

# Bone formation in rabbit cancellous bone defects filled with bioactive glass granules

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We examined new bone formation after filling cancellous bone defects with bioactive glass (BG) in granular form. Cylindrical defects in the trochanter area of 18 rabbit femora were filled with BG granules (diameter 600–830 µm) and compared with similar defects filled with morcellized autogenous bone. New bone formation and surface reaction of BG particles were evaluated by light microscopy, histomorphometry, and scanning electron microscopy. The chemical profile at the bone–material interface was studied by energy dispersive x-ray analysis (EDXA). In the BG group, 41, 32, and 38 percent of the

defects were filled with new bone after 3, 6, and 12 weeks, respectively. The corresponding figures for the autogenous bone group were 36, 29, and 34 percent. The thickness of the reaction layer on the glass surface increased from 82 to 163 µm during the observation periods. An intimate contact without intervening soft tissue between new bone lamellae and BG granules was a constant finding. EDXA showed a chemical continuum between the granules and the new bone. No adverse reactions related to BG were observed. BG is a promising material for filling cancellous bone defects.

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Silica glasses and glass–ceramics have been shown to be both osteoconductive and biocompatible (Hench et al. 1972, Gross et al. 1981, Höland et al. 1985, Kitsugi et al. 1989, Andersson et al. 1992). Previous studies indicate that bioactive glasses and glass ceramics can be used as a bone substitute material (Schepers et al. 1991, Nelson et al. 1994, Neo et al. 1994). In rabbits, we compared bone formation in cancellous bone defects filled with granules of bioactive glass (BG) or morcellized autogenous bone (AB) and assessed the biological response.

## Material and methods

The weight composition of the glass granules is: SiO<sub>2</sub> 53%, Na<sub>2</sub>O 23%, CaO 20% and P<sub>2</sub>O<sub>5</sub> 4%. The glass preparation has been described previously (Andersson et al. 1990). The BG granules had a diameter of 630–800 µm.

The study protocol was approved by the institutional animal study committee. New Zealand white star rabbits (9 males and 9 females; age 10–14 months; weight 3.3–4.6 kg) were premedicated with 1 mg of s.c. atropine. Anesthesia was given in the form of 5 mg/kg of intraperitoneal diazepam, i.m. buprenorphine 0.1 mL/kg and ketamine hydro-

chloride 10 mg/kg. Before operation, an i.m. injection of 50,000 IU/kg benzylpenicillin procaine was given.

A 3 cm incision was made on the lateral side of the proximal femur, the fascia was opened and the periosteum was pushed temporarily aside. A cylindrical hollow trephine drill (Bonefit®, Straumann, Davos, Switzerland) was used to make a hole (diameter 3.5 mm) through the lateral femoral cortex and intertrochanteric cancellous bone of the femur reaching the medial cortex (depth 7 mm) (Figure 1). Drilling was done at a low speed (< 700 rpm) in continuous physiological saline irrigation. The defect on the left side was filled with BG granules soaked in saline (BG group). A separate cortical piece of bone was sawn from the trochanter major and pressed to cover the cortical defect. The wound was closed anatomically in layers with absorbable sutures.

The contralateral control defects (AB group) were drilled similarly. The 2 bone cylinders obtained, including the lateral cortex, were morcellized with a rongeur into approximately the same size as the BG granules and they were used to fill the control hole (Figure 1). No wound infections or other postoperative complications occurred. The animals were killed after 3, 6, and 12 weeks, with 6 animals in each group.

