Comparison of recombinant human bone morphogenetic protein-2-infused absorbable collagen sponge, recombinant human bone morphogenetic protein-2-coated tricalcium phosphate, and platelet-rich fibrin-mixed tricalcium phosphate for sinus augmentation in rabbits

Chul-Hun Kim a, Mi-Ha Ju b, Bok-Joo Kim a*

a Department of Oral and Maxillofacial Surgery, Assistant Professor, Dong-a Univ. College of Medicine, Busan, South Korea
b Department of Pathology, Faculty of Pathology, Dong-a University College of Medicine, Busan, South Korea

Received 7 July 2016; Final revision received 9 January 2017; accepted 10 January 2017
Available online 25 April 2017

KEYWORDS
absorbable collagen sponge; BMP-2; PRF; sinus augmentation; TCP

Abstract Background/purpose: Numerous grafting materials have been used in the bone regeneration of maxillary sinus to obtain a sufficient amount of new bone in implant dentistry. The objective of this study was to compare the potentials of Type I absorbable collagen sponge (ACS) impregnated with recombinant human bone morphogenetic protein (rhBMP)-2, rhBMP-2-coated tricalcium phosphate (TCP), platelet-rich fibrin-mixed TCP for enhancing bone regeneration in sinus augmentation in rabbits.

Materials and methods: The sinus defects were grafted with rhBMP-2+ACS (Group A), rhBMP-2-coated TCP (Group B), and platelet-rich fibrin-mixed TCP (Group C). The specimens underwent decalcification, and were stained for histomorphometric analysis.

Results: There were no significant differences in inflammatory features among the groups 1-week postoperation. In a histomorphometric analysis, the new bone formation ratio showed significant differences between groups at 2 weeks. rhBMP-2+ACS showed a larger and more rapid bone formation area at 2 weeks than those of Groups B and C.

* Corresponding author.
E-mail address: omsbjkim@dau.ac.kr (B.-J. Kim).

http://dx.doi.org/10.1016/j.jds.2017.01.003
1991-7902/© 2017 Association for Dental Sciences of the Republic of China. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Introduction

After tooth loss, edentulous posterior maxillae often present with insufficient alveolar bone quantity and quality, with maxillary sinus pneumatization. To overcome these structural deficiencies and ensure successful implant surgery, most surgeons used to perform maxillary sinus augmentation.

Autogenous bone, known as the gold standard, is a well-established material used to fill insufficient maxillary sinus, because it has osteogenic, osteoinductive, and osteoconductive characteristics. However, some disadvantages and systemic limitations, such as the need for a second surgical site and postoperative morbidity, are well documented. Owing to these limiting characteristics, recent studies have investigated an ideal bone substitute and growth factors to reduce surgical morbidity, and the addition of growth factors and numerous grafting materials to promote bone formation has set high expectations for their clinical potential. Similarly, some studies have focused on early bone formation for early implant loading; reduced sinus augmentation operating time and cost are greatly desired by surgeons for successful implantation and meeting patient expectations.

We previously reported the usefulness of tricalcium phosphate (TCP) as a carrier for recombinant human bone morphogenetic protein (rhBMP)-2 and platelet-rich fibrin (PRF) in sinus augmentation in rabbits, and demonstrated the early bone formation capacity of rhBMP-2-coated TCP and PRF-mixed TCP. However, the use of TCP as a carrier for rhBMP-2 still has limitations because particulate artificial bone can remain in the maxillary sinus surrounded by the Schneiderian membrane, and act as a focus for unwanted infection during the healing period, especially in the event of Schneiderian membrane tearing; if sinus infection is detected after augmentation, the surgeon must remove all of the infected graft materials. Likewise, PRF has early and good bone formation potential, but its use is limited by the recommendation of additional venous blood sampling.

Type I collagen is one of the best rhBMP-2 carriers because of its versatility, high biocompatibility, low immunogenicity, ease of use, and relatively low cost. The first rhBMP-containing products approved by the Food and Drug Administration for the treatment of several spinal disc diseases and open tibial fractures were absorbable collagen sponge (ACS)-based devices impregnated with rhBMP-2. Tripplet et al. conducted a multicenter, randomized, prospective clinical trial and demonstrated the effectiveness and safety of rhBMP-2/ACS compared with bone graft for sinus floor augmentation.

Conclusion: Our histological evaluation demonstrates that Type I ACS can be used as a carrier of rhBMP-2, and rhBMP-2 + ACS showed rapid bone formation, remodeling, and calcification at Week 2 in rabbit.

