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Volumetric, Radiographic, and Histologic Analyses of Demineralized Dentin Matrix Combined with Recombinant Human Bone Morphogenetic Protein-2 for Ridge Preservation: A Prospective Randomized Controlled Trial in Comparison with Xenograft

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Featured Application: Novel technology of bone regeneration.

Abstract: The aim of this study was to evaluate the clinical, volumetric, radiographic, and histologic aspects of autogenous demineralized dentin matrix (DDM) combined with recombinant human bone morphogenetic protein-2 (rhBMP-2) used for ridge preservation, compared to those of deproteinized bovine bone with collagen (DBBC). Following atraumatic extraction, the socket was filled with DBBC, DDM, or rhBMP-2/DDM. Scanned images of dental casts and cone beam computed tomographs (CBCT) were superimposed for the calculation of soft and hard tissue volume alteration. Preoperative and postoperative measurements of the height and width of the alveolar ridge were compared using CBCT images. After 4 months, bone specimens were harvested for histomorphometric assessment. Loss of hard and soft tissue volume occurred at 4 months after extraction and ridge preservation in all groups. No volumetric differences were detected among the three groups before and 4 months after ridge preservation. The reduction in the horizontal width at 5 mm was higher in the DBBC compared to the DDM. Histologically, approximately 40% newly formed bone was founded in rhBMP-2/DDM group. The autogenous dentin matrix used to fill the socket was as beneficial for ridge preservation as conventional xenografts. The combination of rhBMP-2 with dentin matrix also demonstrated appreciable volumetric stability and higher new bone formation compared to DDM alone and DBBC.

Keywords: autografts; bone regeneration; bone morphogenetic proteins; bone substitutes; cone-beam computed tomography; tooth extraction

1. Introduction

Alteration of the alveolar ridge following tooth extraction is unavoidable owing to horizontal and vertical bone resorption [1]. If the alveolar ridge undergoes extensive bone resorption, it may compromise the aesthetic value and function of dental implants. Considerable alveolar bone alteration has been reported to occur during the first year following tooth extraction [2], and two-thirds of this bone resorption was observed in the buccal part. The bone height decreased by approximately

0.8 mm 3 months after extraction, and continued decreasing to 2 mm after 12 months. In contrast, horizontal width resorption occurred more drastically during the first year following extraction [3–5]. To overcome these obstacles, various techniques have been proposed to preserve and maintain the alveolar ridge volume following tooth extraction. This includes bone grafting immediately after tooth extraction using bone biomaterials or substitutes to diminish the extent of volume changes [6–8]. This ridge preservation technique, also known as alveolar socket preservation, socket grafting, or the socket seal technique has been successfully implemented and has been reported to reduce alteration of the socket.

A wide range of biomaterials, including autografts [9], allografts [10], xenografts [11], alloplastic materials such as bioactive glasses [12], autogenous tooth bone grafts [13–15], bioactive materials such as recombinant human bone morphogenetic protein-2 (rhBMP-2) [16], and platelet-rich fibrin (PRF) [17], has been shown to produce positive outcomes for ridge preservation. Autogenous bone is an ideal material for hard tissue defects because it has osteoinduction and osteoconduction and has no immune rejection. However, it has disadvantages in that it causes secondary defects, has a limited amount of harvesting, and inevitable absorption occurs. Allogeneic bone and xenogeneic bone have problems such as infection, immune rejection and high cost. Synthetic bone is considered to be an alternative material, but these materials lack osteoinductive potential, and their osteogenic potential is not satisfactory.

Autogenous tooth-derived demineralized dentin matrix (DDM), has a relatively shorter history of clinical adaptation in comparison with other bone grafts or substitutes. The chemical compositions of dentin and bone are very similar. In addition, about 90% of the dentin organic material consists of collagen fiber, which is mostly type I collagen and plays an important role in calcification. The remaining organic parts are composed of noncollagenous proteins including growth factors, carbohydrates, lipid, etc. [18]. In a previous study, a scanning electron microscopy (SEM) analysis of calcified dentin revealed dentinal tubules, which are thought to contribute to the diffusion of nutrients after grafting by acting as a network [19]. Several recent studies have demonstrated that DDM is well-tolerated when used to fill the ridge defects, indicating promising potential for bone regeneration [13–15,20]. Similarly, the effectiveness of DDM as a bone substitute has already been identified in various clinical circumstances such as the alveolar bone regeneration associated with dental implants, sinus augmentation, and preservation of extraction sockets [13–15,21,22]. In these studies, DDM completely fulfills the criteria for a bone regeneration material, possessing osteoinductive and osteoconductive properties [23]. Moreover, its clinical safety and capability are well-demonstrated. However, published human data, with respect to the therapeutic efficiency of DDM for ridge preservation, is scarce. Only a few studies have investigated the clinical changes following tooth extraction, and socket management, using DDM [13].

A rhBMP-2 is a potent osteoinductive protein which promotes the differentiation of osteoblasts from mesenchymal stem cells and accelerates bone regeneration [24]. The osteoconduction of rhBMP-2 has been widely studied in various bone healing environments, and rhBMP-2 has been shown to heal critical-sized bone defects in animal models and clinical trials [25,26]. In a recent study, rhBMP-2 was used to achieve better bone healing in distraction osteogenesis in an animal study and large mandibular reconstruction defects in clinical trials [27,28].

In the present study, we aimed to evaluate and compare the quantitative alveolar ridge changes before and after ridge preservation using two different biomaterials—deproteinized bovine bone with collagen (DBBC) and DDM—by measuring volumetric and radiographic parameters as well as the histology of grafted socket sites. Moreover, as studies concerning the osteogenic potential of rhBMP-2 are limited and lacking in the context of the ridge preservation technique, we hypothesized that the combination of these two materials (DDM and rhBMP-2) might optimize bone regeneration in the extraction socket. Therefore, the aim of the current study was to analyze the clinical, volumetric, radiographic, and histologic alterations associated with the use of DBBC, DDM, and DDM combined

with rhBMP-2 for the treatment of extraction sockets through the ridge preservation technique over a 4-month period.

2. Materials and Methods

2.1. Enrollment of Patients

A total of 30 patients (16 males and 14 females, aged 27 to 79 years) who needed to undergo extraction were enrolled in this study. The study aims and methods were explained to the patients, and written informed consent was obtained in advance. The study protocol was approved by the Institutional Review Board of Korea University Anam Hospital (IRB number: MD 15013, Registration date: 15 January 2016). The study was performed at the Department of Dentistry, Korea University Anam Hospital. The inclusion criteria were healthy individuals aged 18 or older who needed to undergo extraction of one or more third molars and hopeless teeth (premolar and molar). Reasons for extraction were root fracture, failure of root canal treatment, extensive caries, and moderate to severe periodontitis involving less than 50% of vertical bone loss. The following patients were excluded: those with unstable systemic diseases, uncontrolled metabolic diseases, or a habit of smoking $\geq$10 cigarettes/day; those who had been administered antibiotic drugs or corticosteroids within the last 3 months or who were receiving medication that altered bone healing, such as bisphosphonates; and those with a history of chemotherapy or radiative therapy performed in the oral and maxillofacial region.

