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Bone Marrow Concentrate and Bovine Bone Mineral for Sinus Floor Augmentation: A Controlled, Randomized, Single-Blinded Clinical and Histological Trial—Per-Protocol Analysis

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Purpose: The purpose of this work was to evaluate the potential of substituting autogenous bone (AB) by bone marrow aspirate concentrate (BMAC). Both AB and BMAC were tested in combination with a bovine bone mineral (BBM) for their ability of new bone formation (NBF) in a multicentric, randomized, controlled, clinical and histological noninferiority trial.

Materials and Methods: Forty-five severely atrophied maxillary sinus from 26 patients were evaluated in a partial cross-over design. As test arm, 34 sinus of 25 patients were augmented with BBM and BMAC containing mesenchymal stem cells. Eleven control sinus from 11 patients were augmented with a mixture of 70% BBM and 30% AB. Biopsies were obtained after a 3–4-month healing period at time of implant placement and histomorphometrically analyzed for NBF.

Results: NBF was $14.3\% \pm 1.8\%$ for the control and nonsignificantly lower ($12.6\% \pm 1.7\%$) for the test (90% confidence interval: -4.6 to 1.2). Values for BBM ($31.3\% \pm 2.7\%$) were significantly higher for the test compared with control ($19.3\% \pm 2.5\%$) ($p < 0.0001$). Nonmineralized tissue was lower by 3.3% in the test compared with control (57.6% ; $p = 0.137$).

Conclusions: NBF after 3–4 months is equivalent in sinus, augmented with BMAC and BBM or a mixture of AB and BBM. This technique could be an alternative for using autografts to stimulate bone formation.

Introduction

DURING PHYSIOLOGICAL bone healing, osteoprogenitor cells migrate into the defect, differentiate into osteoblasts, and produce calcified matrix. These osteoprogenitor cells derive from multipotent mesenchymal stem cells (MSCs) that are recruited from bone marrow. MSCs can tolerate low oxygen concentrations and have the potential for osteogenic, chondrogenic, and adipogenic differentiation, depending on the external stimulus by cytokines.^{1–6}

To stimulate bone healing, various grafting techniques have been developed to supply either cytokines, progenitor cells, suitable scaffolds, or any combination of them.^{7–10} Adding cytokines can increase bone formation dynamics.

Adding progenitor cells should optimize the initial physiologic bony regeneration. Scaffolds should ensure volume stability. Our group gained clinical experience with a resorbable biomaterial and expanded osteoblast-like cells.¹¹ The constructs did not preserve shape and volume of the augmented area. This is why in the presented study a rather stable osteoconductive biomaterial was used.

Maxillary sinus floor elevation and secondary implant placement is a standardized procedure and therefore a good clinical model to evaluate bone regeneration.

It is common to combine autologous bone with alloplastic material.^{12,13} Drawback of the procedure is that, to obtain autologous bone, an additional surgical step is needed, causing donor-site morbidity.^{14–16} If alloplastic materials are

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used without addition, normally longer healing times are chosen to compensate for the initial lack of regeneration. This explains why there are no reports about healing periods of less than 6 months when pure alloplastic material is used. In comparison to bone harvesting, the aspiration of autogenous bone (AB) marrow is a minimal invasive puncture, causing less discomfort to the patients.

There are case reports on the efficiency of bone marrow aspirate-derived stem cell transplantation to improve fracture or nonunion healing.^{17–19} The present clinical study histomorphometrically evaluates the bone forming potential of bone marrow aspirate concentrate (BMAC) intraoperatively harvested from the posterior iliac crest and concentrated with a closed system (BMAC) after a short healing time (3–4 months) in sinus lifts of severely atrophied maxillae. As control augmentation served a standard mixture of AB and alloplastic material.

Materials and Methods

Protocol specifications

The clinical study was approved by the ethics committees of the Universities of Freiburg (Germany) and Mainz (Germany). First, the ethics committee regarded treating patients with highly resorbed maxillae (2–3 mm bone height) and a short, previously unreported, healing time of 3–4 months with only bovine bone mineral (BBM) in the view of a superiority design as unethical. This is why it was decided that the control group should be BBM and autologous bone (AB), resulting in a nonsuperiority design.

