Experimental and clinical evaluation of BMP2-CPC graft versus deproteinized bovine bone graft for guided bone regeneration: A pilot study

Hongzhou SHEN1,2*, Yin ZHI1*, Fangxing ZHU1, Jiawen SI1, Jun SHI1 and Steve GF. SHEN1,2,3

1 Department of Oral and Craniomaxillofacial Surgery, Shanghai Ninth People’s Hospital, College of Stomatology, Shanghai Jiao Tong University School of Medicine, Shanghai, 200011, China
2 National Clinical Research Center for Oral Diseases, Shanghai Key Laboratory of Stomatology & Shanghai Research Institute of Stomatology, Shanghai, 200011, China
3 Shanghai University of Medicine & Health Sciences, Shanghai, 201318, China

Corresponding authors, Jiawen SI; E-mail: sjwlyl@163.com, Steve GF. SHEN; E-mail: maxillofacsurg@outlook.com

In this study, we proposed BMP2-incorporated calcium phosphate cement (BMP2-CPC), for application in guided bone regeneration (GBR) and compared the experimental bone restoration performance and clinical alveolar bone reconstruction outcome of BMP2-CPC with those of deproteinized bovine bone (DBB). The animal study indicated that, compared to DBB, which induced the slow ingrowth of new bone, BMP2-CPC induced numerous small growth centers for bone regeneration and facilitated a significant amount of bone regeneration in rabbit calvarial bone defects. Fewer residual graft particles remained in the BMP2-CPC-treated defects than in the DBB-treated defects. The clinical study indicated that BMP2-CPC was similar to DBB in remediying alveolar bone insufficiency and maintaining implant stability. In conclusion, the results of this present study indicate that compared to DBB, BMP2-CPC can significantly enhance in vivo bone regeneration and remodeling in rabbit calvarial bone defects and shows preliminary support on its clinical application in GBR surgeries.

Keywords: Deproteinized bovine bone, Bone morphogenetic protein 2, Calcium phosphate cement, Guided bone regeneration, Alveolar bone reconstruction

INTRODUCTION

In recent years, the possibility of re-establishing the anatomy and function of damaged alveolar bone by GBR has brought remarkable progress to dental implant treatment. The elementary concept of GBR is based on the use of a physical membrane barrier to prevent soft tissues that are unable to form a new connective tissue from contacting the alveolar bone or root surface during healing, creating a protected area allowing local bone regeneration.

Clinically, GBR is often performed with bone grafting in cases with apparent alveolar bone insufficiency. Among the currently available grafts, autogenous bone is undoubtedly the gold standard for grafts due to its superior osteoinductivity and biocompatibility. However, donor site morbidity, potential resorption, increased medication duration and subsequent patient discomfort have always been a concern for clinicians and have significantly limited the clinical application of autogenous bone grafts. Deproteinized bovine bone (DBB), the most frequently used bone graft, is otherwise widely accepted as an alternative grafting material for GBR. However, several inherent disadvantages have been observed with DBB, including the potential risk of infection, lack of osteoinductivity, slow resorption rate and failure to restore large bone defect. According to a long-term follow-up study, DBB was shown to be bio-inert and remained sequestered in bone marrow and fibrovascular tissues up to 10 years. Histological evidence revealed that the healing pattern induced by DBB presents a combination of thin trabeculae and DBB particles. Particulate DBB shows no obvious sign of resorption until 26 weeks after implantation.

Recently, mounting preclinical and clinical evidence has indicated that calcium phosphate (CaP) grafts might be a promising alternative grafting material for GBR. Due to its excellent cellular affinity along with controlled resorption potential, calcium phosphate cement (CPC), one representative of CaP grafts, has been proposed by several studies to be applied in GBR. Previous studies have shown that CPC is a biocompatible graft that mainly serves as an osteoconductive scaffold facilitating the ingrowth of new bone when implanted into bone defects. Controlled resorption of CPC was achieved in several studies by regulating the phase conversion or porosity of CPC. Notably, Grosfeld et al. reported a resorption rate of more than 90% at 26 weeks after implantation by modifying the physical properties of CPC. To improve the osteoinductivity of CPC, bone morphogenetic protein 2 (BMP2) was incorporated and significantly enhanced the osteoinductive property of CPC in a concentration-dependent manner. Recent findings of a long-term observation study implied that BMP2-CPC might be a suitable grafting material for maxillary sinus augmentation, especially for those with...
minimal bone height\(^{20}\). Moreover, the use of BMP2-incorporated CaP grafts in instrumented posterolateral lumbar arthrodesis significantly decreased surgical time and blood loss and facilitated earlier and higher fusion rates than those achieved by autogenous bone grafts\(^{20,21}\).

