Lateral bone augmentation using a titanium mesh combined with the bone substitute and/or collagen membrane on peri-implant dehiscence defects:

An experimental *in vivo* study

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An experimental *in vivo* study

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감사의 글

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학위 과정 동안 끊임없는 도움과 따뜻한 격려로 함께 열심히 달려온 연구원 선생님들께도 진심으로 감사드리며 오늘의 감사한 마음 잊지 않고 오랫동안 간직하겠습니다.

마지막으로 지금의 저를 있게 해주신 사랑하는 우리 가족. 언제나 흔들리지 않고 앞만 보고 달릴 수 있게 해주신 부모님의 든든한 지원과 끊임없는 사랑으로 뒤를 지켜준 우리 언니들에게 진심으로 감사의 마음을 전하고 싶습니다.

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팽경원 올림

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Abstract

Lateral bone augmentation using a titanium mesh combined with the bone substitute and/or collagen membrane on peri-implant dehiscence defects: An experimental *in vivo* study

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Purpose: The aim of this study was to determine the additional effects of collagen membrane (CM) and of synthetic bone substitute (BS) on lateral bone augmentation of peri-implant dehiscence with titanium mesh (TM).

Materials and Methods: Atrophic alveolar ridge was induced in 6 canine mandibles and 5 peri-implant dehiscence were achieved in each hemi-mandible. Bone augmentation was attempted using the following randomly allocated modalities: 1) Control: no treatment, 2) TM group: blood clot covered by TM, 3) TM+BS group: BS covered by TM, 4) TM+CM

group: blood clot covered by TM and CM, and 5) TM+BS+CM group: BS covered by TM and CM. After 16 weeks of submerged healing, radiographic and histomorphometric analyses were performed.

Results: TM exposure occurred in one case in the TM group, one case in the TM+CM group, and two cases in the TM+BS+CM group. Histologically, pseudo-periosteum was observed along the inner and outer surfaces of TM. In general, TM rendered higher values in vertical defect fill and dimension of the augmented hard tissue in comparison to the other treatment groups.

Conclusions: Within limitations of this study, additional use of CM and/or BS did not have an additional benefit on lateral bone augmentation of peri-implant dehiscence with TM.

Keywords: lateral bone augmentation; titanium mesh; resorbable collagen membrane; synthetic bone substitutes; animal experimentation; pseudo-periosteum;

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I. INTRODUCTION

GBR (guided bone regeneration) has been considered as a predictable procedure for alveolar ridge augmentation in the atrophic ridge. Four biologic principles are considered to be needed for successful osteogenesis of GBR; primary wound closure, angiogenesis, space maintenance, and wound stability [1](Wang et al. 2006). To achieve this, various types of barrier membrane have been evaluated and, among them, absorbable

collagen membrane (CM) has become a standard of care in lateral bone augmentation [2](Sanz-Sanchez et al. 2015). CM has excellent biocompatibility, is easy to handle clinically, and does not require secondary surgery [3](Blumenthal et al. 1990), however, has a limitation in that it lacks space maintenance due to its insufficient mechanical characteristics. Thus, it is disadvantageous in the reconstruction of bone defects that are large in size and subject to pressure from soft tissue [4, 5](McGinnis et al. 1998; Oh et al. 2003).

Titanium-mesh (TM) has been reported successful GBR results in recent systematic review, and obviously, this is a barrier membrane that has advantages in space maintenance [6](Briguglio et al. 2019). TM provides excellent mechanical strength, it is easily affixed to the implant fixture, even in the case of large defects. However, one of its limitations is that TM would be insufficient for maintaining cell occlusiveness due to the presence of macro-pores [7-9](Pineda et al. 1996; Kubo et al. 1998; Rakhmatia et al. 2013).

To overcome this limitation, a method of improving cell occlusiveness by covering the occlusive membrane over TM has been proposed [10](Lundgren et al. 1998), however the synergetic effect on this is still controversial. Several preclinical and clinical studies have described limited new bone formation and soft tissue infiltration when occlusive membranes were not overlaid in combined with TM [11, 12](Her et al. 2012; Park et al. 2008). On the other hand, it was reported that additional use of occlusive membrane over TM did not offer added benefit, despite of high exposure rate, for buccal bone preservation in dogs immediate implant placement model [13](Lim et al. 2015). It seems to show contradictory results depending on the experimental model and type of biomaterials.

Therefore, the hypothesis in the present study is that lateral bone augmentation using TM combined with CM and/or synthetic bone substitute (BS) in a chronic narrow ridge would show a better cell occlusiveness and space maintenance, thus it leads to enhanced osteogenesis than TM alone. The aim of this study is to determine the effect of

TM with an adjunctive use of CM and/or BS for lateral bone augmentation in chronic narrow ridge.

II. MATERIALS AND METHODS

This study was approved by the Bioethics Committee with regards to animal selection, management, and surgical procedure at the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Republic of Korea (2016-0165). The article was written in accordance with the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines [14](KilKenny et al. 2010).

1. Animals

Total 6 male mongrel dogs, weighing 35~40 kg and 1-year-old, and having healthy periodontium were used. The animals were reared separately in W2000 mm x D1200 mm x H1500 mm in door runs, ambient room temperature 22 ± 2 ℃, relative humidity 50 ± 10 %, light/dark cycle 12/12 hr, and they were fed a standard pellet dog-food diet (Purina canine lab diet, Cagill, Dangjin, Korea) throughout the study and had ad libitum access to water filtered by the R/O with UV sterilization.

2. Materials for bone augmentation

Five dental implants (Ø 4.3 x 8 mm, Implantium; Dentium, Seoul, Korea) were placed on each side of the mandible in 6 dogs. Except for the control group, the other four groups were treated with TM (CTi-mem B9 type, Neo Biotech, Seoul, Korea). Synthetic biphasic calcium phosphate particulate bone substitute (BS; Osteon III, GENOSS, Suwon, Korea) and cross-linked CM (Collagen Membrane-P, 20x30, GENOSS, Suwon, Korea) were used in combination with TM.

