Prevention of buccal bone resorption using a pamidronate-loaded collagen matrix following immediate implant placement

Joo, Jeungmin

Department of Dentistry

Graduate School

Yonsei University

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Advisor Cha, Jae-Kook

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Joo, Jeungmin

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This Certifies that the Dissertation of Joo, Jeungmin is Approved

Committee Chair	**Jung, Ui-Won**
Committee Member	**Lee, Jung-Seok**
Committee Member	**Cha, Jae-Kook**
Committee Member	**Paik, Jeong-Won**
Committee Member	**Park, Jin-Young**

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Yonsei University
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ABSTRACT

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Aim: The aim of this experimental in vivo pilot study was to evaluate the effect of the local delivery of pamidronate within a collagen membrane on the changes in the buccal soft and hard tissue dimensions at the time of immediate implant placement and whether this effect was influenced by the placement of bone substitutes.

Methods: In six beagle dogs, the distal roots of the third and fourth premolars were extracted, and immediate implants were placed. Treatment groups were randomly allocated to each socket: (i) covering the buccal bone with pamidronate-soaked collagen membrane (BP group), (ii) filling the gap defect with synthetic bone substitute (BS group), (iii) filling the gap defect with synthetic bone substitute and covering the buccal bone with pamidronate soaked collagen membrane (BP/BS group), (iv) no treatment (control group). Intraoral scanning was performed immediately after the surgery and at 20 weeks. Histomorphometric and micro- computed tomography (CT) outcomes were evaluated at 20 weeks.

Results: The micro CT analysis demonstrated that the BP group showed no apparent difference in vertical bone level with residual mesial root area, while control group showed significant buccal bone resorption at the implant site. The histomorphometric analysis demonstrated that the vertical bone level of buccal plate was significantly differed between the BP and control group (0.34 ± 0.93 and 1.27 ± 0.56 mm, respectively; $p = .041$). There was no statistically significant difference in the horizontal ridge width (HRW 1, 2, 3) among the groups. Also, the thickness, height and buccal contours of the soft tissue did not reveal significant changes among the groups.

Conclusions: The local delivery of pamidronate to the outer surface of the buccal wall at the time of immediate implant placement effectively limits buccal bone resorption. The results from the present investigation should be interpreted with caution, as well as its clinical translatability. Further investigation is needed to understand the pamidronate binding and releasing kinetic, as well as the ideal carrier of this drug for its topical application.

Keywords: animal experiment, bisphosphonate, immediate implant placement

1. Introduction

The post-extraction healing of alveolar sockets demonstrates marked volumetric changes of the residual alveolar ridge as the result of the physiological bone remodeling[1]. To limit these dimensional changes, alveolar ridge preservation (ARP) has been evaluated demonstrating significant effects in reducing these changes, mainly on buccal bone[2]. The ARP has demonstrated beneficial effects in maintaining the alveolar ridge volume and facilitating implant placement[3-5]. However, ARP requires time for wound healing and bone formation before implant placement, which delays the overall restorative treatment time[6, 7].

In the previous in vivo pilot study, the antiresorptive effect of the local delivery of bisphosphonates on the extraction socket was demonstrated[8]. In the study, pamidronate soaked collagen mem branes were applied to the outer surface of the buccal plate in fresh extraction sockets and demonstrated less vertical and horizontal bone loss compared to the spontaneous healing. In addition, there were studies corroborating the effect of preventing bone resorption during the healing of extraction sockets by local administration of bisphosphonates[9, 10]. Since the buccal bone resorption is the one of the main problems in the immediate implant, we hypothesized that local delivery of pamidronate may also be beneficial if applied as an adjunct to the immediate implant placement protocol.

The rationale of using bisphosphonates was based on their proven effect on bone biology, inhibiting the osteoclast activity and stimulating their apoptosis[11]. Bisphosphonates initially attach to the hydroxyapatite of the alveolar surface. When osteoclasts resorb the alveolar bone, bisphosphonates are released from the alveolar bone where they are attached. The released bisphosphonates inside the osteoclasts inhibit farnesyl pyrophosphate synthase, a key regulatory enzyme of the pathway producing vital cholesterol and lipids. Consequently, the cellular activity of the osteoclast is reduced, and, as a result, apoptosis is induced[12]. Use of local pamidronate over alveolar bone showed anti-resorptive effects without notable complications[8, 10, 13].

Therefore, the aim of the present in vivo pilot study was to investigate the dimensional changes of buccal soft and hard tissue when pamidronate was locally delivered to the buccal bone upon immediate implant placement with or without bone substitutes.

2. Material & Methods

2.1 Experimental design

This manuscript follows the modified ARRIVE guidelines for in vivo experimental research studies[14]. The investigation was performed in an animal laboratory accredited by the International Association for Assessment and Accreditation of Laboratory Animals at Yonsei Medical Center, Seoul, Korea. Animal selection, management, and the experimental protocol were approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (Permission no. 2018–0007). Based on a previous pilot study, six male beagle dogs aged 12 months and weighing approximately 15 kg were used[8]. All animals were observed for 2 weeks before the study to determine their general health. On the day of surgery, each animal was examined for its general health status for final inclusion.

Treatment allocation was assigned randomly to one of the four groups. Bone level dental implants with diameter of 3.5 mm and length of 10 mm (Superline, Dentium, Suwon, South Korea) were used for implant placement. As graft material, synthetic biphasic calcium phosphate bone substitute (SBS) was used (Osteon III; Genoss, Suwon, South Korea) and as collagen membrane (CM), a cross-linked type I collagen membrane (Collagen Membrane®; Genoss) were used. SBS is a biphasic calcium phosphate consisting of 60% hydroxyapatite and 40% beta tricalcium phosphate. The CM had a size of 5 mm × 5 mm and was soaked in either 15 mg/mL pamidronate (Hanlim, Yongin, Korea) or sterile saline for 10 min prior to coverage of the buccal bone[8].

The first treatment was allocated randomly, and then rotated clockwise in every subsequent dog using the following order:

1. Control Group: Only immediate implant placement without gap filling nor covering with CM

2. BP Group: Immediate implant placement by filling the gap defect with a spontaneous blood clot and covering the buccal bone with pamidronate- soaked CM.

3. BS Group: Immediate implant placement by filling the gap defect with SBS and covering the buccal bone with saline- soaked CM.

4. BP/BS Group: Immediate implant placement by filling the gap defect with SBS and covering the buccal bone with pamidronate-soaked CM.

Post-operative care included the administration of an analgesic (0.2 mg/kg; meloxicam, Boehringer, Ingelheim, Germany) and an antibiotic (20 mg/kg; cefazolin sodium, Yuhan, Seoul, South Korea) once daily until suture removal. The animals were daily monitored by veterinarians throughout the healing process. The surgical wounds were irrigated daily with a 0.2% chlorhexidine solution (Hexamedin, Bukwang Pharmaceutical, Seoul, Korea). Sutures were removed after 10 days, and the dogs were kept on a soft diet during the entire healing period.