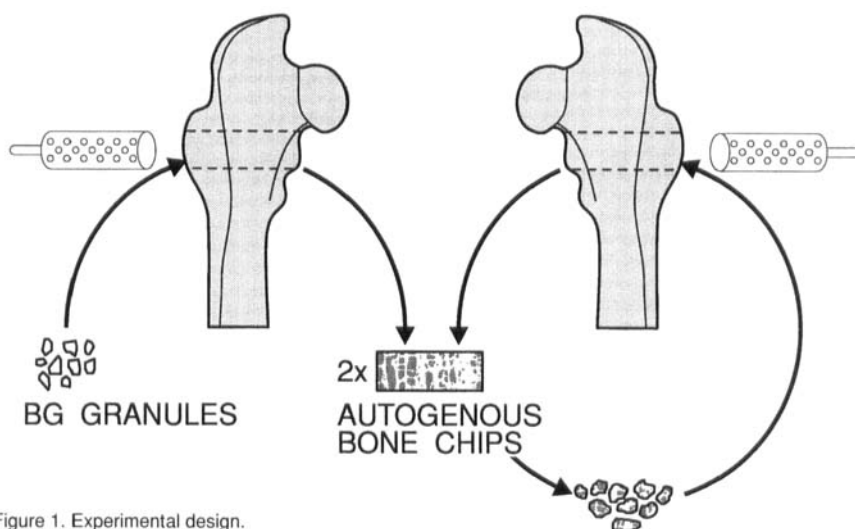


Figure 1. Experimental design.

2-cm long resection blocks of the femurs were fixed in 4% buffered formalin and embedded in methacrylate. Longitudinal sections (15  $\mu$ m) were prepared from the blocks along the mid-axis of the defect, using a cutting-grinding device for undecalcified hard-tissue specimens (Donath and Breuner 1982). The sections were stained with toluidine blue and evaluated by light microscopy. The rest of the blocks were used for scanning electron microscopy (SEM) to study the interface and for energy dispersive x-ray analysis (EDXA) to study the chemical components at the interface. SEM images were by the back-scattered mode. Each EDXA profile was based on linear analysis.

Paper prints of the light microscopy photographs, including the whole defect area and the cortical bone, were prepared at the final magnification of 150 $\times$  and used for histomorphometry. The bone within the defect was colored with red ink. A computerized automatic histomorphometrical analysis program based on color detection (MicroScale TC, Digithurst Ltd, Royston, England) was used to determine the area of new bone and filler in the cancellous bone defect. New bone formation in the sections of the AB group was distinguished histologically from grafted bone on the basis of bone morphology, presence of nuclei and staining characteristics. For control purposes, the amount of bone normally present in the trochanteric bone region was measured from the femurs of 2 rabbits not included in the two study groups.

Differences in bone formation in the defect were statistically evaluated, using two-way analysis of variance. One-way analysis of variance and the

Student's t-test with Bonferroni's correction were used to assess the formation of the reaction layer.

## Results

Macroscopically, the cortical defects healed without any signs of inflammation. One BG and 3 autogenous bone defects were excluded from the study because of failure during processing in the laboratory. Consequently, 17 defects were evaluated in the BG group and 15 defects in the AB group.

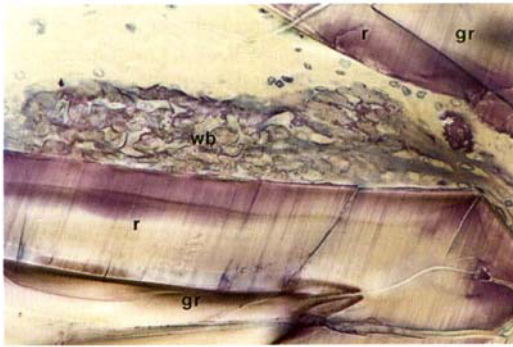
## Histology

A mild inflammatory reaction with some round cells and occasional polynuclear cells was observed in both groups at 3 weeks, but not later. No difference in this respect was found between the 2 groups.

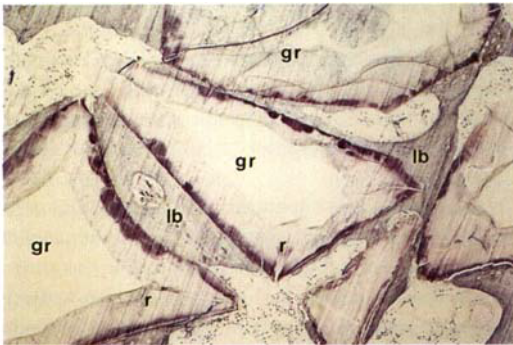
In the BG group, the space between granules was filled at 3 weeks with new bone, fat and some hematopoietic cells and dense collagen fibers. No chondroid tissue was seen. Fibroblast-like cells were usually arranged to follow the surface contour of the granules. Bone repair appeared as immature woven bone growing in intimate contact along the glass surface and between the granules. The newly formed bone became more lamellar with time and filled the whole defect more evenly. The amount of lamellar bone around BG granules increased and finally no woven bone was seen (Figure 2).

