

Article

Octacalcium Phosphate Bone Substitute (Bontree[®]): From Basic Research to Clinical Case Study

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Featured Application: If a two-stage procedure comprising ridge or sinus augmentation using Bontree[®] followed by implant placement is planned, it is generally recommended that implants should be placed approximately 6 months after the healing period. However, as with other types of bone substitute materials, the implantation time can be decided on the basis of the surgeon's clinical experience and patient's condition.

Abstract: Bone grafts used in alveolar bone regeneration can be categorized into autografts, allografts, xenografts, and synthetic bones, depending on their origin. The purpose of this study was to evaluate the effect of a commercialized octacalcium phosphate (OCP)-based synthetic bone substitute material (Bontree[®]) in vitro, in vivo, and in clinical cases. Material characterization of Bontree[®] granules (0.5 mm and 1.0 mm) using scanning electron microscopy and X-ray diffraction showed that both 0.5 mm and 1.0 mm Bontree[®] granules were uniformly composed mainly of OCP. The receptor activator of NF- κ B ligand (RANKL) and alkaline phosphatase (ALP) activities of MG63 cells were assessed and used to compare Bontree[®] with a commercial biphasic calcium phosphate ceramic (MBCP+TM). Compared with MBCP+TM, Bontree[®] suppressed RANKL and increased ALP activity. A rabbit tibia model used to examine the effects of granule size of Bontree[®] grafts showed that 1.0 mm Bontree[®] granules had a higher new bone formation ability than 0.5 mm Bontree[®] granules. Three clinical cases using Bontree[®] for ridge or sinus augmentation are described. All eight implants in the three patients showed a 100% success rate after 1 year of functional loading. This basic research and clinical application demonstrated the safety and efficacy of Bontree[®] for bone regeneration.

Keywords: biomaterial; bone regeneration; bone substitute; dental implant; octacalcium phosphate



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1. Introduction

When teeth are lost, irreversible and gradual resorption of the alveolar bone occurs [1]. The loss of alveolar bone is one of the factors that makes it difficult prosthetically, esthetically, and functionally to restore lost teeth [2]. A sufficiently healthy alveolar bone is one of the main prerequisites for successful dental implant treatment [3]. Therefore, several studies have evaluated the concurrent use of a barrier membrane and graft materials for bone regeneration, which is commonly used to reconstruct resorbed alveolar bone [4].

Currently, bone graft materials used for bone regeneration are divided into autogenous bone grafts, allografts, xenografts, and synthetic bone grafts, depending on their origin. Autografts enable rapid healing without immunity-related problems and infection, and they contain growth factors that can facilitate new bone formation. However, the amount of bone that can be harvested is limited, and some limitations may lead to secondary bony

defects at the donor site [5]. Allografts do not require surgery at the donor site and have osteoinduction and osteoconduction capabilities, but antigenicity- and infection-related problems may arise. In addition, new bone formation depends on the condition of the donor, which is a disadvantage [6]. Deproteinized bovine bone mineral (DBBM) is the most commonly used xenograft material in clinical practice. It has a calcium/phosphorus ratio similar to that of human bone, and its excellent osteoconductivity has been proven. However, xenografts have only osteoconductive ability and high absorption resistance; therefore, new bone formation is slower than that associated with autografts [7]. Synthetic bone graft materials can be mass-produced, their properties can be controlled, and there is no risk of cross-infection, although there are limitations such as low biodegradability depending on the material. Ceramics, tricalcium phosphate (TCP), and hydroxyapatite (HA) are used as synthetic bone materials. Several studies have been conducted on HA and β -TCP, which are calcium phosphate-based biomaterials [8].

Recently, octacalcium phosphate (OCP, $\text{Ca}_8\text{H}_2[\text{PO}_4]_6 \cdot 5\text{H}_2\text{O}$) was developed as a new synthetic bone substitute [9]. OCP is irreversibly converted to sustainable biological apatite under physiological conditions because OCP is a direct precursor of biological apatite [10]. OCP has been proven to be effective for new bone formation because of its high osteogenic capability and rapid bioabsorbability [11]. However, the sintering process changes the original crystal structure of OCP, and it cannot be formed into larger solid masses by sintering. In other words, OCP is generally fragile and difficult to develop as a synthetic bone substitute [12]. Due to these limitations, studies aimed at the commercialization of OCP-based bone substitutes have focused on coating OCP on DBBM [13] or mixing it with collagen [9,12,14]. However, a new synthesis method that does not require sintering was recently developed to mass-produce high-purity OCP with improved physical properties. This made it possible to commercialize an OCP-based synthetic bone substitute material (Bontree[®], HudensBio Co., Gwangju, Korea), which is fully composed of apatite compounds with 80 wt.% OCP and 20 wt.% HA, with improved clinical handling while maintaining the inherent bone regeneration ability of OCP. In this article, we present the *in vitro* and *in vivo* evaluations of Bontree[®]. Its clinical use in three different surgical implant reconstructive procedures is also described.

2. Materials and Methods

2.1. Basic Research

2.1.1. Material Characterization

We purchased 0.5 mm and 1.0 mm diameter granules of the commercial OCP synthetic bone substitute material Bontree[®] (Bontree[®] 0.5-granule and Bontree[®] 1.0-granule, respectively, HudensBio Co., Gwangju, Korea) containing OCP and HA mixed at a weight ratio of 80:20 for experimental evaluation. A commercial biphasic calcium phosphate (BCP) synthetic bone substitute material (MBCP+TM, composed of β -TCP and HA in a 80:20 weight ratio, Biomatlante Sarl, Vigneux de Bretagne, France) was also used for *in vitro* assays. Surface morphology was observed using field-emission scanning electron microscopy (FE-SEM, S-4700 Hitachi, Tokyo, Japan). Phase analysis was conducted using X-ray diffraction (XRD; X'Pert PRO MPD, Malvern Panalytical, Malvern, UK) with a Cu-K α radiation source at a scan speed of 1.0°/min and a step size of 0.026°.