Given the promising potential and aforementioned merits of BMP2-CPC in bone regeneration and bone defect restoration, relevant reports regarding the application of BMP2-CPC in GBR are still very limited. Thus, the purpose of this study was to compare the bone regeneration performance of BMP2-CPC in GBR with that of DBB using rabbit calvarial bone defect models and clinical evaluation parameters. Through this study, we propose BMP2-CPC as an eligible grafting material for application in GBR.

MATERIALS AND METHODS

**Rabbit calvarial bone defect restoration study**

1. Study design

This is a pilot study involving both animals and humans. The *in vivo* osteogenic effect of two commercial biomaterials, BMP2-CPC (Rebone\textsuperscript{TM}, Rebone Biomaterials, Shanghai, China) and DBB (Bio-Oss\textsuperscript{®}, Geistlich Biomaterials, Wolhusen, Switzerland), was evaluated using a rabbit calvarial defect model. The BMP2-CPC material contains 1 mg/g (mg protein/g CPC scaffold) BMP-2. BMP2 was released from BMP2-CPC in a controlled manner, which was previously described in Lin’s study\(^{22}\). The animal study was approved by the Independent Ethics Committee of Shanghai Ninth People’s Hospital affiliated with Shanghai JiaoTong University, School of Medicine and was carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals. Eighteen 3-month-old male New Zealand white rabbits were treated to create a total of 36 calvarial defects. The defects were divided into three groups, depending on the applied treatment: (1) Con (untreated); (2) DBB (defects filled with DBB); and (3) BMP2-CPC (defects filled with BMP2-CPC).

2. Surgical procedures

General anesthesia was induced by injection with a mixture of ketamine (35 mg/kg) and xylazine (5 mg/kg). After the animals were sedated, the surgical sites were shaved and disinfected. A full-thickness incision was made along the midline of the scalp from a midpoint between the base of the ears to approximately 5 cm anteriorly. The skin-periosteal flap was raised to expose the surgical area on both sides of the midline. A 10-mm-diameter trephine bur was used under copious saline solution irrigation to create a bilateral full-thickness calvarial defect. After removal of the trephined bone, the calvarial defects were filled with biomaterials or left empty. Then, the periosteum and skin were closed in layers with a suture line. After surgery, the animals received intramuscular injection with antibiotics 2 times daily for 3 days. Six rabbits were euthanized at 1.5 months after surgery, and the rest were euthanized at 3 months after surgery. The obtained calvarial samples were fixed in 4% paraformaldehyde (PFA).

3. Microcomputed tomography (micro-CT) analysis

The prepared calvarial samples were scanned using a micro-CT imaging system (SkyScan1172, Aartselaar, Belgium). After calibration, the calvarial samples were scanned at 26-μm-thickness sections, 100 kV voltage and 100 μA source current. The region of interest (ROI) was confined to the central 9-mm-diameter region of the 10-mm-diameter defect to avoid including the native bone margins. The newly formed bone in the ROI was evaluated by analysis of bone volume/total volume (BV/TV) and bone mineral density (BMD).

4. Histological examination (undecalcified)

Half of the 3-month samples were randomly selected and embedded without decalcification in methyl methacrylate (MMA) blocks (Merck, Darmstadt, Germany) after dehydration in a series of gradient ethanol (60–100%). Coronal sections encompassing the defect area were sliced and ground with an Exakt cutting and grinding system (Exakt, Wehrheim, Germany). The prepared sections were stained with hematoxylin and eosin (HE) to evaluate new bone formation and biomaterial resorption at the defect site.

