J Clin Periodontol 2005; 32: 1254–1260 doi: 10.1111/j.1600-051X.2005.00792.x

Journal of Clinical Periodontology

Effect of Bio-Oss® with or without platelet-derived growth factor on bone formation by "guided tissue regeneration": a pilot study in rats

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Lioubavina-Hack N, Carmagnola D, Lynch SE, Karring T. Effect of Bio-Oss® with or without platelet-derived growth factor on bone formation by ''Guided Tissue Regeneration'': a pilot study in rats. J Clin Periodontol 2005; 32: 1254–1260. doi: 10.1111/j.1600-051X.2005.00792.x. © Blackwell Munksgaard, 2005.

Abstract

Aim: To investigate the effect of Bio-Oss® with and without the local application of recombinant human platelet-derived growth factor (rhPDGF-BB) on bone formation under Teflon capsules.

Materials and Methods: Eight male, 6-month-old, Wistar strain rats were used in the study. In each animal, the lateral aspect of the mandibular ramus was exposed and small perforations were produced in the bone. A rigid, non-porous hemispherical teflon capsule (diameter 7 mm) was placed on the ramus in both sides of the animals. The capsule placed on the one side of the jaw was filled with Bio-Oss® granules soaked in a solution of PDGF-BB (20 μg/capsule) and autogenous blood prior to placement. The capsules placed on the other side of the jaw were filled with Bio-Oss® granules soaked in autogenous blood only (controls). Four rats were sacrificed after 3 months and the remaining four after 5 months. Undecalcified sections containing the capsule and surrounding tissues were prepared and analysed in the microscope.

Results: Histologic analysis revealed limited amounts of bone formation. Most of the space underneath the capsules was occupied by Bio-Oss® particles surrounded by fibrovascular connective tissue. Given the small sample size statistical analysis was not possible, however, the mean amount of mineralized new bone in the control group (20.8%) appeared to be larger than that in the test group (6.7%). After 5 months the amount of newly formed bone appeared similar in the two groups (23.0% test, 26.0% controls). The Bio-Oss® particles occupied between 31.4% and 41.1% of the capsule area at 3 months and between 34.0% and 34.7% at 5 months. Only particles adjacent to the mandibular ramus were incorporated in newly formed bone.

Conclusion: Limited bone formation was present in the capsules grafted with Bio-Oss® with or without the growth factor.

Key words: Bio-Oss®; bone formation; Guided tissue regeneration; platelet-derived growth factor; rat

Accepted for publication 22 December 2004

Successful clinical outcomes have been reported following implantation of bovine bone-derived anorganic matrix, Bio-Oss® (Geistlich Biomaterials, Wolhusen, Switzerland) in intra-bony periodontal defects (Camelo et al. 1998, Hutchens 1999, Lundgren & Slotte 1999, Camargo et al. 2000, Mellonig 2000), in jaw bone defects associated with dental implants (Zitzmann et al. 1997, 2001, Lorenzoni et al. 1998, Hämmerle & Lang 2001), and in sinus lift procedures (Hürzeler et al. 1997a, Margolin et al. 1998, McAllister et al. 1998, Valentini et al. 1998, Piattelli et al. 1999). The results of other studies in humans (Hislop et al. 1993) and experimental animals (Carmagnola et al. 2000, Carpio et al. 2000, Paolantonio et al. 2001, Stavropoulos et al. 2001), on the other hand, have questioned the favourable effect of Bio-Oss® on bone formation.

Guided tissue regeneration (GTR), often under the term of guided bone regeneration (GBR) or guided bone augmentation (GBA), is widely used for the treatment of different types of bone defects (Dahlin et al. 1988, 1990, Kostopoulos & Karring 1994, Hämmerle et al. 1995, Matzen et al. 1996), and for augmentation of atrophic alveolar ridges (Buser et al. 1990, Cortellini et al. 1993, Lang et al. 1994). During

such procedures, bone grafts or bone substitutes are frequently used to prevent collapse of the barrier membrane and to provide a scaffold for ingrowth of capillaries and pre-osteogenic cells (Hämmerle & Karring 1998). In a study in monkeys, the combination of GBR and the implantation of Bio-Oss® resulted in more bone fill in peri-implant bone defects than GBR alone (Hämmerle et al. 1998). Grafting of Bio-Oss® also enhanced bone formation and osseointegration around implants placed into the sinus cavity in dogs (Wetzel et al. 1995). However, in a histological study in humans, a combined GBR/Bio-Oss® therapy resulted in less bone formation in extraction sockets with buccal dehiscences than GBR alone (Diès et al. 1996).

