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Abstract: **OBJECTIVE** to compare the efficacy of a collagenated synthetic bone substitute (C-SBS) to a particulated synthetic bone substitute (P-SBS) in volume maintenance and new bone formations in a rabbit sinus model. **MATERIALS AND METHODS** Either C-SBS or P-SBS was grafted in both sinuses of 16 rabbits. Four (N = 8) or 12 (N = 8) weeks after the surgery, total augmented volume (TAV) and area (TAA), as well as new bone volume (NBV) and area (NBA), were statistically compared by radiographic and histomorphometric analyses ($p < 0.05$). **RESULTS** The differences in TAV, NBV, TAA and NBA between C-SBS and P-SBS groups at 4 weeks were not statistically significant. The TAV (267.13 ± 62.08 vs. $200.18 \pm 40.32 \text{ mm}^3$) and NBV (103.26 ± 10.50 vs. $71.10 \pm 7.58 \text{ mm}^3$) in group C-SBS were significantly higher than in group P-SBS at 12 weeks ($p < 0.05$). The TAA (19.36 ± 2.88 vs. $14.48 \pm 2.08 \text{ mm}^2$) and NBA (5.43 ± 1.20 vs. $3.76 \pm 0.78 \text{ mm}^2$) in group C-SBS were significantly higher than in group P-SBS at 12 weeks ($p < 0.05$). **CONCLUSIONS** C-SBS grafted in rabbit sinuses demonstrated more favorable outcomes across all outcome measures compared to P-SBS at 12 weeks.

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Dimensional changes of the maxillary sinus augmented with a collagenated synthetic bone block or synthetic bone particulates: A pre-clinical study in rabbits

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Abstract

Objective: to compare the efficacy of a collagenated synthetic bone substitute (C-SBS) to a particulated synthetic bone substitute (P-SBS) in volume maintenance and new bone formations in a rabbit sinus model.

Materials and Methods: Either C-SBS or P-SBS was grafted in both sinuses of 16 rabbits. Four (N = 8) or 12 (N = 8) weeks after the surgery, total augmented volume (TAV) and area (TAA), as well as new bone volume (NBV) and area (NBA), were statistically compared by radiographic and histomometric analyses ($p < 0.05$).

Results: The differences in TAV, NBV, TAA and NBA between C-SBS and P-SBS groups at 4 weeks were not statistically significant. The TAV (267.13 ± 62.08 vs. 200.18 ± 40.32 mm³) and NBV (103.26 ± 10.50 vs. 71.10 ± 7.58 mm³) in group C-SBS were significantly higher than in group P-SBS at 12 weeks ($p < 0.05$). The TAA (19.36 ± 2.88 vs. 14.48 ± 2.08 mm²) and NBA (5.43 ± 1.20 vs. 3.76 ± 0.78 mm²) in group C-SBS were significantly higher than in group P-SBS at 12 weeks ($p < 0.05$).

Conclusions: C-SBS grafted in rabbit sinuses demonstrated more favorable outcomes across all outcome measures compared to P-SBS at 12 weeks.

Keywords: animal experiment; maxillary sinus; synthetic biphasic calcium phosphate; micro-computed tomography; histology

Clinical Relevance

Scientific rationale for the study: A well-maintained volume under the elevated sinus membrane over time is one of the key factors for successful maxillary sinus graft surgery.

Principal findings: When grafted in rabbit sinuses, collagenated soft-type synthetic bone substitutes demonstrated significantly larger total augmented volume and more new bone formation at 12 weeks postsurgery compared to particulated synthetic bone substitutes.

Practical implication: Application of collagenated soft-type synthetic bone substitutes in maxillary sinus augmentation may provide a benefit to the clinicians in terms of space maintenance and de novo bone formation, and this finding needs to be further confirmed in a clinical setting.

1. Introduction

Maxillary sinus augmentation is performed for placing implants in the posterior maxilla exhibiting an insufficient vertical ridge height. The respective long-term effectiveness is reported to be high (Lundgren et al., 2017). Vertical ridge atrophy following tooth extraction usually occurs due to crestal bone resorption and sinus pneumatization, which may make sinus grafting inevitable. Sinus is grafted either by a lateral window or crestal approach, followed by staged or simultaneous implant placement depending on the remaining ridge height (Hong et al., 2017; Zitzmann & Scharer, 1998).

