Ridge preservation using a self – retaining block type bone substitute for extraction sockets with buccal dehiscence defects: an experimental in vivo model

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Directed by Professor Ui-Won Jung

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This certifies that the Doctoral Dissertation
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ABSTRACT

Ridge preservation using a self – retaining block type bone substitute for extraction sockets with buccal dehiscence defects: an experimental in vivo model

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Objective: To evaluate the effect of a self-retaining block type bone substitute (srBB) on the dimensional stability of the horizontal ridge width at the coronal level in a buccal dehiscence model.

Materials and Methods: Four box-shaped bone defects with buccal dehiscence were surgically created in the edentulous mandible (n=6). Experimental biomaterials were randomly allocated to each site: (1) Control group: no treatment, (2) particle type bone substitute (PBS) group, (3) collagenated soft block bone substitute (csBB) group, (4) self-retaining synthetic block bone (srBB) group. All grafted groups were covered with a collagen membrane. The surgical sites were closed to be submerged. After 16 weeks of healing period, clinical, histological, and radiographic analyses were performed.

Results: Three out of six blocks in the srBB group were exposed and fell out during the first postoperative week due to wound dehiscence. Consequently, the remaining three specimens were reclassified as the RsrBB group. The RsrBB group exhibited increased horizontal ridge width compared to the pristine bone width at 2-4 mm below the CEJ, while the other groups showed resorption (augmented width at 2 mm below: 4.2%, 42.4%, 36.2%, and 110.1% in the control, PBS, csBB, and RsrBB groups, respectively). The mineralized bone area was largest in the RsrBB group (4.74 mm^2, 3.44 mm^2, 5.67 mm^2, and 7.77 mm^2 in the control, PBS, csBB, and RsrBB groups, respectively).

Conclusions: The RsrBB group showed the greatest regeneration at the coronal level. These results potentially indicate that a self-retaining block bone substitute could be a good candidate for alveolar ridge preservation, particularly when primary wound closure is maintained.

Keywords: alveolar ridge augmentation, animal model, bone regeneration, bone substitutes, tooth extraction, bone remodeling

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I. INTRODUCTION

Following tooth extraction, physiological changes occur, including alveolar bone resorption caused by the interruption of blood supply to the periodontal ligament and increased osteoclast activity in the socket wall (Araujo et al., 2010; Araújo et al., 2005; Cardaropoli et al., 2003). Notably, the buccal portion, being thin and mainly composed of bundle bone, exhibits a higher absorption rate compared to the lingual/palatal portion. In response to these changes, alveolar ridge preservation (ARP) using bone graft materials has been employed surgically to maintain alveolar ridge dimensions and facilitate future implant placement (Avila-Ortiz et al., 2019; De Risi et al., 2015; MacBeth et al., 2017).

Previous ARP studies have focused on the natural bone remodeling patterns in intact extraction socket models. However, in actual clinical situations, damaged extraction sockets resulting from root canal infection or periodontal disease are more frequently observed, making preservation or augmentation of these sockets more challenging (Koo et al., 2020; Lee et al., 2015; Paeng et al., 2022; Tien et al., 2021).

Among the various types of bone substitutes, particle type bone substitute (PBS) and collagenated soft block bone (csBB) substitute have been widely used for ARP due to ease of management, socket filling capability, good adaptation to irregular defects, and homogeneous distribution. However, compromised volume preservation in the coronal region of the extraction socket can occur, possibly due to mechanical and morphological instability of bone particles under pressure from overlying flaps and temporary dentures. In such cases, even if ARP is performed after tooth extraction, insufficient alveolar bone volume during implant placement may necessitate additional alveolar bone augmentation (Benic and Hämmerle, 2014; Benic et al., 2017b).

To address this limitation, a hard type block bone substitute (BB) with enhanced physical stability has been developed. According to studies conducted on ARP using BB substitute, autografts, which are commonly used, do not elicit immune reactions and exhibit the best effect in terms of osteogenic potential (Precheur, 2007; Trombelli et al., 2002). However, obtaining an autograft requires a secondary surgical at the donor site, increasing the risk of complications and tissue morbidity. Additionally, the increased complexity of the surgery can lead to patient discomfort (Benic et al., 2017a).

Another drawback of BB substitute is the need for fixation, often involving materials like pins, to ensure proper immobilization during healing (Benic et al., 2019; Milinkovic and Cordaro, 2014; Mir-Mari et al., 2016; Roccuzzo et al., 2007). To overcome these limitations, a self-retaining synthetic BB (srBB) has been developed. According to a recent case report, srBB was effective in maintaining total bone volume, including the coronal region (Park et al., 2023). However, qualitative analysis of mineralized tissue to prove its effectiveness in bone regeneration is still insufficient.

The objective of this study was to investigate the effect of srBB in an experimental buccal dehiscence defect model. The primary outcome was to assess the dimensional stability of the horizontal ridge width at the coronal level, and the secondary outcome was to evaluate volume stability and bone regeneration using radiological and histological analyses.

II. MATERIALS AND METHODS

1. Experimental animals

This preclinical in vivo experiment was designed according to the guidelines of ARRIVE. Six male beagle dogs, aged 12 months and weighing about 15 kg, were selected for this study. The dogs were housed in a controlled environment with a temperature maintained at 15-20℃ and humidity of >30%. Throughout the experiment, the animals were maintained a soft diet. Approval for the management of experimental animals and the experimental protocol was granted by the Animal Care and Use Committee at Yonsei Medical Center, Seoul, Korea (Approval no. 2022-0112).

2. Sample size calculation

Prior to commencing the experiment, a sample size calculation was conducted based on a previously published animal study investigating the efficacy of bone block in ARP (Ikawa et al., 2016). It was hypothesized that the horizontal ridge thickness of the srBB group would be 2 mm greater than the control group and 1 mm thicker than the PBS and csBB groups. Utilizing analysis of variance with a significance level of 5% and a power of 95% (standard deviation: 0.8; effect size: 0.884), a minimum sample size of 5 animals per group was determined. Considering a potential dropout rate of 15% and employing a split-mouth design, a total of 6 animals (24 extraction sockets) were deemed necessary for the study.