5. Histological and immunohistochemical examinations (decalcified)

The 1.5-month samples and the remaining 3-month samples were decalcified in 10% ethylene diamine tetraacetic acid (EDTA), dehydrated in a series of gradient ethanol (60–100%), and embedded in paraffin. The samples were sliced from the center of the defects, following the coronal plane of the calvarial bone. Five-micron-thick sections were stained with HE to evaluate bone regeneration at the defect sites. Immunohistochemical staining was performed to evaluate the expression of osteopontin (OPN), osteocalcin (OCN) and vascular endothelial growth factor (VEGF). Following deparaffinizing and rehydrating, the prepared sections were blocked with 3% H\textsubscript{2}O\textsubscript{2}. Then, the sections were subjected to antigen retrieval prior to incubation with primary antibodies. The primary antibodies used in this study are as follows: OPN (Novus Biologicals, Littleton, USA), OCN (Abcam, Cambridge, UK) and VEGF (Abcam). After incubation at 4°C overnight, the sections were treated with biotinylated secondary antibodies and incubated with streptavidin-horseradish peroxidase complex (Thermo Fisher Scientific, Waltham, USA). Then, the sections were incubated with 3,3-diaminobenzidin (DAB) and counterstained with hematoxylin. Images of stained sections were captured under an upright microscope (Nikon, Tokyo, Japan). The integrated optical density (IOD) values of positive areas were measured in five randomly selected fields of vision using ImageJ (National Institutes of Health, Bethesda, USA).
Clinical study

1. Study design
This retrospective study was conducted in accordance with the principles of the Declaration of Helsinki (2013) and approved by the Independent Ethics Committee of Shanghai Ninth People’s Hospital affiliated with Shanghai JiaoTong University, School of Medicine (SH9H-2019-T217-1). The clinical application of BMP2-CPC (Rebone™, Rebone Biomaterials) in maxillofacial surgery and dental alveolar bone regeneration was approved by China Food and Drug Administration (CFDA Certified No. (2013): 34-60199). The subjects were selected from a pool of patients with mild to moderate insufficiency of alveolar bone. Twenty-one patients (7 males and 14 females) who were treated at the Department of Oral and Craniomaxillofacial Surgery, Shanghai Ninth People’s Hospital, from January 2016 to December 2018 were finally enrolled in this study.

The inclusion criteria are as follows: (i) the patients were 18–50 years of age; (ii) the patients received implant placement with simultaneous GBR; and (iii) a single tooth was missing.

The exclusion criteria are as follows: (i) incomplete clinical or radiographic data; (ii) history of periodontal disease; (iii) uncontrolled diabetes mellitus or other systematic disorders; (iv) severe bone defect that needs staged bone augmentation; and (v) a history of smoking or drinking (smoking or drinking frequently in the last 10 years).

The enrolled patients were divided into two groups according to the bone graft used during surgeries. Patients in the DBB group underwent grafting with DBB covered with a resorbable membrane (Bio-Gide®, Geistlich Biomaterials), and patients in the BMP2-CPC group underwent grafting with BMP2-CPC covered with the same resorbable membrane. All surgeries were performed by the same senior maxillofacial surgeon.

2. Radiographic examination
Standardized digital panoramic radiographs obtained before surgery, immediately after surgery and at 3 months after surgery were retrieved for overall evaluation. A routine cone-beam computed tomography (CBCT) examination was performed before surgery, immediately after surgery and at 6 months after surgery. The obtained CBCT data were processed and transformed into three-dimensional views using Simplant Pro Software (Materialise, Leuven, Belgium).

After superimposition of presurgical and postsurgical jaws, the contour of the augmented sites was outlined in transverse CBCT images and was then stacked into three-dimensional models. Parameters related to the augmented sites were measured as follows: (i) volume (V); (ii) vertical height (H); and (iii) buccolingual width (W) located 3 mm to the implant platform (Fig.1). In addition, the three parameters were analyzed in terms of reduction and resorption rate, as illustrated in Table.1.