2.1.2. In Vitro Study

The biological properties of each sample were examined using a human osteoblast MG63 cell line (CTL-1427; ATCC, Manassas, VA, USA). MG63 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO Inc., Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; GIBCO Inc., Grand Island, NY, USA), 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin (Gibco Inc., Grand Island, NY, USA). Prior to *in vitro* testing, Bontree[®] granules were compressed into 15 mm flat-faced tablets using a uniaxial press (Carver Inc., Chicago, IL, USA) for 30 s at a constant pressure of 55 MPa. The cells were incubated in a humidified incubator with 5% CO₂ at 37 °C. MG63

cells were seeded on each surface of the samples at a density of 1×10^4 cells/mL and cultured on the samples for 7 or 14 days. All the samples were sterilized with 70% ethanol solution for 30 min, followed by exposure under UV light for 12 h before MG63 cell seeding.

First, the content of the receptor activator of NF- κ B ligand (RANKL) in the samples was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit. After a predetermined culturing time, the cells on the samples were detached using 0.25% trypsin-EDTA for 4 min at 37 °C, and the suspension containing the detached cells was vortexed and centrifuged at 13,000 rpm for 10 min. Standards and samples (100 μ L) were added to the wells and incubated for 150 min at 25 °C. The wells were washed four times with a diluted wash solution. Next, 100 μ L of biotinylated RANKL detection antibody diluted 80 times was added to each well and shaken gently for 1 h at 25 °C. Then, the wells were washed again with a wash solution diluted 20 times. Next, 100 μ L of streptavidin-HRP conjugate solution diluted 200 times was added to each well, followed by shaking for 45 min at 25 °C, and washing with wash solution diluted 20 times. The wells were incubated with 100 μ L of tetramethylbenzidine (TMB) for 30 min at 25 °C. The reaction was stopped by the addition of 50 μ L of stop solution, and the color developed was measured at 450 nm.

Second, the differentiation of MG63 cells into osteoblasts was examined by an alkaline phosphatase (ALP) activity assay, which measured the transformation of *p*-nitrophenyl phosphate (pNPP, Sigma-Aldrich, Irvine, UK) into *p*-nitrophenol (pNP). After a predetermined culturing time, the cells were lysed in 0.2% Triton X-100 and incubated at 37 °C for 1 h after mixing with 50 μ L of pNPP. The reaction was stopped by the addition of 50 μ L of stop solution, and the color developed was measured at 405 nm.

2.1.3. In Vivo Study

All in vivo animal procedures, including surgical and sacrificial methods, were approved by the Ethics Committee on Animal Experimentation of the Institutional Animal Care and Use Committee of CRONEX (CRONEX-IACUC 201908004). The in vivo bone formation around the bone grafts was examined using a rabbit tibial defect model in 8 week old male New Zealand white rabbits (body weight 2–3 kg, KOSA Bio Inc., Seongnam, Korea). The rabbits were anesthetized using a mixture of xylazine, tiletamine, and a local anesthetic (lidocaine). In each proximal tibia, cylindrical defects of 3×6 mm were created using a hand drill. Two types of bone grafts, Bontree® 0.5-granule and Bontree® 1.0-granule, were placed in each tibial defect, and the soft tissue and skin were sutured carefully. Nongrafted tibial defects were also included as controls. The rabbits were sacrificed after 4 and 12 weeks of implantation.

Tibia extracted with implanted bone grafts were fixed in 10% formaldehyde solution and embedded in resin. The resin blocks were sectioned and stained with hematoxylin and eosin. Microscopic images of the stained sections were obtained using a polarized light Axioskop microscope (Olympus BX51, Olympus, Tokyo, Japan). The bone volume and remaining volume of implanted grafts were measured using ImageJ software (ImageJ version 1.50i, National Institutes of Health, Bethesda, MD, USA).

2.1.4. Statistical Analysis

The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS 23, SPSS Inc., Chicago, IL, USA). Data are presented as the mean $\pm$ standard deviation. For RANKL and ALP activities, Student's *t*-test was used to evaluate the differences. One-way analysis of variance was performed on the in vivo results of new bone and remaining graft volumes, followed by least significant difference post hoc tests. Statistical significance was set at $p < 0.05$.

2.2. Clinical Case Study

Patient Selection

Among the patients who visited the periodontal department of Chosun University Dental Hospital for implant surgery, we selected those who underwent sinus or alveolar ridge augmentation with Bontree[®] 1.0-granule with at least 1 year of functional loading. Patients were excluded if they (i) were not followed up, and (ii) did not have radiographs for evaluation. Three male patients were selected, and their ages ranged from 63 to 77 years (Table 1). This study was approved by the Institutional Review Board (IRB) of Chosun University Dental Hospital in Gwangju, Korea (CUDHIRB-2103-008). All treatment plans and procedures were explained to the patients, and they provided consent for surgery.

Table 1. Characteristics of the patients.

Case	Age	Sex	PMH	Procedure	Position of Implant Placement	ISQ (B/L)	Implant Prognosis
1	77	Male	HTN	Bone augmentation of peri-implant defects	#44i	78/84	Success
					#45i	80/79	Success
					#46i	85/85	Success
					#24i	75/76	Success
2	69	Male	HTNCVD	Vertical ridge augmentation	#26i	71/71	Success
					#27i	75/73	Success
3	63	Male	HTNDM	Sinus and ridge augmentation	#16i	62/75	Success
					#17i	61/71	Success

PMH, past medical history; ISQ (B/L), implant stability quotient (buccal/lingual); HTN, hypertension; CVD, cardiovascular disease; DM, diabetes mellitus.

3. Results

3.1. Basic Research

3.1.1. Material Characterization

Figure 1A shows the FE-SEM micrographs of two different granule sizes of Bontree[®]. At low magnification, both Bontree[®] granules had irregular shapes with clean and rough surface morphologies, and no cracks or defects were found on any Bontree[®] granule surface. High-magnification FE-SEM images clearly showed that micron-sized crystals uniformly covered the surface of the Bontree[®] granules. There were no significant differences between the surface qualities and morphologies of the two Bontree[®] granules.

