

MOLECULAR BASIS FOR ACTION OF BIOACTIVE GLASSES AS BONE GRAFT SUBSTITUTE

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ABSTRACT

Bone grafting procedures are undergoing a major shift from autologous and allogeneic bone grafts to synthetic bone graft substitutes. Bioactive glasses are a group of synthetic silica-based bioactive materials with bone bonding properties first discovered by Larry Hench. They have several unique properties compared with other synthetic bioresorbable bioactive ceramics, such as calcium phosphates, hydroxyapatite (HA) and tricalcium phosphate (TCP). Bioactive glasses have different rates of bioactivity and resorption rates depending on their chemical compositions. The critical feature for the rate of bioactivity is a SiO₂ content < 60% in weight. *In vivo*, the material is highly osteoconductive and it seems to promote the growth of new bone on its surface. In a recent study, the activity of the material was found even to overshadow the effect of BMP-2 gene therapy. *In vivo*, there is a dynamic balance between intramedullary bone formation and bioactive glass resorption. Recent studies of molecular biology have shown that bioactive glass induces a high local turnover of bone formation and resorption. Many osteoporotic fracture patients are candidates for concurrent treatment with bisphosphonates and bioceramic bone graft substitutes. Since osteopromotive silica-based bioactive glasses induce accelerated local bone turnover, adjunct antiresorptive agents may affect the process. However, a recent study showed that an adjunct antiresorptive therapy (zoledronic acid) is even beneficial for bone incorporation of bioactive glass. Based on these observations, bioactive glasses are a promising group of unique biomaterials to act as bone graft substitutes.

Key words: Bone grafts; bioceramics; bioactive glass

INTRODUCTION

The use of synthetic bone graft substitutes seems to be replacing the traditional golden standard of au-

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togenous bone grafting and the current main techniques of bone allografting. Synthetic bone graft substitutes are expected to be a safe and effective option in procedures like filling of fracture defects, resolution of long-bone nonunions, total joint revision surgery and spine fusion. Last year, for the first time, the number of orthopaedic procedures performed in the United States with bone graft substitutes exceeded those done with autologous bone. In Europe, a similar trend is evident especially when new EC directives will significantly affect the practice of local bone banking from donors.

The risk of local complications and morbidity re-

lated to the harvesting of iliac bone graft are not minor factors for a patient, when there are viable options. In addition, the amount of autogenous bone graft available may be the limiting factor for many procedures. Not infrequently, the quality of iliac bone is poor especially in postmenopausal aged females with underlying diseases such as rheumatoid arthritis and osteoporosis.

The use of morselized cancellous bone from fresh-frozen allogeneic femoral heads as well as the use of demineralized bone matrix processed from human cadaver bone have an established role in major bone reconstruction procedures of long bones and pelvis as well as in spinal fusions. As the main benefit, allografts have a favorable biologic influence due to the presence of multiple natural bone-growth stimulating proteins. The use of morselized allografts has inherent limitations and potential complications. Incorporation of allografts can be improved by rinsing the grafts before impaction, probably by washing out immunogenic factors (1). However, the availability of high-quality allogeneic bone has become a limiting factor (2). Most centers have utilized fresh frozen allogeneic femoral heads from their own bone bank (3). In Europe, new EC regulations of quality and safety issues will inevitably change the current intra-institutional and national practices. In the United States, FDA has also issued a request for registration of all tissue banks. Despite vastly improved techniques of sterile procurement, rigid donor criteria and strict culture surveillance, there have been over 60 cases of allograft infections just within the past year, as reported by U.S. Centers for Disease Control and Prevention (CDC). The problem is that sterilization methods tend to degrade either the biologic or biomechanical quality of the allograft tissue. However, despite all the efforts in quality control, the safety of the use of allograft tissue remains a constant issue and the outbreaks of transmitted infections, although extremely rare, seem to dictate the progress towards synthetic graft substitutes.

