- Master`s degree thesis -

Soft and hard tissue changes when socket preservation using rhBMP-2, PRP and Non-Resorbable dPTFE membrane

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Abstract

Objectives: Conventional dentoalveolar osseous reconstruction often involves the use of grafting materials, growth factors and barrier membranes. The purpose of this study was to evaluate soft tissue changes and hard tissue regeneration after extraction socket preservation using rhBMP-2 coated β-TCP/HA, PRP and Non-Resorbable dPTFE membrane.

Material and methods: Total 15 patients were recruited for the clinical study. Among the ones corresponding to proper criteria for selection, Cone beam computed tomography(CBCT) was taken at the baseline and 4 months after treatment for those whose extraction socket was grafted with rhBMP-2 coated β-TCP/HA, PRP and non-resorbable dPTFE membrane. The efficacy endpoints that were used to measure the degree of bone induction included alveolar bone height and 3 measurements of bone width. The Wilcoxon's signed-ranks test was used to determine the significance of the changes($P<0.05$). Keratinized tissue height measurements were taken before tooth extraction and implant installation(after 4 months from baseline) using stent and probe.

Results: Clinically, the keratinized gingiva was mostly preserved. After 4 months, the changes in alveolar bone height of extraction socket were 20.96±9.62mm to 20.75±9.68mm showing almost no change. Changes in alveolar bone width were also measured to determine the minor effects of bone graft on the preservation of alveolar bone. At 25% extraction socket length(ESL), the alveolar bone width was 12.65±2.61mm at the baseline and 12.35±2.59mm at 4 months after treatment. At 50% ESL, the alveolar bone width was 13.11±2.46mm at the baseline and 12.74±2.36mm at 4 months after treatment. And at 75% ESL, the alveolar bone width was 13.38±2.14mm at the baseline and 12.99±2.57mm at the 4 months after treatment. Both the height and width of the alveolar bone of extraction socket were mostly preserved.

Conclusions: The results of this study indicated that the use of rhBMP-2 coated β-TCP/HA, PRP and Non-Resorbable dPTFE membrane predictably led to the preservation of soft and hard tissue in extraction sites.

Key word: socket preservation, rhBMP-2 coated β-TCP/HA, PRP, Non-Resorbable dPTFE membrane.

Ⅰ. Introduction

Ridge preservation means preserving the ridge volume within the envelope existing at the time of extraction(1). Extraction of teeth will be followed undoubtfully by loss in height and width of the alveolar process. It results in a narrowing and shortening of the residual ridge(2).

Bone resorption continues over time, but the most statistically significant loss of tissue contour occurs during the first month after tooth extraction, averaging 3–5mm in width at 6 months(3,4). The mandible will resorb more than the maxilla and the buccal side will lose more volume than the lingual(5).

Based on the systematic review, the group concluded that the reasons for ridge preservation included maintenance of the existing soft and hard tissue envelope, maintenance of a stable ridge volume for optimizing functional and esthetic outcomes and simplification of treatment procedures subsequent to the ridge preservation. Identified indications for ridge preservation are when implant placement is planned later after tooth extraction having some time gap, i.e. when immediate or early implantation is not recommendable, when patients are not available for the immediate or early implant placement, or when primary stability of an implant cannot be obtained and in adolescent people. Other indications are when contouring of the ridge for conventional prosthetic treatment, when provided the cost/benefit ratio is positive or when reducing the need for elevation of the sinus floor. Contraindication for ridge preservation was considered to encompass infections at the site planned for ridge preservation, which cannot be taken care of during the ridge preservation surgery. Also other contraindications are patients radiated in the area planned for ridge preservation and patients taking bisphosphonates(6).

In order to overcome the negative consequences of tooth extraction, various treatment approaches such as immediate implants, graft materials and/or barrier membranes have been advocated and described in the literature. As a conclusion, the majority of the studies show that socket preservation is a suitable technique for socket augmentation with the ability to maintain the ridge dimension to a certain amount(7).

During the past several years, the application of recombinant technologies has included biomimetic devices that stimulate the replacement of anatomic structures. These promote the in vitro or in vivo development of tissue. A group of molecules, the bone morphogenetic proteins(BMPs), members of the transforming growth factors-β superfamily, have been shown to induce heterotopic bone formation(8).

Marx et al. introduced Platelet-Rich Plasma(PRP) and reported clinical, radiographical, and histological increase in bone regeneration rate and bone density(9). They also reported faster stabilization and osteointegration of graft material when used in tooth-extracted socket(10), sinus lift(11), alveolar ridge augmentation(12), alveolar ridge expansion(13). Marx et al. reported faster and more mature bone regeneration in regeneration technique using PRP(14) and Anitu et al. reported that using PRP without membrane showed improved and amplified bone regeneration results(15).

