INTRODUCTION

Among the various factors that cause alveolar bone atrophy, such as trauma, tumors, and aging, the most common causative factor is tooth extraction. Extractions lead to resorption and atrophy of the alveolar ridge with a wide range of dimensional changes that differ among individuals\(^1,2\). Some clinical studies have demonstrated substantial dimensional changes in the surrounding alveolar bone after tooth extraction\(^3-5\). The vertical and horizontal dimensions of the alveolar ridge are important for ideal implant positioning. Therefore, preservation of the alveolar ridge after tooth extraction is an essential component of implant treatment. Socket or ridge preservation models involving various materials (e.g., bioactive glass, demineralized freeze-dried bone allografts, and xenografts) have been reported to effectively prevent alveolar ridge atrophy caused by tooth extraction\(^3,6,7\).

Bovine bone substitute (BBS; Bio-Oss\(^\circledR\); Geistlich Pharma, Wolhusen, Switzerland) has been well studied thus far and has shown efficacy in preserving the extraction socket; however, it is not an entirely ideal material because it is not resorbed after implantation\(^6,10\). Placement of Bio-Oss collagen\((i.e., \ 90\% \mathrm{Bio-Oss} \ \mathrm{granules} \ 10\% \ \mathrm{collagen})\), which promote vascularization and epithelial regeneration) in the socket was insufficient to prevent resorption of the buccal and palatal bone walls in a randomized clinical trial\(^11\). Ideal bone grafting materials should exhibit osteoinductivity and osteoconductivity, mechanical strength, and safety, while ensuring cost-effective application, allowing both resorption and replacement by natural bone. Synthetic bone substitutes are considered effective materials because they can eliminate contamination by unknown factors and avoid differences in inter-lot performance associated with natural products. Typical synthetic bone substitutes currently used in dentistry include hydroxyapatite (HA) and beta-tricalcium phosphate (\(\beta\)-TCP). HA is osteoconductive and biocompatible, and its replacement by natural bone is a considerably slow process\(^12,13\). \(\beta\)-TCP has equivalent osteoconductivity to HA; however, it occasionally causes difficulty in bone mass maintenance because of its rapid resorption\(^14,15\).

Bone grafting materials using HA or \(\beta\)-TCP have been developed and used in some clinical applications; however, an ideal material for maintaining the height and width of sockets and/or ridges has not yet been developed.

Commercially available carbonate apatite (CAP) granules are chemically pure; they are manufactured by a dissolution-precipitation reaction in aqueous solution using a calcite block\(^16,17\) rather than through a sintering process. Notably, CAP has been reported to activate osteoclasts\(^18,19\). Osteoblasts are activated by a signaling factor released from activated osteoclasts, which supports excellent bone formation ability and bone replacement similar to that of autologous bone\(^20\). In transplantation experiments involving rat tibias, low-crystalline CAP showed osteoconductivity greater than that of sintered HA, as well as resorption by osteoclasts and direct bone formation by osteoblasts; these characteristics were similar to those of bone\(^21\). In clinical studies using CAP for maxillary sinus floor elevation and simultaneous implant placement, all patients had normal findings within 1 year postoperatively and exhibited a consistent...
augmentation in bone height. Although BBS, β-TCP, and CAP are reportedly useful for bone formation in animal experiments and in various clinical applications, the ability of CAP to preserve ridges after implant placement has not been determined. Here, we aimed to evaluate the efficacy of bone grafting materials in bone formation for dental implant treatment by assessing early implant stability and preservation of augmented bone after implant placement.

MATERIALS AND METHODS

Experimental animals

The animal selection, management, and surgical procedures were approved by the animal experiment ethics committees of HAMRI (approval no. 18-H076) and Kagoshima University (approval no. D19037). The care of animals to minimize pain and discomfort was in accordance with institution guidelines. Ten healthy male beagle dogs were used in this experiment. The dogs were 10 months old, weighed 8.65–10.10 kg, and had fully erupted permanent dentitions. Prior to the experiment, all dogs were housed individually, and a 1-week acclimatization period was used to ensure standardized environmental factors. All surgical procedures were performed with the dogs under general anesthesia. General anesthesia was performed by intramuscular injection of a combination of the following drugs: 10 mg/kg ketamine (Daiichi Sankyo Propharma, Tokyo, Japan) and 4 mg/kg xylazine (Bayer Yakuhin, Osaka, Japan). Dental infiltration anesthesia was administered at the surgical site using 2% lidocaine hydrochloride (Dentsply Sirona, Tokyo, Japan). The same dentist performed all tooth extraction and implant placement procedures.