The study was conducted in accordance with the Declaration of Helsinki (Fifth Revision, 2004). Patients older than 18 years with need of dental implant placement in the posterior maxilla were eligible if they had a maximum of 4 mm residual alveolar height. They had to be able to comply with study-related procedures, such as returning for follow-up examinations, exercising good oral hygiene, and being able to understand the nature of the proposed surgery. Written informed consent was obtained prior to any study-related procedures. Exclusion criteria were smoking, history

of malignancy, radiotherapy or chemotherapy, pregnancy or nursing, general contraindications for dental or surgical treatment, medications, treatments or diseases, which may have an effect on bone remodeling, bone or connective tissue metabolism, or an allergy to collagen.

Primary parameter of the study was new bone formation (NBF). Secondary parameters were volume of the augmentate and bone height.

Patient population

One hundred thirty-three patients were assessed for the study. Forty patients with 70 atrophied sinus were eligible. As the study was designed to evaluate early NBF, 14 patients (25 sinus) were excluded because of protocol violations, which would have led to NBF bias. The main reason was that the implantation was outside the 3–4 months healing time (Fig. 1). The patients remaining included 45 severely atrophied sinus from 26 patients (age: 56.6 ± 8.0 years; range: 38.9–67.7 years). These patients were treated according to the protocol and were included in the per-protocol evaluation.

Each sinus was randomly assigned to either control or test arm. Randomization envelopes in blocks of six were generated in a 1:2 ratio for test and control. In the test arm, 34 sinus (25 patients) were augmented with BBM (Geistlich Bio-Oss®; Geistlich Biomaterials) and BMAC. Eleven control sinus (11 patients) were augmented with a mixture of 70% BBM and 30% AB harvested from the retromolar area. In 10 patients, the randomization resulted in a split-mouth model in which one side was treated as test arm and the contralateral side was control (Fig. 1 and Table 1). Because of the surgical intervention, neither surgeon nor patient could be effectively blinded.

Harvesting of bone marrow aspirate

The pelvic bone was punctured 2 cm laterocaudally from the superior posterior iliac spine. In a 60 mL syringe, flushed with heparin solution (sodium-heparine, 10,000 U/mL, diluted with NaCl to 1000 U/mL; both from B. Braun) and then filled with 8 mL of citric acid (BMAC-Kit; Harvest Technol-

FIG. 1. CONSORT chart for the clinical trial, starting with the number of screened patients, the number of randomized patients, leading to the number of patients included in the present per-protocol analysis. The patients included in the study can be divided into three groups: patients belonging to only test or control group, and patients with a split-mouth (cross-over) treatment.

TABLE 1. BASELINE VALUES FOR ALL SINUS INCLUDED IN THE EVALUATION

<i>Random no.</i>	<i>Bilateral</i>	<i>Split-mouth</i>	<i>Age</i>	<i>Gender</i>	<i>Volume gain CT scan (mL)</i>	<i>Healing time (months)</i>
Control group						
133	No		38.9	Female	0.80	3.27
136	Yes	Yes (135)	44.0	Male	0.75	2.53
141	Yes	Yes (142)	58.8	Female	0.94	3.97
148	Yes	Yes (149)	66.8	Male	2.03	3.33
150	Yes	Yes (151)	60.3	Female	0.29	3.97
154	Yes	Yes (153)	64.2	Female	1.55	3.03
160	Yes	Yes (161)	57.5	Male	1.53	3.10
162	Yes	Yes (163)	59.0	Male	2.46	3.43
168	Yes	Yes (169)	47.8	Female	1.17	3.10
303	Yes	Yes (301)	56.7	Female	1.67	3.27
306	Yes	Yes (305)	66.4	Female	1.49	3.70
Average			56.4		1.33	3.34
Standard deviation			9.1		0.62	0.42
Test group						
105	Yes		52.7	Female	n.a.	3.40
106	Yes		52.7	Female	n.a.	3.40
112	Yes		49.1	Female	2.17	3.83
113	Yes		49.1	Female	2.36	3.83
114	Yes		47.0	Female	0.63	3.77
115	Yes		47.0	Female	1.84	3.77
116	Yes		61.3	Female	1.13	3.23
117	Yes		61.3	Female	1.64	3.23
122	No		56.2	Female	1.18	3.03
126	Yes		67.7	Male	n.a.	2.90
127	Yes		67.7	Male	n.a.	2.90
130	Yes		45.6	Female	1.82	3.73
131	Yes		45.6	Female	2.49	3.73
135	Yes	Yes (136)	44.0	Male	0.55	2.53
138	No		41.1	Female	1.85	3.57
142	Yes	Yes (141)	58.8	Female	2.76	3.97
144	No		64.8	Female	3.60	3.10
149	Yes	Yes (148)	66.8	Male	1.34	3.33
151	Yes	Yes (150)	60.3	Female	1.51	3.97
153	Yes	Yes (154)	64.2	Female	1.80	3.03
155	Yes		67.7	Female	1.43	3.83
156	Yes		67.7	Female	1.59	3.83
157	Yes		55.7	Male	1.08	3.03
158	Yes		55.7	Male	1.18	3.03
159	No		61.1	Female	1.98	3.67
161	Yes	Yes (160)	57.5	Male	1.54	3.10
163	Yes	Yes (162)	59.0	Male	2.86	3.43
166	No		47.8	Female	1.43	3.97
169	Yes	Yes (168)	47.8	Female	1.84	3.10
301	Yes	Yes (303)	56.7	Female	2.11	3.20
302	Yes		60.4	Female	n.a.	3.20
304	Yes		60.4	Female	n.a.	3.27
305	Yes	Yes (306)	66.4	Female	2.26	3.70
307	No		60.8	Female	2.94	4.00
Average			56.7		1.74	3.43
Standard deviation			7.9		0.69	0.39
Total (control and test groups)						
Average			56.6			3.41
Standard deviation			8.1			0.39