A higher number of studies evaluated various types of bone graft materials for the lateral window approach due to the excessiveness over the crestal approach. Autogenous bone was the first one to be investigated, because of its osteogenic, osteoinductive and osteoconductive properties (Boyne & James, 1980), however, the disadvantages were also reported, such as high surgical morbidity, patients' discomfort and unpredictable resorption rate, resulting in decrease of the grafted site volume (Hallman & Thor, 2008). Therefore, bone substitutes have been considered as an alternative to compensate the limitations of autogenous bone graft (Starch-Jensen, Mordenfeld, Becktor, & Jensen, 2018). Previous studies demonstrated similar new bone formation, but with more volume maintained when comparing xenogeneic or synthetic bone substitutes to autologous bones (De Santis et al., 2017; Lee, Shin, Yun, & Cho, 2017; Starch-Jensen et al., 2018). Xenogeneic and synthetic bone substitutes showed comparable new bone formation (Froum, Wallace, Cho, Elian, & Tarnow, 2008) and volume maintenance (Rodriguez y Baena et al., 2017).

Adding collagen as a cohesive to the xenogeneic or alloplastic bone graft particles has been introduced to generate collagenated soft-type block bone substitutes. Not only they were easier to manipulate (M. G. Araujo, Liljenberg, & Lindhe, 2010), also showed a similar amount of newly formed bone (Benic et al., 2016) and improved volume maintenance (Mir-Mari, Wui, Jung, Hämmerle, & Benic, 2016) compared to the particles. Recently, the advantage of using collagenated bovine bone substitute in volume stability and new bone formation has been reported, when solely grafted underneath the sinus membrane with minor perforation (Paik et al., 2020). However, particulated bone substitutes are still widely chosen in clinics, due to scarce scientific data directly comparing collagenated soft-type block bone substitutes to particulated bone substitutes in the maxillary sinus.

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

2. Materials and Methods

2.1 Ethical statement

The selection and management of experimental animals and the study protocol were approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, South Korea (Approval No. 2017-0331). For the report of the present study, the ARRIVE guideline was followed (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010).

2.2 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 ($1-\beta$) of 90% according to the previous rabbit sinus graft model experiment (Kim et al., 2015).

2.3 Experimental animals, housing and husbandry

Sixteen healthy male New Zealand white rabbits (DooYeol Biotech, Seoul, Korea), with body weight of 2.5–3.0 kg and mean age of 16 weeks, were selected, 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%).

2.4 Experimental procedures

2.4.1 Experimental materials

Biphasic calcium phosphate (BCP) composed of hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP) in the ratio of 3:2 (Osteon 3, Genoss, Suwon, South Korea), was used for group P-SBS at one side. At the contralateral side, a soft-type block composite (Osteon 3 Collagen, Genoss) made of P-SBS containing 6% of pure type I collagen, extracted from porcine tendon by alkaline pre-treatment and enzymatic hydrolysis with pepsin, was grafted in group C-SBS. Both P-SBS and C-SBS were applied in a plastic syringe with a volume of 0.16 cm³.

2.4.2 Surgical procedures

The surgical procedure was performed by an experienced surgeon (U.W.J.) according to the previous study (Choi et al., 2012) (Fig. 1). Under general anesthesia using ketamine hydrochloride (Ketalar, Yuhan, Seoul, South Korea) and xylazine (Rumpun, Bayer Korea, Seoul, South Korea) and local infiltration of 2% lidocaine with epinephrine (lidocaine HCl, Huons, Seoul, South Korea), the incision along the sagittal line of nasal bone was performed, and the full-thickness flaps were raised at both sides. Lateral windows in circular shape, 5.5mm in diameter, were prepared bilaterally on the dorsum

of the sinuses with a trephine bur (C Reamer, Neobiotech, Seoul, South Korea) of the same diameter, and the bone plates were removed and stored in normal saline (0.9% sodium chloride). Schneiderian membranes were carefully elevated to create the space for grafting, and the experimental materials soaked by normal saline were applied based on the randomly assigned group allocation (Supplement table a). After closing the access windows with the removed bone plates, the flaps were primarily closed with a 4-0 resorbable suture material (Monosyn glyconate absorbable monofilament, B-Braun, PA, USA). The animals received antibiotics (enrofloxacin 5 mg/kg, Baytril, Bayer Korea, Seoul, South Korea) and analgesics (ketorolac tromethamine 0.5 mg/kg, Keromin, Hana Pharm, Seoul, South Korea) once a day for 5 days.

