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Lateral onlay grafting using different combinations of soft-type synthetic block grafts and resorbable collagen membranes in dogs

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이 정 태

Abstract

Lateral onlay grafting using different combinations of soft-type synthetic block grafts and resorbable collagen membranes in dogs

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Objectives: To observe the sequential healing of lateral onlay grafts in terms of volumetric and histological changes when using different combinations of synthetic soft-type block bone and resorbable collagen membranes.

Materials and Methods: A lateral onlay graft procedure was applied at the chronic narrow alveolar ridge of the mandible in 15 beagle dogs. The groups were allocated as follows: (i) empty control, (ii) onlay graft using soft-type block bone 1 [hydroxyapatite (HA): β-tricalcium phosphate (β-TCP) = 15:85] and a non-cross-linked collagen membrane (NCCM) (MP-BG group), (iii) onlay graft using soft-type block bone 2 (HA:β-TCP = 60:40) and NCCM (OC-BG group), and (iv) onlay graft using soft-type block bone 1 (HA:β-TCP

= 15:85) and a cross-linked collagen membrane (CCM) (MP-CM group). Volumetric and histomorphometric analysis were performed at 4, 8 and 16 weeks postoperatively.

Results: No clinical complications occurred in any of the groups. The OC-BG group showed significantly larger total augmented volumes than the control and MP-BG groups after 8 and 16 weeks. The areas of new bone were significantly larger in the OC-BG group than the other groups at 16 weeks. The horizontal thickness of the augmented ridge was significantly larger in the OC-BG group than in the control group at 16 weeks. There was no significant difference in the degradation of NCCM and CCM during the experimental period.

Conclusion: The OC-BG group showed superior volume maintenance and osteogenic potential for up to 16 weeks compared to the other groups in an onlay graft model of the dog mandible despite the displacement of the bone graft.

KEY WORDS

animal experiments, soft-type block bone, lateral onlay graft, resorbable collagen membranes, bone healing

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CONTENTS

I. INTRODUCTION

Autogenous block bone has been used in bone augmentation to obtain dimensional stability in bony defects (e.g., vertical and lateral defects) because its volume stability is better than that of particulate bone [1]. However, various complications caused by using an autogenous block bone graft from the oral cavity have been reported, including infections and anatomical changes [2]. Xenogenic and synthetic block bone grafts have therefore been introduced as substitutes for autogenous block bones. Previous studies using hydroxyapatite (HA) block bone have reported a favorable result [3,4], but this has limitations regarding new bone formation and clinical handling [5]. To overcome these drawbacks, a soft type of block bone that comprises HA particles incorporated with a collagen matrix (BHC) has been developed. The inclusion of collagen in a soft block bone contributes to its dimensional stability by maintaining the shape of the bone particles. The collagen in BHC subsequently degrades within 1 - 2 weeks [6], and so soft-type block bone is mainly used in ridge preservation procedures due to their morphological maintenance abilities. In addition, this type of bone substitute is helpful for augmenting one- and two-wall defects [7]. Previous studies had clinically successful results when using a soft-type block bone substitute with a barrier membrane [8,9].

A block-type collagenated alloplastic bone substitute composed of biphasic calcium phosphate (BCP) and collagen was introduced recently. Biphasic calcium phosphate (BCP) is a mixture of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) [10-12]. Degradation rate of BCP could be controlled with changing the ratio

of HA/β-TCP because HA resorbs relatively slowly than β-TCP [13]. The insolubility of HA allows augmented space to maintain. The β-TCP dissolves in calcium and phosphate ions and is ultimately replaced by newly formed bone [14]. Previous studies have found that BCP block exerts favorable effects on bone healing. Kim et al. [15] demonstrated that BCP contributed to bone regeneration and maintaining space for new bone ingrowth over 8 weeks. Another study observed new bone formation when the implant was placed in a BCP block [16]. In addition, Hwang et al. and Pae et al. [17,18] demonstrated that BCP block bone increased both the amount of newly formed bone and the bone density.

Collagen was applied in soft-type block bone for maintaining the volume. The collagen in bone graft materials are from bovine and porcine [19,20]. Bovine collagen is one of the main sources. However allergic reaction was reported in bovine collagen [21-23]. Porcine collagen has been used in periodontal regeneration field including bone regeneration and soft tissue healing [24]. Porcine collagen also has been used for periodontal regeneration of oral defects including function of barrier membrane and bone substitutes [25]. Porcine collagen does not cause much allergic response when used, because it has similarity to human collagen [18].

An ideal barrier membrane would have the characteristics of biocompatibility, space-maintenance ability, tissue integration, cell occlusiveness and ease of manipulation [26]. Resorbable membranes have become the gold standard for lateral bone augmentation procedures because of their excellent biocompatibility [27]. However, resorbable membranes lack stiffness and space-maintenance properties to resistant pressure from the flap [28,29]. A cross-linking procedure

has been introduced to increase the stiffness and slow down the degradation of resorbable membranes [30]. Various types of cross-linked membranes have showed superior mechanical properties and bone healing [31,32]. Cha et al. [6] demonstrated the high biocompatibility and absence of a foreign-body reaction when using a cross-linked membrane. However, Jiménez Garcia et al. [33] found no difference between cross-linked and non-cross-linked membranes in terms of the regenerated bone volume.

The aim of this study was to observe the sequential healing of lateral onlay grafts in terms of volumetric and histological changes when using different combinations of synthetic soft-type block bone and resorbable collagen membranes.

II. MATERIAL AND METHODS

Animals

This preclinical *in vivo* investigation was designed in accordance with the modified ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for preclinical research, including reducing the number of experimental animals, refining procedures to improve animal welfare and replacing the use of animals where possible [34]. Fifteen adult beagle dogs (age, 15 months; weight, 10 - 15 kg) with fully erupted permanent dentition were used. The animal experiments were conducted after a 2-week adaptation period. The experimental procedure and protocols were approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, South Korea (approval no. 2016-0053).

Material preparation

Bone substitutes

Two kinds of soft-type synthetic bone substitutes were used: Osteon III Collagen® (OC; Genoss, Suwon, South Korea and Mastergraft® putty (MP, Medtronic Sofamor Danek, Memphis, TN, USA). OC is composed of BCP comprising 60% HA and 40% β-TCP, and highly purified type I collagen derived from porcine tendon. It is trimmed to a rectangular shape (10 mm × 5 mm × 5 mm) during the manufacturing

process, followed by exposure to gamma radiation. MP is composed of BCP (15% HA and 85% β-TCP) uniformly distributed through highly purified resorbable type I bovine collagen.

