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The Use of Autogenous Bone Mixed with a Biphasic Calcium Phosphate in a Maxillary Sinus Floor Elevation Procedure with a 6-Month Healing Time: A Clinical, Radiological, Histological and Histomorphometric Evaluation

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Abstract: Background: In this study it is evaluated whether autogenous bone mixed with biphasic calcium phosphate (BCP) used in a maxillary sinus floor elevation (MSFE) leads to improved bone formation. Materials and methods: In five patients a unilateral MSFE was performed. Histological and histomorphometric analyses were performed on bone biopsies that were obtained 6 months after MSFE during dental implant surgery. Results: The average vital bone volume was 29.9% of the total biopsy (BV/TV, SD ± 10.1) of which 7.1% was osteoid (OV/BV, SD ± 4.8). The osteoid surface (OS/BS) covered 26.0% (SD ± 13.4) of the bone surface. The BS/TV covered 4.7 mm^2/mm^3 (SD ± 2.3). Compared with previous studies the analyses showed a difference for trabecular thickness (Tb.Th.) and osteoid surface (OS/BS), but not for BV/TV, OV/BV and the number of osteoclasts. Conclusion: MSFE with autogenous bone mixed with BCP shows an amount of newly formed bone that is comparable with the findings from the previously published 6-month study with pure BCP. However, a better distribution of the new bone over the entire biopsy was observed.

Keywords: bone substitute; sinus floor augmentation; maxillary tuberosity

1. Introduction

Lack of vertical bone height in the posterior maxilla limits standard dental implant placement. In order to increase the vertical dimension in the posterior maxilla, a maxillary sinus floor elevation (MSFE) with graft material can be performed. [1,2] MSFE is a predictable preimplant surgical procedure with a high survival rate of the dental implants, exceeding 93.8% [3]. Pjetursson systematically reviewed the success of dental implants placed in combination with MSFE, and reported an implant survival rate after 3 years up to 98.3%, using rough surface dental implants, related to non-augmented jawbone [4].

Due to its osteoinductive, osteoconductive and osteogenic properties autogenous bone is still considered the golden standard as graft material [5–12]. This osteogenic capacity of autogenous bone grafts may be attributed to the presence of bone morphogenic proteins, attracting osteogenic cells from the adjacent tissues, thus mobilizing other growth factors essential for bone regeneration [4].

Bone grafts can be obtained either intraorally or extraorally. Harvesting these bone grafts has drawbacks, such as an extended operating time, donor site morbidity, hospitalization, unpredictable resorption rate of the bone grafts [9,13–15] and sensory disturbances [5,16,17]. Different types of bone substitutes have been developed to overcome these drawbacks (e.g., allograft, xenograft, alloplast and mixtures of different materials) [18,19]. The comparison of bone grafts from different origins has been the subject of study extensively. Meta-analyses have confirmed the superiority of autogenous bone grafts over allografts, xenografts and synthetic bone grafts with respect to new bone formation [20–22]. Ideally, such a bone substitute should be biomechanically stable, capable of degradation within an appropriate time frame, exhibiting osteoconductive, osteogenic and osteoinductive properties, biologically safe, low patient morbidity, volume stable, easy available on the market with low production costs and providing a favorable environment for the entry of blood vessels and bone-forming cells [23–26]. For cranio-maxillofacial purposes, autografts (due to the drawbacks) play a minor role today. In terms of costs and benefits, allografts were the most commonly used bone graft in the United States and xenografts were the most commonly used grafts in Europe [27]. Allografts are tissue grafts from a donor of the same species as the recipient, but not genetically identical, with a risk of immune responses, infection transmission and are known to have high failure rates with long-term use. Additionally, many osteoinductive properties are lost during the manufacturing of allografts [21,28–30]. In Europe, the use of allografts is often abandoned in clinical practice, advised by the Medical Device Regulation [31].

Xenografts are usually of porcine or bovine origin. The use of xenografts involves a number of risks and complications, e.g., disease transmission (Creutzfeldt-Jakob disease), immune responses, foreign body response and chronic inflammation. The production process can lead to a lack of viable cells and reduced osteoinductive properties [32]. For cranio-maxillofacial applications, bovine xenografts are allowed for safe use without reports of transmissible spongiform encephalopathies (TSE) and bovine spongiform encephalopathy (BSE) risk [27,33,34].

Alloplastic (synthetic) grafts are currently most commonly used for their osteoconduction, hardness and acceptability by bone. Most alloplasts consist of hydroxyapatite, a naturally occurring ceramic that is also the primary mineral of bone, or other calcium phosphate compounds, such as β–tricalcium phosphate (β–TCP). Calcium phosphates, like hydroxyapatite (HA), β–tricalcium phosphate (β–TCP) or biphasic calcium phosphate (BCP), a mixture of HA and β-TCP, are osteoconductive, biocompatible and simulate the chemical composition of natural bone. Calcium phosphates do not induce a sustained foreign body response or toxic reaction [35–37]. Hydroxyapatite is, at a physiological pH, the least dissolvable of the naturally occurring calcium phosphates, making it relatively resistant to resorption and suitable for clinical use [9,38–40]. β-TCP does not have osteoinductive properties and resorbs rather quickly, but not necessarily at the same rate as the formation of new bone [11,12,41–44].