2.4.3 Sacrificing of experimental animals

Experimental animals were sacrificed by using an 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.

2.5 Measurements

2.5.1 Radiographic analysis

All 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. Based on the grey scale threshold setting (Supplement table b), total augmented volume (TAV, mm^3) was estimated and regarded as a volume of interest for the measurement of new bone volume (NBV, mm^3), residual graft material volume (RMV, mm^3) and non-mineralized tissue volume (NMV, mm^3) (Lim et al., 2018; Thoma et al., 2017). The proportions of NBV (%NBV), RMV (%RMV) and NMV (%NMV) within TAV were also estimated.

2.5.2 Descriptive histology and histomorphometric analysis

After micro-CT scanning, the block specimens were decalcified with 5% formic acid for 14 days, and paraffin-embedded after dehydration. Paraffin blocks were sectioned coronally along the center of the sinuses with the thickness of 5 μm , and were stained with Masson's trichrome. Digital images of histology were obtained and observed by optical microscopy (BX50, Olympus, Tokyo, Japan). The following parameters were measured by a single examiner (K.R.) using a computer

software (Adobe Photoshop CS5, Adobe Systems, San Jose, CA, USA) after the calibration and training (supplement table), according to the previous study (Yon et al., 2015):

- Total augmented area (TAA, mm²) within the region of interest (ROI), enclosed by the antral bone wall, Schneiderian membrane, and the window.
- Newly formed bone area (NBA, mm²) and the proportion of NBA (%NBA) within the TAA.
- Residual graft material area (RMA, mm²) and the proportion of RMA (%RMA) within the TAA.
- Fibrovascular tissue area (FTA, mm²) and the proportion of FTA (%FTA) within the TAA.

Based on the previous studies, %NBA, %RMA and %FTA were also estimated within the standardized square ROI (1 mm x 1 mm) (Kim et al., 2015) at four different regions, which were near the window, near the bone wall, at the center and near the membrane (Omori et al., 2018).

2.6 Statistical analyses

All of the measurements were statistically analyzed using a computer software SPSS version 23 (IBM, Armonk, NY, USA). Normal distribution was confirmed Kolmogorov-Smirnov test ($p > 0.05$). Paired t-test for intergroup comparison at each time point and independent t-test for intragroup comparison between 4 and 12 weeks were performed ($p < 0.05$).

3. Results

3.1 Clinical outcomes

There was no atypical finding observed during and after the surgery.

3.2. Radiographic analysis

Three-dimensionally reconstructed images revealed that the grafted sinus appeared in a dome-shape at 4 and 12 weeks (Fig. 2). At 12 weeks, TAV, NBV, RMV and NMV were significantly higher in C-SBS group (+67.0, +32.2, +11.3 and +23.5 mm³, respectively; $p < 0.05$) than P-SBS group, while, the differences between the two groups were not significant at 4 weeks. There was no significant difference between C-SBS and P-SBS groups in %NBV, %RMV and %NMV at both, 4 and 12 weeks.

Within group C-SBS, TAV and NMV were comparable at 4 and 12 weeks. Compared to 4 weeks, NBV was significantly higher at 12 weeks (20.1 mm³; $p < 0.05$), whereas RMV was significantly lower (-30.0 mm³; $p < 0.05$). Significantly

higher %NBV (+8.42%) and significantly lower %RMV (-10.1%) was found at 12 weeks compared to 4 weeks ($p < 0.05$), however, %NMV was comparable.

TAV of group P-SBS was significantly lower at 12 weeks (-43.0 mm^3 ; $p < 0.05$) compared to 4 weeks; this was also true for RMV (-36.3 mm^3 ; $p < 0.05$). Even though a higher NBV and lower NMV were found at 12 weeks compared to 4 weeks, both did not show statistical significance. Compared to 4 weeks, %NBV and %RMV at 12 weeks were significantly higher and lower respectively (+10.2% and -10.7%, respectively; $p < 0.05$), while %NMV was similar over time.

All measurements are summarized in Table 1, Figure 2b and 2c.