Resorbable membranes

The cross-linked porcine collagen membrane [CCM; Collagen Membrane-P® (CM), Genoss] and non-cross-linked bovine collagen membrane [NCCM; BioGide®(BG), Geistlich Biomaterials, Wolhusen, Switzerland] were trimmed to a rectangular shape (12 mm × 18 mm). CM consists of type I collagen derived from porcine tendon, and it was chemically cross-linked. The BG consists of type I non-crosslinked collagen derived from bovine.

Study design

For ridge augmentation, a bilateral onlay bone graft model was used in the mandibular premolar area of each dog. Different combinations of bone graft materials and membranes were arranged into the following four groups in one dog that were allocated randomly:

1. Empty control.
2. Onlay graft using MP and BG (MP-BG group).
3. Onlay graft using OC and BG (OC-BG group).
4. Onlay graft using MP and CM (MP-CM group).

The sample size was five at each of the healing periods of 4, 8 and 16 weeks (n=5).

Surgical procedure

All surgical procedures in this study were performed under general anaesthesia.

Surgery I: induction of a chronic narrow ridge

The surgical procedure and animal model, except bone graft materials and healing periods, in this study were the same as the previous studies [6,35]. Supragingival scaling and plaque control were performed to achieve the same oral environment. Surgical procedures were performed in a sterilized operating room while applying both local and general anaesthesia. Three premolars (the second, third and fourth premolars) of both mandibles were extracted to create an edentulous alveolar ridge. The buccal plate of the extraction socket was then removed to induce a narrow ridge (10 mm × 10 mm × 5 mm). Primary wound closure was obtained. After surgery, the dogs were orally administered the antibiotic cephalexin (30 mg/kg; Dong-hwa Pharm., Seoul, South Korea) and the non-steroidal anti-inflammatory drug meloxicam (0.1 mg/kg; Mobic®,Boehringer Ingelheim) for 7 days.

Surgery II: guided bone regeneration performed 8 weeks later

Extraction sites were allowed to heal for 8 weeks, after which a mid-crestal and two vertical incisions were made to expose the alveolar bone of the extracted sites while applying both general and local anaesthesia (Fig. 1a,b). Four groups were assigned to both mandible of each beagle. To provide a blood supply, nine intramarrow perforations were made at the recipient sites (Fig. 1c). The assigned soft-type block graft materials were trimmed to 6 mm × 5 mm × 5 mm and applied to the recipient sites. Resorbable membranes (12 mm

× 18 mm) were applied on each bone substitute and fixed with two pins (Membrane Pin®, Dentium, Seoul, South Korea; Fig. 1d,e) for stabilization. A periosteal releasing incision was made on the buccal flap to achieve primary wound closure of the grafted sites (Fig. 1f). Each animal was euthenized at 4, 8, or 16 weeks postoperatively by administering an overdose of 3% sodium pentobarbital. Experimental specimens including soft tissue were obtained and fixed in 10% buffered formalin for 10days.

Sample size

Sample size was calculated based on estimations from a previous study [6], using the histologic augmented area as the primary variable. The probability of a Type I error was set at 0.05 with achieving 95% statistical power. A sample size of 5 animals was calculated per experimental group considering the drop-out.

Micro computed tomography scanning and histology preparation

Micro computed tomography (micro-CT; SkyScan 1072, SkyScan, Kontich, Belgium) with a resolution of 16 μm (achieved using 130 kV and 60 μA) was used to scan the fixed block specimens. To create three-dimensional shapes, cross-sectionally scanned data were acquired and reconstructed with NRecon software and Dataviewer (Skyscan). The total volume of the augmented area was calculated by integrating data from all cross-sectional images using CTAn software (Skyscan).

Histological and histomorphometric analyses

Each cross-sectionally resected specimen was embedded in acrylic resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany). The central-most section of the experimental site was chosen for histological and histomorphometric analyses. Each specimen was cut to a thickness of 45 μm in the apical - coronal direction. The sections were stained with Goldner trichrome stain and observed with the aid of a digital scanner (Pannoramic 250 Flash III, 3D HISTECH, Budapest, Hungary). Histological and histomorphometric analyses were performed using two image-analysis computer programs (Case Viewer®, 3D HISTECH; Photoshop CC®, Abode, San Jose, CA, USA) by a single experienced investigator (J.T.L.) who was blinded to the surgical procedure and experimental group to which each specimen belonged. For calibrating intraexaminer errors, the same investigator (J.T.L.) performed histomorphometry on 10 sections twice with a 2-week interval. The following histomorphometric areal parameters were measured in square millimeters (Fig. 2):

1. Total augmented area (TA).
2. New-bone area (NBA).
3. Residual-bone-substitute area (RBA).
4. Fibrovascular tissue area (FVA).

Linear measurements in millimeters were also performed. To reduce the measurement errors, the long axis of residual teeth (first premolar and first molar) and the crest of the native bone were defined as the criteria for the linear measurements. The following horizontal linear measurements were also made using the two image-analysis computer programs at the buccal aspect in the direction perpendicular

at 0, 1, 2, 3, 4 and 5 ㎜ to the long axis of residual teeth from the alveolar crest:

1. Augmented tissue thickness (AGT): the distance from the base of the defect to the underneath of barrier membrane at 0,1,2,3,4 and 5 mm apical to the lingual crest (designated as AGT0, 1, 2, 3, 4 and 5, respectively)
2. Newly formed bone thickness (NBT): the thickness of newly formed bone at 0,1,2,3,4, and 5 mm apical to the lingual crest (designated as NBT0, 1, 2, 3, 4 and 5, respectively)

Experimental outcomes

The primary outcome of present study was the TA while the other histomorphometric parameters were measured as secondary variables.

Statistical analysis

Statistical analysis was conducted using standard statistical software (SPSS version 22, IBM, Armonk, NY, USA). The Shapiro-Wilk test was used to test the normality of the distribution. Since the areal measurements were normally distributed, repeated-measures ANOVA test was carried out to detect the differences. The other parameters were not normally distributed, the Kruskal-Wallis test was used to evaluate differences in the total augmented volume and linear measurement of histomorphometric analysis ($P < 0.05$). The threshold for statistical significance was 5%. The post-hoc Bonferroni correction was used for multiple comparisons between the groups.

III. RESULTS

Number of animals analyzed

All 15 dogs were included for radiographic and histomorphometric analysis.

Clinical findings

Surgical wound healing was uneventful during the experimental period at all of the experimental sites, with no complications such as wound dehiscence, severe swelling, or bleeding. During the surgery, both bone substitutes and membranes showed different handling property, that the MP was more malleable than the OC and, the CM was stiffer and less elastic compared to the BG.

Radiographic analysis by micro-CT

Total augmented volume is presented in Table 1. At weeks 4, 8, and 16 the volume was largest in the OC-BG group, and significant changes in volume were observed in the MP-BG group during the healing period (P = 0.048). The OC-BG group showed significantly larger total augmented volumes than the control groups (P = 0.008, 0.008, and 0.008 after 4, 8, and 16 weeks, respectively) and the MP-BG group (P = 0.008 and 0.008 after 8 and 16 weeks, respectively).

