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Fate of bone formed by guided tissue regeneration with or without grafting of Bio-Oss® or Biogran®

An experimental study in the rat

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Abstract

Objectives: The aim of the study was to examine whether bone produced by guided tissue regeneration (GTR) in combination with Bio-Oss® or Biogran® is stable on a long-term basis.

Material and Methods: Fifty-four, 3-month-old Wistar rats were divided into three groups and rigid, hemispherical, teflon capsules were placed with their open part facing the lateral surface of the exposed mandibular ramus (one capsule per animal). In the first group, the capsules were loosely packed with a standardized quantity of Bio-Oss®, in the second group with Biogran®, and in the last group were left empty. After 1 year of healing, the capsules were removed. Six animals from each of the 3 experimental groups were killed immediately after capsule removal (baseline), or 3 or 6 months after re-entry. The volume of (1) newly formed bone, (2) remaining biomaterial particles, and (3) soft connective tissue in the space originally created by the capsule was estimated by a point-counting technique in three to four histological sections, taken by uniformly random sampling.

Results: While considerable bone formation had occurred in the empty control capsules, only limited bone formation was observed in the two test groups. The major portion of the space originally created by the capsules in the test groups was occupied by biomaterial particles embedded in connective tissue. At baseline, the mean volume of newly formed bone occupied 23% of the original capsule space in the animals grafted with Bio-Oss®, 12.6% in those implanted with Biogran®, and 94.1% in those that received empty control capsules. Six months after capsule removal, the corresponding values were 21.5%, 13.2%, and 91.7%, respectively. No statistically significant differences were observed between baseline, and the 3-, and 6-month observation times in terms of bone volume for any of the three treatment groups ($p>0.05$).

Conclusion: Bone produced by GTR with and without implantation of Bio-Oss®, or Biogran®, is stable on a long-term basis, but bone formation is obstructed by implantation of these biomaterials.

Key words: alloplast; bioactive glass; bioglass; Biogran®; Bio-Oss®; deproteinized bovine bone; GBR; GTR; guided bone regeneration; guided tissue regeneration; xenograft

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The principle of ''guided tissue regeneration'' (GTR) has been successfully applied, often under the term ''guided bone regeneration'' (GBR) or ''guided bone augmentation'' (GBA), for the treatment of various types of bone defects and for increasing the bone volume of atrophic alveolar ridges, thereby making the placement of dental implants feasible (for a review, see Hämmerle & Karring 1998).

The GTR technique involves the placement of a physical barrier (membrane) over the bone defect during surgery, so that a secluded space is created and the neighboring soft tissues

are prevented from participating in healing. This ensures that cells with the capacity to regenerate bone populate the defect and/or the created space during the healing process. However, collapse of the barrier during the healing period may compromise the formation of new bone by reducing or eliminating the space available for bone to form (Dahlin et al. 1991, Jovanovic et al. 1992, Sandberg et al. 1993). In order to support resilient barriers and prevent collapse, and at the same time promote bone formation, the GTR technique has often been combined with the placement of bone grafts or bone graft substitutes into the secluded space created by the membrane (for a review, see Hämmerle 1999).

Deproteinized bone of bovine origin (Bio-Oss®, Geistlich AG, Wolhusen, Switzerland) (Hürzeler et al. 1996, Carpio et al. 2000, Mayfield et al. 2001, Zitzmann et al. 2001) or bioactive glass (Biogran®, Orthovita, Malvern, PA, USA) (Schepers et al. 1993, Furusawa et al. 1998, Leonetti et al. 2000, Cordioli et al. 2001) have been used successfully as bone graft substitutes in various types of bone defects, often associated with dental implants, and in sinus lift procedures. However, in recent controlled experimental studies it was shown that limited amounts of new bone were produced by combining the membrane technique with Bio-Oss® or Biogran®, and that implantation of these materials in fact arrested bone formation (Stavropoulos et al. 2001a, b). It remains unknown, however, whether the bone formed with the combined treatment remains stable after the barrier function is terminated (i.e. when the membrane is removed or absorbed).

The aim of the present study, using a discriminating ''capsule model'' in rats, was to examine whether bone produced by GTR in combination with Bio-Oss® or Biogran® is stable on a long-term basis.

Material and Methods

Surgical procedures

A total of 54, 3-month-old, albino rats of the Wistar strain were used in the study and divided into three groups of equal size (18 rats in each group). The animals were anesthetized by a subcutaneous injection of 0.6 ml of Immobilon™ (Pherrovet, Malmö, Sweden). Horizontal incisions were made along the inferior border of the mandible, and by elevating muscle-periosteal flaps, the lateral aspect of the mandibular ramus was exposed. Four holes, 0.5 mm in diameter, placed as corners in a square (with sides of approx. 6 mm, and one side being parallel to the base of the ramus), were made through the ramus with a small round bur. A custom-made rigid, hemispherical Teflon capsule, with an internal diameter of 6 mm and a 1 mm peripheral collar, was placed with its open part facing the lateral surface of the mandibular ramus (one capsule per animal). In the first group,