In the AB group, the transplanted autogenous bone was almost totally resorbed at 3 weeks. Only a few particles of old bone surrounded by osteoclasts were seen. At later stages, no grafted bone was observed.

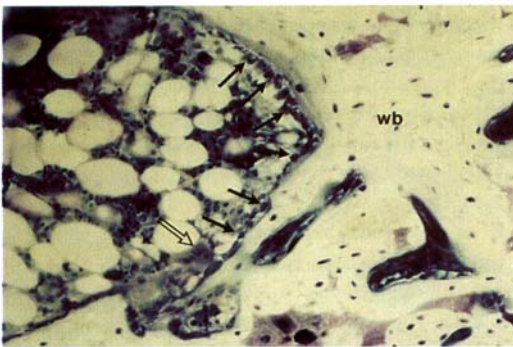
Figure 2. Histological findings (toluidine blue).



At 3 weeks there is a close contact between woven bone (wb) and BG granules (gr). Reaction layer (r) on the surface of the BG granules is seen as a violet zone ( $\times 270$ ).



Lamellar bone (lb) can be seen in close contact with the reaction layer of BG granules at 12 weeks. ( $\times 135$ ).



New bone surrounded by an osteoblast rim (short arrows) is seen in the defect filled with autogenous bone chips at 3 weeks. Note also osteoclast (open arrow) ( $\times 270$ ).

New bone formation occurred irregularly in the defect area. Immature bone was often bordered by a rim of osteoblasts with osteoid formation at 3 weeks (Figure 2). Some chondroid areas were noted. Mostly lamellar bone was found at 6 weeks. At 12 weeks, the bone was more trabecular than at 6 weeks.

Table 1. The amount of new bone and filler material in experimental defects filled with bioactive glass granules (BG group) or with autogenous bone chips (AB group) presented as percentages of the total defect area. Figures are number of specimens, mean SD

	Weeks								
	3			6			12		
<b>BG group</b>									
Bone	4	41	7	6	32	5	5	38	15
BG granule		30	7		28	5		21	6
<b>AB group</b>									
Bone	6	36	15	6	29	7	5	34	18

**Histomorphometry**

There was no difference in bone formation between the 2 groups. In the BG group, BG particles and formed bone together occupied 71, 60, and 59 percent of the defect area at 3, 6, and 12 weeks, respectively (Table 1). The amount of bone normally present in this area of rabbit femur was 20 percent.

A continuous profile of the chemical compounds at the interface between BG and bone was observed with EDXA (Figure 3). SEM revealed that the newly formed bone was in intimate contact with BG granules, without intervening soft tissue (Figure 4).

The reaction layer on the glass surface consisted of a silica-rich and a CaP-rich layer (Figures 2, 3, and 4). In the areas where bone bonded to the surface of the granule, the mean (SD) thicknesses of the reaction layer were 82 (30)  $\mu\text{m}$ , 86 (29)  $\mu\text{m}$ , and 108 (34)  $\mu\text{m}$  at 3, 6, and 12 weeks, respectively. In the areas without bone contact, the corresponding figures were 96 (37)  $\mu\text{m}$ , 112 (33)  $\mu\text{m}$ , and 163 (63)  $\mu\text{m}$ . The increase in the thickness of the reaction layer with

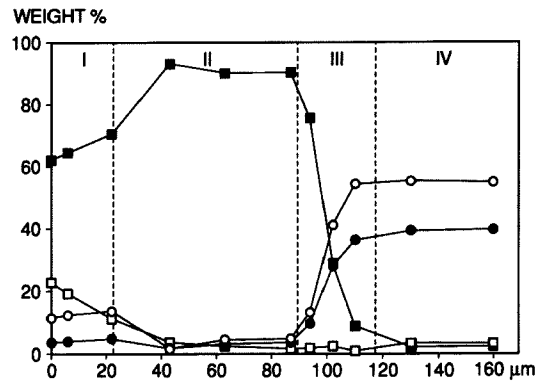


Figure 3. EDXA profiles on the interface at 12 weeks indicate a chemical continuum between bone and BG granule glass. I bulk glass, II Si-rich layer, III CaP-rich layer, and IV bone. ■ SiO<sub>2</sub>, □ Na<sub>2</sub>O, ● P<sub>2</sub>O<sub>5</sub>, and ○ CaO.