A membrane made of high-density polytetrafluoroethylene(dPTFE), designed specifically for use in socket grafting, which does not require primary closure was described in case reports. The successful use

of this material was demonstrated in animal and clinical investigations. Moreover, no primary coverage is necessary, so it is possible to preserve the attached gingiva(16).

The purpose of this study was to evaluate soft tissue changes and the effect on hard tissue regeneration after extraction socket preservation using rhBMP-2 coated β-TCP/HA, PRP and Non-Resorbable dPTFE membrane.

Ⅱ. Materials and Methods

1. Patient selection

This study was performed at the department of periodontology, school of dental medicine, Dankook University, Korea. Participants signed a consent form. Fifteen subjects, 8 males and 7 females, with a mean age of 50 years, were enrolled in the study. The protocol was reviewed and approved by the Institutional Review Board(IRB) of Dankook University in 2013.(IRB NO:H-1301/001/001)

1.1 Inclusion criteria

(1) premolars or molars indicated for extraction with less than 50% of localized alveolar vertical bone loss, (2) patients who agreed to participate in clinical trials

1.2 Exclusion criteria

(1) those who had severe periodontitis with localized alveolar vertical bone loss of more than 50%, (2) those who were currently pregnant or planned to get pregnant within 1 year of the experiment, (3) those who were older than 65 years, (4) those who had recent myocardial infarction or uncontrolled bleeding disorders, (5) those who were contraindicated to minor surgeries, (6) those who had mental illness or suspected mental illness or hypersensitivity to bone graft materials, and (7) those who were classified as inappropriate for clinical trial participation by the clinician due to ethical reasons or other possible impacts on the results of clinical trials.

2. Experimental material

BMP®(rhBMP-2 + β-TCP/HA; Cowell Medi Co, Busan, Korea), and platelet rich plasma(PRP) obtained from patients , and Teflon sheet(Non-Resorbable dPTFE membrane; Cowell Medi Co, Busan, Korea) were used for ridge preservation. For collecting PRP, 10cc of peripheral blood was taken from each volunteer. After that, PRP was put into a test tube that contains 1.5cc of ACDC (anti-coagulant dextrose citrare) solution to prevent clotting. Using a centrifuge (Placon, Oscotec, Korea), collected blood was centrifuged for 3min at 2000g. After the centrifuge, plasma samples of the supernatant were collected using a Gilson pipette and centrifuged for 5 min at 2000g. Afterward, PRP located between the upper part of the platelet diluted plasma and the lower part of RBC cell layer was obtained. Then BMP® and PRP was mixed and gelated.

3. Surgical protocol

Under local infiltration anesthesia(2ml of 2% lidocaine + 1:100,000 epinephrine), intrasulcular incision extending to the adjacent teeth was made and a full-thickness flap was elevated. No vertical releasing incision was made. All surgeries were performed by the same surgeon. Extractions were done using atraumatic technique, and the socket was curetted carefully and irrigated with sterile saline solution. The socket preservation was performed

using a non-resorbable dPTFE membrane alone with rhBMP-2 coated β-TCP/HA mixed with PRP. The flap was repositioned original site and sutured. The membrane was left partially exposed during the healing period. Postoperative care included analgesics, a 3- to 5-day course of oral antibiotics(Augmentin or doxycycline for penicillin-sensitive subjects) and three times daily gum gargle. The membrane and sutures were removed 2 weeks after surgery(Fig 2).

4. Radiological Evaluation

The efficacy of rhBMP-2 coated β-TCP/HA was assessed by the following cone beam computed tomography(CBCT) parameters. For the scan of CBCT, subjects were located in CBCT system(Alphard Vega; Asahi Roentgen, Kyoto, Japan) to adjust the laser beam for vertical and horizontal arrangement, and were scanned within the field of view at 10x10 cm, tube voltages at 80kv, tube current at 7mA, and exposure time at 17 seconds. By this scanning, the digital imagine & communication in medicine(DICOM) image information was composed. The efficacy endpoints that were used to measure bone induction included alveolar bone height(one measurement) and bone width(three measurements at 25%, 50%, and 75% of the extraction socket length [ESL]). ESL means the length from the top of the grafted alveolar ridge to the bottom of extraction socket(Fig 1). These measurements were taken from CBCT scans exposed at baseline and at 4 months following study treatment. A bone height response(4 months minus baseline values) and bone width response(4 months minus baseline values) were calculated.

