Effect of different hydroxyapatite: β-tricalcium phosphate ratios on the osteoconductivity and dimensional stability of biphasic calcium phosphate in the rabbit sinus model

Hyun-Chang Lim

Department of Dentistry

The Graduate School, Yonsei University

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Directed by Professor Seong-Ho Choi

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of Hyun-Chang Lim is approved.

Thesis Supervisor : Seong-Ho Choi

Jung-Kiu Chai

Kyoo-Sung Cho

Yeek Herr

Jong-Hyuk Chung

The Graduate School

Yonsei University

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Abstract

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Hyun-Chang Lim, D.D.S., M.S.D.

Department of Dentistry

The Graduate School, Yonsei University

(Directed by Professor Seong-Ho Choi, D.D.S., M.S.D., PhD.)

Objective: The present study compared the osteoconductivity and the volume stability of biphasic calcium phosphate (BCP) with a high versus a low ratio of beta tricalcium phosphate (β-TCP) relative to hydroxyapatite (HA; i.e., 70:30 vs. 30:70) in the rabbit sinus model.

Material and Methods: ***Experiement1-short and middle term evaluation;*** Bilateral sinus windows were created in eight adult New Zealand white rabbits (2.5–3.5 kg); each sinus in each rabbit was assigned to one of two experimental BCP groups according to the HA:β-TCP ratio. One sinus was grafted with BCP with a high ratio

of β-TCP (i.e., 70:30; TCP70) and the contralateral sinus was grafted with BCP with a low ratio of β-TCP (i.e., 30:70; TCP30). The animals were sacrificed after 2 (n=4) or 8 weeks (n=4) of healing. Biopsy specimens were harvested and evaluated histologically, histomorphometrically, and with microcomputed tomography (micro-CT). ***Experiement2- late term evaluation;*** Experimental protocol was followed expertment1. In five rabbits, sinus augmentation was performed. Calcein green was injected five days before euthanizing. The animals were euthanized 16 weeks postsurgery.

Results: ***Experiement1;*** The total augmented volume (TV) in micro-CT, and the area of new bone (NA) and bone-to-material contact (%NPC) in histologic section did not differ significantly between the two groups at 2 and 8 weeks of healing. The augmented volume did not show significant decrease between 2 and 8 weeks. The residual material was significantly more resorbed in the TCP70 group than in the TCP30 group at both 2 and 8 weeks. Larger amount of multinucleated giant cell was observed in the TCP70 group at both weeks. ***Experiement2;*** Micro-CT analysis revealed that TV and new bone volume (NV) did not show statistical difference between both groups, but the resorption of materials was statistically higher in the TCP70 group than the TCP30 group. Histomorphometrically, TA, NA and the area of residual material (RA), and %NPC did not differ significantly. Trabecular thickness, number and separation were not statistically significant between both groups. Fluorescence with calcein green showed no notable difference between both groups.

Conclusion: Within limitation of this study, the BCPs with HA: β-TCP = 3:7 and 7:3

were demonstrated to be comparably effective in maintaining volume stability and bone formation.

KEYWORDS: bone substitutes, bone tissue engineering, bone regeneration, sinus augmentation

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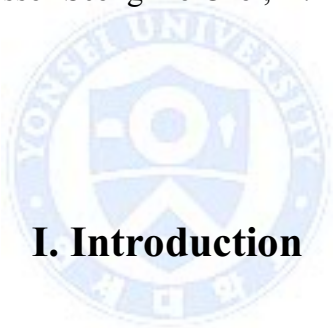

I. Introduction

Autogenous bone has long been considered an ideal material for bone regeneration because of its osteogenic potential, but the amount of tissue that can be harvested from intraoral donor sites is limited and the procedure is associated with problematic morbidity. In a review on sinus augmentation, Pjetursson et al (2008) noted that there was a little difference in survival rate between dental implants placed in sinuses augmented with autogenous bone and those augmented with bone substitute (0.1% vs 1.1%)[1]. Moreover, since there is no clear evidence that autogenous bone should be

chosen over a bone substitute for sinus augmentation[2], various bone substitutes have been used for that procedure.

One of the bone substitutes, biphasic calcium phosphate (BCP), was demonstrated to be effective in sinus augmentation. BCP is composed of slowly-resorbing hydroxyapatite (HA) and fast-resorbing beta tricalcium phosphate (β-TCP). HA stabilizes the augmented space and β-TCP provides the space for de novo bone formation, and so the HA:β-TCP ratio is an important factor determining its reactivity[3,4]. However, the optimum HA:β-TCP ratio has yet to be determined; moreover, it is possible that the optimum HA:β-TCP ratio is site-specific according to the defect type. The most commonly documented HA:β-TCP ratio in BCP for sinus augmentation is 60:40, the resulting outcomes for which are reportedly highly predictable[5-7]; however, this in itself is not strong evidence that this is the optimum ratio. Some studies have also demonstrated that other ratios, such as 70:30 and 30:70, can be used successfully for sinus augmentation[8-11].

Space maintenance is essential for bone regeneration. A higher HA ratio can be advantageous for volume stability, given that the maxillary sinus is constantly exposed to air pressure and that repneumatization has been reported[12,13]. In some cases, repenumatization resulted in protruding implant apex into sinus cavity[12,14]. Conversely, a higher HA ratio may result in a reduction in the space available for de novo bone formation, since HA is highly stable, and either remains unresorbed or is slowly resorbed over a long time[13].

The ideal outcome of regeneration may connote high proportion of newly

regenerated target tissue and low proportion of scaffold. In sinus augmentation, the increased amount of vital new bone may strengthen the long-term predictability in terms of a higher bone-to-implant contact and better load distribution. It was demonstrated that β-TCP has similar characteristics to autogenous bone[15]; accordingly, a higher β-TCP ratio may lead to the production of a greater amount of vital bone.

Some reported unpredictable characteristics of β-TCP. Although β-TCP showed similar behavior to autogenous bone as above mentioned, it was sometimes resorbed rapidly and new bone formation did not keep up with its resorption[15]. Therefore, higher β-TCP ratio may result in undesirable tissue composition in the augmented sinus and, subsequently, may compromise long-term stability.

The above-described situation prompted the present study to compare the volume stability and bone-forming capacity between BCP with a higher ratio of β-TCP (HA:β-TCP ratio of 30:70) and a lower ratio of β-TCP (HA:β-TCP ratio of 70:30) in the rabbit sinus model.

II. Materials and Methods

This dissertation includes separately-conducted two experiments; one was conducted for short- and middle-term evaluation, and the other was for late-term evaluation.

Experiment 1 (Short & middle-term evaluation)

1. Experimental Animals

Eight adult New Zealand white rabbits (2.5–3.5 kg) were used. The selection and management of experimental animals and surgical procedures followed a protocol approved for this study by the Institutional Animal Care and Use Committee, College of Medicine, Yonsei University.

2. Experimental Design

In each rabbit, each of the bilateral sinus windows was assigned to one of two experimental groups according to the composition of the grafted BCP (i.e., the HA:β-TCP ratio): (1) TCP30 (Osteon I, Genoss, Suwon, Korea; HA:β-TCP=70:30, porosity=77%, pore size=300~500 μm) or (2) TCP70 (the contralateral sinus was grafted with Osteon II, Genoss, Suwon, Korea; HA:β-TCP=30:70, porosity=70%, pore size=250 μm).