Materials and methods

Animals and group design

Thirty-six New Zealand white adult female rabbits, aged > 6 months and weighing 2.5–3.5 kg, were used in this study. The animals were housed individually in standard rabbit cages at an ambient 20°C. All of the sinus procedures were performed under general anesthesia, using intramuscular ketamine HCl (50 mg/kg; Ketara; Yuhan, Seoul, Korea) and xylazine (10 mg/kg; Rumpun; Bayer, Seoul, Korea) in a mixture ratio of 5:1 under sterile conditions. The dorsal area of each rabbit’s cranium was shaved before surgery, and the surgical field was prepared with an iodine solution. A midline skin incision was made on the skull, and the periosteum was reflected laterally, exposing the maxilla. Two symmetric ovoid bone defects were then created in the anterior maxilla wall using a round bur under constant irrigation. Special care was taken to avoid injury to the sinus membrane. The defects were grafted with Type I ACS (Ateloplug; Bioland, Chungbuk, South Korea) impregnated with rhBMP-2 (Group A), rhBMP-2-coated TCP (Group B), or PRF-mixed TCP (Group C; Table 1). Each group included 12 rabbits. After obtaining adequate hemostasis, the periosteum was closed with a 4-0 Vicryl suture, and the skin was closed with a 4-0 nylon suture. The animals were given 5 mg/kg gentamycin (Kookie, Seoul, Korea) postoperatively to prevent infection. The postoperative course in all of the cases was uneventful (Figure 1).

The rabbits were killed at 1 week, 2 weeks, 4 weeks, and 6 weeks after surgery, and the six sites of the sinus area were harvested and subjected to histologic examination. All of the experiments were conducted in accordance with the Dong-A University Medical Research Institute’s ethics
guidelines for the treatment and welfare of experimental animals.

**Preparation of graft materials**

ACS impregnated with rhBMP-2 and TCP coated with rhBMP-2

In order to apply the same amount of rhBMP-2 on the two different carriers, we used the rhBMP-2 product that manufactured by Cowellmedi Co. The rhBMP-2 was produced in *Escherichia coli* by genetic engineering. U2OS cells, which express high levels of BMP-2, were used to produce the activated rhBMP-2. The mature forms of rhBMP-2 were cloned from U2OS cells. Finally, dimerized rhBMP-2 was purified by affinity chromatography in a heparin column. Type-I absorbable collagen sponge was soaked with 2 mL 100 μg/mL rhBMP-2 solution by simple injection and divided into four pieces each of 7.6 mm × 12.7 mm in size. By the same pattern, 2 mL 100 μg/mL rhBMP-2 solution was adsorbed on 0.25 g TCP and divided into 0.065 g each for grafting of sinus defects. The final rhBMP-2 concentration of each grafted experimental defect sites was 50 μg/mL.

**Preparation of PRF**

After administering local anesthesia, 9-mL venous blood was drawn from the visible venous vessels in the rabbit ears. Immediately afterward, the dried monovettes (without anticoagulant) were centrifuged at 400g for 10 minutes in a laboratory centrifuge (Gyro 400g; Dong-seo Science Co., Seoul, Korea) in accordance with the manufacturer’s instructions. Thereafter, the PRF clot was cut to a suitable size by scissors.

**Figure 1** (A) Two symmetric ovoid bone defects were created, and both sinus membranes were depressed by the periosteal and sinus membrane elevator for grafting. (B) Grafted maxillary sinus site was harvested and grafted materials are stable (black arrow).

**Figure 2** Traces of the newly formed bone outline using Aperio Technologies Scanscope. NB = newly formed bone; TCP = tricalcium phosphate.
Histology

Each specimen was fixed in 10% formaldehyde solution, decalcified in formic acid for 48 hours, and embedded in paraffin. Serial cross-sections (5 μm) were cut through the larger diameter of the defect and stained with hematoxylin–eosin (HE). The slides were photographed in a virtual slide system (Scanscope CS system; Aperio Technologies, Vista, CA, USA).

Immunohistochemistry and histomorphometric analysis

For detection of the new bone formation property, we performed immunohistochemistry by using osteopontin in Week 4 of each group.