Experimental studies have shown that the GTR technique combined with local application of recombinant human platelet-derived growth factor (rhPDGF) alone or together with insulin-like growth factor (IGF-I) may enhance regeneration of cranial- (Vikjaer et al. 1997), periodontal- (Wang et al. 1994, Cho et al. 1995, Park et al. 1995), and peri-implant-bone defects (Becker et al. 1992). However, the type of growth factor carrier seems to be critical for the outcome of the treatment (Wikesjo et al. 1998). Recent studies in humans and experimental animals have suggested that Bio-Oss® is a suitable carrier for biologically active factors which regulate bone growth (Terheyden et al. 1997, 1999a, b, Jiang et al. 1999, Boyne & Shabahang 2001). Therefore, the aim of the present pilot study was to examine the effect of Bio-Oss® with or without PDGF on bone formation by GTR.

Material and Methods

Eight male, 6-month–old, rats of the Wistar strain were used. The animals were anaesthetized by a subcutaneous injection of 0.2 ml/100 g body weight Immobilon® (Pherrovet, Malmö, Sweden), and percutaneous incisions were made in each rat along the inferior border of the mandible. Following elevation of muscle-periosteal flaps in both sides, the mandibular ramus was exposed and small perforations were produced in the bone with a round bur to create a bleeding bone surface. On each side of the jaw, a specially fabricated, rigid, non-porous, and hemispherical teflon capsule with a diameter of 7 mm (W.L. Gore & Associates, Flagstaff, AR, USA) was fixed to the bone with four titanium miniscrews (Leibinger GmbH, Freiburg, Germany) (Fig. 1), according to a technique described previously (Lioubavina et al. 1999). Prior to placement, the capsule was filled with either Bio-Oss® soaked in a solution of growth factor and autogenous blood (test) or with Bio-Oss® soaked in autogenous blood only (control).

Sixteen vials, each containing 10 μg of purified rhPDGF-BB were used in the study. One hundred microliters of 10 mM acetic acid and 50 μl of autogeneous blood were added directly to each vial immediately prior to use, according to the guidelines of the manufactures. A standard amount of Bio-Oss® granules to be used for each site was determined by filling one of the teflon capsules. Subsequently, these granules were soaked in the solution from two vials for 20 min. in a plastic cup. Immediately prior to placement of a capsule at the test site, chosen at random, all Bio-Oss® granules and the remaining solution in the plastic cup were placed in the capsule. Thus, each capsule at the test sites received 20 μg of PDGF incorporated in Bio-Oss® granules (Fig. 2). The control capsules on the other side of the jaw were filled with Bio-Oss® soaked in autogenous blood only. After fixation of the capsules, the elevated soft tissues were repositioned and sutured with 5–0 Vicryl® (Ethicon, Norderstedt, Germany) sutures. The anaesthesia was terminated by an injection of 0.2 ml/100 g body weight of the antagonist Revivon® (Pherrovet, Malmö, Sweden).

Four rats were sacrificed after 3 months, and the remaining four after 5 months. The mandibles were dissected free and specimens including the capsules and surrounding tissues were fixed in 10% neutral-buffered formalin, dehydrated in alcohol, and subsequently embedded in Technovit® 7200 VLC (Kulzer, GmbH, Bereich Technic, Wehrheim/Ts, Germany). Undecalcified sections were cut through the capsules in a direction perpendicular to the lateral surface of the ramus. Four sections (60–80 μm thick) representing the midportion of each capsule were stained with toluidine blue and subjected to histologic examination.