2.2 Surgical procedure

The surgical procedure used in this experimental in vivo investigation was a modification from previous similar studies. The implant placement intervention was based on Vignoletti et al., while the local delivery of pamidronate was based on Cha et al.[8] and Vignoletti et al.[15]

Following general and local anesthesia, sulcular incisions were made in the third and fourth premolar areas bilaterally, and full- thickness flaps were reflected. Subsequently, the premolars were hemi- sectioned, the distal roots were extracted and immediate implants were placed into the fresh extraction sockets (Figure 1A). The location of the implant platform was levelled with the lingual crestal margin. Then, polyetheretherketone healing abutments, which also served as scan bodies, were connected to enable transmucosal healing. Pulp exposures on the mesial roots were sealed with calcium hydroxide (Dycal, Dentsply Sirona, Charlotte, USA). The gap distance and buccal bone thickness were measured with a probe. Then, according to the treatment assignment, the SBS was applied to the buccal gap and filled up to the bone crest level. The CM was applied on the outer surface of the buccal plate, without covering the entrance of the extraction socket (Figure 1B).

Interrupted sutures were applied for primary flap closure and tissue adaptation around the healing abutment. The mesial side, which was close to the residual root, a 6–0 suture was applied, and on the distal side, which was close to the distal tooth, a 4–0 suture was applied (Monosyn®, B. Braun, Tuttlingen, Germany) (Figure 1C). Sutures were removed after 10 days, and baseline intraoral scanning (Medit i500; Medit, Seoul, Korea) was performed. At 20 weeks postoperatively, intraoral scanning was performed and the animals were sacrificed with an overdose of potassium chloride after full anesthesia. After sacrifice, histologic specimens were obtained and dehydrated in a graded series of ethanol, and embedded in methyl methacrylate for non-decalcified ground sections[16]. Each specimen was sectioned in bucco-lingual direction at the center of the implant fixture along the long axis of the implant, micro polished to a thick ness of 30μm and stained with Goldner's trichrome[17].

2.3 Radiographic analysis of ridge contour between tooth and implant area

A micro-computed tomographic (CT) scan was performed (SkyScan1072, SkyScan, Aartselaar, Belgium) at a resolution 35 μm and reconstructed using three-dimensional software (OnDemand3D, Cybermed, Seoul, Korea). The vertical buccal bone level at the tooth (residual mesial root) (VBL-R) and at the immediate implant area (VBL- I) were measured (Figure 2A). Measurement reference of the vertical bone loss was modified from a previous study[18]. Specifically, cross-sectional micro CT images from each site (the center of the mesial root and the center of the implant) were used, and a line perpendicular to the long axis of the tooth or fixture in each CBCT image was drawn at the most coronal part of the lingual plate. Vertical buccal bone level was defined as the distance between this perpendicular reference line and the most coronal part of the buccal plate. The relative vertical level of buccal bone at the implant sites (rVBL- I) was defined as VBL- I minus VBL- R.

Additionally, to visualize the overall change of the alveolar ridge, the cross- sectional images obtained from the mesial root and center of the implants were superimposed using the mandibular canal and the mandible outline as references [19](Figure S1).

2.4 Histomorphometeric analysis

The methods for the histomorphometric analysis were based on a previous study[20]. All the histomorphometric measurements were analyzed with the image analysis software (Image- Pro Plus, Media Cybernetics, Silver Spring, MD, USA). The changes in vertical bone levels of the buccal plate (VBL) and changes in horizontal ridge width of buccal plate (HRW) were measured using the shoulder of the implant as the landmarks (Figure 2B); The vertical bone loss of the buccal bone plate (VBL) was set as the distance from the implant shoulder to the most coronal point of the buccal bone crest along the implant fixture axis. When the bone crest was located more coronally than the implant shoulder, VBL was measured as a negative value. The horizontal ridge width (HRW) was measured as the distance between the implant fixture and the outer surface of the buccal bone plate. Lines perpendicular to the implant axis were drawn at 1, 2, and 3 mm below the implant shoulder, at which the horizontal ridge width was set as HRW1, HRW2, and HRW3, respectively.

2.5 Evaluation of soft tissue buccal contour change, height, and thickness

To measure soft tissue contour change, a profilometric method was used[21]. In brief, STL files generated from intraoral scans (baseline and 20 weeks) were superimposed using image analysis software (SMOP, Swissmeda, Zürich, Switzerland). The scan abutment and the adjacent teeth were used as landmarks for superimposition (Figure 2C,D). A square of 5 mm × 5 mm was drawn as a region of interest on the buccal gingival surface adjacent to the abutment margin, and then the volume changes between baseline and 20 weeks were calculated as $mm^{3.}$

To measure soft tissue height and thickness, DICOM and STL files at 20 weeks were superimposed. Using the adjacent teeth and the scan abutments as landmarks, the soft tissue contour around the implant of the DICOM and STL files were matched. The soft tissue height and thickness at the implant platform level were measured in cross-section images at the center of the implant using the image processing program (Image J, National Institutes of Health, Bethesda, MD, USA)[21]. A line perpendicular to the implant axis was drawn on the platform level. The soft tissue thickness was set as a distance between the buccal platform of the implant and the buccal soft tissue margin along the line. The soft tissue height was set as a distance between the line that measured soft tissue thickness and the gingival margin on the buccal side of the abutment (Figure 2E).

2.6 statical analysis

Vertical bone level change of the buccal plate was defined as a primary outcome. All measurements were evaluated by two experienced examiners (I.H and Y.K) who were blinded to the group allocation. To assess intra-examiner reliability, each examiner measured the same parameter in separate places, with a two-week interval. The intra-examiner reliability was assessed using the intraclass correlation coefficient (ICC). The ICC for histomorphometric measurements was 0.95 and 0.96, indicating excellent reliability. The ICC for radiographic change was 0.92 and 0.89, and the ICC for soft tissue change was 0.90 and 0.92, respectively, indicating excellent to good reliability. The inter-examiner reliability was also assessed using the ICC. The ICC for histomorphometric measurments was 0.94, radiographic change was 0.91, and soft tissue change was 0.90. The computer software (SPSS version 23, IBM) was used for the statistical analysis. Kruskal-Wallis test was used for testing between groups at each time point, and the Mann–Whitney U test was applied for the tests between time periods within each group.

