

Micro-CT and Histological analysis of Sinus Floor Augmentation with Biphasic Calcium Phosphate and Deproteinized Bovine Bone Mineral: A Randomized controlled clinical trial

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INTRODUCTION

Deproteinized Bovine Bone Mineral (DBBM) is a widely used xenograft, which has well documented clinical outcomes in maxillary sinus augmentation in animal and human studies due to having a slow resorption rate, acting as a scaffold for the growth of new native bone and maintaining the stability of the augmented volume. Biphasic Calcium Phosphate (BCP) is a well-documented synthetic biomaterial, similar in composition and structure to natural bone tissue and good osteoconductive properties. Both BCP and DBBM are suitable for sinus augmentation with comparative results. However, the clinical studies between DBBM and BCP are limited, in particular for lateral window sinus floor elevation.

OBJECTIVE

To compare the micro-CT, and histological analysis between Biphasic Calcium Phosphate (BCP) of 70/30 ratio and deproteinized bovine bone mineral (DBBM) in sinus augmentation.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2020-038) and Trial registration (TCTR20201109003). Patients with residual alveolar bone height less than 3 mm requiring sinus floor elevation and bone augmentation via lateral approach prior to implant placement for single area of upper posterior maxilla at the Faculty of Dentistry, Chulalongkorn University from July 2020 to July 2022 were invited to enroll in the study. The 12 patients who met the criteria and signed informed consent were assigned to either BCP 70/30 ratio (DenaOss-M[®]) for test group (n=6) or DBBM (Cerabone[®]) for control group (n=6) by block randomization.

All sinus floor elevations with lateral approach were conducted under local anesthesia and augmented with the respective biomaterial. Lateral window was covered with a resorbable membrane (Collprotect[®]) (Figure 1a-b). After at least 6 months of healing, patients were scheduled for the bone biopsy and surgical implant placement in the augmented site under local anesthesia. Bone biopsy was collected from the entire augmented bone by using a 2.1-mm-internal-diameter trephine bur (Trephine Ejection Kit, Meisinger[®]). Thereafter, the osteotomy was conducted based on the implant dimensions and the implant manufacturer. The bone biopsies were fixed in 10% neutral buffered formalin at room temperature for 24 hours and then underwent micro-CT and histological processing.

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Micro-CT analysis

The bone specimens were enclosed in small-fitted tubes and proceeded to micro-CT analysis using microtomography system (Scanco μ CT35, SCANCO Medical AG) by one investigator. Bone specimens were scanned and reconstructed at 70 kV/100 μ A/8W in high resolution mode. The threshold, 390-1000, was chosen to segment out graft material and calculate volume segments three-dimensionally based on the volume of the cylinder.

The total volume (TV), bone volume (BV), residual graft material volume (GV), bone volume fraction (BV/TV ratio) and residual graft material fraction (GV/TV) were measured using a 3-dimensional analysis software (Scanco Medical). Then, the non-mineralized connective tissue volume (nMT) was calculated as the relative volume; $TV = BV + GV + nMT$.

Histological analysis

Each specimen was decalcified in 10% EDTA (pH7.4) and embedded in paraffin. The specimens were dehydrated through a series of graded ethanol and clearing with xylene. Bone specimens were embedded in paraffin blocks and sectioned along the longitudinal axis to achieve 5 μ m in thickness, then subjected for hematoxylin-eosin (H&E) staining.

The new bone formation, connective tissue and graft materials remaining area were identified using microscope (Olympus CX41RF, Olympus Corporation) and calculated the percentage of those area to the total area of the specimens using image viewer software (OlyVIA v2.9.1, Olympus).

