J Clin Periodontol 2011; 38: 966–974 doi: 10.1111/j.1600-051X.2011.01754.x

Journal of Clinical Periodontology

Histological evaluation of maxillary sinus floor augmentation with recombinant human growth and differentiation factor-5-coated β-tricalcium phosphate: results of a multicenter randomized clinical trial

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Stavropoulos A, Becker J, Capsius B, Açil Y, Wagner W, Terheyden H: Histological evaluation of maxillary sinus floor augmentation with recombinant human growth and differentiation factor-5-coated β-tricalcium phosphate: results of a multicenter randomized clinical trial. J Clin Periodontol 2011; 38: 966–974. doi: 10.1111/j.1600-051X.2011.01754.x.

Abstract

Objectives: The aim of this prospective, multicenter, randomized clinical trial was to evaluate histologically the outcome of maxillary sinus lift augmentation with a recombinant human growth and differentiation factor-5-coated β-tricalcium phosphate (rhGDF-5/β-TCP) or with a β-TCP and autogenous bone (β-TCP/AB) composite.

Material and Methods: Thirty-one patients requiring unilateral maxillary sinus floor augmentation (residual alveolar bone height <5 mm) were randomly allocated in three treatment groups: (a) rhGDF-5/β-TCP and a 3-month healing period, (b) rhGDF-5/β-TCP and a 4-month healing period, and (c) β-TCP and intra-oral corticocancellous autologous bone (at 1:1) and a 4-month healing period. Cylindrical biopsies were harvested by means of a trephine bur during implant site preparation and evaluated histologically and histometrically.

Results: One patient withdrew from the study before implant placement; 66 implants were inserted in the remaining 30 patients. Four out of 47 (8.5%) implants failed in patients treated with rhGDF-5/β-TCP. The proportion of newly formed bone was similar among groups and averaged 31.4% ($\pm$ 17%) in the rhGDF-5/β-TCP/3-month healing group, 28% ($\pm$ 15.5%) in the rhGDF-5/β-TCP/4-month healing group, and 31.8% ($\pm$ 17.9%) in the β-TCP/AB group. The proportion of remaining β-TCP averaged 12.6% ($\pm$ 14.4%) in the rhGDF-5/b-TCP/3-month group, 6.6% ($\pm$ 6.3%) in the rhGDF-5/b-TCP/4-month group, and 16.5% ($\pm$ 12.3%) in the β-TCP/AB group. The new bone was primarily woven and characterized by slender trabeculae and narrow osteoid zones, and in many instances bone was in contact with residual biomaterial particles. Presence of AB particle remnants was only trivial, while minimal amounts of inflammation were observed only in a few cases.

Conclusion: Sinus augmentation with rhGDF-5/β-TCP resulted in comparable amounts of new bone and of similar quality as those obtained with a β-TCP/AB composite graft.

Key words: autogenous bone; bone substitutes; histology; human; rhGDF-5; sinus augmentation; β-TCP; tissue engineering

Accepted for publication 28 May 2011.

Conflict of interest and source of funding statement

Dr. Björn Capsius is an employee of Scil Technology GmbH, Martinsried, Germany. The rest of the authors declare no conflict of interest with regard to this study.
This research was funded by Scil Technology GmbH.

Bone resorption following tooth extraction or due to advanced periodontal disease, and/or pneumatization of the maxillary sinus may result in insufficient bone in horizontal and/or more frequently, vertical dimension for the placement of dental implants in the posterior maxilla. Augmentation of the maxillary sinus floor (or sinus lift) with bone grafts and/or substitutes is nowadays a standard treatment approach for re-establishing an adequate bone volume in the posterior maxilla.

In bone reconstructive surgery, in general, autogenous bone (AB) is considered as the gold standard, primarily due to its osteogenic potential and remodelling capacity. However, obtaining AB in adequate amounts for a sinus lift procedure most often requires an additional surgical site. In order to avoid the associated morbidity and disadvantages of extensive AB harvesting and also to counteract AB graft resorption, bone substitute materials are used either alone or as adjunct to AB. These biomaterials are mostly osteoconductive and play the role of a space filler to allow new bone in-growth, while when used in combination with AB they expand the volume of available grafting material, i.e. less amount of AB needs to be harvested. Recent systematic reviews have revealed that the nature of the implanted bone graft material, i.e. AB, bone substitute, or combination thereof, does not play a significant role in the clinical success or survival of the implants which are generally comparable with those of implants placed in pristine bone in the maxillary posterior regions ($\geqslant$90% approximately) (Jensen et al. 1998, Tong et al. 1998, Wallace & Froum 2003, Del Fabbro et al. 2004, Pjetursson et al. 2008, Tan et al. 2008, Jensen & Terheyden 2009).