TM measured 12 mm in mesio-distal width and 10 mm in apico-coronal height. TM was applied by mounting it to the implant shoulder and fixating it with a cover cap.

The wings of the TM needed for the stabilization of the graft materials to the bone defect are bendable and can be adapted to the contour of the recipient site.

3. Study design and randomization

Atrophic alveolar ridge was induced in 6 canine mandibles and 5 peri-implant dehiscence were achieved in each hemi-mandible. Bone augmentation was attempted using the following randomly allocated modalities:

- Control group: no treatment
- TM group: blood clots covered by titanium mesh (TM)
- TM+BS group: bone substitute (BS) covered by TM
- TM+CM group: blood clots covered by TM and collagen membrane (CM)
- TM+BS+CM group: BS covered by TM and CM

4. Surgical procedures

All surgical procedures were performed by one experienced surgeon. General anesthesia was induced in the animals by inhalation of isoflurane (Forane® , Choongwae Pharmaceutical, Seoul, Korea), and a subsequent intravenous injection of xylazine (Rompun® , Bayer Korea, Seoul, Korea) in combination with zolazepam + tiletamine (Zoletil® , Virbac, Carros, France).

4.1 Surgery 1 (Creation of edentulous narrow ridge)

Crevicular incisions were made around the mandibular premolars and first molars,

and buccal and lingual flaps were reflected. The teeth were extracted and the buccal bone plate was removed at each alveole to create defects with 5 mm in apico-coronal dimension (Fig. 1a). The flaps were repositioned and sutured using resorbable sutures. During the first post-operative 2 weeks, the dogs were fed a soft diet. After 7 to 10 days, the animals received an intra-venous anesthesia (Rompun® 2mg/kg, Zoletil® 5mg/kg) and the sutures were removed.

4.2 Surgery 2 (Implant placement and GBR)

After 2 months of healing, mucoperiosteal flaps were reflected on each hemi-mandible to visualize the buccal bone, and 5 implants were placed with equal space between each other. After implant placement, a buccal dehiscence defect with 3 mm in apico-coronal height was prepared at each implant site (Fig. 1b-c). After implant placement, TM was bent to fit the outline of the buccal bone and mounted onto the implant shoulder by using a cover cap (Fig. 1d). In the BS groups, BS filled the buccal dehiscence defects under TM (Fig. 1e). In the CM groups, CM was applied to completely cover the TM. The flaps were sutured using single interrupted sutures to allow submucosal healing of all the implant sites.

4.3 Sacrifice

After 4 months of healing, euthanasia was performed on all animals by injecting an overdose of potassium chloride-40 (Daihan®, Seoul, Korea) medication after full anesthesia. The implants and surrounding soft tissues were macroscopically inspected and dissected to obtain samples for radiographic and histologic analyses.

5. Radiographic analyses

The extracted hemi-mandible were fixed in 10 % neutral buffered formalin solution for 10 days followed by micro-computed tomography scanning (micro-CT; Skyscan 1173, SkyScan, Aartselaar, Belgium) with the following settings: 50 μm voxel size, 130 kV acceleration voltage and 60 μA beam current. The scanned data were processed in DICOM format and the area of interest was reconstructed with NRecon reconstruction software (Ver.1.7.4.6, Bruker-microCT, Kartuizersweg 3B 2550 Kontich, Belgium). Radiographic analysis was performed for the 3D visualization of the augmented hard tissue without quantitative measurements. A reconstructed 3D image was used to identify the location of the biomaterials used for implant placement and bone augmentation. The images of TMs were removed through image operation with CtAn software (Ver. 1.19.4.0, Bruker-microCT, Kartuizersweg 3B 2550 Kontich, Belgium). In the 3D images implant were colored in blue and BSs in red by using OnDemand3D software (Cybermed, Seoul, Republic of Korea).

6. Histologic preparation

Bone blocks containing each implant group site were dissected and fixed in 10 % neutral buffered formalin solution. The blocks were dehydrated with ethanol solutions and embedded in resin for ground section. The specimens were sectioned bucco-lingually, and the central sections were selected, reduced to a final thickness of approximately 20 μm, and then stained with Goldner's trichrome stain. Digital images of histology were observed and obtained by optical microscopy (BX50, Olympus, Tokyo, Japan).

7. Histomorphometric analyses

Histomorphometric measurements were conducted by a blinded, experienced

examiner (P.K.W) using a personal-computer-based image-analysis system (Photoshop, Adobe, San Jose, CA, USA). Histomorphometric analyses were performed by measuring the following parameters according to the previous study [15](Jung et al. 2017). The implant fixture platform was used as a reference point. The following measurements were made at the buccal side of implants, as depicted in Fig. 2a-b:

- P-B (mm) : Vertical distance between the implant platform and the first bone-to-implant contact on buccal sides
- P-C (mm): Vertical distance between the implant platform and the most coronal level of bone crest on buccal sides
- Horizontal thickness (HT) of the new bone at 0, 1, 2, 3 and 4 mm below the reference point

The Following parameters were measured within an area of interest (1 mm width x 3 mm height), from the implant platform (Fig. 2b):

- New bone (NB, mm^2): area of newly formed bone
- Residual bone material (RBM, mm^2): area of residual synthetic bone substitute materials
- Fibrovascular tissue (mm^2): area left after subtracting NB and RBM from the area of interest

The thickness of pseudo-periosteum was measured by the vertical direction of the long axis of the implants at 0, 1, 2, 3 and 4 mm below the reference point. The following measurements were made as depicted in Fig. 2c:

- Inner thickness of pseudo-periosteum: The thickness of dense connective tissue located underneath of TM
- Outer thickness of pseudo-periosteum: The thickness of dense connective tissue located on the outer surface of TM

The sum of the inner and outer pseudo-periosteum was considered total thickness of pseudo-periosteum.

8. Statistical analyses

All data was presented in mean and standard deviation values. The statistical analyses were performed using a computer software SPSS version 23 (IBM, Armonk, NY, USA). The test of normality of the data was confirmed Kolmogorov-Smirnov test ($p > 0.05$). Kruskal-Wallis test ($p < 0.05$) was used to determine the differences between each group, and Mann–Whitney test ($p < 0.005$) was performed to compare the differences between each parameter.