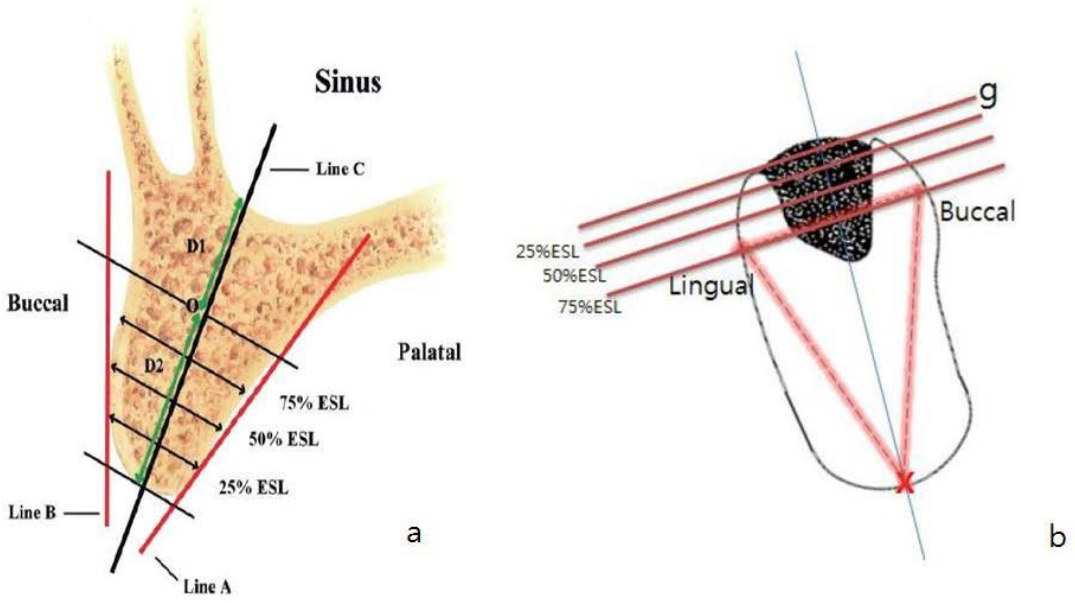

Figure 1. Computed tomography height and width measurements at baseline following tooth extraction. a)Maxilla b)Mandible

5. Keratinized tissue

Clinical evaluation of keratinized gingiva was performed by measuring the distance from the gingival crest to the mucogingival junction at the mid-buccal point, using a periodontal probe(Williams, Premier, USA). Keratinized tissue height measurements were taken before tooth extraction and implant installation
(after 4 months from baseline) using stent and probe.

6. Safety Assessment

Oral wound examinations of the treatment sites were performed at baseline, 14days and 4 months postoperatively to monitor the patients for the occurrence of commonly seen postoperative complications associated with augmentation procedures(edema, erythema, exudate, hematoma, sensory loss, pain, and wound dehiscence). The procedure of clinical study is described in Table 1.

7. Statistical analysis

The difference among each obtained percent values were evaluated by Wilcoxon's signed-ranks test. A p-value of $P<0.05$ was considered statistically significant. These analyses were conducted using SPSS for windows software (Version 12.0:SPSS, Chicago, IL, USA).

ITEMS	Visit 1	Visit2	Visit3	Visit4	sudden Visit
	Screening	Extraction & Socket Preservation	Exam Period		
Days	~ -1	0	14	120	
Patient Selection	○				
Written Consent	○				
Medical & Dental history taking	○				
Clinical Exam	○		○	○	
Panorama Taking	○				
CBCT Taking		○ (Afrer Bone grafting)		○ (Before Implant installation)	
Extraction & Socket Preservation		○			
Stich-Out			○		
Implant installation				○	
Soft tissue Exam		○		○	