Nevertheless, with the intend to replace the need for AB and achieve shorter healing times, potent osteoinductive materials, members of the transforming growth factor-β (TGF-β) superfamily, i.e. recombinant human bone morhogenetic proteins (rhBMPs) and growth and differentiation factors (rhGDFs) have been evaluated for maxillary sinus augmentation (Boyne et al. 1997, 2005, Groeneveld et al. 1999, van den Bergh et al. 2000, Triplett et al. 2009, Koch et al. 2010). In particular, it has been shown in animal experiments (Nevins et al. 1996, Hanisch et al. 1997, Kirker-Head et al. 1997) and clinical studies (Boyne et al. 1997, 2005, Triplett et al. 2009) that rhBMP-2 facilitates placement and sustains osseointegration and loading of implants in augmented sinuses in the majority of cases similarly to AB; in addition, the newly formed bone within the sinus cavity is of comparable quality to that obtained in sinuses grafted with AB or pristine sites. Animal experiments have suggested that also rhBMP-7 (also called as osteogenic protein 1) may have potential in sinus augmentation (Margolin et al. 1998, McAllister et al. 1998, Terheyden et al. 1999, Roldan et al. 2004); however, due to great variability in the results observed in clinical case reports (Groeneveld et al. 1999, van den Bergh et al. 2000), extensive evaluation of this technology has not been pursued.

More recently, another member of the TGF-β superfamily, the rhGDF-5 (also known as cartilage-derived morphogenetic protein-1; CDMP-1, or as BMP-14) has been shown in animal experimental studies to promote bone formation in both ectopic (Spiro et al. 2000, Kakudo et al. 2007) and orthotopic sites (Kuniyasu et al. 2003, Poehling et al. 2006, Yoshimoto et al. 2006), and also in association with dental implants (Simank et al. 2006, Schwarz et al. 2008, Weng et al. 2009, Polimeni et al. 2010). In addition, rhGDF-5-coated onto β-tricalcium phosphate particles (rhGDF-5/β-TCP) was also evaluated for its potential to enhance bone formation in association with maxillary sinus augmentation in both an animal experimental set-up (Gruber et al. 2008, 2009) and in a clinical setting (Koch et al. 2010) with positive results. In particular, the results of this prospective, multicenter, randomized clinical trial (Koch et al. 2010) suggested that rhGDF-5/β-TCP in a coating density of 500 mg rhGDF-5/g β-TCP is equally safe and effective as the use of β-TCP/AB. Nevertheless, equally successful short-term clinical outcomes regarding implant success/survival after sinus augmentation do not necessarily indicate equally successful bone regeneration; implant stability can indeed be achieved with a limited bone-to-implant contact (BIC) only at coronal (i.e. within the pristine bone) portion of the implant. The aim of the present study is to report on the histological and histomorphometrical evaluation of biopsies from the sinuses augmented with rhGDF-5/β-TCP in comparison with those from β-TCP/AB augmented sinuses, harvested in association with this latter clinical trial.

Material and Methods

Thirty-one patients (16 males; average age: 53.8 ± 12.1 years) requiring unilateral sinus floor augmentation with a residual alveolar ridge bone height: 1–5 mm (based on panoramic X-rays) were included in this pilot phase IIa, multicenter, randomized, open, three-arm controlled parallel group study. Detailed information regarding the study, including patient characteristics, sample calculation, clinical and radiographic procedures and results, participating centres, and regulatory authorities involved, are reported in Koch et al. (2010); only the information relevant for the present study is described herein. The clinical trial protocol was approved by all responsible federal and local ethics committees before enrollment of the patients and all patients were fully informed about the scope and study procedures and have signed consent forms before commencement of treatment.

Study materials and groups

The test material comprised of rhGDF-5/β-TCP (coating density: 500 mg rhGDF-5/g β-TCP; Scil Technology GmbH, Martinsried, Germany), hydrated with 0.9% NaCl solution before implantation. A composite (1:1 ratio i.v.) of non-coated β-TCP particles of the same size (Ceraver Osteal, Roissy, France) mixed with corticocancellous autologous bone chips harvested from intra-oral sites – primarily the mandibular angle – was used as control (β-TCP/AB). The patients were randomized according to a computer-generated list into three treatment groups: (a) rhGDF-5/β-TCP and a 3-month healing period, (b) rhGDF-5/β-TCP and a 4-month healing period, and (c) β-TCP/AB and a 4-month healing period.

Surgical procedures

Under local or general anaesthesia and by means of a lateral window approach, the sinus membrane was detached from the bone walls and the buccal window was reflected inwards, the sinus cavity

was filled with the grafting material (i.e. rhGDF-5/β-TCP or β-TCP/AB), and the flaps were repositioned and sutured. The amount of grafting material placed into each sinus varied depending on the amount of augmentation needed to accommodate the implant/s.