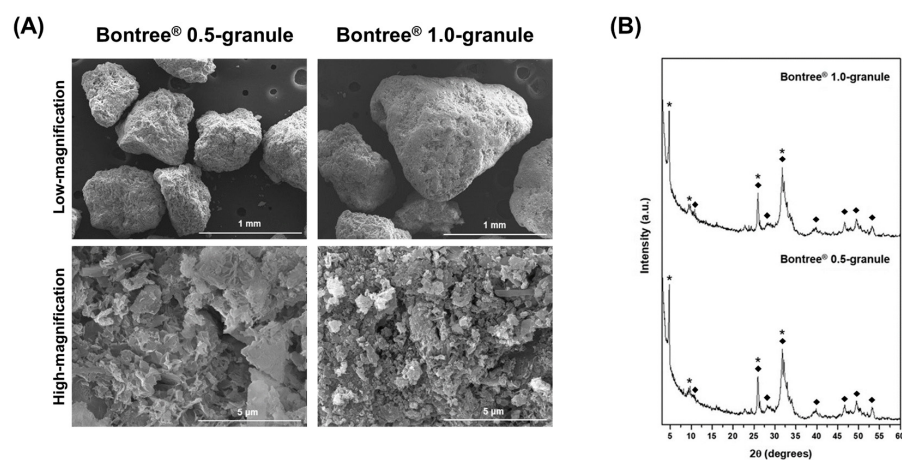


Figure 1. (A) Representative low- and high-magnification FE-SEM images of the Bontree[®] granules: Bontree[®] 0.5-granule and Bontree[®] 1.0-granule refer to Bontree[®] granules with diameters of 0.5 mm and 1.0 mm, respectively. (B) The XRD spectra of the Bontree[®] 0.5-granule and Bontree[®] 1.0-granule. The OCP and HA peaks are indicated by * and ◆, respectively. FE-SEM, field-emission scanning electron microscopy; XRD, X-ray diffraction; OCP, octacalcium phosphate; HA, hydroxyapatite.

Figure 1B shows the XRD patterns of Bontree® 0.5-granule and Bontree® 1.0-granule. XRD was conducted to examine the presence of OCP and HA in Bontree® grafts. The presence of diffraction peaks at $2\theta = 10.8^\circ, 26.3^\circ, 31.8^\circ, 32.2^\circ, 32.9^\circ, 39.8^\circ, 46.6^\circ, 49.3^\circ$, and 53.2° indicated the formation of apatite (HA), while the strongest intensity of the OCP diffraction peak (100) was observed at 4.7° in both samples [15,16]. Phase analysis using XRD showed that OCP is the main constituent of Bontree®, regardless of size.

3.1.2. In Vitro Study

The RANKL and ALP activities of the MG63 cells were assessed using ELISA to assess the role of Bontree® in bone formation and remodeling (Figure 2A,B). For comparison, a commercial biphasic calcium phosphate ceramic (MBCP+™) was also assessed as a reference [17]. Prior to in vitro testing, each specimen was compressed into tablets. Bontree® refers to both Bontree® 0.5-granule and Bontree® 1.0-granule samples.

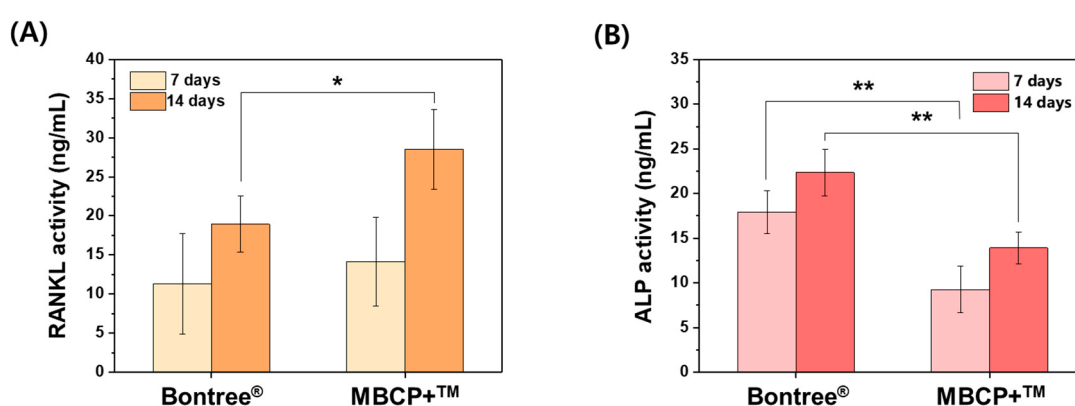


Figure 2. (A) RANKL activity and (B) ALP activity of MG63 cells in Bontree® and MBCP+™ cultures after 7 and 14 days of culture. Statistical significance was set at * $p < 0.05$ and ** $p < 0.01$, respectively. RANKL, receptor activator of NF- κ B ligand; ALP, alkaline phosphatase.

The level of RANKL, an osteoclast differentiation factor [18], was lower in cultures with Bontree® than in those with MBCP+™ during both culturing periods; it was 11.3 ng/mL and 19.0 ng/mL for Bontree® and 14.1 ng/mL and 28.6 ng/mL for MBCP+™ after 7 and 14 days, respectively. In contrast, the activity of ALP, an osteoblast differentiation marker [19], was significantly elevated in cultures with Bontree® (17.9 ng/mL and 22.4 ng/mL at 7 and 14 days, respectively), showing 1.9- and 1.6-fold higher values than in cultures with MBCP+™ (9.2 ng/mL and 13.9 ng/mL at 7 and 14 days, respectively).