3. Surgical Procedure

The animals were anesthetized using an intramuscular injection of ketamine hydrochloride (Ketalar, Yuhan, Seoul, Korea) and xylazine (Rompun, Bayer Korea, Seoul, Korea). The surgical area was shaved, disinfected, and then anesthetized locally with 2% lidocaine (lidocaine HCl, Huons, Seoul, Korea). A skin incision was made along the midsagittal line on the nasal bone, and a full-thickness flap was reflected sufficiently to reveal the lateral antral wall. A trephine bur (outer diameter, 6 mm) was carefully used to create windows under saline irrigation. The bony window was detached and the sinus membrane was gently elevated. Each sinus was grafted with the 0.15g of one or other of the BCPs (i.e., Osteon I into one sinus and Osteon II into the contralateral sinus; Fig. 1). After grafting, the flaps were sutured with 4-0 monosyn (B-Braun, Aesculap, Center Valley, PA, USA). The animals were sacrificed after either 2 weeks (n=4) or 8 weeks (n=4) of healing.

4. Microcomputed tomography analysis

Block sections including the grafted site and the surrounding tissues were harvested and fixed in 10% buffered formalin solution for 10 days. Micro-computed tomography (micro-CT; SkyScan 1072, SkyScan, Aartselaar, Belgium) images of these sections were obtained at a resolution of 35 mm (achieved using 100 kV and 100 mA). The images were stored in DICOM format and reconstructed in three dimensions using commercially available software (On-Demand3D, CyberMed,

Seoul, Korea). The augmented space was color-coded and the total volume of augmented space was calculated.

5. Histologic and histomorphometric Analysis

After obtaining the micro-CT images, the blocks were decalcified in 5% formic acid for 14 days and then embedded in paraffin. The sections were serially sliced at a thickness of 5 μm through the middle of the windows in the antral area, and the two central sections were chosen for histologic analysis. The selected sections were stained with hematoxylin-eosin and Masson's trichrome. Histologic observation and histomorphometric analysis were performed with the aid of a stereomicroscope (MZFLIII, Leica, Wetzlar, Germany), a light microscope (DM-LB, Leica), and an automated image-analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). The following parameters were measured for histomorphometric analysis: the total augmented area (TA), the area of newly formed bone (NA), the area of residual material (RA), the area of fibrovascular tissue (FA), and the new bone : residual particle contact ratio (%NPC). The new bone to residual particle contact ratio was calculated after linear measurement of the perimeter of each residual particle and the contact length between the bone and the particles.

6. Statistics

Commercially available statistical software (SPSS 15.0, SPSS, Chicago, IL, USA)

was used for the statistical analysis. The histomorphometric and micro-CT data are presented as mean±SD values. Paired t-tests were used to evaluate the differences between the TCP30 and TCP70 groups at each healing time point. Student's t-test was used to compare the parameters at 2 and 8 weeks in each group. The level of statistical significance was set at $p<0.05$. In one animal, the sinus assigned to the TCP70 8-week group was excluded from paired t-test analysis because one of its sinuses was severely inflamed, possibly as a result of membrane perforation.

Experiment 2 (Late-term evaluation)

1. Experimental Animals

This study was conducted with approval of the Institutional Animal Care and Use Committee, Yonsei Medical Center. Five adult New Zealand white rabbits (2.5–3.5 kg) were used.

2. Experimental Design

The experimental design was followed Experiment 1.

3. Surgical Procedure

The surgical procedure was followed Experiment 1. In brief, the animals were anesthetized by administrating ketamine hydrochloride (Ketalar, Yuhan, Seoul, Korea) and xylazine (Rompun, Bayer Korea, Seoul, Korea). Shaving, disinfection and local anesthesia (lidocaine HCl, Huons, Seoul, Korea) to surgical area were performed. A mid-sagittal skin incision was made on the nasal bone and lateral antral bone was exposed. Bony windows were made using a trephine bur and the sinus membrane was carefully elevated without tearing. Each sinus was grafted with the 0.2cc of each BCP (Fig. 1). No membrane coverage was performed and the flaps were sutured with 4-0 monosyn (B-Braun, Aesculap, Center Valley, PA, USA).

4. Fluorochrome labeling

The bone-forming activity was evaluated by injecting the fluorochrome calcein green (10 mg/kg, Sigma-Aldrich, St.Louis, US) 5 days before euthanizing the animals at 4 months postsurgery.

5. Microcomputed Tomography Analysis

Block sections of the grafted sites were harvested and fixed in 10% buffered formalin solution. Before histologic processing, micro-computed tomography (micro-CT; SkyScan 1173, SkyScan, Kartuizersweg 3B 2550 Kontich, Belgium) was taken at a resolution of 8.88 μm (achieved using 130 kV and 60 μA). An aluminum filter of 1.0mm in thickness was used. Total exposure time was 500ms and frame averaging was five at each projection. The acquired data was reconstructed using NRecon Software (Ver. 1.6.8.0, Brunker microCT). The region of interest (2240 x 2240 pixel) was established and analyzed. The grey values of threshold for graft particles and newly formed bone was standardized. The range of the values for graft particles and newly formed bone were 90-225 and 55-90, respectively (Fig. 2). The following parameters are measured; total volume of augmentation (TV), the volume of new bone (NV), the volume of residual bone material (RV), the volume of fibrovascular tissue (FV), trabecular thickness (t.Th), trabecular number (n.Th), and trabecular separation (s. Th). The density of NV, RV, and FV were also calculated. Each image

section stored in DICOM format was processed to three-dimensional image using commercially available software (On-Demand3D, CyberMed, Seoul, Korea) and the augmented space was color-coded for visualization.

6. Histologic and histomorphometric analysis

After taking micro-CT, the blocks were embedded in methylmethacrylate resin and polymerized (Exact System, Exact). The resin block was cut at the center of the sinus in an anterior-posterior direction and the final section was 15 □m in thickness. All sections were firstly examined by immunofluorescence microscopy (Leica DM LB, Leica Microsystems, Wetzlar, Germany) and histologic images were captured and saved (cellSens Standard 1.11, Olympus Corporation, Center Valley, PA, USA). Then, the sections were stained with hematoxyln-eosin and the images of stained sections were also saved. Histomorphometric analysis was performed with an automated image-processing system (Photoshop CS5, Adobe systems, CA, USA). The following parameters were measured for histomorphometric analysis: the total augmented area (TA), the area of new bone (NA), the area of residual material (RA), the area of fibrovascular tissue (FA) and the percentage of new bone to graft particle contact (%NPC). Five areas of interest (AOIs; 1.5x1.5mm) were established for regional analysis; the anterior area of the sinus, the posterior area of the sinus, the area adjacent to bony window, the central area, and the area adjacent to Schneiderian membrane. In five AOIs, NA, RA, and FA were also measured.

7. Statistics

All data are presented as mean with standard deviation. Non-parametric Wilcoxon signed rank test was used to evaluate the volumetric, microstructural and histologic differences between the TCP30 and TCP70 groups. The Friedman test with post-hoc Dunn`s correction was used to evaluate histologic difference among AOIs within each sinus. The null hypothesis was rejected at the level of $P < 0.05$ (SPSS 20.0, IBM Corporation, Armonk, NY, USA).

III. RESULTS

Experiment 1 (Short & middle-term evaluation)

1. Clinical Findings

Minor membrane perforation occurred in one sinus in the TCP70 8-week group, as described above. The membrane elevation was carefully extended so as to protect the perforated area, and grafting was performed. No special treatment was conducted to repair the perforation. None of the other experimental animals suffered any adverse event.

2. Micro-CT finding

The augmented sinus had an inverse dome-like shape that was elongated in the sagittal direction. The gross morphology of the augmented sinuses was similar in all animals irrespective of the group or healing time (Fig. 2). A well-defined osteotomy was observed at 2 weeks, the appearance of which was maintained at 8 weeks. The BCP particles were uniformly distributed within the augmented space.

