Dimensional change of maxillary sinus augmented with collagenated synthetic bone block compared to synthetic bone particulates: a pre-clinical animal experiment in rabbits

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Dimensional change of maxillary sinus augmented with collagenated synthetic bone block compared to synthetic bone particulates: a pre-clinical animal experiment in rabbits

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Table of Contents

List of figures

List of tables

Abstract

Dimensional change of maxillary sinus augmented with collagenated synthetic bone block compared to synthetic bone particulates: a pre-clinical experiment in rabbits

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Objective: to compare the efficacy of collagentated soft type synthetic bone substitute (C-SBS) to particulated synthetic bone substitutes (P-SBS) in volume maintenance and new bone formation in a rabbit sinus graft model.

Material and methods: Either C-SBS or P-SBS was grafted in both sinuses of 16 rabbits. Four weeks (N=8) or 12 weeks (N=8) after the surgery, radiographic and histomorphometric analyses were conducted. Statistical significance level was 5%.

Results: Total augmented volume and area less decreased in C-SBS group compared to P-SBS group over time. Total augmented volume of C-SBS group (267.13±62.08 mm^3)

was significantly higher than that of P-SBS (200.18±40.32 mm^3), as well as new bone volume (103.26±10.50 mm^3 vs. 71.10±7.58 mm^3) at 12 week (p<0.05). Total augmented area and new bone area of C-SBS group (19.36±2.88 mm^2 and 5.43±1.20 mm^2, respectively) were significantly higher than those of P-SBS group (14.48±2.08 mm^2 and 3.76±0.78 mm^2, respectively) at 12 weeks (p<0.05)

Conclusion: Collagenated synthetic bone substitutes grafted in rabbit sinuses demonstrated higher augmented volume and area and more new formation at 12 weeks, compared to the particulated synthetic bone substitutes.

Keywords: animal experiment; maxillary sinus; bone regeneration; synthetic biphasic phosphonate; microcomputed tomography; histology.

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I. INTRODUCTION

Maxillary sinus augmentation has become one of the most commonly performed surgery for placing implants at the ridges in the posterior maxilla having an insufficient vertical height, and it is thought to be reliable in terms of long term effectiveness (Lundgren et al., 2017, Raghoebar et al., 2019). Since vertical ridge atrophy following extraction usually occurs due to crestal bone resorption and sinus pneumatization, there have been some previous publications suggesting alveolar ridge preservation (ARP) to prevent both vertical shrinkage of crestal bone and coronal migration of sinus floor (Cha et al., 2019b, Levi et al., 2017, Lombardi et al., 2018), however, sinus grafting may be inevitable if the patient received extraction only. It is conducted either by lateral window approach or crestal approach, and lateral window approach is suggested rather than crestal approach when the sinus floor needs to be elevated more than 4mm (Zitzmann and Scharer, 1998), which can be performed before the implant placement (Wood and Moore, 1988) or simultaneously (Jung et al., 2010, Hong et al., 2017).

A number of studied previously evaluated various kinds of bone graft material which can be used for lateral window approach. The first publication of sinus floor elevation in the history by Boyne et al. introduced autogenous bone grafting, once considered as a gold standard, since it has osteogenic, osteoinductive and osteoconductive properties (Boyne and James, 1980, Nkenke and Stelzle, 2009). On the contrary, there were also previous studies reporting disadvantages of autogenous bone such as high surgical morbidity increasing patients' discomfort and unpredictable resorption rate decreasing volume of the grafted site (Nkenke et al., 2004, Hallman and Thor, 2008, De Santis et al., 2017, Danesh-Sani et al., 2017). Therefore the idea of using bone substitutes has been proposed (Starch-Jensen et al., 2018, Cha et al., 2019a). Previous studies demonstrated comparable new bone formation and more volume maintained when xenogenic or synthetic bone substitutes (SBS) such as biphasic calcium phosphate composed of hydroxyapatite and tricalcium phosphate were used compared to autologous bones (Starch-Jensen et al., 2018, Corbella et al., 2016, Lee et al., 2017, De Santis et al., 2017), and when comparing two types of substitutes each other, one was not superior to the other one in sinus augmentation (Rodriguez y Baena et al., 2017, Froum et al., 2008). Furthermore, since there are some patients having a concern from the risks of disease transmission and host immune activation (Kim et al., 2013, Fernandez et al., 2015), the need of using SBS in graft procedures is getting increased over time.