3.3 Descriptive histology

At 4 weeks, 6 out of 8 sinuses in each group demonstrated their windows closed with newly formed bone, while the rest of the windows was not completely closed. In both groups, the 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 blood vessels. Most of the newly formed bone was in immature woven bone state and found between the graft materials (Fig. 3). At 12 weeks, sinus windows of both groups were completely closed with the new bone bridge. Newly formed bone could be seen throughout the entire space showing a matured state with a lamellar pattern. The remaining C-SBS and P-SBS particles were smaller in size (Fig. 4).

3.4 Histomorphometric analysis

All measurements are summarized in Table 2 and Figure 5.

3.4.1 Histomorphometric analysis within the total augmented area

At 4 weeks, TAA, NBA, RMA and FTA of C-SBS group were larger than P-SBS group, however, statistical significance was observed in FTA only ($+2.64 \text{ mm}^2$; $p < 0.05$). %NBA and %RMA were slightly lower in group C-SBS compared to group P-SBS, while %FTA in group C-SBS was significantly higher than group P-SBS (+4.66%; $p < 0.05$). At 12 weeks, C-SBS group showed significantly larger values for TAA, NBA, RMA and FTA compared to group P-SBS (+4.88, +1.67, +1.32 and +1.85 mm^3 , respectively; $p < 0.05$). Among %NBA, %RMA and %FTA, only %NBA in group C-SBS was significantly higher than group P-SBS (+2.06%; $p < 0.05$).

C-SBS group demonstrated a similar TAA at 4 and 12 weeks, while NBA and %NBA were significantly higher at 12 weeks compared to 4 weeks (+1.57 mm³ and +8.60%; $p < 0.05$). RMA and %RMA were comparable between 4 and 12 weeks. FTA was lower at 12 weeks compared to 4 weeks with no statistical significance, whereas %FTA at 12 weeks was significantly lower than 4 weeks (-6.83%; $p < 0.05$). In P-SBS group, TAA and FTA at 12 weeks were lower compared to 4 weeks, but the differences were not significant. NBA and %NBA at 12 weeks were higher than 4 weeks, but statistical significance was only observed in %NBA (+4.67%; $p < 0.05$). Significantly lower RMA and %RMA were observed, when comparing 12 weeks to 4 weeks (-1.34 mm³ and -4.76%; $p < 0.05$).

3.4.2 Histomorphometric analysis at four separate regions

Both C-SBS and P-SBS groups showed smallest %NBA at the center, while larger %NBA near the window, bone walls and membrane was seen at the center at 4 weeks. Between the two groups, %NBA at each region was not significantly different at 4 weeks, however, significantly larger %RMA and smaller %FTA were observed near the membrane in P-SBS group compared to C-SBS group (+15.3% and -13.1%, respectively; $p < 0.05$). At 12 weeks, all proportions were similar between the two group, except for %NBA of C-SBS group near the bone walls which was significantly larger than P-SBS group (+7.25%; $p < 0.05$). Compared to 4 weeks, larger %NBA and smaller %FTA were observed at 12 weeks in both groups, however, statistical significance was found only in %FTA of C-SBS group near the window and membrane (-8.88% and -16.6%, respectively; $p < 0.05$) and %FTA of P-SBS group at the center (-12.2%; $p < 0.05$).

4. Discussion

The present study investigated the dimensional change and new bone formation of augmented maxillary sinus over time following grafting with C-SBS or P-SBS. TAV and TAA changed over time to 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 TAV and TAA 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 compared to group P-SBS, when compared at each time point. Comparing 12 weeks to 4 weeks, greater NBV and NBA were found in C-SBS group (+24.2% and +45.3%, respectively), compared to P-SBS group (+12.0% and +7.42%, respectively).

In the radiographic analysis, TAV of C-SBS group was similar between 4 and 12 weeks, whereas that of P-SBS group was significantly lower at 12 weeks compared to 4 weeks. At 12 weeks, both groups showed a significantly higher NBV and lower RMV compared to 4 weeks. This finding is partly similar to the previous researches, which demonstrated higher NBV and lower RMV as well as lower TAV and NMV at a later time point as the postoperative swelling got resolved (Kim et al., 2015; Lim, Zhang, Lee, Jung, & Choi, 2015). This might be 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 at 12 weeks in C-SBS group, while it decreased in P-SBS group. This difference might have resulted from the collagen portion of C-SBS, however, it is difficult to draw such a conclusion since it is still debatable whether the collagenated bone substitutes would have an advantage over the particulates in optimization of the grafted space (Alayan, Vaquette, Saifzadeh, Hutmacher, & Ivanovski, 2016; M. Araujo, Linder, Wennstrom, & Lindhe, 2008; Chang et al., 2015). The micro-CT data of the present study should be cautiously interpreted regarding the limitation in discriminating various kinds of tissues (Iida, Baba, Botticelli, Masuda, & Xavier, 2020; Iida et al., 2018). Nonetheless, since micro-CT is capable of three-dimensional volume measuring while the histology can only show two-dimensional view, it might be useful to conduct both analyses to reinforce each other.