In previous studies a mixture of 60% HA and 40% β-TCP (BCP) as graft material in an MSFE procedure demonstrated sufficient bone (re)generation after 6 months for placement of dental implants, although remnants of BCP could still be observed, indicating that the process of bone substitution was not yet completed [12,45]. After 9- and 12-months healing time, a high bone formation was still observed and remnants of BCP particles could still be detected [46]. A significant lower total bone volume is found for each biomaterial or combination of different graft materials compared to autogenous bone [11,47].

This study was based on the use of an autogenous bone graft, harvested from the maxillary tuberosity, in an MSFE procedure, as the golden standard [10]. Though, if autogenous bone graft volume is insufficient, a bone substitute can be supplemented to achieve sufficient graft volume for

completion of the MFSE procedure, thereby avoiding a second surgical intervention and minimizing donor site morbidity. Referring to previous studies on the use of BCP's only [12,46], it would be interesting to further study the use of a mixture of autogenous bone and Straumann® Bone Ceramic (SBC), a BCP (Straumann Holding AG, Basel, Switzerland).

Therefore, the purpose of this study was to determine whether a mixture of autogenous bone and BCP in an MSFE procedure leads to an improved bone formation compared to an MSFE with pure BCP and ideally a total remission of BCP remnants in the entire augmented MSFE area, eventually leading to a sufficient bone structure, qualitatively and quantitatively, for dental implant placement. Five subsequent patients were evaluated clinically, radiologically, histologically and histomorphometrically after a 6-month healing period. The results were compared with the results of the previously reported studies with pure BCP (no autogenous bone added), after 6-, 9- and 12-month healing time, which were conducted according to the same study protocol [12,46].

2. Materials and Methods

2.1. Study Population

Five subsequent, healthy patients (3 males and 2 females), with a partially edentulous posterior maxilla with vertical dimensions of less than 8 mm but preferably more than 4 mm, requiring dental implants for dental rehabilitation, were included in this study and underwent a unilateral MSFE procedure 6 months before the dental implants were placed. The average age was 61 years (range: 51–70).

The study was performed in accordance with the principles of the Declaration of Helsinki. Since the study involved a Conformité Européenne (CE)-marked device (biphasic calcium phosphate) being used for its intended purpose (use as carrier material for bone augmentation in sinus floor elevation procedures) and the harvested material is regarded as surgical waste, no specific regulatory approval from a medical ethical committee was required. Patients provided written consent before the study-related MSFE and dental implant procedures were undertaken. Biopsies were retrieved during dental implant surgery, with trephine drills, implicating the tissue in the hollow drill is considered surgical waste and no extra inconvenience to the patient.

2.2. Maxillary Sinus Floor Elevation Procedure

A unilateral two-stage MSFE was performed as described by Tatum [2] and similar to the previously reported 6-month and 9–12-month studies with pure BCP [12,46]. MSFE surgery was performed under local anesthesia. All patients took amoxicillin 500 mg orally, 4 times daily during 7 days, starting one day preoperatively. Oral hygiene was performed with 0.12% chlorhexidine-digluconate 3 times daily for two weeks. The autogenous bone graft was harvested from the maxillary tuberosity at the implant site with mallet and chisel and grinded in small pieces. Before filling the created area at the sinus bottom, the bone graft was mixed in equal proportions with BCP granules (60% HA and 40% β–TCP, Straumann® Bone Ceramic, Straumann Holding AG, Basel, Switzerland). No collagen membrane was placed to cover the lateral window [48] (Figure 1A–E).

2.3. Dental Implant Surgery and Biopsy Retrieval

The dental implants were placed six months after the MSFE procedure. Implant osteotomies were made and biopsies were obtained from the previously grafted area at the planned dental implant positions, using trephine drills with an external diameter of 3.5 mm and internal diameter of 2.5 mm (Straumann® trephine drill) with copious irrigation of sterile saline. In the five patients 11 standard plus, regular neck, soft tissue level Straumann® SLA dental implants with a diameter of 4.1 mm and a length of 10 or 12 mm were placed. (Figure 1F). The dental implants were left to integrate in a non-submerged unloaded fashion. A panoramic radiograph was taken immediately after dental implantation to allow postoperative radiological evaluation. After 10–14 days the Gore-Tex® (W.L.

Gore and Associates, Newark, DE, USA) sutures were removed and, if needed, provisional prosthetics were adapted to the new situation. Loading of the dental implants was prohibited for three months. After osseointegration of the implants, a restorative dentist fabricated and placed the superstructures.