As the collagenated soft type block bone substititues (xenografts or alloplasts) have been introduced for easier manipulation of the graft materials, not only they were tested in guided bone regeneration (GBR) (Sun et al., 2018, Benic et al., 2017) and ARP (Araujo and Lindhe, 2009, Araujo et al., 2008, Kim et al., 2017, Cha et al., 2019b), but they were also evaluated in sinus graft (Kim et al., 2015, Lim et al., 2015a, Alayan et al., 2016b, Alayan et al., 2016a, Alayan and Ivanovski, 2018, Lambert et al., 2011, Lambert et al., 2013, Paik et al., 2020), showing the similar effectiveness in terms of new bone formation compared to the particulates. Furthermore, there were previous studies investigating the potential of collagenated synthetic block bone as a carrier for growth factors such as bone morphogenetic protein-2 in animal sinus graft models (Kim et al., 2015, Lim et al., 2015a). Collagenated bone substitutes may provide an advantage to the clinicians in the aspect of easier handling of the material and shorter chair time (Araujo et al., 2008, Araujo et al., 2010, Araujo and Lindhe, 2009), and it has also been assumed that collagenated form of bone graft might have a benefit over particulates when it is used in sinus graft under perforated Schneiderian membrane (Alayan et al., 2016b, Paik et al., 2020). Still, however, particle types of bone graft material are widely chosen in clinics, because the publications which evaluated the performance of collagenated soft type block bone substitutes are scarce. Especially, while a few previous researches compared collagenated xenografts to particle types grafted in sinus (Alayan and Ivanovski, 2018, Alayan et al., 2016b, Alayan et al., 2016a), there is no study which compared collagenated and particulated synthetic bone substitutes in sinus augmentations as far as the authors are aware of.

Therefore, the aim of the present study was to evaluate the efficacy of collagenated soft type synthetic bone substitute (C-SBS) compared to particulated synthetic bone substitutes (P-SBS) in terms of new bone formations and grafted volume maintenance in a rabbit sinus graft model.

II. MATERIALS AND METHODS

1. Ethical statement

The present experiment was followed the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines checklist (Kilkenny et al., 2010). The selection and management of experimental animals and the study protocol were approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Republic of Korea (2017-0331).

2. Sample size calculation

The required sample size of eight per each group was estimated based on a power calculation with a significance level (α) of 5% and power (1–ß) of 90% according to the previously published rabbit sinus graft model experiment which used similar study design (Kim et al., 2015).

3. Experimental animals, housing and husbandry

Sixteen healthy male New Zealand white rabbits (DooYeol Biotech, Seoul, Korea) with mean body weights of 2.5–3.0 kg were selected for the experiments, and both maxillary sinuses of each animal, total 32 sinuses, were grafted. Animals were raised under standard laboratory conditions with appropriate feeding and housed at room temperature (15–20°C) and humidity (> 30%).

4. Experimental materials

P-SBS (Osteon 3, Genoss, Suwon, Republic of Korea), biphasic calcium phosphate (BCP) composed of hydroxyapatite and β-tricalcium phosphate in the ratio of 6:4 was used for P-SBS group at one side. At the contralateral side, C-SBS (Osteon 3 Collagen, Genoss), a soft-type block composite made of P-SBS containing 6% of porcine type I collagen, was grafted for C-SBS group. Both P-SBS and C-SBS were applied in a plastic syringe with a volume of 0.16cc.

5. Surgical procedures

The surgical procedure was performed according to the previous studies from our research group (Choi et al., 2013, Kim et al., 2015, Yon et al., 2015). Under general anesthesia and additional local infiltration of lidocaine (lidocaine HCl, Huons, Seoul, Republic of Korea), full-thickness flaps were raised at both sides, followed by the incision along the sagittal line of nasal bone. Lateral windows in circular shape, 5.5mm in diameter, were prepared bilaterally on the dorsum of the sinuses, and the bony windows were removed and stored in saline. Schneiderian membranes were carefully elevated to create the space for grafting, and the experimental materials were applied based on the randomly assigned group allocation (C-SBS or P-SBS). After closing the window entrances with the removed bony window discs, the elevated flaps were primarily closed with a 4-0 resorbable suture material (Monosyn glyconate absorbable monofilament, B-Braun, PA, USA) (Fig. 1). The animals received antibiotics (5 mg/kg Baytril, Bayer Korea) and analgesics (0.5 mg/kg ketorolac, Keromin, Hana Pharm, Seoul, Korea) once a day for 5 days and the suture materials were removed at 7 days postsurgery.

6. Sacrificing of experimental animals

Experimental animals were sacrificed by using applying overdose of general anesthesia after either 4 or 12 weeks, 8 animals each respectively. The operated sites including the grafted sinus and surrounding tissue were dissected to obtain the samples for radiographic and histologic analyses.

7. Radiographic analysis

All collected samples were fixed in 10% formalin for 10 days, followed by taking micro-computed tomography (micro-CT; Skyscan 1173, SkyScan, Aartselaar, Belgium) under a resolution of 14.91 μm (130 kV and 60 μA). Three-dimensional image was achieved and visualized by reconstruction of the micro-CT data, and total augmented volume (TAV, mm^3) was estimated based on the previously published study (Yon et al., 2015). New bone volume (NBV, mm^3), residual graft material volume (RMV, mm^3) and non-mineralized tissue volume (NMV, mm^3) were also measured.

8. Descriptive histology and histomorphometric analysis

After micro-CT scanning, the block specimens were decalcified with 5% formic acid for 14 days, the specimens were paraffin-embedded after dehydration. Paraffin blocks were sectioned coronally along the center of the sinuses with the thickness of 5μm and were stained with Masson’s trichrome. Digital images of histology were observed and obtained by optical microscopy (BX50, Olympus, Tokyo, Japan), and histomorphometric analysis were performed by measuring the following parameters according to the previous study (Yon et al., 2015):

- Total augmented area (TAA, mm^2) enclosed by the antral bony wall, Schneiderian membrane, and the window.
- Newly formed bone area (NBA, mm^2) within the TAA.
- Residual graft material area (RMA, mm^2) within the TAA.
- Fibrovascular tissue area (FTA, mm^2) within the TAA.