Whether SBS was collagenated or not, less new bone was found in the center area compared to the other regions at 4 weeks. At 12 weeks, an increased new bone at the center could be seen. This ossification pattern seems obvious because the osteogenic factors mainly arrive from the native bone at the base of the sinus and Schneiderian membrane (Cho et al., 2014; Jakob et al., 2010), implying that adding collagen to the SBS does not have a benefit in acceleration of ossification in the center area. Significantly higher %FTA and lower %RMA were observed in C-SBS group compared to P-SBS group at 4 weeks near the membrane, which became similar between the two groups at 12 weeks. This finding reflects that the graft particles were more scattered in P-SBS group, while those in C-SBS group were more aggregated at the early stage, which might be logical regarding the mechanical property of C-SBS. Replacing the bone plates over the access window in both groups might have been advantageous in volume maintenance and ossification near the window and bone walls, however, previous animal researches which investigated the effect of bone plate repositioning reported diverse results. When compared to the coverage with resorbable collagen membrane, a previous research reported a superior result of bone plate repositioning in new bone formation (Moon et al., 2014), whereas other study did not show significant difference in both volume maintenance and new bone formation (Omori et al., 2018).

Despite its biocompatibility, xenogeneic collagen may inhibit tissue integration, especially when it is cross-linked (Bornstein, Bosshardt, & Buser, 2007; Schwarz et al., 2008). In histology of the present study, C-SBS group did not present atypical appearance, which might be owing to that the collagen added to C-SBS was native without cross-linking. This finding is similar to the previous experiments which evaluated collagenated xenograft (Alayan et al., 2016; M. G. Araujo & Lindhe, 2009). In previous studies, when collagenated xenogeneic bone block was grafted, augmented area decreased over time due to the resorption of the collagen (Chang et al., 2015), and new bone formation was delayed compared to the particle types in the early stage (1 and 5 weeks) (Lambert et al., 2013). Other research, however, presented consecutive histologic images showing well-maintained space even after the spontaneous collagen absorption (M. G. Araujo et al., 2010). Moreover, collagenated xenograft showed a similar performance in an animal sinus graft model in terms of hard tissue regeneration and space maintenance compared to the xenograft particulates combined with autogenous bone (Alayan et al., 2016). The present finding demonstrated that TAA of C-SBS group seemed well-preserved over time, and NBA and %NBA were comparable between the two groups at 4 weeks in histology.

β -TCP is responsible for the recruitment of the cells having osteogenic potential, whereas the slow-resorbing HA maintains the volume by compensating for the fast degradation of β -TCP (Yamada, Heymann, Bouler, & Daculsi, 1997). It might be assumed that a higher ratio of HA compared to β -TCP could be disadvantageous in new bone formation. It was, however, proven that ossification was comparable between the BCP having higher ratio of HA and that having lower ratio of HA (Lim et al., 2015). %NBA at 12 weeks in C-SBS and P-SBS groups (27.91% and 25.85% in mean, respectively) of the present study, significantly higher compared to 4 weeks, did not differ from the previously reported results, which was over 20% at the time point of later than 8 weeks when particulated (Lim et al., 2015) and collagenated substitutes were grafted (Lambert et al., 2013).

C-SBS group showed higher values of TAA, NBA and RMA compared to group P-SBS at both time points, whereas the differences between the two groups were only significant at 12 weeks. TAA was relatively well-preserved in C-SBS, which might be owing to the significantly increased NBA and a limited change in RMA. In group P-SBS, however, a slightly higher NBA and significantly lower RMA were found at 12 weeks compared to 4 weeks. Consequently, P-SBS demonstrated lower TAA (- 13.1%) than that of C-SBS group (- 4.72%) at 12 weeks compared to 4 weeks, similar to the results in radiographic analysis. Given that FTA and %FTA in C-SBS group were significantly higher than P-SBS group at 4 weeks and %FTA in C-SBS group was significantly lower at 12 weeks compared to 4 weeks, newly formed bone tissue might have resided the spaces which was previously dominated by fibrous tissue, and this speculation could be supported by the biological mechanism described in the previous study with serial histologic evaluation (M. G. 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.