Histometric measurements

In each histological section the following parameters were measured:

1. total area of the capsule space (mm^2),
2. percentage of the total capsule area occupied by (a) new mineralized bone; (b) bone marrow; (c) Bio-Oss® particles; and (d) fibrous connective tissue,
3. (a) number; (b) area (mm^2); and (c) circumference (mm) of Bio-Oss® particles present in the capsules,
4. percentage of the circumference of Bio-Oss® particles presenting a direct contact with newly formed bone.

All measurements were performed at a magnification of × 50 using a point counting technique. A lattice comprising 100 cross points was superimposed

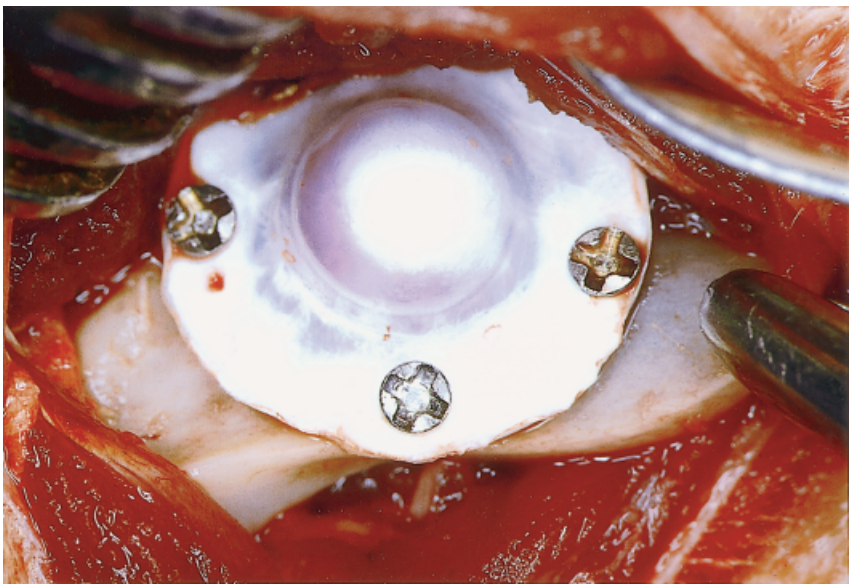

Fig. 1. Rigid teflon capsule placed on the mandibular ramus and fixed with titanium miniscrews.

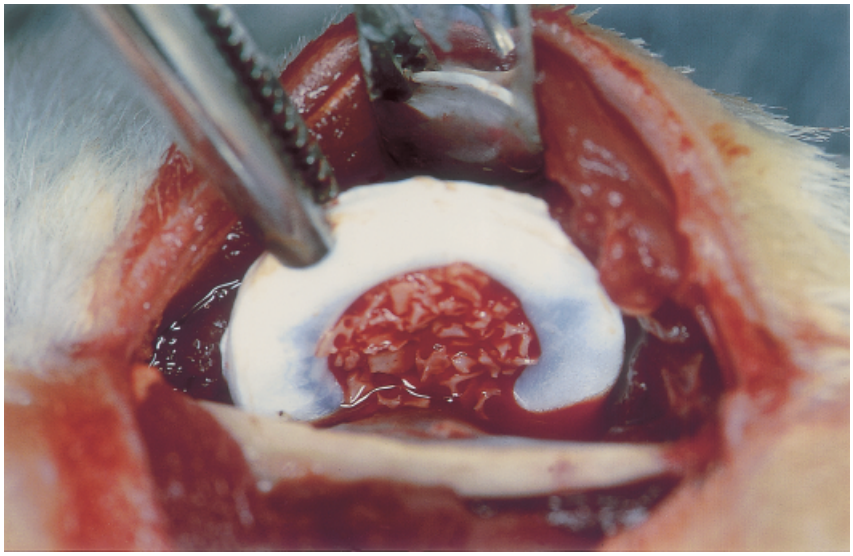

Fig. 2. Capsule filled with Bio-Oss® and autogenous blood prior to installation.