Biopsy harvesting

Core bone biopsies were harvested with a trephine bur (2 mm internal ∅, 13 mm long) during implant site preparation 3 or 4 months (rhGDF-5/β-TCP/3 month; rhGDF-5/β-TCP/4 month and β-TCP control, respectively) after sinus floor augmentation. Implant number and positioning was according to the prosthetic reconstruction treatment plan. For every implant placed in the augmented area, one biopsy cylinder was obtained. The biopsy at the implant site with the least preoperative bone height was primarily chosen for analysis. Biopsy preparation depth never exceeded the appropriate depth required for the planned implant length at each specific site.

The biopsy samples were first stored inside the trephine burs in a 10% buffered formaldehyde solution. After some time for fixation, the bone cores were pushed carefully out of the trephine burs, while registering the apico-coronal orientation of the cores for future reference during evaluation. Then, the biopsy cores were processed for undecalcified section preparation (cutting–grinding technique) (Donath & Breuner 1982) and the central longitudinal ~40 μm thick obtained sections were stained with toluidine blue.

Histological evaluation and histomorphometry

Two experienced evaluators (A. S. and H. T.), blinded with respect to treatment group, examined independently the biopsies while viewing them on a LCD flat screen with live streaming of images captured by a digital camera adapted to the light microscope (Olympus DH 50, Olympus Denmark AS, Ballerup, Denmark). In order to avoid differences in the relative contribution of pristine tissues within the biopsy cylinder due to variation in the residual alveolar bone height from implant site to implant site, the analysis regarded only the portion of the biopsy representing the newly formed tissues. First, the margin between pristine bone and newly formed tissue inside the sinus was histologically identified according to staining behaviour and visible bone lamellae, and the length of the portion of the biopsy representing pristine bone was measured at the 25th, 50th, and 75th percentile of section width. The averaged length was compared with the radiographic residual bone height measured on the pre-surgical orthopantomogram, and if it differed >1 mm the biopsy was re-evaluated until identification of the pristine/regenerated bone was achieved. Then the area fraction of the various tissues was estimated by means of semiautomatic segmentation process using the Leica QWin Software (Leica Mikrosysteme, Wetzlar, Germany). The quality and maturity of the bone formed within the sinus cavity was graded by means of

Table 1. Number of biopsies in the three treatment groups according to category describing the quality and maturity of the newly formed bone, and evaluating evidence of inflammation, foreign body reaction associated with the β-TCP particles, or osteoclast-like cells within the regenerated bone

Category	*N*			Total *N*	Total %
	rhGDF-5/β-TCP 3 month	rhGDF-5/β-TCP 4 month	β-TCP/AB		
Quality and maturity*					
0	–	–	–	–	–
1	3	2	2	7	23.3
1–2	1	1	0	2	6.7
2	2	2	2	6	20
2–3	0	1	1	2	6.7
3	1	1	3	5	16.7
3–4	0	1	1	2	6.7
4	1	1	0	2	6.7
4–5	1	0	0	1	3.3
5	1	1	1	3	10
Inflammation†					
0	9	7	9	25	83.3
1	0	1	1	2	6.7
1–2	1	0	0	1	3.3
2	0	1	0	1	3.3
3	0	1	0	1	3.3
4	–	–	–	–	–
5	–	–	–	–	–
Foreign body†					
0	3	5	3	11	36.7
0–1	0	0	2	2	6.7
1	6	2	4	12	40.0
1–2	0	0	1	1	3.3
2	1	2	1	4	13.3
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
Osteoclasts†					
0	1	0	1	2	6.7
1	6	4	5	15	50.0
1–2	2	2	2	6	20.0
2	0	4	1	5	16.7
3	1	0	1	2	6.7
4	–	–	–	–	–
5	–	–	–	–	–

*Six-point scale categories describing quality and maturity of the new bone: 0 = no bone, 1 = few woven bone trabeculae with minimal contact to β-TCP, 2 = woven bone trabeculae with osteoblasts and broad osteoid, β-TCP present, 3 = lamellar bone trabeculae, narrow osteoid, β-TCP present, 4 = areas of Haversian bone, β-TCP grossly degraded, 5 = bone completely remodeled, β-TCP not present, 1–2, 2–3, and 4–5 categories indicate that biopsies were better described by combining both the respective categories of the six-point scale.

†Six-point scale categories evaluating inflammation, foreign body reaction, and osteoclast presence: 0 = absent, 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = severe; 0–1 and 1–2 indicate that biopsies were better described by combining both the respective categories of the six-point scale.

rhGDF-5, recombinant human growth and differentiation factor-5-coated; β-TCP, β-tricalcium phosphate; AB, autogenous bone.

a six-point scale, while histological evidence of: (1) inflammation, (2) foreign body reaction associated with the β-TCP particles, and (3) presence of osteoclasts within the regenerated bone was recorded using another six-point scale (please refer to footnote in Table 1). Histomorphometrical evaluation included measurement of the total area of regenerated (i.e. harvested from the sinus) tissues (TRT) and of the total area of regenerated mineralized bone in mm^2, as well as estimation of area fractions (as % of TRT) of newly formed mineralized bone tissue, bone marrow, dense fibrous connective tissue, and remaining β-TCP.

