Bioactive glass versus hydroxylapatite in reconstruction of osteochondral defects in the rabbit

Jouni T Heikkilä[1], Allan J Aho[1], Antti Yli-Urpo[2], Örjan H Andersson[3], Heikki J Aho[4] and Risto-Pekka Happonen[5]

We studied osseointegration of a bioactive glass (BG) and hydroxylapatite (HA) in rabbit femur epiphyseal and metaphyseal regions. 17 BG and 24 HA cones implanted in defects through arthrotomy were analyzed. The holes for implants were drilled through distal femur joint surfaces. The cartilage wound repaired generally by fibrous tissue. Histomorphometry showed that 61, 78, and 79 percent of BG surface was covered by bone at 3, 6, and 12 weeks, respectively. The corresponding figures for HA were 47, 67, and 78 percent. Chemical bonding between bone and implants of both types was confirmed by scanning electron microscopy (SEM) and energy-dispersive x-ray analysis (EDXA). Formation of a calcium phosphate-rich layer on the surface BG implant was demonstrated by EDXA. Our results indicate that the osseointegration rate of bioactive glass does not differ from that of hydroxylapatite.

Turku University, Departments of [1]Surgery and [4]Pathology at the Central Hospital, [2]Prostchodontics and [5]Oral and Maxillofacial Surgery at the Institute of Dentistry, and Åbo Akademi University, Department of [3]Chemical Engineering, Åbo Akademi University, Turku Finland
Correspondence: Dr. J T Heikkilä, Department of Surgery, University of Turku, SF-20520 Turku, Finland
Tel +358–21 611 611. Fax 21 612 284
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Hydroxylapatite (HA) is the most thoroughly studied bone substitute material (Jarcho 1981). HA has been used clinically as a filler of bone defects and also as coating material on orthopedic prosthesis (Bucholz et al. 1988, Cook et al. 1988). There are, however, some problems in crystallizing apatite with constant qualities. Furthermore, HA has low strength, and its attachment to a metal surface is uncertain. For these reasons, the search for alternative bioactive materials which are able to bind to the surrounding tissue has continued (Neo et al. 1992). Bioactive glasses (BG), glass-ceramics and CaP-ceramics are both bioactive and biocompatible (Gross and Strunz 1985, Höland et al. 1985, Kitsugi et al. 1989). A common characteristic of these materials is that a biologically active hydroxyl-carbonate apatite (HCA) layer forms on the reactive surface if immersed in simulated body fluid or implanted in living tissue (Hench et al. 1972).

BGs are silicate glasses containing sodium, calcium, and phosphate as main components. They bind to bone by a surface layer of HCA which forms through chemical reactions on the glass surface (Hench and Paschall 1973). The chemical bonding of BG and bone has been shown by several investigators (Ito et al. 1987, Andersson et al. 1992). Very little is known about the reactions of BG in subchondral bone and cartilage. The purpose of this study was to examine BG implants in the treatment of osteochondral defects in rabbit femur.

Material and methods

The implants

Implant cones (length 9.0 mm, diameters 4.0 and 2.5 mm) were manufactured from a bioactive glass and hydroxylapatite to fit the dimensions of the drill used in the operation (Figure 1). The composition of BG (designated as S56.5P4) given in weight percentages was: SiO_2 56.5, NaO_2 27.0, CaO 12.0, P_2O_5 4.00, and Al_2O_3 0.50. The Ca/P ratio of the sintered hydroxylapatite was 1.67. HA was produced by a slurry technique to form compositionally uniform material (Nordström and Karlsson 1990).