Randomization numbers from 100 to 169 originate from the Freiburg center, and no. 300 onward from the Mainz center.

ogies Corporation), 52 mL of bone marrow was collected. According to the instructions of the manufacturer, bone marrow cells were isolated in 15 min directly in the operating room by using the BMAC system (Bone Marrow Procedure Pack; Harvest Technologies Corporation).

Colony-forming unit assay

To evaluate the number of MSCs, a probate amount of aspirate was used for cell count (Sysmex) and continuous growth from selected patients. Cells were plated on 96-well

plastic plates (Becton Dickinson) and cultured in bone marrow-derived mesenchymal stem cells medium (Cambrex) in a humidified atmosphere of 95% air and 5% CO₂ at 37°C for 7 days. Each sample was plated at a density of 10,000 nucleated cells per cm². The medium was initially changed after 24 h and then every second day. Nonadherent cells were washed from the culture. MSCs were selected on the basis of plastic adhesion. Colonies were counted under an inverted phase-contrast microscope at a final magnification of 50 ×.

Sinus augmentation procedure

Under general anesthesia or local anesthesia, a mucoperiosteal flap was raised to expose the lateral wall of the maxillary sinus. A bone window of 1.5 cm² was outlined with a round burr at 800 rpm with constant saline irrigation. Then, the Schneiderian membrane was detached from the sinus floor and lifted. Test sites were augmented with a combination of BBM (Bio-Oss® 0.25–1 mm; Geistlich Pharma AG) and BMAC with autologous thrombin made from venous blood (Thrombin Kit; Harvest Technologies Corporation). The thrombin was needed to clot the BMAC solution around the BBM. Three milliliters of bone marrow concentrate and 1 mL of autologous thrombin solution were added with a two-chamber syringe to 2 g of biomaterial with a volume of 4 cm³.²⁰ The biomaterial was applied according to clinical needs.

Control sites were augmented with a mixture of BBM (70%) and milled autologous bone (AB) (30%). No blood was added to the mixture. AB was obtained from the retromolar area as described by Sauvigne *et al.*²¹

A collagen membrane (Bio-Gide®; Geistlich Pharma AG) was placed over the facial sinus wall to cover the graft. The mucoperiosteal flap was replaced and closed with resorbable suture material (Vicryl 4-0; Ethicon). Patients were discharged from the hospital the same day. After an average healing period of 3.41 ± 0.39 months, second-stage implant placement was performed under local anesthesia. Cylindrical bone biopsies from the augmented maxillary sinus were taken with a trephine burr (Gebr. Brasseler GmbH & Co. KG) under 600 rpm and constant cooling.

Volume rendering

For 29 test sinus and 9 control sinus (Table 1), cone beam digital volume tomography (dental CT) was recorded because of clinical needs (Scanora [Soredex] and Promax [Planmeca]). After each 2 mm slice was manually outlined, the volume of the augmentation at the time of implant insertion was calculated by the program VoXim 4.3 (© IVS Solutions AG).