3.1.3. In Vivo Study

On the basis of the results of in vitro testing, Bontree® 0.5-granule and Bontree® 1.0-granule were selected for further evaluation of new bone formation in vivo. Figure 3A shows low- and high-magnification histological images of transverse cross-sections stained using hematoxylin and eosin; the cortical bone tissue stained light red or pink, and collagen fibers stained pale pink; the black areas represent the implanted Bontree® grafts [20]. To examine the size effect of bone grafts, two Bontree® grafts (Bontree® 0.5-granule and Bontree® 1.0-granule) were assessed in this in vivo study. At both 4 and 12 weeks after surgery, bone regeneration was observed in the histological images without any sign of local inflammation or rejection of the bone graft. In the control group (i.e., nongrafted defect), a small amount of new bone tissue was formed only at the edge of the tibial defect, and fibrous connective tissue and bone marrow nearly filled the entire tibial defect after 4 weeks of surgery. In contrast, defects filled with the Bontree® 0.5-granule and Bontree® 1.0-granule showed a significant amount of newly formed bone tissues that were uniformly distributed over the entire tibial defect and even in the bone marrow cavity. Notably, in the case of the Bontree® 1.0-granules, thick and dense new bone tissues were consistently

generated between the graft granules, while relatively thin and less dense new bone tissues were formed with Bontree[®] 0.5-granules. Quantitative analysis of the new bone region (Figure 3B) showed $21.6\% \pm 5.3\%$ and $26.0\% \pm 8.2\%$ of new bone volumes after 4 weeks of grafting Bontree[®] 0.5- and 1.0-granules, respectively, which were significantly higher than that of the control ($11.2\% \pm 2.9\%$) ($p < 0.01$).

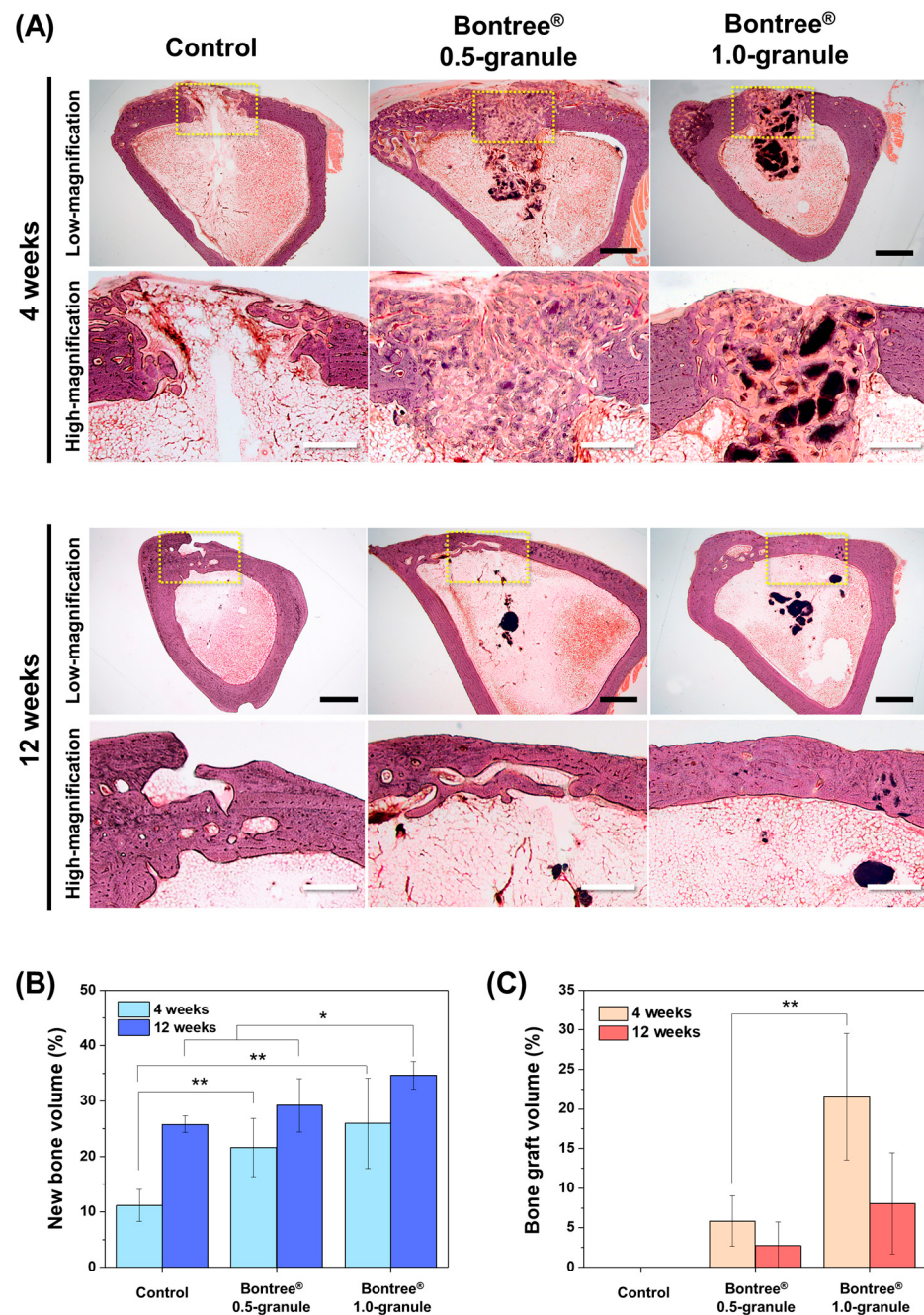


Figure 3. (A) Representative histological images with low- and high-magnifications of the bone graft implantation regions in a rabbit tibia 4 and 12 weeks after surgery. Magnified regions are marked with yellow rectangles in the low magnification images. Black and white bars indicate 2 mm and 1 mm, respectively. Percentages of (B) new bone and (C) bone graft volumes compared with the tibial defect volume at 4 and 12 weeks after surgery. Statistical significance was set at * $p < 0.05$ and ** $p < 0.01$, respectively.

The histological differences between the samples decreased with increasing implantation time. Twelve weeks after surgery (Figure 3A), all samples exhibited complete sealing

of the bone defects; the tibial defects were fully filled with newly formed compact bone, and there was no open gap at the side of the cortical bone defect regions. However, in the control and Bontree® 0.5-granule groups, regenerated bone tissues were not fully dense, and they showed macroscopic inner pores within the bone structures. In contrast, tibias grafted with Bontree® 1.0-granule showed a fully dense regenerated bone structure at the wound site, and they showed good histomorphological continuity with adjacent old bone tissues. From the quantitative results (Figure 3B), the Bontree® 1.0-granule group showed significantly higher new bone volumes ($34.5\% \pm 2.5\%$) than the control ($25.8\% \pm 1.5\%$) and Bontree® 0.5-granule ($29.2\% \pm 4.8\%$) groups ($p < 0.05$).

