Placement of Autogeneic Bone Chips or Bovine Bone Mineral in Guided Bone Augmentation: A Rabbit Skull Study

Christer Slotte, DDS, PhD[1]/Dan Lundgren, DDS, PhD[2]/Patricia Miranda Burgos, DDS[3]

Purpose: *Our primary aim was to use a rabbit guided bone augmentation model to evaluate whether use of autogeneic bone grafts or bovine bone mineral (BBM) combined with a space-making barrier enhances bone augmentation compared with a barrier alone.* ***Materials and Methods:*** *Sixteen rabbits were studied. In each rabbit, 2 titanium cylinders, each with 1 titanium lid, were placed subcutaneously in perforated slits made in the cortical bones, with their open ends facing the parietal bones. One cylinder was left empty and the other was filled with either autogeneic skull bone chips or BBM. Bone labels were injected after 4 and 11 weeks. After 12 weeks, the animals were sacrificed to obtain ground sections for histology and histomorphometry.* ***Results:*** *Significantly more tissue was augmented in the 2 test groups than in the control group. Most of the autografts were resorbed, leaving only minute amounts in the upper third of the cylinders. Slender new bone trabeculae were distributed mainly from the contiguous bone plate that had no contact with the remaining graft material. In the BBM group, most of the BBM remained evenly distributed in the cylinder. In the upper third of the cylinder, the BBM was surrounded by soft connective tissue, while in the lower two thirds, mainly mineralized bone enclosed the BBM. Equal amounts of mineralized bone were found in both test groups. Comparisons of contact between bone and BBM on one hand and bone and bone cylinder wall on the other revealed that the greatest bone contact was with the BBM in the lower third of the cylinder. In the middle and upper third of the cylinder, bone-BBM contact and bone–cylinder wall contact were similar. Fluorescent label intensity was higher in the autograft group than in the BBM group. In all 3 groups the intensity of the early label was similar to that of the late label, indicating that the graft materials do not seem to retard mineralization.* ***Discussion:*** *BBM was found to promote as much new bone as did autogeneic bone. In addition, BBM appears to have at least the same osteoconductive properties as titanium, provided BBM is contained in a stable environment.* ***Conclusions:*** *Placement of autogeneic bone or BBM in conjunction with a stiff space-making barrier generated more tissue than a barrier only. In this model, autogeneic bone chips and BBM augmented similar amounts of new mineralized bone.* (More than 50 references) INT J ORAL MAXILLOFAC IMPLANTS 2003;18:795–806

Key words: *autologous transplanation, bovine bone mineral, guided bone augmentation, rabbit, skull bone*

Guided tissue regeneration (GTR) is generally perceived as a procedure wherein space-making membrane barriers that give preference to desirable tissue-matrix-producing cells are used in the regeneration of tissue.[1–7] The procedure has been shown to generate bone both in animal models[8–12] and clinically.[13–15] No consensus has been established, however, on the precise definition of guided bone augmentation (GBA). Strictly speaking, GBA is the creation of new bone through the guidance of bone cells to an area beyond the original skeletal envelope (outer or inner).[12]

Osteoconduction is generally perceived as a 3-dimensional process of ingrowth of capillaries, perivascular tissue, and osteoprogenitor cells from the recipient bed into the structure (framework) of an implant or bone graft.[16] A framework porosity of 100 to 200 µm has been shown to be optimal for ingrowth of bone within the skeletal envelope.[17,18]

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Bone autografting, for which there are various approaches, is considered to be the gold standard for bone reconstruction.[19–24] Few investigations have, however, studied bone autografts in conjunction with GBA.[23,25–29] Autogeneic bone grafting usually requires a second operation site and may cause various degrees of morbidity in the donor area. Also, when the amount of donor bone is small, as in the maxillofacial area, the gain in bone volume may be minor. Moreover, free (unvascularized) block autografts may undergo partial necrosis and resorption because of prolonged ischemia and insufficient subsequent revascularization.[20,30-34] Hence, the degree of osseointegration and the stability of a titanium implant placed in a grafted area may be limited. The use of particulated autogeneic bone grafts (bone chips) is one approach for overcoming some of the problems described above. The nutrition of cells within the graft may be facilitated if the bone graft is split into smaller pieces, initially by diffusion through the blood clot and subsequently through newly formed vessels. In addition, milling or morseling the bone may promote the release of osteoinductive substances within the bone matrix, enhancing new bone formation. On the other hand, small bone particles that are not rigidly fixated and therefore undergo micromotion may inhibit bone formation.[35]