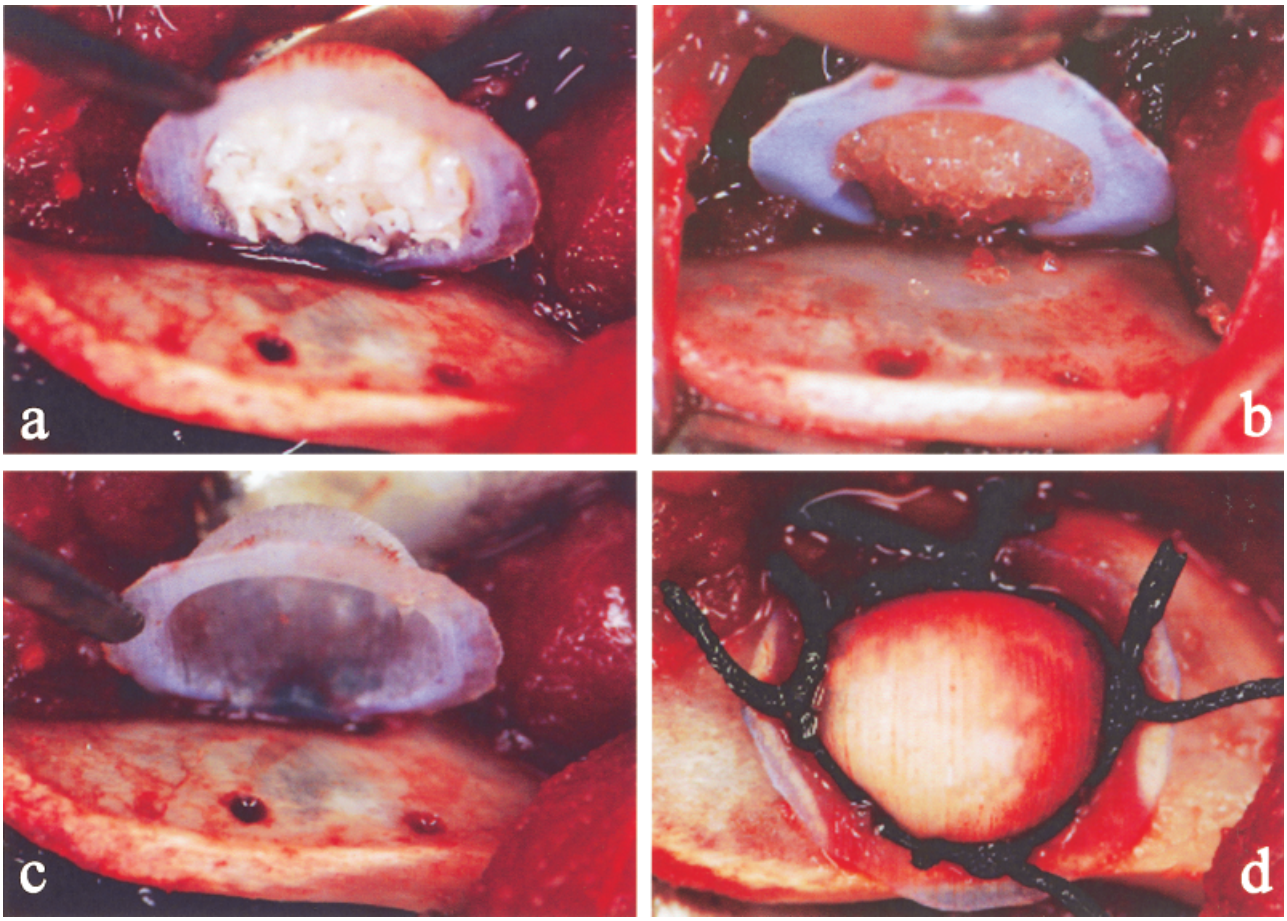

Fig. 1. Test capsules filled with a standardized quantity of Bio-Oss®(a) or Biogran®(b), and a control (''empty'') capsule (c), are placed on the lateral surface of the mandibular ramus and fixed by means of silk sutures (d).

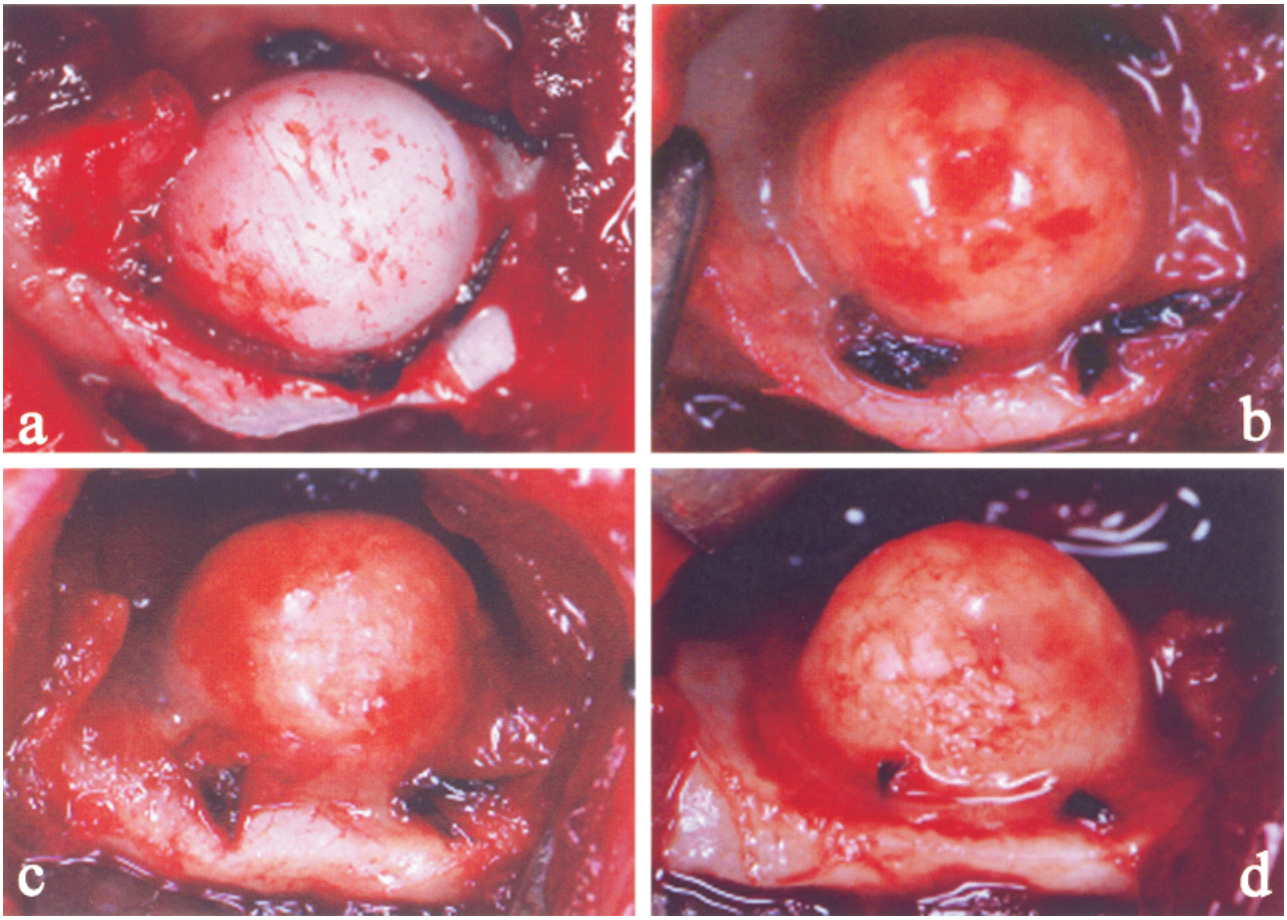

Fig. 2. After 1 year in place (a), the capsule is removed and it can be seen that a dome-shaped bone-like tissue has formed under the capsules implanted with Bio-Oss® (b) or Biogran® (c), and under the empty control capsule (d).