3. Clinical evaluation
Clinical evaluation of the implants was performed according to the recorded implant survival status and implant stability quotient (ISQ) values at 6 months after surgery. ISQ value was measured using the Ostell ISQ® device (Integration Diagnostics AB, Göteborg, Sweden). The readings were taken along and perpendicular to the implant axis. The averages of these readings were taken as a representative value for each implant. The success of the treatment was evaluated on the basis of

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Clinical study

Table 1 Analysis formulas

|                          | Volume                   | Vertical height | Buccolingual width |
|--------------------------|--------------------------|-----------------|-------------------|
| Reduction value          | V_{im} - V_{6m}          | H_{im} - H_{6m} | W_{im} - W_{6m}   |
| Resorption rate          | (V_{im} - V_{6m})/V_{im} × 100% | (H_{im} - H_{6m})/H_{im} × 100% | (W_{im} - W_{6m})/W_{im} × 100% |

V_{im}: immediate volume, V_{6m}: 6-month volume;
H_{im}: immediate vertical height, H_{6m}: 6-month vertical height;
W_{im}: immediate buccolingual width, W_{6m}: 6-month buccolingual width.
the recorded complications (postsurgical pain, infection, graft rejection, nerve dysfunction, etc.) and the absence or looseness of the implants during follow-up.

Statistical analysis
All quantitative measurements are presented as the mean±standard deviation and were analyzed using SPSS 22.0 (IBM, Armonk, USA). For the in vivo study, an unpaired t-test was performed to identify differences between the two groups. The multigroup data were analyzed by one-way or two-way analysis of variance (ANOVA) followed by Tukey’s post hoc analysis. The Mann-Whitney test was used to detect differences in the clinical data. The significance level was set as *(p<0.05) or **(p<0.01).

RESULTS

In vivo study
1. Micro-CT analysis
Figure 2a shows a small amount of newly formed bone located in the margin of the defects of the Con group. A mixed hard tissue composed of newly formed bone and implanted grafts filled the defects of the DBB and BMP2-CPC groups. In contrast to the poor bone regeneration in the Con group, BV/TV was significantly increased in
the DBB and BMP2-CPC groups, and, accordingly, BMD was also increased in the two groups (Figs. 2b, c). No significant differences in BV/TV and BMD were found between the DBB and BMP2-CPC groups.

2. Histological examination (undecalcified)

Figure 3a shows that fibrous tissues filled the defects of the Con group with no obvious sign of bone formation, whereas signs of tissue healing were found in defects treated with DBB or BMP2-CPC. The healing patterns of the DBB and BMP2-CPC groups manifested as a mixture of newly formed bone and graft particles constituting the major part surrounded by fibrous tissues. The newly formed bone was in close contact with graft particles, revealing good biocompatibility of DBB and BMP2-CPC. Compared to no treatment, the implantation of DBB or BMP2-CPC induced a significant increase in bone formation, and in particular, BMP2-CPC induced the highest production of new bone among the three groups (Fig. 3b). Residual graft particles were observed in the defects of both groups at 3 months after surgery. Quantitative analysis indicated that compared to the amount of DBB, little BMP2-CPC remained in the defects, revealing an improved remodeling pattern of BMP2-CPC (Fig. 3c).

3. Histological examination (decalcified)

In the Con group, the histological characteristics were similar between 1.5 months and 3 months after surgery. The samples of the Con group were mainly characterized by a filled soft tissue presenting a large amount of collagen fibers oriented parallelly to the surface of the defect, in which we observed the presence of fibroblasts, blood vessels and a few inflammatory cells. A very narrow band of newly formed bone presented in the margin of the defects at 3 months after surgery. In the DBB and BMP2-CPC groups, the tissue occupying the defects exhibited some similar histological characteristics. The implanted graft particles were surrounded by vascularized fibrous tissue that housed varying amounts of scattered newly formed bone. Abundant capillaries were distributed in the fibrous tissues, and multinucleated giant cells (MNGCs) were observed around the graft particles (Fig. 4a). Notably, two distinct patterns of bone regeneration contributed to the different bone healing processes in the DBB and BMP2-CPC groups. In the DBB group, the amounts of newly formed bone that progressed from the margin of the defect to the center was significantly higher than that observed in the Con group (Figs. 4a, b). In the BMP2-CPC group, the implanted graft particles formed numerous small growth centers for bone regeneration and were then encapsulated by newly formed bone. The numerous growth centers merged into a bone bridge occupying a large part of the defect (Fig. 4a). Quantitative analysis of the new bone area indicated that among the three groups, BMP2-CPC induced the highest amount of new bone (Fig. 4b). Compared to DBB, BMP2-CPC induced a delicate network of trabeculae at 1.5 months after surgery. The immature trabecular structure was remodeled into a well-organized structure that possessed thick trabeculae and small medullary spaces covered by a two-layer cortical bone-like structure at 3 months after surgery (Fig. 4a).