9. Statistical analyses

All of the measurements were statistically analyzed using a computer software SPSS version 23 (IBM, Armonk, NY, USA). Normality of the data was confirmed Kolmogorov-Smirnov test ($p > 0.05$). Paired t-test ($p < 0.05$) was used to determine the differences between P-SBS and C-SBS groups at each time point (4 and 12 weeks), and independent t-test ($p < 0.05$) was performed to compare the differences between 4 and 12 weeks within each group.

III. RESULTS

1. Clinical outcomes

There was no Schneiderian membrane perforation observed during the surgery in all surgeries. All animals did not show uneventful healing not complication after the surgical procedures.

2. Radiographic analysis

Three dimensionally reconstructed images revealed that the grafted sinus appeared in a dome-shape and the window was partially closed in both groups at 4 and 12 weeks (Fig. 2). TAV was greater in C-SBS group compared to P-SBS group at each time point, as well as NBV, RMV and NMV, however, the statistical significance between two groups was only found in TAV, NBV and RMV at 12 weeks (267.13 ± 62.08 mm^3 vs. 200.18 ± 40.32 mm^3 in TAV; 103.26 ± 10.50 mm^3 vs. 71.10 ± 7.58 mm^3 in NBV; 47.27 ± 13.92 mm^3 vs. 36.01 ± 18.25 mm^3 in RMV; $p < 0.05$). The ratios of NBV, RMV and NMV did not differ significantly between C-SBS and P-SBS groups at both, 4 and 12 weeks ($p > 0.05$).

Within C-SBS group, TAV and NMV were comparable between 4 and 12 weeks ($p > 0.05$). Compared to 4 weeks, NBV at 12 weeks significantly increased (83.16 ± 13.29 mm^3 vs. 103.26 ± 10.50 mm^3, $p < 0.05$), whereas RMV significantly decreased at 12 weeks (77.27 ± 10.20 mm^3 vs. 47.27 ± 13.92 mm^3, $p < 0.05$). The ratios of NBV and RMV was significantly different between two time points (31.16 ± 2.16% vs. 40.01 ± 7.41% in %NBV; 29.51 ± 2.74% vs. 19.15 ± 8.95% in %RMV; $p < 0.05$), however, the ratio of NMV was comparable ($p > 0.05$).

Unlike in C-SBS group, TAV of P-SBS group significantly decreased over time (243.20 ± 23.27 mm^3 at 4 weeks vs. 200.16 ± 40.32 mm^3 at 12 weeks; $p < 0.05$). RMV significantly decreased at 12 weeks (72.33 ± 4.09 mm^3) compared to 4 weeks (36.01 ± 18.25 mm^3; $p < 0.05$), however the increase of NBV and decrease of NMV did not show statistical significance ($p > 0.05$). The ratios of NBV and RMV at 4 weeks was significantly increased (26.29 ± 5.02% vs. 36.50 ± 6.80%; $p < 0.05$) and decreased (29.97 ± 3.21% vs. 19.23 ± 11.04%; $p < 0.05$), while the ratio of NMV was similar ($p > 0.05$), compared to 12 weeks.

All measurements are summarized in Table 1.

3. Descriptive histology

At 4 weeks, 6 out of 8 sinuses in each group demonstrated their windows closed with newly formed bone, while rest of the windows were not completely closed. In both groups, Schneiderian membranes were intact, and bone graft materials were scattered within the space created under the elevated sinus membrane and encapsulated by fibrous tissue with the blood vessels. Most of the newly formed bones were in immature state of woven bone and found between the residual bone grafts. The osteoclastic activity could be observed on the outer border of the C-SBS and P-SBS particles, and osteoblasts resided at the outer margin of the newly formed bone, which were mainly observed near the window and Schneiderian membrane (Fig. 3).

At 12 weeks, sinus windows of both groups were completely closed with the new bone bridge, and new bone formation could be seen throughout the entire space underneath the elevated membrane with the lamellated state. Remaining C-SBS and P-SBS particles became smaller in size, and more blood vessels embedded within fibrous tissue were observed between the particles (Fig. 4).

4. Histomorphometric analysis

At 4 weeks, TAA, NBA, RMA and FTA of C-SBS group were larger than that of P-SBS group, however, the statistical significance was observed only in FTA (10.46 ± 2.03 mm^2 vs. 7.81 ± 1.65 mm^2; $p < 0.05$). The ratios of NBA and RMA were slightly lower in C-SBS group compared to P-SBS group ($p > 0.05$), while the ratio of FTA of C-SBS group was significantly higher than that of P-SBS group (51.25 ± 3.42% vs. 46.59 ± 4.48%; $p < 0.05$).