Histological observations

Week 4

There were no adverse inflammatory reactions associated with either bone substitutes or resorbable membrane in all groups. In the OC-BG group, the cortical bone layer of the basal bone was resorbed by osteoclast-like cells, and the remaining bone particles near the basal bone were in contact with the aligned new bone. Resorbable membrane was well defined over the entire augmented area (Fig. 3a,d). The histological findings in the MP-CM group were similar to those in the OC-BG group. There were fewer MP particles in the MP-CM group than OC particles in the OC-BG group. Some MP particles were found within the fibrovascular tissue (Fig. 3c,f). Most of the specimens in the OC-BG and MP-CM groups showed a convex ridge contour, while only one specimen in the MP-BG group.

NCCM and CCM showed differences in angiogenesis inside the membranes. In NCCM, blood clots and fibrin networks had infiltrated into the membranes. A dense layer of amorphous collagen fibrils were observed through the CCM, whereas larger interstices and collagen fibrils were distributed in the NCCM (Fig. 6a-d).

Week 8

In the OC-BG group, the augmented area was still well-maintained and bone remodeling was in progress with activity of osteoclast-like cells. There were newly formed capillaries within the BG membrane (Fig. 4a,d). On the other hand, in the MP-BG group, the external form of the augmented area was not maintained. There were a few

bone particles at the bone defect sites. Osteoblast-like cells were observed on the surfaces of newly formed immature bone with a reversal line (Fig. 4b,e). The histological features in the MP-CM group were similar to those in the MP-BG group. Most of the bone substitutes had disappeared in the MP-CM group, however the remaining MP particles were tightly attached to the newly formed woven bone. Osteoblast-like cells were observed on the surfaces of newly formed immature bone with a reversal line. Many capillaries were evident within the CM (Fig. 4c,f).

Blood vessels were observed in both NCCM and CCM membranes. Periosteum-like tissues(PT) were formed underneath the membranes (Fig. 6e-h).

Week 16

The dome-shaped augmented area was well maintained in the OC-BG group at 16 weeks. A large amount of residual OC particles was maintained at the OC-BG group. Newly formed matured bone was observed between OC particles, and the BG membrane remained (Fig. 5a,d). In the MP-CM and MP-BG groups, the residual particles were dispersed along the bone bed. Both CM and BG membrane were still observed, but not as clear as 8 weeks. A small amount of MP particles was found with the new bone (Fig. 5b-f).

Both NCCM and CCM membranes are highly resorbed and have larger interstices than their original form. Collagen fibrils of both membranes were shorter and thinner than those at 4 and 8 weeks. PT were also observed (Fig. 6i-l).

Histomorphometric analysis

The results of the histomorphometric analysis are summarized in Tables 2 and 3.

Week 4

The statistical significance was found in TA (P = 0.003), however there were no significant differences in post hoc comparisons. NBA and FVA were significantly larger in the OC-BG group than the control group (P = 0.003 and 0.004, respectively). FVA in the MP-CM group was also larger than the control group (P = 0.001). The horizontal linear measurement of the augmented area was significantly larger in the MP-CM group than the control group (P = 0.008).

Week 8

TA and FVA was significantly larger in the OC-BG group than the control (P = 0.003 and P = 0.004, respectively), while NBA was significantly larger in the OC-BG group than the MP-BG group (P = 0.007). AGT0 in the MP-BG group differed significantly from that in the control group (P = 0.008). In addition, AGT1, AGT2, and AGT3 were significantly larger in the OC-BG group than in the control group (P = 0.008 for each). NBT1, AGT0, AGT1, and AGT2 differed significantly between the MP-CM and control groups (P = 0.008 for each).

Week 16

TA was significant larger in the OC-BG group than in the control group at 16 weeks (P = 0.007), and NBA was significantly larger in

the OC-BG group than the control and MP-BG groups (P = 0.006 vs. control, P = 0.007 vs. MP-BG). AGT0, AGT1, AGT2, and AGT3 were significantly larger in the OC-BG group than the control group at 16 weeks (P = 0.008). Similarly, AGT1, AGT2, and AGT3 differed significantly at 16 weeks (P = 0.008). (Table 3).

IV. DISCUSSION

The present study evaluated the sequential healing of lateral onlay grafts using different combinations of soft-type synthetic block bone and resorbable collagen membranes in terms of volumetric and histological changes. The histological results indicated that the total augmented volume and amount of new bone in the OC-BG group were about two times larger than those in the other groups at 4, 8, and 16 weeks. NBT and AGT in the OC-BG group were also significantly larger than those in the other groups. The data obtained in this study indicate that the lateral onlay graft with soft-type block bone and resorbable collagen membranes is helpful for reconstructing a defect area and increasing the volume for implant placement. In particular, the OC-BG group showed superior volume maintenance and osteogenic potential compared to the other groups in our chronic dehiscence defect model.

TA differed significantly between the OC-BG group and the other groups in this study. It is considered that the constituents of the bone graft materials contributed to these results. The HA:β-TCP ratio could affect the resorption rate, since HA has a slow resorption rate while β-TCP has a faster resorption rate, and so the absorption rate is mainly adjusted by changing their ratio [36]. In the present study, the β-TCP proportion was 85% in MP compared to 15% in OC. The results of this study suggest that a high proportion of β-TCP had an unfavourable effect on maintenance of the bone volume in the chronic dehiscence defects.

The present study found that the technique used to maintain the bone graft materials seemed to markedly affect the results. Two pins

were used in each defect to mechanically fix the collagen membrane. A previous study found that applying pins for membrane fixation achieved primary stability of the bone materials [37]. The use of soft-type block bone and resorbable collagen membranes with two pins may have contributed to the primary stability observed in the present study. However, there were three cases in which displacement of bone graft materials occurred at 4, 8 and 16 weeks in the OC-BG group. It could be assumed that the bone was dispersed into the space between the two pins. These drawbacks could be overcome by increasing the number of pins or placing the pins closer to the bony surface. In spite of the dissipation of bone materials, total augmented volume in the OC-BG group was larger than that in the other groups.

In this study, CCM showed that the shape was well maintained at 4 weeks compared to NCCM. It means that CCM has superior physical properties [31]. However, there was no significant difference in the degradation of NCCM and CCM during the experimental period. This result is consistent with previous studies [6,38]. It seems that CCM and NCCM had barrier function until 16 weeks, because remnants of membrane were observed. Considering that thin collagen fibrils with large interstices appeared, NCCM might be degraded prior to CCM. While NCCM has drawback which is its rapid biodegradation, NCCM had favorable bone regeneration in this study.