Though rabbits are considered proper for maxillary sinus graft experiment (Stübinger & Dard, 2013), since the extraoral access window opening is preferred than the intraoral osteotomy due to easier manipulation, the window locates near the roof but rather far from the floor, opposite to the clinical situation (Lim et al., 2015). Further experiments with larger animals or clinical studies might be needed to verify the findings of the present study. Moreover, the present study tried to demonstrate the early healing with woven bone (4 weeks) and matured state with lamellar bone (12 weeks) regarding the rabbits' metabolism, three to four times faster than humans (Choi et al., 2012), however, more insights could have been presented if the observation at other time points were additionally conducted.

5. Conclusions

C-SBS grafted in rabbit sinuses resulted in higher augmented volume and area, as well as more newly formed bone, at 12 weeks compared to P-SBS.

Conflict of interests

The authors declare that they have no conflict of interests.

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Figure legends

Figure 1. Clinical photographs. After the incision made along the sagittal line of nasal bone and the full-thickness flaps reflection, lateral windows in circular shape, 5.5mm in diameter, were prepared bilaterally and removed gently to avoid the damage of the underlying Schneiderian membrane (a). Graft materials were applied based on the group allocation after sinus membrane elevation, P-SBS for one side (b) and C-SBS for contralateral side (c). The grafted sites were covered with the circular bone windows (d) and primarily closed state (e).

Figure 2. Radiographic analysis. (a) Representative micro-CT images. Three dimensionally reconstructed micro-CT images of 4 weeks (upper row) and 12 weeks (lower row) 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 row) and 12 weeks (lower row). Ant., anterior; Post., posterior; Lat., lateral; Med., medial. (b) Volume measurements (mm^3) of new bone (NBV), residual graft materials (RMV) and non-mineralized tissue (NMV). (c) The proportion of NBV (%NBV), RMV (%RMV) and NMV (%NMV) within TAV.

Figure 3. Representative histology at 4 weeks. Black squares (1 mm x 1 mm) shown in the slides with low magnification (x 20, scale bar = 1 mm) (a and b), indicate the separated regions (window, bone walls, center and membrane), which were highly magnified (x 100, scale bar = 200 μm). Well-augmented sinuses are observed in both C-SBS (a) and P-SBS (b) groups, showing more newly formed bones near the window, bone walls and membrane, compared to the center area. W: near the window; B1 and B2: near the bone walls; C: center area; M: near the membrane; NB, new bone; RM, residual bone graft material; FT, fibrous tissue; Asterisk, blood vessel.

Figure 4. Representative histology at 12 weeks. Black squares (1 mm x 1 mm) shown in the slides with low magnification (x 20, scale bar = 1 mm) (a and b), indicate the separated regions (window, bony walls, center and membrane), which were highly magnified (x 100, scale bar = 200 μm). Compared to 4 weeks, both C-SBS (a) and P-SBS (b) groups shows an increased newly formed bone with lamellated state, which is found throughout the augmented sinus including the center area as well as near the window, bone walls and membrane. W: near the window; B1 and B2: near the bone walls; C: center area; M: near the membrane; NB, new bone; RM, residual bone graft material; FT, fibrous tissue; Asterisk, blood vessel.

Figure 5. Histomorphometric analysis. (a) Histomorphometric analysis within the total augmented area (mm^2). (b) The proportion of new bone area (%NBA), residual graft material area (%RMA) and fibrovascular tissue area (%FTA) within the total augmented area. (c) The %NBA, %RMA and %FTA at the separated regions (near the window, near the bone walls, at the center and near the membrane) within the standardized region of interest (1 mm x 1 mm).