Bone substitutes may be used in augmentation procedures when the supply of autogeneic bone is limited. The use of bone substitutes in augmentation procedures can *(1)* maintain the space available for tissue ingrowth by preventing barrier collapse, *(2)* enhance osteoconduction by forming a porous framework, and *(3)* prevent wound contraction by stabilizing the blood clot and subsequent provisional matrix. Bone substitutes may also serve as possible carriers for bone-inducing growth factors. The use of Bio-Oss (Geistlich Biomaterials, Wolhusen, Switzerland), a bone substitute of bovine bone mineral (BBM), in the reconstruction of defects within the skeletal envelope[36–40] or augmentation beyond the skeletal envelope[41–47] has been studied extensively (for an extensive review, see Carmagnola[48]). Recently, it has been shown in animal augmentation models that BBM has a greater capacity to promote calvarial tissue than the negative control.[49,50] However, a previous GBA study by Slotte and Lundgren[51] found that placement of BBM on the rat calvaria resulted in lower amounts of new mineralized bone compared with the negative control and also seemed to delay the mineralization of the augmented tissue. Stavropoulos and coworkers[52] had similar findings in a recent GBA study in the rat mandible. In the Slotte and Lundgren[51] study, ingrowth of soft tissue from the sagittal suture in the experimental space could not be prevented. Also, the elasticity of the silicone material of the device might have increased the micromotion of the barrier and, in turn, hindered hard tissue formation. In the study by Stavropoulos and coworkers,[52] instability resulting from the elasticity of barrier-anchoring sutures and strain from the overlying jaw muscles might have caused micromotion or displacement of the barrier, resulting in ingrowth of suprabony connective tissue into the experimental space.

Thus, in the studies discussed above, it is possible that *(1)* the choice of experimental sites and *(2)* the design of the devices influenced the results. A stiff barrier designed to exclude the ingrowth of suprabony tissue may overcome some of these problems. Also, use of a larger species of experimental animal with a larger bone plate devoid of sutures in the experimental area may improve experimental studies of GBA.

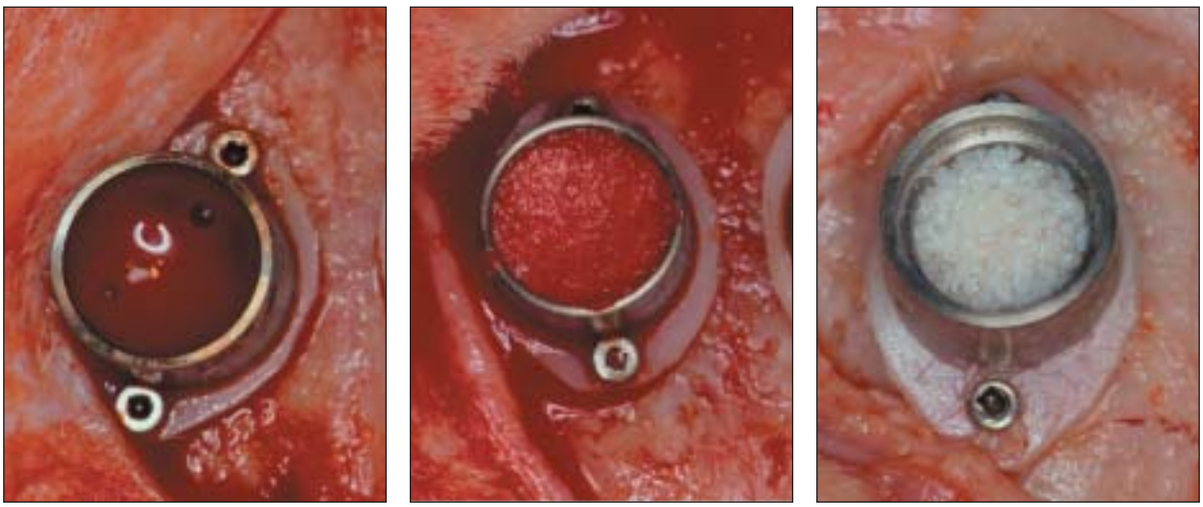
The aims of the present investigation were to use a rabbit GBA model to *(1)* evaluate whether the use of autogeneic bone grafts or BBM combined with a space-making barrier enhance bone augmentation compared with a barrier alone; *(2)* assess the degree of contact between augmented bone and the supplied material; *(3)* assess the degree of contact between augmented bone and the barrier wall; and *(4)* assess the degree of mineralization in early and late stages of wound healing.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Research Ethics Committee at Göteborg University, Göteborg, Sweden. The housing and care of the experimental animals followed the routines at the Laboratory for Experimental Biomedicine at Göteborg University. For a comprehensive description of anesthesia, surgery, and the augmentation device, the reader is referred to other papers from this laboratory.[53,54]

Animals and Anesthesia

Sixteen female adult (9-month-old) New Zealand white rabbits weighing 3 to 4 kg were used in the experiment. General anesthesia was induced by the injection of a mixture of 10 mg/mL fluanisone and fentanyl citrate (0.315 mg/mL, Hypnorm, Janssen Pharmaceutica, Brussels, Belgium) intramuscularly and diazepam (5 mg/mL, Stesolid Novum, Dumex, Copenhagen, Denmark) intraperitoneally. In addition, a local anesthetic (20 mg/mL, Xylocain, Astra, Södertälje, Sweden) was given subcutaneously.



Figs 1a to 1c The titanium cylinders, including *(left)* a control cylinder, *(center)* a test cylinder filled with autogeneic bone chips, and *(right)* a test cylinder filled with bovine bone material, in place on the parietal bones minus the titanium lids. The contiguous bone was perforated. No attempt was made to add blood to the cylinder space. Each rabbit received 1 test and 1 control cylinder.