the capsules were loosely packed with 0.025 g of Bio-Oss® before placement, in the second group 0.045 g of Biogran® were used, and in the last group empty (control) capsules were placed (Fig. 1a–c). The capsules were fixed on the ramus by means of interrupted 4.0 silk sutures (Ethicon, 2000 Norderstedt, Germany) through the four holes in the bone (Fig. 1d). The soft tissues were repositioned over the capsule and sutured with mattress 4.0 Vicryl sutures (Ethicon, 2000 Norderstedt, Germany). The anesthesia was terminated by a subcutaneous injection of 0.6 ml of the antagonist Revivon™ (Pherrovet, Malmö, Sweden). During the experiment, the animals were fed ad libitum with standard laboratory food pellets.

After 1 year the experimental sites were re-entered, the capsules were removed, and the soft tissues were repositioned and sutured over the tissue formed under the capsule. After the re-entry operation, the animals were positioned with their back on a custom-made gypsum-base and their heads were kept in place with a silicon-made bite index. Then, radiographs of the head were made using standardized exposure parameters (Kodac Ektaspeed, Eastman Kodak Company, Rochester, NY, USA; 60 kV, 10 mA, focus film distance 10 cm). Prior to the re-entry operations, six animals from each of the three experimental groups were randomly allocated for killing immediately after capsule removal (baseline), or after 3 or 6 months.

Histomorphometry

After the killing of the animals, the tissue formed under the capsules including surrounding tissues were dissected free and fixed in 10% neutral buffered formalin, dehydrated in a series of ascending concentrations of alcohol, and embedded in glycolmethacrylate (Technovit 7200 VLC, Kulzer GmbH, Bereich Technic, Wehrheim/Ts, Germany). Undecalcified sections of approximately 200 μm thickness were obtained perpendicular to the lateral surface of the ramus, and with a random direction. The sections were then reduced to a thickness of 20–30 μm by means of the Exact™ (Exact-Apparatebau, D-200 Norderstedt, Germany) sawing–grinding technique (Donath & Breuner 1982). Finally, every second section was stained with a modified Goldner's trichrome stain and the rest of the sections with van Gieson's picro fuchsin stain.

From each specimen, three to four sections were systematically, uniformly randomly sampled for analysis. By means of a computer-assisted stereological toolbox (C.A.S.T.-Grid®, Olympus Denmark A/S, Albertslund, Denmark) connected to a BH-50 Olympus light microscope via a video camera and a frame grabber, a grid of test points was superimposed on the section image. The space originally created by the capsule was delineated (traced) on the computer screen by means of a mouse. Data were generated by counting separately test points ''hitting'' (i.e. superimposed on) the capsule area, newly formed bone (bone trabeculae and marrow spaces), biomaterial