Histological evaluation

Bone biopsies were fixed in formalin for 48 h, rinsed with water, and dehydrated in serial steps of alcohol (70%, 80%, 90%, and 100%), remaining for 3 days in each concentration. Then, samples were infiltrated with resin for 2 weeks (Technovit 7200 VLC; Heareus Kulzer). The resin was polymerized in a UV light chamber for 10 h. After hardening of the polymer, two sections of 300–400 µm thickness were cut parallel to the trephine axis (Microslice; IBS). Sections were placed on acrylic slides (Maertin) and reduced to a thickness

of 100 µm on a rotating grinding plate (Struers). Specimens were stained with Azur II and Pararosanilin.

Histologic examination was performed with a light microscope (Axiovert 135; Zeiss). Pictures were digitally stored. BBM, AB, and NBF were manually marked on the screen (biomaterial = green; old bone = yellow; newly formed bone = red) while the actual specimen could be viewed under the microscope. Because of the short healing time, the local bone was clearly distinguishable from the augmentation site. Histomorphometric analysis was achieved by detection of RGB colors with the computer software AnalySISD Soft Imaging system (Olympus Europa GmbH). Only the augmentation site was evaluated for the histomorphometrical analysis and was marked as region of interest before. The differentiation between BBM particles, AB, and NBF tissue was possible, because osteocytes can be detected in the lacunae of NBF and AB. NBF was stained darker than AB and shows lamellar bone formation. The overall evaluated area was divided into BBM, newly formed bone, old bone, and nonmineralized tissue.

Proof of multipotency

MSCs obtained from the BMAC were amplified and differentiated into three cell lineages according to Pittenger *et al.*²² The lines were tested for their multipotent stem cell character in their ability to differentiate into chondrogenic, adipogenic, and osteogenic phenotypes. Adipocytes were stained with oil red O, a lipophilic red dye. Chondrogenic potential was confirmed by immunostaining with mouse anti-human aggrecan antibodies for aggrecan (counterstaining DAPI; Sigma) and collagen type II (both from Sigma). Osteogenic cells were tested for their expression of high levels of alkaline phosphatase (counterstaining with neutral red; both from Sigma) and collagen type I (counterstaining with Mayer's Hemalum solution; Merck), and calcification was assessed by van Kossa staining.

Statistical analysis

This study is a pilot study, as no data could be identified to give a thorough base for a power analysis. For the parameters new bone (NBF), BBM, and nonmineralized tissue, values were expressed in percentage of the evaluated area.

Data were analyzed by a linear mixed effect model with power weighting to correct for unequal variances.²³ Error bars giving standard errors and *p*-values are based on tests of linear contrasts. This model correctly handles correlation in the partial cross-over design used in this study.

Results

Twenty-six of 40 randomized patients (45 sinus) were treated according to the protocol including all visits in the set time window and were included in the per-protocol analysis (Fig. 1). Patients were treated between May 2006 and March 2008. The most prevalent reason for patient dropout/removal from evaluation was late implant placement outside the 3–4-month interval given for the healing time. Removal of these patients from the analysis was necessary to evaluate early bone formation, which is the aim of the study.

All patients from the test arm (BBM + BMAC) recovered well from the surgical procedure. No major intraoperative complications occurred. Occasional rupture of the sinus

membrane was treated by placing a membrane (Bio-Gide; Geistlich Pharma AG) over the rupture. No occurrence of pain, hematoma, or infection at any time after bone marrow aspiration and sinus floor augmentation in the postoperative time were recorded. In the control group (BBM+AB), one patient suffered from an injury of the inferior alveolar nerve, which resulted from the harvesting of the retromolar bone. Two patients of this group had an infection of the bone harvesting site. All noticed complications healed within reasonable time and standard care.

Healing time

Average healing time was 3.46 ± 0.43 months for the test group treated with BMAC and BBM, which was very similar to the control group (3.34 ± 0.42 months) treated with AB and BBM (Table 1).

Volumetric analysis

For 39 samples (28 test, 11 control), the radiographic bone volume was determined (Table 1). Radiologic gain and persistence of augmented bone height was statistically higher for the test (1.74 ± 0.69 mL) than for the control group (1.33 ± 0.62 mL) ($p=0.02$; 95% confidence interval of the difference: 0.13 to 1.04 mL).

Histological analysis

Specimens obtained from both the test and control sites were histologically similar (Fig. 2). No signs of inflammation were detected. Vital bone tissue containing osteocytes inside the bone lacunae was observed in the newly formed osseous lamellae. The biomaterial could be easily identified by its size, shape, and color in comparison to NBF or the preexisting local bone. NBF appeared dark red than BBM particles in the Azur II Pararosanilin staining (Fig. 2). The newly formed bone connected the biomaterial particles and stabilized the grafted complex. NBF was also observed in the macropores of the BBM particles. Blood vessels could be detected throughout the specimens, showing that the blood supply is ensured throughout the whole augmentate. Generally, the biomaterial with the NBF was well integrated in the surrounding local bone (Fig. 2). The maturity of the newly NBF was visually assessed. All specimens showed mainly woven bone, with regions of bone maturation and partially lamellar structure (Fig. 2).