3. Experimental materials

Three types of synthetic bone grafts comprised of 60% Hydroxyapatite (HA) and 40% Beta-Tricalcium phosphate (β-TCP), with calcium/phosphorus ratios ranging from 1.5 to 2.0, were utilized for ridge augmentation in the damaged extraction sockets. The manufacturing details for each material are outlined as follows (Figure 1):

- PBS: OSTEON™ 3 (Genoss, Suwon, South Korea) is a particulate bone graft material featuring a particle size range of 0.5 to 1.0 mm, with particles exhibiting a porosity of less than 80%. The macropore size measured 200–400 μm, while the micropore size was <10 μm.
- csBB: OSTEON™ 3 Collagen (Genoss, Suwon, South Korea) represents a soft type of block bone, comprising a mixture of particle-type BCP with 8% type I collagen. The dimensions were 5 mm in diameter and 5 mm in height.
- srBB: OSTEON™ 3 Block (Genoss, Suwon, South Korea) is a self-retaining synthetic block bone graft material fabricated from OSTEON™ 3, featuring dimensions of 5 mm in diameter and 5 mm in height, with size adjustments achievable using a trephine bur.
- CM: Collagen Membrane-P (Genoss, Suwon, South Korea) is a chemically crosslinked collagen membrane utilizing 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as the crosslinking agent. The dimensions measured 20 × 30 mm (width × length).

Figure 1. Three types of biomaterials used in this study.

4. Surgical procedure

Prior to surgery, a comprehensive assessment of the general health status of the animals was conducted, and oral health was optimized through supragingival scaling and plaque control measures. On the day of surgery, intravenous injections of Alfaxan (2 mg/kg; Jurox, Rutherford, Australia) and Tomidin (0.02 mg/kg; Provet Veterinary Products, Istanbul, Turkey) were administered for anesthesia induction, followed by maintenance with Isoflurane 2% (Hana Pharm, Kyonggi-Do, Korea). Local anesthesia using 2% lidocaine HCl with epinephrine 1:100,000 (Kwangmyung Pharm) was then applied to the surgical site. A crevicular incision was made from the second premolar to the fourth premolar, and a mucoperiosteal flap was raised to expose the alveolar ridge. The third and fourth premolars were hemisectioned, followed by bilateral extraction of the mesial roots. Remaining pulp tissue in the distal root was cleared, and calcium hydroxide paste was applied and sealed with resin. Periosteal releasing incisions were made to facilitate flap advancement for proper primary closure. The buccal bone plate of the extraction socket was measured to be 5 × 5 mm (mesio-distal × apico-coronal) using a probe and surgically removed to create four bone dehiscence defects per animal (Figure 2a). To minimize bias and account for site-specific variations, a randomized group allocation was employed. Materials were assigned as follows:

1. Control group: no treatment.
2. PBS group: Onlay graft using PBS and CM.
3. csBB group: Onlay graft using csBB and CM.
4. srBB group: Onlay graft using srBB and CM.

The PBS was soaked in saline, while csBB and srBB were applied directly to the defect site without soaking (Figure 2b). srBB was tightly fitted into the defect site, and CM was trimmed into a rectangular shape measuring 10 × 30 mm (width × length), soaked in saline, and adapted over the bone substitutes (Figure 2c). Primary wound closure was achieved using absorbable sutures (Monosyn 4/0, B. Braun Aesculap AG & Co KG, Tuttlingen, Germany) (Figure 2d), with sutures removed after 1 week. Postoperatively, dogs received subcutaneous injections of Cefazolin (30 mg/kg, Chong Kun Dang Pharmaceutical Corp., Chungcheongnam-do, Korea) and Keromine (0.5 mg/kg, Hana Pharm, Kyonggi-Do, Korea), as well as oral administration of Cefalexin Hydrate (30 mg/kg, Boryung Pharmaceutical Co. Ltd, Kyonggi-Do, Korea) and Meloxicam (0.2 mg/kg, Boehringer Ingelheim Ellas A.E., Koropi, Greece) for 7 days. After a 16 weeks healing period, euthanasia was performed via intravenous injection of Tramadol (5 mg/kg, Aju Pharm Co. Ltd., Seoul, Korea) and Potassium Chloride (20 ml, Dai Han Pharm Co. Ltd., Seoul, Korea) (Figure 2e). The jaw was incised, and block specimens were collected from the surrounding hard and soft tissues, including the experimental site, then fixed in 10% neutral-buffered formalin for 10 days to prevent damage.

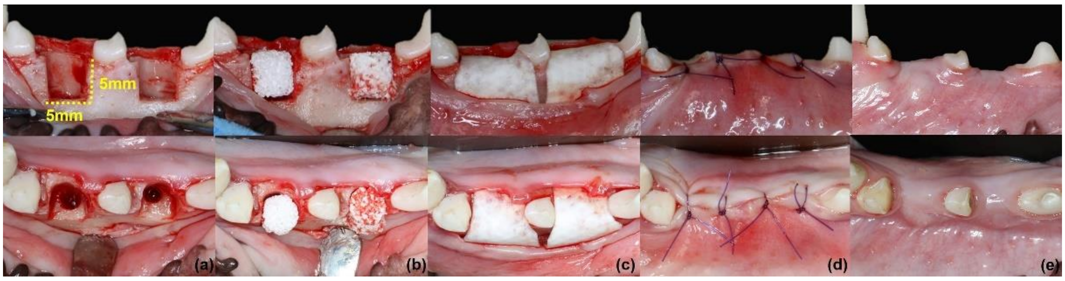

Figure 2. Clinical photographs of surgical procedure; (a) bone defect, (b) bone graft with biomaterial, (c) membrane coverage, (d) suture, (e) after 16 weeks of healing.

5. Radiographic analyses

The fixed block specimens underwent scanning using a micro computed tomography (CT) system (SkyScan 1072, SkyScan, Aartselaar, Belgium) with a resolution of 35 µm (100 kV and 100 µA). The scanned data were converted to DICOM format, and 3D software (OnDemand3D, Cybermed, Seoul, South Korea) was used to visualize the region of interest. Cross-sectional micro-CT images were obtained by bucco-lingual sectioning using 3D software. The graft site was sectioned from the central 2.5 mm portion of the 5 mm horizontal defect created, while the distal root was sectioned from the center-most part of the tooth. Assuming the remaining distal root has the same bucco-lingual shape as the extracted mesial root, the distal root site served as a baseline reference for the pre-extraction situation. Two images were aligned based on the alveolar bone shape using computer software (Adobe Photoshop 22.4.3, Adobe Systems, San Jose, CA, USA). Five horizontal lines perpendicular to the remaining distal root axis were drawn in parallel at 1 mm intervals from the cementum enamel junction (CEJ) to 5 mm below (Figure 3a). The following parameters were measured by a single investigator (Y.H.K.) who was blinded to the group allocation:

1. Horizontal ridge width:

- Augmented ridge width (AW; as a percentage): Calculated as a percentage of the pristine ridge width value in the augmented ridge width value.