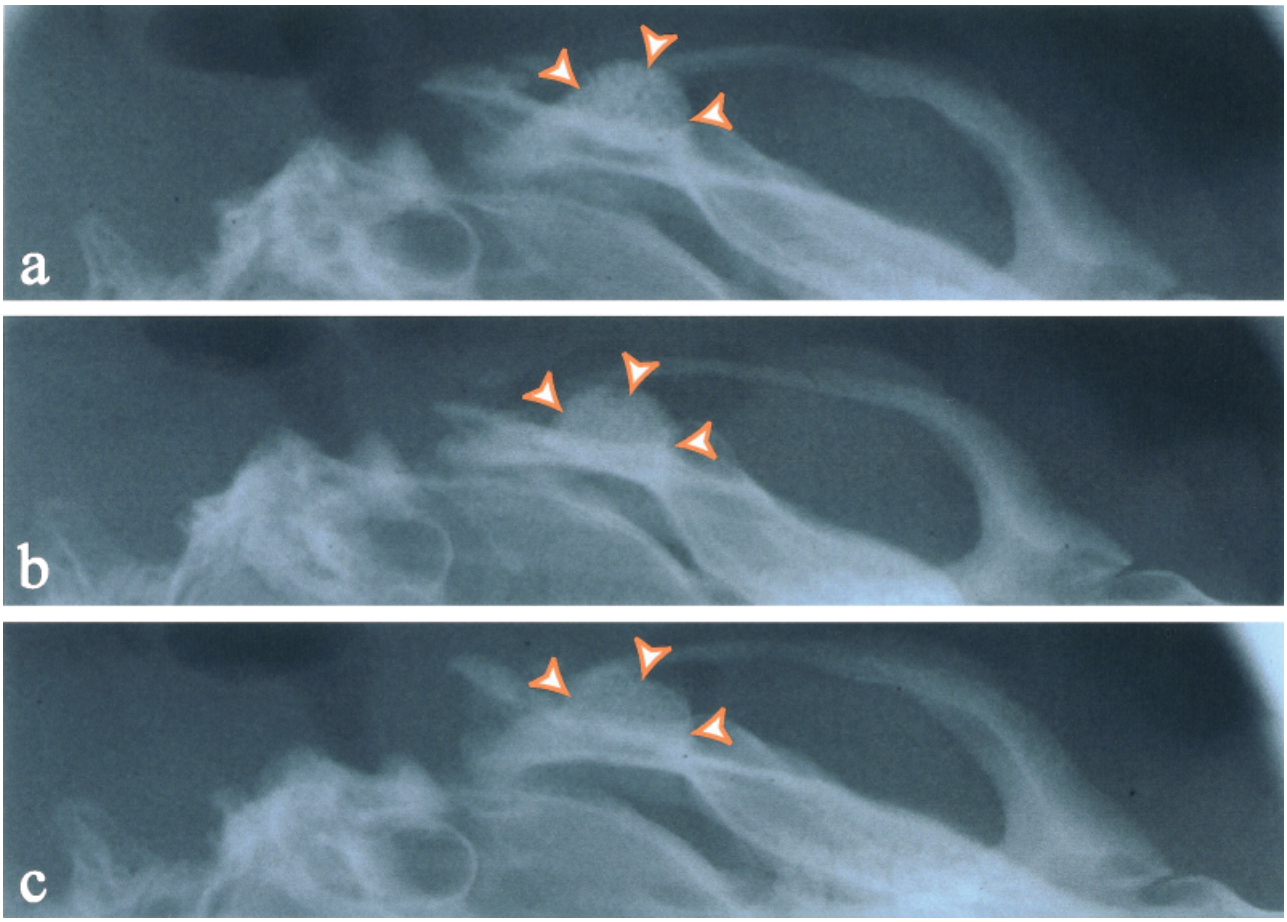

Fig. 3. Radiographs after 1 year: dome-shaped formations (arrowheads) of similar size and with a homogenous radio-opacity comparable to that of the neighboring craniofacial skeletal structures had formed on the lateral surface of the mandibular ramus in (a) Bio-Oss®- and (b) Biogran®-implanted animals, and in the control animals (c).

particles, and loose connective tissue (inside the capsule area) (Gundersen & Jensen 1987). The data for each specimen were expressed as total volume, and as percentage of the space originally created by the capsule for each of the above-mentioned parameters. The volume of the space created by the hemispherical capsules was calculated by the mathematical formula for the volume of a hemisphere ($V_{(caps)} = \pi\delta^3/12$, where δ is the internal diameter of the capsule). Additionally, the height of the newly formed bone (corresponding to the width of the ramus) was measured linearly at the midpoint of the base of the traced capsule area, in the most central section of each specimen. The same researcher (A.S.) made all measurements.

Table 1. Mean bone height in mm (CV) and mean tissue fill in % (CV) of the space originally created by the capsule for bone, particles, and connective tissue

	N	Bone			Fill %	
		Height	Fill %	*p*	Particles	Con. Tissue
Baseline						
Bio-Oss®	6	1.4 (0.40)	23.1 (0.16)	0.004*	39.9 (0.15)	37.0 (0.2)
Biogran®	6	0.5 (0.40)	12.6 (0.66)	0.004†	35.0 (0.39)	52.4 (0.03)
''empty''	5	2.6 (0.10)	94.1 (0.03)		–	5.9 (0.1)
3 months						
Bio-Oss®	6	0.9 (0.60)	19.6 (0.16)	0.002*	38.8 (0.15)	41.6 (0.02)
Biogran®	5	0.8 (0.30)	11.2 (0.66)	0.01†	44.5 (0.39)	44.3 (0.01)
''empty''	6	2.1 (0.10)	94.9 (0.17)		–	5.1 (0.04)
6 months						
Bio-Oss®	4	1.3 (0.30)	21.5 (0.16)	0.02*	41.6 (0.15)	36.9 (0.02)
Biogran®	5	0.9 (0.30)	13.2 (0.66)	0.01†	44.3 (0.39)	42.5 (0.01)
''empty''	5	2.1 (0.10)	92.6 (0.02)		–	7.4 (0.03)

*Bio-Oss® versus empty,
†Biogran® versus empty, analyzed by the Mann–Whitney test.
CV: coefficient of variation.

Statistical analysis

The differences within each experimental group at the various observation times were analyzed with the Kruskal–Wallis test for non-paired observations, while the differences between the experimental groups at each observation time were tested with the Mann–Whitney test for non-paired observations.

The accuracy and the reproducibility of bone volume estimations by the method of analysis used in the present experiment was documented previously in studies using capsules of similar size

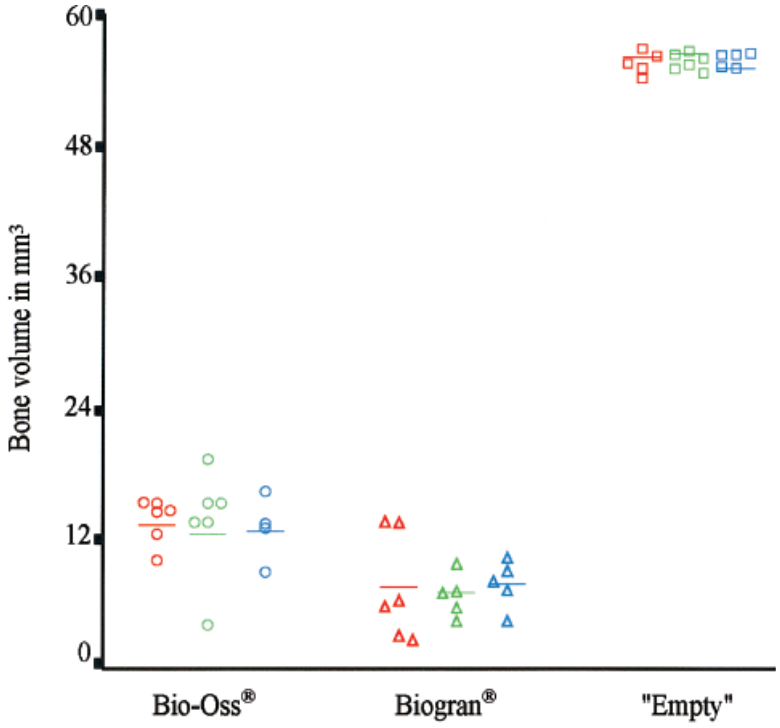

Fig. 4. Volumes of newly formed bone in each control and test capsule at baseline (red), after 3 months (green), and after 6 months (blue). Horizontal lines indicate mean values.

(Stavropoulos et al. 2001a, 2003). The capsules, however, in those studies were not removed prior to the preparation of the specimens, while in the present experiment they were removed (i.e. no capsules are present in the histological sections). Since all data are generated with relation to and expressed in percentage of the capsule volume, it was mandatory to evaluate whether the area originally created by the capsules is identified (traced) correctly on the sections. This was done by counting the number of points ''hitting'' the traced capsule space in the most central section of each specimen and multiplying it by the corresponding ''area per point''. Possible differences in the traced capsule areas between the experimental groups were analyzed with the Kruskal–Wallis test for non-paired observations.