Histomorphometrical analysis

An overview of the estimated values and the 90% confidence intervals of NBF, biomaterial, and marrow space are displayed in Figure 3. The histological values for each sinus are given in Table 2.

New bone formation. Average NBF was $14.3\% \pm 1.8\%$ in the control group and $12.6\% \pm 1.7\%$ in the test group. The difference was not statistically significant ($p=0.333$) with a 90% confidence interval for the difference of -4.6% to 1.2% for specimen obtained from test sites, giving a 95% non-inferiority limit of not more than 4.6% for bone formation of the test treatment with respect to the control treatment.

Biomaterial. The measured fraction of BBM was significantly higher for the test arm ($31.3\% \pm 2.7\%$) compared with

control ($19.3\% \pm 2.5\%$; $p<0.0001$); the difference resembles the mixing ratios of the test group (100% BBM) and the control group (70% BBM).

Marrow space. The remaining marrow space of the augmentate ($57.7\% \pm 2.3\%$) in the control arm was 3.3% nonsignificantly lower than the test arm (54.4 ± 2.2 ; $p=0.137$).

Colony-forming unit assay

The anticoagulated aspirate with 8 mL citric acid contained $17.2 \times 10^3 \pm 13.5 \times 10^3$ white blood cells (WBC)/ μ L. The BMAC process resulted in $79.4 \times 10^3 \pm 45.8 \times 10^3$ WBC/ μ L with 41.4 ± 15.6 colony-forming units (CFUs)/ 1×10^6 mononuclear cells.

Proof of multipotency

The cells taken from the BMAC procedure could be subsequently selected as plastic-adhered cells and successfully differentiated into adipocytes (as shown by oil red O staining), chondrocytes (as shown by aggrecan immunostaining and collagen type II activity), and osteoblasts (as shown by calcification, alkaline phosphatase, and collagen type I activity). This demonstrates their multipotency and proves them to be MSCs (Fig. 4).

Discussion

The regeneration of bone requires an adequate scaffold as well as cells that are capable to attach to the scaffold, proliferate, and differentiate. The dynamics of bone healing are controlled by growth factors, such as bone morphogenetic proteins. To promote these requirements, several strategies for bone and tissue regeneration have been developed. However, in the case of sinus floor elevation, the most frequently applied technique is the augmentation with alloplastic biomaterials, such as β -tricalciumphosphate (β -TCP) or hydroxyapatite (HA). In addition to being a common procedure, sinus floor elevation presents a good model to any clinical evaluation of augmentation materials, as bone formation occurs in an enclosed space, in which it can occur with a minimum of external factors. Further, a two-step procedure, with delayed implant placement, can provide researchers with bone biopsies, to monitor the bone development at a given moment.

Consequently, many bone substitutes have been evaluated in sinus floor procedures. BBM is one of the most widely used scaffolds employed in sinus augmentation procedures.^{24–26} It has similar physical properties to human cancellous bone, both in its morphological structure and its mineral composition.²⁷ Scientific studies have shown that BBM only remodeled to a minimal amount and, therefore, ensures the augmentation volume.²⁸ The higher BBM content could be responsible for the higher volume in the test arm. This result is in accordance with the animal trial by Gutwald *et al.* in which sinus augmentations with AB showed less volume than augmentations with BBM and MSCs.²⁰ Often autologous bone chips, providing preosteoblasts as well as autologous growth factors, are added to increase the kinetics of the initial bone formation. As a result, AB is still the most widely applied augmentation material.¹²

FIG. 2. Histological analysis of both test and control specimens. Overview of a whole obtained specimen: (A) control, (B) test site. (C, D) Control and test sites at $\times 5$ magnification. (C) The network of newly formed bone around the autogenous bone and bovine bone mineral (BBM) particles. The autogenous bone is in the process of being resorbed and replaced by newly formed bone. (D) A similar network of newly formed bone around BBM particles. No indication of BBM resorption can be seen. (E, F) Control and test site at $\times 40$ magnification. Both show areas of woven bone and indication of early remodeling into lamellar bone.