Each of the extraction sockets were randomly assigned into one of three therapeutic groups (Figure 1):

(a) Group A: Bio-Oss® Collagen (Geistlich, Wolhusen, Switzerland) graft (n = 10)
(b) Group B: DDM graft (n = 10)
(c) Group C: DDM graft combined with rhBMP-2 (rhBMP-2/DDM) (n = 10)

2.2. Randomization Procedure

After being enrolled, the patients were randomly assigned to the three groups. A random number table was used to assign participants. We selected 27 sequential random numbers from an arbitrary point in the table. The first 9 numbers were assigned to Group A, the next 9 to Group B, and the next 9 to Group C, and these assignments were sorted in ascending order. This procedure created a random order of consecutive treatment assignments. Sealed and opaque numbered envelopes containing the process assignments were prepared, and the order of envelopes was matched to the assigned schedule. This process was performed by one person (S.S.I.). Analyses of epithelialization, volume, radiological and histological results proceeded to the blind state at each analysis.

2.3. DDM and rhBMP-2 Preparation

Before ridge preservation, previously extracted third molars were soaked in 75% ethyl alcohol and cleaned by removing old restorative materials, pulps, periodontal tissue, and attached remnants. The root portion was particularly selected for DDM and broken into fragments of diameter ranging from 0.5 mm to 1 mm (Auto BT, Korea Tooth Bank, Seoul, Korea). These particles of teeth were stored in a solution of distilled water and hydrogen peroxide, dehydrated, defatted, decalcified, and then stored in room temperature until further use. In order to evaluate them as carriers for the rhBMP-2 (CowellBMP, Cowellmedi, Busan, Korea), DDM was selected and 2 mg/mL of rhBMP-2 was added to 0.03 g of DDM by the dip dry method.

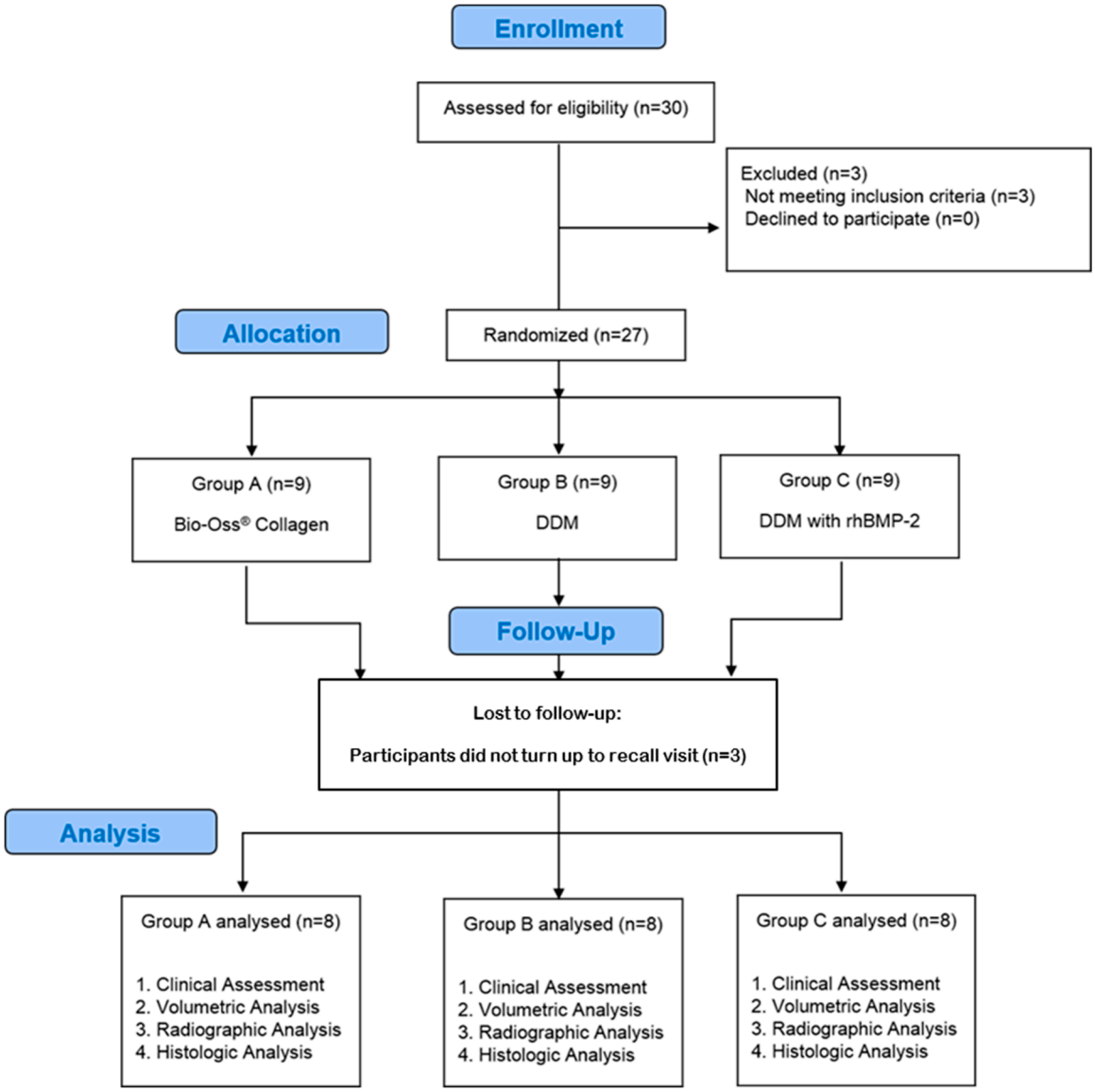

Figure 1. Study flowchart showing the participants enrolled in this study.

2.4. Surgical Protocol

Following anesthetization of the region of extraction, the tooth was extracted carefully using a flapless technique by either of the 2 oral and maxillofacial surgeons (S.-H.J., T.-H.J.). Extraction was performed using periotomes and surgical forceps to minimize the mechanical trauma caused to the buccal bone. For atraumatic extraction of molars, the diverged roots were separated by a straight bur before extraction. The extraction sockets were gently debrided and rinsed with saline, and granulation tissue was removed.

The extraction sockets were then filled with Bio-Oss® Collagen (Group A), DDM (Group B), or rhBMP-2/DDM (Group C). At the openings of these sockets, an absorbable atelocollagen plug (Rapiplug®, Dalim Tissen, Seoul, Korea) was applied and secured with cross-mattress sutures using 5-0 non-resorbable nylon (Ethicon Inc., Somerville, NJ, USA). Postoperatively, all patients were prescribed analgesics (nonsteroidal anti-inflammatory drugs) and antibiotics (amoxicillin plus clavulanic acid or clindamycin) for 5 to 7 days following ridge preservation. Sutures were removed after 7 to 14 days and patients were called for follow-up clinical examinations after 1 day, 1 week, 2 weeks, and 1 month. The graft and subsequent processes were done by one surgeon (S.-H.J.).