- Buccal bone resorption (BR; in millimeters): The subtraction value between the pristine bone ridge width and augmented ridge width.

2. Vertical ridge height (in millimeters):

- CEJ-BC: Vertical distance between the cementum enamel junction and the most coronal portion of the bone crest.

- DBC-GBC: Vertical distance between the most coronal portion of the buccal bone crest at the distal root and the most coronal portion of the buccal bone crest at the grafted site.

- DLC-GLC: Vertical distance between the most coronal portion of the lingual bone crest at the distal root and the most coronal portion of the lingual bone crest at the grafted site.

6. Histological and histomorphometric analyses

The block specimens were decalcified and embedded in paraffin. Sections were prepared by bucco-lingual sectioning, with slices resected from the middle of each distal root and from the most central section of the grafted site, resulting in one slice each. The final thickness of the sections was 3 μm. Histological slides were stained with Masson's trichrome and observed using a polarized light microscope (BX51; Olympus Research Systems, Tokyo, Japan) and computer software (CaseViewer, 3DHISTECH, Budapest, Hungary). Histomorphometric measurements were conducted by a single experienced investigator (Y.H.K.) blinded to the group assignment, utilizing software program (Adobe Photoshop, Adobe Systems, San Jose, CA, USA). Images were superimposed similarly to radiographic analysis, and a horizontal line was drawn at 5 mm below the CEJ. Subsequently, a vertical line was drawn on the tooth's lingual bone wall of the pristine bone, encompassing all grafted buccal areas within the line, defined as the region of interest (ROI; all in square millimeters) (Figure 3b).

The following terms were utilized for histomorphometric analysis:

- New bone (NB): Area of newly formed mineralization bone.
- Residual bone materials (RBM): Area of remaining graft materials.
- Fibrovascular tissue (FVT): Area of non-mineralization tissue.

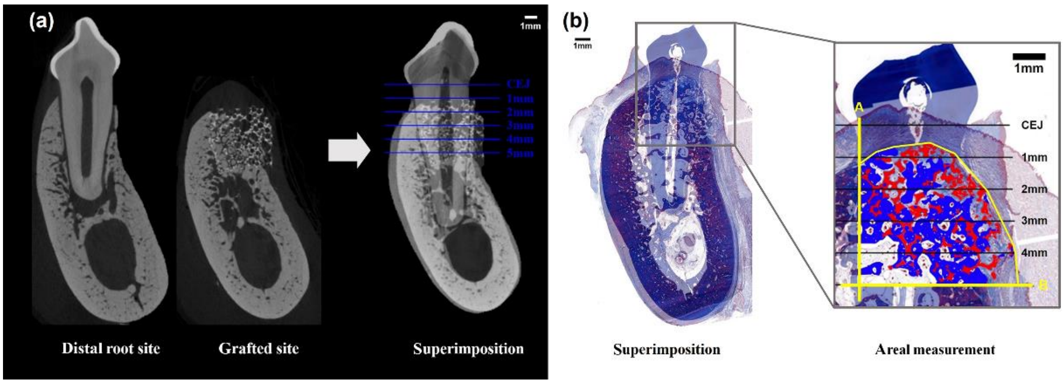

Figure 3. Methods for the analyses of radiographic and histological measurements; (a) liner measurement in radiographic analysis, Bucco-lingual sections of the distal root and the grafted site were superimposed. Five parallel horizontal lines were drawn at 1 mm intervals perpendicular to the remaining distal root axis, starting from the CEJ and extending 5 mm below (scale bar = 1 mm). (b) areal measurement in histological analysis, The images were superimposed same as radiographic analysis, and a horizontal line was drawn 5 mm below the CEJ. A vertical line was then drawn on the lingual bone wall of the original bone, and all grafted buccal areas within that line were measured as the ROI (scale bar = 1 mm).

7. Statistical analysis

The statistical analysis was performed using standard software (SPSS version 29.0, IBM Corporation, USA). Due to the sample size of 6 or fewer for all measurement data, assuming a normal distribution was not appropriate. Therefore, non-parametric methods were utilized. The data underwent analysis using the Friedman's test, followed by post hoc analysis using the Wilcoxon signed-rank test was conducted to assess the differences between each group. Statistical significance was determined when the *p*-value was less than 0.05.

III. RESULTS

1. Clinical findings

All six dogs maintained good health throughout the experimental period. Most of the surgical sites healed without complications. However, 1 week of post-surgery, except for the control group, wound dehiscence was observed at four sites in the PBS group, five sites in the csBB group, and four sites in the srBB group, accompanied by clinical signs such as redness and swelling. Specifically, three sites in the srBB group exhibited severe exposure and partial loss of the grafted bone substitute material. However, all experimental sites underwent spontaneous healing within 4 weeks.

2. Comparison between sites with and without exposure

The specimens were subjected to histological and radiographic analysis to compare the remaining bone substitutes with the failed specimens. Preservation or increase in ridge dimension of the grafted area was observed when the bone substitute materials remained intact and without dehiscence. However, when exposed, no significant differences were noted in all parameters compared to the control group. Consequently, analysis of the srBB group was conducted solely for the remaining three specimens, renamed as the RsrBB group. Comparative results for the RsrBB group compared to the other groups are presented in tables and figures, while the data for all specimens of the srBB group are provided in appendix 1, 2, and 3.