Implantation

In rabbits 20 BG and 25 HA cones were implanted in the right distal femur, epi- and metaphyseal regions under general anesthesia. For control purposes, 15 additional holes were left without implants. The rabbits (13 female and 10 male New Zealand White Star rabbits; age 6–12 months; weight 2.9–4.2 kg) were premedicated with 1 mg of s.c. atropine. Anesthesia was accomplished with 5 mL of intraperitoneal diaze-

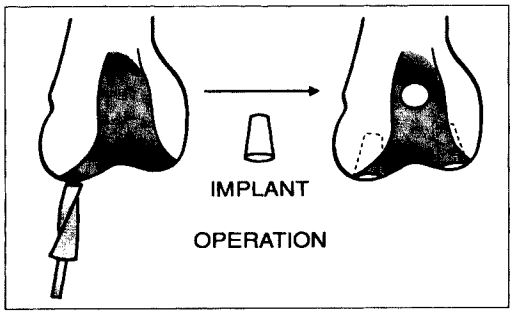


Figure 1. Experimental design.

pam, i.m. buprenorphine 0.1 mL/kg and ketamine hydrochloride 10 mg/kg i.m. Before the operation, an injection of benzylpenicillin procaine 50,000 IU/kg was given.

The operation area was shaved and cleaned with chlorhexidine gluconate (Hibitane) and physiological saline. An adhesive plastic cut-through operation barrier was applied. A medial arthrotomy was made on both knees through a 3 cm long medial incision. The patella was dislocated laterally, and 3 holes were made on the load-bearing cartilage surface, 1 under the patella and 2 on the condyles (Figure 1), using a special drill (Frialit 4-0, Friedrichsfeld GmbH, Mannheim, Germany). Drilling was done with low speed (< 700 rpm) in abundant, continuous saline irrigation. The cones were randomly implanted by a press-fit technique into drilled holes on the level of the subchondral bone. Additional control holes were made without implantation. The arthrotomy was closed with absorbable sutures. The animals were killed after 3, 6, and 12 weeks with pentobarbital and carbon dioxide.

Evaluation

The resected knees were evaluated macroscopically and radiographically in 2 perpendicular planes, paying attention to signs of inflammation and infection and to changes in cartilage and bone around the implants.

41 implants (17 BG and 24 HA) were analyzed, whereas 4 implants were excluded for the following reasons: 1 knee with 3 implants was lost because of infection and 1 implant was lost because of patella dislocation. The numbers of BG implants evaluated at 3, 6, and 12 weeks were 6, 4, and 7, respectively. The corresponding numbers for HA implants were 10, 4, and 10, and for control holes 5, 4, and 6. Wound infection was observed in 3 knees, all of which healed well without signs of arthritis.

The specimens were fixed in 4% buffered formalin and embedded in plastic. 20-µm sections were prepared through the longitudinal axis of the implants, using a cutting-grinding method developed for undecalcified hard tissue specimens (Donath and Breuner 1982). Sections were stained with toluidine blue and evaluated independently by 2 examiners by light microscopy. Additional 150-µm sections were processed for scanning electron microscopy (SEM) and energy-dispersing x-ray analysis (EDXA). A computerized analysis system was used to evaluate the amounts of different tissue components at the interface between the implant and host tissue on histological sections and the amount of bone on the surface of implants on SEM pictures.

The analysis of differences in osseointegration between the 2 biomaterials determined at 3 observation periods was assessed with 2-way ANOVA. The statistical analysis for inter-observer variation in measurement of bone contact was done by calculating the mean difference between the 2 observers, its 95 percent confidence interval (CI) and calculating the limits of agreement as the mean difference ± 25D (Bland and Altman 1986). The Pearson correlation coefficient was also calculated for the measurements of the 2 observers. Statistical computing was performed with SAS statistical software.

Results

All implants were incorporated into host bone by visual inspection. Slight redness of synovium and increased fluid content in the knee synovium as a sign of inflammation were noted in some animals in connection with both implant materials used. These changes were more prominent after 3 weeks than at 6 or 12 weeks. The color of cartilage in a small circular area around the implant was more pale than cartilage further away. Radiographic examination did not reveal any osteolytic changes around the implants.