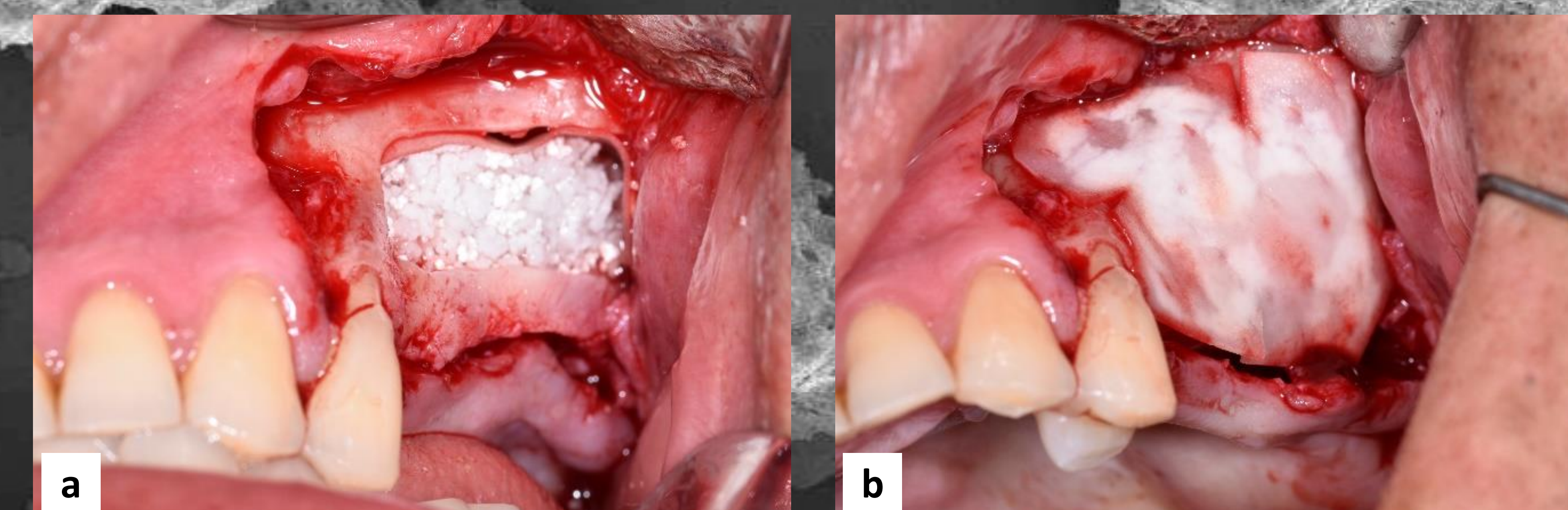


Figure1 a) Sinus floor elevations (Lateral approach) and augmentation with the respective biomaterial to either BCP 70/30 ratio (DenaOss-M®) for test group (n=6) or DBBM (Cerabone®) for control group (n=6), b) Lateral window was covered with a resorbable membrane (Collprotect®).

Statistical analysis

All collected data were analyzed using the Statistical Package for Social Science version 22.0 (SPSS Inc). Normality of the distribution of the data at baseline was assessed with Shapiro-Wilk test. The difference between the means of the micro-CT, histological results were assessed for statistical significance among three groups by the Independent-Samples Kruskal-Wallis Test and Dunn's post hoc analysis with confidence interval of 95%.

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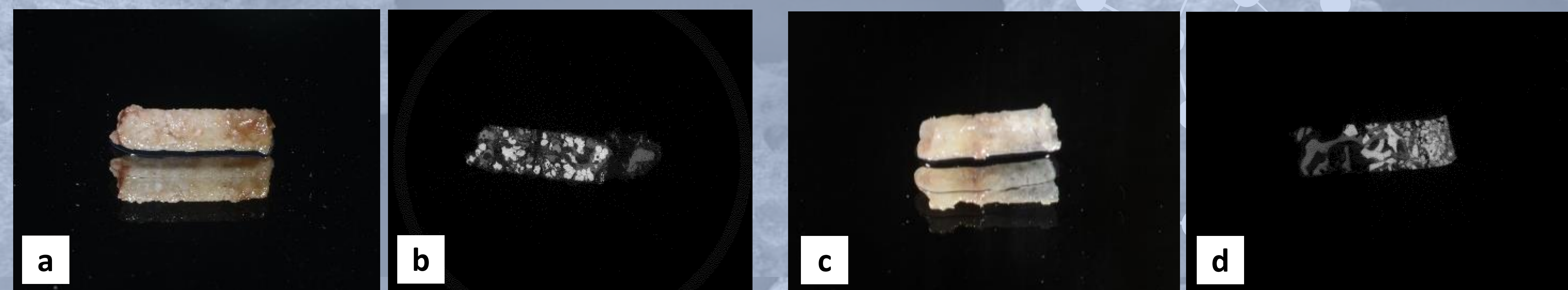


Figure 2 The trephined bone samples and corresponding micro-CT images of augmented sinus using BCP (a, b) and DBBM (c, d).

The micro-CT analysis	Group		p-value
	BCP 70/30 ratio	DBBM	
Total volume (TV, mm ³)	50.60 ± 32.46	46.19 ± 17.48	.776
Bone volume (BV, mm ³)	23.03 ± 19.32	18.50 ± 6.52	.606
Ratio of bone volume to total volume (BV/TV ratio)	0.4151	0.4097	.941
The residual graft material volume (GV, mm ³)	4.85 ± 3.81	6.13 ± 5.30	.641
Ratio of residual graft material to total bone volume (GV/TV ratio)	0.0997	0.1441	.431

Table 1 The micro-CT analysis for the total volume (TV), bone volume (BV), the residual graft material volume (GV), the ratio of bone volume to total volume (BV/TV ratio) and the ratio of residual graft material to total volume (GV/TV ratio) of both groups (mean ± standard deviation).