3. Results

3.1 Clinical analysis

Following implant placement, there were no significant differences in the buccal gap distance and the buccal bone thickness (Figure 3). During the post-operative healing period, a single dog showed delayed soft tissue healing on BP, BP/BS, and control site at 2 weeks, which healed well subsequently. At 20 weeks post-op, every implant in all dogs demonstrated clinically healthy condition with a normal probing depth of around 3 mm and minimal bleeding on probing. All implants were well-osseointegrated with periotest values <−2 (Figure 4). All six dogs were included in the analysis.

3.2 Micro CT analysis

In the control group, there was significant buccal ridge resorption in the immediate implant area compared to the residual mesial root area (Figure S1A). Due to buccal bone resorption, there was notable dehiscence on the immediate implant sites. In contrast, buccal bone was maintained on the residual mesial root area. In the BP group, there was no significant difference between the alveolar ridge dimensions at the site of the immediate implant compared to the residual mesial root (Figure 1B). In BS and BP/BS groups, also, there was also no significant difference between alveolar ridge dimensions at the site of the immediate implant compared to the residual mesial root (Figure 1C,D). In both groups, grafted SBS were observed inside the gap between the buccal plate and the implant. In addition, there were residual SBS outside of the extraction socket.

The VBL-R, VBL-I, and rVBL-I are presented in Figure 5. There were no significant differences in VBL-R in the control and all experimental groups. In the BP group, VBL-I was significantly lower than the control group (1.17 ± 1.54 and 3.47 ± 1.14, respectively, $p < .05$), while there were no significant differences in the VBL- I of BS and BP/BS compared to the control group. Similarly, rVBL-I was significantly lower in the BP group compared to the control group (0.29 ± 1.37 and 2.29 ± 0.66. respectively, $p < .05$).

3.3 Histologic and histomorphometric analysis

Representative histologic slides of each group are shown in Figure 6 and Figure S2. In the control group, buccal dehiscence of the implant was observed with notable resorption in the coronal one- third of the buccal bone. (Figure 6A). In contrast, the BP group showed maintained buccal bone with a thick and dense cortical buccal bone plate, similar to its lingual plate (Figure 6B). The outer surface of the buccal plate showed limited bone remodeling and matured bone structure was observed at the inner surface of the preserved buccal bone (Figure 6E). The inner and outer surfaces of the buccal plate showed continuity of cortical bone without showing differences from the cortical basal bone area. A pamidronate- loaded collagen membrane, which was placed on the outer surface of the buccal plate, was resorbed without the identification of any remnant in the histological observations. The implant was well osseointegrated with new bone deposition observed along its surface (Figure 6F). The buccal gap area was filled with new bone with abundant blood vessels and its entrance was covered with a bone bridge between the implant and the buccal bone plate.

In the BS group, the outer and inner surface of the buccal plate showed bone remodeling with bone resorption (Figure 6C). The outer surface of the buccal plate was resorbed and new bone accumulation was observed at the inner surface of the buccal plate. The bundle bone of the inner surface was fully resorbed. New bone formation was found around the SBS particles placed in the buccal gap. Some SBS particle was found outside of the socket encapsulated by fibrous tissue without inflammation. The BP/BS group showed a similar ridge shape to the BS group (Figure 6D). Like the BS group, the buccal plate of the BP/BS group showed remarkable bone remodeling with resorption of the outer surface and bone accumulation of the inner surface. The pamidronate- loaded collagen membrane, which was placed on the outer surface of the buccal plate was resorbed without remnant. Newly formed bone was observed around the SBS and implant surface. The histomorphometric results are described in Figure 7. The VBL was significantly different between the BP and control group (0.34 ± 0.93 and 1.27 ± 0.56 mm, respectively; $p = .041$). However, there was no significant difference in VBL between the control group and the BS or BP/BS groups. There was no statistically significant difference between all experimental groups and control group in HRW1, HRW2, and HRW3.

3.4 Soft tissue analysis

The soft tissue thickness, soft tissue height, and buccal ridge contour volume changes are described in Table S1. There was no significant difference in soft tissue thickness, height, and buccal ridge volume change among groups.

4. Discussion

Based on a previous study demonstrating that local delivery of pamidronate inhibits the resorption of the buccal plate in the extraction socket healing, this study aimed to assess whether local delivery of pamidronate could inhibit the resorption of the buccal plate after immediate implant placement in fresh extraction sockets. The present in vivo investigation demonstrated that the local delivery of pamidronate on the outer surface of the buccal plate significantly prevented the buccal plate resorption during the healing of immediate implants. However, there was no significant difference between the local delivery of pamidronate with synthetic bone substitute and the sole use of synthetic bone substitute on buccal plate resorption of the immediate implant.

In the immediate implants, the resorption of the buccal plate causes dehiscence of the implant, which results in biological and aesthetic complications, so to prevent buccal plate resorption, immediate implant with socket grafting to fill the gap with bone substitute has been applied[22, 23]. In the previous in vivo study, the socket grafting with immediate implant using collagenated bone substitute showed approximately 0.1 mm of vertical bone loss on the buccal, whereas immediate implant without grafting showed 1.3 mm of vertical bone loss on the buccal[24]. Similarly, in the present study, the local delivery of pamidronate reduced the vertical buccal bone loss to 0.16 mm, while the control group showed 2 mm of vertical bone loss on buccal.

A thin buccal wall thickness of <2 mm is also vulnerable to buccal resorption after immediate implant placement[25]. However, a previous review revealed that the majority of maxillary anterior teeth have <1 mm of buccal bone thickness[26]. Since the esthetic complication is critical on maxillary anterior teeth, such a thin buccal wall places a great burden on the clinician to place immediate implants. In the present study, despite of the thin buccal wall thickness of 0.69 mm, the local delivery of pamidronate could effectively maintained the buccal plate after immediate implant placement.

In previous studies, the use of biphasic calcium phosphate and crosslinked collagen membranes have shown favorable outcomes in guided bone regeneration and when applied together with immediate implant placement[27, 28]. Also, biphasic calcium phosphate and crosslinked collagen membrane used have the advantage of allowing the addition of drugs or growth factors during the manufacturing process[29, 30]. Future studies should explore the use of these biomaterials as drug carriers, specifically the study of the kinetics of local pamidronate when seeded in biphasic calcium phosphates and crosslinked collagen membranes.

However, interestingly, the concomitant use of SBS and pamidronate could not prevent buccal plate resorption in the present study. This might be explained by the delay of new bone regeneration in the extraction socket when bone substitutes are applied. In spontaneous socket healing, new bone

fills quickly starting from the apical of the extraction socket[31]. In the healing of the ARP site, the bone substitutes act as a scaffold, but the bone substitute itself seems to delay new bone formation in the early healing phase. In the situation of the BP/BS group, while the local delivery of pamidronate inhibits resorption of the buccal plate, rapid new bone regeneration is necessary to fill up the gap defect and support the buccal bone plate.