Regarding bone graft degradation, Bontree® 0.5-granule and Bontree® 1.0-granule showed apparent degradation over 12 weeks (Figure 3C). Four weeks after surgery, large amounts of bone graft remained inside the tibial defects in both groups, and the retention rates were $5.8\% \pm 3.2\%$ and $21.5\% \pm 8.0\%$ for the Bontree® 0.5-granule and Bontree® 1.0-granule groups, respectively. In contrast, at the end of the in vivo test (week 12), the bone grafts were significantly degraded, showing only several coarse remnants in the marrow cavities in both groups. The retention rates were $2.7\% \pm 3.0\%$ and $8.1\% \pm 6.4\%$, respectively, which were 2.2 and 2.7 times lower than those at week 4, respectively.

3.2. Clinical Case Study

3.2.1. Description of Three Cases

All surgical procedures were performed by a single skilled periodontologist (W.-P.L.). Gargling was performed for 1 min using a 0.12% chlorhexidine solution before each surgery. After local anesthesia with 2% lidocaine containing epinephrine (1:100,000), bone was exposed by elevating a full-thickness flap after an alveolar ridge incision and a vertical incision with a #15 surgical blade. After curettage of the inflamed tissue, decortication was performed using a #330 carbide burr for bleeding. To facilitate the sticky bone substitute material condition, Bontree® and the entire blood harvested from the surgical site were mixed.

After each surgery, antibiotics (Augmentin 625 mg, Ilsung Pharm. Co., Seoul, Korea) and analgesics (aceclofenac 100 mg, Dona-A ST, Seoul, Korea) were administered orally for 7 days. In addition, the patients were advised to rinse the oral cavity with 0.12% chlorhexidine twice a day for 2 weeks. Complete stitch-out was performed 2 weeks after surgery. The implant stability quotient (ISQ) of each implant was measured at the second stage of implant surgery approximately 4 months after implantation.

The characteristic surgical procedure for each case is described below.

Case 1

A 77 year old man visited the clinic for implant placement in the 44–46 region. After local anesthesia, when implants (TS III SA®, Osstem, Seoul, Korea) were inserted, one implant thread was partially exposed buccally. Therefore, the guided bone regeneration (GBR) procedure was performed on the peri-implant dehiscence defect using a mixture of Bontree® and whole blood, followed by adaptation of a collagen barrier membrane (Ossix Plus®, Datum Dental Biotech, Lod, Israel). During the second-stage implant surgery performed 4 months after implantation, sufficient horizontal bone augmentation was observed around the implants. The final prosthesis was inserted approximately 6 months after implantation (Figure 4).

Case 2

A 69 year old man visited the clinic for bone augmentation and implant placement in the 24–27 region. As a severely atrophic alveolar ridge was expected, vertical ridge augmentation was performed first using Bontree® mixed with whole blood and a titanium mesh (Jeil Medical, Seoul, Korea) prefabricated on a 3D-printed model. The first stage of implant surgery for 24, 26, and 27 was performed 6 months after ridge augmentation. High primary stability was achieved at the time of implant (Luna®, Shinhung Co., Seoul,

Korea) placement, and sufficient buccal and lingual marginal bone width was confirmed. Four months after implantation, vestibular loss and a lack of buccal attached mucosa were observed with vertical bone loss. Therefore, an apically positioned flap was performed simultaneously with the second-stage implant surgery, followed by the application of an absorbable periodontal dressing (Reso-Pac[®], Hager & Werken GmbH & Co., KG, Germany). The final prosthesis was provided approximately 6 months after implantation (Figure 5).

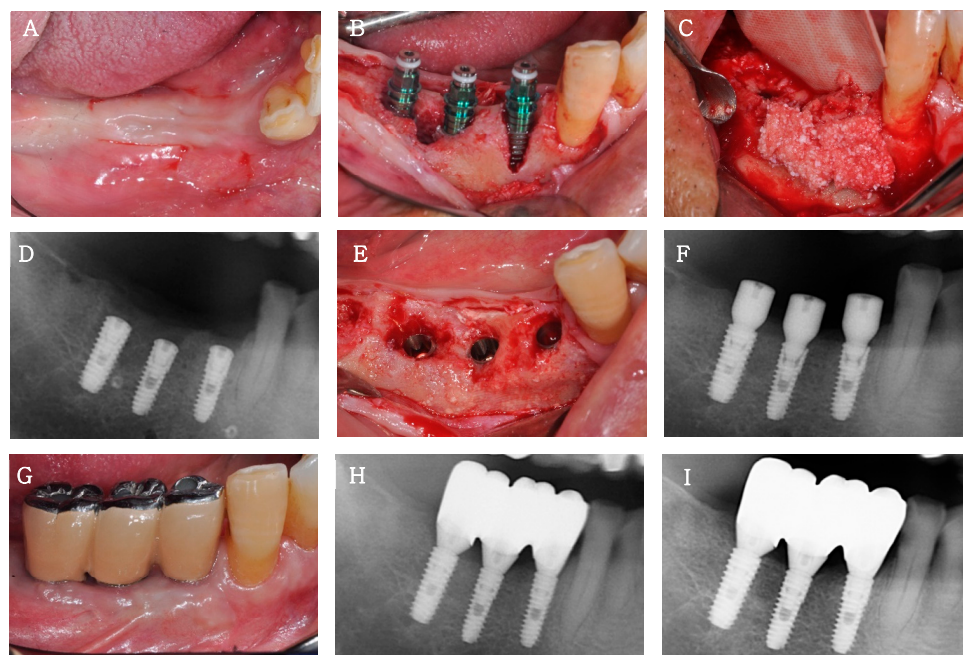


Figure 4. Clinical case 1. (A) Clinical view before bone augmentation and implantation of 44, 45, and 46. (B) Peri-implant dehiscence defects at 44 and 45. (C) Bone augmentation at 44 and 45 using Bontree[®] and collagen barrier membrane. (D) Radiograph after bone augmentation and implantation. (E) Clinical view after flap elevation during the second-stage implant surgery 4 months after implantation. (F) Radiograph after the second-stage implant surgery. (G,H) Clinical and radiographic views of the final prosthesis 6 months after implantation. (I) Radiograph at 1 year after loading.