If the results of the two examiners differed $>5\%$ as regards the relative fraction of newly formed bone (absolute difference), the biopsies had to be re-evaluated from scratch in a common session by both examiners until consensus was reached.

Statistical analysis

For the analysis, the mean value of the entries of the two examiners was used. If a third joint histopathologic evaluation was performed, then the values of this evaluation were used for those particular specimens. Summary statistics and the one-sided Wilcoxon test were used to describe/analyse the data by means of the SPSS 13.0 software (SPPS Inc., Chicago, IL, USA). The level of significance level was set at $p<0.05$.

Results

One patient belonging in rhGDF-5/β-TCP/3-month healing group withdrew from the study before implant placement surgery (i.e. no biopsy was harvested) for reasons not related with the study. In nine patients (29%) a minor perforation of the Schneiderian membrane was observed during the augmentation surgery, and was covered with a collagen membrane (BioGide, Geistlich, Wolhusen, Switzerland) before grafting; five of those patients belonged to the rhGDF-5/β-TCP/4-month healing group. In general, large variation was observed in biopsy length and height of pristine bone among patients. In 15 cases, the biopsies had to be re-evaluated jointly by both examiners during an additional session, because there were differences in identification and positioning of the pristine/regenerated bone margin at the first separate evaluation by each of the examiners. This difficulty in identifying the pristine/regenerated bone margin was due to: (1) great resemblance of the old and newly formed bone tissue and uniform trabecular structure; (2) fracturing of the biopsy cylinder either during the clinical procedures or during the histological preparation and poor reassembly of the pieces. Finally, in a small number of specimens, some areas of thermal damage of the bone tissue – apparently due to trephine biopsy harvesting – was observed; nevertheless, this necrosis did not preclude histomorphometrical evaluation of these specimens.

The results of the histological and histometrical evaluation are presented in Tables 1 and 2. In a few cases, the situation could not be described with only one category of the six-point scale and two categories were used. These ''dual categories'' are reported jointly with the existing scales. Representative overview histomicrographs from the three treatment groups are presented in

Table 2. Mineralized bone, bone marrow, dense fibrous connective tissue, and residual β-TCP fraction means ± SD (range) in the three treatment groups

	N			*P*
	rhGDF-5/β-TCP 3 month	rhGDF-5/β-TCP 4 month	β-TCP/AB	
No. of biopsies	10	10	10	
New bone	31.4 ± 17.0 (13.4–62.3)	28.0 ± 15.5 (0.3–49.7)	31.8 ± 17.9 (13.3–63.6)	NS
Bone marrow	41.5 ± 16.5 (11.3–65.7)	40.3 ± 16.6 (0.0–56.7)	28.2 ± 17.3 (5.9–52.8)	NS
Dense fibrous CT	14.6 ± 13.4 (1.2–47.8)	25.1 ± 30.4 (0.0–94.3)	23.5 ± 19.8 (3.0–49.3)	NS
β-TCP	12.6 ± 14.4 (0.0–44.9)	6.6 ± 6.3 (0.0–17.0)	16.5 ± 12.3 (0.0–35.7)	NS

rhGDF-5, recombinant human growth and differentiation factor-5-coated; β-TCP, β-tricalcium phosphate; AB, autogenous bone.

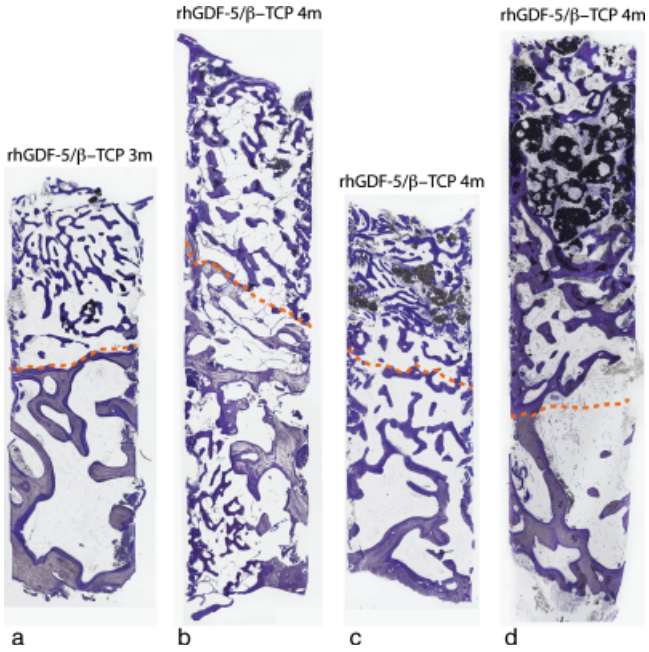

Fig. 1. Representative histomicrographs of biopsies from sinuses implanted with recombinant human growth and differentiation factor-5-coated β-tricalcium phosphate (rhGDF-5/β-TCP) harvested after 3 (a) or 4 months (b–d). The orange dashed line indicates the pristine/regenerated bone margin. The newly formed bone is characterized by immature trabeculae with minimal contact to the β-TCP particles. Large variation regarding new bone density and the amount of residual biomaterial is observed. A tendency for the residual β-TCP to aggregate towards the apical portions of the biopsy (b and c) and/or cluster in large numbers embedded in connective tissue (d) was observed. Toluidine blue staining. Original magnification: × 14.