The volume of total augmentation was calculated from micro-CT images. The total augmented volumes in the TCP30 group were 115.83±11.99 and 94.35±20.74 mm3 at 2 and 8 weeks, respectively; the corresponding values in the TCP70 group were 114.35±4.08 and 92.03±36.12 mm3. There was no statistical significance between the groups at each healing time point. There appeared to be a reduction in

volume over time, but the difference was not statistically significant. A relatively high standard deviation was noted in both groups at 8 weeks (Table 1).

3. Histologic Findings

At 2 weeks

The augmented area was convex in cross-section at 2 weeks in both the TCP30 and TCP70 groups. The bone material was well maintained under the elevated sinus membrane in all cases. The window areas were still not healed with bone at this time point; instead, there was a fibrous tissue bridge between opposing window edges. Ingrowth of new bone was observed along the surface of the sinus wall near the windows. Very little bone-like tissue was observed in the central area in both groups. New bone had formed adjacent to the sinus membrane and in contact with the graft particles in both groups. The border between the particles and the new bone was rougher in the TCP70 group than in the TCP30 group, and a larger number of multinucleated giant cells was observed near the Schneiderian membrane and the center of the augmented area in the TCP70 group (Fig. 3, 4).

At 8 weeks

The augmented areas in all groups were well maintained at 8 weeks, and the quantity of new bone in the augmented areas was increased compared to that at 2 weeks. Although the windows were not still bridged with new bone, a substantial amount of new bone had formed between the edges of the window in both the TCP30

and TCP70 groups. One sample in the TCP70 group presented with loss of sinus membrane integrity due to unhealed membrane perforation and significant inflammation throughout almost the entire cavity. The two groups differed in terms of residual material. While in the TCP30 group the residual particles maintained its structure well, with slowly resorbing characteristics at its surface, those in the TCP70 group was relatively reduced and partitioned. In both groups, substantial new bone formation was observed in both the central and Schneiderian membrane areas. The new bone was in close contact with BCP particles in both groups, and reversal lines were observed. Numerous osteocytes were observed in the newly formed bone tissue. The new bone in the TCP30 group was bridged along the surface of the BCP particles, forming a linear bony outline partially surrounding the particles. The new bone in the TCP70 group exhibited not only a linear pattern but also a sprouting pattern, producing small islands, and tended to be embedded in soft tissue more than in the TCP30 group. The borders of the new bone against particles were rougher in TCP70 than in TCP30. In the TCP70 group, the number of multinucleated giant cells in the center and near the Schneiderian membrane was reduced, but still higher compared to the TCP30 group (Fig. 5, 6).

4. Histomorphometric Analysis

The histometric findings are summarized in Table 2 and 3. Although both the TA and RA appeared to have decreased over the observation period in both groups, the

differences were not statistically insignificant. There was a statistically significant increase in the NA in both groups over the observation period.

Neither TA nor NA differed significantly between the TCP30 and TCP70 groups at both 2 and 8 weeks. The RA was significantly smaller in the TCP70 group than in the TCP30 group at both healing time points.

The %NPC was found to have increased significantly in both groups over time. There was no statistically significant difference in this ratio between the TCP30 and TCP70 groups at either 2 or 8 weeks.

Table 1. Total augmented volume at 2 and 8 weeks. The data are mean±SD values in mm^3.

	TCP30	TCP70
2 weeks	115.83±11.99	114.35±4.08
8 weeks	94.35±20.74	92.03±36.12

There was no statistically significant difference between the groups at each time point, or between the time points within each group.

TCP30, biphasic calcium phosphate (BCP) with a hydroxyapatite (HA):beta tricalcium phosphate (β-TCP) ratio of 70:30; TCP70, BCP with an HA:β-TCP ratio of 30:70.

Table 2. Total augmented area (TA), area of newly formed bone (NA), and area of residual material (RA) at 2 and 8 weeks.

	TA (mm^2)		NA ($mm^{2)}$		RA (mm^2)	
	TCP30	TCP70	TCP30	TCP70	TCP30	TCP70
2 weeks	16.32±4.03	14.46±1.55	0.91±0.26	0.89±0.28	6.46±0.72	4.40±0.32§
8 weeks	14.43±2.81	13.06±3.71	3.11±1.28¶	3.06±0.89¶	5.67±0.85	2.66±0.58§

§Statistically significant difference compared to corresponding TCP30 group

¶Statistically significant difference compared to corresponding 2-week group

Table 3. The percentage of new bone to graft particle contact ratio (%NPC) at 2 and 8 weeks.

	TCP30	TCP70
2 weeks	9.63±3.23	4.44±2.78
8 weeks	39.33±9.43	39.49±6.12

There was no statistically significant difference between the groups at each time point, or between the time points within each group.

Experiment 2 (Late-term evaluation)

1. Clinical Findings

During surgery, Schneiderian membrane perforation was not observed. After 16-week healing, no animal showed any inflammatory sign.

2. Micro-CT finding

In the sinus cavity, dome shaped augmentation consisting of newly formed bone and residual particles were observed. Bony window was almost healed by new bone and corticalization was evident with some depression. New bone and residual particles looked well-blended regardless of location in the augmented sinus (Fig. 7).

Volumetric and microstructural analyses were presented in Table 4 and 5. There was no statistical difference in TV and NV between both groups. Compared to the TCP30 group, RV was significantly lower and FV was greater in the TCP70 group ($P<0.05$). The t.Th, n.Th and s.Th were not statistically different between both groups.

3. Histologic Findings

In both groups, Schneiderian membrane was elevated in a dome shape. Bony window was mostly healed with new bone. New bone formation in the augmented sinus was almost even aside from the locations of the augmented sinus. Compared to the TCP70 group, new bone fragment in the TCP30 group was larger. Almost all

particles were closely in contact with new bone. Some particles adjacent to Schneiderian membrane were not still incorporated with new bone and just contacted with the membrane. The resorption of graft particles showed distinct difference. Like the appearance of new bone, graft particles in the TCP30 were by far larger and bulkier than those in the TCP70 group. The particles in the TCP70 group looked more scattered (Fig. 8, 9).

4. Histomorphometric Analysis

Histomorphometric results in the whole augmented sinus were presented in Table 6. There were no statistical differences in TA, NA, RA, FA, and %NPC between both groups.

The histomorphometric results of AOIs were shown in Figure 10, 11 and 12. Within each group, only FA in the TCP70 group between anterior and membrane area showed statistical difference. NA in the anterior, center, window and membrane areas did not show statistical difference between groups, but posterior area of TCP70 was significantly greater than that of TCP30. RA in the all AOIs of the TCP30 group was greater than that of the TCP70 group, and statistical difference was found in the posterior, center and membrane areas. FA did not show statistical difference in all AOIs even though FA was greater in all AOIs of the TCP70 groups than in the those of the TCP30 group.

5. Fluorochrome labeling

The fluorescence of NB tissue and graft particles was varied in both groups, and no clear difference was not detected. A few luminous streaks were observed near the particles in both groups, and the brighter fluorescence was frequently found in the Harvesian canal. (Fig. 13).

Table 4. TV, the volume of new bone (NV), the volume of residual material (RV), the volume of fibrovascular tissue (FV) at 16 weeks. The data are mean±SD values in mm^3.

	TV (mm^3)	NV (mm^3)	RV (mm^3)	FV (mm^3)
TCP30	219.94±18.47	56.97±9.92	70.88±7.72	92.09±19.51
TCP70	210.35±16.85	65.93±13.01	33.39±7.13*	111.13±18.98*

*Statistically significant compared to the TCP30 group

TV, total volume of augmentation; NV, the volume of new bone; RV, the volume of residual bone material; FV, the volume of fibrovascular tissue.

