new bone and 13.7% residual particles) compared to previous studies. 26, 27

In this study, DBBM showed consistent osteoconductive effects, representing significantly greater mean areas (P < .05) of new bone and residual graft particles, with less remaining fibrous tissue than ICA or SDA. The results also showed that ICA and SDA were transformed into new bone rapidly through osteoinductive mechanisms, but they were gradually surrounded by cellular and vascular fibrous tissues. However, the remaining particles of DBBM led to the direct deposition of anastomosing bony trabeculae more than with ICA and SDA in the 4 to 6 months after grafting.

CONCLUSION

Overall, since demineralized bovine-derived bone mineral (DBBM) resulted in the most favorable osteoconductive effect in extraction sockets prior to dental implantation, it may be applicable to bone defects associated with severe periodontitis. DBBM may succeed in maintaining volume during healing (for example, with one-wall defects), whereas irradiated cancellous allograft and solvent-dehydrated allograft, which are rapidly resorbing materials, might be helpful for intact bone defects, such as three-wall defects and fresh extraction sockets.

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REFERENCES