III. RESULTS

1. Clinical outcomes

During 16 weeks of healing, mucosal dehiscence with TM exposure occurred in one case in the TM group, in one case in the TM+CM group, and in two cases in the TM+BS+CM group. The first exposure was observed at the 3rd week of healing period at the TM+BS+CM group. In the 7th week of healing period, size of wound dehiscence was increased to the nearby test group (TM+CM group) without an inflammatory response. However, at the 8th week of healing period, an inflammatory reaction was observed in two dogs, including the early-exposed site. Each exposed site in the TM group, the TM+CM group, and TM+BS+CM group presented an inflammatory reaction thus at each of these sites the TM was removed and exchanged with a healing abutment (Fig. 3). Subsequently, none of the exposed sites revealed adverse symptoms. The other one of the TM+BS+CM groups exposed also at the 8th week of healing, however, had no symptoms of inflammation. At the time of sacrifice, the tissue of the exposed site was slightly reddish around the exposure, but no infection sign was seen.

In the final analysis, all specimens could be included in the analysis data without any missing groups. 3 out of 4 cases of early TM exposure cases occurred in the 2nd premolar area, where the ridge was narrower than the posterior region.

2. Radiographic findings

The 3D micro-CT images of all animals were presented in Fig. 4a. Images of removing TM using software was presented in Fig. 4b, through this, it was possible to observe the remaining mineralized tissue and scattered BS underneath the TM. The

dehiscence defects were partially covered by mineralized tissue. Although the titanium wing at the margin of TM was designed to enhance graft localization, the particles of BS were scattered and migrated to adjacent experimental groups. This phenomenon occurred in 2 out of 6 cases of the control group, 5 out of 6 cases of the TM group, and 3 out of 6 cases of the TM+CM group by the adjacent TM+BS or TM+BS+CM group.

3. Histologic findings

All of histologic images were presented in Fig. 5. Showing that all implants were successfully osseointegrated. However, in the control group, the dehiscence defects were still remained in the buccal side. TM was connected to implant and maintain the augmented space formed on the buccal side, and it was filled with newly formed bone, residual BS (in the TM+BS or TM+BS+CM group) and connective tissue. In the TM+CM and TM+BS+CM groups, the residual CM was detected above the TM in half of the specimens. The BS particles were present on the dehiscence defect, and in some specimens, it was connected to each other by new bone. Complete resolution of dehiscence defects through re-osseointegration was observed in one specimen in the TM group, two specimens in the TM+BS group, and one specimen in the TM+BS+CM group. The remaining specimens were partially recovered by newly formed bone in the test groups.

Dense connective tissue, called pseudo-periosteum was observed along the outer and inner surface of the TM in all test groups. These two layers were linked by end of TM, which could be inferred those two layers are the same structure. There was a difference in the direction of the collagen fiber according to the additional use of CM. In the TM and TM+BS groups, these connective tissue fibers, running in a direction parallel to the TM, were interposed to each other through pores, a plaid pattern can be seen due to the connective tissues that penetrate. However, in the TM+CM group and TM+BS+CM group, this grid pattern is hardly observed (Fig. 6). No inflammatory tissue was observed within the pseudo-periosteum, and there were few blood vessels in these layers, and no

mineralized structure was observed.

4. Histomorphometric analyses

The histomorphometric measurements are presented in Table 1. On average, TM group reached more vertical defect fill compared to the other groups (P-B; 52.2±46.6 [TM], 87.5±69.3 [TM+BS], 85.3±21.2 [TM+CM], 84.0±56.5 [TM+BS+CM], 105.6±10.1 [Control], P-C; 35.3±61.7 [TM], 81.6±78.7 [TM+BS], 80.3±20.0 [TM+CM], 76.7±55.7 [TM+BS+CM], 104±9.1 [Control]). However, there was no statistical difference in P-B and P-C among all groups.

The inclination of the horizontal bone thickness was presented in Fig. 7. All horizontal bone thickness, including the control group, increased as it went further apically. However, no significant difference was observed among all groups.

The new bone ratio within the region of interest in the test groups was greater than that of the control group (32.1 %, 17.0 %, 11.8 %, 15.5 % and 0.3 % for TM group, TM+BS group, TM+CM group, TM+BS+CM group and control group, respectively). The RBM ratio decreased in the TM+BS+CM group than the TM+BS group; however, no statistically significant difference was shown.

The pseudo-periosteum was histologically assessed at the inner and outer of TM. The control group which didn't use TM was excluded from this measurement. The mean thickness were thinner than 1mm both in inner and outer (Inner; 0.8±0.4 [TM], 0.7±0.4 [TM+BS], 0.8±0.4 [TM+CM], 0.5±0.4 [TM+BS+CM], Outer; 0.3±0.2 [TM], 0.2±0.2 [TM+BS], 0.1±0.1 [TM+CM], 0.2±0.1 [TM+BS+CM]) ($p > 0.05$). Except the TM+BS group, all the other group had 1 exposure site which lead to removal of TM. These 3 specimens were excluded from the inner/outer measurements but included to total thickness. The mean of total pseudo-periosteum thickness were generally approach 1mm except the TM+BS+CM group (Total; 1.0±0.6 [TM], 0.9±0.5 [TM+BS], 0.9±0.5 [TM+CM], 0.6±0.5

[TM+BS+CM]). However, there was no statistical differences in the thickness of pseudo-periosteum among the test groups ($p < 0.05$).

IV. DISCUSSION

The aim of the present study was to determine the synergetic effect of TM with an adjunctive use of BS and/or CM for lateral bone augmentation in chronic narrow ridge. Considering the difference in the orientation of pseudo-periosteum fibers between the groups with and those without CM, the additional use of CM seems to improve the cell occlusiveness of TM. However, this did not lead to enhancing the amount of bone ingrowth within the augmented region. The use of BS in addition to the TM did not promote the new bone formation. This finding might be explained through the frequently observed migration of bone substitute particles due to the uncontained bone defect morphology and the insufficient rigidity of TM.