Table 5. Trabecular thickness (t.Th), trabecular number (n.Th) and trabecular separation (s. Th) at 16 weeks. The data are mean±SD values in mm.

	t.Th (mm)	n.Th (1/mm)	s.Th (mm)
TCP30	0.06±0.01	4.15±0.34	0.24±0.02
TCP70	0.06±0	5.11±0.95	0.24±0.04

There was no statistically significant difference between the groups.

t.Th; trabecular thickness, n.Th; trabecular number, s. Th; trabecular separation.

Table 6. TA, NA, RA, the area of fibrovascular tissue (FA) and %NPC at 16 weeks. The data are mean±SD values in mm^2.

	TA (mm^2)	NA (mm^2)	RA (mm^2)	FA (mm^2)	NPC (%)
TCP30	60.81±12.19	13.7±6.23	28.56±5.75	18.5±4.07	82.63±16.23
TCP70	62.84±13.74	14.03±5.53	24.02±4.67	24.79±7.53	77.72±18.03

There was no statistically significant difference between the groups.

TA; the total augmented area, NA; the area of new bone, RA; the area of residual material, FA; the area of fibrovascular tissue, %NPC; the percentage of new bone to graft particle contact.

IV. DISCUSSION

The balance between the slowly resorbing HA and fast-resorbing β-TCP in BCP is thought to determine the amount of new bone formation at graft sites. The stability of HA acts to maintain the augmented space, while β-TCP promotes bone formation within that space. Some authors have labeled β-TCP a temporary bone replacement[16]. The maxillary sinus has strong potential for bone regeneration, and this potential can be maximized with the use of active biomaterials. It is plausible that increasing the β-TCP ratio would increase the amount of vital bone by which stabilizes the augmented volume. On the contrary, an increasing ratio of β-TCP may compromise the volume stability due to unpredictable resorption rate; sometimes too rapid to keep up with new bone formation. Such should be elucidated in a long term for vouching the longevity of sinus augmentation. Therefore, in the present experiments, a higher ratio of β-TCP (HA:β-TCP ratio of 30:70) and a lower ratio of β-TCP (HA:β-TCP ratio of 70:30) were grafted in the rabbit sinuses and evaluated at 2 (short-term), 8 (middle-term) and 16 weeks (late-term).

It has been reported that β-TCP has similar characteristics to autogenous bone in terms of its new bone formation and resorption properties[15]. Jensen et al (2006) demonstrated that β-TCP grafting with an expanded polytetrafluoroethylene membrane results in substantial new bone formation at 8 weeks in the mandibles of minipigs, which is not statistically different to that achieved with autogenous bone

grafts (57% vs 55%), and that the β-TCP and autogenous bone particles had almost completely resorbed by 4 weeks. In sinus augmentation, β-TCP also yielded comparable bone formation to autogenous bone. Szabo et al (2005) demonstrated that the percentage of new bone in sinuses grafted with β-TCP was comparable to that in sinuses grafted with autogenous bone (36% vs 38%)[17].

However, the reported performance of β-TCP appears to be somewhat contradictory. Von Arx et al (2001) reported that the amount of new bone obtained with pure phased TCP and barrier membrane was inconsistent in a lateral-ridge augmentation model[18], which may indicate that the degradation of TCP may be too fast to support bone formation. Similar inconsistencies were also reported in sinus grafting. Zijderveld et al (2005) reported a low percentage of new bone in lateral core biopsy samples in β-TCP-grafted sinuses (17±5%) compared to autogenous-bone-grafted sinuses (41±10%)[16]. In another study, Zijderveld et al (2009) demonstrated a significant resorption of β-TCP 5 years after sinus augmentation[19]. The graft height reduced 57.8%~ 62.2% in comparison to the originally augmented amount. Rapid replacement or resorption of biomaterials may render them unsuitable for sinus grafting or where long-term scaffolding is required in the graft.

The findings for HA in BCP reciprocate the inconsistent outcomes of β-TCP, suggesting that BCP with a low HA ratio may provide both volume stability and sufficient bone formation. In a long-term study on costal defects in sheep, the resorption and bone formation achieved with an HA:β-TCP ratio of 30:70 did not differ significantly from that obtained with a ratio of 50:50[20]; however, the 30:70 ratio

resulted in better integration in the bone-remodeling process, implying that a higher β-TCP ratio in BCP may accelerate bone formation. In a study evaluating the efficacy of an HA:β-TCP ratio of 30:70 in the human maxillary sinus, the new bone, residual material, and marrow spaces were found to be comparable to those of a study using a ratio of 60:40[11,21]. Indeed, the 30:70 ratio was found to perform well even in lateral ridge augmentation[22,23]; the BCP particles were well integrated with the NB and the dental implants exhibited a high bone-to-implant contact ratio with the regenerated bone.

However, the higher ratio of β-TCP appeared not to result in greater amount of new bone[24,25], which is contradictory to the theoretical concept of BCP. In the present experiments, the amount of new bone did not differ significantly between the TCP70 and TCP30 groups at all healing time intervals. Yang et al. (2014) also observed that there was no statistically significant difference in augmented area between BCP with 60% HA and BCP with 20% HA in rabbit calvarial defects[25].

This finding may be explained by the osteoconductive mechanism of BCP and the cellular response to the HA: β-TCP ratio. Dissolution of BCP apatite crystals in the superficial layer is known to facilitate reprecipitation of an apatite layer, which leads to bone formation on the surface of the BCP particles[26]. Furthermore, physiochemical dissolution during the resorption of calcium phosphate releases calcium ions into the grafted area, and the resulting elevated level of calcium ions may act as a reservoir that promotes bone formation away from the grafted particles[27]. Conversely, the content of TCP has been reported to be inversely correlated with the degree of viable

cell adhesion. Higher concentrations of TCP decrease the attachment of osteoblasts and monocytes to the BCP particles[28,29], which is in agreement with the smaller bone islands observed in the present TCP70 group. Furthermore, an increased number of multinucleated giant cells was observed adjacent to the sinus membrane in the TCP70 group at 2 weeks. The rapid dissolution of TCP in BCP may provoke an inflammatory reaction in the initial phase, thus delaying wound healing, even though multinucleated osteoclasts are related to bone formation via their coupling with osteoblasts. Likewise, Hong et al. observed an increased number of multinucleated osteoclasts in β-TCP- and BCP-grafted (HA:β-TCP ratio of 60:40) extraction sockets, and a pronounced inflammatory reaction at the initial phase[30].

The rabbit sinus model is generally considered to be appropriate for evaluating bone regeneration due to anatomical similarity and almost equivalent air pressure to the human sinus[31]. However, the healing conditions differ between these two species. The bony area in the rabbit sinus, where the osteotomy is made, is not the sinus floor, but the roof. Accordingly, the graft is ultimately located on the membrane. The difference in the final graft position between the rabbit and human sinuses may negatively contribute to new bone formation by micro-vibrating membrane from the ventilation of the sinus. Moreover, considering that rabbit sinus can accommodate smaller amount of graft material compared to human sinus, the pressure per graft in the rabbit may be bigger than in human. However, shorter bone remodeling period of rabbit (e.g. 6 weeks and 17 weeks for rabbits and human, respectively) make it possible to evaluate long-term outcomes at a relatively short interval.

Statistically significant new bone formation was observed in both BCP groups over time (between 2 and 8 weeks). Lambert et al (2011) evaluated the percentage of new bone in the augmented area with BCP in a rabbit sinus model[13]. At 1 week, 5 weeks, and 6 months of healing, the percentage of new bone increased significantly, being 0.3±0.1%, 12.9±2.2% and 21.5±3.8%, respectively. At 16 weeks of this study, new bone formation was highly homogenous in all portions of sinuses. The microstructural index (t.Th, n.Th and s.Th) at this period did not differ significantly in both groups. In fluorochrome staining, both TCP30 and TCP70 showed varying degrees of fluorescence with a few bright streaks near particles; it was hard to determine which BCP had more sustained and strong bone-forming activity.