At 12 weeks, C-SBS group showed significantly larger TAA, NBA, RMA and FTA (19.36 ± 2.88 mm^2, 5.43 ± 1.20 mm^2, 5.33 ± 1.00 mm^2 and 8.59 ± 1.48 mm^2, respectively) than P-SBS (14.48 ± 2.08 mm^2, 3.76 ± 0.78 mm^2, 4.01 ± 0.81 mm^2 and 6.74 ± 0.83 mm^2, respectively; $p < 0.05$). Among the ratios of NBA, RMA and FTA, only that of NBA in C-SBS group was significantly higher than P-SBS (27.91 ± 4.31% vs. 25.85 ± 3.46%; $p < 0.05$).

C-SBS group demonstrated similar TAA at 4 and 12 weeks ($p > 0.05$), while NBA and the ratio of NBA significantly increased at 12 weeks (5.43 ± 1.20 mm^2 and 27.91 ± 4.31%) compared to 4 weeks (3.86 ± 0.86 mm^2 and 19.31 ± 4.72%; $p < 0.05$). RMA and the ratio of RMA were comparable between 4 and 12 weeks ($p > 0.05$). FTA decreased at 12 weeks compared to 4 weeks with no statistical significance ($p > 0.05$), whereas the ratio of FTA at 4 weeks was significantly different from that at 12 weeks (51.25 ± 3.42% vs. 44.42 ± 3.33%; $p < 0.05$). In P-SBS group, TAA and FTA decreased over time, but the differences were not significant, and so was the ratio ($p > 0.05$). Comparing 4 weeks and 12 weeks, the increase in NBA and the ratio of NBA was found (3.50 ± 0.67 mm^2 vs. 3.76 ± 0.78 mm^2; 21.18 ± 4.01% vs. 25.85 ± 3.46%), but statistical significance was only observed in the ratio ($p < 0.05$). RMA and the ratio of RMA at 12 weeks decreased compared to 4 weeks significantly (5.35 ± 0.80 mm^2 vs. 4.01 ± 0.81 mm^2; 32.21 ± 3.51% vs. 27.42 ± 3.26%; $p < 0.05$). All measurements are summarized in Table 2.

IV. DISCUSSION

The present study investigated the dimensional change of augmented maxillary sinus over time following grafting C-SBS or P-SBS. Total augmented volume and area changed over time in a limited extent in C-SBS group (+ 1.42% in volume and – 4.72% in area), while the changes were more prominent in P-SBS group (- 17.7% in volume and - 13.1% in area), and the volume and area measured at each time point (4 and 12 weeks) were higher in C-SBS group compared to P-SBS group. New bone formation of C-SBS group was also superior than that of P-SBS group, when they were compared at each time point, and more increases of newly formed bone volume and area over time were found in C-SBS group (+ 20.10 mm^3 and + 1.57 mm^2, respectively) compared to P-SBS group (+ 7.60 mm^3 and + 0.26 mm^2, respectively).

In the radiographic analysis, TAV of C-SBS group was comparable between 4 and 12 weeks, whereas that of P-SBS group significantly decreased. Both groups showed a significant increase of NBV and significant decrease of RMV over time. This finding is partly similar to the previous reports but also partly opposite, since the previous studies demonstrated the increase of NBV and decrease of RMV over time with the decreased TAV and NMV at a later time point as the postoperative swelling got resolved (Kim et al., 2015, Lim et al., 2015b). It might be considered due to more increase of NBV and less decrease of RMV observed in C-SBS group compared to P-SBS group, which might have induced significant differences in TAV, NBV and RMV between the two groups at 12 weeks. NMV also slightly increased significantly at 12 weeks in C-SBS group, while it decreased in P-SBS group. It might have resulted from the collagen portion of the graft material, however, it is difficult to draw such a conclusion since it is still controversial whether the collagenated soft-type bone substitutes would have an advantage over the particulates in optimization of the grafted space or not (Araujo et al., 2008, Chang et al., 2015, Alayan et al., 2016b).

Whether SBS was collagenated or not, new bone formation was predominant near the window and elevated Schneiderian membrane, while relatively less new bone was found in the middle area at 4 weeks. At 12 weeks, an increase in newly formed bone in the middle area could be seen. This pattern of ossification is considered to be obvious since the osteogenic factors mainly come from the native bone at the base of the sinus and Scheiderian membrane (Cho et al., 2014, Jakob et al., 2010), and it seems that adding collagen to the SBS does not have a benefit in acceleration of ossification in the center area.

A previous study demonstrated that the augmented area might decrease over time due to the resorption of the collagen portion when collagenated xenogenic bone block was grafted (Chang et al., 2015). On the other hand, there were previous publications which presented well-maintained space with consecutive histologic images even after the spontaneous collagen absorption (Araujo et al., 2010) and which reported that collagenated xenograft showed similar performance in an animal sinus graft model in terms of hard tissue regeneration and maintenance of augmented space compared to the xenograft particulates combined with autogenous bone particles (Alayan et al., 2016b). There was also a previously published study which reported that collagenated bovine bone mineral delayed de novo bone formation compared to the particulated bone grafts in the early stage (1 and 5 weeks) (Lambert et al., 2013). In the present study, total augmented space of C-SBS group seemed well-preserved over time, and comparable amount and ratio of newly formed bone was found in both groups at 4 weeks, in histology.