Experimental Device

A turned cylinder of commercially pure titanium with an inner diameter of 6 mm and an inner height of 4.5 mm was used as the experimental chamber. The roof of the cylinder was an occlusive titanium lid. A Delrin frame (DuPont, Kista, Sweden) was snapped in an outer circumferential slit at the base of the cylinder. The device was fixed to the skull bone with 2 stainless steel miniscrews placed in the Delrin frame (Figs 1a to 1c).

Bone Graft and Bone Substitute

Cortical autogeneic bone was harvested from the skull of each rabbit using a bone grafting device (mx-grafter, Maxilon Laboratories, Hollis, NH). This device comprises a blade with a cutting edge and a chamber where collected bone is stored. A light downward pressure on the blade placed on the bone surface and a gentle raking motion were used to collect thin shavings of bone.

Cancellous particles (0.25 to 1 mm long) of Bio-Oss BBM were used as a bone substitute. During manufacturing, the bovine bone is deproteinized in an alkaline and heat treatment that leaves the original lamellar and porous bone structure unchanged.

Surgery

Surgery was conducted under aseptic conditions. After the vertex was shaved and disinfected, a full-thickness flap was raised to expose the calvaria. With a trephine, a 0.5-mm-deep circular slit that did not cross the cranial sutures was prepared in each parietal bone. Within the area bordered by this slit, the cortical bone plate of both sites was perforated with 7 evenly distributed holes using a round bur with a diameter of 1.2 mm. A titanium cylinder was placed in each slit and attached to the bone with 2 miniscrews through the Delrin frame. Each rabbit received 1 test and 1 control cylinder. Which side (left or right) would be the test side and which the control was determined randomly, as was the choice of graft material. The test cylinders were gently filled with either autogeneic bone chips or BBM, and the control cylinders were left empty. The preparation of the bone and the filling procedure resulted in various degrees of bleeding in the cylinders. No other attempts were made to fill the devices with blood.

The titanium lids were press-fitted onto the inner shelf near the top of the cylinders. The skin flaps were relocated and attached by Vicryl sutures (Ethicon, Somerville, NJ). No attempt was made to cover the cylinders with the periosteum. After the operation, the animals were given antibiotics in a single intramuscular injection (300 mg/mL Penovet [benzylpenicillin procaine], Boehringer Ingelheim, Hellerup, Denmark). A single dose of analgesic (0.3 mg/mL Temgesic [buprenorphine hydrochloride], Reckit & Colman, Hull, United Kingdom) and saline was given subcutaneously. The animals were held in separate cages for 2 weeks and thereafter kept together until the end of the experiment. Fluorochrome bone labels were given as single injections subcutaneously: 25 mg/mL oxytetracycline at 25 mg/kg body weight (Sigma, St Louis, MO) after 4 weeks and 30 mg/mL alizarin complexone at 30 mg/kg body weight (Sigma, St Louis, MO) after 11 weeks.

Specimen Preparation

The animals were sacrificed after a healing period of 12 weeks by intramuscular injections of Hypnorm followed by intravenous injections of a solution of pentobarbitol (100 mg/mL, Apoteksbolaget, Uppsala, Sweden). The soft tissues of the experimental area were removed, and biopsy specimens of the parietal bones comprising the 2 titanium cylinders were taken en bloc and fixated by immersion in 4% buffered formaldehyde.

The specimens were dehydrated in a graded series of alcohol and embedded in plastic resin (Technovit 7200 VCL, Kulzer, Wehrheim, Germany). Vertical sections through the centers of the cylinders were cut using a sawing and grinding technique (the Exakt system; Exakt Apparatebau, Norderstedt, Germany). Each section was ground to a thickness of about 100 µm and microradiographed. The specimens were ground to 50 µm and 20 µm to assess the thickness that best allowed the study of the fluorochrome intensity. In the final step, the sections were ground to 10 µm and stained with 1% toluidine blue and 1% pyronin G.

Analyses

Bone labels were revealed best in the 20-µm sections. The intensity of the fluorochrome bone labels within and outside the titanium cylinders and also in the contiguous bone plate was assessed using a modified scale originally suggested by Puranen[20]: 0 = no fluorescence in the assessed area, 1 = scattered fluorescence within the assessed area, 2 = distinct fluorescence in the assessed area, and 3 = intense fluorescence throughout the assessed area.