FIG. 3. Graphic presentation of the histomorphometric analysis. Shown are the mean values with the standard deviation for new bone, BBM, and marrow space. The lower part of the graph shows the comparison of the test and control groups with 90% confidence intervals.

This combination of AB and BBM has been investigated in previous histological sinus floor elevation studies. Yildirim *et al.* first evaluated the bone formation in sinus floor elevations performed with a mixture of AB and BBM in 13 sinus floor elevations and found $18.9\% \pm 6.4\%$ NBF after a 7.1-month healing period.²⁹ In a second study, Yildirim *et al.* examined, under similar conditions, sinus floor elevation procedures with only BBM and venous blood in 15 sinus of 11 patients and found $14.7\% \pm 5.0\%$ NBF after a healing time of 6.8 months.³⁰ Therefore, the addition of AB to the BBM yielded an advantage of 4.2% NBF, which can be translated in a delay in the healing time of a couple of months. Similarly, Thorwarth *et al.* wanted to further quantify this effect in the bone formation dynamics.³¹ They have used a minipig model, showing that in the first 8 weeks BBM with 25% AB added has superior bone forming kinetics compared with BBM alone. For longer healing times, this difference in formed bone quantity narrow were no longer detectable after 3 months. Arguably, these data cannot be translated one to one from minipigs to man, but should be regarded as a guideline that the initial bone formation is significantly higher if AB is added. In a previous animal trial, Sauerbier *et al.* found 49% more bone formation per time when bone marrow-derived MSCs were attached to BBM with fibrin glue.³² BBM and fibrin glue without cells served as control. This means that the clot alone was not responsible for the improved NBF.

Several tissue engineering approaches have been tried to stimulate bone formation. Similar to the results presented here, the addition of *in vitro* cultured MSCs has proven to

stimulate osteogenesis.^{33,34} Preclinical and clinical studies have demonstrated the ability of bone marrow-derived stem and progenitor cells to regenerate various tissues, including bone.³⁵ Shayesteh *et al.* evaluated clinical and histological findings in six patients and suggested that the addition of *in vitro* cultivated MSCs to HA/TCP may enhance the bone formation and allow implant placement after sinus floor augmentation.³⁶ Ueda *et al.* evaluated the use of tissue-engineered bone with *in vitro* cultured MSCs and subsequently differentiated osteoblasts, platelet-rich plasma, and β -TCP as grafting materials for sinus lift procedures and simultaneous implant placement in a study with 20 implants in six patients.³⁷ A mean increase of 7.3 ± 4.6 mm in bone height was seen in radiographic findings at 12 months after the surgery. The same research group used tissue-engineered bone in 14 patients for sinus lift and onlay grafts. This *in vitro* cultured bone induced bone formation and osseointegration of the placed dental implants.³⁸ To bypass the problem of selection, multiplication, and differentiation, most of the mentioned studies selected MSCs, multiplied them, and then differentiated them to the osteogenic lineage. As this is unreasonable to do in daily practice, an approach was chosen in which bone marrow aspirate or its concentrate is used unselected and undifferentiated, and therefore can be harvested and transplanted during the same surgical procedure. Based on the hypothesis that the number of MSCs is secondary, the individual potential of MSCs to survive high stress and chemotactically attract osteogenic progenitor cells will dominate. As the augmentation material within the sinus is far from local blood supply, it can be assumed that a natural selection procedure will be applied to the cells. As blood and oxygen supply are reestablished, the MSCs could then unfold their full potential.³⁹ In clinical settings, it is ethically difficult to prove survival of transplanted cells. Smiler *et al.* described a small number of cases in which bone marrow aspirate from the iliac was placed onto biocompatible scaffolds. The procedure successfully regenerated bone in sinus augmentations and particulated onlay grafts of the maxilla.⁴⁰

The data presented here did not show a correlation between the number of obtained MSCs from each BMAC procedure and the amount of bone formation seen in the histologies.¹⁹ The correlation, seen by others is probably masked by the variability of the potential of the MSCs among the patients.⁴¹ The BMAC procedure resulted in a 4.6-fold increase of WBC from $17.2 \times 10^3 \pm 13.5 \times 10^3/\mu\text{L}$ to $79.4 \times 10^3 \pm 45.8 \times 10^3/\mu\text{L}$ with 41.4 ± 15.6 CFUs/L $\times 10^6$ mononuclear cells. This means an estimated 2465.7 colony-forming cells/augmented 1 cm^3 .