The complications that may occur after bone graft are as follows: swelling and edema, bleeding, subcutaneous bleeding, hematoma, infection or abscess, graft area exposure, absorption. Mild and moderate swelling, edema, and bleeding can be resolved by medication and proper hemostasis. However, in the event of severe swelling, tenderness, infection or abscess, the study was stopped and the bone graft was removed, followed by additional antibiotic administration and lavage. Later, re-grafting was considered. If the graft materials were exposed within 24 to 48 h, suture was considered immediately. If the graft materials were exposed by 2 to 3 cm or more, or 2 to 3 days later, systemic antibiotics and chlorhexidine rinses were performed twice a day.

2.5. Clinical Assessment of Epithelialization

Clinical measurements were recorded at 1 day, 1 week, 2 weeks, and 1 month postoperatively and assessed as scores of epithelialization, graded as follows:

- Grade 1: non-existent
- Grade 2: covering less than one-quarter of the wound surface
- Grade 3: covering less than half the wound surface
- Grade 4: covering more than three-quarters of the wound surface
- Grade 5: normal or complete covering of the wound

2.6. Volumetric Assessment of Soft and Hard Tissue

2.6.1. Volume Calculation for Soft Tissue/Stone Cast Measurement

The three-dimensional (3D) structure of the socket was measured and analyzed with dental cast before (baseline) and 4 months (follow-up) after ridge preservation. Stone casts were prepared based on vinylpolysiloxane impressions obtained before extraction and 4 months after the ridge preservation. For the assessment of changes in the dimension of the soft tissue at the ridge preservation sites, the stone casts were scanned with a 3D scanner (Cerec 3, Sirona Dental Systems GmbH, Bensheim, Germany), and the images were exported as stereolithography (STL) files. Matching and superimposition of 3D models of preoperative and postoperative sockets were conducted to minimize image distortions, and the data were analyzed using Geomagic Studio Software (3D system Inc., Rock Hill, SC, USA). The buccal surfaces of the central incisors and distal surfaces of the most posterior teeth were used as fixed reference points for the superposition of the different images (Figure 2). The region of interest was designated 10 mm below the cementoenamel junction and proximal height of contour of adjacent teeth and the differences in the volume of soft tissue in the targeted area were calculated. To determine the preoperative and postoperative soft tissue volumes accurately, the crown portions of extracted teeth were removed from the digital images. The total soft tissue volume of extraction sockets was measured in cubic mm (mm^3) using a volumetric analysis tool (Figure 3).

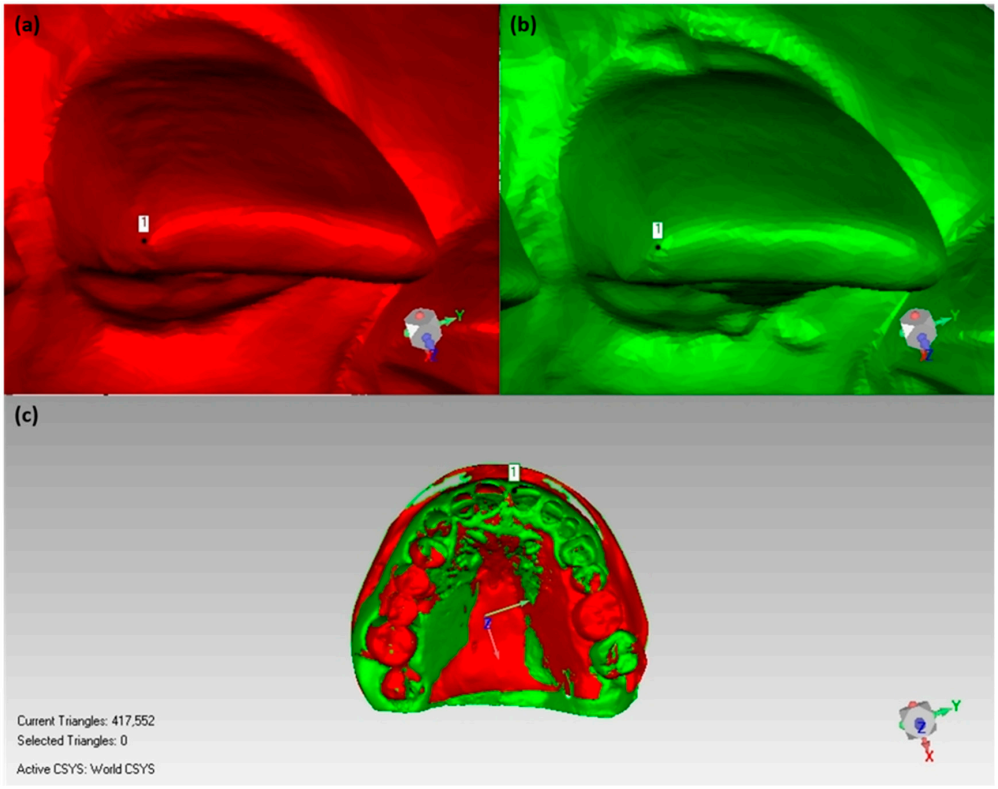

Figure 2. Matching and superimposition of preoperative and postoperative models for soft tissue volume calculation. The preoperative (**a**) and postoperative (**b**) cast scanned images were superimposed (**c**) by fixed reference points: the buccal surfaces of the central incisors and the distal surfaces of the most posterior teeth.

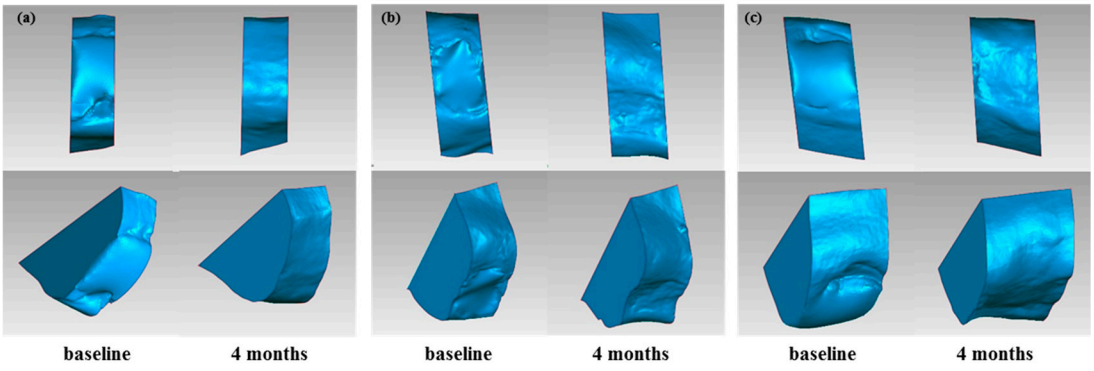

Figure 3. Soft tissue volume calculation. The scanned images were superimposed for volumetric quantification of soft tissue alteration. The three-dimensional rendered images of occlusal view were cropped and measured in cubic mm (mm^3) via Geomagic Studio Software (3D system Inc., Rock Hill, SC, USA): (**a**) Group A; (**b**) Group B; (**c**) Group C.