Histologic examination and morphometric assessments of the sections were made with a Leitz Aristoplan microscope (Leitz, Wetzlar, Germany) equipped with a Leitz Microvid Morphometric System and connected to a personal computer. The morphometric measurements were made on 1 vertical section through the center of the titanium cylinder from each site. The following measurements were made:

1. The total experimental area as bordered by the inner surface of the cylinder, the lid, and the outer surface of the parietal bone (expressed in mm^2) and the height of the cylinder wall (mm)
2. The total area of augmented tissues (mm^2)
3. The area of mineralized bone (mm^2)
4. The area (mm^2) and perimeter (mm) of remaining autograft/BBM particles
5. The nonmineralized tissue area (mm^2)
6. The mean height of the augmented tissues *(a)* at the center of the cylinder and *(b)* along the inner surface of the cylinder wall (mm)
7. The amount of contact between mineralized bone and the inner wall of the cylinders (mm)
8. The amount of contact between newly formed bone and remaining graft material (mm and percent)

These measurements enabled the authors to calculate the percentage of tissue fill, bone density, bone-titanium wall contact, and bone-graft contact. Area measurements were considered representative of volumetric values.[55]

The histomorphometric measurements were made by authors CS and PMB. No attempt was made to mask readings of the sections because of the obvious differences in appearance between the test and control areas. Intraexaminer precision was tested by making triplicate readings of augmented tissues 1 week apart in 32 sites. The Friedman test found no significant differences between these measurements ($P = .747$). Interexaminer correlation was evaluated by making area and length measurements in a subset of 45 different structures in 16 sections. The paired Student t test showed a correlation of 0.997 between examiners ($P < .001$). Difference in the mean and SD of the readings between the examiners was –0.10 ± 0.89 with a confidence interval (CI) of –0.36 to 0.17, $P = .463$.

Statistical Analyses. A statistical software package (SPSS, Chicago, IL) was used. The Mann-Whitney test was run for group analysis of fluorochrome uptake. Analysis of histomorphometric measurements was made using the 1-way analysis of variance (ANOVA). The Levene statistic was used to test homogeneity of variances, and the Bonferroni multiple comparison test was used post hoc to determine the location of significant differences. Wilcoxon's signed rank test was used for paired comparisons within groups. Significance for the analyses was set to $P < .05$.

RESULTS

All animals recovered well, and the skin wounds healed uneventfully. At biopsy specimen harvesting, all cylinders were found to be stable and in the same position as at placement. The cylinders contained varying amounts of newly formed tissue with no signs of inflammation or ingrowth of suprabony connective tissue. In the control group the augmented tissue comprised slender bone trabeculae distributed in abundant nonmineralized tissue with the same appearance as the contiguous bone marrow. Newly formed bone was found mainly along the cylinder wall and on the contiguous bone surface

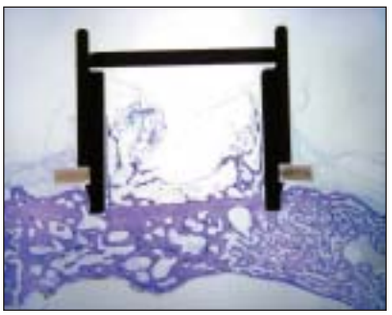


Fig 2 Transverse cross-section through the center of a control cylinder with the augmented bone tissue and the subjacent parietal bones. The cylinder is partly filled with slender bone trabeculae and abundant nonmineralized tissue. The tissue is higher along the inner wall than at the center of the cylinder (toluidine blue /pyronin G; original magnification × 1.4).

Fig 3a Representative specimen from the autogeneic bone chip group. Most of the graft material had been resorbed, and only small amounts of thin shavings were left in the uppermost part of the cylinder. The experimental space was almost totally filled with new tissue. Slender bone trabeculae were evenly distributed in the augmented tissue (toluidine blue /pyronin G; original magnification × 1.4).

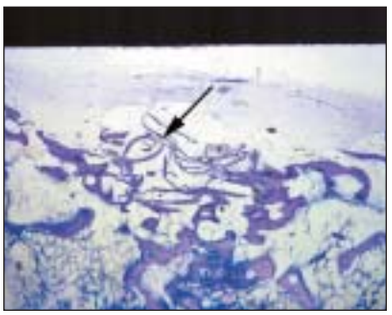
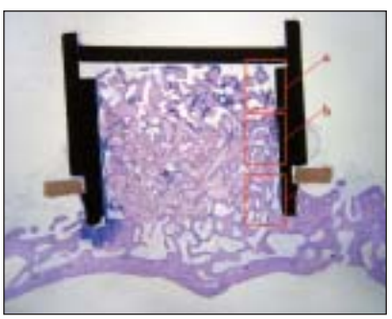