Table1. Procedure of clinical study

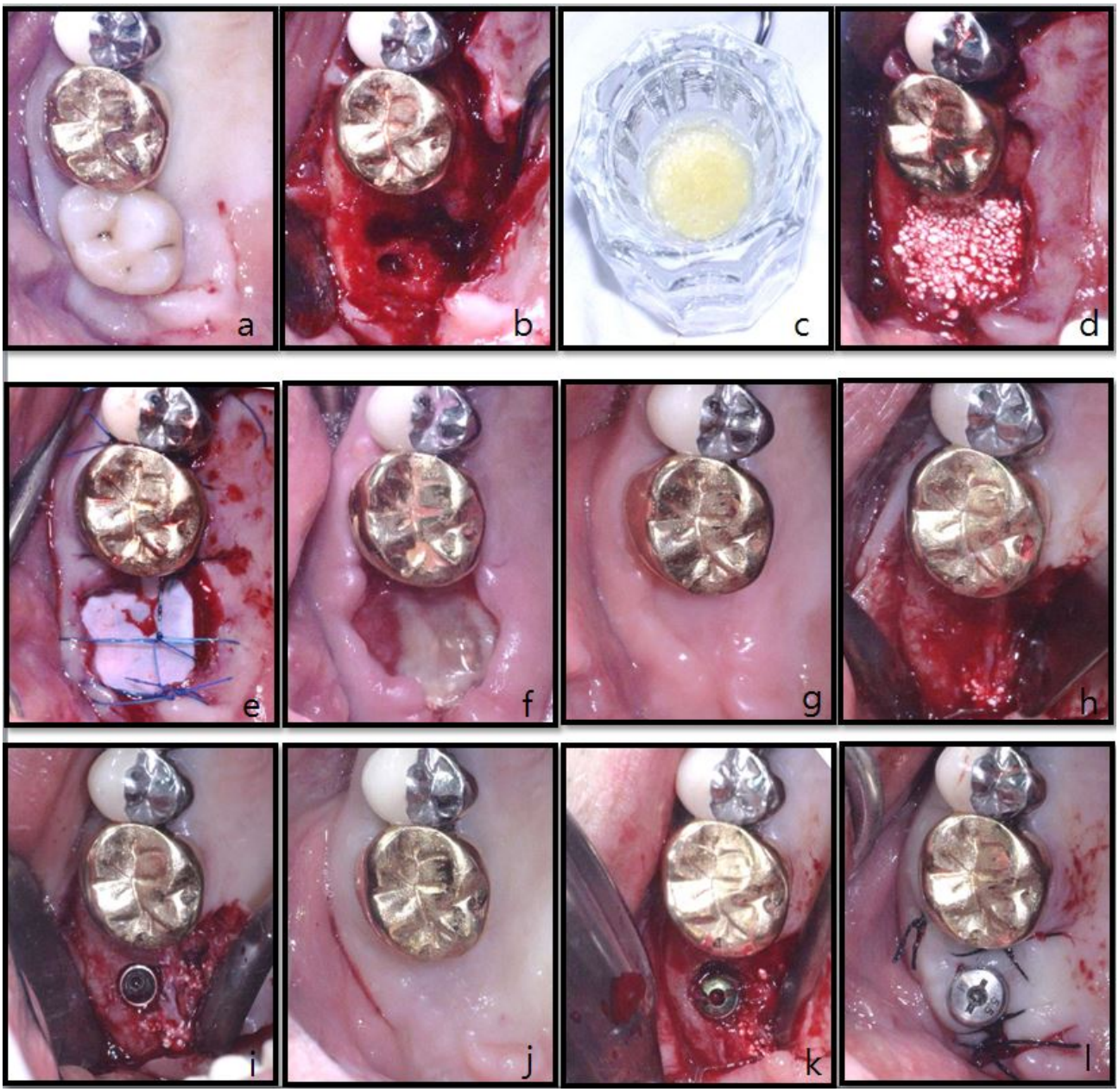

Figure 2. .a)Pre-OP b)Flap reflection c)BMP® mixed with PRP

d)Membrane placed e)Membrane remove f)After 4 months

g)Flap reflection h)Implant installation i)After 3 months

j)Flap reflection k)Healing abutment connection l) Bone grafting

Ⅲ. Results

No subjects were withdrawn or lost to follow-up. None of the subjects enrolled in this study reported any unusual pain or discomfort, abscess, swelling, or allergic reactions during the course of treatment. Membranes were left partially exposed after surgery. No sign of acute inflammation, exudate or pain was detected. After membrane retrieval, non-epithelialized soft tissue was found in the areas previously covered by the membrane. This tissue was completely reepithelialized clinically within 4weeks after membrane removal. Nevertheless, a slight but clearly distinguishable difference in color compared to the adjacent mucosa persisted(Fig 2).

Clinical evaluation of keratinized gingiva was performed by measuring the distance from the gingival crest to the mucogingival junction at the mid-buccal point, using a periodontal probe(Williams, Premier, USA). It was recorded pre-operatively and after soft tissue healing. The change of keratinized tissue height is shown in Table 2. The ratio was 0.8 for the two subjects(mandible) and 1.0 for the thirteen subjects. At baseline, the mean width of the keratinized tissue was 5.3mm and after 4 months, the mean height of the keratinized tissue was 5.2mm. Clinically, the whole keratinized gingiva was preserved(Fig 3).