These results are quite different from previous *in vivo* study which was designed similarly with the present study. It was reported that the combination of TM and CM showed significantly greater bone regeneration compared to TM or CM alone when used in peri-implant bone defects at 6 months [16](Li et al. 2018). This difference can be explained by the differences of experimental model. In this study, a box-type surgically made bone defect was used, which is thought to more favorable on the localization of bone substitutes. Likewise, Shin et al. (2013) reported enhanced bone regeneration according to the additional use of CM in a rabbit calvarial model, which also indicated that contained defects [17]. The chronic defect used in the present study is a model that reproduces the clinical situation after tooth extraction, and it is considered a more challenging for lateral bone augmentation because the graft material constantly slides toward the apical side. Thus, it would be explained that the difference in graft localization according to the defect types maximizes the effect of TM in GBR.

Previous preclinical studies with similar results to our studies have also been reported. Lim et al. (2015) evaluated the effect of overlaying TM with CM in immediate

implant placement and described no benefit for mucosal healing and buccal bone preservation with high exposure rate [13]. Another study demonstrated that combined use of CM in GBR with TM did not show improvement in new bone quality in rat femur model [18](Borges et al. 2020). Both studies have used non-cross-linked CM which was resorbed within 8 weeks after transplantation [19](Tal et al. 2008). Therefore, cross-linked CM that lasted longer with superior mechanical properties was used in the present study, under the assumption that rapid absorption of CM could reduce the synergetic effect with TM, however, the results were not different from previous studies.

The histologic presence of pseudo-periosteum in GBR has been reported in previous studies. Generally, this is dense connective tissue fibers with low cellularity, and it was reported that this could be formed in the area of non-resorbable membranes [20-22](Dahlin et al. 1998; Corinaldesi et al. 2007; Simion et al. 2007). In addition, Cha et al. (2017) described in a human autopsy case report that when GBR was performed in peri-implant dehiscence defect using resorbable CM, pseudo-periosteum-like tissue was found even after 5 years [23]. However, why it appears and the clinical role of this tissue still remains unclear. Previous studies referred to this phenomenon as fibrointegration due to the micromovement of the membrane, and thus demonstrated that the thicker the thickness of pseudo-periosteum, the worse new bone quality [24](Cucchi et al. 2019). In addition, another study reported that cell-occlusiveness and degradation rate of membrane might affect the amount of soft tissue underneath the membrane [9](Rakhmatia et al. 2013). In the present study, all TM used group showed pseudo-periosteum not only underneath but also outer surface of TM, and there was no statistical difference in thickness among groups. Although it was found that the direction of the collagen fibers interposed in the TM pores was different according to the additional use of CM, this did not lead to the difference in thickness of pseudo-periosteum and the amount of new bone. This result was different from previous studies, and further studies would be needed.

From the reconstructed 3D image in the radiographic analysis, the dislocation of

BS particles was frequently observed in all groups. As one of the reasons for this phenomenon would be high flexibility of TM used in this study. Although the mechanical property of TM are solid and rigid, it was fixed only on top of the implant with cover cap. Therefore, this phenomenon seems to have occurred as it was not enough to withstand the pressure from soft tissue and micromovement in the bucco-lingual direction occurs. Due to this phenomenon, there were few cases where BS particles were found even in groups that did not use BS, which could be a limitation of this study.

Nevertheless, the use of TM showed superior bone augmentation compared to the negative control group. When compared with the previous study of GBR using absorbable CM fixed with pins in the same experimental model, the TM group showed comparable results in terms of P-B and P-C and new bone thickness [15](Jung et al. 2017).

V. CONCLUSION

Within the limitation of this study, additional use of CM and BS combined with TM did not have an additional benefit in lateral bone augmentation. Further studies are needed to investigate the mechanism of GBR and the use of bone substitutes, membranes and meshes for the treatment of uncontained bone defects.

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TABLES

Table 1. Histometric measurements (mm; mean ± standard deviation).

(a)		Treatment modality				
Parameter	**Unit**	**1. Control** (n=6) Mean ± SD (Q1,M,Q3)	**2. TM** (n=6) Mean ± SD (Q1,M,Q3)	**3. TM+BS** (n=6) Mean ± SD (Q1,M,Q3)	**4.TM+CM** (n=6) Mean ± SD (Q1,M,Q3)	**5. TM+ BS+CM** (n=6) Mean ± SD (Q1, M, Q3)
P-B	**mm**	3.17 ± 0.30 (2.8, 3.2, 3.5)	1.57 ± 1.40 (0, 0.9, 3.3)	2.63 ± 2.08 (0, 3.2, 4.4)	2.56 ± 0.64 (1.3, 2.7, 3.1)	2.52 ± 1.70 (0.4, 2.1, 5.2)
P-B	**%**	105.61 ± 10.16 (92.3, 107.7, 116)	52.28 ± 46.63 (0, 30, 110)	87.56 ± 69.37 (0, 107, 146.6)	85.36 ± 21.26 (43, 91.3, 102.4)	84.06 ± 56.57 (14.6, 69.3, 173)
P-C	**mm**	3.12 ± 0.27 (2.8, 3.2, 3.5)	1.06 ± 1.85 (-2.2, 0.9, 3)	2.45 ± 2.36 (-0.9, 3.2, 4.4)	2.41 ± 0.60 (1.2, 2.4, 2.8)	2.30 ± 1.67 (0.4, 2.0, 5.2)
P-C	**%**	104 ± 9.01 (92.3, 105.7, 116)	35.33 ± 61.78 (-72.3,30,100)	81.67 ± 78.74 (-30.7, 107, 146.7)	80.39 ± 20.04 (41.3, 81.3, 94.3)	76.76 ± 55.72 (14.7, 68.3, 172.9)
HT0mm	**mm**	0 ± 0.00 (0, 0, 0)	0.20 ± 0.49 (0, 0, 1.2)	0.54 ± 0.99 (0, 0, 2.5)	0 ± 0.00 (0, 0, 0)	0 ± 0.00 (0, 0, 0)
HT1mm	**mm**	0 ± 0.00 (0, 0, 0)	0.38 ± 0.78 (0, 0, 1.9)	1.09 ± 1.27 (0, 0, 2.9)	0 ± 0.00 (0, 0, 0)	0.01 ± 0.03 (0, 0, 0.1)
HT2mm	**mm**	0 ± 0.00 (0, 0, 0)	0.72 ± 0.68 (0, 0.5, 1.7)	1.75 ± 1.28 (0, 1.4, 3.5)	0.24 ± 0.60 (0, 0, 1.5)	0.98 ± 1.12 (0, 0, 2.2)
HT3mm	**mm**	0.10 ± 0.16 (0, 0, 0.4)	1.07 ± 0.76 (0.4, 0.6, 2.1)	2.37 ± 1.37 (0, 2.5, 3.7)	0.96 ± 0.46 (0.4, 0.8, 1.5)	2.04 ± 1.29 (0.7, 1.2, 3.9)
HT4mm	**mm**	0.81 ± 0.28 (0.4, 0.8, 1.2)	2.18 ± 0.57 (1.3, 2.1, 2.9)	2.78 ± 1.11 (0.6, 3.0, 3.7)	1.93 ± 0.80 (1.0, 1.7, 2.8)	2.25 ± 1.55 (0.4, 1.7, 4.3)
NB	**%**	0.37 ± 0.65 (0, 0, 1.6)	32.13 ± 33.41 (0, 23.1, 89.4)	17.01 ± 25.42 (0, 1.3, 54.3)	11.84 ± 18.81 (0.3, 1.9, 49.4)	15.51 ± 17.21 (0, 6.6, 44.1)
RBM	**%**	0	0	6.92 ± 5.42 (0.3, 7.0, 16.2)	0	4.03 ± 4.58 (0, 1.6, 11.3)
FVT	**%**	95.90 ± 4.23 (87.8, 96.8, 99.0)	67.45 ± 33.53 (10.6, 71.0, 100)	71.15 ± 24.08 (42.8, 62.2, 94.3)	85.41 ± 20.54 (44.1, 90.6, 98.1)	70.60 ± 15.15 (55.9, 66.8, 98.1)