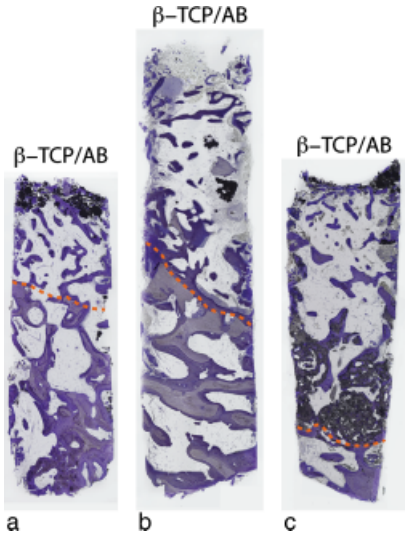

Fig. 2. Representative histomicrographs of biopsies from sinuses implanted with β-tricalcium phosphate and autogenous bone (β-TCP/AB) harvested after 4 months. The orange dashed line indicates the pristine/regenerated bone margin. The newly formed bone is characterized by immature trabeculae with minimal contact to the β-TCP particles. Large variation is observed regarding the amount of residual biomaterial, while only few remaining AB particles are observed. Toluidine blue staining. Original magnification: × 14.

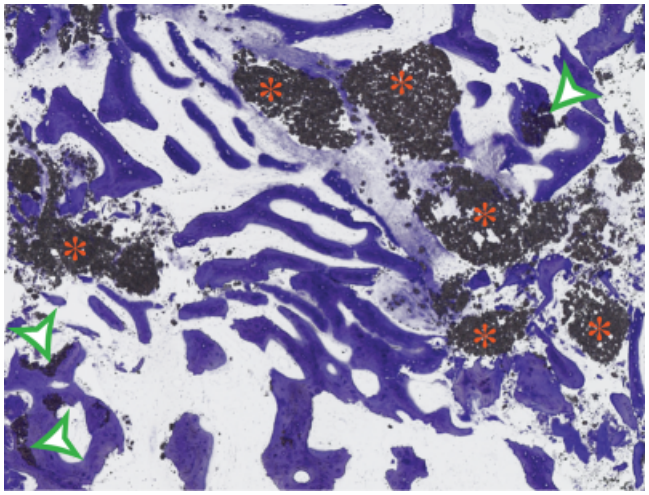

Fig. 3. Detail of Fig. 1c. Recombinant human growth and differentiation factor-5-coated β-tricalcium phosphate (rhGDF-5/β-TCP) 4-month group. The newly formed bone is rather dense and characterized by immature trabeculae primarily with no contact to the β-TCP particles (asterisks). Only occasionally are biomaterial particle fragments embedded within the new bone (arrowheads). Toluidine blue staining. Original magnification: × 42.

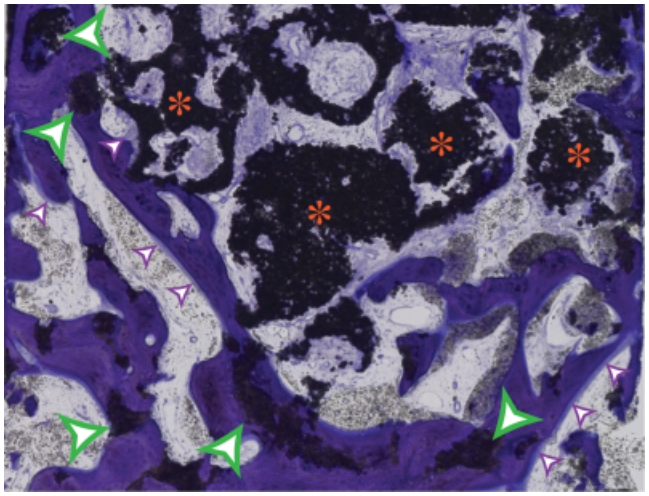

Fig. 4. Detail of Fig. 1d. Recombinant human growth and differentiation factor-5-coated β-tricalcium phosphate (rhGDF-5/β-TCP) 4-month group. The newly formed bone is characterized by immature trabeculae primarily with no contact to the β-TCP particles (asterisks), and only occasionally were biomaterial particle fragments embedded within the new bone (green arrowheads). Clusters of large numbers of β-TCP particles are embedded in connective tissue. Narrow and broad osteoid zones are observed (violet arrowheads). Toluidine blue staining. Original magnification: × 42.