Significant resorption of graft material over time was observed in the TCP70 group, in agreement with earlier animal studies using β-TCP[15,18,32]. The stability of the augmented volume is an important factor for the long-term survival of dental implants. In the present study, the sinuses augmented with TCP70 were also well maintained in terms of volume and area throughout the observation period. This might indicate that the augmentation can be maintained with a low proportion of HA in BCP.

One of the parameters of interest after grafting procedures is the %NPC. A high contact ratio infers a stable network of bone and biomaterial, which can be interpreted as strong osteoconductivity and sufficient stability for implant placement. The %NPC was calculated almost 40% and 80% at 8 and 16 weeks for both groups, respectively, indicating that the integration potency with new bone tissue of the BCPs with two different HA:β-TCP ratios are very similar. However, histologic difference of the

TCP30 and the TCP70 groups may result in different clinical situations. Two scenarios can be thought. The bulkier new bone in the TCP30 group may generate greater primary stability when implant is installed, or the network of smaller new bone and residual material in the TCP70 group may achieve larger bone-to-implant contact

Jensen et al stated that the ideal biomaterial for maxillary sinus grafting is not necessarily an ideal material for other types of bony deficiency[15], which in turn means that different types of defect may have different biomaterial requirements. In the pursuit of preferable biomaterials for sinus grafting, two BCPs with different compositions (HA:β-TCP ratio of 30:70 and 70:30) were evaluated in rabbit model. Within limitation of this study, the volume stability and osteoconductive capacity of the two BCPs were comparably effective, and it can be conjectured that both BCPs can be successfully used in sinus augmentation. However, it should be confirmed in larger number of samples and prospective clinical study.

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Figure Legends

Figure 1. Clinical photographs of the surgery. (a) Two osteotomies were prepared and the sinus membrane was carefully elevated. (b) The sinuses were then grafted with biphasic calcium phosphate with a hydroxyapatite (HA):beta tricalcium phosphate (β-TCP) ratio of 70:30 (TCP30) on one side and an HA:β-TCP ratio of 30:70 (TCP70) on the contralateral side.

Figure 2. Color-coded reconstructed microcomputed tomography image of the grafted sinuses at 8 weeks. Blue color, TCP70; red color, TCP30

Figure 3. Histologic observations in the center and Schneiderian membrane areas in the TCP30 group at 2 weeks. NB, new bone; RM, residual material; black arrows, osteoclasts (left, Masson's trichrome stain, original magnification ×100; right, hematoxylin-eosin stain, original magnification ×200).

Figure 4. Histologic observations in the center and Schneiderian membrane areas in the TCP70 group at 2 weeks. Black arrows, osteoclasts (left, Masson's trichrome, original magnification ×100; right, hematoxylin-eosin, original magnification ×200).

Figure 5. Histologic observation of the center and Schneiderian membrane areas in the TCP30 group at 8 weeks. Yellow arrows, bone-forming fronts; stars, reversal line

(left, Masson's trichrome, original magnification ×100; right, hematoxylin-eosin, original magnification ×200).

Figure 6. Histologic observation of the center and Schneiderian membrane areas in the TCP70 at 8 weeks. Black arrow, osteoclast; yellow arrow, bone-forming front; yellow star, reversal line (left, Masson's trichrome, original magnification ×100; right, hematoxylin-eosin, original magnification ×200).

Figure 7. Micro CT view of the TCP30 (A, B, C) and the TCP70 groups (D, E, F). (A, D), three-dimensional reconstruction; (B, E), coronal views; (C, F), sagittal views.

Figure 8. Histologic observation of the TCP30 (A) and TCP70 groups (B). Hematoxylin-eosin stain, original magnification x 12.5.

Figure 9. Histologic observation of the AOIs in the TCP30 (A - E) and TCP70 group (F – J). Hematoxylin-eosin stain, original magnification x 100

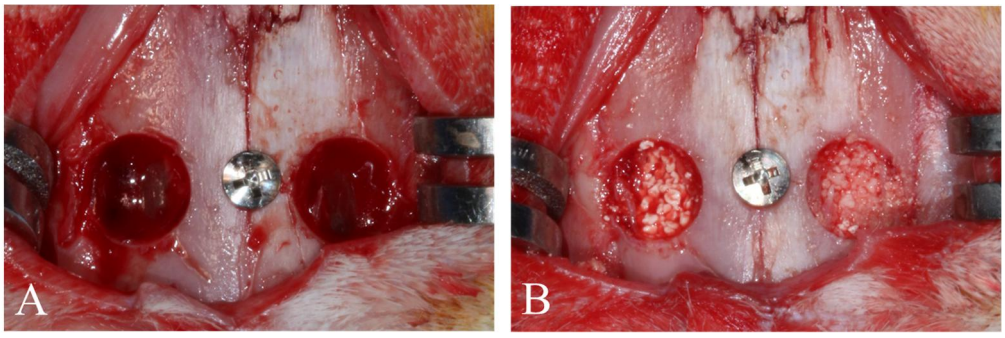
Figure 10. The area of new bone (NA) in the AOIs. *statistical significance (P<0.05).

Figure 11. The area of residual material (RA) in the AOIs. *statistical significance (P<0.05).

Figure 12. The area of fibrovascular tissue (FA) in the AOIs. *statistical significance (P<0.05).

Figure 13. Comparison between histologic section stained using Hematoxylin-eosin and corresponding fluorochrome labeled section in the TCP30 (A, B) and TCP70 groups (C, D). original magnification x 12.5.

Figures



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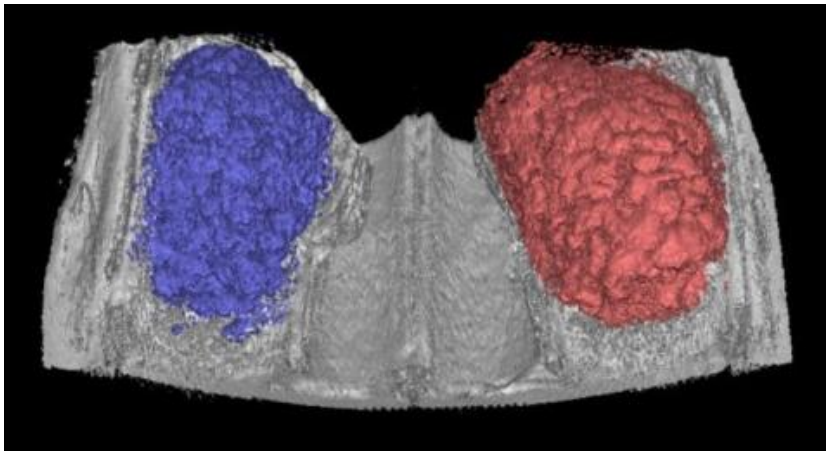