2.6.2. Bone Volume Calculation/Radiographic Assessment

Changes in hard tissue volume were compared between baseline and 4 months after extraction using radiographs. Three-dimensional cone beam computed tomography (CBCT; KaVo 3D eXam, Biberach, Germany) was performed at baseline (before extraction) and at 4 months after ridge preservation. The regions of the postextraction socket/ridge preservation sites were determined to be encircled by four reference points: the proximal heights of the contour of the two adjacent teeth, the most apical point of the alveolar socket, and the marginal bone crest. The vertical line was set

parallel to the tooth axis of the tooth before extraction, and the horizontal line was set perpendicular to the vertical line. The targeted ridge preservation area was cropped and subsequently analyzed using segmented surface model superimpositions (Figure 4). Thus, volumetric alteration was compared between the two scans at baseline and after 4 months of healing.

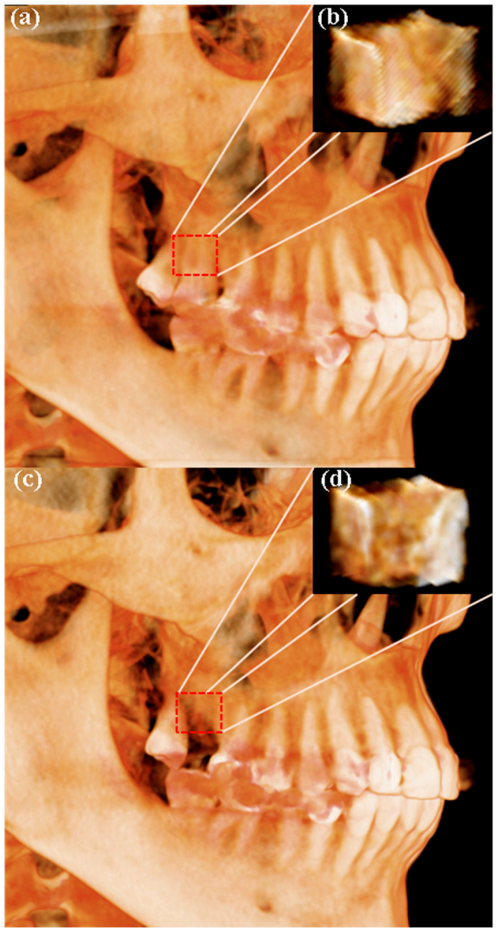

Figure 4. Volumetric analysis of hard tissue. The three-dimensional cone beam computed tomography (CBCT) images were taken at baseline (**a**) and at 4 months after ridge preservation (**c**). The regions of the postextraction socket/ridge preservation sites were determined. The cropped preoperative hard tissue (**b**) and postoperative hard tissue (**d**) volumes were measured in cubic mm (mm^3) via InVivoDental software, Version 5.0 (Anatomage Inc., San Jose, CA, USA).

2.7. Radiographic Analysis Using CBCT

To measure the alteration of the alveolar ridge height and width, consecutive CBCT images were acquired at baseline (before extraction) and at 4 months after ridge preservation. All CBCT images were acquired in identical conditions, and all of the radiographic measurements and analyses were performed by one examiner (I.-S.S.) using InVivoDental software, Version 5.0 (Anatomage Inc., San Jose, CA, USA). All measurements were performed based on imaginary lines parallel and perpendicular to the vertical and horizontal reference lines previously described in the literature [29]. The buccal and lingual ridge heights were measured from the most apical point of the alveolar socket to the height of the crest (Figure 5a). The ridge width was calculated at 1, 3, and 5 mm below the highest crest in the coronal plane (Figure 5b). The data points and lines were determined on the baseline CBCT images and duplicated onto the 4-month follow-up CBCT to accurately quantify the height and width of the alveolar ridge (Figure 6).

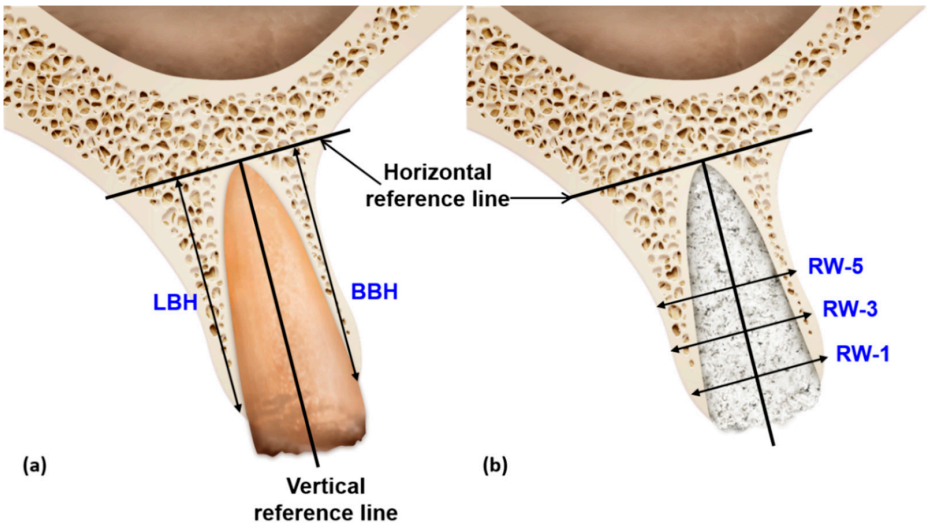

Figure 5. Schematic drawings of radiographic landmarks for measurement of the height and width of the alveolar ridge on CBCT images. The vertical reference line passed through the middle of the alveolar socket from the apex, and the horizontal reference line was drawn perpendicular to the vertical line. (**a**) The buccal and lingual bone heights were measured based on these reference lines relative to the marginal crest and root apex. (**b**) The horizontal ridge width was recorded at 1, 3, and 5 mm from the highest marginal crest.