4. Immunohistochemical staining

The expression of the osteogenesis-related markers OPN, OCN and VEGF was investigated by immunohistochemical staining, and the results are presented in Fig. 5. Compared to the expression of OPN in the Con group, the expression of OPN in the DBB
and BMP2-CPC groups was slightly increased with no significance at 1.5 months after surgery; however, the expression in the BMP2-CPC group was significantly increased at 3 months after surgery. Compared to no treatment, the implantation of BMP2-CPC led to a significant increase in OCN expression over the 3 months after surgery, whereas DBB had no significant influence on the expression of OCN. VEGF production was significantly higher in the BMP2-CPC group than in the Con group, while this increasing trend was not apparent in the DBB group until 3 months after surgery (Fig. 5b).

Fig. 5 Immunohistochemical results.

a). Representative images of immunohistochemical staining for OPN, OCN and VEGF (200×); b). Quantitative analysis of the expression of OPN, OCN and VEGF in each group (1.5 months after surgery); c). Quantitative analysis of the expression of OPN, OCN and VEGF in each group (3 months after surgery).
Clinical study
1. General information
Of the 26 patients initially enrolled in this study, 2 were excluded because of poor quality imaging data, and 3 were excluded because of incomplete clinical data. For the remaining enrolled patients, 10 patients with a mean age of 32.30±6.94 years were included in the DBB group, and 11 patients with a mean age of 33.91±7.87 years were included in the BMP2-CPC group. Uneventful healing was achieved in all patients, and no major complications or implant failure was recorded. The mean ISQ values were 72.50±4.46 for the DBB group and 71.73±6.06 for the BMP2-CPC group. Implant stability was acceptable in both groups, and no significant difference was found between the DBB and BMP2-CPC groups (Fig. 6).

2. Radiographic evaluation
The immediate panoramic radiographs show that the implantation of DBB or BMP2-CPC effectively remedies local alveolar bone insufficiency, rendering sufficient bone support for later osseointegration of implants. The 3-month panoramic radiographs indicate that all implants were successfully osseointegrated, as seen by close bone-to-implant contact at the implant surface from the alveolar crest to the apical end (Fig. 7a).

The measurement results of the parameters

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\begin{array}{cccc}
\text{Parameter} & \text{DBB} & \text{BMP2-CPC} & p \text{ value} \\
V_{im} (\text{cc}) & 0.50±0.07 & 0.44±0.08 & 0.114 \\
H_{im} (\text{mm}) & 7.02±1.43 & 7.82±1.39 & 0.197 \\
W_{im} (\text{mm}) & 8.01±2.13 & 8.79±2.38 & 0.349 \\
V_{6m} (\text{cc}) & 0.43±0.06 & 0.38±0.05 & 0.072 \\
H_{6m} (\text{mm}) & 6.22±1.51 & 7.06±1.44 & 0.197 \\
W_{6m} (\text{mm}) & 6.88±1.77 & 7.35±2.01 & 0.654 \\
\end{array}
\]

\(V_{im}:\) immediate volume, \(V_{6m}:\) 6-month volume;
\(H_{im}:\) immediate vertical height, \(H_{6m}:\) 6-month vertical height;
\(W_{im}:\) immediate buccolinguinal width, \(W_{6m}:\) 6-month buccolinguinal width.
related to the augmented sites indicated no significant difference between the DBB and BMP2-CPC groups postsurgically (Table 2). The alteration pattern of the parameters revealed a decreasing trend in volume, vertical height and buccolingual width over the 6 months after surgery. Figure 8a demonstrates that there were no significant differences in the reduction in volume, vertical height and buccolingual width between the DBB and BMP2-CPC groups. Similarly, no significant difference was detected in the resorption rates of volume and vertical height between the DBB and BMP2-CPC groups. However, statistical analysis indicated a higher resorption rate of buccolingual width (16.42±2.00%) for the BMP2-CPC group compared to the 14.04±2.03% for the DBB group (Fig. 8b). This finding was supported by the evidence derived from CBCT images in which we observed an apparent reduction in the buccolingual width at 6 months after surgery compared to the buccolingual width at 1 month after surgery (Fig. 7b).