The Aperio Technologies Scanscope CS system is useful for calculating new bone formation areas on HE-stained slides. The simple calculation involves only drawing the newly formed bone outlines. Slides in each group were photographed by virtual slide system microscopy (×100), and six slides from each group were selected in Week 2, Week 4, and Week 6. To calculate the new bone formation area, four sites were randomly selected for each slide, and 0.884 mm × 0.684 mm photographs were collected (Figure 2). In this study, we applied two statistical methods to the significance testing of each group. The dependent variables of the control and experimental groups were averages and standard deviations. The difference between the dependent variables in each group for Week 2, Week 4, and Week 6 was analyzed by two-way analysis of variance and the Kruskal–Wallis test. The collected data were analyzed using SPSS Win 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Gross observations

All of the animals recovered from the operation and healed uneventfully until the end of the experiment. Group A (rhBMP-2+ACS), Group B (rhBMP-2+TCP), and Group C (PRF+TCP) were all well adapted in critical bone defects. Generally, Groups A and C at Week 2 showed more rigid and well-osteointegrated graft material. However, after 4 weeks, there was no apparent differentiation of rigidity among the groups.

Histological findings

HE staining

One week after grafting, there was normal inflammatory reaction and no new bone formation in any of groups (Figure 3). At Week 2, Groups A, B, and C showed slight aggregations of collagen fibers with new bone formation, with greater effects in Groups A and C. Groups A and C exhibited osteoblast proliferation occurring in the early stage of new bone formation, and Group A showed more new bone formation than Group B or C (Figure 4). By Week 4, each experimental group manifested advanced bone formation and calcification; in Group B, bone formation was observed but was relatively small in volume. By Week 6, all of the experimental groups showed more advanced calcification or complete calcification. Thus, Groups A and C showed early initiation of bone formation and remodeling in Week 2.

Immunohistochemistry and histomorphometric analysis

In Week 4, all of the groups were represented a large amount of osteopontin staining around grafted TCP and newly formed bone that indicates osteoconductive and
osteoinductive properties of the grafted materials (Figure 5).

In Week 2, osteoblast proliferation was observed in all experimental groups at the early stage of new bone formation. The areas of newly formed bone in each group after 2 weeks, 4 weeks, and 6 weeks were measured. In Week 2, the mean extent of bone formation in the Groups A, B, and C was 29.6%, 12.9%, and 17.6%, respectively, and that in Week 4 was 30.5%, 25.8%, and 31.2%, respectively. After 6 weeks, the results were 42.5%, 31.2%, and 40.6%, respectively (Table 2, Figure 6). Kruskal–Wallis statistical analysis was performed and the results are presented (Table 3, Figure 7). We further compared the bone formation area of the three experimental groups using two-way analysis of variance. There was a statistically significant difference between subject (group:week) effect ($F = 14.894$, $P < 0.001$) and a difference in the marginal means of bone formation area, especially at Week 2, in each group (Table 4).

**Discussion**

The addition of growth factors (rhBMP-2, platelet-derived growth factor, transforming growth factor-β, insulin-like...
growth factor, etc.) and use of numerous grafting materials to promote bone formation in sinus augmentation have become routine procedures in implant dentistry. After the first report of sinus floor augmentation by Boyne and James,11 many researchers reported variable outcomes from numerous grafting materials and techniques. There was a consensus conference12 on the sinus floor lift procedure in 1996. Probably the most important conclusion reached was that the most effective grafting material is autologous bone. Nevertheless, the requirement of two surgical areas leads many surgeons and patients to refuse this treatment. Owing to these morbidities, numerous grafting materials were developed, such as freeze-dried bone allograft (FDBA), demineralized FDBA, β-TCP, and xenograft. Currently, PRF and rhBMP-2 are the most widely studied and used materials in implant dentistry for regeneration of bone defect sites.6,8,13

The bone morphogenetic proteins (BMPs) are members of the transforming growth factor superfamily, which were first described by Urist16 after observing ectopic bone formation in a rodent model from implanted devitalized cadaveric bone. After Urist,16 many researchers reported that BMPs are one of the most potent local growth factors for induction or stimulation of bone formation in instances of skeletal defects and fracture.17,18 In 2004, rhBMP-2 was approved for adjuvant use in open tibia fractures, and in March 2007, it was approved “as an alternative to autogenous bone graft for sinus augmentations, and for localized alveolar ridge defects associated with extraction sockets.”13

For successful bone regeneration and new bone formation by BMPs, suitable carriers that retain certain amounts of BMPs at application sites are required. An ideal carrier is

![Figure 6](image6.png)