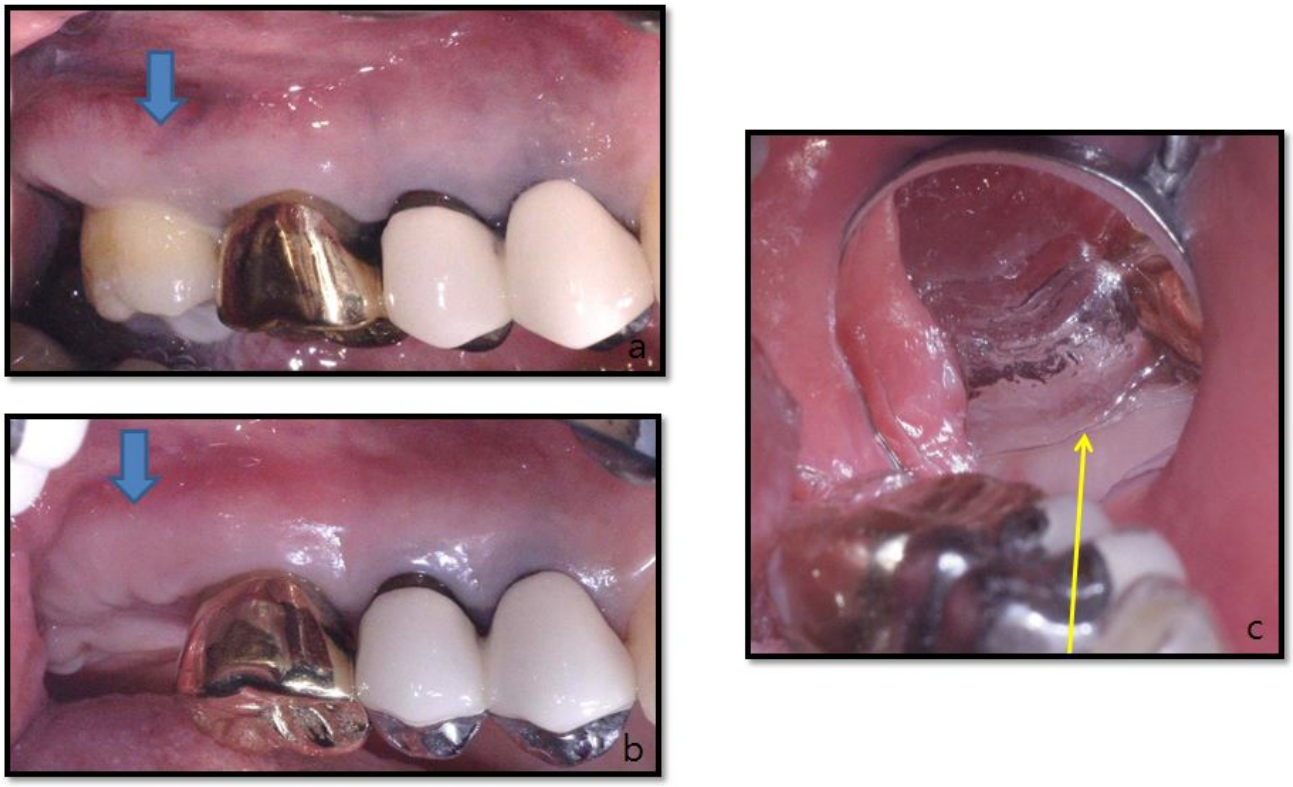

Figure 3. a)Pre-OP b)After 4 months c)Stent placement

Patient No.	Site	height of keratinized tissue		
		Baseline (mm)	Post-surgery(mm)	Ratio
1	#46	5	4	0.8
2	#47	5	5	1
3	#37	5	5	1
4	#17	5	5	1
5	#46	3	3	1
6	#37	5	5	1
7	#14	5	5	1
8	#25	5	5	1
9	#46	8	8	1
10	#17	6	6	1
11	#16	5	5	1
12	#46	5	4	0.8
13	#14	7	7	1
14	#16	5	5	1
15	#16	6	6	1
Mean		5.3	5.2	0.973

Table2. Dimensional change of keratinized gingiva before and after surgery.

After 4 months, the changes in alveolar bone height of extraction socket were 20.96±9.62mm to 20.75±9.68mm showing almost no change. The Wilcoxon's signed-ranks test was used to compare the changes between the baseline and 4 months after treatment, and the differences were statistically not significant(Table 3). Changes in alveolar bone width were also measured to determine the minor effects of bone grafts on the preservation of alveolar bone. At 25% ESL, the alveolar bone width were 12.65±2.61mm at the baseline and 12.35±2.59mm at 4 months after treatment. At 50% ESL, the alveolar bone width were 13.11±2.46mm at the baseline and 12.74±2.36mm at 4 months after treatment, and at 75% ESL, the alveolar bone width were 13.38±2.14mm at the baseline and 12.99±2.57mm at the 4 months after treatment. Both the height and width of the alveolar bone of extraction socket were mostly preserved. The Wilcoxon's signed-ranks test was used to compare the changes between the baseline and 4 months after treatment, and the differences were statistically significant(Table 4).

Group		Average	SD	p-value†
Bone Height	Base line	20.96	9.62	0.098
	4 months after treatment	20.75	9.68	

Table 3. Evaluation of the efficacy in maintaining alveolar bone height

**:P<0.05,* ***:P<0.01,* †:Wilcoxon's signed-ranks test

Group		Average	SD	p-value†
25% ESL	Base line	12.65	2.61	0.001**
	4 months after treatment	12.35	2.59	
50% ESL	Base line	13.11	2.46	0.001**
	4 months after treatment	12.74	2.36	
75% ESL	Base line	13.38	2.14	0.001**
	4 months after treatment	12.99	2.57	