Surgical procedure

Figure 1 shows the experimental protocol used in this study. All defects were designed to minimize the impact on mastication and to ensure a consistent experimental model among the dogs. Because the width of mandibular bone becomes narrow toward the mesial region in dogs, a defect model was created at the left and right fourth premolar sites to ensure defect alignment. After bilateral extraction of the fourth premolar, an identical box-type dehiscence defect (10-mm mesiodistal diameter, 4-mm buccolingual diameter, and 5-mm depth) was surgically prepared at the site of extraction using a fissure bur (Fig. 1C). One of the following four treatment modalities was applied to each defect: control (sham operation; no grafting material), β-TCP (Cerasorb M®, Curasan, Frankfurt, Germany), BBS (Bio-Oss®, or CAP (Cytrans® M size, GC, Tokyo, Japan) (Fig. 1D). Implant placement was performed 7 weeks after the defect had been filled with the designated material. A dental implant (3.0 mm in Ø and 8 mm in length) (SETIO® Plus, GC) was placed at the height of the central bone margin of the regenerated bone. A single specialist with 20 years of experience in implant treatment performed all implant placements using a uniform procedure. The insertion
Fig. 2 Method used to measure the vertical height of the bone regeneration site. X-rays were taken at 5 weeks after implant placement (12 weeks after grafting material placement). Distortion of the X-ray photograph was corrected by referring to the position of the cementoenamel junction of the adjacent tooth and the length of the placed implant. In Fig. 2, (A) indicates a line connecting the cementoenamel junction of both adjacent teeth, while (B) indicates the implant platform level. (A-B) indicates the distance between (A) and (B). (C) indicates the bone margin at 5 weeks after implant placement (12 weeks after grafting material placement); (A-C) indicates the distance between (A) and (C). (A-B) and (A-C) indicate the shortest distance between each line. (D) indicates the sum of the implant fixture length and the healing abutment height. The actual length of (D) was 11 mm. The actual values of (A-B) and (A-C) were calculated using the ratio of the length on the X-ray photograph of (D) to the actual length of (D). Because the implant platform was matched with the bone level at the implant placement as shown in Fig. 1E, subtracting the value of (A-B) from the value of (A-C) represented the reduced bone level during the 5 weeks from implant placement to sacrifice. A smaller value obtained from these calculations indicated greater ridge preservation.

Insertion torque during implant placement and implant stability measurement
The insertion torque during implant placement was measured using an implant micromotor (GC implant motor IM-III; GC), which can measure in-use torque data. Implant stability was measured by resonance frequency analysis (Ossstell®, Integration Diagnostics, Göteborg, Sweden) and recorded using the implant stability quotient (ISQ) at implant placement and 5 weeks after implant placement. The evaluation was based on a report suggesting that no implant with an ISQ exceeding 60 had previously failed.

Histologic examination and histometric analysis
Five weeks after implant placement, the dogs were euthanized with an overdose of thiamylal sodium (Nichi-Iko Pharmaceutical, Toyama, Japan) (≥50 mg/kg) and their mandibles were collected. Immediately after collection, the samples were fixed with 10% neutral buffered formalin (FUJIFILM Wako Pure Chemical, Osaka, Japan). After tissue fixation, the non-decalcified samples were embedded in methyl methacrylate to prepare polished specimens with a thickness of 25–40 μm in the mesiodistal direction. All specimens were subjected to Villanueva–Goldner staining, showing mature and immature bone in green and red, respectively; histologic examinations and histometric analyses were then performed using an optical microscope. The ratio of the length of mature bone in contact with the implant to the length of the implant was used as an indication of bone formation around the implant.

Statistical analysis
The mean and standard deviation were calculated, and one-way analysis of variance followed by Tukey’s test was used to compare variables among the four groups. Statistical analyses were performed using SPSS Statistics for Windows, version 25 (IBM, Armonk, NY, USA).