The origins of the collagen in bone graft materials may also affect the result of the study [19,20]. In the present study, two types of origin for collagen were used for soft-type block bone (porcine derived collagen for the OC and bovine derived collagen for the MP). Bovine collagen is one of the main sources used in biomaterial fields,

but one of its disadvantages is its association with allergic responses [21-23]. The drawbacks of bovine collagen can be overcome by instead using porcine collagen. A previous study demonstrated that only minimal allergic reactions were induced by porcine collagen, which is due to porcine collagen being very similar to human collagen [39].

The lateral onlay graft model is unfavourable and challenging defect because it involves a non-contained defect characterized by continuous pressure from the flap and the presence of cortical bone on the marginal defect [40]. According to the histomorphometric measurements made in this study, most of the NBT values (0.00～2.87 mm) appeared to indicate a low rate of new bone formation, which is in agreement with previous findings [11,12]. However, in terms of clinical relevance, studying the lateral onlay graft model is important since this model is commonly applied in clinical cases.

V. CONCLUSIONS

The OC-BG group showed superior volume maintenance and osteogenic potential up to 16 weeks compared to the other groups in an onlay graft model of the dog mandible despite the displacement of the bone graft MP appears to lose its potential to maintain volume over time, even when used in combination with a cross-linked collagen membrane.

VI. TABLES AND FIGURES

Table 1. Total augmented volume by radiographic analysis (mm^3)

Group		4 weeks	8weeks	16weeks	
					P value
Control	Mean (SD)	7.57 (2.31)	4.04 (2.12)	5.76 (3.53)	0.114
	Median	6.45	4.53	4.54	
MP-BG	Mean (SD)	9.94 (3.13)	5.01 (0.94)	5.80 (2.61)	0.048[b]
	Median	10.92	4.91	4.96	
OC-BG	Mean (SD)	25.36 (21.20)	26.56 (14.73)	33.43 (23.46)	0.059
	Median	15.64	21.67	23.05	
MP-CM	Mean (SD)	19.37 (10.26)	9.68 (8.67)	9.34 (3.56)	0.677
	Median	15.44	6.21	8.39	
P value		0.010[a]	0.010[a]	0.006[a]	
MP-BG vs Control		0.310	0.421	0.841	
OC-BG vs Control		0.008[c]	0.008[c]	0.008[c]	
MP-CM vs Control		0.032	0.151	0.151	
MP-BG vs OC-BG		0.016	0.008[c]	0.008[c]	
MP-BG vs MP-CM		0.095	0.310	0.151	
OC-BG vs MP-CM		0.690	0.056	0.016	

MP: Mastergraft® putty, OC: Osteon III Collagen®, CM: Collagen membrane-P®, BG: BioGide®

a: Significant different at the same observation period. (Statistical significance level was 5%, $P<0.05$)

b: Significant different at the same group. (Statistical significance level was 5%, $P<0.05$)

c: Significant different between two groups. (Statistical significance level was 0.83%, $P<0.008$)

Table 2. Areal measurement of histomorphometic analysis (mm^2)

	TA	NBA	RBA	FVA
4 weeks	Mean (SD)			
Control	2.17 (0.63)	0.71 (0.13)	0.00 (0.00)	1.46 (0.63)
MP-BG	6.35 (2.29)	1.11 (0.53)	0.57 (0.79)	4.66 (1.88)
OC-BG	14.44 (9.39)	**2.01 (0.59)**[a]	2.75 (3.49)	**9.68 (5.72)**[a]
MP-CM	13.93 (2.92)	1.47 (0.53)	1.57 (1.38)	**10.89 (1.48)**[a]
P value	**0.003**[*]	**0.004**[*]	0.159	**0.001**[*]
8 weeks	Mean (SD)			
Control	1.77 (0.76)	0.45 (0.28)	0.00 (0.00)	1.32 (0.74)
MP-BG	3.67 (1.27)	0.41 (0.15)	0.10 (0.14)	3.15 (1.12)
OC-BG	**12.21 (5.37)**[a]	**1.58 (0.83)**[b]	2.31 (1.74)	**8.32 (3.46)**[a]
MP-CM	6.42 (5.04)	0.66 (0.31)	0.72 (1.07)	5.04 (3.81)
P value	**0.003**[*]	**0.004**[*]	**0.009**[*]	**0.005**[*]
16 weeks	Mean (SD)			
Control	1.90 (0.36)	0.61 (0.20)	0.00 (0.00)	1.29 (0.21)
MP-BG	2.78 (0.82)	0.68 (0.22)	0.04 (0.03)	2.06 (0.66)
OC-BG	**14.56 (9.92)**[a]	**3.31 (2.08)**[a,b]	2.07 (2.12)	9.18 (6.07)
MP-CM	6.23 (2.23)	1.19 (0.24)	0.28 (0.15)	4.76 (2.23)
P value	**0.005**[*]	**0.003**[*]	**0.020**[*]	**0.006**[*]

TA, total augmented area; NBA, new bone area; RBA, residual bone substitute area; Fibrovascular tissue area, FVA

MP: Mastergraft® putty, OC: Osteon III Collagen®, CM: Collagen membrane-P®, BG: BioGide®

*: Significant different at the same observation period. (Statistical significance level was 5%, $P<0.05$)

a: Significantly different from control group in the same healing period. (Statistical significance level was 0.83%, $P<0.008$)

b: Significantly different from MP-BG group in the same healing period. (Statistical significance level was 0.83%, $P<0.008$)

c: Significantly different from OC-BG group in the same healing period. (Statistical significance level was 0.83%, $P<0.008$)

Table 3. Linear measurement of histomorphometic analysis (mm)