**DISCUSSION**

Alveolar bone insufficiency resulting from congenital deformity, trauma, tumor or systemic diseases poses a major challenge for dental implantation. To overcome alveolar bone insufficiency, GBR surgeries with a combination of bone grafts and membranes are advocated to facilitate local bone regeneration and provide bone support for dental implantation. To date, various bone grafting materials have been developed, among which, DBB is widely accepted as an alternative grafting material for GBR. DBB is a xenogenic bone derivate that undergoes a low-heat (300°C) chemical extraction by which all of its organic components are removed, and only the inorganic bone structure permitting attachment of osteoprogenitor cells and deposition of bone matrix is maintained. Mounting evidence has shown that DBB is capable of facilitating some extent of periodontal regeneration as well as bone regeneration when applied in GBR. Lindgren et al. found that sinus augmentation grafted with DBB induced a 32±18.0% area of newly formed bone and resulted in an implant survival rate of 96.8% at 3 years after surgery. Long-term observation results indicated that DBB was successful in the maintenance of volumetric stability of reconstructed alveolar bone. However, DBB is still unable to fully meet the clinical requirements for bone grafting materials. To address these problems, a variety of synthetic bone grafting materials, such as CaP grafts, bioactive glasses and hydroxyapatite, have been developed and explored for GBR. However, these bone grafting materials still showed several disadvantages, including weak mechanical properties, low osteoinductivity, uncontrolled resorption rates and undesirable byproducts. Given the unmet need in clinical practice, we herein proposed an alternative bone graft, BMP2-CPC, for application in GBR and compared the in vivo bone regeneration performance and clinical bone reconstruction outcome of BMP2-CPC with those of DBB using rabbit calvarial bone defect models and clinical evaluation parameters.

As demonstrated in the present and previous animal studies, the osteoconductive event of DBB manifested as ingrowth of new bone from the margin to the center of the defects. In contrast, BMP2-CPC induced a different pattern of new bone formation characterized by scattered small growth centers for bone regeneration in the entire defect area, which was likely attributed to the superior osteoinductivity of BMP2-CPC. Generally, the osteoinductive capability of BMP2-CPC is due to the incorporation of BMP2 and the release of calcium and phosphate ions. The robust osteogenic effect...
induced by BMP2-CPC markedly accelerated the bone regeneration process, leading to more new bone formation than that induced by DBB. Wang et al. reported that the osteoinductive efficacy of BMP2-incorporated CaP grafts was even comparable to that of autogenous bone. Interestingly, although the histomorphological results indicated an enhanced bone regeneration effect induced by BMP2-CPC, this merit could not be revealed by the micro-CT results. This discrepancy might result from the similar inorganic composition of DBB (or BMP2-CPC) to that of natural bone, which makes DBB (or BMP2-CPC) difficult to distinguish from newly formed bone in radiographic images. Thus, to be exact, this study evaluated the micro-CT examination of the bone formation pattern of a mixed hard tissue composed of newly formed bone and implanted grafts rather than the actual outcome of new bone formation in the defect sites. In addition to exhibiting enhanced osteoinductivity, BMP2-CPC exhibited an improved bone remodeling pattern compared to that of DBB. Generally, manipulating the physical, chemical or biological properties of BMP2-CPC could optimize its resorption rate to match the rate of bone regeneration. At 3 months after the surgery, the BMP2-CPC group showed well-organized new trabeculae bone regeneration. At 3 months after the surgery, the BMP2-CPC group showed well-organized new trabeculae bone regeneration. Given the low osteoinductivity and slow resorption rate of DBB, which were far below the rate of bone regeneration, our animal study results indicated that the bone regeneration performance of BMP2-CPC was superior to that of DBB.