Case 3

A 63 year old man visited the clinic for sinus augmentation and implant placement in the 16–17 region. Because oroantral communication (OAC) was expected on radiographic evaluation, vertical ridge augmentation with simultaneous sinus floor elevation was planned. After a piezoelectric lateral bony window osteotomy and sinus membrane elevation, sinus grafting was performed with a mixture of Bontree[®] and whole blood, followed by bony window replacement. Next, vertical ridge augmentation was performed with Bontree[®] mixed with whole blood and a titanium-reinforced d-PTFE membrane (Cytoplast[®] Ti-250, Osteogenics Biomedical, Lubbock, TX, USA). A single-stage implant surgery for 16 and 17 was planned 6 months postoperatively, because the radiograph showed sufficient hard tissue volume in the 16–17 region, indicating the resolution of OAC. A core biopsy was conducted before drilling at site 16 for implant placement. The biopsy was harvested through the alveolar process at a depth of 10 mm using a trephine bur with an inner diameter of 2 mm. High primary stability was obtained when the implants (Superline[®], Dentium Co. Ltd., Seoul, Korea) for 16 and 17 were placed, and sufficient buccal and lingual marginal bone width was also confirmed. The harvested specimens were fixed using paraformaldehyde in 4% buffered saline, followed by demineralization. The specimens were processed into paraffin blocks, and a microtome was used for micro-sectioning. Next, hematoxylin and eosin staining was performed. Four months after

implantation, modified periosteal fenestration [21–23], which we first suggested as a free gingival graft alternative, followed by the application of an absorbable periodontal dressing, was performed because of the loss of attached mucosa buccally. The final prosthesis was inserted 6 months after the single-stage implant surgery (Figure 6).

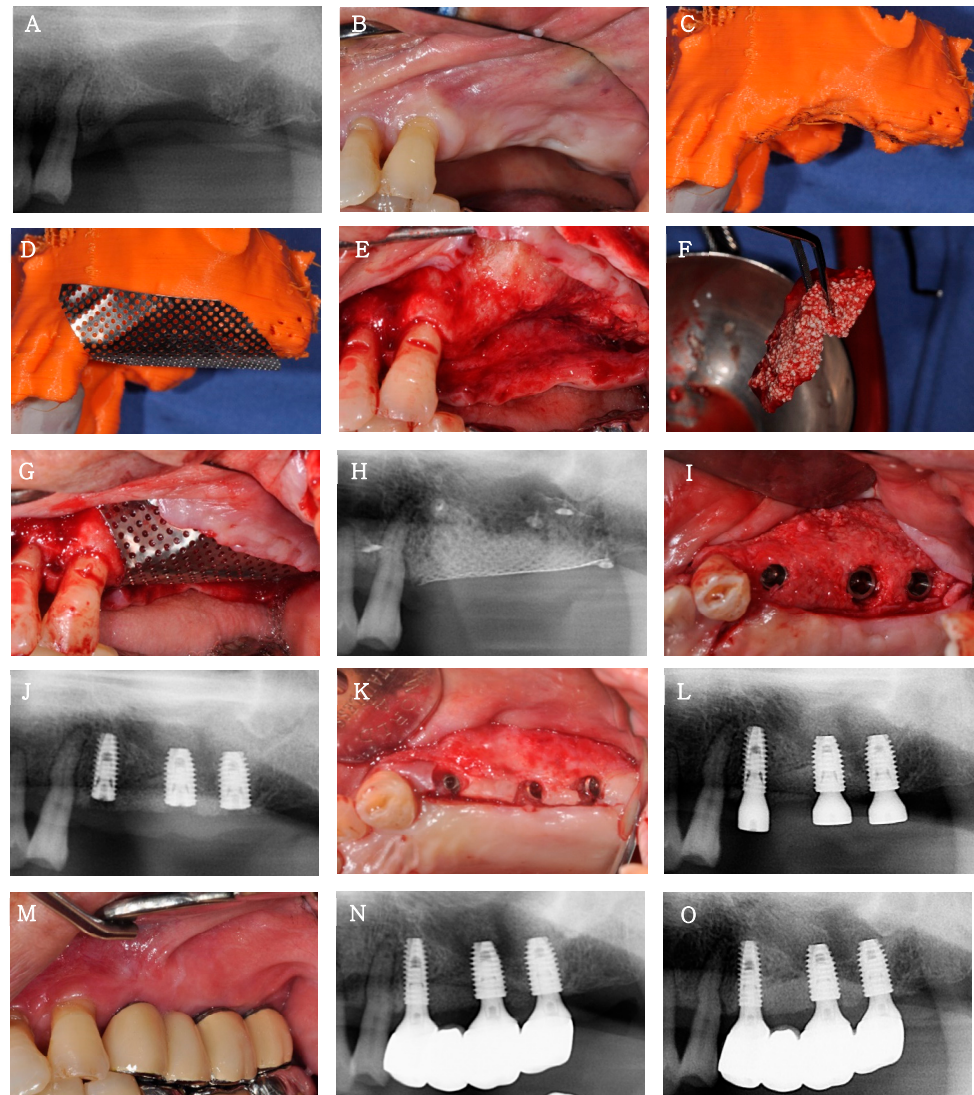


Figure 5. Clinical case 2. (A,B) Clinical and radiographic views before vertical ridge augmentation in the 24, 26, and 27 regions. (C) Three-dimensional printed model of the severely atrophic posterior maxilla. (D) A prefabricated titanium mesh on the 3D-printed model before clinical application. (E) Buccal view after flap elevation. (F) Bontree[®] mixed with whole blood. A sticky bone graft with good manipulability is observed. (G) Buccal view of vertical ridge augmentation using Bontree[®] and a titanium mesh. (H) Radiograph after vertical ridge augmentation. (I) Occlusal view of 24, 26, and 27 implants placed 6 months after vertical ridge augmentation. (J) Radiograph after the first-stage implant surgery. (K) Occlusal view of the second-stage implant surgery using apically positioned flap and punching technique 4 months after implantation. (L) Radiograph after the second-stage implant surgery. (M,N) Clinical and radiographic views of the final prosthesis 6 months after implantation. (O) Radiograph at 1 year after loading.