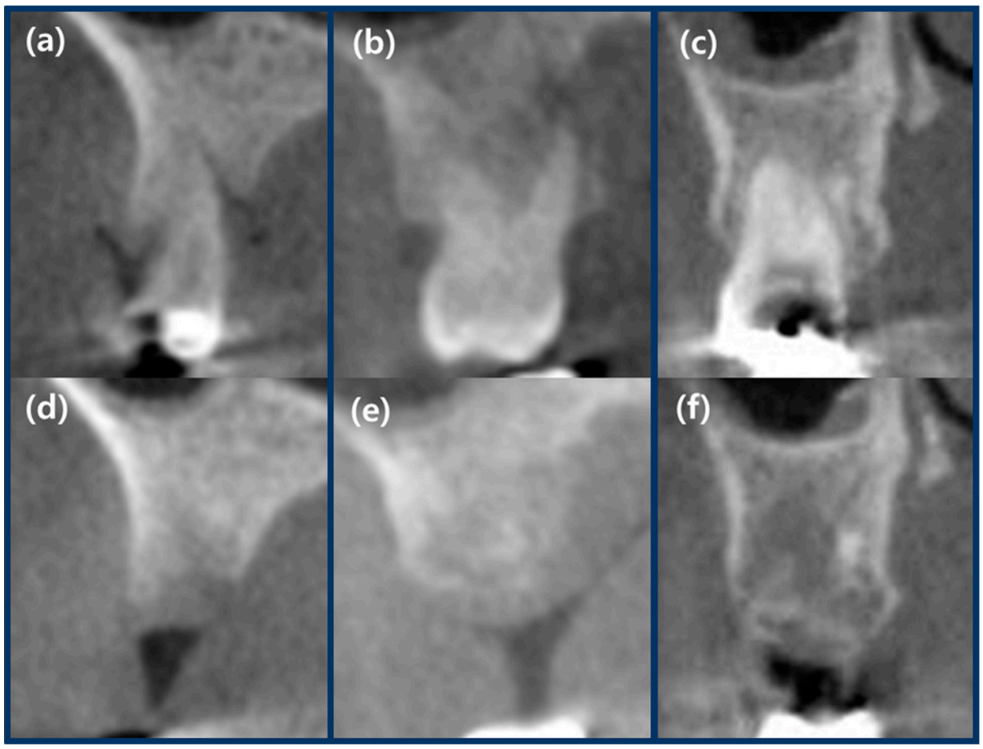

Figure 6. Cross-sectional images of the baseline and 4 months after ridge preservation. (**a**–**c**) Preoperative CBCT images of Groups A, B, and C, respectively. (**d**–**f**) Four months after extraction and ridge preservation for the Bio-Oss collagen, demineralized dentin matrix (DDM), and recombinant human bone morphogenetic protein-2 (rhBMP-2)/DDM groups. The buccal bone height (BBH), lingual bone height (LBH) and horizontal ridge width at 1, 3, and 5 mm from the alveolar crest (RW-1, RW-3, RW-5) were measured from the reference lines shown in Figure 5.

2.8. Histologic and Histomorphometric Evaluation

After 4 months of healing, patients returned for a postoperative examination (obtaining impressions for cast preparation, and CBCT imaging) and re-entry surgery of the ridge preservation site. Core biopsies were obtained from the center of the ridge preservation site with a 3-mm diameter trephine drill. The bone core was immediately placed in 4% formalin and kept immersed for 2 days and then decalcified in 10% ethylenediaminetetraacetic acid (EDTA) for 4 weeks. Samples were embedded in paraffin, sectioned, and then stained with Masson's trichrome and with Hematoxylin and Eosin. Histomorphometric evaluation of the samples was performed under a light microscope (Olympus BX51, Olympus Co., Tokyo, Japan) using the image analysis software Kappa image base metro (Kappa Opto-Electronics, Gleichen, Germany). Sections were examined at magnifications of 40× and 100×. The grafted area, newly formed bone area, and soft tissue area were measured and expressed as percentages of the total sample area.

2.9. Statistical Analyses

The sample size was calculated using the statistical software G*Power (Version 3.1.9, Kiel, Germany) [30]. The estimated effect size given for comparison of the groups was 2.049, based on a previous study [15]. It was determined that a sample set of nine participants per group would be required to recognize a significant difference regarding the ridge width of the CBCT, with 80% power and a 5% confidence level. To achieve a sufficient sample size for radiographic examination required, therefore, that there were at least 9 patients in each group. Considering a possible dropout rate of 10% during the study period, a sample size of 10 subjects per group (30 subjects in total) was used.

The parameters were measured and are presented as means ± standard deviation, and/or medians. A p-value of <0.05 was considered indicative of statistical significance. Differences in clinical, volumetric, radiographic, and histologic parameters between 3 different graft groups at baseline and at 4 months were analyzed using Kruskal-Wallis tests. When a parameter was found to be significantly different across groups using the Mann–Whitney test ($p < 0.017 = 0.05/3$), Bonferroni's post hoc test was performed to determine intergroup differences. With regard to the time schedule, differences for all parameters between the baseline and the 4-month follow-up were evaluated by Wilcoxon signed-rank tests (between treatment stages). Statistical analyses were performed using SPSS software (Version 22, IBM Software, Armonk, NY, USA).

3. Results

The study population consisted of 30 participants aged 27 to 79 years with one or more third molars and hopeless teeth. Twenty-seven patients were considered eligible for this experiment; three patients were excluded due to noncompliance with the study protocol (Figure 1 and Table 1). There was 1 patient with swelling and pain in Group A, 2 patients with pain in Group B, 1 patient with bleeding in Group C, and no infection or other complications in all other patients.

Table 1. Demographic data of patients.

Patient Information	Group A	Group B	Group C
Number	8	8	8
Age (years)	49.75 ± 17.21	46.63 ± 18.12	45.38 ± 15.62
Men/women	5/3	4/4	5/3
Biotype (thin/thick)	4/4	2/6	5/3
Grafted site (premolar/molar)	3/5	0/7	1/6

3.1. Ostoperative Healing Assessment

The degrees of epithelialization are shown in Figure 7. There were no statistically significant differences between the three groups with respect to the epithelialization score at 1 day, 1 week, 2

weeks, and 1 month after ridge-preservation. At 1 month post-treatment, complete epithelialization was observed in all groups without any significant differences ($p > 0.05$). Wound healing was typically uneventful following tooth extraction, ridge preservation, and re-entry surgery with no signs of infection or other clinical symptoms in all patients.