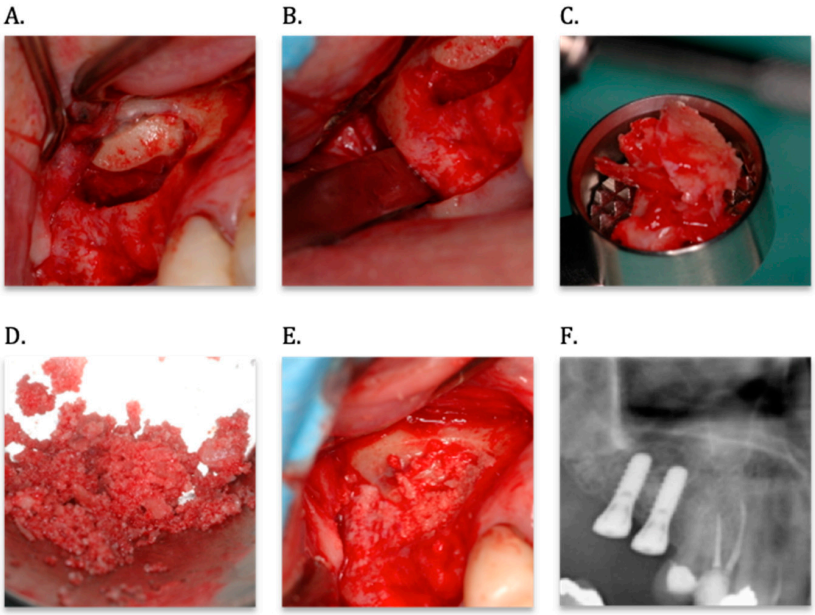


Figure 1. Maxillary sinus floor elevation (MSFE) procedure using a mixture of autogenous bone from the maxillary tuberosity and a biphasic calcium phosphate (BCP). (**A**) The preparation of the top hinge door in the lateral window of the right maxillary sinus. (**B**) Harvesting of an autogenous bone graft from the maxillary tuberosity with a chisel at the same surgical side and during the same procedure. (**C**) The harvested bone graft is grinded in smaller pieces by means of a bone mill. (**D**) The milled bone graft is mixed with a biphasic calcium phosphate (Straumann® Bone Ceramic 60:40). (**E**) Area between the lifted lid and the maxillary sinus floor is filled with the mixed bone graft. (**F**) Radiograph taken directly after the insertion of two Straumann® SLA dental implants in the augmented right posterior maxilla (6 months after MSFE).

2.4. Clinical Evaluation

One experienced oral and maxillofacial surgeon (C.M.T.B.) assessed clinically all 11 inserted dental implants for good primary stability. At abutment connection the osseointegration was tested with a 35 N cm torque. All placed Straumann® SLA dental implants resisted the applied 35 N cm torque.

2.5. Radiological Evaluation

According to the same study protocol as previously reported [12,46], panoramic radiographs were taken at patient's intake (T0); immediately after the MSFE procedure (T1); immediately after dental implant placement (T2); 1 year after dental implant placement (T3) and 5 years after dental implant placement (T4). On the panoramic radiographs changes in tissue height (mm) of the grafted area were measured at the implant sites on all time points. An average magnification of 1.25 was taken into account to calculate the actual tissue heights.

2.6. Biopsy Processing and Analyses

Bone biopsies were prepared for histology according to previously described procedures [49]. In short, the biopsies were fixed overnight in 4% phosphate-buffered formaldehyde and transferred to alcohol 70% [50]. After dehydration, the bone specimens were embedded without prior decalcification in methyl methacrylate supplemented with 20% dibutylphtalaat and 0.008 g/mL Lucidol. The biopsies were cut into 5 µm longitudinal sections (Polycut S., Leica microtome type sm2500s, Leica, Wetzlar,

Germany). Goldner's trichrome staining was used to evaluate bone mass indices and osteoid surface [51]. Tartrate resistant acid phosphate (TRAP) staining was performed to visualize osteoclasts. Von Kossa staining was performed to visualize mineralized tissue.

2.7. Qualitative Histological Analysis

Qualitative assessment included screening the presence of BCP (Straumann® Bone Ceramic) remnants, clearly visible in the Von Kossa staining and a judgment of the vitality of the bone tissue. Moreover, the tissue was screened for inflammatory infiltrate. Three independent observers detected semi-quantitatively BCP particles and classified the particles into quartiles (<25% of BCP, 25%–50%; of BCP, 50%–75% of BCP and >75% of BCP).

2.8. Quantitative Histomorphometric Analysis

Quantitative measurements were performed semiautomatically using a digitizer and image analysis software (Osteomeasure, Atlanta, GA, USA). Since it was difficult to distinguish the exact border between augmented and native bone, histomorphometric measurements were executed over the total section of the biopsy, including newly formed and native bone. The parameters were measured in consecutive fields of a complete section, in four 150 µm-separated sections throughout the biopsy, covering a total measured area of 60 mm^2. Nomenclature was used according to the American Society for Bone and Mineral Research (ASBMR) nomenclature committee [52].