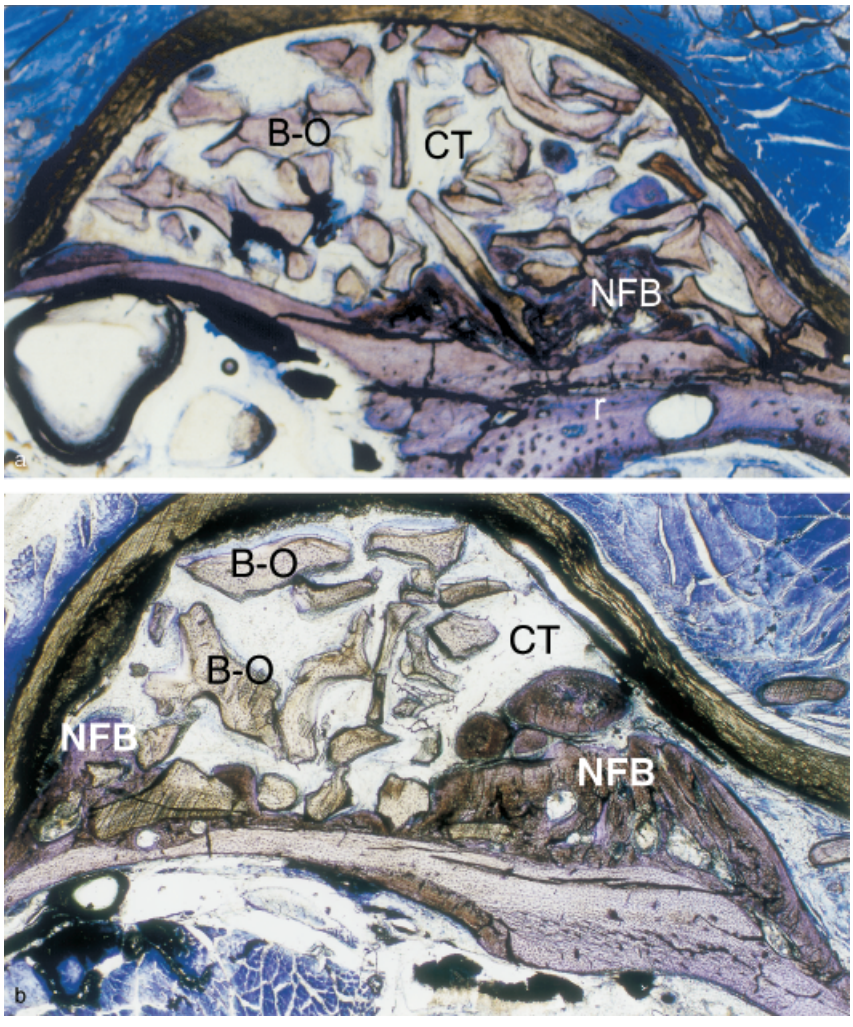

Fig. 3. (a) Microphotograph of a test capsule with Bio-Oss® (B-O) and PDGF at 3 months. Small amounts of the newly formed bone (NFB) are formed adjacent to the ramus. (b) A control capsule with Bio-Oss® (B-O) at 3 months. Note a greater amount of NFB at the edges of the capsule. r, ramus; CT, connective tissue (toluidine blue, magnification × 25).

over the specimen, and the various tissues in the capsule were identified. Bone marrow was identified by the presence of adipocites, while connective tissue was identified by the presence of collagen fibres and fibroblasts. Mean values for the different parameters were calculated for each animal, treatment modality and healing time. Because of the small number of experimental animals in each treatment group a comparative statistical analysis was not performed.

Results

Healing occurred uneventful in all animals.

Histological observations

Three- month specimens

Within the four animals evaluated, new bone formation was observed only on the surface of the mandibular ramus, and in particular at the edges of the capsule in all groups. Small amounts of new bone were found in the test capsules with Bio-Oss® and rhPDGF-BB (Fig. 3), while there appeared to be more bone in the control capsules with only Bio-Oss®. In both test and control specimens, the newly formed mineralized bone had the appearance of a parallel-fibred type of bone showing signs of ongoing re-modelling. Small bone marrow spaces containing adipocites were occasionally observed within the new mineralized bone. The remaining area of the capsule was occupied by fibrous connective tissue and Bio-Oss® particles of various size. The particles located adjacent to the ramus were surrounded by newly formed mineralized bone, while those located further away from the host bone were embedded in connective tissue (Fig. 4).