The SPSS 10.0 (SPSS Inc., Chicago, IL, USA) statistical program was used for all analyses.

Results

Healing following the surgical procedures was uneventful in all the animals. Two animals grafted with Bio-Oss® (belonging to the 6-month group) and 2 animals implanted with Biogran® (one belonging to the 3- and one to the 6-month group) died prior to the end of the study from reasons not related to the experimental procedures.

Clinical findings

At re-entry for removal of the capsules after 1 year, it was observed that dome-shaped bone-like tissue had formed on the lateral surface of the mandibular ramus (under the capsules) in all the specimens (Fig. 2), except for two control specimens (one belonging to the baseline and one to the 6-month group), where the coronal part of the dome was lacking. These two specimens were excluded from the calculations. The new tissue felt rather hard by palpation in all three experimental groups. In the Bio-Oss® grafted animals, graft particles incorporated in the newly formed dome-shaped tissue were distinguishable (by their color and pattern). The examination of the radiographs revealed that dome-shaped formations of similar size, with a homogenous radio-opacity comparable to that of the neighboring craniofacial skeletal structures, had formed on the lateral surface of the mandibular ramus in all three experimental groups (Fig. 3).

Histological findings

Histometric analysis showed that there were no statistically significant differences between the different observation times regarding the traced capsule area (data not shown). One year after capsule placement (i.e. at capsule removal), limited amounts of new bone were present in the specimens implanted with either Bio-Oss® (23.1 %) or Biogran® (12.6%), while abundant amounts of new bone (94.1%) were observed in the control specimens (i.e. where empty capsules were used) (Table 1 and Fig. 4). A small, but significant ($p = 0.03$) difference in bone formation in favor of Bio-Oss® when compared with Biogran® was observed, but only at baseline. The histological features of the tissues formed under the capsules (Figs 5–7) and the amounts of bone at capsule removal (baseline) (Table 1 and Fig. 4) were similar to those observed 3 and 6 months after capsule removal.

In the Bio-Oss®-grafted animals, the newly formed bone in the space originally created by the capsule was in continuity with the pre-existing host bone, and had a dense appearance with few marrow spaces (Fig. 5). The new bone had grown in direct contact with the Bio-Oss® particles, which in many cases were totally incorporated in bone. However, the newly formed bone was confined to the lower part of the dome-shaped tissue, extending on average 0.9–1.4 mm (Table 1) from the lateral surface of the ramus at the various observation times. In most of the specimens, bone formation had occurred to a slightly higher level at the periphery than in the central part of the dome-shaped tissue. The major part of the tissue, within the space originally created by the capsule, consisted of graft particles embedded in connective tissue. The osteocyte lacunae in the graft particles were empty and there were no signs of progressive resorption of the particles (Fig. 5d). In only a few cases were osteoclast-like cells encountered adjacent to the Bio-Oss® particles. A layer of dense fibrous connective tissue lined the entire periphery of the dome, clearly separating it from the surrounding soft tissues (muscles etc.).

In the specimens of the Biogran®-implanted animals, the newly formed bone in the space originally created by the capsule was in continuity with the host bone, extending on average 0.5–0.9 mm (Table 1) from the lateral surface of the