The biopsy was examined for the following parameters:

Parameters evaluating vital bone mass/bone structure:

- Vital bone volume (BV/TV): percentage of the total section that is vital bone tissue (%).
- Bone surface (BS/TV): BS expressed as a fraction of the total vital bone volume (mm^2/mm^3).
- Thickness of bone trabeculae (Tb.Th; µm).

Parameters evaluating bone turnover:

- Osteoid volume (OV/BV): percentage of the vital bone tissue section that is osteoid (%).
- Osteoid surface (OS/BS): osteoid-covered surfaces expressed as the percentage of the total BS (%), to measure new vital bone formation.
- Osteoid thickness (O.Th; µm)
- Number of osteoclasts (N.Oc/BPM) per mm^2 total area.

2.9. Statistical Analysis

Results are expressed as the mean plus or minus standard deviation. The results of this study were compared to the results of previously reported experiments, which were conducted in our institution in a similar manner by use of a non-parametric Kruskal–Wallis test.

3. Results

3.1. Clinical Evaluation

All five patients responded identical. None of them displayed postoperative infections, neither after the MSFE procedure nor after the placement of dental implants. During the insertion of the dental implants, it was observed that the graft material was well vascularized. Although there was a clear demarcation between the grafted area and the original bone of the alveolar process, there was continuity between the graft and the original bone. Although bone substitute particles could still be recognized in the retrieved tissue specimen, the drill remained stable during implant bed preparation. Clinically, all particles appeared to be well integrated in newly formed tissue. All dental implants osseointegrated well and could be loaded with fixed prostheses three months after implant surgery. There was no loss of dental implants during the 5-year follow-up.

3.2. Radiological Evaluation

The results of the alveolar tissue height measurements on panoramic radiographs in time are shown in Table 1. On average an 8.7 mm (SD ± 1.6) increase in height of the grafted area was accomplished using the mentioned MSFE.

Table 1. Alveolar tissue height measurements on panoramic radiographs (in true mm) in five patients in whom a maxillary sinus floor elevation (MSFE) procedure was performed with a mixture of autogenous bone from the maxillary tuberosity and Straumann® Bone Ceramic (60:40) and 6 months healing time.

Patient (*N*)	Gender/Age	Implant Site	T0	T1	Increase	T2	T3	T4
1	M/53	15	8.0	16.2	8.2	16.1	16.2	16.0
		16	6.1	14.1	8.0	15.0	15.0	15.0
2	M/70	16	6.8	14.8	8.0	16.0	13.7	15.6
		17	3.6	12.4	8.8	14.0	13.3	12.1
3	M/68	14	5.6	11.5	5.9	12.1	11.3	10.4
		15	4.6	14.1	9.5	14.1	13.0	12.9
		16	4.3	14.1	9.8	14.2	12.7	12.6
4	F/64	15	9.3	17.1	7.8	16.8	14.1	14.0
		16	6.0	14.9	8.9	16.9	15.8	15.7
5	M/51	16	9.0	18.1	9.1	17.6	16.2	NA
		17	5.8	18.0	12.2	16.0	16.7	NA
Mean	61.2	-	6.3	15.0	8.7	15.3	14.4	13.8
SD	-	-	1.9	2.1	1.6	1.6	1.7	1.9

M, male; F, female; age in years at biopsy retrieval; tissue height corrected for magnification (×1.25) on panoramic radiograph; T0: (native bone height) preoperative alveolar bone height; T1: directly after MSFE procedure; T2: immediately after dental implant placement (6 months after MSFE); T3: 1 year after dental implant placement; T4: 5 years after dental implant placement; NA: not available.

The measured tissue height appeared to be stable between 1- and 5-years follow-up (Figure 2). The results of the present study (6-month mixed graft) when compared with the results of our former MSFE procedures with pure BCP, also show a stable gain in tissue height (a total of four studies).

An overview of the results of the present study (6-months mixed graft) and the results of previously reported studies with pure BCP after 6-, 9- and 12 months healing time [12,44] is shown in Table 2. Comparing the results, the gained tissue height appears to be stable in all four studies.

Table 2. Alveolar tissue height measurements on panoramic radiographs (in true mm), overview of the mean values of the 6-month mixed graft group, autogenous bone mixed with Straumann® bone ceramic (60:40), compared to the 6-, 9- and 12-month results with pure Straumann® bone ceramic (60:40), as previously published, in a maxillary sinus floor elevation (MSFE) procedure. Tissue height corrected for magnification (×1.25) on panoramic radiographs.

Patient Group	T0	T1	Increase	T2	T3	T4
6-month mixed	6.3	14.8	8.7	15.3	14.4	13.8
6-month (*)	6.5	15.2	8.7	14.6	13.4	NA
9-month (**)	6.4	13.9	7.5	14.1	13.3	13.2
12-month (**)	4.4	13.8	9.3	13.6	13.4	13.8

T0: (native bone height) preoperative alveolar bone height; T1: directly after MSFE procedure; T2: immediately after dental implant placement; T3: 1 year after dental implant placement; T4: 5 years after dental implant placement; NA: not available. * Study by Frenken et al. (2010) [12]; ** Study by Bouwman et al. (2017) [46].