RESULTS
Quality of regenerated bone produced with each bone grafting material
The quality of regenerated bone after ridge preservation with each bone grafting material was evaluated by measuring the insertion torque during implant placement and the ISQ values immediately after implant placement. The insertion torque during implant placement tended to be higher in the CAP group; however, there was no significant difference among the groups (Fig. 3A). There were no significant differences in ISQ values immediately after implant placement (Fig.
Fig. 3 Insertion torque during implant placement and implant stability immediately after implant placement.  
(A) The insertion torque during implant placement was measured using an implant micromotor. There were no significant differences in the insertion torque among the groups (mean±standard deviation, n=5).  
(B) Implant stability immediately after implant placement was measured by resonance frequency analysis and recorded as the ISQ. There were no significant differences in the ISQ values among the groups (mean±standard deviation, n=5).

Fig. 4 Representative X-rays.  
Representative X-ray photographs 5 weeks after implant placement (12 weeks after grafting material placement). (A) Control; (B) β-TCP; (C) BBS; and (D) CAP groups.

5 weeks after implant placement  
(12 weeks after grafting material placement)

Control

β-TCP

BBS

CAP

Fig. 5 Vertical height of the reduced bone level.  
The vertical height of the reduced bone level 5 weeks after implant placement was calculated from X-rays.  
(B) In the BBS transplanted group, the reduced bone level around the implant 5 weeks after implant placement was significantly greater than in the other groups (mean±standard deviation, n=5) *p<0.05.

3B). In all groups, the mean ISQ values were >60.

Evaluation of bone level after ridge preservation  
Figure 4 shows representative X-ray images taken 5 weeks after implant placement (12 weeks after grafting material placement). As shown in Fig. 1E, the implant platform matched the top of the formed bone. Therefore, the reduced bone level 5 weeks after implant placement [(A-C)-(A-B)] was evaluated by measuring the distance of the bone level around the implant from the implant platform. The reduced bone level around the implant in
Implant stability at 5 weeks after implant placement was measured by resonance frequency analysis and recorded as the ISQ. There were no significant differences in the ISQ values among the groups (mean±standard deviation, \( n = 5 \)).

**DISCUSSION**

In the present study, we investigated the efficacy of CAP, \( \beta \)-TCP, and BBS in forming bone during dental implant treatment by assessing early implant stability and preservation of augmented bone after implant placement in a canine model of mandibular defects. Importantly, we found no significant differences in insertion torque during implant placement, regardless of the preservation materials used. This indicated that there were no differences in the quality of the formed bone; however, CAP tended to have a high insertion torque, suggesting that it may have contributed to earlier bone formation than the other materials. The bone level around the implant 5 weeks after implant placement was significantly lower in the BBS group. This result is consistent with a report that BBS alone has poor bone formation ability *in vivo*\(^2\). However, in this study, we found no significant differences in ISQ values among the groups, either at implant placement or at 5 weeks after implant placement. We expected that the ISQ values would significantly differ according to changes in the quantity and/or quality of regenerated bone in our model. However, because of the robust bone regeneration, we did not observe differences in the ISQ values among the groups. Furthermore, the ISQ values exceeded 60 in all groups at implant placement and at

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**Fig. 6** Implant stability at 5 weeks after implant placement.

**Fig. 7** Representative microscopy photographs.

The mature bone-to-implant contact ratios calculated from microscopy photographs are shown. There were no significant differences in the bone-to-implant contact ratio among the groups (mean±standard deviation, \( n = 5 \)).

**Fig. 8** Bone-to-implant contact ratio.

*Implant osseointegration with regenerated bone and mature bone level after implant placement*

Figure 6 shows the ISQ values 5 weeks after implant placement. There were no significant differences in ISQ values among the groups; the mean ISQ values were >60 in all groups. Figure 7 shows representative microscopy photographs of specimens with Villanueva–Goldner staining. The BBS group tended to have a lower ratio of mature bone regeneration around the implant; however, the mature bone-implant contact rates were not significantly different between the groups (Fig. 8).
5 weeks after implant placement. It has been reported that osseointegration is achieved even with immediate loaded implants when the ISQ value exceeds 54\textsuperscript{25}. In this study, all implants had sufficient initial fixation to withstand immediate loading.