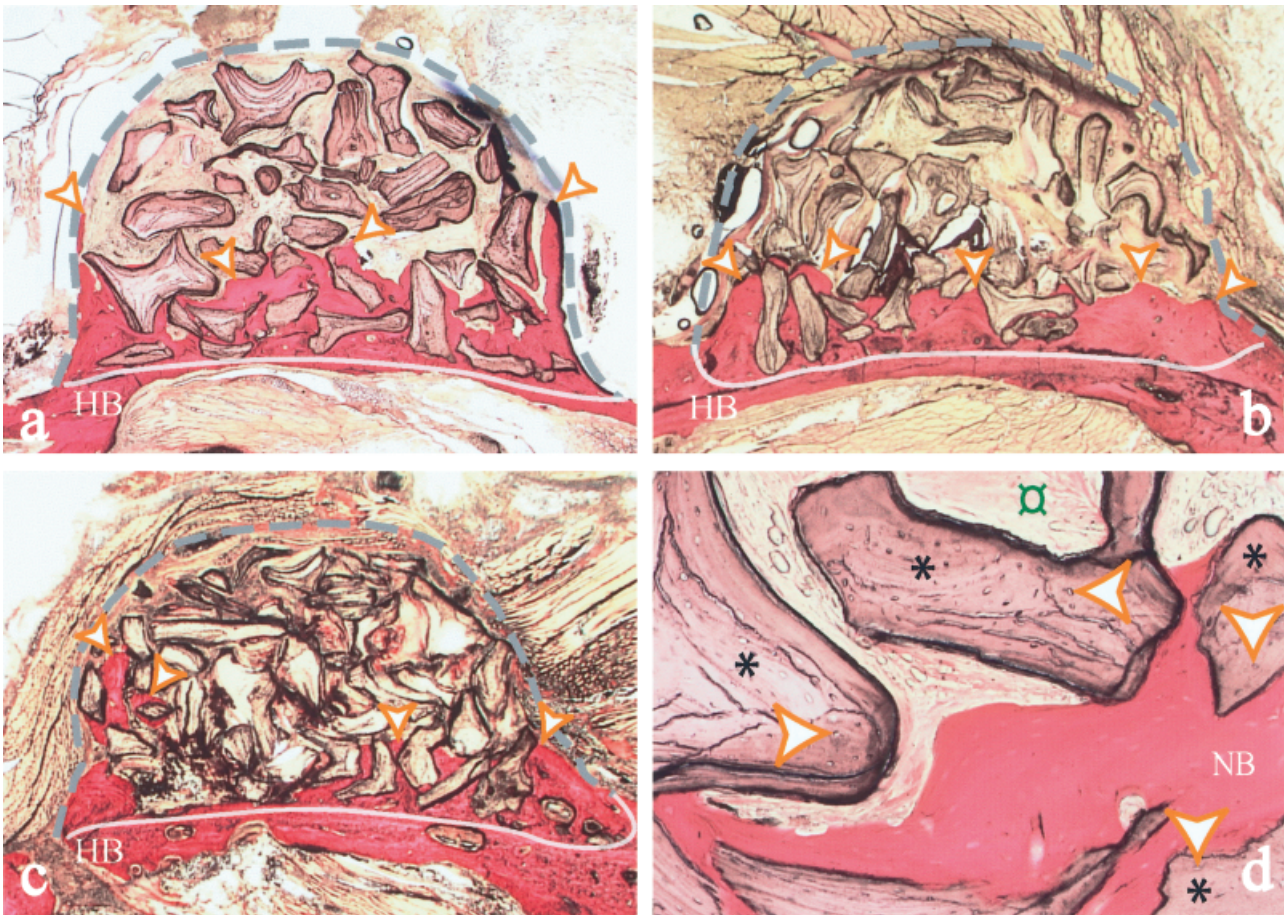

Fig. 5. Photomicrographs of tissues formed in Bio-Oss®-grafted capsules at capsule removal after 1 year (a), and after 3 months (b) and 6 months (c): new bone formation (arrowheads) is limited and confined to the lower part of the tissue formed under the capsules (dashed line). The major portion of the tissue consists of graft particles embedded in connective tissue. It can be seen in the high magnification (d) of (a) that the new bone (NB) has grown into direct contact with Bio-Oss® particles (*), and that the particles, partly embedded in connective tissue (¤) contain empty osteocyte lacunae (arrowheads). The white line (in a–c) delineates the host bone surface (HB). Van Gieson's picro fuchsin stain.

ramus at the various observation times. The new bone had a dense appearance with few marrow spaces (Fig. 6) and had grown in contact with the bioactive glass particles, which in many cases were totally incorporated in bone. The newly formed bone was confined to the very lower part of the dome-shaped tissue, while the major part of the tissue, within the original capsule space, consisted of biomaterial particles embedded in connective tissue. A large number of particles had cracks/fissures on their surface, and a few of them presented an internal excavation. This internal cavity communicated with the surrounding tissue through the cracks/fissures, and was filled with connective tissue. Occasionally, bone had grown inside the cavities and/or inside the cracks of particles embedded in or located near the newly formed bone (Fig. 6d). A layer of dense fibrous connective tissue lined the entire periphery of the dome-shaped tissue.

In the control specimens, new bone had formed in continuity with the host bone, extending on average 2.1–2.6 mm (Table 1) from the host bone at the various observation times. The new bone consisted of lamellar mature bone with a trabecular appearance and marrow spaces with fat cells (Fig. 7). In the baseline specimens, the new bone had a dome-shaped configuration, and seemed to match the internal surface of the hemispherical capsules almost precisely (Fig. 7a). In the 3- and especially in the 6-month specimens, the dome-shaped bone tissue appeared somewhat flattened (Figs 7b and 7c), and some reduction had occurred in the height of the new bone as compared with baseline (Table 1). Areas undergoing physiological remodeling could be identified in all 3 observation groups, and a layer of dense fibrous connective tissue lined the surface of the new bone (Fig. 7d), like in the two other experimental groups.

Discussion

The present study demonstrated that bone formed by means of GTR alone or combined with Bio-Oss® or Biogran® implantation remains stable on a long-term basis. This finding corroborates the results of a previous study showing that bone tuberosities formed on the lateral aspect of the rat mandible under originally empty teflon capsules are stable at least 1 year after membrane removal (Lioubavina et al. 1999). In that study a small (4–8%), but statistically significant, reduction in the amount of newly formed bone was observed at 3 months after capsule removal, while no further resorption occurred up to 12 months. In the present study, some remodeling of the bone formed under the empty control capsules had obviously occurred after capsule removal, but the amounts of bone at 3 and 6 months after capsule