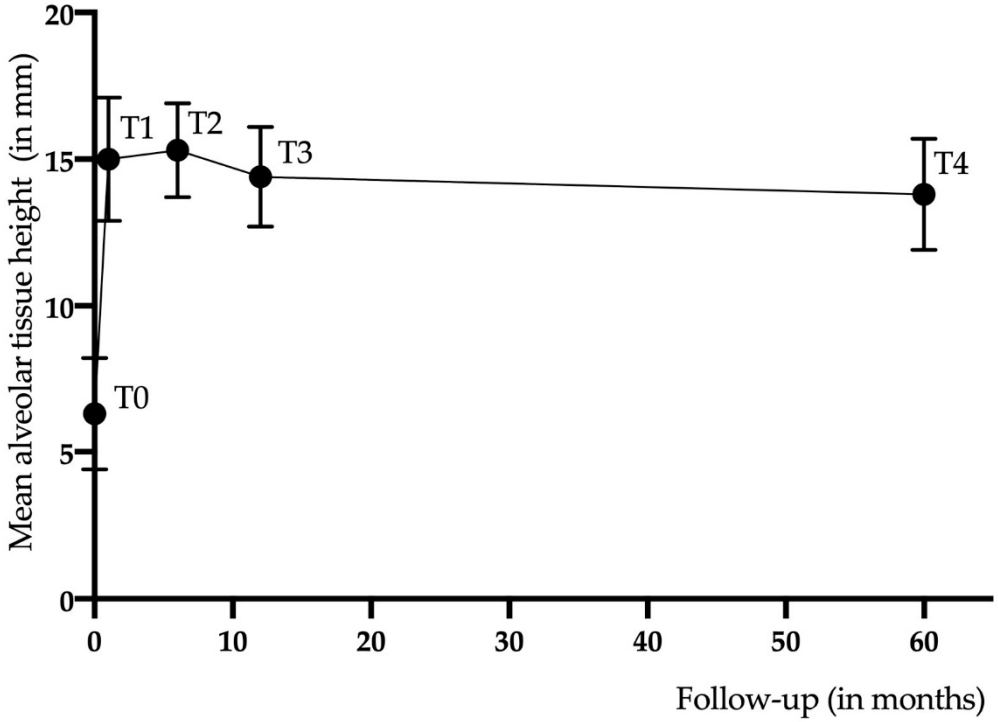


Figure 2. Mean alveolar tissue height (in true mm) over a 5-year period in five patients in whom a maxillary sinus floor elevation procedure was performed with autogenous bone from the maxillary tuberosity and Straumann® bone ceramic (60:40) with 6 months healing period. T0: (native bone height) preoperative alveolar bone height (SD ± 1.9); T1: directly after a maxillary sinus floor elevation (MFSE) procedure (SD ± 2.1); T2: immediately after dental implant placement (6 months after an MSFE; SD ± 1.6); T3: 1 year after dental implant placement (SD ± 1.7); T4: 5 years after dental implant placement (SD ± 1.9).

3.3. Qualitative Histological Evaluation

The histological evaluation in six biopsy specimens was executed on the complete section, comprising native bone, newly formed bone and residual graft material. BCP particles were scattered and detected throughout the entire biopsy from caudal to cranial (Figures 3 and 4a). The BCP particles were surrounded by connective tissue, osteoid islands and newly formed bone. The newly formed bone comprised of both woven and lamellar bone; it appeared as vital bone tissue containing osteoblasts, osteoid covering the border of BCP and osteocytes inside bone lacunae (Figure 4b). No inflammatory cells in the tissue adjacent to the bone substitute particles were found during histological analysis. Bone marrow-like tissue, including blood vessels, was observed in between the bone trabeculae. Fragments of the BCP particles as shown by Von Kossa staining showed in four biopsies <25% of BCP, in one biopsy 25%–50% of BCP and in one biopsy 50%–75% of BCP. The presence of >75% of BCP fragments was not detected.

3.4. Quantitative Histomorphometric Evaluation

Table 3 shows the individual histomorphometric indices. An average vital bone volume of 29.9% (BV/TV) was measured in the complete biopsies (SD ± 10.1) of which 7.1% (OV/BV, SD ± 4.8) was osteoid. The osteoid surface (OS/BS) covered 26.0% (SD ± 13.4) of the bone surface. The BS/TV covered 4.7 mm^2/mm^3 (SD ± 2.3). As can be read from Table 3, Patient #4 had extremely high values for trabecular bone volume (BV/TV and Tb.Th.). This high BV/TV is in accordance with the high T0 value, most likely caused by an oblique section of the cortical maxillary sinus wall (Table 1). A low bone surface (BS/TV: 2.2 mm^2/mm^3) and a high osteoid thickness (O.Th: 481.9 µm) were observed in the bone biopsy of patient #5 (Table 3).