As previous studies have demonstrated (Lim et al., 2015b, Kim et al., 2015), the tendency of increase in newly formed bone and decrease in graft materials at the same time was also observed in both groups of the present study. The SBS used for this study was BCP composed of hydroxyapatite and β-tricalcium phosphate in the ratio of 6:4. It is well known that β-tricalcium phosphate is responsible for the recruitment of the cells having osteogenic potential and slow-resorbing hydroxyapatite maintains the volume by compensating the fast degradation of β-tricalcium phosphate (Yamada et al., 1997). It might be assumed that higher hydroxyapatite with lower β-tricalcium phosphate could have a disadvantage in terms of de novo bone formation, however, it has been previously proven that new bone forming efficacy was comparable between the BCP having higher ratio of HA and that having lower ratio of HA (Lim et al., 2015b). When compared to the previous studies, the ratio of NBA at 12 weeks in C-SBS and P-SBS groups (27.91% and 25.85% in mean, respectively) of the present study, which increased significantly compared to 4 weeks, did not differ from the previously reported results (over 20%) at the time point of more than 8 weeks (Lim et al., 2015b, Lambert et al., 2011, Lambert et al., 2013).

C-SBS group showed larger TAA, as well as NBA, RMA and FTA, than those of P-SBS group when compared at each time point, and the differences between the two groups were significant at 12 weeks. TAA was relatively well-preserved in C-SBS, which is thought to be owing to the significantly increased NBA and a limited change in RMA. In P-SBS group, on the contrary, a minimal increase of NBA was found, whereas RMA decreased significantly at 12 weeks compared to 4 weeks. Consequently, P-SBS demonstrated more decrease of TAA (- 13.1%) over time than that of C-SBS group (-4.72%), similar to the results in radiographic analysis. Given that FTA of C-SBS group was significantly higher than P-SBS group at 4 weeks and it significantly decreased at 12 weeks compared to 4 weeks, it seemed that newly formed bone tissue resided the spaces which was previously dominated by fibrous tissue. It could be speculated by the biological mechanism described in the previous study with serial histologic evluation (Araujo et al., 2010). Collagen degradation, which is known to start from a week after the grafting, might have occurred rapidly by the activity of hydrolytic enzymes in C-SBS group, forming more fibrous tissue with osteogenic potential around the graft materials. This phenomenon, however, might have not appeared in P-SBS group.

V. CONCLUSION

Collagenated synthetic block bone substitutes grafted in rabbit sinuses resulted in higher augmented volume and area, as well as more newly formed bones, at 12 weeks compared to the particulated synthetic bone substitutes. It is necessary to verify these findings in the clinical settings.

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TABLES

Table 1. Radiographic analysis (mean ± standard deviations)

Healing period		TAV	NBV	RMV	NMV
4 weeks					
C-SBS group	Volume (mm^3)	263.38 ± 39.18	**83.16 ± 13.29**†	**77.27 ± 10.20**†	102.94 ± 21.19
	Ratio (%)	100	**31.59 ± 2.16**†	**29.50 ± 2.74**†	38.90 ± 3.94
P-SBS group	Volume (mm^3)	**243.19 ± 23.27**†	63.50 ± 11.33	**72.33 ± 4.09**†	107.36 ± 25.53
	Ratio (%)	100	**26.29 ± 5.02**†	**29.97 ± 3.21**†	43.74 ± 6.97
12 weeks					
C-SBS group	Volume (mm^3)	**267.13 ± 62.08***	**103.26 ± 10.50***,†	**47.27 ± 13.92***,†	**116.60 ± 68.01***
	Ratio (%)	100	**40.01 ± 7.41**†	**19.45 ± 8.95**†	40.55 ± 15.81
P-SBS group	Volume (mm^3)	**200.18 ± 40.32***,†	**71.10 ± 7.58***	**36.01 ± 18.25***,†	**93.07 ± 47.85***
	Ratio (%)	100	**36.50 ± 6.80**†	**19.23 ± 11.04**†	44.28 ± 14.24

* (bold): significantly different between C-SBS and P-SBS groups at each time point ($p < 0.05$)

† (bold): significantly different from 4 and 12 weeks at each group ($p < 0.05$)

Table 2. Histomorphometric analysis (mean ± standard deviations)

Healing period		TAA	NBA	RMA	FTA
4 weeks					
C-SBS group	Area (mm^2)	20.32 ± 3.14	**3.86 ± 0.86**†	5.99 ± 1.17	**10.45 ± 2.02***
	Ratio (%)	100	**19.31 ± 4.72**†	29.42 ± 2.49	**51.25 ± 3.42***,†
P-SBS group	Area (mm^2)	16.67 ± 2.38	3.50 ± 0.67	**5.35 ± 0.80**†	**7.81 ± 1.65***
	Ratio (%)	100	**21.18 ± 4.01**†	**32.21 ± 3.51**†	**46.59 ± 4.48***
12 weeks					
C-SBS group	Area (mm^2)	**19.36 ± 2.88***	**5.43 ± 1.20***,†	**5.33 ± 1.00***	**8.59 ± 1.48***
	Ratio (%)	100	**27.91 ± 4.31***,†	27.16 ± 2.76	**44.42 ± 3.32**†
P-SBS group	Area (mm^2)	**14.48 ± 2.08***	**3.76 ± 0.78***	**4.01 ± 0.81***,†	**6.74 ± 0.83***
	Ratio (%)	100	**25.85 ± 3.46***,†	**27.42 ± 3.26**†	46.72 ± 2.99