(b)

	Statistical analysis*									
parameter	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
P-B	0.132	0.818	0.026	0.394	0.485	0.485	0.485	0.394	0.937	1
P-B (%)	0.132	0.818	0.026	0.394	0.485	0.485	0.485	0.394	0.937	1
P-C	0.009	0.699	0.009	0.132	0.240	0.240	0.394	0.394	0.818	0.589
P-C (%)	0.009	0.699	0.009	0.132	0.240	0.240	0.394	0.394	0.818	0.589
HT0mm	0.699	0.180	1	1.000	0.394	0.699	0.699	0.180	0.180	1
HT1mm	0.180	0.180	1	0.699	0.589	0.180	0.310	0.180	0.240	0.699
HT2mm	0.065	0.015	0.699	0.180	0.180	0.240	0.818	0.041	0.394	0.310
HT3mm	0.002†	0.026	0.002†	0.002†	0.132	0.937	0.093	0.065	0.589	0.180
HT4mm	0.002†	0.026	0.026	0.093	0.093	0.589	0.937	0.093	0.589	0.818
NB (%)	0.026	0.180	0.015	0.132	0.394	0.394	0.394	0.589	1	1
RBM (%)	1	0.002†	1	0.065	0.002†	1	0.065	0.002†	0.394	0.065
FVT (%)	0.180	0.015	0.180	0.015	0.818	0.485	0.937	0.485	0.937	0.240

Vertical measurements of fBIC at buccal site. P-B = vertical distance between the fixture platform and the most bone-implant contact level; P-C = vertical distance between the fixture platform and the most coronal portion of bone crest; TM = titanium mesh, BS = bone substitute, CM = collagen membrane. HT = Horizontal thickness of new bone thickness at 0, 1, 2, 3 and 4 mm apical to the implant platform. Areal measurements of ROI (1 mm wide x 3 mm height) from the implant platform. NB = areas of newly formed bone; RBM = areas of residual bone materials; FVT = areas of fibrovascular tissue.

*Results of Mann-Whitney test.

†Statistically significant.