Five-month specimens

Increased amounts of new bone were observed in the 5-month test and control specimens as compared with the 3-month specimens (Figs 5 and 6). The new bone was always in continuity with the mandibular ramus and presented the characteristics of lamellar bone with secondary osteons and bone marrow. New bone formation was confined to the portion of the capsule near the ramus. The central and upper portions of the capsule were occupied by connective

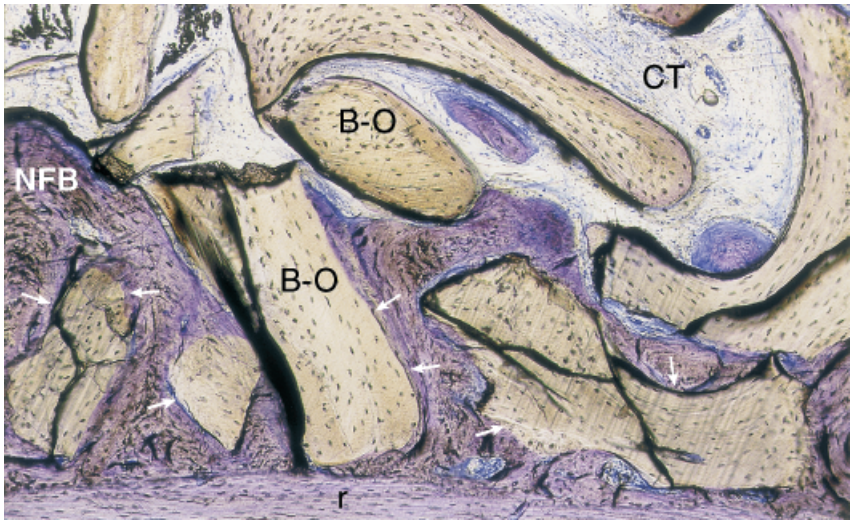

Fig. 4. Microphotograph of a test capsule at 3 months. Note the Bio-Oss® particles (B-O) in the newly formed mineralized bone (NFB) adjacent to the host bone (arrows) and in the connective tissue (CT) further away from the ramus (r) (toluidine blue, magnification × 100).

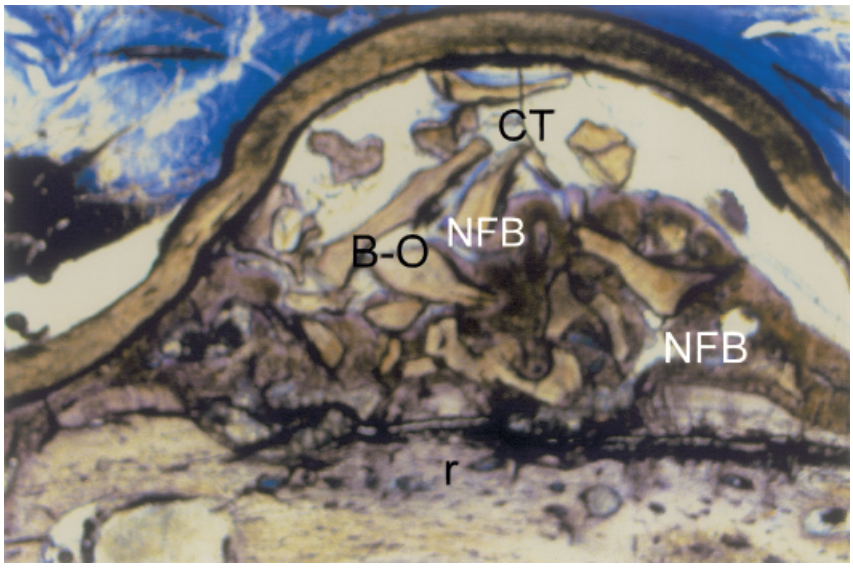

Fig. 5. Microphotograph of a test capsule with Bio-Oss® (B-O) and PDGF at 5 months. Increased amounts of the newly formed bone (NFB) are seen inside the capsule. r, ramus; CT, connective tissue (toluidine blue, magnification × 25).