* (bold): significantly different between C-SBS and P-SBS groups at each time point (p < 0.05)

† (bold): significantly different from 4 and 12 weeks at each group (p < 0.05)

FIGURES

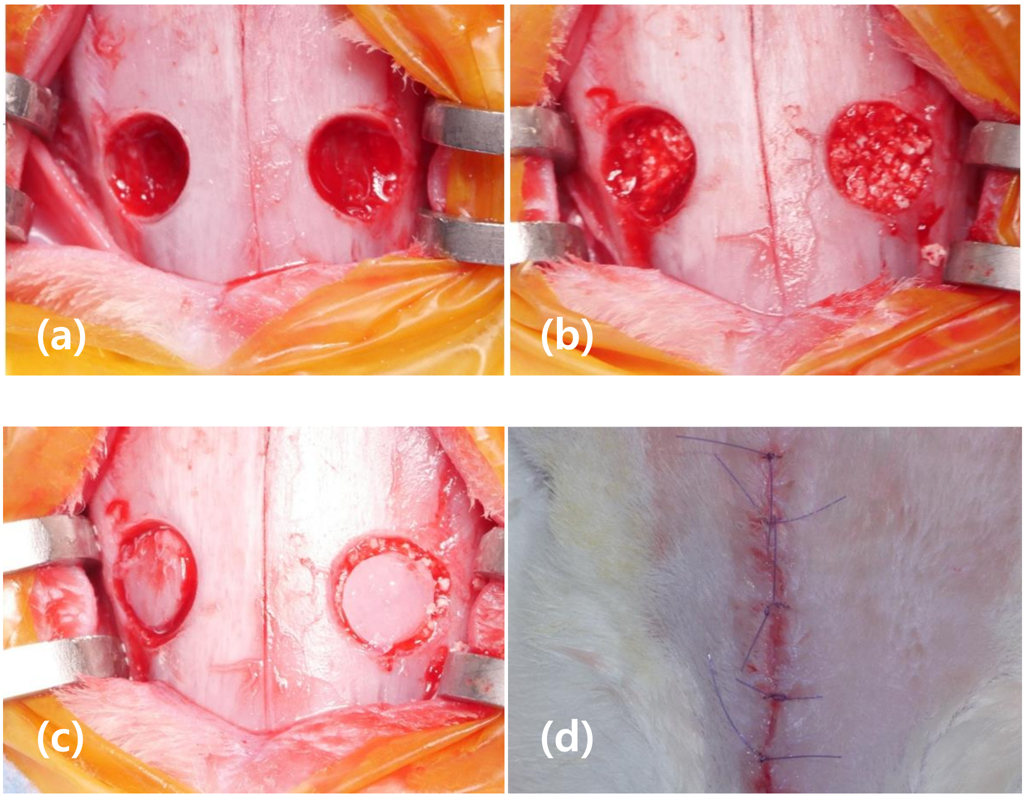

Figure 1. Clinical photographs.

Circular windows of 5.5 mm diameter removed from both sinuses (a). Graft materials applied each side based on the group allocation after membrane elevation (b). Grafted sites covered with bony windows (c). Primarily closed state (d).

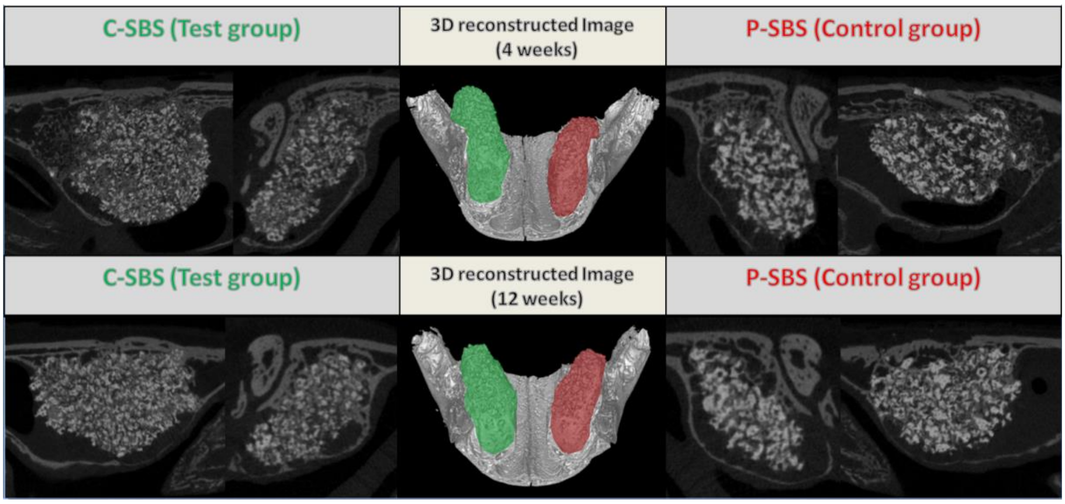

Figure 2. Representative micro-CT images.