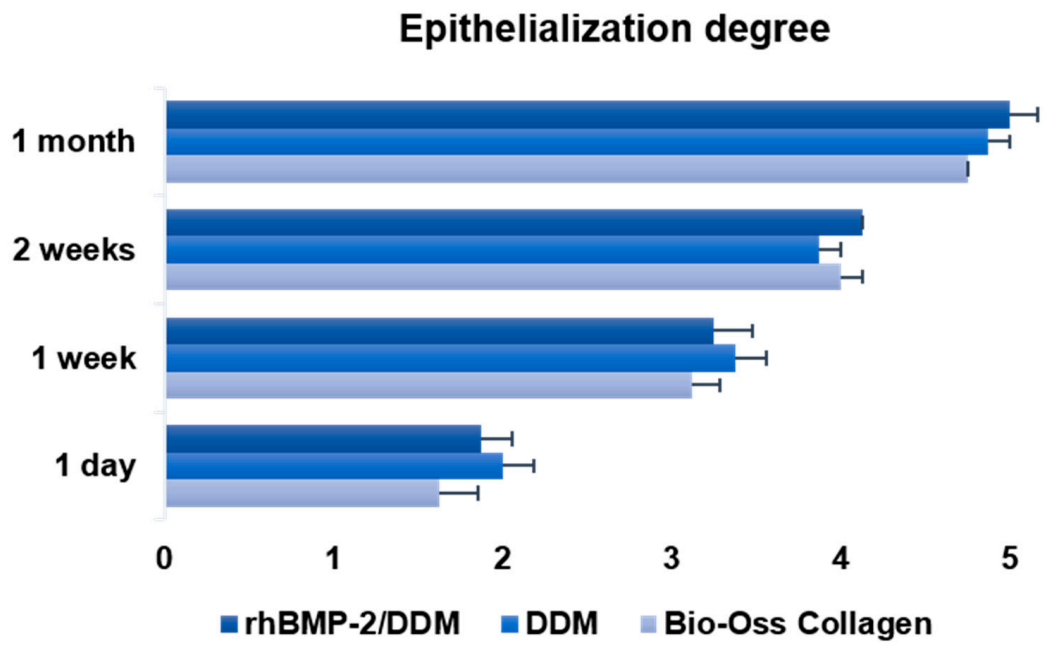

Figure 7. Degree of epithelialization.

3.2. Results of Volumetric Analysis

To verify soft tissue volume changes in extraction sockets at 4 months, impressions were obtained and casts were fabricated. The scanned images were superimposed for volumetric quantification of soft tissue alteration (Table 2). With respect to the hard tissue bone volume, 3D CBCT images were superimposed and measured using software. The Bio-Oss® Collagen, DDM, and rhBMP-2/DDM groups exhibited baseline soft tissue volumes of 1343.68 ± 328.12, 1430.45 ± 201.42, and 1430.94 ± 242.67 mm^3, respectively. At the evaluation conducted 4 months after extraction, the soft tissue volume decreased in all groups (Table 2) ($p < 0.05$). Similarly, the hard tissue volume significantly changed after extraction/ridge preservation, compared to the baseline (Table 2) ($p < 0.05$). However, no statistically significant intergroup differences in soft and hard tissue volume were observed between baseline and the 4-month follow-up.

Table 2. Results of the volumetric measurements.

	Group A (Bio-Oss Collagen)			Group B (DDM)			Group C (rhBMP-2/DDM)		
	Baseline	**4 Months**	**Ratio**	**Baseline**	**4 Months**	**Ratio**	**Baseline**	**4 Months**	**Ratio**
Soft Tissue Volume (mm^3)									
Mean ± SD	1343.68 ± 328.12	1117.53 ± 271.5	83.39 ± 5.52	1430.45 ± 201.42	1162.90 ± 198.8	80.99 ± 4.34	1430.94 ± 242.67	1171.64 ± 04.88	82.09 ± 7.91
Median	1500.55	1210.51	84.60	1421.00	1202.67	80.61	1383.36	1163.68	83.69
Min	739.98	624.91	75.11	1004.39	757.39	75.40	1182.92	815.08	68.70
Max	1655.29	1402.85	91.18	1629.35	1383.86	86.98	1743.84	1443.54	91.10
	$p < 0.05$ *			$p < 0.05$ *			$p < 0.05$ *		
Hard Tissue Volume (mm^3)									
Mean ± SD	848.38 ± 426.26	637.25 ± 316.11	75.55 ± 9.79	1179.75 ± 84.32	892.75 ± 367.17	86.12 ± 7.22	780.13 ± 417.88	617.88 ± 370.63	78.35 ± 11.01
Median	712.00	567.50	78.51	1314.50	973.50	87.18	672.00	463.00	78.34
Min	402.00	302.00	63.31	626.00	491.00	76.51	283.00	214.00	65.90
Max	1439.00	1088.00	91.79	1413.00	1371.00	97.03	1312.00	1254.00	95.58
	$p < 0.05$ *			$p < 0.05$ *			$p < 0.05$ *		

Values are expressed as mean ± SD in mm^3 and ratios calculated by Post/Pre. * Indicates statistically significant differences between preoperative and postoperative volumes ($p < 0.05$, the Wilcoxon signed-rank test).

3.3. Results of Radiographic Analysis

The results of the radiographic analysis shown in Table 3 describe the alteration of alveolar bone height and width from baseline to 4 months after ridge preservation. The average buccal bone heights (BBH) were 8.34 ± 2.50 mm, 9.18 ± 2.14 mm, and 8.78 ± 1.71 mm for the Bio-Oss® Collagen, DDM, and rhBMP-2/DDM groups, respectively. There was no significant difference in the ridge height of the buccal plate between the three groups. During the re-entry surgery performed 4 months later, the BBHs were 7.21 ± 2.05 mm, 8.21 ± 1.88 mm, and 7.95 ± 1.73 mm for the Bio-Oss® Collagen, DDM, and rhBMP-2/DDM groups, respectively. As in the previous case, there were no significant differences between groups. The mean lingual bone height (LBH) exhibited a similar trend, with no statistically significant difference between the three differently grafted groups 4 months after tooth extraction.

The alterations in the alveolar ridge width at 1, 3, and 5 mm below the marginal crest (RW-1, RW-3, RW-5) were measured to be 1.68 ± 1.11 mm (15.73%), 1.24 ± 0.65 mm (10.47%), and 0.50 ± 0.19 mm (3.93%) for Group A (Bio-Oss® Collagen); 0.78 ± 0.41 mm (6.23%), 0.49 ± 0.47 mm (3.43%), and 0.20 ± 0.15 mm (1.25%) for Group B (DDM); and 1.54 ± 0.74 mm (12,69%), 0.79 ± 0.54 mm (6.24%), and 0.32 ± 0.21 mm (2.39%) for Group C (rhBMP-2/DDM). At the levels of 1 and 3 mm, the groups did not show significant differences in either the postoperative ridge width or the level of decrease in width. At the 5 mm level, the decrease in ridge width was significantly different between Groups A (0.50 ± 0.19 mm) and B (0.20 ± 0.15 mm) ($p < 0.017$). All radiographic parameters were balanced among the three groups at baseline, except for the RW-5.

Table 3. Alveolar bone heights and widths in the three groups (A, B, and C) at baseline and 4 months following ridge preservation.