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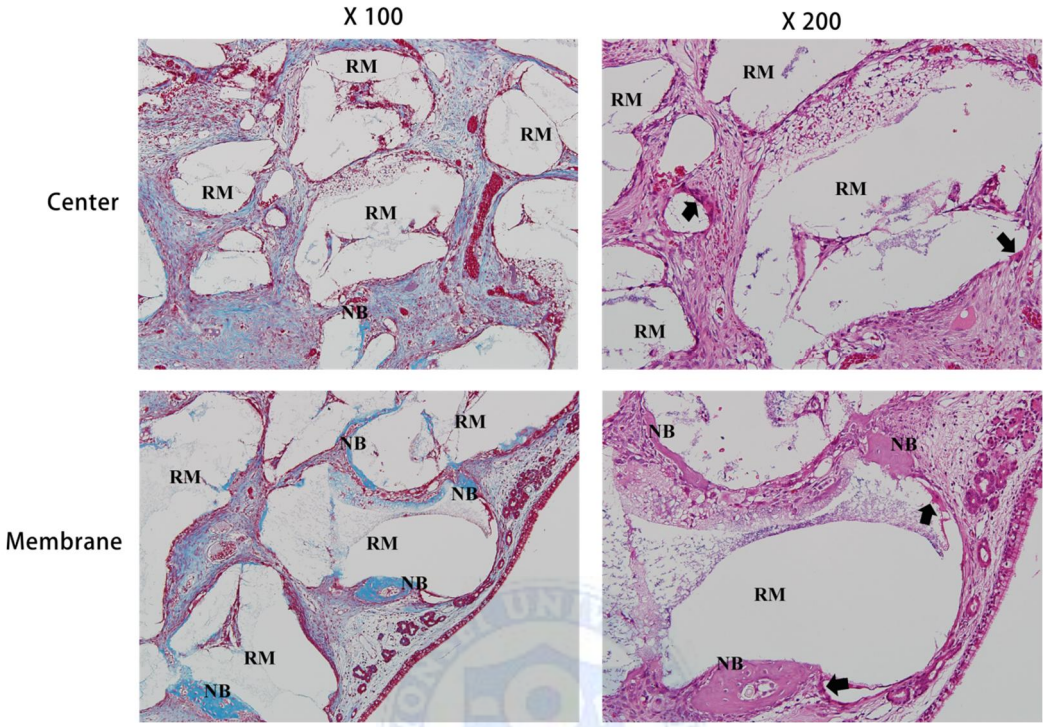

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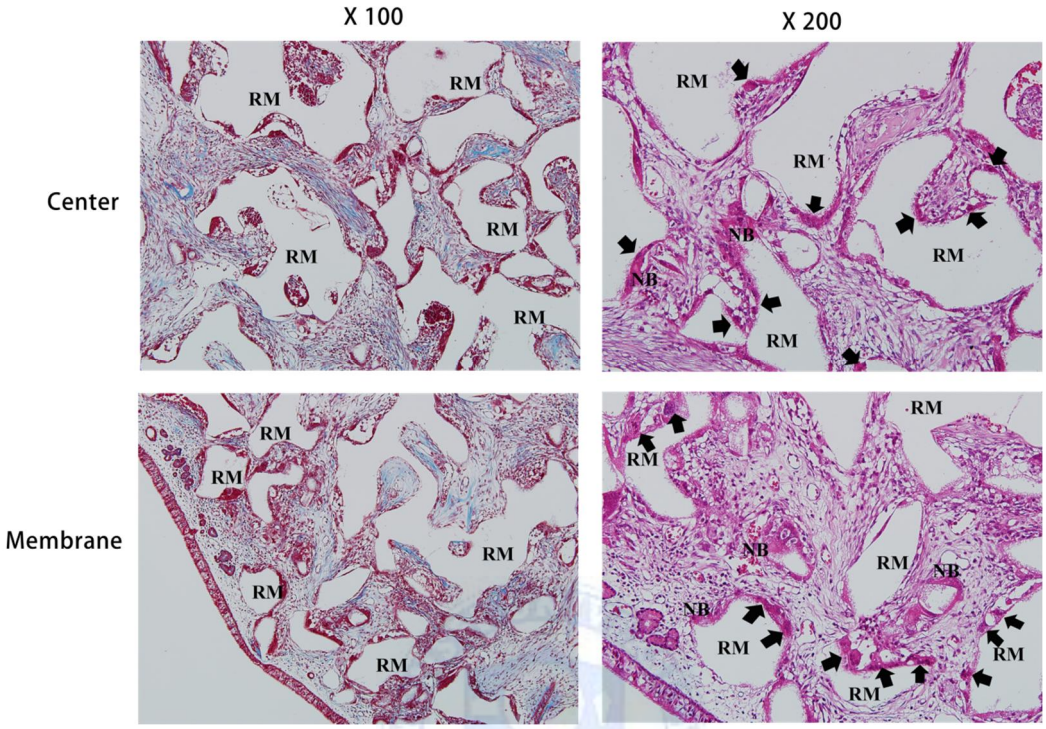

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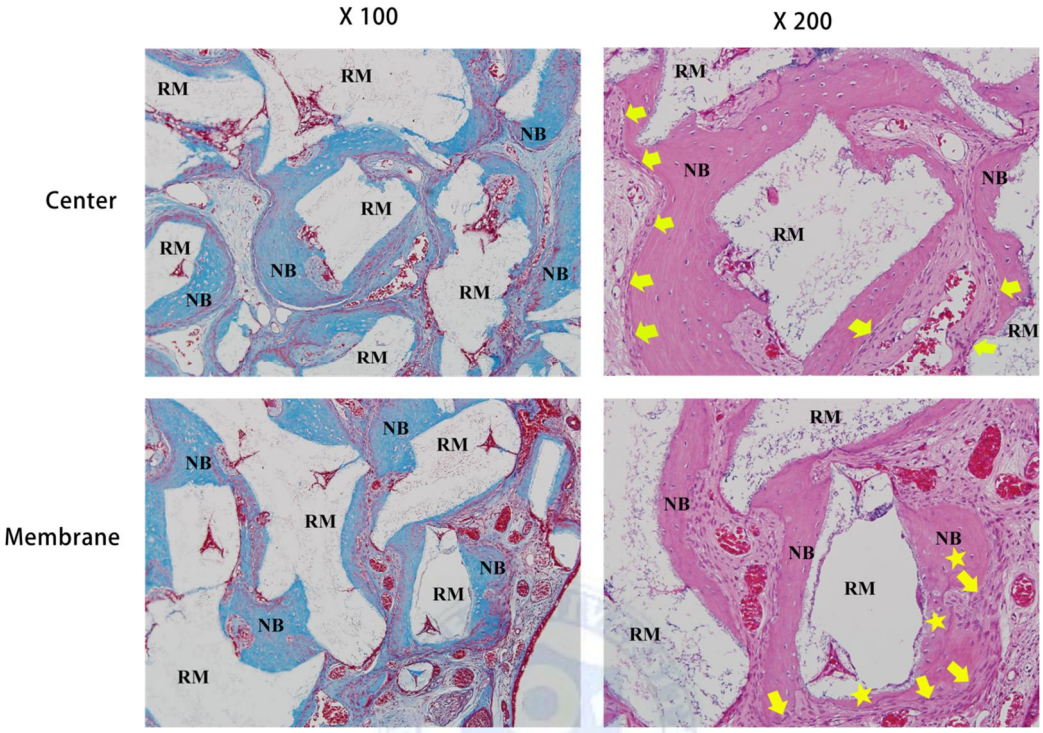