Three dimensionally reconstructed micro-CT images of 4 weeks (upper) and 12 weeks (lower) are shown in the center column showing well-augmented sinuses in a dome shape: the green and red colors represent C-SBS group and P-SBS group, respectively. Images in the left and right columns are of C-SBS group and P-SBS group, respectively, at 4 weeks (upper) and 12 weeks (lower).

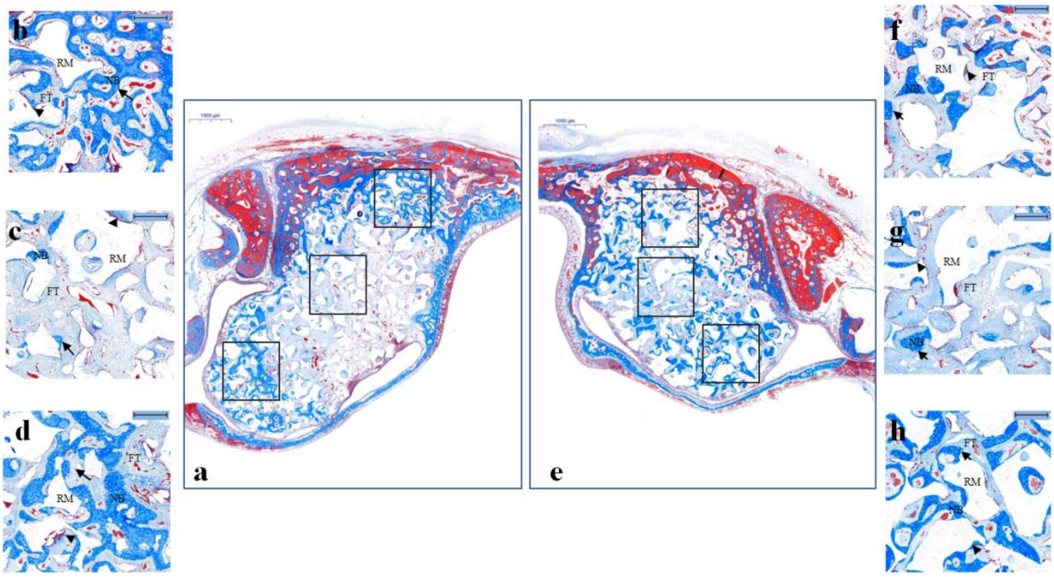

Figure 3. Representative histology at 4 weeks.

Black squares (1mm x 1mm in size) shown in the slides with low magnification (x 20, scale bar = 1 mm) (a and e), indicate the three different regions (membrane, center and window), and they were highly magnified (x 100, scale bar = 200 μm) (b-d and f-h). Well-maintained grafted sites are observed in both the C-SBS group (a) and P-SBS group (e). NB, RM and FT represents the newly formed bone, residual bone graft materials and fibrous tissue consisted of dense connective tissue and blood vessels, respectively. Black arrows and black triangles indicate osteoblasts and osteoclasts, respectively.

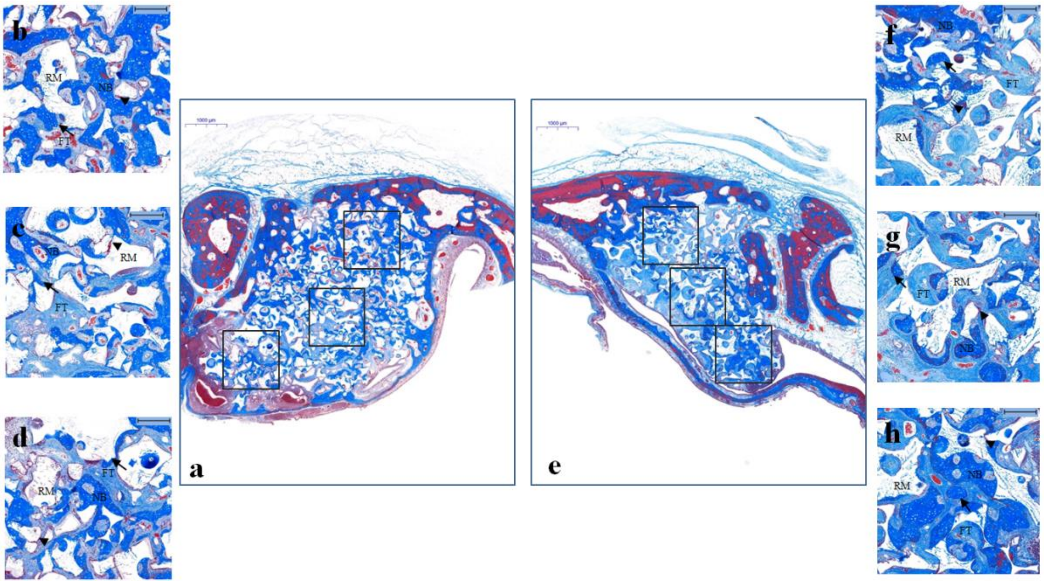

Figure 4. Representative histology at 12 weeks.