RESULTS

All maxillary sinus augmentation procedures were performed without perforation of the Schneiderian membrane and a total of 12 bone specimens from both two groups were collected from the entire augmented bone at the size of the intended implant length and processed for micro-CT and histologic analysis.

Six male and six female patients were operated (the average age was 56.75±13.55 years old with no significant difference between two groups). Seven specimens of augmented bone were harvested from first molar and five specimens from second molar site of maxilla at 9-10 months.

Micro-CT analysis

The micro-CT showed new bone formation and remaining graft particles for both groups (Figure 2a-d). The means of total volume (TV), bone volume (BV) and residual graft material volume (GV) in the BCP group was 50.60, 23.03 and 4.85 mm³ respectively, whereas the same was 46.19 mm³, 18.50 mm³ and 6.13 mm³, for the DBBM group respectively. The average ratio of bone volume to total volume (BV/TV) for BCP group was 0.4151 (41.51%) and the average ratio of residual graft material to total volume (GV/TV) was 0.0997 (9.97%). The same in the DBBM group was BV/TV of 0.4097 (40.97%) and GV/TV of 0.1441 (14.41%), respectively. No statistically significant difference was found between the two groups for all parameters (p-value >0.05) (Table 1).

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RESULTS

Histological analysis

The slide of mid-section along the longitudinal axis was selected for the analysis using microscope (Olympus CX41RF, Olympus Corporation) and quantified using image viewer software (OlyVIA v2.9.1, Olympus). The mean percentage of new bone area to the total area was 31.43% in BCP group and 30.09% in the DBBM group. The residual graft particles occupied 40.76% of the area in the BCP group, whereas 45.06% in the DBBM group. There was no statistically significant difference in any of the outcomes between the two groups (p-value >0.05) (Table 2).

Group	The Percentage of proportion to total area		
	The new bone formation area (%)	The connective tissue area (%)	The graft materials remaining area (%)
BCP 70/30 ratio	31.43 ± 14.36	27.81 ± 19.02	40.76 ± 16.05
DBBM	30.09 ± 9.86	24.84 ± 17.80	45.06 ± 17.19
p-value	1.000	.749	.631

Table 2 The histological analysis for the percentage of new bone formation, connective tissue, and graft materials remaining area to total area of both (mean ± standard deviation).

DISCUSSIONS

The present clinical trial was the first attempt to investigate and compare radiographic and histological outcomes as well as gene expression in sinus augmented with BCP of 70/30 ratio and DBBM through the lateral window approach.

Both micro-CT and histological showed similar outcomes for both groups. The average ratio of BV/TV was slightly higher in BCP group and the area corresponding to new bone was slightly greater in the BCP augmented biopsies but without reaching a level of statistical significance.

The results of this study appear similar to previous studies (12,16,20) where outcomes of sinus augmentation with DBBM and 60/40 of BCP were assessed at 6-8 months post-surgery, with the exception of one study (11) which showed higher newly formed bone and lesser residual graft material when BCP was used. A meta-analysis (21) supported the findings of similar outcomes with regards to new bone and residual particles volume between the two biomaterials. However, this meta-analysis included studies with both low and high HA/TCP ratio.

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DISCUSSIONS

In a split mouth study utilizing both micro-CT and histology in five patients, de Lange et al.⁽²²⁾ compared outcomes of sinus augmentation with 60/40 of BCP and DBBM. Although the new bone volume and residual particle volume, degree of bone mineralization, amount of osteoclast and osteocyte were similar, bone biopsies of the BCP group had relatively more osteoid than DBBM group, still however, not reaching a difference of statistical significance. A similar study by Oh et al. in 2019⁽¹⁶⁾ also suggested a trend of higher new bone fraction and lower volume of remaining bone graft for BCP, without again reaching significant difference.

The results of this study should be seen under the limitations of the methodology. All re-entry biopsies and implant placement surgeries were planned at 6 months post-sinus augmentation, however due to pandemic and other restrictions arising from COVID-19, the respective procedures were conducted at time points ranging from 9-10 months after the sinus augmentation.

CONCLUSIONS

Based on micro-CT and histological analysis, the use BCP with HA/ β -TCP ratio of 70/30 or DBBM particles resulted in similar outcomes in terms of new bone formation and volume of residual graft.

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