Biphasic calcium phosphate is composed of hydroxyapatite and beta-tricalcium phosphate is composed of hydroxyapatite beta- tricalcium phosphate[32]. Hydroxyapatite serves as a scaffold and is associated with osteoconductivity. Beta-tricalcium phosphate is relatively soluble, thus increasing the concentration of calcium ions in the surrounding area, which may have osteoinductive potential. In fact, previous animal experiments have reported that this osteoinductive potential can lead to ectopic bone formation[33]. Since the biphasic calcium phosphate used in the present study consists of 60% hydroxyapatite and 40% beta-tricalcium phosphate, the higher proportion of hydroxyapatite is associated with a lower resorption rate[32] and higher osteoconductivity and lesser osteoinductivity, which might be related to delayed bone regeneration. This fact may imply that the concomitant use of bone substitutes and pamidronate may limit the effect on the maintenance of the buccal plate as it has been shown with the sole use of bisphosphonate.

There is no significant difference in physiological changes after tooth extraction whether the immediate implant is placed or not[34]. In a previous study comparing immediate implant placement with simultaneous bone augmentation and delayed implant placement after ARP, there was no difference in buccal bone thickness in long term[35]. In the pilot study with local delivery of pamidronate to the extraction socket in a similar manner to the present study, vertical bone loss was limited to 0.52 ± 0.39 mm, and the horizontal bone width measured at 1 mm below the lingual crest was significantly wider in the test group compared to the control group (4.68 ± 0.62 vs 3.44 ± 0.55 mm, $p = 0.008$)[8]. In the present study, local application of the pamidronate with immediate implant placement showed limited vertical bone loss of 0.34 ± 0.93 mm. In line with previous studies, inhibition of extraction socket resorption through local delivery of pamidronate was not affected by immediate implant placement.

In a previous pilot study, collagen membrane soaked in 15 mg/mL of pamidronate completely covered the entrance of the socket, and the entrances of the extraction sockets were incompletely bridged with newly formed bone[8]. In the study applying the collagenated porcine bone substitute soaked in 90 mg/mL of pamidronate on the extraction socket, bone regeneration of the coronal part of the extraction socket was delayed or impaired[10]. Since pamidronate inhibits bone remodelling by inhibiting osteoclasts, it may deteriorate bone regeneration inside the extraction socket. In this study, collagen membrane soaking 15 mg/mL of pamidronate was applied only to the buccal plate area, which has relatively low concentration of pamidronate and narrower application compared to previous studies. The entrances of the extraction socket in the present study were fully covered with bone bridge and implant fixture. It was able to minimize inhibition of the bone remodelling inside the extraction socket through more localized application of bisphosphonate compared to previous

studies. However, further research is still needed on the appropriate dose and release of drug according to the graft material used for carrier.

The present study has clear limitations, mainly since it was conducted in an animal model, in which the effects of local pamidronate on bone physiology might be different in humans and the restriction to use a large number of animals may result in an underpowered experimental study insufficient to achieve statistical significance in some of the comparisons. Also, the surgical sites of the two implants were close, so a single flap from the distal of the second premolar to the mesial first molar covered both surgical sites. Accordingly, post-operative factors like swelling could have influenced the displacement of bone substitutes in adjacent implants assigned to the different study groups. Similarly, released pamidronate from the collagen membrane could have influenced the adjacent study group. These disturbances might have worked as a factor for the lack of statistical significance. Secondly, delivery of pamidronate was performed by soaking the pamidronate injection into the collagen membrane. It is uncertain how much pamidronate was soaked into the collagen membrane and ultimately released to the buccal bone. Thus, the clinical application of the findings in the study should be interpreted with caution.

5. Conclusion

In conclusion, the local delivery of pamidronate when applied with a biphasic calcium phosphate bone substitute and a cross- linked bioabsorbable collagen membrane around immediately placed dental implants in fresh extraction sockets, reduced the vertical and horizontal resorption of the buccal plate in this in vivo experimental model, and therefore, seemed to enhance the maintenance of the alveolar ridge dimensions. Meanwhile, there was no significant difference in buccal wall resorption regardless of local delivery of pamidronate in the case of the bone substitute applied. The results from the present investigation should be interpreted with caution, as well as its clinical translatability. Further investigation is needed to understand the pamidronate binding and releasing kinetic, as well as the ideal carrier of this drug for its topical application in alveolar bone for both ARP and immediate implant procedures.

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FIGURE LEGENDS

Figure 1. Overall surgical procedure is described. (A) After hemisectioning the premolars, the distal roots were carefully extracted. (B) After implant placement at the fresh extraction socket, synthetic bone substitutes (SBS) were grafted at the gap and collagen membrane (CM) either soaked onto pamidronate or saline was placed on buccal. (CM, white dotted circle; SBS, yellow dotted circle) (C) Primary flap closure was attempted around the healing abutment

SBS, synthetic bone substitute; CM, collagen matrixs

Figure 2. Schematic drawing of the measurements. (A) Micro-CT measurement; The lines perpendicular to the long axis of the implant and tooth (Blue line) were drawn on the most coronal part of the lingual plate (Yellow dotted line). Vertical buccal bone level of residual tooth (VBL-R) and implant area (VBL-I) were measured as the distance between the yellow dotted line and the most coronal part of the buccal plate (White arrow). (B) Histometric measurement; The vertical bone level of the buccal bone plate (VBL) and horizontal ridge width at 1, 2 and 3 mm below the lingual crest (HRW 1/2/3, respectively) were measured. Implant shoulder was used as the landmark. (C, D) Analysis of buccal ridge contour and soft tissue; The STL images of baseline (green) and 20 weeks (yellow) were superimposed. The square of 5 × 5 mm was drawn as a region of interest (orange) on the buccal. Volume change on buccal contour (blue) was analyzed (D). (E) The acquired DICOM and STL files at 20 weeks were superimposed, and the soft tissue thickness (*) and height (**) were measured.

Figure 3. Buccal gap distance (A) and buccal plate thickness (B) of the experimental and control groups in the baseline ($n = 6$ for each group). There was no significant difference in buccal gap distance and buccal plate thickness among groups.

Figure 4. Every implant in all dogs demonstrated clinically healthy condition with normal probing depth of around 3 mm, and minimal bleeding on probing. All implants were well-osseointegrated. (A) BP and BS group (B) BP/BS and control group (C) BS and BP/BS group (D) control and BP group.

BP, bisphosphonate; BS, bone substitute

Figure 5. The result of micro-CT analysis ($n = 6$ for each group). VBL-I and rVBL-I of the BP group were significantly lower than the control group (VBL-I, Vertical buccal bone level of implant area; VBL-R, Vertical buccal bone level at residual tooth area; rVBL-I, Relative vertical bone level of implant area).