3. Radiographic analyses by micro-CT

The radiographic measurement data are presented in figure 4 and 5, table 1 and 2, and appendix 1 and 2. Horizontal ridge width changes (mean [median]) at respective levels were compared between groups (Figure 5). At AW1, the RsrBB group indicated the highest mean value, while the buccal bone in the control group showed no maintenance (14.0 [0.0], 7.1 [0.0], and 42.2 [50.8]% in the PBS, csBB, and RsrBB groups, respectively). Similar results were observed for levels AW2-4, with the RsrBB group showing over-augmented width compared to pristine bone, while the other groups exhibited some degree of resorption (AW2: control group, 4.2 [0.0]%; PBS group, 42.4 [45.3]%; csBB group, 36.2 [30.9]%; RsrBB group, 110.1 [107.6]%; AW3: control group, 40.0 [39.8]%; PBS group, 73.3 [78.8]%; csBB group, 69.5 [73.4]%; RsrBB group, 122.3 [109.5]%; AW4: control group, 52.6 [52.1]%; PBS group, 91.5 [92.8]%; csBB group, 83.9 [81.6]%; RsrBB group, 122.5 [115.3]%). At AW5, all test groups (104.0 [104.1], 93.8 [91.8], and 101.0 [104.6]% in the PBS, csBB, and RsrBB groups, respectively) exhibited higher values than the control group (59.5 [59.3]%). Additionally, buccal bone resorption followed a similar trend as the augmented width value. Comprehensive data are provided in table 1 and appendix 1.

The vertical ridge height measurement data are presented in table 2 and appendix 2. Regarding CEJ-BC, the RsrBB group exhibited the least amount of change compared to the other groups. DBC-GBC values in the control group were the largest, primarily attributable to buccal bone resorption, whereas the RsrBB group demonstrated the smallest value due to more favorable ridge maintenance. Additionally, only minor differences were observed between all groups for DLC-GLC.

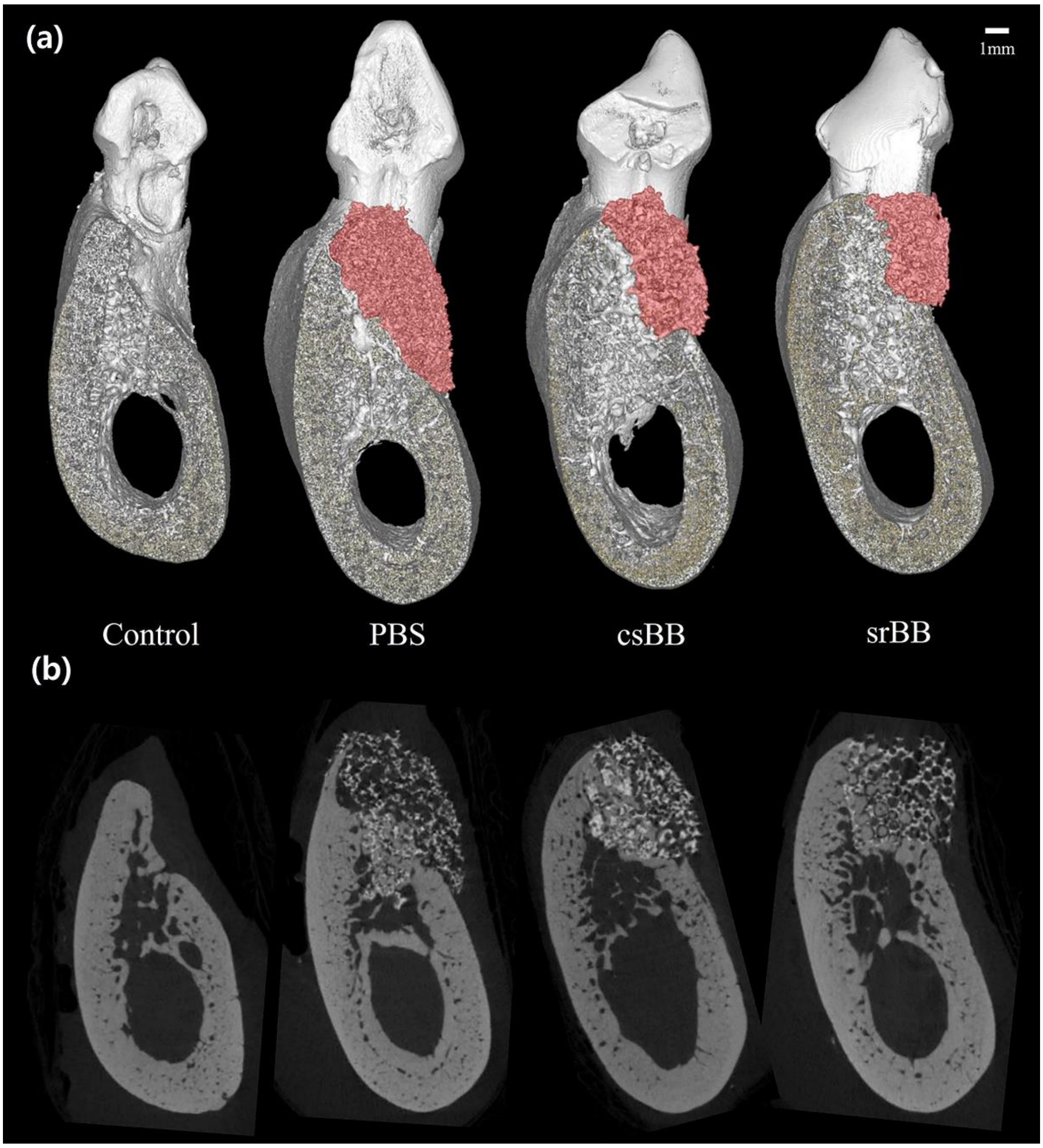

Figure 4. Micro-CT images of a bucco-lingual section at 16 weeks after grafting; (a) 3D coloring image, red portion showed the residual bone material, (b) 2D section of micro-CT images (scale bar = 1 mm).