The BBS used in this study is composed of low-absorbable demineralized bovine bone and is one of the most clinically studied xenografts. The BBS has demonstrated good results when used as a bone grafting material for medial bone defects\textsuperscript{26,27}. However, because the BBS alone has poor bone formation ability \textit{in vivo}\textsuperscript{24}, sufficient vertical bone recovery may not be achieved in patients with large buccal bone defects\textsuperscript{28}. Although β-TCP is presumed to become solubilized \textit{in vivo}, thereby releasing calcium and phosphorus to form apatite\textsuperscript{29}, occasional difficulties in bone mass maintenance occur because of its rapid resorption\textsuperscript{14,15}. CAP activates osteoclasts to form bone\textsuperscript{18,19}, and it has been reported that human bone marrow cells cultured on CAP have higher expression of osteoblast differentiation markers, such as type I collagen, alkaline phosphatase, osteopontin, and osteocalcin, than those cultured on HA\textsuperscript{30}. In an experimental analysis, CAP granules were found to elicit a greater degree of early-stage new bone formation in the cortical portion of a bone defect in the rabbit femur than commercial BBS\textsuperscript{32}. Following transplantation of CAP, HA, and β-TCP into dog mandibles for comparison of bone formation ability, CAP formed a large volume of regenerated bone when compared with other materials at 12 weeks after transplantation\textsuperscript{16}. CAP formed vertical bone earlier than β-TCP, HA, or BBS in a three-wall bone defect model in the canine mandible\textsuperscript{33}. In these reports, CAP showed excellent osteoconductivity. Thus, although the effectiveness of BBS and β-TCP in promoting bone healing has already been described, CAP is proven to be an effective alternative to these materials. However, only bone formation ability was investigated; changes in bone level after implant placement were not determined.

In this study, in which samples were collected at only one time point after implant placement, there was a significant difference in outcome in terms of the bone level around the implant at 5 weeks after implant placement. There were no significant differences among the CAP, β-TCP, and control groups, and there was no evidence that CAP and β-TCP inhibited bone formation during ridge preservation or implant osseointegration. The reduced bone level around the implant in the BBS group was significantly greater than that in the other three groups. This result differed from previous reports in which BBS has demonstrated good results when used as a bone grafting material for medial bone defects\textsuperscript{26,27}. Because the BBS was visible on the surface of the material grafting site at the time of implant placement, we matched the surface with the implant platform. However, physiological bone was not formed on the surface of the transplanted site in the BBS group, and the BBS, which was surrounded by fibrotic tissue rather than bone, may have remained. In this study, bone formation around the implant could be analyzed by histological examination, enabling this condition to be identified. However, clinically, it has been suggested that implant placement at highly crystalline apatite filling sites, such as the BBS, is at risk of incorrect implant placement depth. Further research is needed to clarify why bone was not formed; however, it is suggested that the BBS may inhibit bone regeneration in environments where bone is easily regenerated. In this study, the implant was placed 7 weeks after transplantation of the bone grafting material, and the tissue was collected 5 weeks later. Because CAP has been reported to form bone more rapidly than other bone grafting materials\textsuperscript{31,32}, evaluation of the regenerated bone quantity and/or quality at earlier time points may demonstrate clear differences among the materials. Additionally, β-TCP is rapidly resorbed and may not support continued maintenance of bone mass\textsuperscript{14,15}. Because the samples were not continuously collected and analyzed in this study, future studies should perform histological examinations over time to accurately evaluate bone formation at the defect site with each bone grafting material. Synthetic bone graft materials containing CAP may avoid contamination due to unknown factors and differences in performance among product lots, as well as consistent material quality. However, a more tightly controlled animal model is necessary to determine the effectiveness of each material in terms of changes in bone level before and after implant placement.

**CONCLUSIONS**

In this study, dental implants placed in bone regenerated by transplantation of three bone grafting materials, including BBS, CAP, and β-TCP, achieved sufficient initial fixation and subsequent osseointegration. CAP and β-TCP were comparable in terms of their efficacy to preserve the bone level around the implant after implant placement, and these two materials were significantly more efficacious than the BBS. Highly crystalline apatite, such as the BBS, formed less bone in the socket preservation model than other bone grafting materials.

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