Figure 6. Representative histologic slides of the control and the experimental groups. (A) Control group, Notable resorption in the coronal one-third of the buccal bone was observed. (B) BP group. The BP group showed a maintained buccal plate of the ridge, which clearly differed from the control. (C) BS group. Some grafted bone substitutes were found outside of the socket encapsulated by fibrous tissue without inflammation (D) BP/BS group. High magnification image of the BP group was presented (E) The outer surface of the buccal plate showed limited bone remodeling, and matured bone structure (yellow arrows) was observed in the inner surface of the buccal plate. (F) New bone accumulation was observed along the fixture surface. The buccal gap area was filled with new bone with abundant blood vessels (*).

Figure 7. Results of histomorphometric analysis (n = 6 for each group). (A) The VBL was significantly different between the BP and control group. (B) Horizontal ridge width. There was no significant difference in HRW1, 2, and 3 among the groups (VBL, Vertical bone level of buccal plate; HRW 1/2/3, Horizontal ridge width at 1, 2 and 3 mm below the lingual crest)

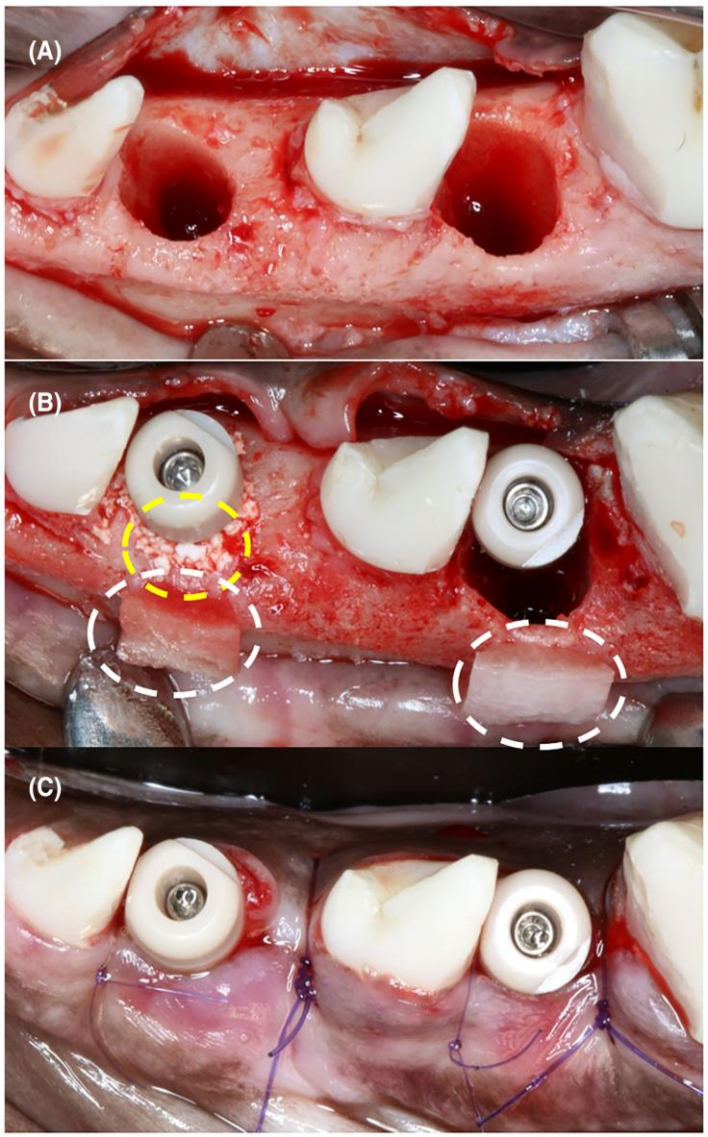
Supplement Figure 1. Superimposition of CT images of the residual mesial root and implant area. (A) control group, (B) BP group, (C) BS group, (D) BP/BS group.

green; alveolar bone of mesial root area, blue; alveolar bone of immediate implant area, cyan; overlapped area of alveolar bone of mesial root area (green) and immediate implant area (blue), yellow: overlapped area of implant fixture and cyan area, red; bone substitute, white line; line perpendicular to the tooth axis

Supplement Figure 2. Supplemental histologic slides of the experimental groups (A,B,C; low magnification, D,E,F; high magnification). (A, D) BP group, BP group with smaller buccal gap also showed limited remodeling of the buccal plate, (B,E) BS group, due to the buccal plate resorption, SBS grafted in buccal gap was exposed. New bone regeneration was observed around the SBS remained in the buccal gap. (C,F) BP/BS group, In BP/BS group with the larger buccal gap, more bone regeneration was observed around the SBS.

FIGURES

Figure 1



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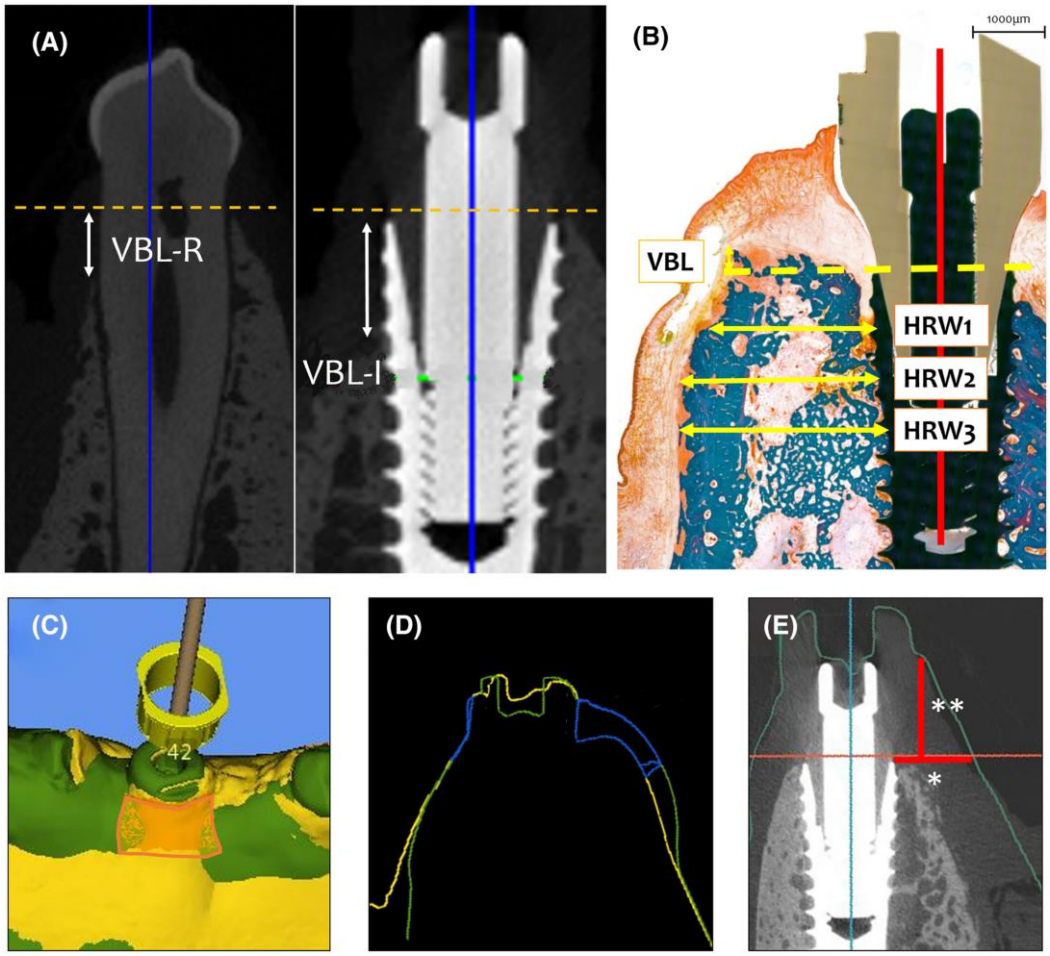