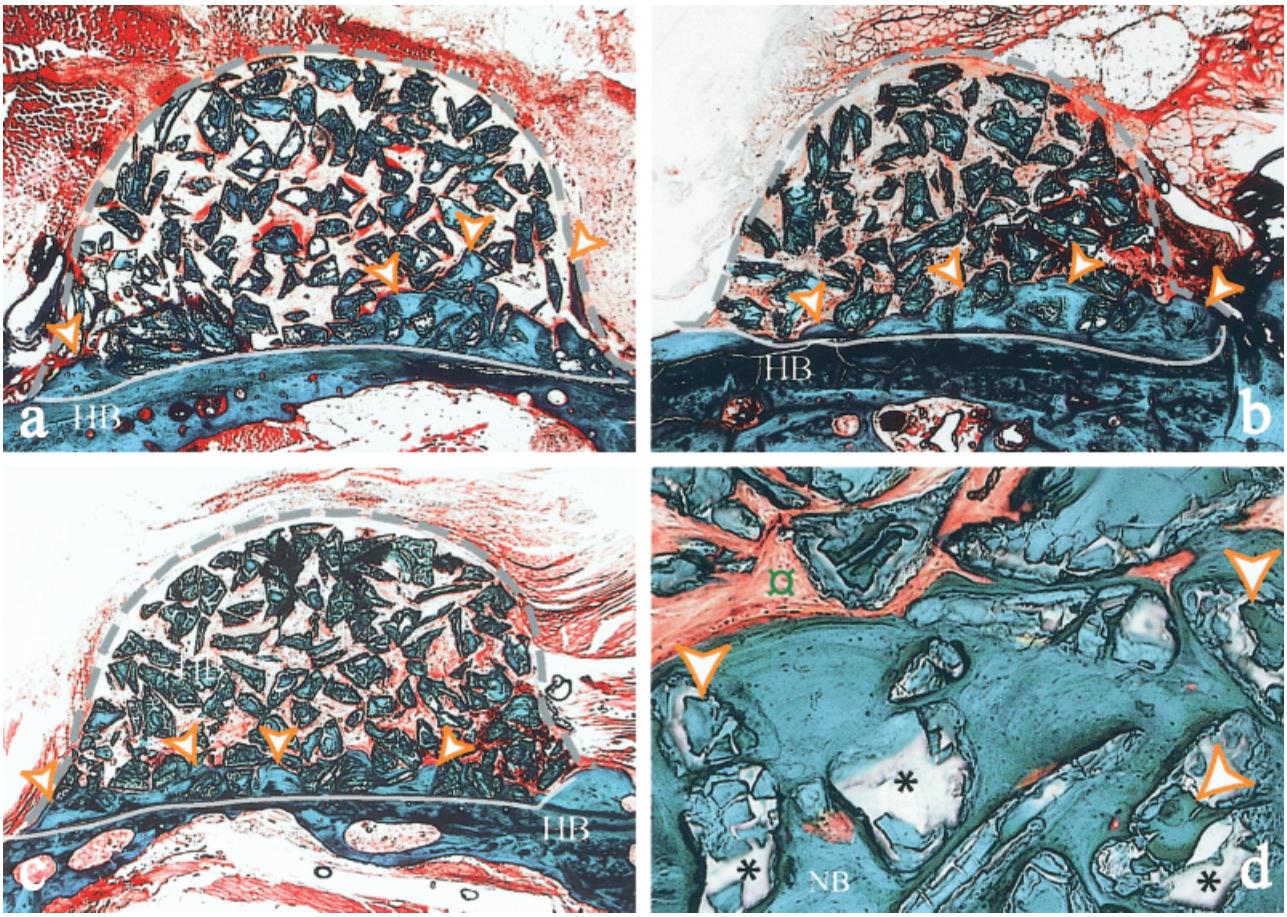

Fig. 6. Photomicrographs of tissues formed in Biogran®-implanted capsules at capsule removal after 1 year (a), and after 3 months (b) and 6 months (c): new bone formation (arrowheads) is limited and confined to the very lower part of the tissue formed under the capsules (dashed line). The major portion of the tissue consists of biomaterial particles embedded in connective tissue. It can be seen in the high magnification (d) of (a) that the new bone (NB) has grown in direct contact with Biogran® particles (*). New bone can also be observed inside cavities and/or inside cracks (arrowheads) in the particles, but some particles are completely surrounded by connective tissue (¤). The white line (in a–c) delineates the host bone surface (HB). Modified Goldner's trichrome stain.

removal did not differ significantly from that observed at baseline. The discrepancy between the observations of Lioubavina et al. (1999) and those in the present study regarding some early resorption of the newly formed bone may be due to the fact that the capsules in the former study were removed after 6 months of healing, while in the present study the capsules stayed in place for 1 year, resulting in a more mature bone than that in the study of Lioubavina et al. (1999). This view is supported by the findings in a study in dogs, where barrier removal after 6 months of healing resulted in partial resorption of the regenerated bone, while ongoing bone modeling activity was observed when the membranes were retained for 15 months (Buser et al. 1995).

An important finding in the present study was that the amount of bone formed inside empty control capsules was significantly larger than that formed in the Bio-Oss®- or Biogran®-implanted capsules. This observation is in agreement with the results of two recent controlled experimental studies, where implantation of Bio-Oss® (Stavropoulos et al. 2001a) or Biogran® (Stavropoulos et al. 2001b) combined with GTR was found to interfere with bone formation. In those studies, capsules similar to those used in the present experiment and implanted with Bio-Oss® or Biogran® presented statistically significant less new bone compared with originally empty (control) capsules after 4 months of healing. In the study examining Bio-Oss®, the newly formed bone occupied only 12% of the total capsule space in the grafted specimens versus 39% in the empty control capsules (Stavropoulos et al. 2001a). In the study examining Biogran®, the newly formed bone occupied only 7% of the total capsule volume in the implanted specimens versus 38% in the controls (Stavropoulos et al. 2001b). The major portion of the capsules in those studies was occupied by biomaterial particles embedded in loose fibrovascular connective tissue similar to that observed in the present study. It was speculated in these studies that if longer observation periods were used, the connective tissue inside the capsules might gradually have transformed into new bone. This view was based on reports suggesting that the formation of new mineralized bone by GTR is always preceded by the formation of abundantly vascularized connective tissue in the membrane-protected space (Schenk et al. 1994, Hämmerle et al. 1995, 1996). The lack of active bone formation in all implanted specimens in the present experi-