BIOACTIVE GLASSES

Bioactive glasses are a group of synthetic silica-based bioactive materials with unique bone bonding properties first discovered by Hench in the early 1970s (4). Bioactive glasses have different rates of bioactivity depending on their chemical compositions. The critical feature for bioactivity is a SiO_2 content < 60% in weight. The most rapid bonding is achieved with bioactive glasses containing 45–52% in weight of SiO_2 . These glasses form a chemical bond to bone (Fig. 1), but also to soft tissue. Glasses with a SiO_2 content 55–60% in weight react more slowly, show long-lasting bioactivity, and do not bind soft tissues (5). The first bioactive glasses were based on a simple four-component system of SiO_2 , Na_2O , CaO and P_2O_5 . These glasses (e.g. Bioglass® 45S5 and S53P4) have been used in various clinical applications (presented below) in solid and particular forms. However, these "first generation" bioactive glasses show a tendency to crystallization during the manufacturing of glasses

into different shapes at a high temperature. To overcome this disadvantage, novel bioactive glasses based on the $\text{Na}_2\text{O-K}_2\text{O-MgO-CaO-B}_2\text{O}_3\text{-P}_2\text{O}_5\text{-SiO}_2$ system have been developed (6). This gave the possibility to manufacture bioactive glasses into microspheres, fibers and porous implants, and provides an additional advantage for clinical use.

BIOACTIVE GLASS INCORPORATION WITH BONE

In vivo studies applying a modified bone healing model of bone marrow ablation of rat tibia have shown that filling of intramedullary space with bioactive glass microspheres results in significant intramedullary new bone formation and high local bone turnover (7, 8).

During the first two weeks of primary bone response, relatively undifferentiated mesenchymal tissue surrounds the bioactive glass microspheres. The tissue differentiates into an immature woven bone structure. Interestingly, the filling of the medullary space with bioactive microspheres seems to even inhibit the primary bone response seen at 2 weeks in the empty controls left to heal without bioactive glass filling. Obviously, the massive filling of the medullary space with bioactive glass microspheres acts as a mechanical barrier for primary bone response. The osteopromotive effect of bioactive glass microspheres becomes evident after the primary bone response (abundant formation of immature woven new bone, followed by its fast resorption) subsides (4–8 weeks). At the period of new bone remodelling, the new bone matures to lamellar bone, which is filling inter-microsphere spaces and partly covering the microspheres (Fig. 2). Histomorphometric analysis have revealed that bioactive glass filling induces a constant time-related increase of new bone, while in the empty controls left to heal without filling the amount of new bone peaked at 2 weeks and decreased thereafter (Fig. 3). Eight weeks after the operation, the defects filled with bioactive glass contained approximately twice as much bone than the empty controls left to heal without filling and 35.6–55.8% of the outer perimeter of bioactive glass microsphere was in contact with ongrown new bone (affinity index).

The BEI-SEM imaging with EDXA mineral analysis have demonstrated the presence of silica and CaP phosphate reaction layers on surface of the bioactive glass microspheres, and a direct contact between the CaP layer and the ongrown new bone (Fig. 1).

There is a dynamic balance between intramedullary bone formation and bioactive glass resorption. After implantation in the medullary cavity, the area occupied by bioactive glass microspheres decreases by approximately half by 8 weeks, but the total area occupied by bioactive glass and intramedullary new bone remains the same (approximately 36% of the cross-sectional area of intramedullary space). The most intense resorption of microspheres occurs during the first two postoperative weeks. This phenomenon suggests an interesting balance between bioactive glass dissolution and new bone formation. The