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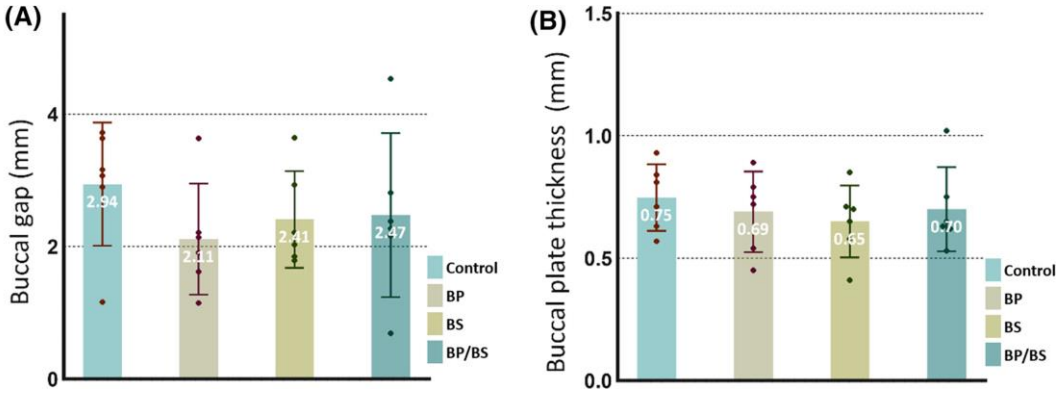

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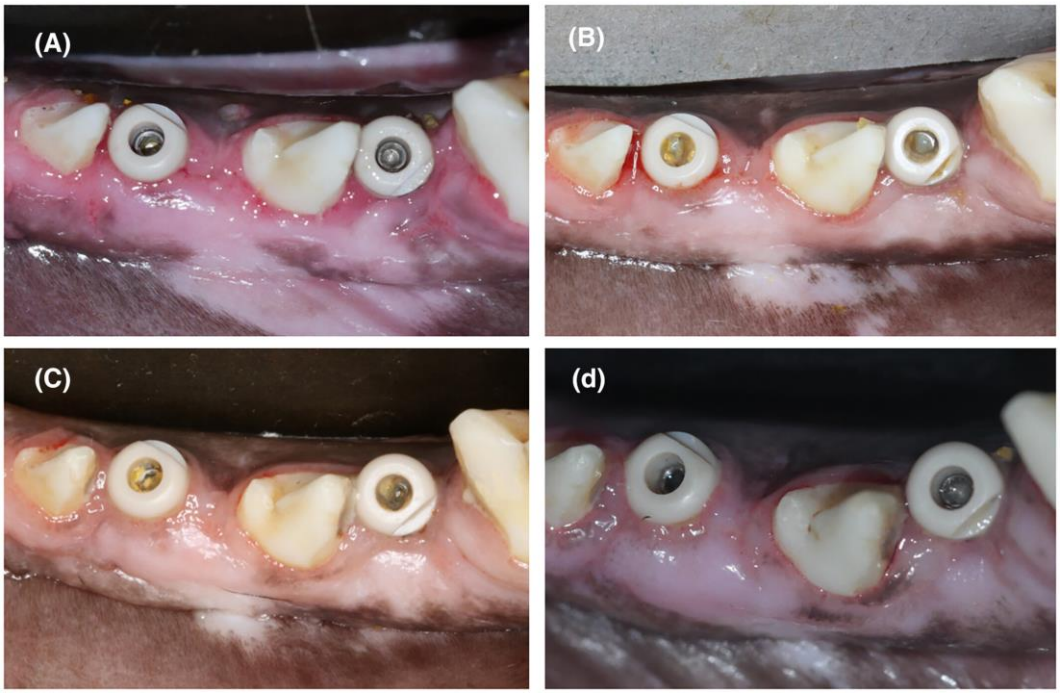

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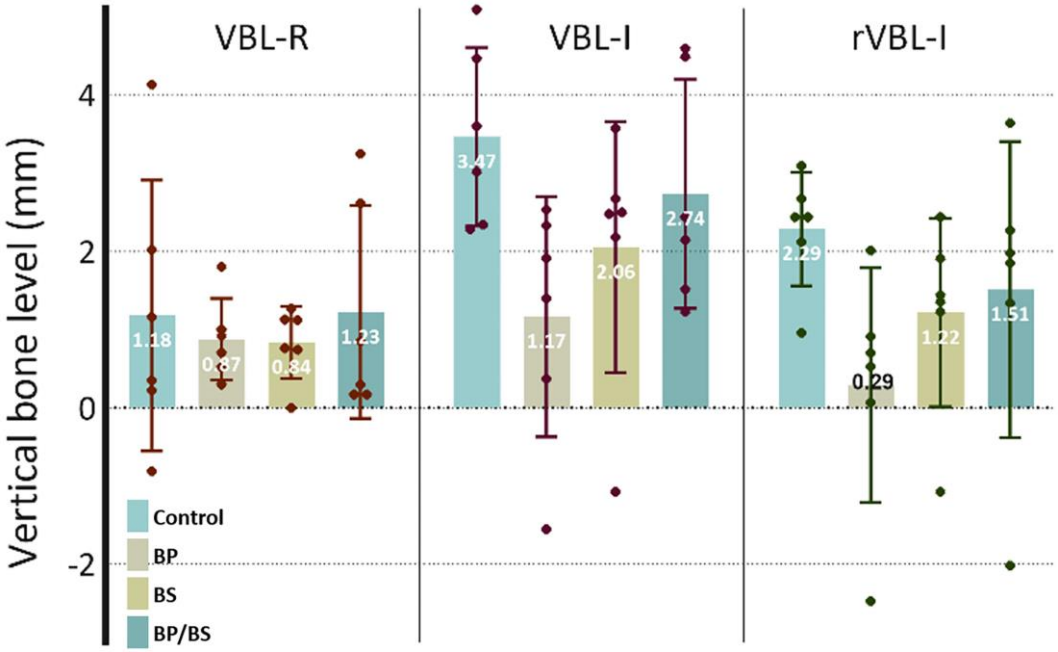