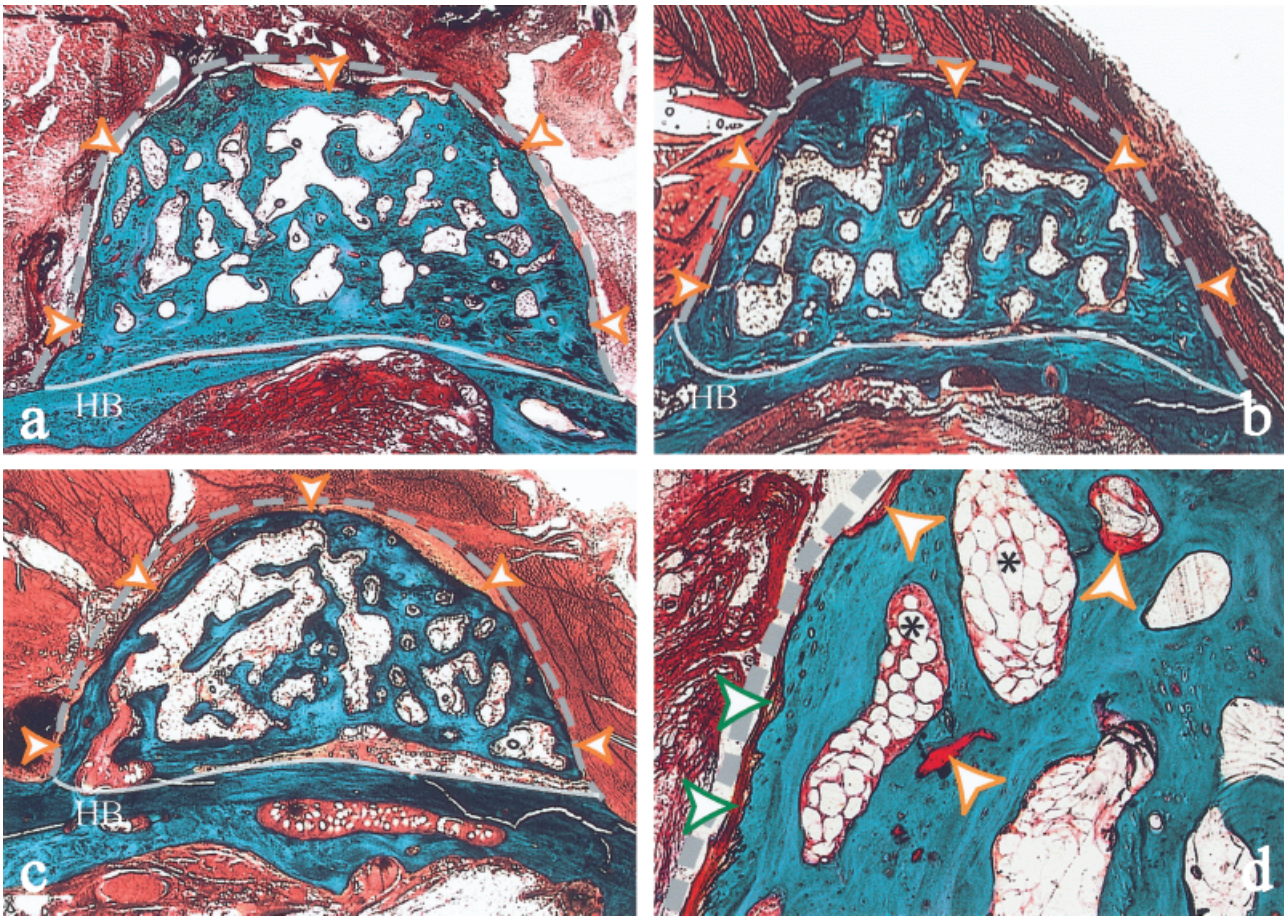

Fig. 7. Photomicrographs of tissues formed in control capsules at capsule removal after 1 year (a), and after 3 months (b) and 6 months (c): new bone (arrowheads) occupies entirely the capsule space at all observation times (dashed line). It can be seen in the high magnification (d) of (a) that the new bone has a trabecular appearance with marrow spaces with fat cells (*). Areas undergoing remodeling with osteoid formation can be identified (orange arrowheads), and a layer of dense fibrous connective tissue is lining the surface of the new bone (green arrowheads) formed under the capsule (dotted line). The white line (in a–c) delineates the host bone surface (HB). Modified Goldner's trichrome stain.

ment indicates that such connective tissue does not transform into bone even after a long period of time, which in turn means that Bio-Oss® and Biogran® in fact obstruct bone formation. This conclusion is in agreement with the observations in other studies (Wheeler et al. 1998, Al Ruhaimi 2001, Carmagnola et al. 2002, 2003).

In the specimens grafted with Bio-Oss®, the newly formed bone was always in continuity with the host bone and extended only a limited distance from the pre-existing bone surface. In the rest of the space originally created by the capsule (i.e. at a larger distance from the host bone), the graft particles remained embedded in fibrovascular connective tissue. In recently published animal and human studies, a similar ''pattern'' of bone formation was observed after grafting of various types of bone defect with Bio-Oss® (Slotte & Lundgren 1999, Paolantonio et al. 2001, Carmagnola et al. 2002, 2003). Apparently, new bone formation in Bio-Oss®-filled defects tends to be confined to the vicinity of the host bone even with prolonged periods of healing. Therefore, it is reasonable to presume that a narrow defect filled with Bio-Oss® may heal completely, while a wider one may not. This, in turn, may explain why Bio-Oss®-grafted defects in some experimental studies were reported to be filled up with new bone (Klinge et al. 1992, Berglundh & Lindhe 1997). The defects in those studies may possibly have had sufficiently small dimensions to be filled out by the limited bone formation occurring adjacent to the host bone. This assumption, along with the finding in the present study that bone formed in association with Bio-Oss® remains stable on a long-term basis, may also explain the reported long-term clinical success of implants placed in combination with Bio-Oss® with or without GTR (Hürzeler et al. 1996, Valentini & Abensur 1997, Tawil & Mawla 2001, Mayfield et al. 2001, Zitzmann et al. 2001). A similar assumption, regarding bone formation in Biogran®-filled defects, can be made. Specimens implanted with Biogran® in the present study presented a pattern of bone formation similar to that observed in the specimens grafted with Bio-Oss®.

The amount of Bio-Oss® or Biogran® particles embedded in connective tissue and bone did not differ significantly from one observation time to the other in the present study, and osteoclast-like cells adjacent to the biomaterial particles were encountered only in a few specimens. This observation is in accordance with the results of several animal studies that failed to show a reduction of the amount of implanted Bio-Oss® over time (Fukuta et al. 1992, Jensen et al. 1996), and with the finding of large amounts of graft particles 44

months (Skoglund et al. 1997) and 6 years (Schlegel & Donath 1998) after implantation in humans. Similarly, it has been shown in experimental studies that Biogran® particles occupy a large portion of the grafted space (Wheeler et al. 1998, Al Ruhaimi 2001), even after 24 months of healing (Schepers & Ducheyne 1997), although the number or size of the particles may decrease over time. Recently, it was reported that Biogran® particles occupied 10–15% of core biopsies taken 9–12 months after sinus lifts procedures in humans (Cordioli et al. 2001), but a rather fast degradation and/or resorption of this material has been reported previously (Schepers et al. 1991, Furusawa et al. 1998).

In the present study, new bone (%) in the area originally created by the capsule was defined as bone trabeculae and marrow spaces. Since Bio-Oss® and Biogran® are barely resorbable materials and occupy a substantial portion of the capsule space, it could be argued that the volume of biomaterial particles incorporated into new bone should have been included in the calculation of the percentage of bone fill in the test capsules. However, it is questionable whether this tissue blend can be considered as equal to true bone, and even if it were included, the amount of bone fill in the Bio-Oss®- and Biogran®-implanted capsules would still be smaller than that in the control capsules.

In the present experiment, no differences were identified between the three experimental groups regarding the appearance and the quality of the dome-shaped tissues formed under the capsules, neither clinically at re-entry during barrier removal nor radiographically, despite the fact that there were substantial quantitative and qualitative differences with respect to bone formation. This finding strongly indicates that assessment of treatment results following the use of bone grafts or bone graft substitutes (with or without GTR) by clinical observations (e.g. clinical measurements, sounding, re-entry etc.) and/or radiographs are unreliable. On the basis of the results of the present study, it can be concluded that bone produced by GTR with and without implantation of Bio-Oss® or Biogran® is stable on a long-term basis, but implantation of Bio-Oss® and Biogran® in a membrane-protected space obstructs bone formation.

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