From the triangle of bone formation, made up of matrix, cells, and growth factors, only the growth factors can increase the overall kinetics of bone formation, as seen in the study by Jung *et al.*⁴² The addition of autogenous cells can only overcome the initial lack phase leading to a higher early bone formation when compared with augmentations performed with bone substitutes. In the present study, the BMAC biomaterial mixture showed NBF comparable to the autologous bone biomaterial composition. BMAC could therefore compensate partially for the normally added AB and eliminate the lack phase seen by augmentations with only bone substitutes.

Consequently, the aim of the presented autologous BMAC approach is to achieve a bone formation rate comparable to

TABLE 2. HISTOMORPHOMETRIC DATA OF EACH INDIVIDUAL SINUS

Random no.	No. of slides	New bone	BBM	Marrow space	Healing time (months)
Control group					
133	2	14.8	29.6	54.0	3.27
		16.3	24.6	52.9	
136	3	10.4	11.9	77.7	2.50
		21.6	3.5	67.4	
		18.6	1.5	71.4	
141	2	8.9	7.6	67.9	3.97
		10.7	6.3	66.8	
148	2	9.9	28.4	61.7	3.33
		8.6	30.5	60.9	
150	2	13.4	15.4	71.2	3.97
		17.0	7.2	75.8	
154	1	6.5	31.9	51.1	3.03
160	2	21.4	9.2	69.4	3.10
		16.7	13.2	70.1	
162	2	11.0	34.1	54.9	3.43
		12.8	40.5	46.7	
168	2	14.4	23.2	60.5	3.10
		18.4	20.6	58.5	
303	2	12.7	29.4	42.4	3.20
		13.3	30.3	34.0	
306	4	13.9	19.2	53.3	3.70
		13.6	13.0	59.0	
		6.9	23.7	57.0	
		7.3	27.5	57.0	
Intercept		14.3	19.3	57.7	
Standard deviation error		1.8	2.7	2.3	
Test group					
105	3	31.5	34.2	34.3	3.40
		29.6	35.8	34.6	
		39.2	33.1	27.7	
106	2	34.1	26.0	39.9	3.40
		26.2	35.8	38.0	
112	2	21.6	35.7	42.7	3.83
		24.9	31.9	44.0	
113	2	34.9	24.3	40.8	3.83
		26.3	25.3	48.4	
114	2	20.5	23.7	55.8	3.77
		17.7	18.8	63.5	
115	2	1.5	38.5	60.2	3.77
		1.0	41.7	57.3	
116	2	12.5	39.5	48.0	3.23
		11.8	37.3	50.9	
117	2	19.0	29.1	51.9	3.23
		15.9	32.1	52.0	
122	2	10.1	40.6	49.3	3.03
		8.2	45.3	46.5	
126	1	3.7	38.8	57.5	2.90
127	1	9.3	37.4	53.3	2.90
130	3	11.6	23.2	65.2	3.73
		10.6	26.3	63.1	
		11.1	27.5	61.4	
131	4	10.9	36.4	52.7	3.73
		7.6	36.5	55.9	
		14.8	18.4	66.8	
		11.7	22.8	65.5	
135	3	14.9	9.7	75.4	2.50
		6.3	29.9	63.8	
		6.7	31.7	61.6	
138	6	24.6	8.1	67.3	3.57

TABLE 2. (CONTINUED)