Figure 6

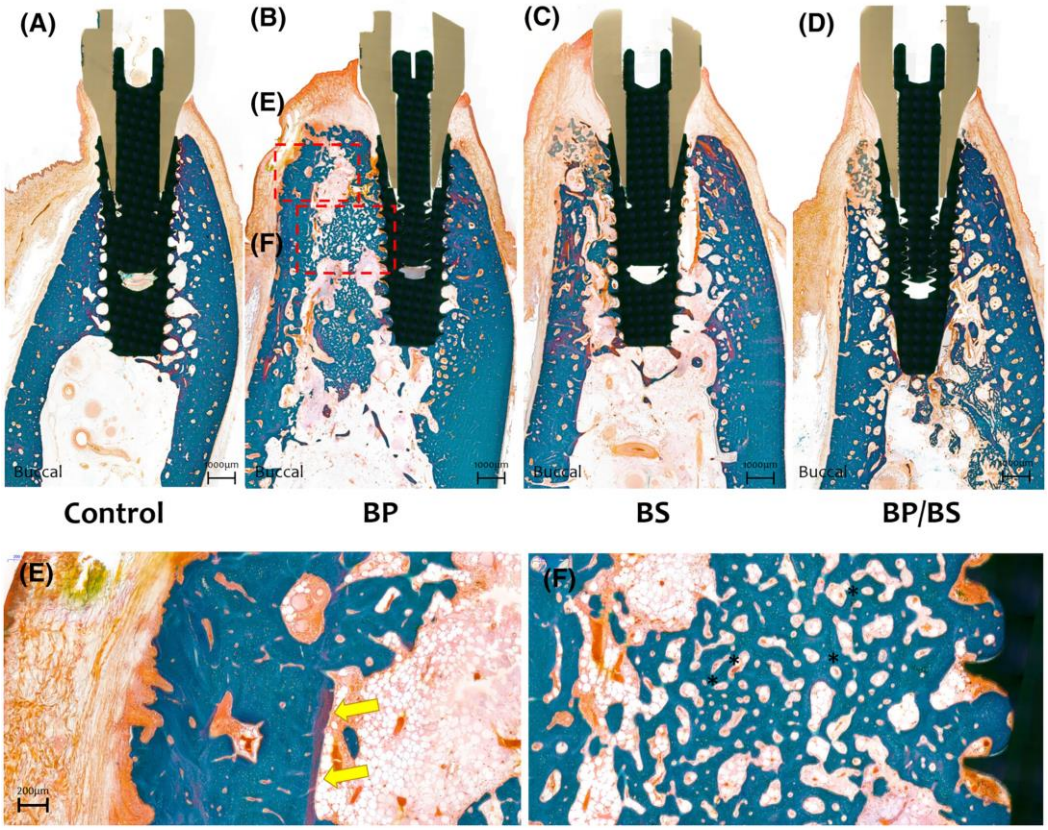


Figure 7

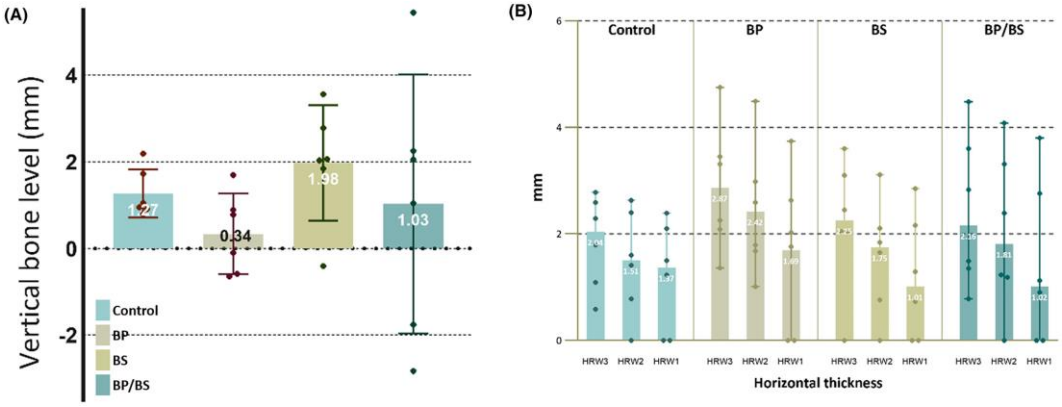
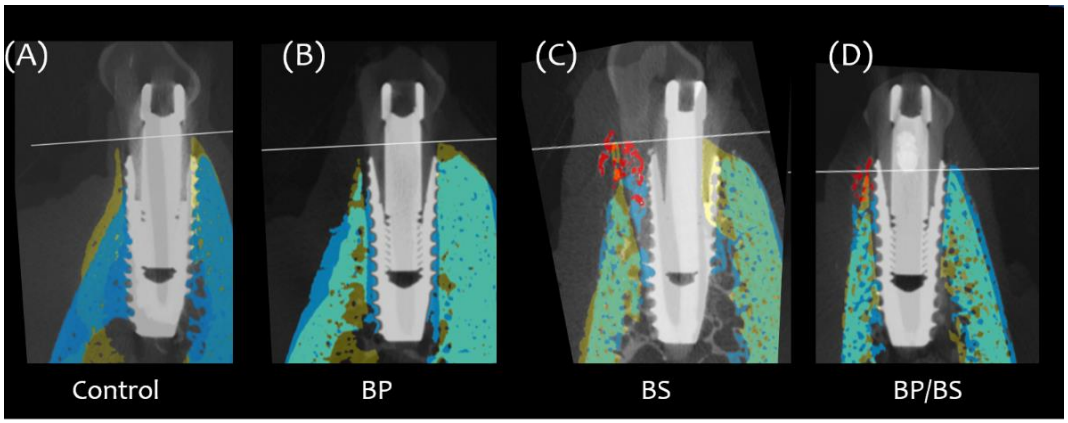