Table 4. Evaluation of the efficacy in maintaining alveolar bone width

*:*P<0.05*, **:*P<0.01*, †:Wilcoxon's signed-ranks test

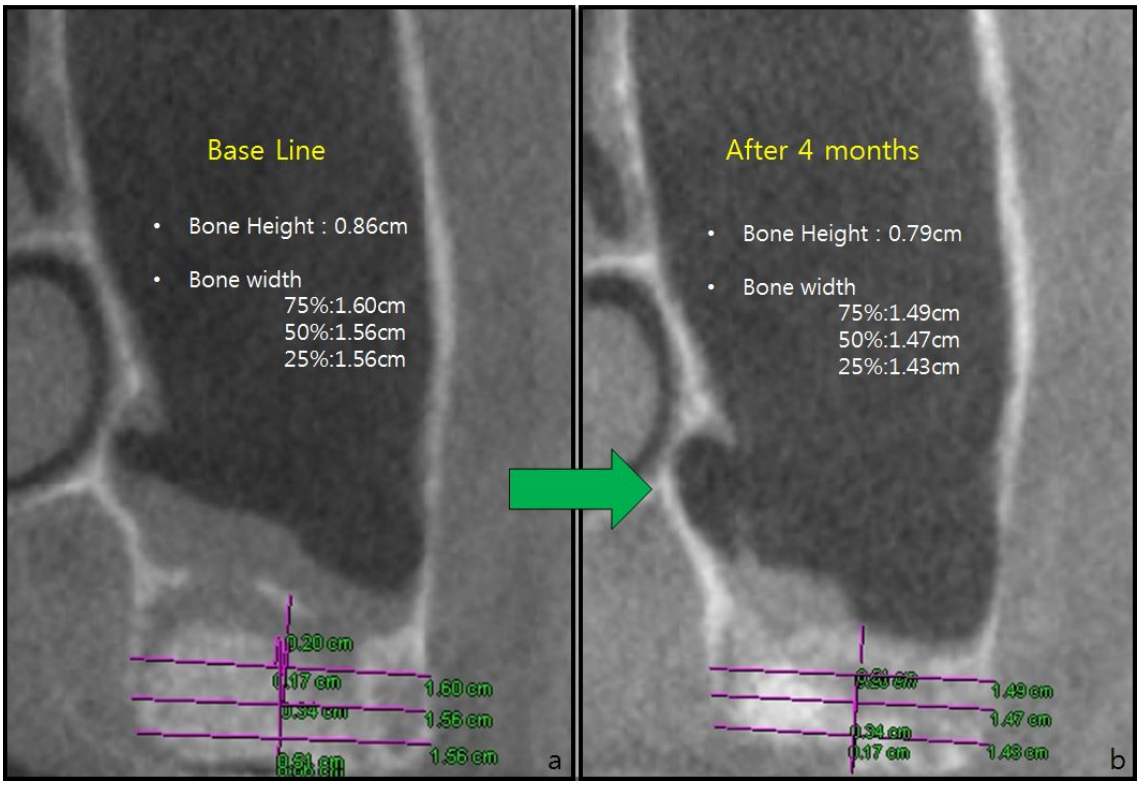

Figure 4. a)CBCT(Base line) b)CBCT(4 months after treatment)