Black squares (1mm x 1mm in size) shown in the slides with low magnification (x 20, scale bar = 1 mm) (a and e), indicate the three different regions (membrane, center and window), and they were highly magnified (x 100, scale bar = 200 μm) (b-d and f-h). Well-maintained grafted sites are observed in both the C-SBS group (a) and P-SBS group (e). NB, RM and FT represents the newly formed bone, residual bone graft materials and fibrous tissue consisted of dense connective tissue and blood vessels, respectively. Black arrows and black triangles indicate osteoblasts and osteoclasts, respectively.

국문요약

콜라겐 함유 합성골 블록형 이식재와 입자형 합성골이식재를 이용한 상악동 골이식 후 부피 유지능의 비교 평가: 토끼에서의 방사선학적 및 조직계측학적 연구

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상악동 골이식술은 상악 구치부 무치악 치조제에서의 임플란트 식립 시 수직 고경이 충분히 확보되지 않을 경우 흔히 이용되는 술식으로, 지금까지 선행 연구들을 통해 다양한 종류의 골 이식재의 효능이 평가되었다. 입자형 골이식재에 콜라겐 성분을 추가하여 제조되는 콜라겐 함유 블록형 골이식재는, 조작 편이성 측면에서 큰 이점을 제공하고, 골유도 재생술과 치조제 보존술에서 입자형 골이식재와 비교하였을 때 유사한 신생골 형성능과 부피 유지능 보이는 것으로 보고된 반면, 아직 상악동 골이식술에서의 효능 비교를 진행한 연구는 아직 많지 않다. 따라서, 본 전임상 실험은, 토끼 상악동에 콜라겐 함유 합성골 블록형 이식재와 입자형 합성골이식재를 적용하였을 때의 부피 유지능과 신생골 형성능을 방사선학적 및 조직계측학적 방법을 통해 비교 평가하고자 한다.

건강한 토끼 16마리가 실험을 위해 준비되었고, 실험 재료로는 콜라겐 함유 합성골 블록형 이식재 (collagenated synthetic bone substitutes, C-SBS)

와 입자형 합성골이식재 (particulated synthetic bone substitutes, P-SBS)가 이용되었으며, 두 이식재는 모두 hydroxyapatite와 β-tricalcium phosphate의 화합물인 biphasic calcium phosphate로 구성되었다. 절개 후 판막 거상을 통해 양측 상악동 부위를 노출시키고, 5.5 mm 직경의 trephine bur를 이용하여 형성한 window를 제거한 뒤, 상악동막을 거상하였다. 거상된 상악동막 하방으로 군 배정 (C-SBS군과 P-SBS군)에 맞춰 골이식재를 적용하였고, 이식부위에 window를 재위치시킨 뒤 일차 봉합을 시행하였다. 정해진 시기에 맞춰 수술 4주 후 (8 마리) 또는 12 주 후 (8 마리)에 실험동물을 희생 후, 채득된 시편은 마이크로 컴퓨터 단층 촬영 (micro-computed tomography, micro-CT)을 통한 방사선학적 관찰과 마송 삼색 (Masson trichrome) 염색 후의 조직학적 및 조직계측학적 평가에 이용되었다, 모든 계측치에 대한 통계적 검정을 시행하였고, 통계적 유의차의 기준은 5%로 설정하였다.

방사선학적 분석 결과, 전체 이식 부피의 시간에 따른 변화가 C-SBS군에서는 거의 나타나지 않은 반면 ($p > 0.05$), P-SBS군에서는 유의한 감소가 관찰되었다 ($p < 0.05$). 12주 째에 C-SBS군은 P-SBS군에 비해 유의하게 높은 전체 이식 부피와 신생골 부피를 보였고, 잔존 골이식재의 부피 역시 유의하게 높은 것으로 확인되었다 ($p < 0.05$). 조직학적으로 두 군은 비슷한 양상의 신생골 형성을 보였는데, 4주 째에서 두 군 모두 골형성능이 더 높은 window 부근과 거상된 상악동막 부근에서 더 활발한 신생골 형성을 보인 반면 중심부위에서는 상대적으로 적게 형성된 신생골을 관찰할 수 있었다. 12주 째에서는 두 군 모두 신생골 형성이 이식 부위 전반에 걸쳐 관찰되었다. 조직계측학적 평가 결과, 전체 이식 면적은 4주 째와 12주 째에서 모두 C-SBS군이 P-SBS군보다 더 컸으나, 통계적 유의차는 12주 째에서만 관찰되었다 ($p < 0.05$). 방사선학적 계측 결과와 비슷하게, 전체 이식 면적과 신생골 면적 그리고 잔존 골이식재의 면적 모두 C-SBS군이 P-SBS군에 비해 통계적으로 유의

하게 더 큰 것이 확인되었다 ($p < 0.05$).

결론적으로, 본 연구를 통해 콜라겐 함유 합성골 블록형 이식재가 입자형 골이식재와 비교하였을 때, 상악동 골이식술에서 더 나은 이식 부피 및 면적 유지능과 신생골 형성능을 보임을 확인할 수 있었다. 이러한 결과는 향후 적절히 통제된 임상 연구를 통해 검증되어야 할 것이다.