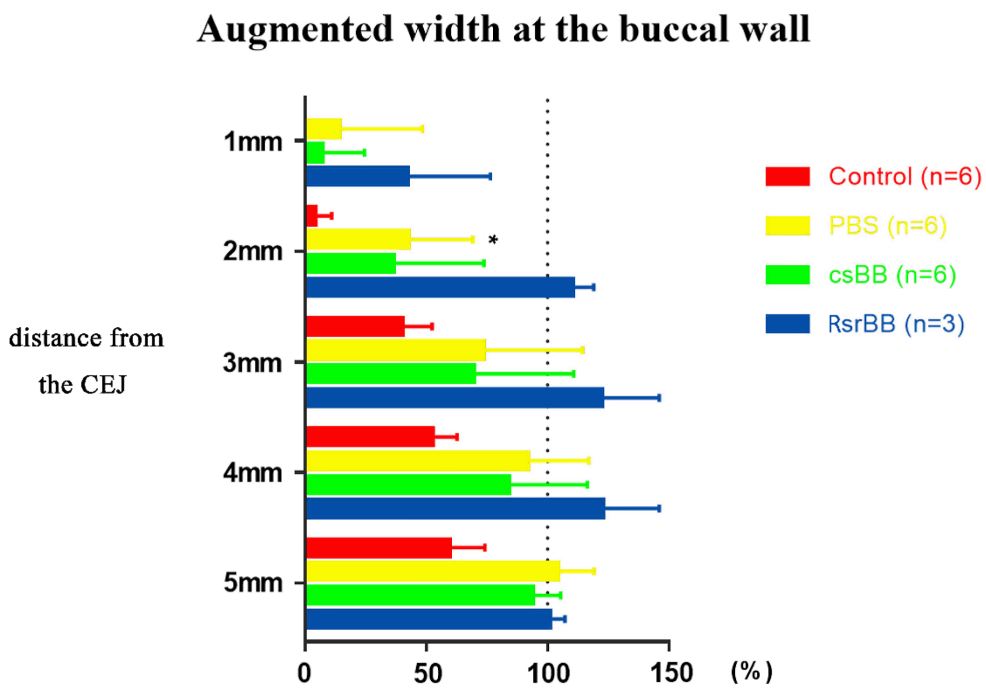

Figure 5. Comparison between the width of pristine buccal bone and grafted buccal bone. Each parameter was measured at 1, 2, 3, 4 and 5 mm below of the CEJ.

* Significantly different from the control group ($p < 0.05$)

Table 1. Horizontal ridge width measurements in micro-CT (mean, median, [Q1 – Q3]; mm).

	Control (n = 6)	PBS (n = 6)	csBB (n = 6)	RsrBB (n = 3)
BR1	NA†	0.4, 0.4†† (0.4 – 0.4)	0.8, 0.8†† (0.8 – 0.8)	-0.1, 0.1 (-0.2 – 0.1)
BR2	2.4, 2.4 (2.2 – 2.6)	1.5, 1.3 (0.9 – 2.3)	1.2, 0.7 (0.1 – 1.8)	-1.3, -1.5 (-1.7 – -1.0)
BR3	1.8, 1.7 (1.6 – 1.9)	0.2, 0.3 (0.2 – 0.7)	0.5, 0.7 (-0.6 – 0.8)	-1.6, -1.6 (-1.9 – -1.2)
BR4	1.8, 1.6 (1.3 – 2.1)	0.2, -0.2 (-0.7 – 0.6)	0.6, 0.7 (-0.6 – 1.2)	-1.7, -2.1 (-2.2 – -1.4)
BR5	1.7, 1.6 (1.1 – 2.1)	-0.4, -0.5 (-0.8 – -0.4)	0.1, 0.2 (0.0 – 0.4)	-0.5, -0.7 (-0.8 – -0.4)

Note: Buccal bone wall resorption of pristine bone at 1, 2, 3, 4 and 5 mm from below CEJ. Positive values indicate resorption of the buccal bone plate; BR, the distance between the buccal bone plate of the grafted site and the buccal bone plate of the distal root.

Abbreviations: PBS, particle bone substitute; csBB, collagenated soft block bone; RsrBB, remaining self-retaining block bone.

† The alveolar bone crest height of the pristine bone did not reach BR1 in all subjects.

†† The alveolar bone crest height of the pristine bone did not reach BR1 in all subjects except one site.

Table 2. Vertical ridge height measurements in micro-CT (mean, median, [Q1 – Q3]; mm).

	Control (n = 6)	**PBS (n = 6)**	**csBB (n = 6)**	**RsrBB (n = 3)**
CEJ - BC	2.2, 2.2 (1.9 – 2.5)	1.4, 1.5 (1.1 – 1.6)	1.9, 1.8 (1.5 – 2.3)	0.7, 0.6 (0.6 – 0.8)
DBC - GBC	4.6, 4.8 (3.9 – 5.2)	2.2, 2.0 (1.4 – 2.9)	2.4, 3.0 (1.4 – 3.2)	-0.1, 0.0 (-0.2 – 0.1)
DLC - GLC	2.3, 2.2 (1.9 – 2.4)	1.3, 1.3 (0.7 – 1.9)	1.6, 1.8 (1.4 – 1.9)	1.1, 1.4 (1.0 – 1.4)

Note: Vertical measurements of bone resorption. Positive values indicate resorption of the buccal bone plate.

Abbreviations: PBS, particle bone substitute; csBB, collagenated soft block bone; RsrBB, remaining self-retaining block bone; CEJ-BC, vertical distance between the cementum enamel junction and the most coronal portion of bone crest; DBC-GBC, vertical distance between the most coronal portion at the distal tooth of buccal bone crest and the most coronal portion at the augmented ridge of buccal bone crest; DLC-GLC, vertical distance between the most coronal portion at the distal tooth of lingual bone crest and the most coronal portion at the augmented ridge of lingual bone crest.

4. Histologic and histomorphometric analyses

The histomorphometric images are presented in figure 6. Crest shoulders were observed in the cervical region of some samples. However, crest shoulder occurrence was noted when grafting on a narrow alveolar ridge and was observed in all groups, thus not affecting the analysis results. Across all test groups, greater bone formation was evident closer to the defect margins, with more fibrous tissue observed nearer to the covering flap. In the case of RsrBB, the block bone with macro pores effectively maintained space, with new bone formation occurring inside the pores. Rapid formation of new bone occurred, with maturation progressing. Additionally, abundant blood vessel formation was identified within the pores, particularly in areas distant from the defect margins. Woven bone with irregularly arranged cells transitioned into mature bone, characterized by concentric osteocytes and Haversian canals. Osteoblast and osteoclast activity were observed around the residual bone substitute materials. Conversely, groups csBB and PBS predominantly exhibited fibrous tissue around the remaining bone substitute materials, except for some regions near the borders of the defect margins. New bone formation was comparatively delayed compared to the RsrBB group.