tissue and Bio-Oss® granules. Only the granules adjacent to the mandibular ramus were either completely or partially incorporated in bone, while the granules located further away from the host bone were surrounded by a fibrous connective tissue (Fig. 7). No signs of resorption of the granules were observed.

Histometric measurements

The mean inner cross-sectional area of the capsules was about 10 mm^2 in both the test and control group.

At 3 and 5 months, a similar number of Bio-Oss® particles was present in the test and control specimens (range 25.4–30.9) (Table 1). In the test specimens grafted with Bio-Oss® and rhPDGF-BB, the percentage of circumference of Bio-Oss® particles that was in direct contact with newly formed bone increased from 1.2% to 12.8%, from 3 to 5 months. In the capsules with Bio-Oss® alone, the corresponding increase was from 12.2% to 17.8% (Table 1).

At 3 months the amount of mineralized newly formed bone was considerably lower in the test group (6.7%) than in the control group (20.8%) (Table 2), but after 5 months it was similar in the two groups (23.0% and 26.0%, respectively). The percentage of bone marrow increased from 0.2% to 1.7% in the test group, and from 1.9% to 4.8% in the control group from 3 to 5 months. A decrease in the percentage of connective tissue amounting to about 10% occurred in both groups. The Bio-Oss® particles occupied 41.1% and 34.7% of the capsule area in the Bio-Oss® and rhPDGF-BB group at 3 and 5 months which was similar to that found in the group with Bio-Oss® alone (31.4% and 34.0%).

Discussion

The results of this pilot study demonstrated the presence of limited bone formation in capsules grafted with Bio-Oss® with or without rhPDGF. The small sample size does not allow statistical comparison between groups but at 3 months there appeared to be less new bone including bone marrow (NFB+BM) in the test rhPDGF-BB/Bio-Oss® sites than in the control Bio-Oss® sites (6.9% and 22.8%, respectively, Table 2). However, after 5 months the total amount of mineralized bone with bone marrow ((NFB+BM) was similar in the test (24.7%) and control (30.8%) sites. The limited osteogenesis after 3 months may have been responsible for the small percentage of new bone seen in direct contact with the Bio-Oss® particles measured at that observation time. It might also be suggested that the initial pH value of the growth factor solution was not optimal for new bone formation.

The observations of the present study are in disagreement with those of studies in dogs where the treatment of periodontal defects with a rhPDGF-BB solution and GTR resulted in significantly more bone and periodontal regeneration than with GTR alone (Cho et al. 1995, Park et al. 1995). Similarly, in recent histological and clinical studies in humans (Camelo et al. 2003, Nevins et al. 2003), local application of rhPDGF-BB solution on the previously conditioned root surface and subsequently on DFDBA in intra-bony or Class II furcation defects resulted in substantial periodontal and bone regeneration. The rhPDGF-BB/DFDBA treatment without GTR led to significantly more bone formation than Bio-Oss®/GTR therapy, suggesting that bone allografts may be a suitable carrier of growth factors. However, a 9-month observation period used in those studies