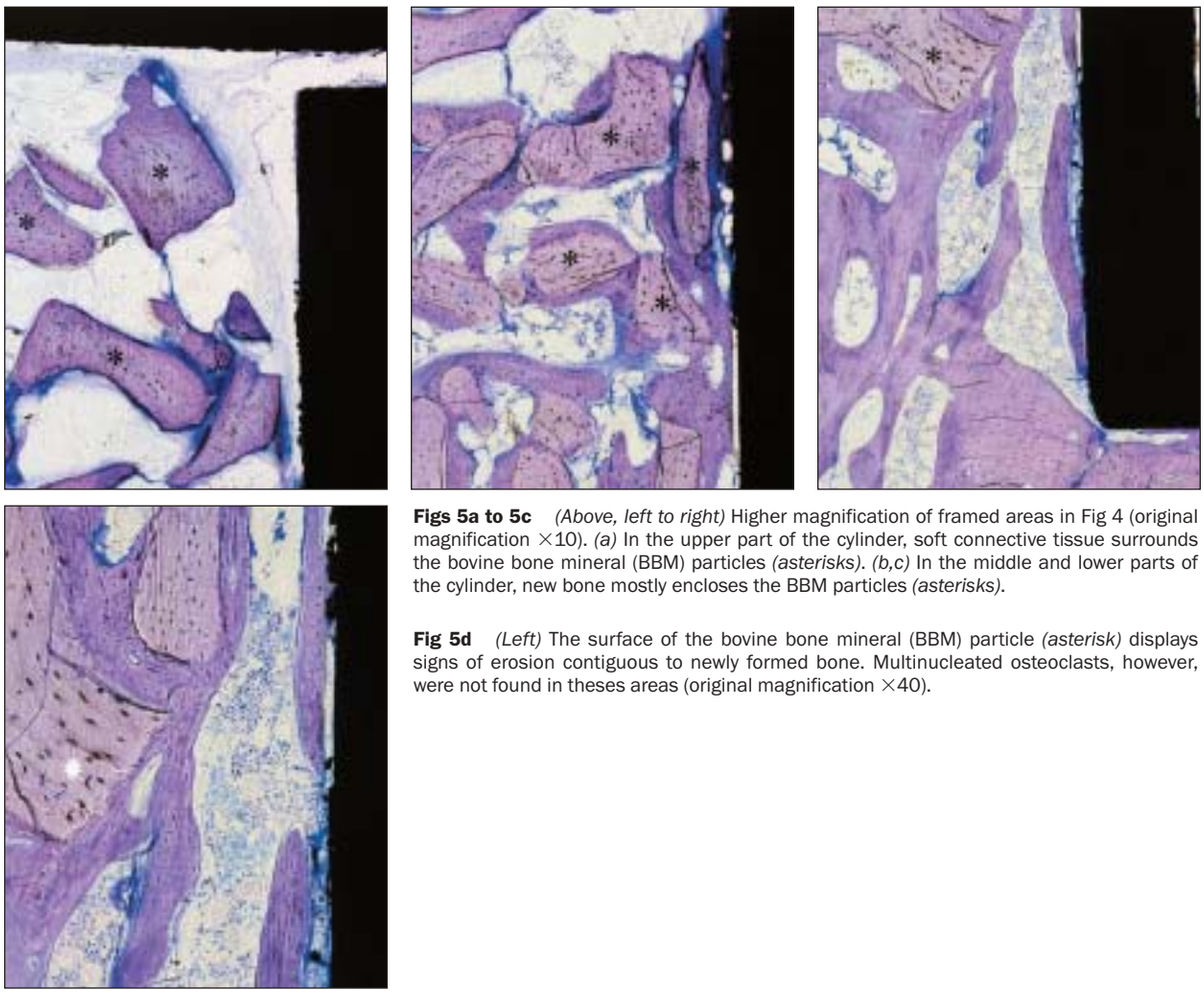
Fig 3b Higher magnification of residual autogeneic bone particles in the uppermost part of the cylinder. The graft bone seems to be necrotic. Contact with newly formed bone is sparse (original magnification ×4).

Fig 4 Representative specimen from the bovine bone mineral (BBM) group. The experimental space was almost totally filled with new tissue. Most of the BBM seemed to remain and was evenly distributed in the new tissue (toluidine blue /pyronin G; original magnification × 1.4).



(Fig 2). Significantly more tissue was augmented in the 2 test groups than in the control group ($P < .001$) (Figs 3 and 4). About 93% of the experimental space in the bone autograft group was filled with new tissue, 94% in the BBM group, and 55% in the control group. Most of the autogeneic bone had been resorbed. Minute amounts of necrotic bone were found encapsulated in nonmineralized, marrow-like tissue in the uppermost part of the experimental space (Fig 3b). Slender bone trabeculae were distributed evenly in the experimental space. Contact between bone and autograft remains was sparse. Most of the bone substitute material in the BBM group seemed to be intact and evenly distributed within the augmented tissue. In the upper third of the cylinder, the BBM was mostly surrounded by nonmineralized tissue, while in the lower two thirds, the BBM was mainly enclosed by mineralized bone (Figs 5a to 5c). Multinucleated giant cells were regularly found in the nonmineralized tissue close to the BBM particles, while in areas with mineralized bone in contact with BBM, no such cells could be identified despite signs of surface erosion of the BBM material (Fig 5d).