Random no.	No. of slides	New bone	BBM	Marrow space	Healing time (months)
		23.7	5.2	71.1	
		13.7	33.1	53.2	
		17.2	34.8	48.0	
		21.9	28.2	49.9	
142	4	16.1	31.7	52.2	3.97
		6.4	21.0	72.6	
		12.5	22.0	65.5	
		11.2	28.0	60.8	
		5.1	42.1	52.8	
144	8	19.1	14.8	66.1	3.10
		23.3	14.2	62.5	
		13.6	35.8	50.6	
		13.6	27.7	58.7	
		14.7	17.6	67.7	
		17.8	24.7	57.5	
		13.9	32.9	53.2	
		11.9	29.2	58.9	
149	2	12.1	32.2	55.6	3.33
		10.4	34.2	55.4	
151	4	7.4	27.5	65.1	3.97
		8.4	32.8	58.7	
		11.4	39.8	48.8	
		12.6	36.3	51.1	
153	6	23.8	13.5	62.7	3.03
		12.8	33.0	54.2	
		8.4	40.5	51.1	
		11.4	41.2	47.4	
		6.7	31.9	61.4	
		2.7	35.7	61.6	
155	2	16.8	33.9	49.3	3.83
		17.6	33.1	49.3	
156	2	16.9	36.1	47.0	3.83
		15.5	33.8	50.7	
157	1	0.2	37.2	62.6	3.03
158	1	8.6	31.2	60.2	3.03
159	2	12.2	39.6	48.2	3.67
		14.8	42.8	42.4	
161	1	2.1	35.0	62.9	3.10
163	2	11.1	36.7	52.2	3.43
		2.4	38.7	58.9	
166	2	17.2	29.1	53.7	3.97
		21.7	24.1	54.2	
169	2	10.9	27.3	61.8	3.10
		15.3	34.8	49.9	
301	2	16.9	36.3	46.8	3.20
		15.0	36.4	48.6	
302	2	8.9	48.3	42.8	3.27
		7.2	46.8	46.0	
304	2	3.7	39.6	56.7	3.27
		4.7	37.6	57.7	
305	4	9.1	30.7	60.2	3.70
		8.5	34.0	57.5	
		10.8	43.0	46.2	
		6.6	37.0	56.4	
307	2	19.0	27.4	53.6	4.00
		21.6	22.0	56.4	
Intercept		12.6	31.3	54.4	
Standard deviation error		1.7	2.7	2.2	

Number of slides evaluated per randomized sinus and the intercept resulting from the contrast table of the mixed model (lme) in combination with the standard error are given.

BBM, bovine bone mineral.

(continued)

FIG. 4. Test for multipotency of the colony-forming units obtained from the bone marrow aspirate concentrate procedure. Phenotype and staining used: (A) mesenchymal stem cells from colony-forming units: HE staining; (B) adipocytes: oil red O staining; red indicates intracellular fat droplets; (C) chondrocytes: aggreCAN immunostaining (positive is red, DAPI counterstaining is blue); (D) chondrocytes: collagen type II (brown staining is positive, counterstaining with Mayer's Hemalum solution); (E) osteoblasts: blue staining is positive for alkaline phosphatase; (F) osteoblasts: collagen type I activity (reddish-brown staining); (G) calcification by osteoblasts: van Kossa staining. Dark brown staining indicates calcified tissue.

BBM with autologous bone. To test this hypothesis, the study was designed to measure the initial bone formation kinetics and not primarily the maximal bone formation. Therefore, early time point after a healing phase of only 3–4 months was chosen.

NBF over both groups was very similar. The biopsies of the control group showed $14.3\% \pm 1.8\%$ NBF, and those of the treatment group with BBM and BMAC resulted in $12.6\% \pm 1.7\%$ NBF. Statistically, the newly formed bone of the test group was equivalent to that of the control group, which indicates that the NBF of the BMAC/BBM mixture is equivalent to the NBF of AB/BBM after 3–4 months, indicating good early bone formation. Bone formation after 14.8 ± 0.7 weeks was with $17.7\% \pm 7.3\%$ even more in the BMAC–biomaterial side than the $12.0\% \pm 6.6\%$ in the bone–biomaterial side in a split-mouth trial of Rickert *et al.* on 11 patients ($p=0.026$).⁴³ The reason for the higher NBF in the BMAC group in the study by Rickert *et al.* is not completely clear. There the patients were even older (60.8 ± 5.9 years) than in the present study (56.6 ± 8.0 years). The autologous bone particles of the control group were smaller in Rickert's study than in the presented multicentric study in which 6 different surgeons treated 70 sinus. In the Rickert study 1 surgeon did the complete surgery on 22 evaluated sinus.

The present study supports the data of Minamide *et al.*, who found in a histomorphometric rabbit study no differences between the spinal fusion treatment using autologous bone mixed with HA and bone marrow-derived MSCs and HA.⁴¹ Presently, augmentations performed with BBM in a blood clot are left for a longer healing time, compared with augmentations preformed with AB.^{29,30}

As the bone formation is equivalent in both groups, the option presented here of adding BMAC to a biomaterial reduces the healing time or osseointegration time of the implant and therefore leads to a reduced treatment time and earlier dental rehabilitation of the patient when compared with BBM alone.^{29,30}

Conclusion

BMAC associated with BBM can regenerate equivalent amount of new bone when compared with autologous bone mixed with BBM even after a short healing time of 3–4 months. The BMAC mixture seems to fully compensate the benefit known for AB in early bone formation. Therefore, BMAC/BBM is a new treatment option almost as convenient as the treatment with only biomaterials and as potent as with AB.

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