Time	Group	NBT0	NBT1	NBT2	NBT3	NBT4	NBT5	AGT0	AGT1	AGT2	AGT3	AGT4	AGT5
4 weeks	Control	0.30 (0.43)	0.27 (0.40)	0.33 (0.38)	0.32 (0.43)	0.31 (0.26)*	0.09 (0.08)	0.56 (0.82)	0.70 (1.03)	0.73 (1.26)	0.81 (1.23)	0.37 (0.30)	0.30 (0.07)
Mean (SD)	MP-BG	0.05 (0.03)	0.27 (0.08)	0.25 (0.18)	0.21 (0.18)	0.49 (0.26)	0.26 (0.26)	0.64 (0.53)	0.78 (0.69)	1.02 (0.89)	1.33 (0.67)	0.85 (0.25)	0.56 (0.22)
	OC-BG	0.13 (0.11)	0.44 (0.22)	0.32 (0.10)	0.62 (0.44)	1.01 (0.75)	0.61 (0.38)	1.67 (1.49)	1.91 (1.57)	2.29 (1.84)	1.91 (1.43)	1.35 (0.71)	0.84 (0.62)
	MP-CM	0.26 (0.41)	0.41 (0.33)	0.58 (0.44)	0.40 (0.50)	0.56 (0.29)	0.13 (0.18)	0.76 (0.42)	1.40 (0.73)	2.00 (0.66)	2.55 (0.88)	1.98 (0.63)[a]	1.40 (0.32)
8 weeks	Control	0.12 (0.08)	0.06 (0.04)	0.12 (0.12)	0.13 (0.09)	0.10 (0.06)*	0.02 (0.02)	0.08 (0.08)*	0.22 (0.12)	0.24 (0.18)	0.36 (0.26)	0.11 (0.09)*	0.12 (0.04)
Mean (SD)	MP-BG	0.05 (0.08)	0.21 (0.13)	0.06 (0.07)	0.19 (0.15)	0.00 (0.00)*	0.00 (0.00)	0.80 (0.26)[a]	1.07 (0.69)	1.00 (0.38)	0.57 (0.29)	0.18 (0.00)*	0.00 (0.00)
	OC-BG	0.65 (0.67)	1.13 (1.12)	0.82 (0.82)	0.76 (0.51)	0.25 (0.54)	0.00 (0.00)*	1.40 (1.04)	1.81 (0.81)[a]	2.30 (0.94)[a]	1.87 (0.75)[a]	0.93 (0.72)	1.17 (0.00)
	MP-CM	0.13 (0.11)	0.51 (0.47)[a]	0.41 (0.22)	0.29 (0.23)	0.30 (0.15)	0.00 (0.00)	0.72 (0.31)[a]	0.84 (0.18)[a]	0.93 (0.44)[a]	0.96 (1.02)	1.52 (0.69)	1.07 (1.98)
16 weeks	Control	0.38 (0.68)	0.20 (0.19)	0.17 (0.12)	0.14 (0.05)	0.11 (0.04)	0.16 (0.09)	0.10 (0.10)	0.15 (0.08)	0.16 (0.07)	0.19 (0.07)	0.23 (0.06)	0.21 (0.12)
Mean (SD)	MP-BG	0.08 (0.09)	0.17 (0.05)	0.18 (0.09)	0.20 (0.16)	0.12 (0.13)†	0.19 (0.00)	0.34 (0.20)	0.34 (0.15)	0.49 (0.18)[a]	0.50 (0.34)	0.37 (0.35)	0.24 (0.00)
	OC-BG	0.29 (0.39)	1.51 (1.67)	1.59 (1.89)	1.64 (2.72)	2.87 (2.40)	3.24 (0.76)	1.03 (0.88)[a]	1.07 (0.40)[a]	1.52 (0.79)[a]	1.81 (1.64)[a]	1.02 (0.49)	0.23 (0.29)
	MP-CM	0.24 (0.27)	0.37 (0.22)	0.33 (0.28)	0.17 (0.10)	0.12 (0.09)†	0.36 (0.23)	0.46 (0.37)	0.61 (0.13)[ab]	0.77 (0.35)[ab†]	1.10 (0.35)[a†]	1.18 (0.74)	1.25 (0.01)

MP: Mastergraft® putty, OC : Osteon III Collagen®, CM : Collagen membrane-P®, BG: BioGide®

NBT, newly formed bone thickness at 0, 1, 2, 3, 4 and 5 mm apical from the alveolar crest (designated as NBT-0, -1, -2, -3, -4 and -5, respectively);

AGT, augmented tissue thickness at 0, 1, 2, 3, 4 and 5 mm apical from the alveolar crest (designated as NBT-0, -1, -2,

-3, -4 and -5, respectively);

a: Significantly different from control group (Statistical significance level was 0.83%, $P<0.008$).

b: Significantly different from MP-BG group (Statistical significance level was 0.83%, $P<0.008$).

c: Significantly different from MP-CM group (Statistical significance level was 0.83%, $P<0.008$).

∗: Significantly different between 4 and 8 week groups (Statistical significance level was 1.67%, $P<0.017$).

† : Significantly different between 4 and 16 week groups (Statistical significance level was 1.67%, $P<0.017$).

‡ : Significantly different between 8 and 16 week groups (Statistical significance level was 1.67%, $P<0.017$).

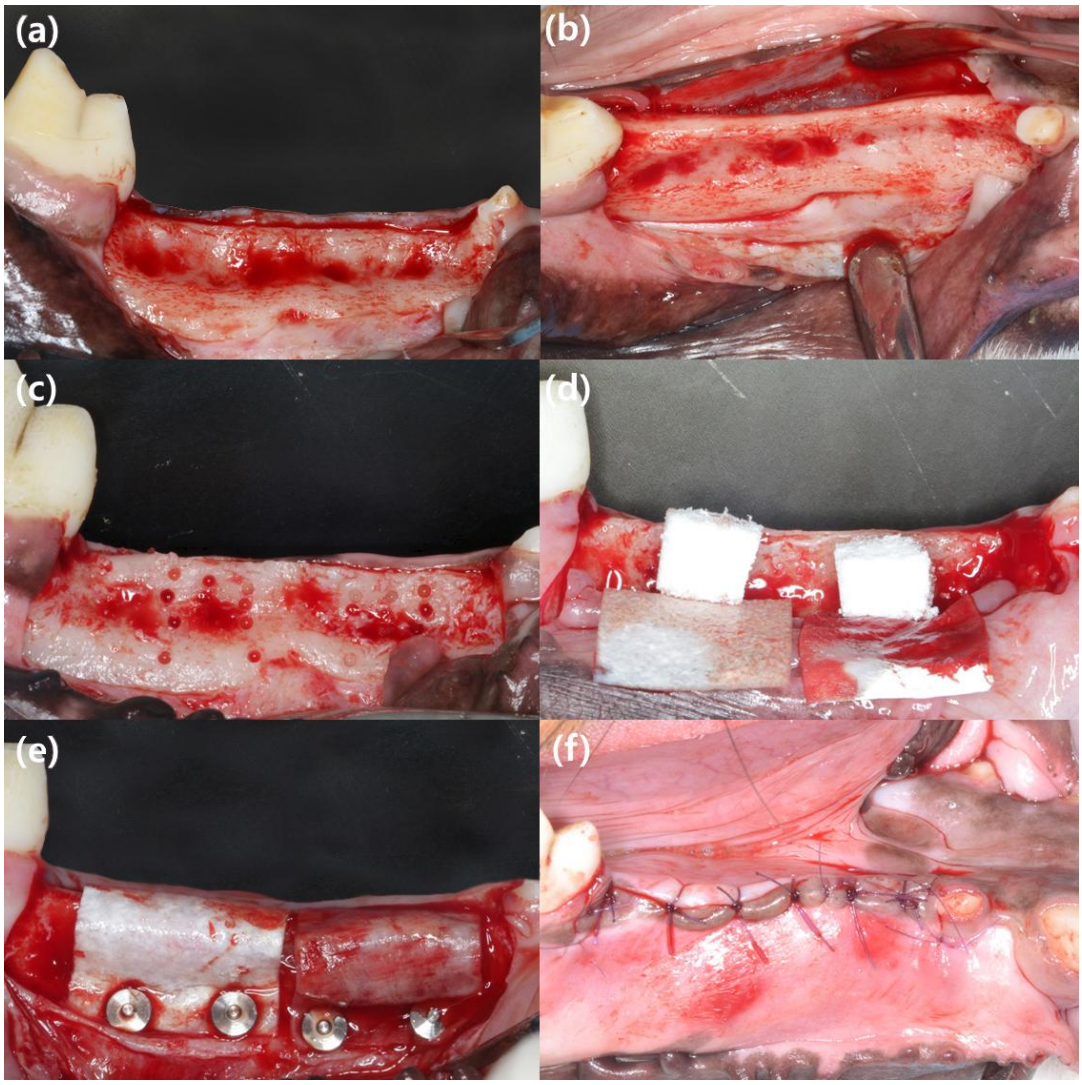

Fig. 1. Clinical photographs of the experimental sites.