Results of the morphometric measurements are presented in Figs 6 to 8 and Tables 1 and 2. Similar amounts of mineralized bone were found in the autograft and the BBM groups. The BBM group had significantly more mineralized bone than did the control group ($P = .049$). The cylinders in the bone autograft group contained significantly more nonmineralized tissue than the cylinders of the other 2 groups ($P < .001$). Significantly more residual BBM than autogeneic bone was found ($P < .001$). In the control group, the augmented tissue consistently reached higher along the cylinder walls than in the center (3.4 ± 0.7 mm versus 2.4 ± 0.8 mm, $P = .003$). In the test groups the height of the tissue along the cylinder walls was significantly higher than in the control group: 4.7 ± 0.3 mm in the bone autograft group and 4.8 ± 0.3 mm in the BBM group ($P < .001$). The height of augmented tissue in the centers of the test cylinders did not differ significantly from the cylinder wall values (4.6 ± 0.4 mm in the bone autograft group and 4.7 ± 0.3 mm in the BBM group). The average bone–cylinder wall contact was 50.8% ± 13.2% in the bone autograft group. The corresponding values were 45.5%



Figs 5a to 5c *(Above, left to right)* Higher magnification of framed areas in Fig 4 (original magnification ×10). *(a)* In the upper part of the cylinder, soft connective tissue surrounds the bovine bone mineral (BBM) particles *(asterisks)*. *(b,c)* In the middle and lower parts of the cylinder, new bone mostly encloses the BBM particles *(asterisks)*.

Fig 5d *(Left)* The surface of the bovine bone mineral (BBM) particle *(asterisk)* displays signs of erosion contiguous to newly formed bone. Multinucleated osteoclasts, however, were not found in theses areas (original magnification ×40).

± 13.0% in the BBM group and 46.4% ± 20.5% in the control group. The differences between the groups were not statistically significant.

The perimeters of remaining bone graft/BBM materials and their contact with mineralized bone are shown in Fig 7 and Table 2. The perimeter of autograft remains found in the upper third of cylinder space was significantly larger than that found in the lower two thirds (P = .012). The bone-autograft contact in the lower third was significantly lower than in the upper third (P = .012). The perimeter of remaining BBM was significantly smaller in the lower third than in the middle third of the cylinders (P = .036), while no significant differences were found between the upper and lower and the upper and middle thirds. Bone-BBM contact was significantly lower in the upper than in the middle and lower thirds of the cylinders (P = .012). Comparisons of the degree of bone contact with the cylinder wall on the one hand and with BBM on the other revealed similar values in the middle and upper thirds of the experimental space, while in the lower third, the degree of bone contact was significantly higher with BBM than with the cylinder (Fig 8). When total experimental areas were compared, bone contact values with BBM and with the cylinder wall were similar.

The uptake of fluorescent label was generally weak. No differences could be found between label intensity within the cylinders and the contiguous bone plate (data not shown). Neither could any differences be found between the uptake of the 4-week label (oxytetracycline) and that of the 11-week label (alizarin complexone) within the cylinders. A significantly higher median value for both labels, however, was found in the autograft group than in the BBM group (P = .040). Label uptake within the titanium cylinders is presented in Fig 9.

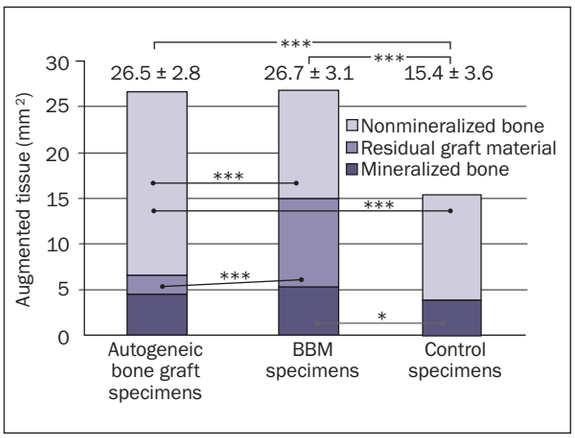


Fig 6 Results of morphometric measurements of the area of augmented tissues. *P = .049; **P < .001 (1-way ANOVA); BBM = bovine bone mineral.

Table 1 Mean ± Standard Deviation Values (in mm²) of Area Measurements Illustrated in Fig 6

	Autogeneic bone graft specimens	BBM specimens	Control specimens
Nonmineralized tissue	20.1 ± 2.6	12.0 ± 2.7	11.6 ± 3.5
Residual graft material	1.9 ± 1.9	9.4 ± 1.1	—
Mineralized bone	4.6 ± 1.4	5.3 ± 0.8	3.9 ± 1.4

BBM = bovine bone mineral.

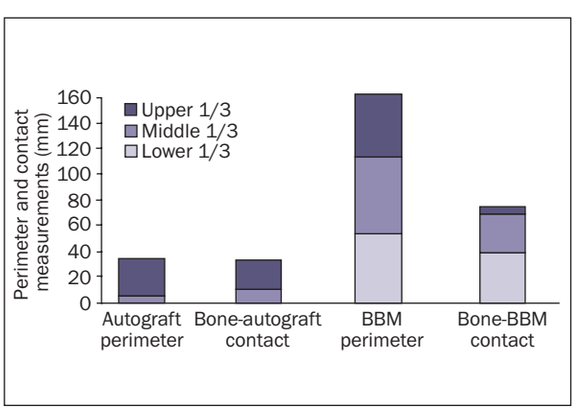


Fig 7 Results of morphometric perimeter measurements of residual autogeneic bone graft and BBM materials and their contact with newly formed bone. BBM = bovine bone mineral.