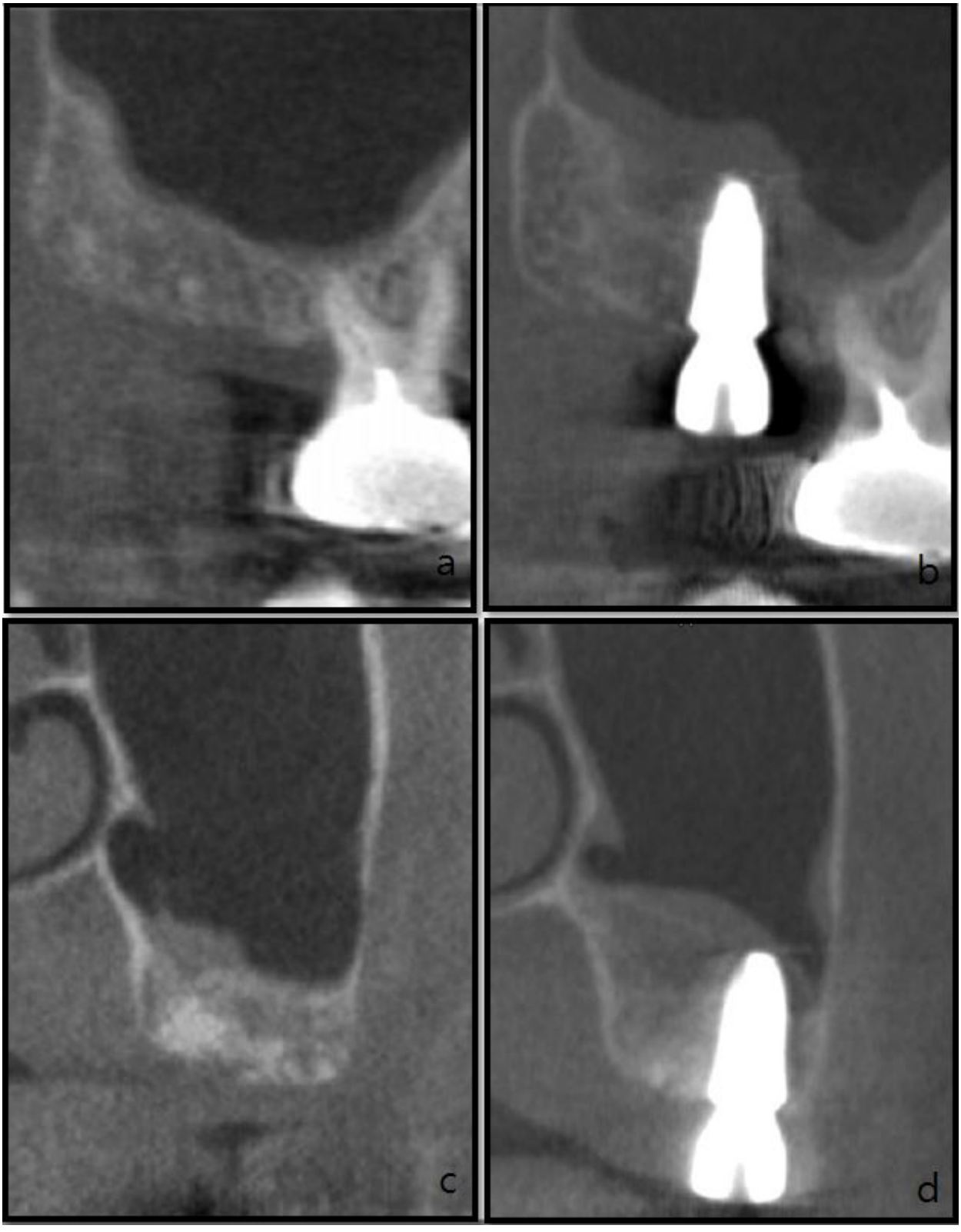

Figure 5. a)CBCT(4 months after treatment) b)CBCT(Implant installation with crestal approach, 7 months after treatment) c)CBCT(4 months after treatment) d)CBCT(Implant installation with crestal approach, 7 months after treatment)

Ⅳ. Discussion

Conventional dentoalveolar osseous augmentation procedures for creating bone volume for dental implants often involve the use of grafting materials with or without barrier membranes to foster selective cell and tissue repopulation.

The clinical and histologic efficacy seems to be dependent on the type, source, biocompatiblity, and ability to maintain volume(17,18). The rationale for utilizing

several of these materials to promote osseous regeneration of the alveolus has been the presence of osteoinductive proteins(19,20).

BMPs have been shown to be responsible for post-fetal bone induction, including normal bone remodeling healing and repair(21-23). The potential therapeutic utility of rhBMP-2 in orthopedic and craniofacial reconstruction has been investigated. Preclinical studies evaluated induction and repair of bony defects in a variety of indications(24-27).

Alloplastic bone substitutes or ceramic implants such as hydroxyapatite(HAP)

and tricalcium phosphate(TCP) have been investigated extensively because they are composed of minerals similar to natural bone tissue(28-34). Moreover, they are osteoconductive and do not elicit immunogenicity. Porous beta-tricalcium phosphate(β-TCP) is well known as a biodegradable material with good osteoconductive capacity and demonstrated clinical efficacy(35). Some researchers have attempted to add bone inducing capacity to β-TCP by combining this material with rh-BMP-2 to accelerate bone healing(36-39). Most of these studies have showed successful results by using β-TCP itself as a BMP carrier. To be effective as carriers for rhBMP-2, it is necessary for the ceramics to adsorb this protein. It is noteworthy that over 90% of rhBMP-2 was adsorbed to all biphasic calcium phosphate(BCP) ceramics except 75% HAP–25% TCP at the end of 30 min incubation. Therefore, these BCP ceramics could work as good delivery system for rhBMP-2(40).

A variety of protocols for the preservation of extraction sockets has been described previously, such as the use of membranes, grafting materials, or a combination of both. The use of a grafting material may also be helpful in preventing a possible collapse of the membrane. Expanded PTFE(ePTFE) membranes have high surface roughness, which facilitates adhesion of bacteria. Thus, primary closure over the membrane needs to be achieved to avoid exposure to the oral environment and resulting in bacterial colonization because the resulting inflammation can impair the treatment outcome(41,42). Furthermore, the removal of ePTFE membranes often necessitates a second surgical procedure. To avoid this, bioabsorbable membranes made from different materials can be used. However, these require primary closure to avoid premature degradation, which is often not easily achievable when covering extraction sites. Herein lies a significant advantage of dPTFE membranes; the membrane is impenetrable for bacteria because of its surface characteristics. And no primary coverage is necessary, since there is no need for releasing incisions or additional freeing of the flap, thereby facilitating the surgical procedure and enhancing the esthetic outcome and preserving the attached gingiva by not changing the mucogingival junction. In addition, because of the comparatively smooth surface, dPTFE membranes can

usually be removed without an additional surgical procedure
(43,44).