FIGURES

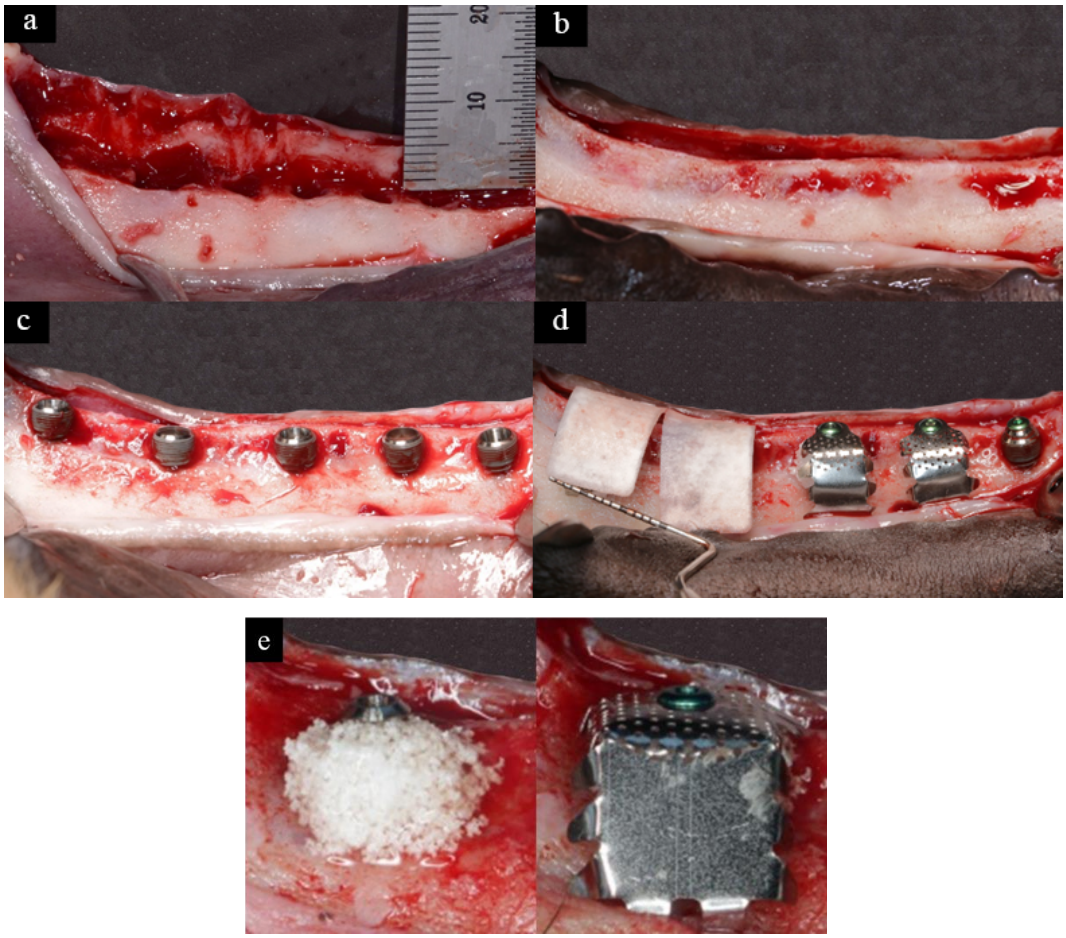

Figure 1. Clinical photographs of surgical intervention.

Clinical photographs of creation of experimental narrow ridge (a) and implant placement and GBR protocol (b – e) at Surgery 2.

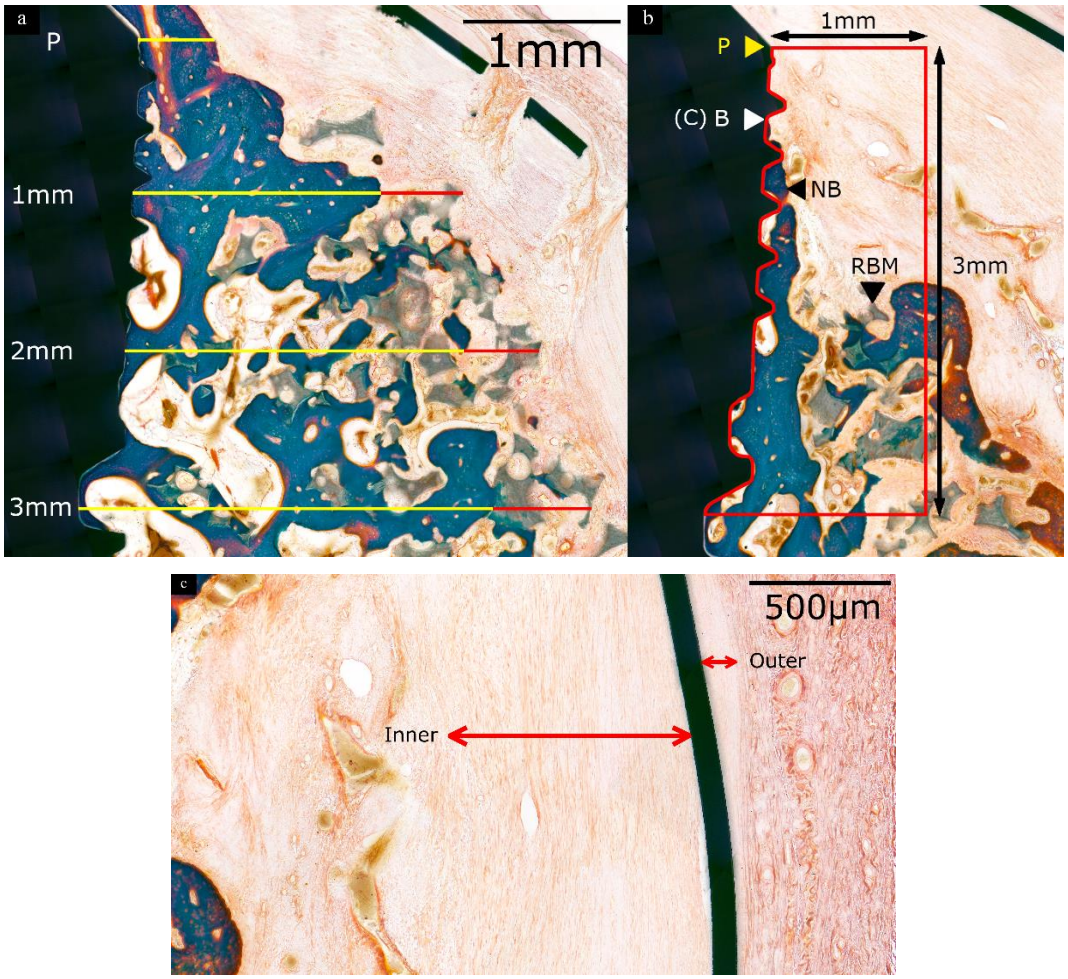

Figure 2. Histomorphometric measurements.

(a) Horizontal linear measurements. P, implant platform. (b) Areal measurements and Vertical linear measurements. Red box, region of interest (1 mm x 3 mm); NB, area of new bone; RBM, area of residual bone material; B, the most coronal level of bone-to-implant contact; C, the most coronal level of bone crestal. (c) Horizontal linear measurements. Inner; inner pseudo-periosteum, Outer; outer pseudo-periosteum.

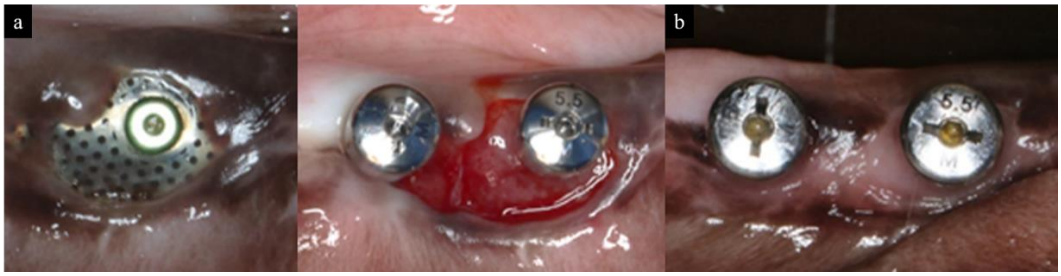

Figure 3. Clinical findings.