Histology

A mild chronic inflammatory reaction consisting of lymphocytes and macrophages was seen at 3 weeks around both implant materials. No neutrophiles were observed. The inflammation did not differ from that seen in connection with empty holes. The inflammation had subsided at 6 weeks. No foreign body reaction was seen. At 3 weeks, some vascular granulation tissue with a tendency towards fibrosis, as well as woven bone, was seen around BG and HA implants. At 6 and 12 weeks, the implants were mostly covered by mature lamellar bone. A tight contact was observed between the implants and bone. The amount of bone in contact with the implant increased with time in both groups.

Table 1. Osseointegration rate of bioactive glass and hydroxyl apatite implants as detected by light microscopy (LM) and scanning electron microscopy (SEM). Osseointegration is given as percentages from the total length of the implant surface line within the bone tissue. Mean *SD*

Observation period		Osseointegration rate (percent)			
		Bioactive glass		Hydroxylapatite	
3 weeks	LM	60	25	47	*21*
	SEM	51	*23*	38	*13*
6 weeks	LM	78	*12*	67	*13*
	SEM	59	*29*	56	*20*
12 weeks	LM	79	10	78	20
	SEM	73	*14*	67	12

At 12 weeks, 79 percent of BG-implants and 78 percent of HA-implants were covered by bone (Table 1). Neither the group effect nor the interaction effect of the group and period was significant. Repair of the control holes occurred by variable amounts of fibrous tissue and bone. No cartilage was seen. Repair of all control holes was incomplete.

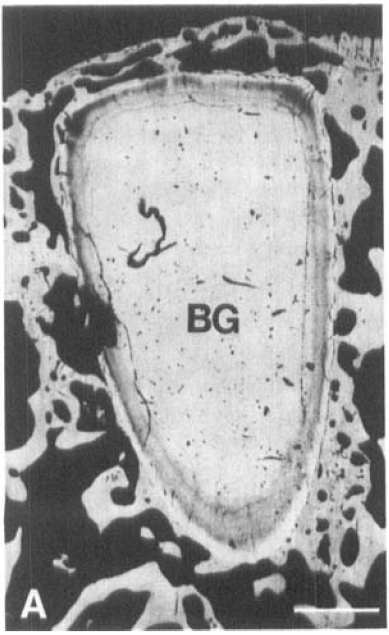
The cartilage defect on top of the implant was mostly covered by fibrous and granulation tissues in both groups at 3 weeks. Around the defect, fibrous tissue grew also under the normal joint cartilage. Some bone formation occurred on the top of the implants. After 6 weeks, fibrocartilage with a few areas morphologically resembling hyaline cartilage were present. Mature bone grew from the edges of the defect over the implants. After 12 weeks, bone coverage increased on the top of the implants and fibrous tissue was partly replaced by woven bone. Cartilage formation was seen more often on the top of HA (8/24) than BG (2/17) implants.

The inter-observer evaluation of the histomorphometric method used showed that the mean difference in bone connection between the 2 independent examiners was 5.85 (95%CI 2.2–9.5). The limits of agreement, mean difference ± 2 SD, were −17.0 to 28.7. The Pearson correlation coefficient was 0.87.

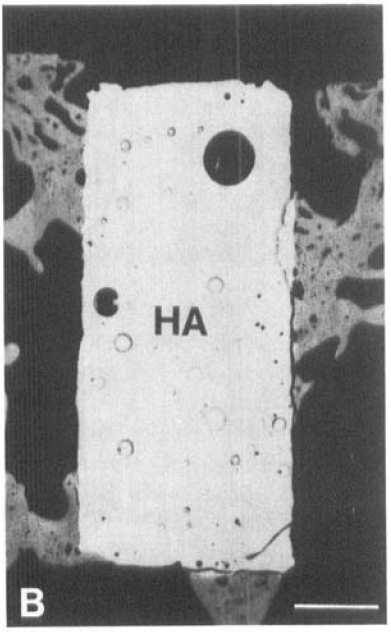
SEM and EDXA

At 12 weeks, 73 percent of the surface of BG-implants and 67 percent of HA-implants were covered by bone in SEM (Figure 2, Table 1). The difference in osseointegration between the 2 types of implants was not significant. EDXA analysis of the reaction layer within the glass showed formation of a Si-rich subsurface layer and a Ca,P-rich surface layer (Figure 3). The change in all the components was continuous through the interface between host bone and BG implant. No differences were found in CaO and P_2O_5 levels between HA-implant and the surrounding bone.