(a - c) A full-thickness flap was elevated, and the bone bed was prepared by perforating the cortical bone. (d,e) The assigned bone substitutes and membranes were applied. The left block is Mastergraft® putty (MP) and the right block is Osteon III Collagen® (OC). The bone substitutes were covered by membranes [left: Collagen Membrane-P® (CM), right: BioGide®(BG)] that were stabilized using two pins. (f) Suturing.

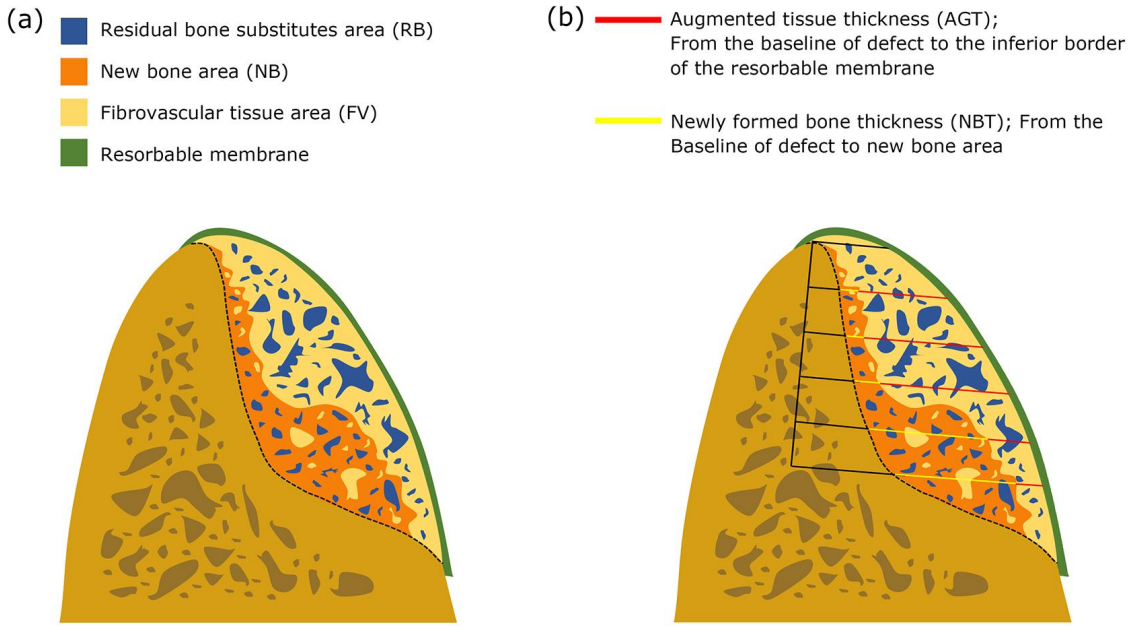

Fig. 2. Schematic drawings of the histomorphometric analyses.

(a) The total augmented area (TA) was demarcated by the base of the mandibular bone (black dotted line) and the inferior border of the barrier membrane (green line). The new-bone area (NBA), residual-bone-substitute area (RBA) and fibrovascular tissue area (FVA) were measured within the augmented area.

(b) Schematic drawings illustrating the linear measurements. Yellow line indicates the augmented tissue thickness (AGT), measured from the base of the defect to the inferior border of the resorbable membrane. Red line indicates the newly formed bone thickness (NBT), measured from the base of the defect to the area of new bone.

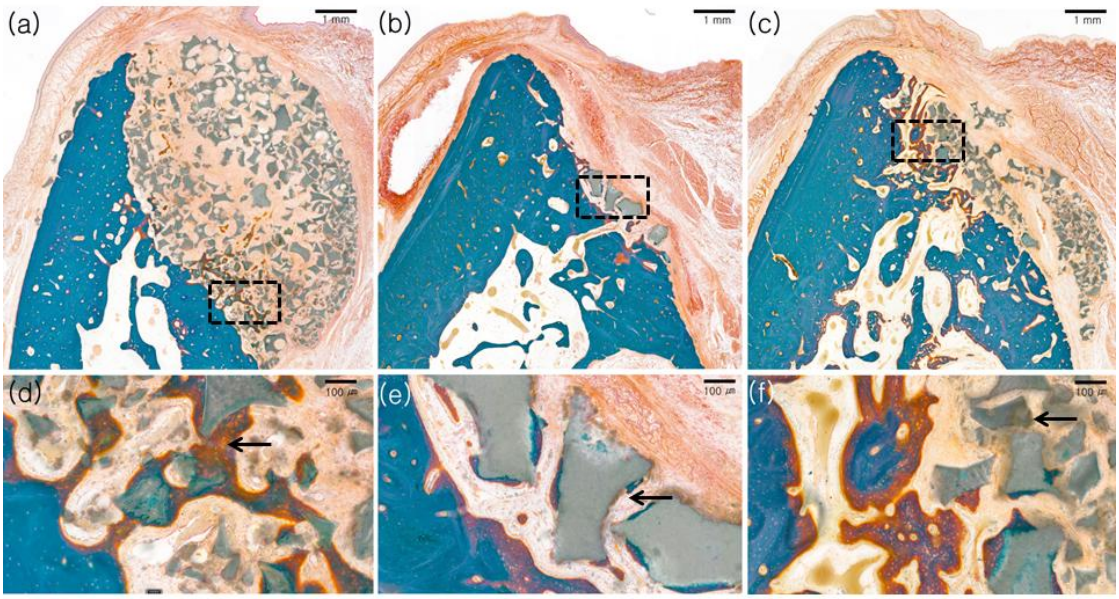

Fig. 3. Histological images obtained at 4 weeks after staining with Goldner trichrome. (a - c) Low-magnification images from the OC-BG, MP-BG and MP-CM groups (scale bar = 1 mm). (d - f) High-magnification images of the areas highlighted in panels a - c (scale bar = 100 mm). (d) The remaining OC particles were in contact with the aligned newly formed bone (arrow). (e) Osteoclast-like cells (arrow) were observed on the surface of the MP particles. (f) Residual bone particles were observed within the fibrovascular tissue (arrow).