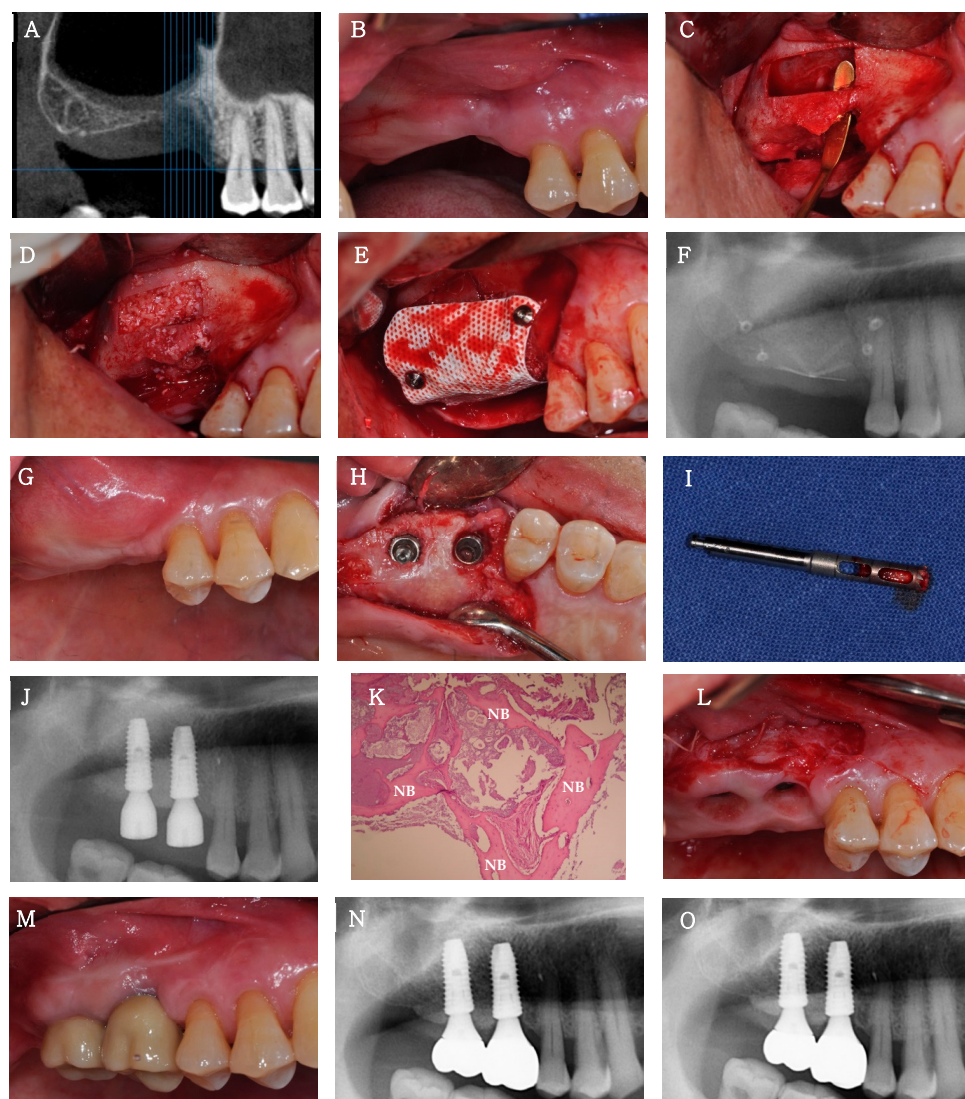


Figure 6. Clinical case 3. (A,B) Clinical and radiographic views before alveolar ridge and sinus augmentation in the 16 and 17 regions. (C) Buccal view after a piezoelectric bony window osteotomy and sinus membrane elevation. An oroantral communication is seen. (D) Sinus graft with Bontree[®]. (E) Vertical ridge augmentation with Bontree[®] and titanium-reinforced d-PTFE membrane after the replacement of the bony window. (F) Radiograph after sinus and ridge augmentation. (G) Buccal view before placement of implants 16 and 17, 6 months after sinus and ridge augmentation. A coronally positioned mucogingival junction line is observed. (H) Occlusal view of implants 16 and 17 immediately after placement. (I) The core biopsy in the 16 region using a trephine bur. (J) Radiograph after the single-stage implant surgery. (K) Histological examination. New bone formation (NB) is observed with no inflammatory tissue (H&E-stained; original magnification $\times 100$). (L) Buccal view after performing a modified periosteal fenestration 4 months after the single-stage implant surgery. (M,N) Clinical and radiographic views of the final prosthesis 6 months after the single-stage implant surgery. (O) Radiograph at 1 year after loading.

3.2.2. Clinical and Radiological Findings

A total of eight implants were placed in three patients who underwent sinus or alveolar ridge bone grafting with Bontree[®]. None of the patients had postoperative complications other than slight swelling at the surgical site. At 4 months after implantation, the ISQ values were >60 for all implants, indicating good implant stability (Table 1). All eight implants in all three patients were followed up for at least 12 months after functional loading, and the success rate, which was evaluated on the basis of the International

Congress of Oral Implantologists Pisa Consensus implant health scale [24], was 100% (Table 1). The radiograph at the 1 year follow-up showed integration of the implant with the regenerated bone and no bone loss or peri-implant radiolucency. No decrease in graft height was observed on any radiograph, and healthy peri-implant mucosa was established around all implants during the 1 year loading period.

3.2.3. Histological Findings

Histological analysis at the site of 16 in case 3, where sinus and ridge grafting was performed with Bontree[®], revealed the deposition of newly formed bone around the residual synthetic bone graft and satisfactory incorporation of the newly formed bone with the residual synthetic bone graft. No foreign body reactions or inflammatory signs were detected (Figure 6K).