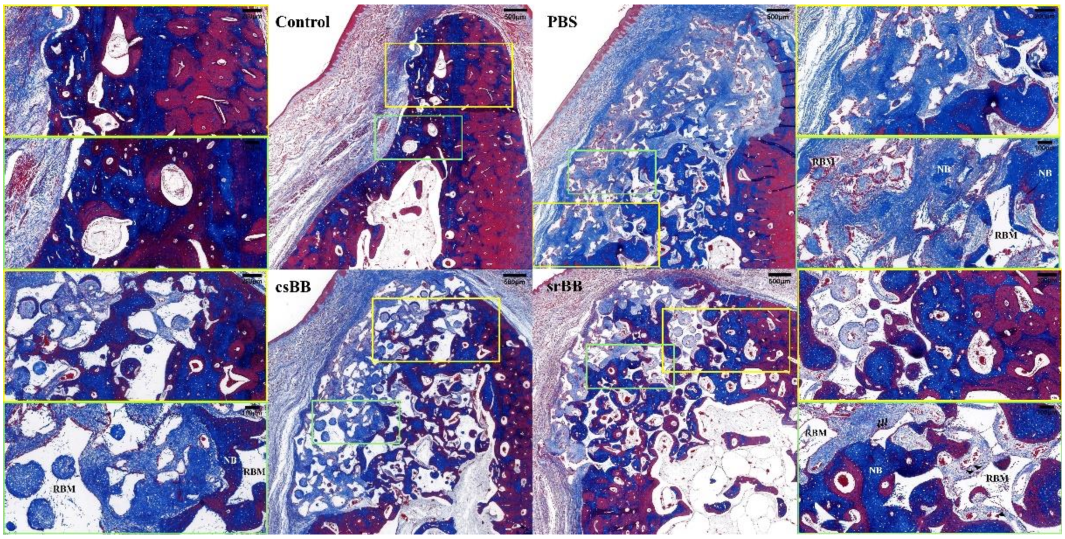

Figure 6. Histomorphometric images of a bucco-lingual section at 16 weeks after grafting. Lower-magnification histological photographs were presented aligned in the center (scale bar = 500 μm). High-magnification photographs were presented within yellow and green boxes for each group (yellow box; scale bar = 200 μm, green box; scale bar = 100 μm). Yellow box showed the new bone formation of defect margin boundary and green box showed the maturation of the new bone. NB, new bone; RBM, residual bone materials; arrow, osteoblast; arrow head, osteoclast.

The histometric areal measurement data are outlined in table 3 and appendix 3. In the RsrBB group, the largest values were observed for the new bone area and the residual bone material area. Additionally, the fibrovascular tissue area was also highest in the RsrBB group compared to the other groups. Regarding the total area, the RsrBB group showed a substantial difference compared to the other groups. However, these differences did not reach statistical significance.

Table 3. Histometric areal measurements (mean, median, [Q1 – Q3]; mm^2).

	Control (n = 6)	PBS (n = 6)	csBB (n = 6)	RsrBB (n = 3)
Total (mm^2)	6.8, 6.6 (5.4 – 7.6)	8.8, 8.8 (6.5 – 10.2)	9.9, 8.6 (7.1 – 11.5)	16.1, 17.9 (15.1 – 17.9)
NB (mm^2)	4.7, 5.2 (4.5 – 5.8)	1.3, 1.1 (0.4 – 1.6)	3.7, 3.5 (2.5 – 5.3)	4.9, 3.6 (3.5 – 5.5)
RBM (mm^2)	NA	2.1, 2.0 (1.1 – 2.9)	2.4, 1.6 (1.0 – 3.4)	2.9, 3.2 (2.6 – 3.4)
FVT (mm^2)	2.0, 1.4 (1.0 – 1.8)	5.4, 5.1* (4.5 – 6.3)	4.3, 4.2 (3.1 – 5.4)	8.3, 7.3 (7.0 – 9.1)

Note: Areal measurements of ROI (5 mm height × most buccal point between lingual wall of the tooth) from the CEJ.

Abbreviations: PBS, particle bone substitute; csBB, collagenated soft block bone; RsrBB, remaining self-retaining block bone; NB, areas of newly formed bone; RBM, areas of residual bone materials; FVT, areas of fibrovascular tissue.

* Significant difference from the control group ($p < 0.05$).

IV. DISCUSSION

The aim of this preclinical model experiment was to assess the dimensional stability of a newly developed srBB compared to conventional biomaterials in an experimental damaged extraction socket model. Group RsrBB demonstrated the highest volume stability at the coronal level 16 weeks after surgery, particularly when the graft material was secured under the mucosa.

In the radiographic assessment, the control group maintained approximately 50% of the socket dimension even at a depth of 4 mm below the CEJ, while the other three groups showed much better preservation horizontally. These findings suggest that ARP procedures effectively maintain horizontal ridge dimensions irrespective of the material used, compared to spontaneous healing (Araújo and Lindhe, 2005; Cha et al., 2019). However, maintaining the dimension of the most coronal part of the extraction socket appears to rely on the stability of the grafting material. When grafting particulated or collagenated soft blocks without rigid fixation, there is a risk of losing positional stability during flap closure, leading to apical migration of the graft material and limiting dimensional maintenance (Benic and Hämmerle, 2014; Thoma et al., 2017). The srBB, being accurately positioned by size adjustment to fit the defect type, allows for ridge width maintenance up to the bone crest without additional fixation. This is supported by a less favorable horizontal dimension observed in the PBS and csBB groups compared to the RsrBB group, despite an over-augmentation found at the 2–4 mm levels below the CEJ. Moreover, unlike PBS and csBB, where the grafting materials undergo collapse, srBB remains in an unabsorbed state until implant placement. Clinically, this might lead to less GBR needed at implant placement.

In a previously performed experimental study (Benic et al., 2022) implants were placed in semi-saddle type bone defects, followed by GBR using particulate deproteinized bovine bone mineral (DBBM), block DBBM, or equine block bone. Outcomes were assessed after 4 months, revealing significantly higher augmented ridge dimensions with block types compared to the particle type. In a randomized controlled clinical trial (Benic et al., 2022), 20 patients received particulate bone substitute materials, while the remaining 20 patients received GBR with soft block bone substitute material. Measurements of the horizontal dimension of augmented hard tissue at the implant shoulder level after 6 months favored the soft block over the particulate bone substitute material. However, space maintenance decreased over time, consistent with findings in the present study using the same materials.