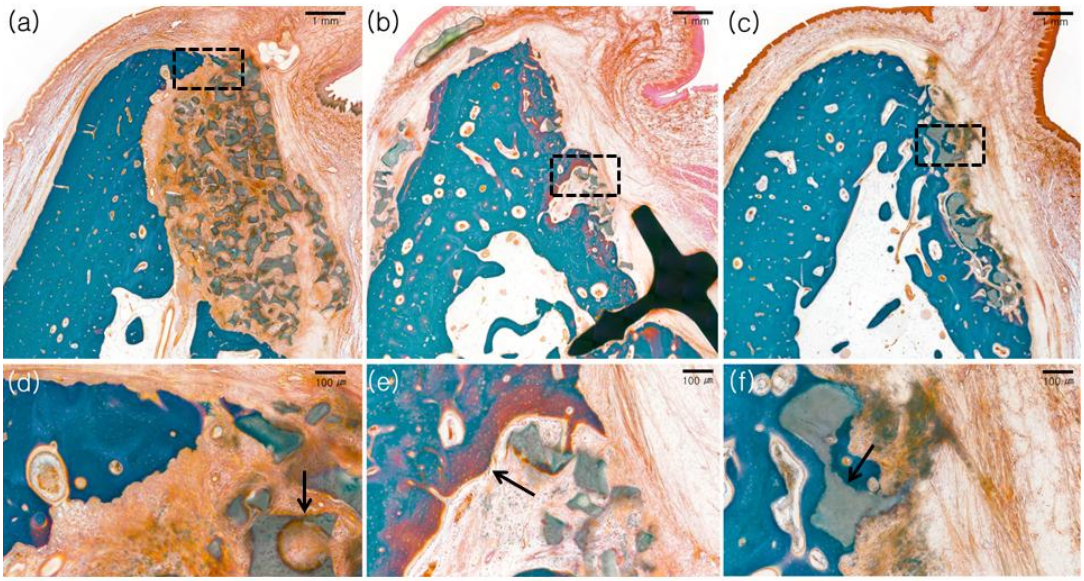

Fig. 4. Histological images obtained at 8 weeks after staining with Goldner trichrome. (a - c) Low-magnification images from the OC-BG, MP-BG and MP-CM groups (scale bar = 1 mm). (d - f) High-magnification images of the areas highlighted in panels a - c (scale bar = 100 mm). (d) Bone resorption was still in progress with osteoclast activity (arrow). (e) Osteoblasts (arrow) were observed on the surface of newly formed immature bone with a reversal line. (f) The remaining MP particles were tightly attached to the newly formed woven bone (arrow).

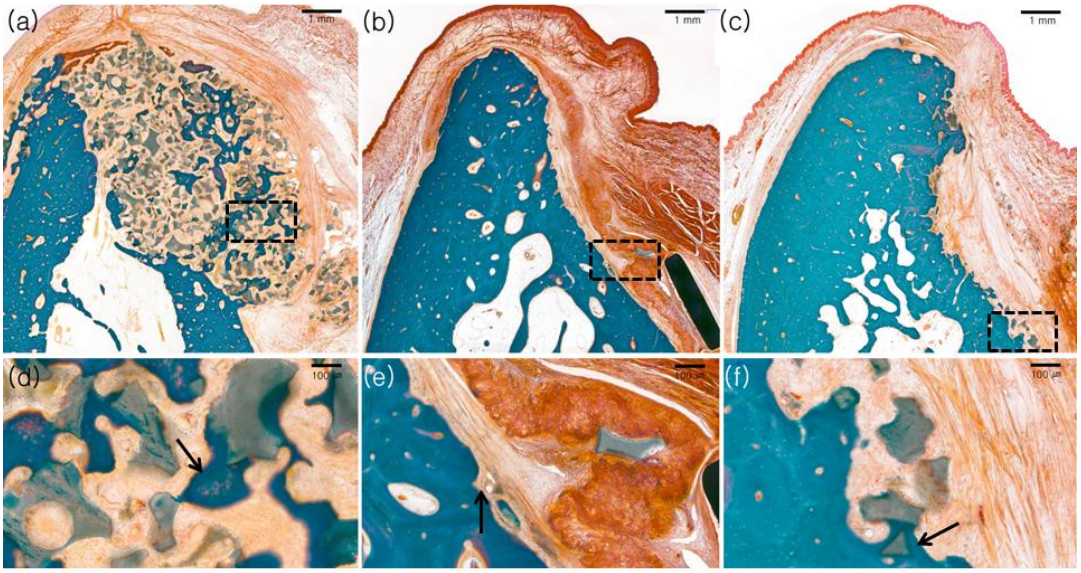

Fig. 5. Histological images obtained at 16 weeks after staining with Goldner trichrome. (a - c) Low-magnification images from the OC-BG, MP-BG and MP-CM groups (scale bar = 1 mm). (d - f) High-magnification images of the areas highlighted in panels a - c (scale bar = 100 mm). (d) The remaining OC particles were tightly attached to the matured bone (arrow). (e) A small amount of MP particles was observed (arrow). (f) Matured bone was observed between the MP particles (arrow).