Figs 1 and 2. In general, the newly formed bone was characterized by immature trabeculae with minimal contact to the β-TCP particles, with or without presence of broad osteoid zones (Figs 3 and 4). Biomaterial particle fragments embedded within the new bone were, in fact, only occasionally observed (Figs 3–5). Lamellar bone was observed in only a few cases and complete degradation of the β-TCP was a rare finding, irrespective treatment group. In fact, somehow large variation was observed among and within groups regarding the amount of residual biomaterial (Figs 1 and 2). Nevertheless, a tendency for more mature trabeculae was observed in the β-TCP/AB group compared with the rhGDF-5/β-TCP groups (Fig. 5). New bone formation was more or less uniform across the biopsy, i.e. similar amounts of bone formation were observed near the pristine bone and in the apical portions of the biopsy towards the elevated sinus mucosa. Occasionally, a tendency for the residual β-TCP to aggregate towards the apical portions of the biopsy (Figs 1b,c, and 2a) or cluster in large numbers embedded in connective tissue (Fig. 1d) was observed; however, these findings did not seem to be treatment-group specific. Regarding the β-TCP/AB group, only few remaining AB particles, void of cells, were observed either embedded within the newly formed bone or surrounded by connective tissue (Fig. 5). Furthermore, the histological evaluation showed that mostly minimal signs of inflammation were observed in 16.7% of the analysed biopsies; in the rest 83.3% no signs of inflammation were detected. Similarly, no or minimal signs of foreign body reaction against the biomaterial particles were observed in the vast majority of evaluated biopsies (83.3%). On the other hand, multinuclear osteoclastic cells were present in all except two of the evaluated biopsies. The presence of multinuclear osteoclastic cells was judged as minimal – indicative of physiological remodelling – in 50% of all biopsies and only moderate in 6.7% of the cases.

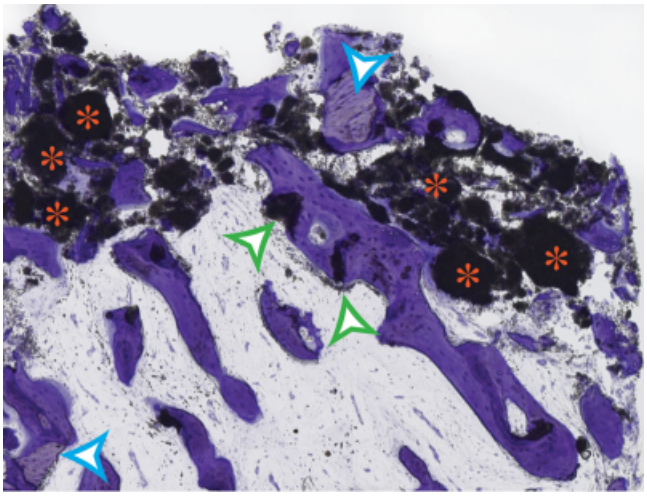

Fig. 5. Detail of Fig. 2a. β-Tricalcium phosphate and autogenous bone (β-TCP/AB group). The newly formed bone is characterized by immature trabeculae primarily with no contact to the β-TCP particles (asterisks), and only occasionally were biomaterial particles embedded within the new bone (green arrowheads). Only few remaining AB particles (blue arrowheads) void of cells and entrapped within the newly formed bone are observed. Toluidine blue staining. Original magnification: × 56.

The histomorphometric evaluation showed that, the amount of new mineralized bone fraction was similar in the three treatment groups and occupied approximately one-third of the total area of harvested regenerated tissues. The rhGDF-5/β-TCP/4-month healing group showed a slightly lower average amount of newly formed mineralized bone fraction (28.0 ± 15.5) compared with the rhGDF-5/β-TCP/3-month and β-TCP/AB treatment groups (31.4 ± 17.0 and 31.8 ± 17.9, respectively). This finding is due to a single patient where almost no bone had formed; if this patient is excluded from the analysis the mean percentage of newly formed bone in the rhGDF-5/β-TCP/4-month healing group rises to over 30%. No statistically significant differences ($p > 0.05$) were observed among the groups regarding any of the evaluated parameters (Table 2).

Discussion

The results of the present study showed that implantation of rhGDF-5/β-TCP particles for sinus augmentation facilitated similar amounts of bone formation as those obtained after implantation of a β-TCP and AB composite graft. A point of concern related with the uncritical acceptance of reported bone fraction values regards the true spatial distribution of the newly formed bone within the sinus cavity. Average bone fraction values do not necessarily denote a uniform density throughout the length of the biopsy, i.e., the height of augmentation. In the present study, new bone formation was seemingly more or less uniform across the biopsy, i.e. similar amounts of bone formation were observed near the pristine bone and in the apical portions of the biopsy towards the elevated sinus mucosa, in all three treatment groups.