The change in horizontal dimension also influenced the vertical dimension. Due to the more favorable horizontal ridge width in the RsrBB group, particularly at the coronal aspect, both the CEJ-BC and DBC-GBC were higher compared to the other three groups. However, the DLC-GLC showed a different trend, remaining similar among all four groups. In a recently published randomized clinical trial comparing soft block bone and particulate bone substitute materials, incomplete filling of defects occurred over time due to the loss of material stability (Benic et al., 2022). This appears logical, as the lingual side of the extraction socket, damaged in the present experiment, remained intact, suggesting consistent spontaneous bone remodeling across all groups. Meanwhile, the buccal side was influenced by the type of bone substitute material grafted.

The histological analysis revealed that the RsrBB group exhibited larger areas of new bone formation and residual bone graft material compared to the other three groups. This finding aligns with a prior animal study involving block type bone grafts, which similarly demonstrated increased new bone formation and minimal degradation of the bone graft material (Schwarz et al., 2008). The present study demonstrated that block bone grafts promote blood supply through micro vessels due to their porous structure with micro and macro pores. Additionally, hydroxyapatite (HA) serves as a primary constituent of bone, offering stability as a resilient scaffold that does not degrade, while beta-tricalcium phosphate (β-TCP) is recognized for its high biocompatibility. These material attributes, combined with the inherent stability of block bone, facilitate the promotion of new bone formation. The initiation of bone formation occurs within the material at the interface between the block bone and the pristine bone. As a result, the volumetric stability of the grafted site is sustained even after degradation of the substitute material.

In the present experiment, attempts were made to achieve primary closure of the socket entrance in all groups, and mucosa thickness was evaluated in consideration of bone graft exposure. However, wound dehiscence occurred in a considerable number of sites across all groups except the control group within one week. During ARP, insufficient soft tissue volume at the extraction socket entrance can complicate soft tissue management during flap closure. Additionally, controlling dogs chewing behavior at the surgical site can present challenges and potentially jeopardize the success of the graft. Despite soft tissue exposure in both the PBS and csBB groups, graft materials remained well preserved. However, in the srBB group, half of the specimens (3 out of 6) experienced graft material loss. While primary wound closure is typically advocated for successful guided bone regeneration (Wang and Boyapati, 2006), prior studies have suggested that it may not always be necessary in ARP cases involving particulated or collagenated soft block bone substitutes (Barone et al., 2013; Choi et al., 2017). These findings suggest that favorable healing can occur spontaneously, even with exposure, when such particulated or collagenated soft type block bone substitute materials were used. Conversely, in block bone grafts, once exposed, it affects the entire grafting material, resulting in nearly complete loss.

As mentioned in previous research (Thoma et al., 2019; Thoma et al., 2018), soft tissue dehiscence can occur after block bone grafting. This may lead to post-surgical infections and subsequent micromovements of the block bone due to the partial loss of soft tissue coverage over the grafting material. However, in the PBS and csBB groups, it is expected that micromovements were minimal, as particulated or collagenated soft block bone substitutes integrate rapidly with the surrounding fibrovascular tissue, enhancing stability (Song et al., 2020). Additionally, in the RsrBB group, such occurrences were rare, as immobilization of the block bone through rigid fixation is one of the most important prerequisites for successful integration into the recipient site (Caneva et al., 2017; Raghoebar et al., 2006; Silva et al., 2022). Therefore, this suggests the importance of maintaining primary closure of the extraction socket entrance after ARP using a block bone. Primary wound closure may ensure the stability of the grafting material and support the immobilization of srBB.

There are several limitations in this study that require attention. Firstly, this study was designed with a minimal sample size, and the loss of bone blocks in some specimens reduced the sample size in the srBB group, significantly reducing the statistical power of this study. Therefore, further clinical studies with larger sample sizes are necessary to validate the current findings. Secondly, histological analysis in this study utilized the paraffin-embedded decalcification process. However, considering the benefits of resin embedding for morphometric observations and immunohistochemistry for quantitative analysis, the use of these methods in histological analysis will be considered in future studies. Thirdly, in a clinical setting, additional factors need to be considered for the application of srBB. While in this study, the defect size and block bone material were standardized, allowing for friction-based positioning without additional fixation. However, in clinical situations where the defect is much larger than the block size, relying solely on friction to properly position srBB can be challenging. Therefore, it is proposed to secure srBB by utilizing friction on the severely damaged wall side, and then applying particulate bone substitute between srBB and the opposite intact bony wall to properly stabilize the defect's configuration. Further research is needed to investigate the effectiveness of this proposed application method.

V. CONCLUSION

The srBB group demonstrated the highest volume stability at the coronal level. These findings would potentially suggest that self-retaining block bone substitute might be a good candidate for alveolar ridge preservation given that a primary wound closure is maintained.

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APPENDICES

Appendix 1. Horizontal ridge width measurements in micro-CT (RsrBB; mm)

	dog no.1	dog no.2	dog no.3	dog no.4	dog no5	dog no.6
AW1 (mm) [%AW1]	2.4 [71.4]	2.0 [50.8]	0.2 [4.6]	0.0 [0.0]	0.0 [0.0]	0.0 [0.0]
AW2 (mm) [%AW2]	4.6 [120.1]	5.3 [107.6]	5.1 [102.8]	0.0 [0.0]	0.1 [2.5]	0.0 [0.0]
AW3 (mm) [%AW3]	5.8 [149.7]	6.4 [109.5]	6.0 [107.7]	0.0 [0.0]	1.8 [38.9]	0.0 [0.0]
AW4 (mm) [%AW4]	6.0 [148.8]	7.5 [115.3]	6.2 [103.5]	2.1 [42.5]	2.5 [51.4]	1.8 [43.2]
AW5 (mm) [%AW5]	4.6 [104.6]	6.5 [93.7]	6.4 [104.6]	3.3 [60.8]	3.5 [69.0]	3.3 [65.3]
BR1 (mm) [%BR1]	0.1 [3.0]	-0.4 [-10.8]	0.1 [2.8]	0.0 [0.0]	0.0 [0.0]	0.0 [0.0]
BR2 (mm) [%BR2]	-1.8 [-47.1]	-1.5 [-30.6]	-0.5 [-9.2]	0.0 [0.0]	2.7 [67.3]	0.0 [0.0]
BR3 (mm) [%BR3]	-2.3 [-58.7]	-1.6 [-27.3]	-0.8 [-14.1]	0.0 [0.0]	2.4 [53.2]	0.0 [0.0]
BR4 (mm) [%BR4]	-2.2 [-54.4]	-2.1 [-31.7]	-0.8 [-12.7]	1.7 [33.9]	2.2 [46.8]	2.1 [51.7]
BR5 (mm) [%BR5]	0.0 [0.0]	-0.7 [-10.0]	-0.8 [-13.4]	1.3 [23.6]	1.6 [31.0]	1.8 [34.7]