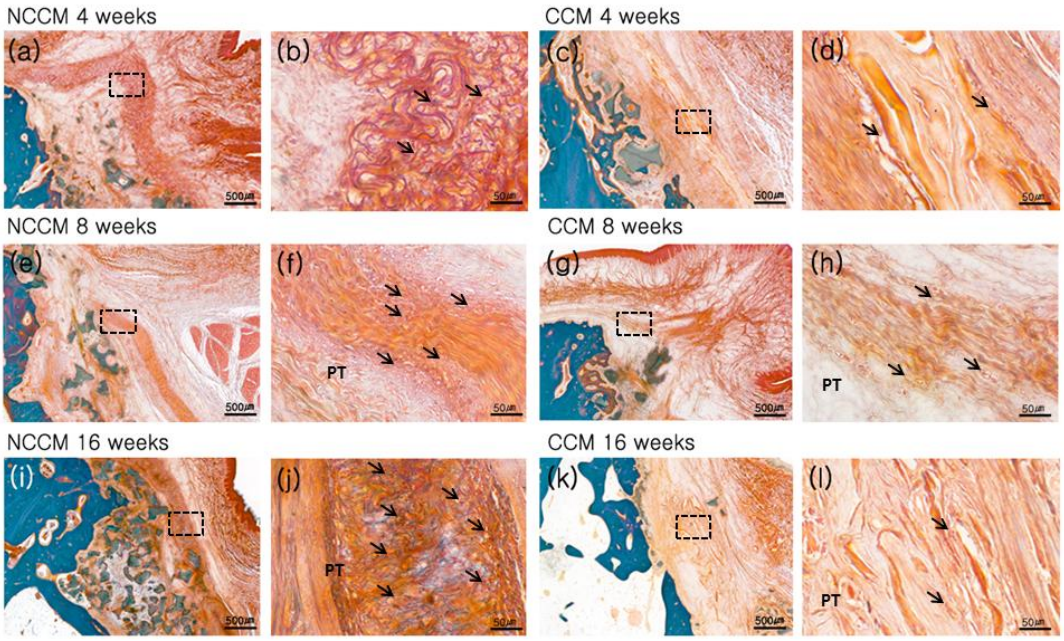

Fig. 6. High-magnification photomicrographs of NCCM and CCM at 4, 8 and 16 weeks (Goldner trichrome stain; arrows, blood vessels). (a,b) Blood clots and fibrin networks had infiltrated into the membranes at week 4 (scale bar = 500 and 50 ㎛). (c, d) A dense layer of amorphous collagen fibrils were observed through the CCM at week 4 (scale bar = 500 and 50 ㎛). (e-h) Blood vessels were observed in both NCCN and CCM membranes. Periosteum-like tissues(PT) were formed underneath the membranes at week 8 (scale bar = 500 and 50 ㎛). (i-l) Both NCCM and CCM membranes are highly resorbed and have larger interstices than their original form. Collagen fibrils of both membranes were shorter and thinner than those at 4 and 8 weeks (scale bar = 500 and 50 ㎛).

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국문초록

성견에서 온레이 타입 수평골 이식시 흡수성차단막과 연질 블록형 골이식재의 효과

이 정 태
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(지도교수 김 성 태)

1. 연구 목적

이 연구는 수평적인 골소실 부위에 온레이 타입으로 골이식을 시행 시, 사용되는 연질 블록형 골이식재와 흡수성 차단막 효과를 평가 하는 것이다.

2. 연구 방법

15 마리 성견의 양측 하악 P2~4를 발치한 후, 협측 부위 치조골에 수평골 (10mm x 10mm x 5mm) 삭제를 시행하였다. 8주간의 치유기간 이후 골결손 부위를 형성한 후 양측에 2개씩 흡수성 차단막과 두 가지 타입의 연질 블록형 골이식재를 이용하여 온레이 타입의 수평골 이식을 시행하였다.

골이식시 다음과 같이 네 가지 군으로 분류하였다.
대조군: 골이식술을 시행하지 않음. MP-BG 군: 첫 번째 타입의 연질 블록형 골이식재 [hydroxyapatite (HA): β-tricalcium phosphate (β-TCP) = 15:85]와 비가교성 흡수성 차단막(Non-cross-linked collagen membrane: NCCM) 사용, OC-BG 군: 두 번째 타입의 연질 블록형 골이식재 (HA:β-TCP = 60:40)와 비가교성 흡수성 차단막 (NCCM)을 사용, MP-CM 군: 첫 번째 타입의 연질 블록형 골이식재와 가교성 흡수성 차단막 (Cross-linked collagen membrane: CCM)을 사용.
차단막은 두 개의 핀으로 고정하였다. 4, 8, 16주의 치유기간 후 희생시켜 조직 계측학적 분석을 위한 시편을 제작하였다. 치조제의 부피변화를 측정하기 위해서 콘빔형 전산화 단층촬영 (Cone-beam computed tomography scans)을 하였다. 또한, 조직 샘플을 레진포매한 후, Goldner trichrome stain 염색을 시행하였다. 염색된 조직샘플에 대한 전산화 스캔을 시행하였고, 스캔된 이미지를 바탕으로 조직학적 및 조직형태학적인 계측 (Histological and histomorphometric analyses)을 하였다. 이미지를 토대로 증가된 총 영역 [Total augmented area (TA)], 새롭게 형성된 치조골의 영역 [New-bone area (NBA)], 잔존된 이식재 영역 [Residual-bone-substitute area (RBA)], 섬유성 혈관 조직 영역 [Fibrovascular tissue area (FVA)]을 구분하여 분석하였다. 선형측정 (Linear measurement)을 위하여, 잔존 치아의 치축을 기준으로 치조골정에서부터 치근단까지 가상선을 설정하였다. 이 선위에 0, 1, 2, 3, 4, 5㎜ 위치에서 가상선의 수직으로 수평선을 설정하여, 증가된 총 조직의 길이 [Augmented tissue thickness (AGT)]와 새롭게 형성된 치조골의 길이 [Newly formed bone thickness (NBT)]를 측정하였다.

3. 결과

모든 군에서 임상적인 부작용은 없었다. 8 주와 16 주에서, OC-BG 군은 치조제 부피가 대조군 및 MP-BG 군보다 유의하게 높았다. 또한 새로운 치조골의 형성을 관찰하기 위해서 시행된 조직형태학적인 검사 결과 OC-BG 군의 TA값이 8주와 16주에서 대조군 보다 유의하게 높은 수치를 보였다. 특히 16주에서는 OC-BG의 NBA값은 대조군과 MP-BG군 보다 유의하게 높았다. 선형계측 분석결과 8주와 16주에서 OC-BG 군은 대조군에 비해, AGT1, AGT2, 그리고, AGT3의 수치에서 높은 유의성을 보였다. MP-CM군은 대조군에 비해 8주에서 NBT1, AGT0, AGT1, AGT2, AGT3의 값에서 유의성 있는 차이를 보였다. 4주와 8주 사이 MP-BG군에서는 NBT4와 AGT4 값에서, OC-BG군에서는 NBT5 값에서 유의성 있는 차이가 관찰되었다. MP-BG군과 MP-CM군에서 NBT4값은 4주와 비교했을 때, 8주에서 유의하게 감소하였다. 특히 MP-CM군은 AGT2와 AGT3의 값이 4주보다 16주에서 많이 감소하였다.

CCM과 NCCM의 흡수정도는 실험기간동안 유의성 있는 차이를 보이지 않았다. 4주에서 NCCM은 CCM에 비해서 차폐막안으로 혈관의 신생정도가 활발하게 나타났다. CCM은 NCCM 보다 차폐막의 형태가 보다 잘 유지되는 것을 볼 수 있었다. 16주에서는 두 차폐막 모두 많은 양이 흡수가 되었다.

4. 결론

이 연구에서 온레이 타입 수평골 이식시 연질 블록형 골이식재와 흡수성 차단막을 함께 사용하는 경우에 더 많은 신생골이 형성되었고, 골재생의 양과 질이 높아지는 결과를 보였다.

주요어: 연질 블록형 골이식재, 흡수성 차단막, 온레이 타입 수평골이식, 치조골 회복, 전임상실험

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