Figure S1



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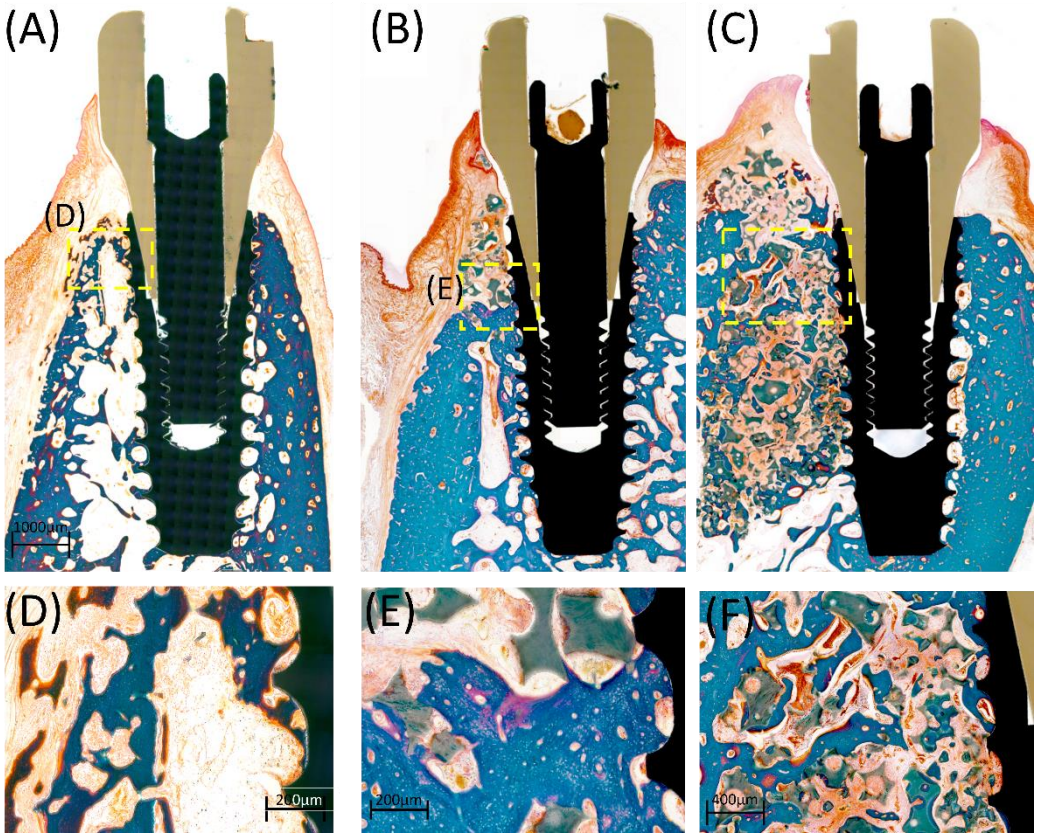

Tables

mean ± SD	*Linear measurement (mm)*		*Volumetric measurement (mm^3)*
	Soft tissue height	Soft tissue thickness	Buccal volume change
Control (n=6)	3.88 ± 1.03	4.11 ± 1.40	23.82 ± 19.48
BS group (n=6)	3.69 ± 2.00	3.30 ± 1.25	21.61 ± 6.90
BP group (n=6)	3.37 ± 1.95	3.02 ±1.63	25.18 ± 8.11
BP/BS group (n=6)	3.61 ± 1.52	3.98 ± 2.02	25.30 ± 11.98

Supplement table 1. Evaluation of soft tissue thickness and height at 20 weeks, and buccal ridge contour volume change between baseline and 20 weeks

Abstract in Korean

즉시 임플란트 식립 후 파미드로네이트 함유 콜라겐 기질을 활용한 협측 골 흡수 방지에 관한 전임상연구

본 연구는 생채 내에서 수행된 선행 연구로써 연구의 목적은, 즉시 임플란트 식립 후 파미드로네이트를 적용한 콜라겐 멤브레인을 국소적으로 적용했을 때 협측 연조직 및 경조직의 차원 변화에 미치는 영향을 평가하는 것이었으며, 이 효과가 골이식재의 사용에 의해 영향을 받는지를 확인하는 것이었다.

성견 6마리를 대상으로 양측 하악 제3, 제4 소구치의 원심 치근을 발치하고 즉시 임플란트를 식립하였다. 발치와는 무작위로 다음과 같은 순서로 배정되었다: (i) 아무런 처치를 하지 않은 대조군(control군), (ii) 파미드로네이트를 적용한 콜라겐 멤브레인을 협측골에 위치시킨 군(BP군), (iii) 합성 골이식재로 결손부를 충전한 군(BS군), (iv) 합성 골이식재로 결손부를 충전하고, 파미드로네이트를 적용한 콜라겐 멤브레인을 골에 위치시킨 군(BP/BS군). 수술 직후와 20주 후 구강스캔을 시행하였으며, 20주 후 조직형태계측 및 Micro CT 분석을 수행하여 평가하였다.

Micro CT 분석 결과, BP군은 잔존 근심 치근 부위와 수직적 골 높이에서 유의한 차이가 없었으나 대조군은 임플란트 식립 부위에서 협측 골 흡수가 유의하게 나타났다(그림.1).

조직학적 분석 결과, 대조군에서는 협측골의 치관측 1/3 부위에서 현저한 골흡수가 관찰된 반면, BP군에서는 협측골이 잘 유지되어 있어 대조군과 뚜렷한 차이를 보였다. BS군에서는 일부 이식된 골대체재가 발치와 외부에서 섬유성 조직으로 둘러싸인 상태로 관찰되었으며, 염증 반응은 나타나지 않았다. BP/BS군의 고배율 조직학적 소견에서는 협측골의 바깥쪽 표면에 제한적인 골 재형성이 나타났고, 안쪽 표면에서는 성숙된 골 구조가 관찰되었다. 또한 임플란트 표면을 따라 신생골이 형성되어 있었으며, 협측 간극은 풍부한 혈관을 포함한 신생골로 충전된 양상을 보였다(그림.2).

조직형태계측학적 분석 결과에서는 BP군과 대조군 간 협측골의 수직적 골 높이가 통계적으로 유의한 차이를 보였다(BP군: 0.34 ± 0.93 mm, 대조군: 1.27 ± 0.56 mm; p = .041). 그러나 수평 치조골 폭(HRW 1, 2, 3)은 각 군 간에 통계적으로 유의한 차이가 없었다(표.1).

결론적으로 즉시 임플란트를 식립할 때 협측골 외측에 파미드로네이트를 국소적으로 적용하면 협측골의 흡수를 효과적으로 억제할 수 있었다. 하지만 연구 여러 한계점이 존재하기 때문에 연구 결과에 대한 신중한 해석이 필요하며, 임상에 적용할 때에도 주의가 필요하다. 향후 파미드로네이트가 뼈에 어떻게 결합하고 방출되는지에 대한 연구가 더 필요하고, 이 약물을 국소적으로 적용시 적합한 전달체에 대해서도 추가적인 연구가 필요하다.

핵심되는 말: 동물실험, 비스포스포네이트, 즉시 임플란트 식립