Appendix 2. Vertical ridge height measurements in micro-CT (RsrBB; mm)

	dog no.1	**dog no.2**	**dog no.3**	**dog no.4**	**dog no5**	**dog no.6**
CEJ - BC	0.6	0.6	1.9	3.1	1.2	3.7
DBC-GBC	0.8	0.4	1.4	2.9	2.2	0.7
DLC-GLC	-0.9	0.5	1.4	3.3	3.6	4.1

Appendix 3. Histometric areal measurements (RsrBB; mm^2)

	dog no.1	dog no.2	dog no.3	dog no.4	dog no5	dog no.6
Total	17.9	18.0	12.3	3.2	4.9	3.2
NB	3.5	7.5	3.6	2.5	3.8	1.0
RBM	3.6	3.2	2.0	0.0	0.0	0.3
FVT	10.8	7.3	6.8	0.6	1.2	2.0

ABSTRACT (KOREAN)

발치와 부위의 협측 열개형 골 결손부에 대한 자가 고정 블록형 골이식재의 치조제 보존 효과: 전 임상 연구

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권 윤 희

치아 발치 후 발생하는 생리학적 변화 중에서 가장 대표적인 것은 치조골 흡수이다. 이러한 변화에 대응하기 위해 발치 직후 골 이식 재료를 사용하는 치조제 보존술이 시행되고 있다. 과거의 치조제 보존술 연구는 손상되지 않은 발치와 모델에서 자연적인 골 재형성에 초점을 맞추었지만, 실제 임상 상황에서 만나게 되는 다양한 유형의 파괴된 발치와를 통한 골이식 후의 치유 과정에 대한 근거는 제한적이다. 치조제 보존술에 사용되는 다양한 종류의 골이식재 중 입자형 골이식재와 콜라겐 혼합형 골이식재가 불규칙한

결함에서도 균일하게 분포한다는 장점을 가지며 널리 사용되었으나, 여러 물리적 압력으로 의한 발치와 치관부에서의 부피 보존이 저하되는 단점으로 인해, 물리적 안정성을 갖춘 블록형 이식재가 사용되어 왔다. 하지만 블록형 이식재는 치유기간 동안 적절한 고정을 유지하기 위해 핀과 같은 추가 재료를 사용해야 한다는 단점이 있어 자가 고정 블록형 골이식재가 개발되었다. 그러나, 이에 대한 연구는 제한적이기 때문에, 본 연구에서는 발치와 부위의 협측 열개형 골 결손부에 자가 고정 블록형 골이식재를 사용한 후 치관부에서의 부피 보존 효과와 골 재생효과를 평가하는 것을 목적으로 하였다.

6 마리 비글견 하악 양측의 제 3, 4 소구치를 편측 절단하고 근심 치근을 발거한 후, 외과적으로 5 × 5mm 크기의 협측 열개형 골 결손부를 총 4 개 형성하였다. 대조군(Control)에는 어떠한 처치도 하지 않았고, 실험군 1(PBS)에는 HA 와 β-TCP 가 60:40 비율로 구성되어 있는 합성골 중 입자형 이식재를 이식하였고, 실험군 2(csBB)에는 합성골 이식재에 콜라겐을 혼합한 소프트 블록형 합성골을 이식하였으며, 실험군 3(srBB)에는 자가 고정 블록형 합성골 이식재를 이식하였다. 모든 실험군에는 콜라겐 차폐막을 사용하여 골 이식재를 덮어 치조제 보존술을 시행하였다. 16 주의 치유기간을 거친 후 수술 부위의 방사선학적 및 조직학적 분석을 시행하였다. 치근을 발거하지 않은 원심 측과 치조제 보존술을 시행한 근심 측의 협-설 단면을 중첩하여, 방사선학적 분석에서는 백악법랑경계 기준 1, 2, 3, 4 및 5mm 하방에서의 각 치조제의 수평 폭 변화를 계측했으며, 조직학적 분석에서는 치조제의 면적 변화량을 계측하였다. 실험군과 대조군의 통계적 비교는 Friedman’s 검정으로 시행하였다 ($p < 0.05$).

자가 고정 블록형 이식재를 사용한 그룹은 6 개의 이식 부위 중 3 개 부위에서 수술 후 첫 주에 노출이 발생하여 이식재가 탈락하였다. 이식재가 남아있는 3 개 부위는 치조제의 두께가 보존되거나 증가하는 것이 관찰되었으나, 이식재가 탈락한 3 개 부위는 모든 매개변수에서 대조군과 비교하여 유의미한 차이가 관찰되지 않았다. 따라서 이식재가 남은 3 개 부위에 대하여 RsrBB 로 그룹을 재구성하였다. 방사선학적 분석을 통해 확인한 결과, 백악법랑경계 아래 2-4mm 부위에서 자가 고정 블록형 이식재를 사용한 경우에는 원래 뼈 폭에 비해 증가한 수평 능선을 보여준 반면, 다른 그룹들에서는 흡수를 보였다(백악법랑경계 2mm 하방에서 보존된 치조제 폭: 대조군, PBS, csBB 및 RsrBB 그룹에서 각각 4.2%, 42.4%, 36.2% 및 110.1%). 또한, 무기질화된 뼈의 면적은 자가 고정 블록형 이식재를 사용한 경우 가장 크게 나타났다(대조군, PBS, csBB 및 RsrBB 그룹에서 각각 4.74mm^2, 3.44mm^2, 5.67mm^2 및 7.77mm^2).

결론적으로 자가 고정 블록형 이식재를 사용한 경우 치관부에서 가장 높은 부피 안정성 및 골 재생효과를 보여주었다. 따라서 자가 고정 블록형 이식재를 사용할 시 일차 상처 봉합이 유지되는 한 치조제 보존술의 좋은 이식재 후보가 될 수 있음을 시사한다.

핵심되는 말: 치조제 보존술, 동물 실험, 골 재형성, 골이식재, 발치