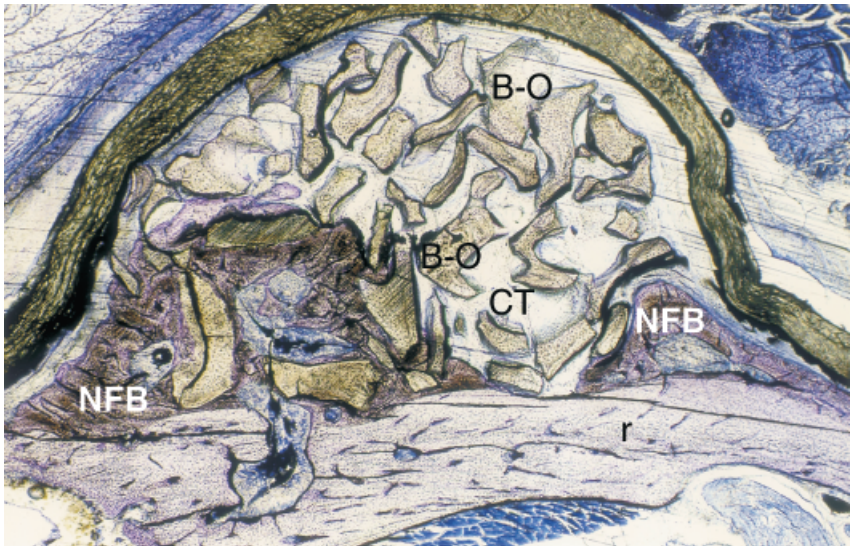

Fig. 6. Microphotograph of a control capsule with Bio-Oss® (B-O) and blood at 5 months. The newly formed bone (NFB) is seen in direct contact with the ramus (r). Most of the Bio-Oss® particles are surrounded by connective tissue (CT) (toluidine blue, magnification × 25).

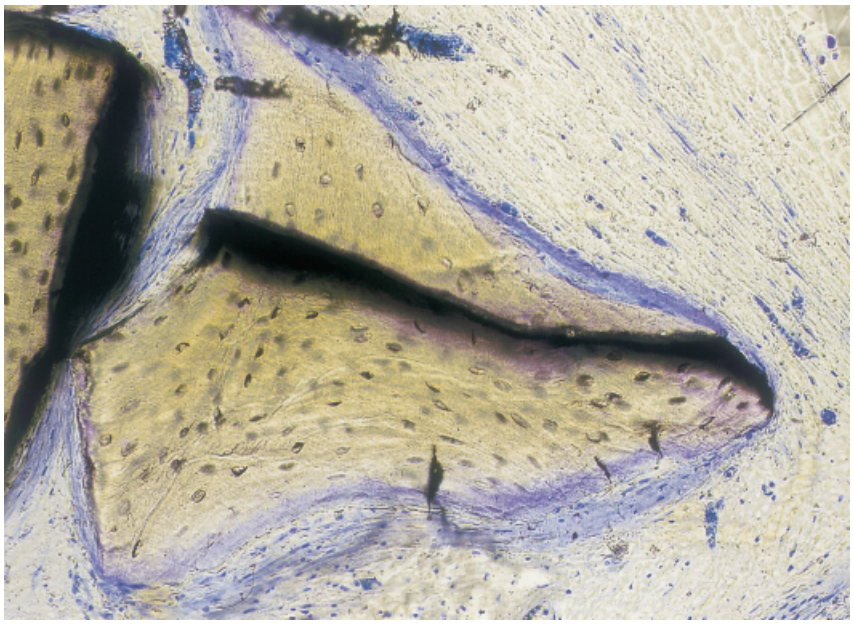

Fig. 7. Microphotograph of a Bio-Oss® particle encapsulated in fibrous connective tissue in a test capsule at 5 months (Toluidine Blue, magnification × 200).

does not permit to evaluate the short-term effect of the rhPDGF-BB on bone formation. Furthermore, it cannot be excluded that the conditioned root surfaces may also have served as an additional slow release delivery system of PDGF-BB, thereby enhancing periodontal regeneration.

The findings of the present study are also in disagreement with the results of other reports, in which rhPDGF-BB/IGF-I delivered in methyl cellulose gel enhanced bone regeneration in periodontal (Lynch et al. 1989, 1991b, Giannobile et al. 1994, Howell et al. 1997), and peri-implant bone defects (Lynch et al. 1991a) in dogs, monkeys, or humans, when used without the membrane technique. It was suggested that in such non-membrane covered defects, the carriers were disintegrated fast by cells penetrating into the wound, thus resulting in release of 96% of the growth factors within 4 days after application (Lynch et al. 1991b).