TM exposure was occurred at the 8th week of healing period (a). The exposed TM was removed and exchanged to a healing abutment (b).

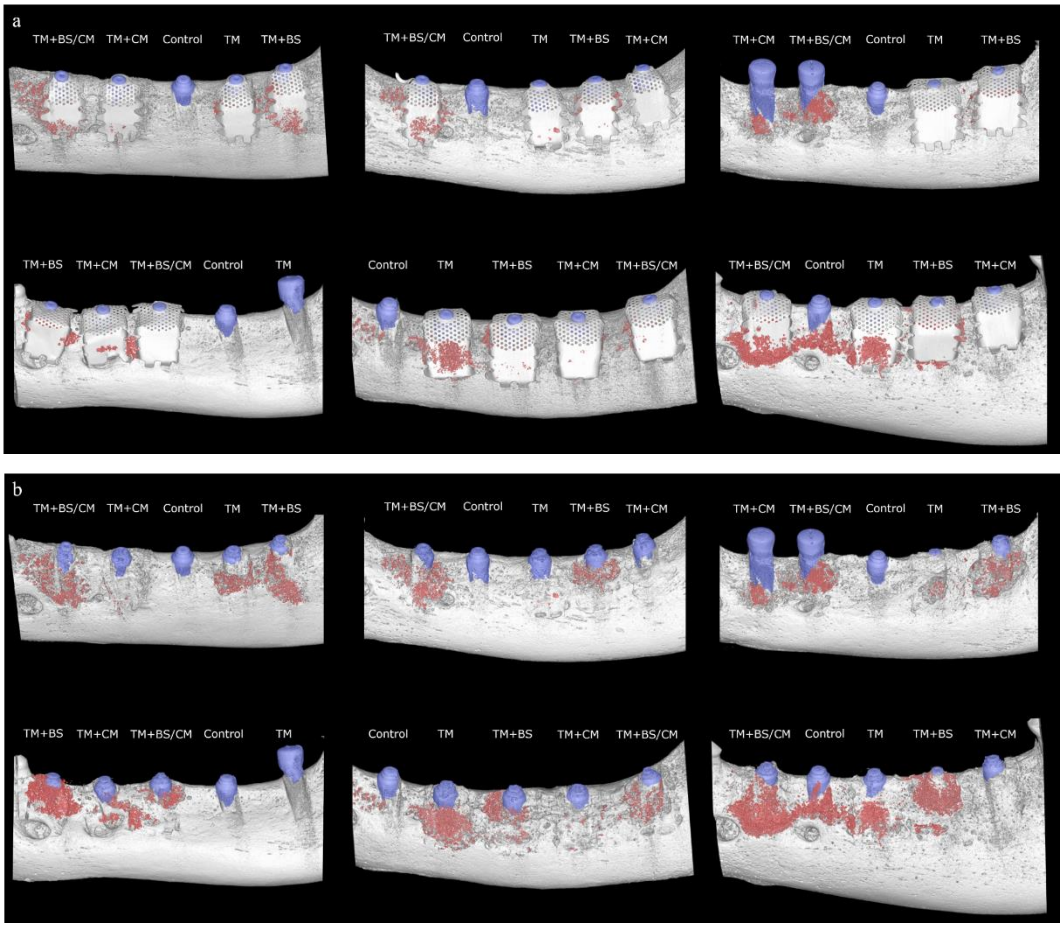

Figure 4. 3D micro-CT images.

(a) The hemi-mandible region of the experimental animal model was imaged using 3D micro-CT. Blue area, implant surface; Red area, residual bone materials. (b) 3D micro-CT image after removal of the TM using CtAn software.

Figure 5. Histological photomicrographs.

Yellow box, removed the titanium mesh and exchanged to a healing abutment since the inflammatory reaction occurred.

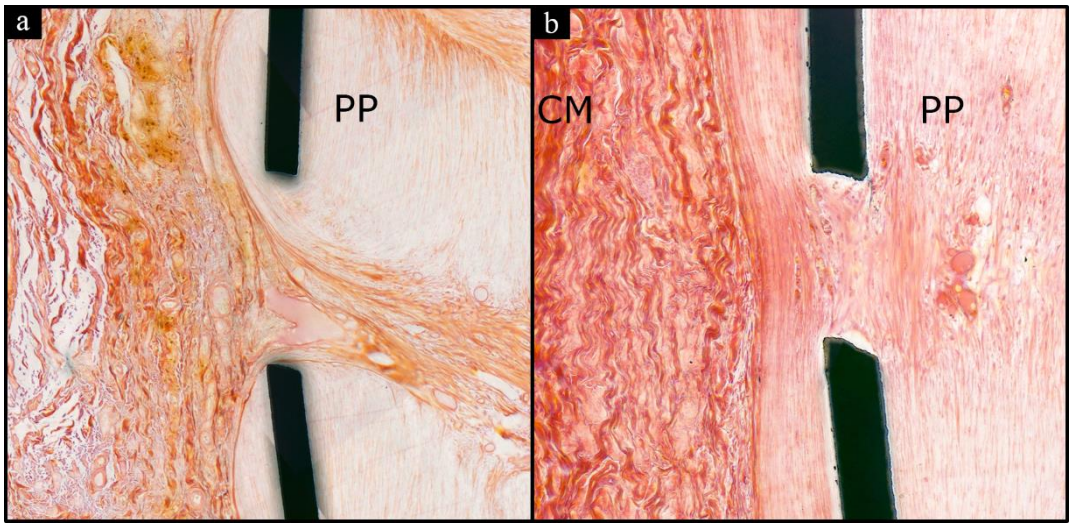

Figure 6. Histologic images of pseudo-periosteum.

Higher magnifications of the pseudo-periosteum. (a) TM group. PP, pseudo-periosteum. (b) TM+CM group. CM, residual of the CM.