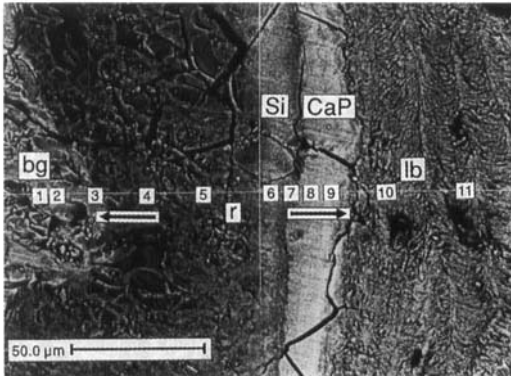


Figure 4. SEM picture of the interface between a BG granule and new bone shows an intimate contact at 12 weeks. bg bulk glass, r reaction zone consisting of Si-rich and CaP-rich layers, lb new lamellar bone. Numbers in the picture correspond to the same sites of analysis with EDX presented in Figure 3. Bar 50  $\mu\text{m}$ .

time was significant both in areas covered by bone ( $p < 0.005$ ) and in soft tissue ( $p < 0.0001$ ).

## Discussion

A material is bioactive if it elicits a biologically beneficial tissue response after implantation. In the case of bioactive glasses, a reaction layer consisting of calcium phosphate-rich and silica-rich zones is formed on the surface of the glass. The calcium phosphate layer in the top of this reaction layer is responsible for the bone-bonding seen in SEM as a continuum of the chemical components at the interface. Bioactive glasses have been shown to be biocompatible and nontoxic (Hench et al. 1972, Wilson et al. 1981, Hench and Wilson 1984). Our findings indicate that BG granules are bioactive and osteoconductive. The growth pattern of bone advancing from the periphery of the defects inward was similar to that observed previously using different bone substitute materials (Viikari and Aho 1963, Schepers et al. 1991, Neo et al. 1994).

To our knowledge, no histomorphometric studies have been published comparing bioactive glass granules with autogenous bone as a filler material. In a study on reconstruction of mandibular alveolar ridge, more bone was obtained with calcium polyphosphate bioceramic than with autogenous bone graft (Nelson et al. 1994). In our experiment there was no significant difference in the amount of new bone formation between cancellous bone defects filled with BG or AB.

The defect used in our study was smaller than the critical size for a unicortical defect in the rabbit, i.e., a defect which does not heal during the life time of the animal (Hollinger and Kleinschmidt 1990). The amount of new bone obtained in both groups exceeded the normal amount of bone in this area of the rabbit femur. It is likely, however, that the amount of bone in the AB group will decrease with time, as a result of bone remodeling, according to Wolff's law. No resorption of BG was seen during the observation periods. Whether the amount of new bone achieved in the BG group will remain unchanged needs studies with longer observation times.

The morcellized autogenous bone graft was found to resorb quickly. At 3 weeks, only small remnants of the autograft could be identified. The vitality of bone cells in autogenous bone grafts depends on nutrition. If the bone graft is cut into small pieces, more cells survive because the increased surface area facilitates the nutrition of cells. Consequently, the formation of new bone but also the resorption of transplanted bone are enhanced (Phemister 1914, Burchardt 1987, Springfield 1987).

New bone binds chemically to implanted BG particles through the formation of silica-gel and calcium phosphate layer on the surface of the glass, as shown by EDXA. A similar EDXA profile has previously been observed between bone and cones of a similar type of glass (Heikkilä et al. 1993). The reaction layer of the glass granules became thicker with the length of the observation period. The bone formed in contact with bioactive glass seemed to slow down the thickening of the reaction layer, but not to stop it completely. A similar finding concerning the formation of the calcium phosphate layer in the reaction layer has been presented recently (Aho et al. 1993, Neo et al. 1994). Although the thickness of the reaction layer increased, no signs of resorption of the BG granules were observed histologically during the follow-up time up to 12 weeks. However, the amount of BG filler in the defect was smaller at 12 weeks than at 3 weeks. This may be due to the slow dissolution of BG. The observation periods we used are too short to draw any conclusions on this matter.

Our findings indicate that granular BG glass is a promising material for filling cancellous bone defects. However, the biomechanical properties of the glass-bone composite need to be elucidated.

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