Figure 2. SEM picture of implants and surrounding bone at 12 weeks. Bar 750 μm.



Bioactive glass implant.



Hydroxylapatite implant.

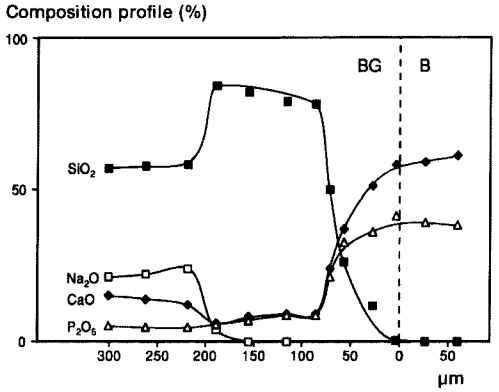


Figure 3. Composition profiles detected by EDXA analysis across the interface between bioactive glass (BG) and bone (B) at 12 weeks.

Discussion

The main observations of this study were good biocompatibility, bioactivity and osteoconductivity of both BG and HA. Both materials were confirmed to bond chemically to bone by SEM/EDXA. There was no significant difference in bone formation between the 2 implant groups. Reactivity and bioactivity of BG can be regulated by changing their chemical composition (Hench and Paschall 1973, Andersson et al. 1992), while the regulation of biological properties of HA is more restricted (Jarcho 1981). This makes BGs interesting materials for studies of various clinical applications in orthopedics.

Constantly lower amounts of bone in contact with the surface of BG and HA implants was found by SEM than by light microscopy. However, this difference was not statistically significant. The very thin layer of bone in contact with the implant cannot be separated from the calcium phosphate layer formed on the surface of the implant by SEM. These 2 layers are easy to distinguish by light microscopy because structural characteristics of bone can be seen. The computerized analysis system used for histomorphometry proved reliable, as shown by a good correlation coefficient between the 2 independent examiners.

Hench (1988) has reported 100 percent osseointegration for a BG (Bioglass®), and Gross and Strunz (1982) 96 percent for a glass-ceramic (Ceravital®) in compact bone of rabbit tibia at 12 weeks. Results of Gross and Strunz (1985) have indicated osseointegration of 85–95 percent for various glasses and glass ceramics. Less favorable results have been presented by Barth (1986) and Gross and Strunz (1985). Using titanium fibers coated with Bioglass® Barth (1986) achieved better results with uncoated than coated fibers. This may be due to the coating process used. In the study of various glasses in the diaphysis of rat femur, Gross and Strunz (1985) found that bone-bonding in connection with ceramic implants was impaired by reduced solubility and too high amounts of metal oxides. In our study, the implants were inserted into cancellous bone. Thus, the osseointegration rate of 79 percent for the BG used is comparable to the figures mentioned.

Cartilage repair on top of HA implants has been studied by Chiroff et al. (1977) who found a hyaline cartilage layer over repaired bone in the area of the cartilage defect on the implant. In our experiment, bone formation on top of the implant with some fibrocartilage was seen. Hyaline cartilage formation was scanty. The changes in cartilage around and on top of the implants were similar to those seen in arthrosis.

The solubility of bioactive glass can be reduced by addition of Al_2O_3 (Hench 1988, Ohura et al. 1992) which may accumulate at the glass surface and inhibit the calcium phosphate formation (Andersson et al. 1990) and mineralization of osteoid (Gross and Strunz 1985, Ohura et al. 1992). However, the bioactive glass used in our study, containing 0.5 weight percent Al_2O_3, caused no disturbance in the mineralization of osteoid. This agrees with our previous results showing that up to 1.5 weight percent Al_2O_3 can be used without interfering with mineralization (Andersson et al. 1988).

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