	Group A			Group B			Group C		
(mm)	**Baseline**	**4 Months**	**Δ**	**Baseline**	**4 Months**	**Δ**	**Baseline**	**4 Months**	**Δ**
BBH	8.34 ± 2.50	7.21 ± 2.05	1.14 ± 0.81	9.18 ± 2.14	8.21 ± 1.88	0.97 ± 0.39	8.78 ± 1.71	7.95 ± 1.73	0.82 ± 0.36
LBH	8.34 ± 2.54	7.70 ± 2.52	0.65 ± 0.37	9.26 ± 2.09	8.50± 2.06	0.76 ± 0.29	8.61 ± 1.65	8.12 ± 1.78	0.50 ± 0.22
RW-1	10.68 ± 2.41	9.00 ± 2.76	1.68 ±1.11	12.52 ± 1.68	11.74 ± 1.57	0.78 ± 0.41	12.14 ± 1.42	10.60 ± 1.90	1.54 ± 0.74
RW-3	11.84 ± 2.28	10.60 ± 2.61	1.24 ± 0.65	14.29 ± 1.84	13.80 ± 1.80	0.49 ± 0.47	12.67 ± 1.99	11.87 ± 2.03	0.79± 0.54
RW-5	12.71 ± 2.27	12.20 ± 2.24	0.50 ± 0.19*	15.94 ± 1.88 *	15.74 ± 1.89 *	0.20 ± 0.15	13.38 ± 1.80 †	13.06 ± 1.73†	0.32 ± 0.21

Values are presented as mean ± SD in mm. A vs. B: * $p < 0.017$, A vs. C: † $p < 0.017$. Differences in BBH, LBH, and RW-1, 3, and 5 at baseline and at 4 months were analyzed using Kruskal–Wallis tests (between the three groups) and Wilcoxon signed-rank tests (between treatment stages). * † Different letters indicate statistical significance under Bonferroni correction. Δ is the net change 4 months after extraction and ridge preservation, calculated by Pre − Post. BBH; buccal bone height, LBH; lingual bone height, RW-1; horizontal width at 1 mm, RW-3; horizontal width at 3 mm, RW-5; horizontal width at 5 mm.

3.4. Histologic and Histomorphometric Analysis

Bone specimens obtained from the ridge preservation sites of 18 patients (six patients for each of the three groups) did not show any inflammatory response around Bio-Oss® Collagen and DDM. All grafted sites indicated the presence of newly formed bone, remaining parts of tooth, bone graft materials, and connective tissues (Figure 8). The new bone comprised ~22% (Group A), 33% (Group B), and 39% (Group C) of the total area, respectively, with no statistically significant differences between the groups, as determined by the Kruskal–Wallis test ($p > 0.05$) (Table 4).

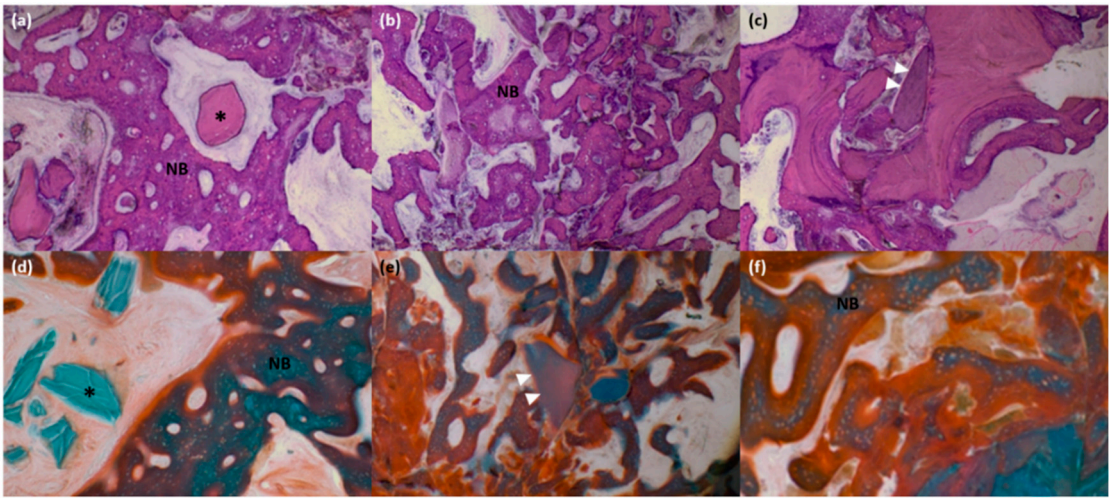

Figure 8. Histomorphometric measurement at 4 months after ridge preservation. Section was stained with Hematoxylin and Eosin (H&E) and Masson's trichrome with magnification x 100. (**a**,**d**) Bio-Oss collagen group. (**b**,**e**) DDM group. (**c**,**f**) rhBMP-2/DDM group. NB: Newly formed bone, asterisks: residual Bio-Oss particle, arrowheads: residual dentin particle.

Table 4. Histomorphometric analysis of 3 different groups at 4 month reentry.

Ridge Preservation Sites	Number	Group A	Group B	Group C
Grafted area (%)	$n = 6$	13.20 ± 9.79	10.72 ± 9.83	11.02 ± 12.72
New bone area (%)	$n = 6$	22.00 ± 11.01	32.88 ± 14.48	39.09 ± 15.30
Soft tissue area (%)	$n = 6$	64.80 ± 10.11	56.40 ± 8.58	49.88 ± 11.14

4. Discussion

In this randomized prospective study using serial stone cast scans and CBCT images obtained at baseline and at a 4-month follow-up assessment, we evaluated the alteration of alveolar morphology after ridge preservation following tooth extraction. We assessed the effectiveness of autogenous tooth bone graft, either alone (DDM only) or in combination with rhBMP-2 (rhBMP-2/DDM), on ridge preservation, compared to conventional bovine xenografts (DBBC). To the best of our knowledge, this is the first randomized controlled study to compare rhBMP-2/DDM and xenografts in human subjects. Similar clinical trials using DDM as a scaffold for rhBMP-2 have recently been published, but most of these studies were conducted on animals [31,32].

Natural reduction of the alveolar ridge after tooth extraction can be demanding, and it is challenging to rehabilitate the aesthetic and functional value of the implants. Since the clinical purpose of extraction socket is mainly implant placement, it is fundamental for clinicians to minimize postextraction bone atrophy. The ridge preservation technique includes the placement of biomaterials and substitutes in an extraction socket to prevent ridge atrophy. Many studies on ridge preservation have documented the structural changes that occur around the alveolar ridge following ridge preservation [13,33–35]. It is well-known that ridge preservation can limit and delay alveolar bone alteration [36,37]. Not all studies have reported beneficial effects from reducing alveolar ridge atrophy [36,38] which implies that ridge atrophy occurs in almost all the extraction sockets regardless of ridge preservation. In this study, we reported negative postextraction remodeling changes (approximately 80% of original soft tissue volume) in all three groups after tooth extraction accompanied with ridge preservation ($p < 0.05$). These results are consistent with those obtained in previous studies which have shown that horizontal bone loss of approximately 30~60% and vertical bone loss of approximately of 10~20% is inevitable after 6 months [39]. Despite the ridge preservation technique, postextraction bone atrophy appears to be entirely unavoidable [40].