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Table 1. Radiographic analysis (mean $\pm$ standard deviations)

Healing period		TAV	NBV	RMV	NMV
4 weeks					
C-SBS group	Volume (mm ³)	263.38 $\pm$ 39.18	83.16 $\pm$ 13.29[†]	77.27 $\pm$ 10.20[†]	102.94 $\pm$ 21.19
	Proportion (%)	100	31.59 $\pm$ 2.16[†]	29.50 $\pm$ 2.74[†]	38.90 $\pm$ 3.94
P-SBS group	Volume (mm ³)	243.19 $\pm$ 23.27[†]	63.50 $\pm$ 11.33	72.33 $\pm$ 4.09[†]	107.36 $\pm$ 25.53
	Proportion (%)	100	26.29 $\pm$ 5.02[†]	29.97 $\pm$ 3.21[†]	43.74 $\pm$ 6.97
12 weeks					
C-SBS group	Volume (mm ³)	267.13 $\pm$ 62.08[*]	103.26 $\pm$ 10.50^{*,†}	47.27 $\pm$ 13.92^{*,†}	116.60 $\pm$ 68.01[*]
	Proportion (%)	100	40.01 $\pm$ 7.41[†]	19.45 $\pm$ 8.95[†]	40.55 $\pm$ 15.81
P-SBS group	Volume (mm ³)	200.18 $\pm$ 40.32^{*,†}	71.10 $\pm$ 7.58[*]	36.01 $\pm$ 18.25^{*,†}	93.07 $\pm$ 47.85[*]
	Proportion (%)	100	36.50 $\pm$ 6.80[†]	19.23 $\pm$ 11.04[†]	44.28 $\pm$ 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 ($p < 0.05$)

C-SBS, collagenated soft-type synthetic bone substitute; P-SBS, particulated synthetic bone substitute.

TAV, total augmented volume; NBV, new bone volume; RMV, residual graft material volume; NMV, non-mineralized volume.

Proportion (%) of NBV, RMV and NMV, calculated within TAV.

Table 2. Histomorphometric analysis (mean $\pm$ standard deviations)

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

(a) Measurement within the total augmented area

Healing period			Window	Bone walls	Center	Membrane
4 weeks	C-SBS group	%NBA	31.25 $\pm$ 7.30	37.13 $\pm$ 10.41	15.13 $\pm$ 6.96	22.13 $\pm$ 9.95
		%RMA	28.88 $\pm$ 10.84	20.38 $\pm$ 9.72	35.13 $\pm$ 7.57	27.38 $\pm$ 5.01[*]
		%FTA	39.88 $\pm$ 9.45[†]	42.50 $\pm$ 8.73	49.63 $\pm$ 8.75	50.50 $\pm$ 8.25^{*,†}
	P-SBS group	%NBA	29.50 $\pm$ 10.24	33.00 $\pm$ 6.67	11.00 $\pm$ 5.35	20.00 $\pm$ 8.73
		%RMA	25.75 $\pm$ 12.28	21.63 $\pm$ 5.64	41.50 $\pm$ 9.87	42.63 $\pm$ 4.74[*]
		%FTA	44.75 $\pm$ 11.76	45.38 $\pm$ 9.62	47.50 $\pm$ 9.75[†]	37.38 $\pm$ 8.83[*]
12 weeks	C-SBS group	%NBA	36.63 $\pm$ 7.85	40.75 $\pm$ 8.71[*]	22.88 $\pm$ 8.08	31.00 $\pm$ 11.15
		%RMA	32.28 $\pm$ 5.78	23.50 $\pm$ 9.34	32.63 $\pm$ 6.76	35.13 $\pm$ 11.79
		%FTA	31.00 $\pm$ 5.29[†]	35.75 $\pm$ 6.18	44.50 $\pm$ 8.96	33.88 $\pm$ 3.23[†]
	P-SBS group	%NBA	35.25 $\pm$ 8.73	33.50 $\pm$ 8.91[*]	24.00 $\pm$ 7.45	26.50 $\pm$ 7.17
		%RMA	29.38 $\pm$ 7.17	27.25 $\pm$ 6.76	40.71 $\pm$ 7.26	37.50 $\pm$ 8.05
		%FTA	35.38 $\pm$ 5.24	39.25 $\pm$ 9.25	35.29 $\pm$ 6.09[†]	36.00 $\pm$ 5.71

(b) Measurements within the separate regions

* (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 ($p < 0.05$)

C-SBS, collagenated soft-type synthetic bone substitute; P-SBS, particulated synthetic bone substitute.

TAA, total augmented area; NBA, new bone area; RMA, residual graft material area; FTA, fibrovascular tissue area

Proportion (%) of NBA (%NBA), RMA (%RMA) and FTA (%FTA) within TAA (Table 2a) and the standardized region of interest of 1 mm x 1 mm (Table 2b).

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