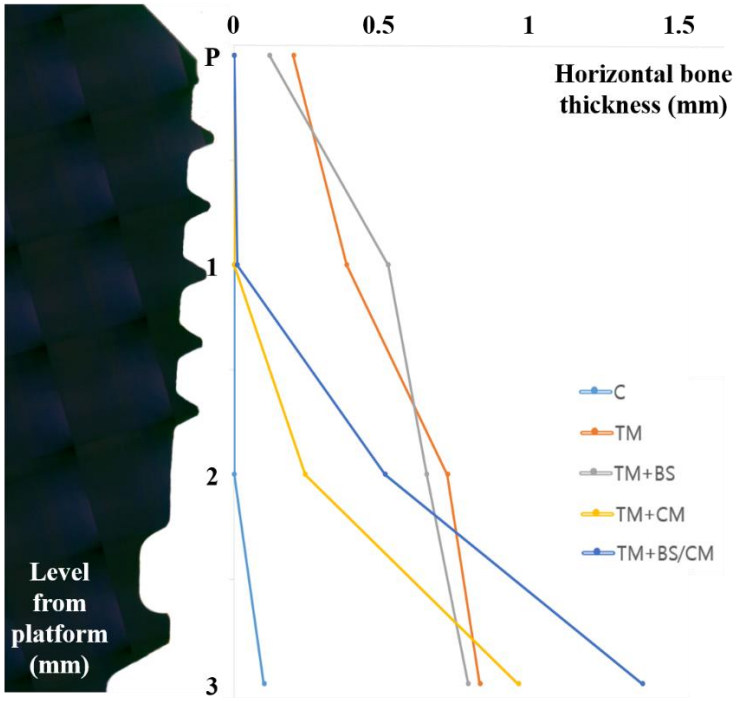

Figure 7. Horizontal bone thickness.

The horizontal bone thickness (x-axis) relative to the depth from the fixture platform (y-axis).

국문요약

임플란트 주위 열개형 골 결손 부위에 티타늄 막을 사용한 수평적 골 증강 술식 시 합성골 이식재 및 흡수성 차단막 추가 이식 여부에 따른 골재생 양상 평가

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치주 질환으로 인하여 치아를 발거한 후 별다른 처치 없이 오랜 기간이 지나게 되면 치조골이 위축되는 현상이 관찰된다. 이러한 무치악 상태의 환자에게 임플란트 식립을 위해 치조골을 재건하는 다양한 술식들이 있는데, 그 중 골유도재생술은 현재 임상에서 가장 널리 사용되고 있다. 해당 술식을 성공적으로 시행하기 위하여 필수적으로 요구되는 조건은 1차 수술 부위의 봉합 상태, 혈관신생, 공간 유지 및 수술 부위의 안정성이다.

흡수성 차단막은 제거를 위한 이차 수술이 필요 없으나 공간 유지 기능이 떨어져 술 후 예측이 어렵고, 티타늄 막은 기계적 물성이 우수하여 공간

유지 기능이 뛰어나지만, 다공성 구조로 인해 연조직의 개재를 효과적으로 막기 어렵다는 단점이 존재한다. 고로, 넓은 결함 부위에서 효과적으로 공간을 유지할 수 있는 티타늄 막과 합성골 이식재에 세포 차단성이 있는 흡수성 콜라겐 막을 함께 사용할 경우 신생골 형성이 더욱 증진 될 것이라는 가설을 수립하였다.

따라서, 본 연구는 실험적으로 유도된 무치악부에 티타늄 막을 사용하여 수평 골 증대술을 시행할 때 흡수성 콜라겐 막 및 합성골 이식재를 병행하여 사용할 경우의 효능을 임상적, 그리고 조직학적으로 분석하고자 한다.

총 6 마리의 성견을 대상으로 하악 편 측을 무작위로 배정하여 제 2, 3, 4번 소구치와 제 1 대구치를 발거하여 실험적으로 무치악 상태를 유도하였다. 이후 선정된 하악 편 측에 5 개의 임플란트를 식립 하였다. 각 임플란트는 티타늄 막, 흡수성 콜라겐 막, 합성골 이식재를 교차적으로 사용하는 다음의 군에 맞게 이식술을 시행하였다: 1) 대조군(아무 처치를 하지 않는 군), 2) TM군(티타늄 막으로 공간 유지하는 군), 3) TM+ BS군(합성골 이식재를 이식한 뒤 티타늄 막을 이용한 군), 4) TM+ CM군(티타늄 막으로 공간을 유지하고 위에 콜라겐 막을 덮은 군), 5) TM+ BS+ CM군(합성골 이식재를 이식한 뒤 티타늄 막과 콜라겐 막을 함께 사용한 군). 이식술로부터 16 주간의 치유기간을 거친 뒤 실험 동물을 희생하였고, 임상적 분석과 조직학적

분석을 통해 각 실험군 간의 결과를 비교하였다.

연구 결과 아무 처치도 하지 않은 대조군에 비해 실험군 모두 수직적 및 수평적 골 증강은 관찰되었지만, 통계적으로 유의한 차이를 보이진 않았다. 실험적으로 유도된 임플란트 주변 결함 영역(임플란트 상방 플랫폼으로부터 너비 1 mm x 깊이 3 mm)에서 신생골이 자라난 백분율 수치를 보았을 때에도 각 군별 유의한 차이는 없었다(0.37 %[대조군]; 31.78 %[TM군]; 17.09 %[TM+ BS군]; 12.03 %[TM+ CM군]; 15.89 %[TM+ BS+ CM군]). 대조군을 제외한 티타늄 막을 사용한 모든 실험군에서 가성 골막이 티타늄 막을 얇게 감싸는 형상으로 관찰되었다.

결론적으로, 본 연구에서 골 증강을 위한 수평 골 재건 술식 시 티타늄 막에 흡수성 콜라겐 차단막 및 합성골 이식재를 병행하여 사용하여도 티타늄 막만 사용한 부위에 비해 더 많은 신생골이 형성되는 결과로 이어지진 않았다.

핵심되는 말: 수평골재건술; 티타늄 막; 흡수성 콜라겐 차단막; 합성골 이식재; 동물 실험; 가성 골막