As no group of patients augmented with only β-TCP was included in the present study, direct conclusions on the relative contribution of GDF-5 coating to the amount of bone regeneration observed herein might be difficult to draw. Nevertheless, in several animal studies (Poehling et al. 2006, Gruber et al. 2008, Weng et al. 2009) using a variety of models (rats, mini-pigs, and dogs, respectively), where rhGDF-5/β-TCP implanted sites were directly compared with control sites receiving only β-TCP, consistently better results in terms of new bone formation was obtained in the GDF-5 treated ones. For example, sinus augmentation with rhGDF-5/β-TCP resulted in larger amounts of new bone formation and BIC compared with sinuses augmented with β-TCP, in a mini-pig model (Gruber et al. 2008). Furthermore, the amount of bone formation observed in the rhGDF-5/β-TCP implanted groups in the present study is much larger to that reported in a recently published meta-analysis of histomorphometric data from human biopsies from sinus augmentation procedures involving a variety of bone grafts and bone graft substitutes, including β-TCP (Handschel et al. 2009). In this latter report, biopsies from sinuses implanted with only β-TCP were found to contain on average ca. 25% new bone at early healing times (4–9 months) after the augmentation. Thus, it seems reasonable to assume that the increased amount of bone formation observed after the short-healing time (3–4 months) in the present study was due to the coating of biomaterial particles with the growth factor.

In recently published animal studies, the bone forming potential of rhGDF-5/β-TCP was also compared directly to that of a β-TCP/AB composite graft (Gruber et al. 2009, Weng et al. 2009). Gruber et al. (2009) placed a single implant with simultaneous sinus grafting with rhGDF-5/β-TCP (400 or 800 μg/g) or a β-TCP/AB (1:1 ratio) composite graft, again in mini-pigs and with a split-mouth design. Histological analysis after 12 weeks of healing revealed significantly larger amounts of newly formed bone in the GDF-5 implanted sinuses comparing to the amounts of bone obtained in the β-TCP/AB group. Furthermore, BIC was significantly enhanced in test sites compared with controls. Similarly, in a study using a box-type peri-implant defect in the mandibles of dogs (Weng et al. 2009) larger amounts of new bone formation after rhGDF-5/β-TCP implantation comparing to defects treated with β-TCP/AB were observed. In contrast to those findings, no difference was found in the present group of patients among treatment modalities in terms of mineralized bone fraction within the harvested biopsies. The observed discrepancy between the present study and the above experiments may be due to inherent differences of the models used (human *versus* animals). Animals bred for experimental purposes have less variation in the anatomy (e.g., similar dimensions and shape of the sinus cavity) and metabolism comparing to humans; systematic variation in the shape and size of the augmented sinuses (e.g., narrow *versus* wide in a bucco-lingual direction) among the groups in the present study cannot be excluded. In context, although the amount of grafting material placed into each sinus varied depending on the amount of augmentation needed to accommodate the implant/s (i.e. total GDF-5 dose varied among sites), the possibility that larger defects (sinus

volumes) would need disproportionally larger total dose of GDF-5 than smaller defects to achieve similar results cannot be excluded.

Lack of difference in terms of bone formation among the groups herein, on the other hand, should probably not be attributed to the relatively short healing period of histological evaluation. In an in vitro evaluation of the β-TCP carrier used in this study, it was demonstrated that almost the entire amount of rhGDF-5 was released from the carrier within the first 7 days (Poehling et al. 2002), and in the previously described animal study of (Gruber et al. 2008), indeed no differences in the amount of bone formation or BIC were observed between a short *versus* a long observation period (4 *versus* 12 weeks) in the rhGDF-5/β-TCP implanted sites. In this context, it must also be mentioned that longer healing times would most likely not result in larger amounts of bone formation neither in the β-TCP/AB group. In a very recently published systematic review of histomorphometric data from human biopsies, no significant differences in the amount of bone were observed in biopsies harvested after 4.5–9 months or >9 months from sinuses augmented with β-TCP/AB (Klijn et al. 2010).

As mentioned earlier, AB is generally considered the gold standard in bone reconstructive surgery. One might speculate that, grafting of AB would accelerate bone formation and/or result in larger amounts of new bone as compared with what could be achieved with the use of only bone substitutes, due to concomitant transplantation of osteogenic cells and growth factors within the grafted mass. However, the results reported in the literature seem not to support such an assumption, at least regarding β-TCP. In the above mentioned systematic review of (Klijn et al. 2010) AB indeed yielded much larger amounts of mineralized bone and in a shorter period, comparing to what observed with most of the evaluated bone substitutes, but the differences were not significant regarding β-TCP or β-TCP/AB. A similar result was also found in the systematic review of (Handschel et al. 2009), where biopsies from sinuses augmented with only AB or only β-TCP were evaluated. In addition, it should also be mentioned that the ideal bone density for successful implant osseointegration is still to be defined. Thus, based on (a) the results of these systematic reviews, (b) the results of the animal studies of Gruber et al. (2008;2009), where rhGDF-5/β-TCP augmented sinuses showed larger amounts of bone formation comparing to those implanted with β-TCP/AB or β-TCP, and (c) those of the present evaluation, where rhGDF-5/β-TCP implantation resulted in similar amounts of bone with those achieved with β-TCP/AB grafting, it seems reasonable to suggest that the particular rhDGF-5/β-TCP material used herein could eliminate the need for AB grafting in sinus augmentation procedures. Nevertheless, this assumption needs to be confirmed in a larger scale clinical study including the long-term observation of the treatment outcome.