Table 2 Mean ± Standard Deviation Values of Perimeter and Bone-Graft Contact Measurements Illustrated in Fig 7

	Autograft perimeter	Bone-autograft contact	BBM perimeter	Bone-BBM contact
Upper 1/3	28.6 ± 25.1	4.2 ± 1.6	48.7 ± 13.5	5.7 ± 6.9
Middle 1/3	5.0 ± 9.3	1.9 ± 3.5	60.5 ± 6.9	31.2 ± 13.9
Lower 1/3	0.4 ± 1.1	0.3 ± 0.8	53.1 ± 6.6	37.9 ± 5.5

*P = .012; **P = .036 (Wilcoxon signed rank test).
BBM = bovine bone mineral.

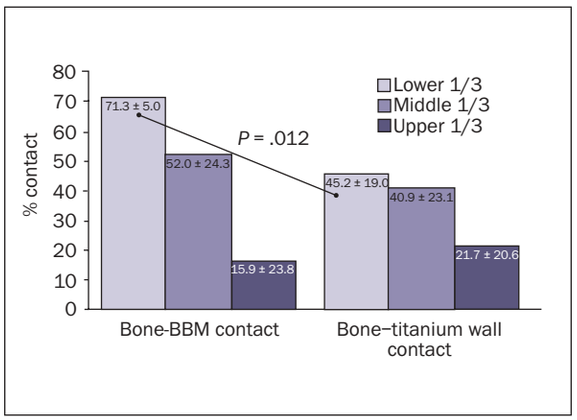


Fig 8 Bone-BBM contact and bone–titanium wall contact. Mean bone-BBM contact was 46.4% ± 13.3%. Mean bone–titanium wall contact was 45.5% ± 13.0%.

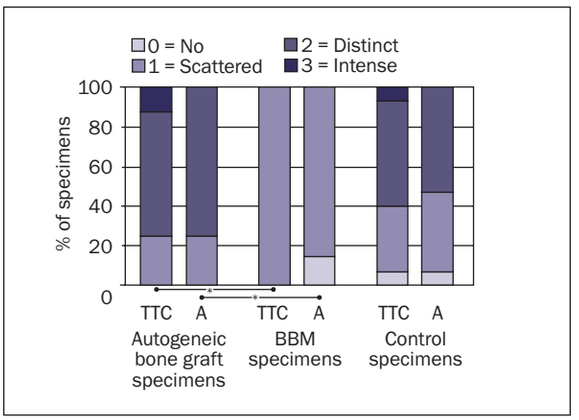


Fig 9 Frequency distribution of fluorochrome label intensity in the 3 experimental groups. *$P < .04$ (Mann-Whitney test). BBM = bovine bone mineral; TTC = oxytetracycline; A = alizarin complexone.

DISCUSSION

The finding that the adjunct of autogeneic bone or BBM in a GBA procedure promotes tissue augmentation supports findings in previous reports.[49–52] Furthermore, the BBM was found to have the capacity to promote as much new bone as particulate bone autografts and more bone than nongrafted control cylinders. The latter finding is in conflict with the results of a previous GBA study from the authors' laboratory[51] in which lower amounts of mineralized bone were found in the BBM group than in the control group. As discussed earlier, this may be related to the design of the device and the choice of experimental animal. The titanium cylinder that was used in the present model effectively prevented ingrowth of suture tissue and suprabony tissue and was also, in contrast to the silicone material used in the previous study, less sensitive to pressure and micromotion.[35,56–58]

In a recent GBA study in the rat mandible, significantly less bone was found in the BBM group than in the control group after 2 and 4 months of healing.[52] The authors concluded that BBM seems to delay bone formation and is also less osteoconductive than the barrier material itself. In that study, a dome-shaped Teflon barrier was anchored to the mandibular bone with sutures. Jaw muscle strain, combined with the instability of the anchoring sutures over time, may have caused micromotion of the barrier. This may also have disrupted the peripheral seal, thereby enabling the ingrowth of suprabony connective tissue. These circumstances might have influenced the outcome of the procedure. This explanation is supported by findings in a recent ridge augmentation study in the dog.[48] In that study, the premolars in the mandible were extracted and 1-wall bone defects were created. BBM mixed with fibrin glue was placed facing the intact lingual bone plate. Three months later titanium implants were placed in the augmented tissue. No new bone was found at the marginal-buccal aspect of the implants. The authors suggested that masticatory forces may have negatively influenced the initial wound healing by causing micromotion of the BBM particles. In addition, since no barrier was used to seal the graft material, ingrowth of suprabony connective tissue was possible.