Marx et al. introduced method to manufacture PRP in which mean platelet concentration is about 3~4 times and reported increase in bone generation rate and bone density, clinically, radiographically, and histologically. Furthermore, addition of thrombin and calcium in PRP activates platelet to secrete a-granule's contents which contain platelet derived growth factor and TGF-β. Thrombin and calcium initiate coagulation and cause PRP gel which is clinically useful and it is known to enhance effects of autografts and bone replacement(45).

In this study, to assess the major effects of the bone graft material in preserving the alveolar bone, alveolar bone height at baseline and 4 months post-treatment were compared by measuring bone height in the cross sectional CT images. To assess the minor effects of the bone graft material, changes in alveolar bone width at 25% ESL, 50% ESL and 75% ESL were compared using cross-sectional CT images at baseline and 4 months post-treatment. In addition, clinical observation was performed to evaluate keratinized tissue height. In the systemic review of previously reported alveolar bone dimensional changes of post-extraction sockets in humans, amount of change in alveolar bone height was –1.67±1.11mm and change in alveolar bone width was –3.87±0.82mm(46). In this study, less resorption was observed in both alveolar bone height and width. The results from present study indicate that a novel method to recreate the alveolar ridge in order to support a dental implant was efficacious. The 4 months time had minimal effect on vertical ridge height or horizontal ridge width changes. Implants were successfully placed at all sites, irrespective of the 4 months time. When sinus augmentation was needed, preserved ridge dimension made it possible to avoid lateral approach and to perform crestal approach. Therefore, sinus augmentation and implant installation were possible at the same time, shortening the treatment period(Fig 5). Likewise, necessity for GBR in mandible decreased. And the whole keratinized gingiva was mostly preserved.

Many clinicians would prefer to completely preserve the original ridge contours. This did not occur in this study, where only an intrasocket graft was used. This is in agreement with findings in previous studies where guided bone regeneration was used. Original contours were preserved, however, and even slightly augmented, when an additional extrasocket graft was overlaid on the buccal and coronal portion of the alveolus. This indicates that an additional extrasocket buccal and coronal overlay graft may be essential in the maxillary anterior if the original esthetic contours are to be preserved. Therefore, the ridge preservation technique needed may be different for posterior versus anterior sites, where it is preferable to preserve the convexities of the original root prominences: the intrasocket graft alone may be adequate for posterior sites, while both an intra- and extrasocket graft may be preferred in the maxillary anterior(47).

Evian et al. published a study on the healing process of years after tooth extraction. Complete bone filling is almost completed by 16 weeks after tooth extraction(48). However, in the current study, extraction sites are not completely filled with bone. Because grafting materials may have interfered with normal extraction socket healing. Araujo et al. reported use of the β-TCP graft may

in fact have retarded bone formation in the model used. The reason for this 'delayed' healing is presently not understood but may be related to a high local concentration of Ca2+ and PO3-4 that may have been detrimental to osteoblast function as suggested by Yuan et al.(49). At longer time in vivo, β-TCP became more resorbable ; the circulation inside the implants decreased with the bone formation. So, Ca2+, PO3-4 ions accumulated in local sites and a high Ca2+, PO3-4 concentration was reached. The high Ca2+, PO3-4 concentration was detrimental to cells including osteoblast, osteocyte, macrophages and also not suitable for osteoclast resorption function. As a result, no bone remodeling and no bone marrow formation occurred in β-TCP implants. Furthermore, the acidic microenvironment caused by dissolution made the formed bone demineralize(50).

Osteogenic differentiation of the cells was evaluated by means of alkaline phosphatase(ALP) activity quantification. Optimal pH for ALP activity was reported approximately pH of 10(51).

In this study, when implant were placed after 4 months, bone quality was not

satisfactory. However, bone quality of 2 patients whose implants were placed

after 6 months, was relatively satisfactory. So, further studies are necessary to evaluate whether another rhBMP-2 carrier leads to enhancement of the treatment outcome. This study had small number of subjects and short study period. Also since there was no histological investigation by biopsy, there were limitations for evaluating accurate repair mechanism. Therefore, further studies for these limitations are needed in the future.

Ⅴ. Conclusion

The results of this study indicated that the use of rhBMP-2 coated β-TCP/HA, PRP and Non-Resorbable dPTFE membrane predictably led to the preservation of soft and hard tissue in extraction sites. However grafting materials retarded bone formation.

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