At this point, it should be mentioned that direct comparison of bone fraction data from the various studies should be made with care not only due to variations in the ratios of AB:biomaterial and evaluation times, but also due to differences among studies regarding the sites of biopsy harvesting and, especially, in the methods of histomorphometrical evaluation. A strict evaluation protocol was followed in the present study, including blind evaluations and joint sessions by the histopathologists in case of >5% discrepancy in bone values, in order to ensure evaluation of biopsy portions representing just the tissues formed within the sinus cavity; such a meticulous evaluation process is usually missing and in many reports, in fact, bone tissue fractions include also various portions of the residual alveoral ridge.

In this context, the ideal grafting material should be completely replaced by new bone with time, of course, without any negative influence (i.e. reduction) in the achieved augmentation volume. Presence of osteoclasts within the regenerated bone herein was scored largely as limited/moderate, indicating a more or less physiological remodelling and apparent stability of the newly formed tissue. Of course, solely morphological evaluation and lack of immunohistochemical staining specifically for osteoclast detection may have overestimated osteoclast presence, by recognizing wrongly giant cells as osteoclasts. Nevertheless, slight overestimation, if any, of osteoclast presence would only have limited influence in the interpretation of the results. Gradual degradation over time leading to complete elimination of the implanted β-TCP after 12–18 months has been reported previously in association with sinus augmentation procedures (Szabo et al. 2001, Zerbo et al. 2004). In the patients included herein some residual β-TCP was observed in all groups. Nevertheless, the relative fraction of β-TCP was lower in the rhGDF-5/β-TCP treatment groups, despite the fact that at baseline the β-TCP/AB group only contained approximately 50% of β-TCP comparing to 100% in the former groups. This finding can be the result of a rapid bone growth and tissue expansion in the rhGDF-5 group, which pushed the residual carrier material apically or to the sides of the augmentation, i.e. to areas not represented by the harvested biopsy. However, it is more likely this observation suggests a higher degradation rate within the rhGDF-5/β-TCP treatment groups comparing to the β-TCP/AB group, and is in agreement with previous studies (Wikesjo et al. 1994, 2002, Koo et al. 2007) where other growth factors have been shown to accelerate degradation of the biomaterials used as carrier technologies.

A critical aspect when considering candidate biomaterials for sinus augmentation, in addition to an osteoconductive/osteoinductive potential, is the safety profile, i.e. clinical complications/adverse reactions as well as tissue reactions. In the present multicenter, randomized clinical trial study no serious clinical complications or side effects, or relative to the laboratory analysis were observed for any of the patients (Koch et al. 2010). In addition, the histopathological analysis in general showed only minimal signs of inflammation or foreign body reaction towards the biomaterial. In perspective, use of rhBMP-2 for sinus augmentation has yielded comparable bone formation as that observed after AB grafting, however, adverse reactions manifested as excess facial swelling have been frequent (Boyne et al. 1997, Triplett et al. 2009).

In conclusion, the results of the present study showed that sinus augmentation with rhGDF-5/β-TCP resulted in comparable amounts of new bone and of similar quality as those obtained with a β-TCP/AB composite graft. Based on these results and the discussion above, it could be suggested that this particular rhGDF-5/β-TCP material could eliminate the need for AB grafting in sinus augmentation procedures. Nevertheless, this assumption needs to be confirmed in a larger scale clinical study including the long-term observation of the treatment outcome.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. CONSORT 2010 Flow Diagram.

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Clinical Relevance

Scientific rationale for the study: Emerging pre-clinical evidence indicates that rhGDF-5 adsorbed onto a particulate β-TCP) carrier may enhance bone formation. The objective of this study was to provide a histologic and histometric record of bone regeneration in man following maxillary sinus augmentation with rhGDF-5/β-TCP compared with that following grafting of an AB/β-TCP composite.
Principal findings: Sinus augmentation with rhGDF-5/β-TCP resulted in comparable amounts of new bone and of similar quality as those obtained with a β-TCP/AB composite graft.
Practical implications: rhGDF-5/β-TCP appears a promising candidate technology that could eliminate the need for AB grafting in sinus augmentation procedures. This assumption needs to be confirmed in a larger scale clinical study including the long-term observation of the treatment outcome.