In this study, extraction was done by two surgeons. However, atraumatic extraction was performed with minimal damage to the bone during extraction, and there was no difficulty in extraction. Therefore, it is considered that this did not significantly influence the study outcomes. Study outcomes may be affected by extraction causes such as pulpitis and periodontitis. In order to control for this bias, patients with severe periodontitis with a vertical bone loss of more than 50% were excluded from the study and the inflammatory tissue was debrided with the extraction.

In this study, 3D volumetric alteration of both soft and hard alveolar tissue was recorded at baseline and 4 months after extraction, and comparative analyses were performed between the DBBC-treated group, DDM-treated group, and the group treated with DDM and rhBMP-2. From a quantitative perspective, we did not observe any significant differences in volume between the three groups at 4 months postoperation ($p > 0.05$). Although our study did not include a control group, this result implies that the DDM groups exhibited similar changes in ridge volume as the conventional DBBC grafting. Consequently, this result may indicate that DDM grafting on the extraction socket might lead to lesser alveolar atrophy compared to cases without postextraction grafting. According to radiographic assessment performed in this study, there were no statistical differences between the three groups in vertical bone height. Similarly, previous studies did not show statistical differences between DBBC and DDM in vertical bone height after 6 months of ridge preservation [14]. The 4-month postoperative ridge width at 1 mm (RW-1) from the marginal crest was significantly reduced in the DBBC group (9.00 ± 2.76 mm), the DDM group (11.74 ± 1.57 mm), and the rhBMP-2/DDM group

(10.60 ± 1.90 mm), with no significant differences between these three groups. Similarly, at the level of 3 mm (RW-3), the level of ridge alteration was not statistically different among the groups. However, at the level of 5 mm (RW-5), the Bio-Oss® Collagen group exhibited significantly greater loss in width (0.50 ± 0.19 mm), compared to Group B (0.20 ± 0.15 mm) treated with DDM. Nevertheless, the apical portion of the alveolar socket (RW-5) is less important and dynamic, as the majority of the apical portion of the buccal bone plate is lamellar bone [41]. The marginal portion of the crest (RW-1 and RW-3 mm) is more critical in the bone remodeling cascade due to the bundle bone. In accordance with these results, the loss of ridge width can be reduced by ridge preservation with DDM, considering it equivalent to DBBC. Previous studies have also documented that DDM and deproteinized bovine bone can act as favorable biomaterials to preclude atrophic ridge alteration after tooth extraction [14].

A range of biomaterials used for ridge preservation have been documented and categorized in the literature [42]. Among them, the emerging autogenous dentin materials were successfully used for various clinical situations including guided bone regeneration (GBR), sinus grafts, and ridge preservation [20,21]. Many studies have indicated that manipulated dentin materials can act as osteoconductive or even osteoinductive substitutes for common graft biomaterials [31]. In addition, this novel DDM material has been applied as an rhBMP-2 carrier as well as a bone substitute. The bone healing process proceeds with the formation of blood clots, migration and proliferation of mesenchymal stem cells, granulation tissue formation, angiogenesis, proliferation of fibroblasts, and collagen synthesis. Thus, mesenchymal stem cells are essential for the bone healing process, and mesenchymal stem cell markers such as CXCR4, CD106, CD146, OCT-4, NANOG, CD34, CD146 and CD105 are upregulated at bone healing sites [43]. rhBMP-2 has been utilized to stimulate osteoblastic proliferation from mesenchymal stem cells and accelerates bone regeneration [24]. Although numerous bone morphogenetic protein (BMP) carriers have been tested, such as collagen, demineralized bovine bone, and hydroxyapatite, these carriers have several shortcomings, such as rapid resorption, lack of long-term maintenance of volume, and incompetent release of BMP. Processed demineralized dentin is already recognized as a rhBMP-2 carrier, since it facilitates induction of new bone formation and helps to release BMP slowly [44]. A recent animal study by Kim et al. demonstrated that DDM dissolves rhBMP-2 more gradually and steadily than other tricalcium phosphates (TCP) and bovine bone, establishing that particulated DDM is the most effective BMP carrier [31]. Other studies have reported that adding rhBMP-2 to DDM ensures the formation of new bone and cartilage, and DDM is absorbed and replaced by vital new bone tissue approaching almost 80% of the total volume, as indicated by histomorphometric assessment [23,45]. The current study was undertaken to evaluate, using a histomorphometric assessment, whether the addition of rhBMP-2 to DDM leads to superior bone regeneration in comparison with DDM alone or conventional DBBC. We observed relatively higher vital bone regeneration without statistical differences in the rhBMP-2/DDM group (39.09 ± 15.30%) and DBBC (22.00 ± 11.01%) and DDM only (32.88 ± 14.48%) groups ($p > 0.05$). In a previous study, it was reported that newly formed bone was obtained in 35.00% of the DBBC group and 31.24% in the DDM group after 6 months of ridge preservation [14]. In this study, core biopsy was performed 4 months later, so the results of the two studies were slightly different. However, the proportions of newly formed bone, soft tissue, and grafted area between the two studies were similar in the DDM group. Dentin and bone have similar compositions, consisting of 18% collagen, 70% hydroxyapatite, and 2% noncollagenous protein [24]. Besides the type I collagens in dentin, microporous dentinal tubules can seize BMP solution and widen the contact area with proteins which can facilitate the continuous binding and release of BMPs [19,44].

Several studies have reported the use of rhBMP-2/collagen [46], TCP [47], deproteinized bovine bone [48], and other materials, such as enamel matrix derivatives [48], in ridge preservation techniques. However, the use of autogenous tooth materials with rhBMP-2 in human studies is rare, and only a few animal studies have demonstrated histologic assessment of rhBMP-2/DDM. In the present histologic result, DDM particles were well-incorporated and surrounded by newly formed bone, and a greater area of newly formed bone (39.09%) was reported in the rhBMP-2/DDM group.

This present study provides clinical, volumetric, radiologic, and histomorphometric evidence to suggest that DDM with/without rhBMP-2 might be a favorable alternative to conventional bone biomaterials used in the ridge preservation technique. However, the limitations of this study are that the study was not performed on the same tooth and was performed on various teeth, including anterior and posterior teeth. In addition, this study lacks appropriate negative controls, and the sample size was insufficient to support the clinical application of DDM and rhBMP-2. Further research is required to investigate the precise mechanism of rhBMP release from DDM, and studies of the same tooth are needed. In addition, more clinical and histomorphometric data are required to determine the effectiveness and sustainability of this novel material in the context of the ridge preservation technique.

5. Conclusions

The application of rhBMP-2/DDM into an extraction socket results in higher new bone formation compared with the use of DDM alone or DBBC at 4 months after ridge preservation. However, volumetric alteration of the postextraction socket was completely unavoidable regardless of the graft materials. Within the limitations of the present study, we validated that DDM has great potential to be used as a BMP carrier and shows appreciable alveolar ridge maintenance and formation of new bone in the ridge preservation site following tooth extraction.

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