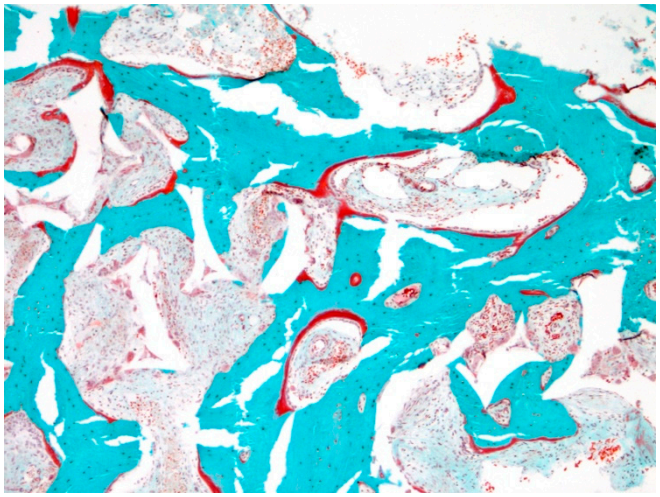
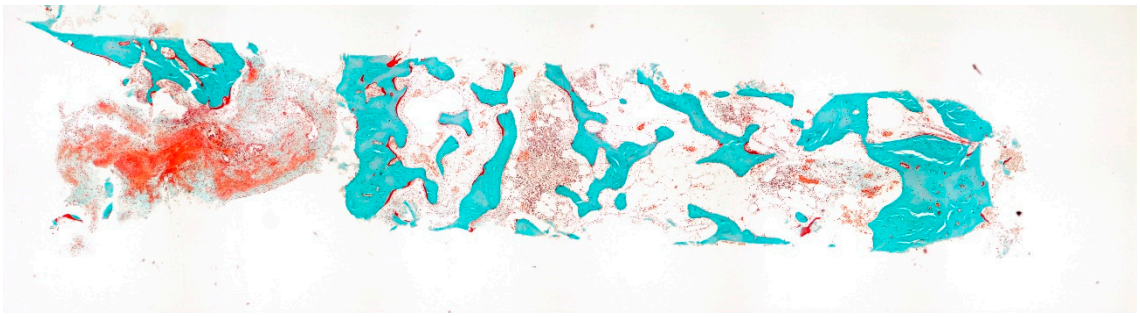
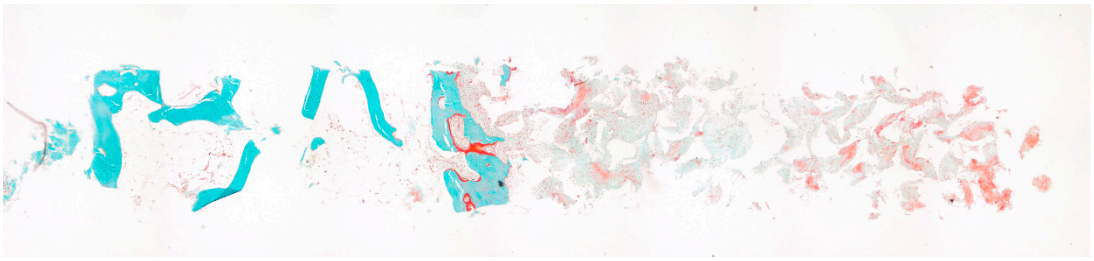


Figure 3. Increased bone formation following the shape of the grafted particles in a maxillary bone biopsy after a maxillary sinus floor elevation procedure from a patient with autogenous bone mixed with Straumann® bone ceramic (60:40) after 6 months healing time, stained with Goldner trichrome staining. No Howship's lacunae could be detected on the characteristic outlines of the calcium phosphate particles. (original magnification ×100).



(**a**)



(**b**)

Figure 4. (**a**) Overview of an example of a bone biopsy from the maxilla of a patient, 6 months after a maxillary sinus floor elevation procedure using a mixture of autogenous bone and Straumann® bone ceramic (60:40), stained with Goldner trichrome staining. Bone is scattered throughout the entire biopsy (original magnification ×10). (**b**) Overview of a not previously shown bone biopsy from the maxilla of a patient, 6 months after a maxillary sinus floor elevation procedure using pure Straumann® bone ceramic (60:40) as studied by Frenken et al. [12], stained with Goldner trichrome staining. Bone formation at the first 3 mm immediately cranially from the former floor of the maxillary sinus (original magnification ×10).

Table 3. Histomorphometric evaluation of the six biopsies from five patients in whom a maxillary sinus floor elevation (MSFE) procedure was performed with a mixture of autogenous bone from the maxillary tuberosity and Straumann® bone ceramic (60:40) after 6 months healing time.