**Figure 6** Quantitative analysis of bone formation area. At Week 2, Group A showed higher bone formation area than Groups B and C. ACS = absorbable collagen sponge; BMP = bone morphogenetic proteins; PRF = platelet-rich fibrin; TCP = tricalcium phosphate.

| Table 3 Krukskal–Wallis test of bone formation area. |
|---------|---------|---------|
| Group Wk 2 | Wk 4 | Wk 6 |
| A  | 0.179 ± 0.009 | 0.185 ± 0.011 | 0.257 ± 0.014 |
| B  | 0.078 ± 0.009 | 0.156 ± 0.017 | 0.189 ± 0.015 |
| C  | 0.107 ± 0.012 | 0.189 ± 0.009 | 0.246 ± 0.015 |
| $\chi^2$ | 14.149 | 9.789 | 11.942 |
| P  | <0.001 | <0.007 | <0.003 |

Values indicate mean ± standard deviation; unit: mm².

![Figure 7](image7.png)

**Figure 7** Krukskal–Wallis test of bone formation area. All groups showed increasing bone formation patterns to Week 6. In particular, Group A (rhBMP-2+ACS) showed earlier peak bone formation than Group B or C. ACS = absorbable collagen sponge; BMP = bone morphogenetic proteins; PRF = platelet-rich fibrin; TCP = tricalcium phosphate.
Table 4

| Source          | Type III sum of squares | Mean square | F      | P       | Partial η2 |
|-----------------|-------------------------|-------------|--------|---------|------------|
| Corrected model | 0.160 ^a                | 8           | 0.020  | 95.898  | <0.001 0.945 |
| Intercept       | 1.660                   | 1           | 1.660  | 7955.258 | <0.001 0.994 |
| Group           | 0.040                   | 2           | 0.020  | 96.548  | <0.001 0.811 |
| Wk              | 0.107                   | 2           | 0.054  | 257.257 | <0.001 0.920 |
| Group × wk      | 0.012                   | 4           | 0.003  | 14.894  | <0.001 0.570 |
| Error           | 0.009                   | 45          | 0.000  |         |            |
| Total           | 1.830                   | 54          | 0.169  | 95.898  | <0.001 0.945 |
| Corrected total | 0.169                   | 53          | 0.169  | 95.898  | <0.001 0.945 |

ANOVA = analysis of variance.
^a R^2 = 0.945 (Adjusted R^2 = 0.935).

not only nonimmunogenic and bioresorbable, but also provides a three-dimensional structure as a scaffold for new bone formation, and is easy to use. Various carriers have been introduced and developed. Owing to its good biocompatibility and osteointegrative property, TCP (Ca₃(PO₄)₂), a synthetic bone-promoting biomaterial, has been extensively applied and investigated as a biodegradable bone replacement for repairing various shapes and sizes of bone defects caused by trauma, tumor resection, or skeletal abnormalities. TCP is gener-

**References**

1. Sharan A, Madjar D. Maxillary sinus pneumatization following extractions: a radiographic study. Int J Oral Maxillofac Implants 2008;23:48–56.
2. Kim JW, Cho MH, Kim SJ, Kim MR. Alveolar distraction osteogenesis versus autogenous onlay bone graft for vertical augmentation of severely atrophied alveolar ridges after 12 years of long-term follow-up. Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:540–9.
3. Rickert D, Slater JJ, Meijer HJ, Vissink A, Raghoebar GM. Maxillary sinus lift with solely autogenous bone compared to a combination of autogenous bone and growth factors or (solely) bone substitutes. A systematic review. Int J Oral Maxillofac Surg 2012;41:160–7.
4. Kim YK, Kim SG, Park JY, Yi YJ, Bae JH. Comparison of clinical outcomes of sinus bone graft with simultaneous implant