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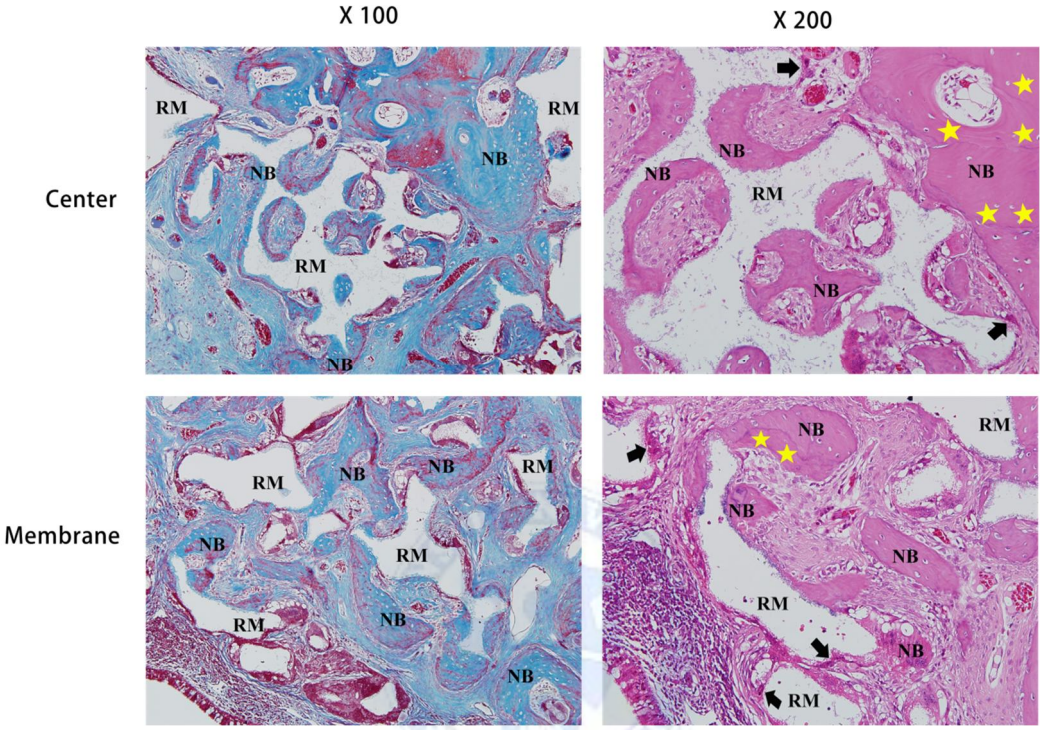

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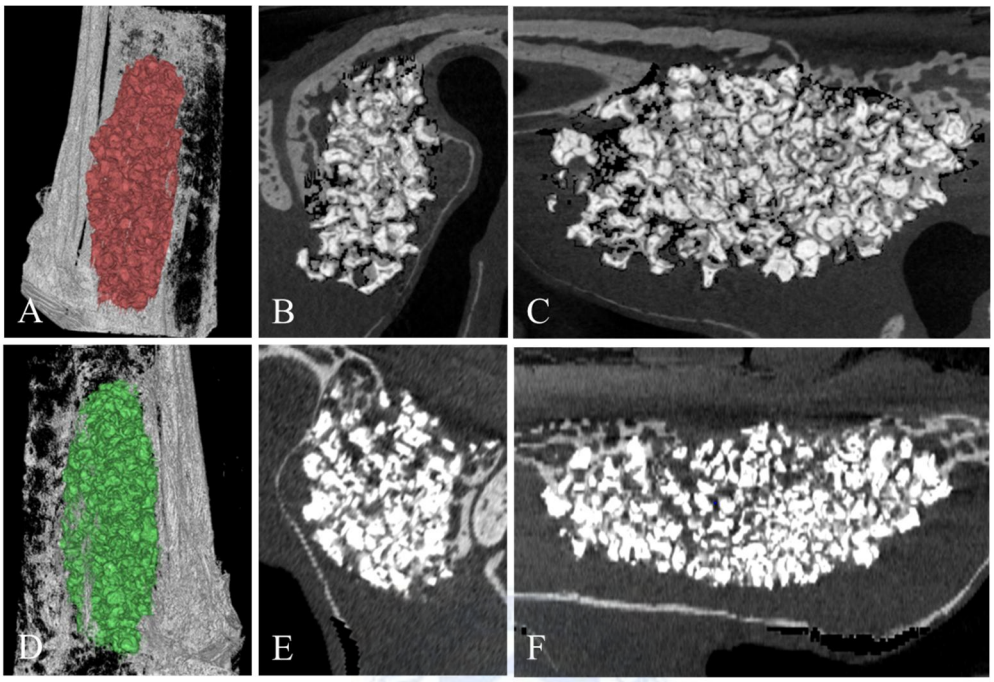

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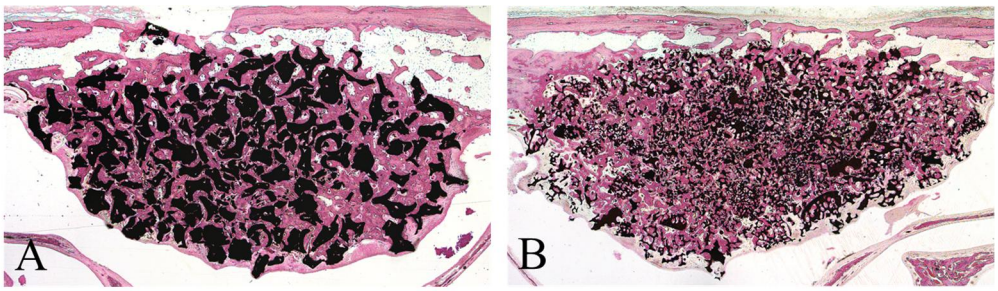

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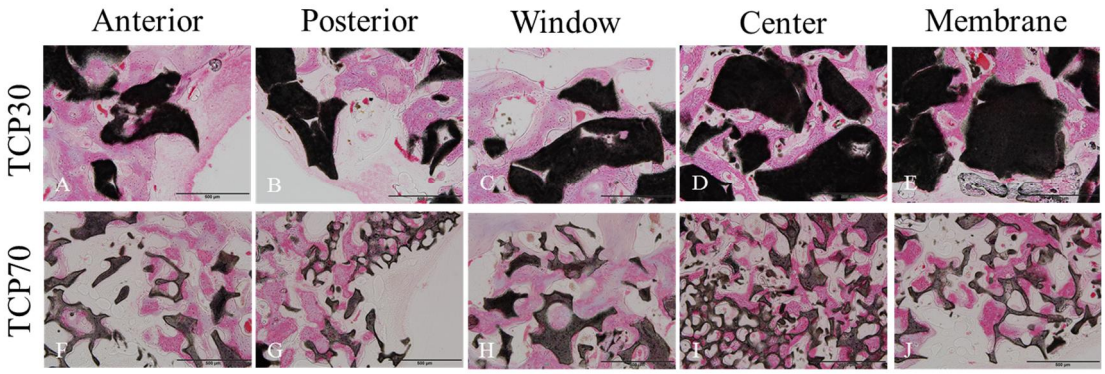
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Figure 9.

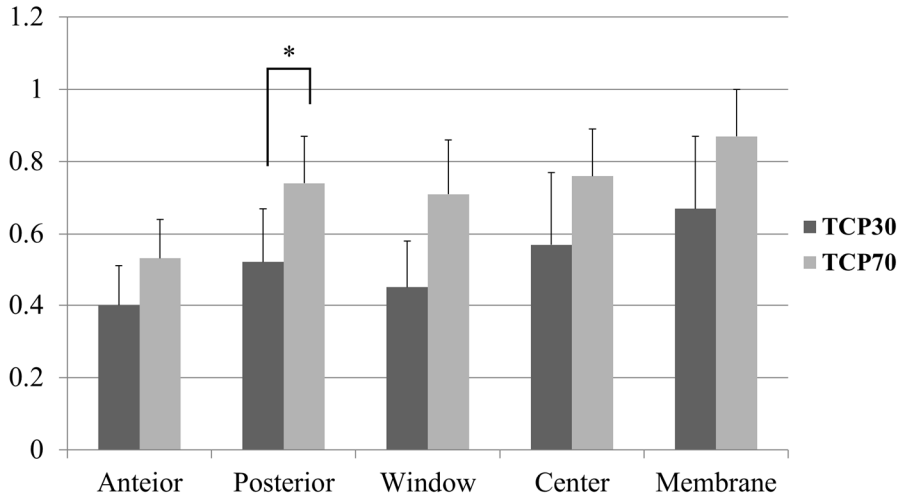
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Figure 10.