Patient (*N*)	Gender/Age	Biopsy Location	BV/TV (%)	BS/TV (mm^2/mm^3)	Tb.Th (µm)	OV/BV (%)	OS/BS (%)	O.Th (µm)	N.Oc/BPM $1/mm^2$
1	F/53	16	40.5	2.4	335.8	6.9	38.8	342.2	0.62
2	M/70	16	29.2	6.8	85.5	3.2	11.1	10.7	1.52
3	M/68	14	19.7	6.9	57.5	7.6	17.9	11.6	-
-	-	15	29.3	6.5	91.2	3.5	12.9	11.7	2.54
4	F/64	15	42.4	3.5	290.0	5.1	35.3	97.5	1.91
5	M/51	16	18.3	2.2	163.4	16.3	39.8	481.9	1.58
mean	-	-	29.9	4.7	170.6	7.1	26.0	159.3	1.79
SD	-	-	10.1	2.3	116.6	4.8	13.4	203.5	0.5

M, male; F, female; age in years at biopsy retrieval; BV/TV: vital bone volume/total volume; BS/TV: bone surface/total volume; Tb.Th: trabeculae thickness; OV/BV: osteoid volume/vital bone volume; OS/BS: osteoid surface/bone surface; O.Th: osteoid thickness; N.Oc/BPM: number of osteoclasts per bone perimeter.

Table 3 shows the individual histomorphometric indices. An average vital bone volume of 29.9% (BV/TV) was measured in the complete biopsies (SD ± 10.1) of which 7.1% (OV/BV, SD ± 4.8) was osteoid. The osteoid surface (OS/BS) covered 26.0% (SD ± 13.4) of the bone surface. The BS/TV covered 4.7 mm^2/mm^3 (SD ± 2.3). As can be read from Table 3, Patient #4 had extremely high values for trabecular bone volume (BV/TV and Tb.Th.). This high BV/TV is in accordance with the high T0 value, most likely caused by an oblique section of the cortical maxillary sinus wall (Table 1). A low bone surface (BS/TV: 2.2 mm^2/mm^3) and a high osteoid thickness (O.Th: 481.9 µm) were observed in the bone biopsy of patient #5 (Table 3).

An overview of the histomorphometric findings from the present study (6-months mixed graft) and previously reported studies with pure BCP after 6-, 9- and 12 months healing time is shown in Table 4. A non-parametric Kruskal–Wallis test showed significant differences between OS/BS ($p = 0.0233$) and Tb.Th. ($p = 0.0244$). Other tested histomorphometric indices (BV/TV, OV/BV, and N.Oc/BPM) were not different.

Table 4. Histomorphometric evaluation of the total section: overview of the mean values of the 6-month mixed graft group (autogenous bone mixed with Straumann® bone ceramic (60:40)) compared to the 6-, 9- and 12-month results with pure Straumann® bone ceramic (60:40), as previously published, in a maxillary sinus floor elevation procedure.

Patient Group	BV/TV (%)	SD ±	BS/TV (mm^2/mm^3)	SD ±	Tb.Th (µm)	SD ±	OV/BV (%)	SD ±	OS/BS (%)	SD ±	O.Th (µm)	SD ±	N.Oc/BPM $1/mm^2$	SD ±
6-month mixed	29.9	10.1	4.7	2.3	170.6	116.6	7.1	4.8	26.0	13.4	159.3	203.5	1.79	0.5
6-month (*)	27.3	4.9	4.5	1.1	132.1	38.4	7.5	4.3	41.3	28.5	13.3	4.7	1.1	1.3
9-month (**)	35.2	9.5	4.2	1.9	224.7	150.0	8.8	3.8	42.4	12.1	93.9	135.8	1.8	1.1
12-month (**)	28.2	3.2	8.3	1.3	66.7	5.4	3.4	2.5	8.2	5.3	13.6	1.0	***	-

BV/TV: vital bone volume/total volume; BS/TV: bone surface/total volume; Tb.Th: trabeculae thickness; OV/BV: osteoid volume/vital bone volume; OS/BS: osteoid surface/bone surface; O.Th: osteoid thickness; N.Oc/BPM: number of osteoclasts per bone perimeter. * Study by Frenken et al. (2010) [12]; ** Study by Bouwman et al. (2017) [46]; *** N.Oc/BPM not measured as an insignificant number of osteoclasts were available.

4. Discussion

Addition of Straumann® bone ceramic (60:40; BCP) to autogenous bone did not seem to improve the outcome after 6 months healing time compared to the use of pure BCP in an MSFE, as demonstrated by histomorphometric analyses in the five patients included in the present study. Histomorphometric indices show a high variability, which in most cases can be explained individually. For instance, an oblique section of the cortical maxillary sinus wall could explain, to some extent, the variability mentioned. Compared to autogenous bone, for each biomaterial or combination of graft materials in a maxillary sinus floor elevation (MSFE) procedure a significant lower BV/TV was found (reference

value 41% for autogenous bone) [53]. The autogenous bone graft, in the present study was harvested from the maxillary tuberosity, at the same side as the MSFE procedure was performed, implicating no extra or at least far less morbidity for the patient. However, the disadvantages of a second surgical procedure of harvesting a bone graft should also be taken into account. This disadvantage remains in the situation of a mixed graft, consisting of autogenous bone and a bone substitute.