In two companion papers, Schmid and associates[49] and Hämmerle and associates[50] studied BBM in combination with GBA using a rabbit calvaria model. Occlusive polylactic acid barrier domes were placed bilaterally on the parietal bones and fixated with miniscrews. Each rabbit received 1 test dome (BBM + peripheral blood) and one control dome (peripheral blood only). The animals were sacrificed 1 month and 2 months after surgery. It was found that the test domes contained significantly more new tissue and mineralized bone after 1 month than did the control domes. At 2 months, however, the tissue gains in the control domes had accelerated and reached levels similar to those in the test group. The amount of mineralized bone was significantly higher in the control group at 2 months. However, when the area of BBM was excluded, the fractions of mineralized bone were similar. These findings indicate that BBM initially increased both the tissue-forming rate and formation of mineralized bone; they also indicated, as judged at the 2-month observations, that this effect subsequently declines.

The 2 bone materials used in the present study behaved differently regarding amount and distribution of residual material in the experimental space. In the autograft group, most of the material was

resorbed, and only small amounts of thin bone shavings remained in the uppermost part of the augmented tissue. Sparse or no contact with new bone was found. In the early healing phase, unresorbed bone chips (thin shavings) may nevertheless have been tissue-conductive, since significantly more nonmineralized tissue was found in the autograft group. Osteogenesis may have arisen from bone-forming cells that survived within the graft.[59] Also, soluble factors in the autogeneic bone graft may have had an osteoinductive effect on mesenchymal-type cells from the contiguous marrow space. Since the amounts of mineralized bone in the control and test cylinders were similar, such events, if present, did not promote formation of mineralized bone under the present experimental conditions. In the BBM group, most of the BBM remained, although it was slightly condensed. Most of the BBM particles in the lower and middle thirds of the cylinder had been osseointegrated, while most in the upper third had been fibrointegrated, perhaps as a result of micromotion and wound contraction. It has been suggested that rigidly fixed block autografts are superior to bone chips which, despite a greater ability to release growth factors, may break down, resorb too fast, and also undergo micromotion.[60,61] A porous bone block may therefore be advantageous because of its higher internal stability.[60,61]

The nonmineralized tissue found in the experimental space had the same morphologic appearance in all groups and resembled the marrow tissue in the contiguous bone. Most likely, cells that had differentiated and subsequently formed mineralized bone in the cylinders had migrated from the marrow space. It may also be that osteoprogenitor cells still resided in the nonmineralized augmented tissue. Thus, prolonged healing may have resulted in larger proportions of mineralized bone.

Compared with the native parietal bone, the augmented bone tissue area was double in the test groups, although the mineralized bone density was around 20%. This finding is in accordance with the findings in other investigations.[10,11,50,51,53,54,62–64] The time factor will, of course, greatly influence the amount of augmented bone. Whether there is a limit to the amount of bone that can be augmented in a GBA procedure is at present unknown. The extent of bone fill and density in large spaces beyond the skeletal envelope certainly is related to site-specific factors, such as the availability of bone-forming cells, the vascular supply, the content of growth factors and cytokines, and the intensity and rate of metabolic activity.[65,66] The influence of such factors remains to be studied.

The surfaces of the BBM particles displayed signs of erosion contiguous to the newly formed bone (Fig 5d). Whether this was a result of resorptive cell activity could not be established because of the absence of multinucleated cells in these areas. Bone resorption, however, can take place under the influence of mononuclear as well as multinuclear cells.[67–69] There is also some debate concerning whether osteoclasts are always multinucleated.[70–72]

In the control group, a higher level of tissue was found along the cylinder wall than in the center of the cylinders. This finding supports the solid base theory, ie, that a stiff biocompatible material facilitates bone growth in an osteoconductive manner.[73] Stavropoulos and associates[52] found that the osteoconductive capacity of the barrier itself was superior to that of the BBM particles (although this finding was not supported by morphometric data). In the present study, similar or better bone contact with the BBM particles than with the cylinder wall was found in the lower two thirds of the cylinder space. It seems that BBM itself has at least the same conductive properties as titanium, provided BBM is contained in a stable environment.

The intensity of the 2 bone labels, delivered 4 and 11 weeks postoperatively, was the same in the BBM group and the control group, which indicates that xenogeneic hydroxyapatite graft material might not have a delaying effect on mineralization. However, the intensity of the labels in the autograft group was found to be higher than in the BBM group, similar to the findings of Puranen.[20] He studied label uptake in femoral sites in rabbits receiving autogeneic bone grafts (fresh, frozen, or preserved in whole blood) and allogeneic bone grafts (frozen). Puranen found that the label intensity was higher in the animals that had received fresh autografts than in animals that had received preserved bone grafts. As in the present study, Puranen found that fresh autografts were remodeled at an earlier stage. He also found that fresh autografts were better incorporated than preserved autografts, which was not found in this study. The reason for this difference is unclear. Because of the generally weak intensity of the labels in the present study, these findings must be interpreted with caution. Methodologic problems may have influenced the results (concentration of labels, route of distribution, influence of formalin fixation, etc).[74] Moreover, in the authors' opinion, a mixture of graft material, new bone, and soft tissue—as in the present study—most likely hampers reading and interpretation of labels.

CONCLUSIONS

Placement of autogeneic bone or bovine bone mineral in conjunction with a stiff space-making barrier promoted more tissue than placement of a barrier only. In this model, autogeneic bone chips and bovine bone mineral resulted in similar amounts of new mineralized bone.

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