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Acknowledgments**

This study was supported by a grant of Dong-A University Medical Research Institute.
placement: 4-month and 6-month final prosthetic loading. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:164–9.
5. Lee JY, Kim SG, Moon SY, Lim SC, Ong JL, Lee KM. A short-term study on immediate functional loading and immediate nonfunctional loading implant in dogs: histomorphometric evaluation of bone reaction. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:519–24.
6. Kim BJ, Kwon TK, Baek HS, et al. A comparative study of the effectiveness of sinus bone grafting with recombinant human bone morphogenetic protein 2-coated tricalcium phosphate and platelet-rich fibrin-mixed tricalcium phosphate in rabbits. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2012;113:583–92.
7. Hong SB, Kim JS, Shin SI, Han JY, Herr Y, Chung JH. Clinical treatment of postoperative infection following sinus augmentation. J Periodontal Implant Sci 2010;40:144–9.
8. Visser R, Arrabal PM, Becerra J, Rinas U, Cifuentes M. The effect of an rhBMP-2 absorbable collagen sponge-targeted system on bone formation in vivo. Biomaterials 2009;30:2032–7.
9. Geiger M, Li RH, Friess W. Collagen sponges for bone regeneration with rhBMP-2. Adv Drug Deliv Rev 2003;55:1613–29.
10. Tripelett RG, Nevins M, Marx RE, et al. Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. J Oral Maxillofac Surg 2009;67:1947–60.
11. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg 1980;38:613–6.
12. Boeck-Neto RJ, Gabrielli M, Lia R, Marcantono E, Shibli JA, Marcantonio Jr E. Histomorphometrical analysis of bone formed after maxillary sinus floor augmentation by grafting with a combination of autogenous bone and demineralized freeze-dried bone allograft or hydroxyapatite. J Periodontol 2002;73:266–70.
13. McKay WF, Peckham SM, Badura JM. A comprehensive clinical review of recombinant human bone morphogenetic protein-2 (INFUSE Bone Graft). Int Orthop 2007;31:729–34.
14. Lee J, Susin C, Rodriguez NA, et al. Sinus augmentation using rhBMP-2/ACS in a mini-pig model: relative efficacy of autogenous fresh particulate iliac bone grafts. Clin Oral Implants Res 2013;24:497–504.
15. Choukroun J, Dass A, Simonpieri A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:299–303.
16. Urist MR. Bone: formation by autoinduction. Science 1965;150:893–9.
17. Wozney JM, Rosen V. Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. Clin Orthop Relat Res 1998;346:26–37.
18. Yamaguchi A, Komori T, Suda T. Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1. Endocr Rev 2000;21:393–411.
19. Saito N, Okada T, Toba S, Miyamoto S, Takaoka K. New synthetic absorbable polymers as BMP carriers: plastic properties of poly-D-L-lactic acid-polyethylene glycol block copolymers. J Biomed Mater Res 1999;47:104–10.
20. Winn SR, Uludag H, Hollinger JO. Carrier systems for bone morphogenetic proteins. Clin Orthop Relat Res 1999;367:95–106.
21. Uludag H, D’Augusta D, Golden J, et al. Implantation of recombinant human bone morphogenetic proteins with biomaterial carriers: a correlation between protein pharmacokinetics and osteoinduction in the rat ectopic model. J Biomed Mater Res 2000;50:227–38.
22. Kim KN, Yang JE, Jang JW, Sasikala B, Beng W, Kim IK. Morphometric analysis on bone formation effect of β-TCP and rhBMP-2 in rabbit mandible. J Korean Assoc Oral Maxillofac Surg 2010;36:161.
23. Uludag H, D’Augusta D, Palmer R, Timony G, Wozney J. Characterization of rhBMP-2 pharmacokinetics implanted with biomaterial carriers in the rat ectopic model. J Biomed Mater Res 1999;46:193–202.
24. Ogose A, Kondo N, UmezU H, et al. Histological assessment in grafts of highly purified beta-tricalcium phosphate (OSferion) in human bones. Biomaterials 2006;27:1542–9.
25. Damien CJ, Parsons JR. Bone graft and bone graft substitutes: a review of current technology and applications. J Appl Biomater 1991;2:187–208.
26. Hollinger JO, Brekke J, Gruskin E, Lee D. Role of bone substitutes. Clin Orthop Relat Res 1996;324:55–65.
27. Jeong SM, Lee CU, Son JS, Oh JH, Fang Y, Choi BH. Simultaneous sinus lift and implantation using platelet-rich fibrin as sole grafting material. J Cranio maxillofac Surg 2014;42:990–4.
28. Moon JW, Sohn DS, Heo JU, Shin HJ, Jung JK. New bone formation in the maxillary sinus using peripheral venous blood alone. J Oral Maxillofac Surg 2011;69:2357–67.
29. Bassi AP, Pioto R, FaSerani LP, Canestraro D, Fontao FG. Maxillary sinus lift without grafting, and simultaneous implant placement: a prospective clinical study with a 51-month follow-up. Int J Oral Maxillofac Surg 2015;44:902–7.
30. Sohn DS, Moon JW, Moon KN, Cho SC, Kang PS. New bone formation in the maxillary sinus using only absorbable gelatin sponge. J Oral Maxillofac Surg 2010;68:1327–33.