4. Discussion

In this study, the safety and efficacy of Bontree[®] for bone regeneration were demonstrated using in vitro and in vivo experiments. In addition, in patients with bony defects in the alveolar ridge or maxillary sinus, oral rehabilitation using dental implants was possible after ridge or sinus augmentation using Bontree[®].

Although OCP is a promising bone graft material with excellent biological properties, there are only a few commercially available OCP-based products, such as Ti-Oss[®] (Chiyewon, Guri, Korea) or Bonarc[™] (Toyobo Co. Ltd., Osaka, Japan) bone grafts. However, even these products are not fully composed of Ca- and P-containing apatites; rather, they consist of a combination of OCP with naturally occurring bovine bone materials or collagens. In contrast, the recently introduced OCP bone graft, Bontree[®], is fully composed of apatite compounds with 80 wt.% OCP and 20 wt.% HA, and it exhibits a commercially acceptable quality, defect-free homogeneous surface morphology, and relatively uniform size distribution (Figure 1A). In addition, numerous micron-scale crystals covering the entire surface of Bontree[®] granules are beneficial for increasing the rate of dissolution and resorption of Ca and P ions under physiological conditions, thereby enabling active mineralization on their surfaces.

Short-term in vitro and long-term in vivo studies were performed to investigate the bone formation ability of Bontree[®]. In the in vitro experiments, RANKL and ALP were used as key markers for bone formation. RANKL is a transmembrane protein that controls the differentiation, maturation, and activation of osteoclasts, which results in bone resorption during remodeling, whereas ALP is an enzyme secreted by osteoblasts and is involved in the mineralization and calcification of newly formed bone tissues. Therefore, the suppressed RANKL activity of MG63 cells is closely associated with reduced osteoclastic differentiation and bone resorption, while the improved ALP activity indicates better osteoblastic differentiation and new bone formation on the bone grafts [19,25,26]. If the bone graft material has insufficient ability to regenerate bone tissues, pores between the bone grafts allow infiltration of soft tissues that hinder the formation of new bone [27,28]. Moreover, the soft tissue is unable to provide sufficient mechanical support to the host bone and prevents the escape of bone grafts from the implanted site [29]. As shown in Figure 2A,B, Bontree[®] exhibited significantly higher values of ALP activity than MBCP⁺[™] at 12 days of MG63 cell culturing, and a significant amount of newly formed bone tissues appeared uniformly distributed over the entire region of the Bontree[®] 0.5-granule- and Bontree[®] 1.0-granule-grafted tibial defects at 4 weeks after surgery. In previous studies, OCP showed outstanding osteoconductive and osteoinductive properties owing to its unique physicochemical properties [30]. When degraded and converted to HA, it releases numerous inorganic PO₄³⁻ and Ca²⁺ ions, which are known to promote osteoblastic differentiation and maturation better than HA, as evidenced by the increased ALP activity (Figure 2B) [31]. In addition, OCP is beneficial for the adsorption of bone-forming molecules and proteins, which could further increase the osteogenic differentiation of cells and initiate bone regeneration [32].

In terms of the size effect of bone grafts on bone regeneration, macroscopic bone grafts generally provide more free space than their microscopic counterparts for cells to move into and attach to, thereby promoting vascularization and ingrowth of newly formed bone tissue into the bone grafts [33]. As shown in Figure 3A,B, Bontree[®] 1.0-granule was associated with greater amounts of newly formed bone tissues than the Bontree[®] 0.5-granule at both 4 and 12 weeks. According to the results of in vitro and in vivo experiments, the Bontree[®] 1.0-granule has a high potential as a commercially applicable novel bone graft material, and it was selected for the evaluation of clinical significance.

On the basis of the results of this basic research, we attempted to perform alveolar ridge or sinus augmentation using Bontree[®] 1.0-granule during dental implant surgery. Eight implants in three patients showed a 100% success rate after 1 year of functional loading. During the surgical procedure, clinical and systemic complications, such as pain, severe inflammation, or significant infection, were not observed. In Case 1, GBR was performed on the peri-implant dehiscence bony defects using Bontree[®] and an absorbable collagen barrier membrane, and clinically successful horizontal hard tissue gain was observed after 4 months. In Cases 2 and 3, vertical ridge or sinus augmentation with Bontree[®] was performed 6 months before implant placement, sufficient horizontal/vertical bone width and height were obtained clinically, and sufficient implant primary stability ≥ 40 N was achieved. All ISQ values measured approximately 4 months after implant placement were >60 , indicating successful secondary implant stability. In addition, no characteristic marginal bone loss around the implant was observed on radiographs for up to 1 year after loading. The clinically and radiographically successful bone regeneration with Bontree[®] can be attributed to the excellent new bone formation ability unique to OCP. In other words, OCP seems to provide a nucleus for promoting osteogenesis and creates numerous starting points for ossification, unlike HA and β -TCP [34]. In addition, Kawai et al. [14] reported that OCP-based bone grafts achieved comparable osseointegration to autologous bone grafts in a study of the mandible in a canine model. However, due to the limitations inherent to retrospective studies, the number of samples was too small, and we cannot confirm the clinical characteristics of Bontree[®] only on the basis of the three cases included in this study. In addition, the follow-up period of approximately 1 year may be too short to evaluate the success rate of the implants. Therefore, as a follow-up study, comparative studies with other bone substitute materials, long-term studies, and prospective studies using Bontree[®] in a much larger number of cases are needed.

5. Conclusions

According to our basic research and clinical applications, Bontree[®] showed significantly higher ALP activity than a commercial biphasic calcium phosphate ceramic (MBCP+TM). Although both Bontree[®] 0.5-granule and Bontree[®] 1.0-granule are mainly composed of OCP distributed uniformly regardless of size, the Bontree[®] 1.0-granule has a higher potential for new bone formation. The clinical cases had predictable and successful outcomes, which demonstrated the safety and efficacy of Bontree[®] in alveolar ridge or sinus augmentation.

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