It has previously been shown that the creation of a secluded space by a GTR-device adjacent to the surface of existing bone results in substantial bone formation (Kostopoulos et al. 1994, Lundgren et al. 1995, Hämmerle et al. 1996, Lioubavina et al. 1999). It was presumed that the enhancement of this bone forming process by stimulating osteogenic cells with growth factors may improve the predictability of GTR in cases where complications such as infection, membrane exposure or escape of undesirable tissue underneath the membrane may compromise the results. An osteopromotive effect of rhPDGF-BB in combination with GTR treatment of calvarial defects has been demonstrated in studies in rabbits and rats. Application of this growth factor in a methyl–cellulose gel into the membrane protected defect (Vikjaer et al. 1997) or release of the growth factor into the defect during degradation of a resorbable membrane (Chung et al. 1997, Park et al. 1998) resulted in significantly more new bone formation than GTR alone.

In the present study, the Bio-Oss® particles occupied 41.0% and 35.0% of the capsule space, which means that more than 50.0% of the initial space was available for new bone formation. Although an increase in the amounts of mineralized new bone was observed in the test (from 6.7% to 23.0%) and control (from 20.8% to 26.0%) groups between 3 and 5 months, these amounts did always comprise less than 50.0% of the initial capsule area. In previous studies in the rat, using the same expermental design as in the present study, empty capsules of a similar size placed on the mandibular ramus became completely filled with new bone after 4–6 months (Kostopoulos et al. 1994, Lioubavina et al. 1999). Thus, the findings of the present experiment suggest that neither the growth factor nor the grafted Bio-Oss® enhanced bone formation in the capsules.

In all specimens only Bio-Oss® particles which were located close to the host bone were either completely or partially incorporated in new bone. Most of the space underneath the capsules was occupied by Bio-Oss® particles surrounded by fibro-vascular connective tissue. This observation corroborates those in studies where grafting of Bio-Oss® into dome-shaped membranes placed on the rat calvaria (Slotte & Lundgren 1999) or mandible (Stavropoulos et al. 2001) resulted in considerably less bone formation after 2 and 4 months of healing than in capsules without grafting material.

It ought to be emphasized that the capsule model with a paired design used

Table 1. Number of Bio-Oss® particles present in the capsules and the percentage of their circumference that was in contact with newly formed bone

	rhPDGF/Bio-Oss®		Bio-Oss®	
	3 months	5 months	3 months	5 months
Number	30.9 (6.1)	29.0 (13.1)	25.5 (10.0)	25.4 (4.2)
% contact	1.2 (1.6)	12.8 (12.6)	12.2 (11.9)	17.8 (21.9)

Mean values (± SD).
rhPDGF, recombinant human platelet-derived growth factor.

Table 2. Histometric assessments of the percentage (mean ± SD) of connective tissue (CT), Bio-Oss® (B-O), newly formed bone (NFB) and bone marrow (BM)

	RhPDGF/B-O		B-O	
	3 months	5 months	3 months	5 months
CT	51.9 (6.4)	40.4 (10.5)	45.9 (4.3)	35.2 (15.0)
B-O	41.1 (5.0)	34.7 (10.7)	31.4 (13.0)	34.0 (8.2)
NFB	6.7 (2.3)	23.0 (12.3)	20.8 (10.0)	26.0 (15.0)
BM	0.2 (0.2)	1.7 (1.5)	1.9 (2.8)	4.8 (5.5)

in the present study, showed previously continuous bone formation in the capsule within 4–6 months (Kostopoulos et al. 1994, Lioubavina et al. 1999). In this experimental model a similar and well-defined space for bone formation in a test and control site is created, thus allowing an osteopromoting role of various growth factors and grafting materials to be evaluated in this confined environment. Further research should be conducted under well-controlled experimental conditions to answer the question whether the placement of recombinant growth factors in a proper carrier can in fact promote bone formation by GTR.

Acknowledgements

This investigation was supported by research grants from W. L. Gore & Associates, Flagstaff, AR, USA and from Geistlich Biomaterials, Wolhusen, Switzerland.

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