In a previous clinical study, Lin et al. found that BMP2-CPC significantly shortened the bone healing time and improved the bone healing quality when applied in orthopedic surgeries, which indicates the possibility of applying BMP2-CPC in GBR. The clinical study was carried out to further evaluate the feasibility of applying BMP2-CPC in GBR by comparing the performance of BMP2-CPC with that of DBB. Generally, BMP2-CPC was as good as DBB in remodeling alveolar bone insufficiency in our clinical study. The vertical height and buccolingual width augmented by DBB or BMP2-CPC provided sufficient bone volume for dental implantation, which was important for the long-term stability of implants. No major complications, such as infection, graft rejection, absence or looseness of implant, was reported in the medical records, indicating a satisfactory clinical outcome in both the DBB and BMP2-CPC groups. The ISQ results demonstrated that both the BMP2-CPC and DBB groups yielded satisfactory implant stability for occlusal rehabilitation. The stable implants indicated that both BMP2-CPC and DBB contributed to the good osseointegration of implants in the grafted area. The radiographic evaluation results demonstrated that the effect of bone augmentation was similar between the DBB and BMP2-CPC groups, and the augmented sites exhibited a shrinking bone profile in both the DBB and BMP2-CPC groups over the 6-month follow-up. CBCT quantification indicated that BMP2-CPC was similar to DBB in preserving the volume and vertical height augmented by GBR. Moreover, the volume and vertical height alteration patterns of the augmented sites between the DBB and BMP2-CPC groups were in line with the findings reported by previous clinical studies. Compared to the 41.62±6.97% volumetric resorption at autogenous bone grafted alveolar sites after 6 months of healing, both the 13.38±8.62% and 13.11±7.96% resorption rates of DBB and BMP2-CPC, respectively, were satisfactory. Regarding the buccolingual width, the resorption rate of BMP2-CPC was significantly higher than that of DBB. This interesting phenomenon might result from BMP2-stimulated osteoclastogenesis through upregulating the expression of receptor activator of nuclear factor-κ B ligand (RANKL) and decreasing the expression of osteoprotegerin (OPG) in BMP2-treated osteoblasts. Moreover, some preclinical studies indicated that the mechanical pressure from the overlying soft tissue greatly contributed to the contour reduction of the augmented sites. Because the main component of DBB, hydroxyapatite, exhibits high stability and low resorption behavior, the prominent labial/buccal contour augmented by BMP2-CPC was very vulnerable to soft tissue pressure and bone resorption at the labial/buccal side. In such cases, the trend of resorption of BMP2-CPC dominated the buccolingual alteration pattern of the augmented sites and compromised the stability of buccolingual width in the BMP2-CPC group. Nevertheless, despite the slightly higher reduction in buccolingual width in the BMP2-CPC group, large quantities of alveolar bone were reconstructed in both groups and provided enough bone support for dental implantation.

Although animal models are generally superior to in vitro studies in reproducing the complex in vivo environment, there are still several limitations in animal studies, including apparent differences in the anatomical differences, host response and disease development between animal and humans. Thus, the rabbit calvarial models cannot fully simulate the anatomical, physiological, biomechanical and functional environment of human stomatognathic system. Any comprehensive and final point-to-point conclusions drawn from animal models still need to be validated in clinical studies. The present results derived from the clinical study should be interpreted with caution due to the small number of subjects and the short follow-up time. To this end, a randomized controlled clinical trial with larger samples and long-term observation will be performed to gain a clear understanding in the future. Particularly, related prospective study is also needed in the future to obtain more solid evidence with higher rank in the hierarchy of evidence. Moreover, although
we adopted CBCT measurement in this study to avoid errors caused by the linear measurement in panoramic radiographs, radiographic examination is still limited in discovering minor alterations in bone structure. Thus, clinical histological examination is required to investigate the actual outcome of bone regeneration in future study.

In conclusion, the results of this present study indicate that compared to DBB, BMP2-CPC can significantly enhance in vivo bone regeneration and remodeling in rabbit calvarial bone defects and shows preliminary support on its clinical application in GBR surgeries. However, long-term studies with larger sample size are still required in the future to further confirm the application efficacy of BMP2-CPC in alveolar bone regeneration.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (81600827, 81570947, 81771036); National Key R&D Program of China (2017YFB1104100); Science and Technology Commission of Shanghai Municipality (17410710500); Pudong New Area Commission of Health and Family Planning (PW2016E-1).

DISCLOSURE OF INTEREST

No conflicts of interest

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