Since the procedures in this study were similar to the procedures in the previously reported studies using pure BCP as a bone substitute in an MFSE procedure [12,32], it was possible to compare the histomorphometric findings. This analysis showed a difference for trabecular thickness (Tb.Th.) and osteoid surface (OS/BS) but not for the other parameters (BV/TV, OV/BV, and N.Oc/BPM). The osteoid surface suggested a gradual decrease over healing time for pure BCP while the 6-months mixed graft group did not fit in this pattern, which suggests a different healing pattern. Surprisingly, the presence of autogenous bone in the graft does not seem to result in a higher bone volume at 6-months healing time. According to Klijn et al. [9], a healing time of 6 months may not show the ultimate favorable effect in bone regeneration procedures.

In this study, no dental implants were lost during the 5-year follow-up. A minimum native bone height of 4 mm was chosen as a prerequisite to ensure primary stability and high survival rates of the dental implants [12,46] after an MSFE procedure with a bone substitute, regardless of the type of graft used. Klijn et al. indicated that the measured bone volumes are higher in the first stage of healing (first 4.5 months) and in the later stages of healing after 9 months, which indicates that our study design of 6 months healing time does not entirely demonstrate the advantage of adding a bone substitute to autogenous bone. It should, however, be stressed that an MSFE procedure using pure autogenous bone provides a higher vital bone volume after 6 months if a block graft is used compared to a particulate graft or a mixed graft of autogenous bone with a bone substitute [9]. This is also confirmed by meta-analyses on histomorphometric outcome of bone substitutes and autogenous bone grafts in various combinations. Altogether, autogenous bone (and if needed a mixture of autogenous bone with BCP) is superior in terms of the amount of the newly formed bone.

The low number of patients ($n = 5$) in this study was considered a limitation since quantitative histomorphometric data may be less reliable. Indeed, the histomorphometric indices do not always show a clear difference between pure BCP and autogenous bone mixed with BCP. However, qualitative assessment, in contrast, demonstrated consistently a different distribution of bone throughout all biopsies. In the 6-months mixed graft group bone matrix was scattered throughout the entire biopsy with a slightly less dense trabecular pattern in the centers of the grafted area, while in the 6-months pure BCP group a concentration of bone formation at the first 3 mm immediately cranially from the former floor of the maxillary sinus and less bone in the center of the graft was seen. This might be beneficial for bone-to-implant contact (BIC).

Dental implants are endosseous implants, which implicates that the implant should be anchored in and surrounded by vital bone for a stable result with a high (and long lasting) survival rate. In that respect the presence of vital bone over a larger area in grafted sites is important for the longevity of the dental implants to be inserted later. In this study, a minimal native bone height of 4 mm was required to achieve a good primary stability after the MSFE procedure. Using 100% autogenous bone in the MSFE procedure could shorten the healing time to 4 months and would result in a better BIC. If no or not enough autogenous bone is available, alternatively a bone substitute could be added in the MSFE procedure to achieve a good BIC. However, longer healing times (6-, 9- or 12 months studies) should be respected. In our previous studies with pure BCP the optimal healing time seemed to be 9 months, based on the BV/TV, Tb.Th. and O.Th. observed [45]. In cases with less than 4 mm native bone height, an autogenous bone graft is preferably used in an MSFE procedure. If the availability of autogenous bone is limited, it may be considered to use a mixture of autogenous bone and bone substitute to achieve on the one hand a larger graft volume and on the other hand a larger bone volume, that approximates the bone volume in case a full autogenous bone graft is used. The newly formed bone in the present mixed graft study was observed throughout the entire biopsy, suggesting a large

BIC after 6 months. However, the consequences of these findings for dental implant survival still have to be unraveled.

5. Conclusions

Based on clinical, radiological, histological and histomorphometric analyses in this study with five patients, the use of an autogenous bone graft mixed with a biphasic calcium phosphate (BCP) in a maxillary sinus floor elevation procedure does not result in a higher bone formation, compared with the results of previously reported studies with pure BCP. However, a better distribution of new bone was seen throughout the entire augmented area, which might eventually improve the bone-to-implant contact.

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Abbreviations

ASBMR	American Society for Bone and Mineral Research
BCP	biphasic calcium phosphate
BIC	bone-to-implant contact
BPM	bone perimeter
BV/TV	bone volume/total volume
BS/TV	bone surface/total volume
C.M.T.B.	Christiaan M. ten Bruggenkate
CE	Conformité Européenne
HA	hydroxyapatite
MSFE	maxillary sinus floor elevation
NA	not available
N.Oc	number of osteoclasts
O.Th	osteoid thickness
OS/BS	osteoid surface/bone surface
OV/BV	osteoid volume/bone volume
SBC	Straumann® Bone Ceramic
Tb.Th	trabecular thickness
TRAP	tartrate resistant acid phosphate
β–TCP	β–tricalcium phosphate

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