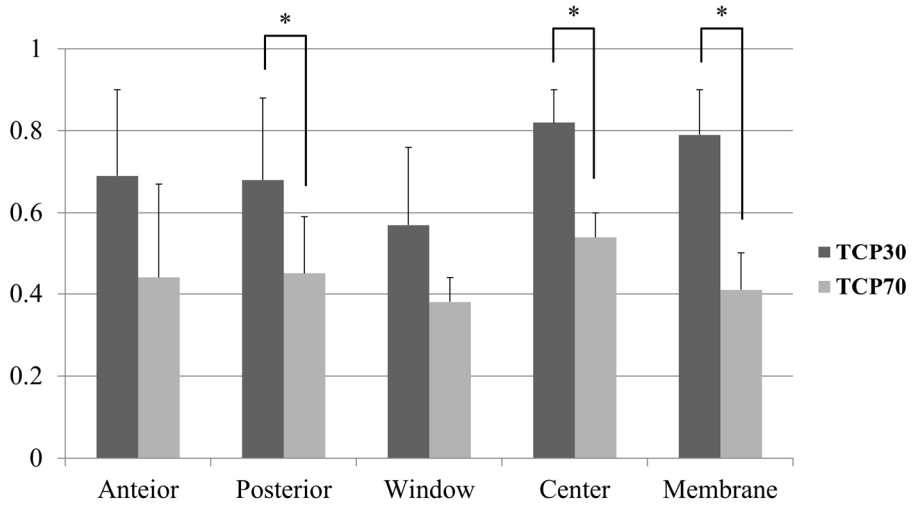
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Figure 11.

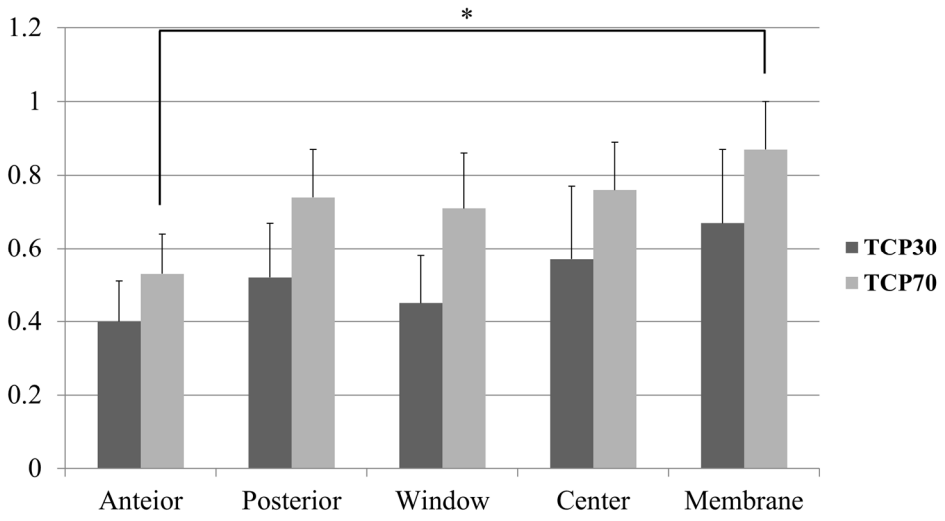
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Figure 12.

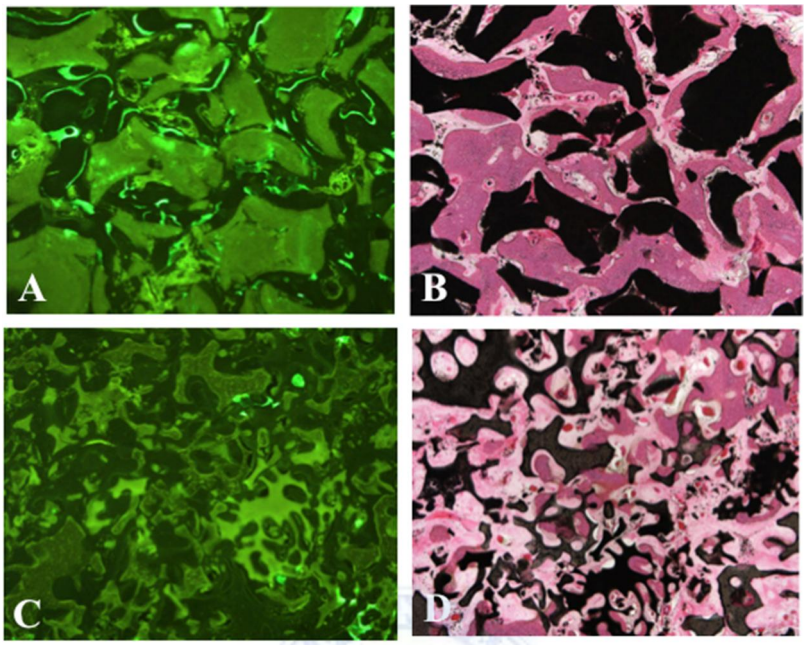

Figure 13.

국문요약

가토 상악동 모델에서 이상인산칼슘의 골전도능과 체적 안정성에 대한 수산화인회석과 베타 제삼인산칼슘의 비율의 영향

< 지도교수 최성호 >

연세대학교 대학원 치의학과

임 현 창

목적: 이 연구의 목적은 서로 다른 수산화인회석과 베타 제삼인산칼슘의 비율로 이루어진 이상인산칼슘의 골전도능과 체적 안정성을 토끼 상악동에서 평가하고자 함이다.

재료 및 방법: *실험 1*; 8마리의 토끼에서 상악동 거상술이 시행되었다. 한쪽 상악동에는 수산화인회석과 베타 제삼인산칼슘의 비율이 3:7인 이상인산칼슘 (TCP70)이 반대편 상악동에는 수산화인회석과 베타 제삼인산칼슘의 비율이 7:3인 이상인산칼슘 (TCP30)이 이식되었다. 실험 동물은 2주와 8주에 각각 4마리씩 희생되었으며 방사선학적, 조직계측학적 분석이 시행되었다. *실험2*; 5마리의 토끼에 실험1과 같은 방법의 상악동 거상술이 시행되었다. 16주 후에 실험동물이 희생되었으며, 희생 5일전 calcein

green이 정맥주사 되었다. 방사선학적, 조직계측학적 분석이 시행되었다.

결과: *실험 1*; 단층 촬영 영상에서 2주, 8주 모두에서 두 군 간 총 증대된 부피는 차이가 없었으며, 두 군 모두 2주와 8주 사이에 통계적으로 유의성 있는 변화를 나타내지 않았다. 조직 계측학적으로 2주와 8주 모두에서 두 군 사이에 골생성량, 골과 재료 사이의 접촉율은 차이를 보이지 않았으며, 골생성량은 2주보다 8주에서 유의성있게 증가하였다. TCP70군은 TCP30군에 비해 유의성 있는 재료의 흡수가 나타났으며, 다핵형 거대세포가 더 많이 관찰되었다. *실험2;* 방사선학적 분석에서 총 증대된 부피, 골생성량은 두 군 간 차이가 없었으나 재료의 흡수는 TCP70에서 유의성 있게 더 많이 관찰되었다. 두 군간 골소주의 두께, 수, 분리도에서도 차이를 보이지 않았다. 조직계측학적으로 총 증대면적, 골생성면적, 이식재가 차지하는 면적, 골과 재료 사이의 접촉율은 차이를 보이지 않았다. Calcein green으로 인한 형광발색에 있어 TCP30과 TCP70 중 어느 것이 더 골형성이 활발하게 일어나는지는 명확히 판단할 수 없었다.

결론: 이 연구에서 사용된 두 비율의 이상인산칼슘은 상악동에 이식되었을 때 골형성능과 체적 안정성에 있어 효과적으로 사용될 수 있을 것이다.

핵심되는 말: 골재생, 골대체제, 골조직공학, 상악동 증대술