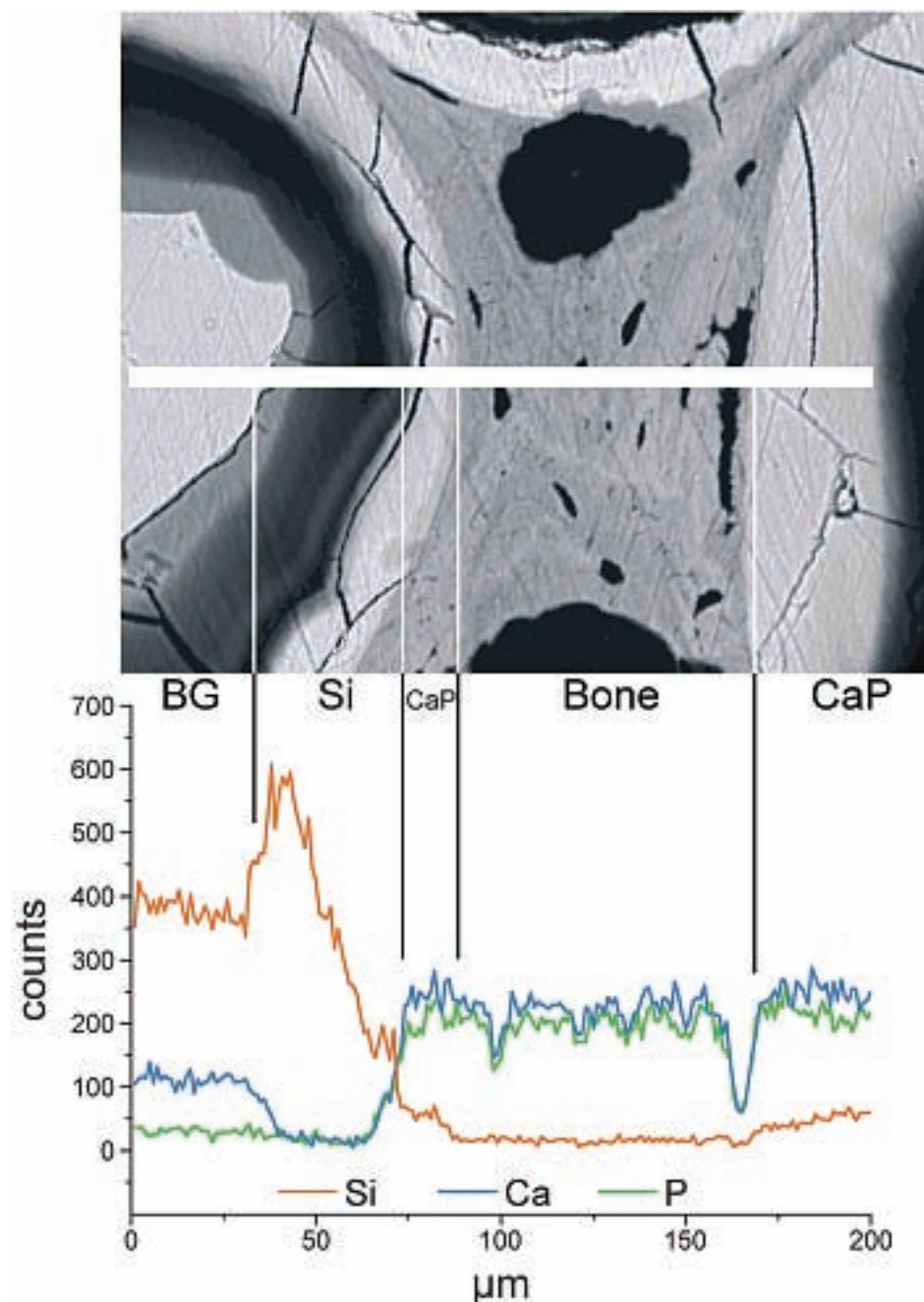


Fig. 1. BEI-SEM imaging of bonding between reaction layers of bioactive glass and new bone aligned with the corresponding EDXA profiles. The profiles demonstrate the contents of selected elements (Si, P, and Ca)(expressed as counts) as a function of their distance from the interface (expressed as μm). The white Ca-P zones of the microsphere surfaces are in intimate contact with intervening new bone. Redrawn from Välimäki et al. Bone 2006;38:432–443. (25)

finding fits well into the mechanism of balanced gradual dissolution of the bioactive glass matrix, and the synthesis of new bone on its surface.

There is a difference in the healing tendency of the cortical window between the empty controls and the bioactive-glass-filled defects. Healing of the cortical window has been shown to be incomplete in most of the bioactive-glass-filled defects, while bone bridging across the window is observed in virtually all defects left to heal without filling. Previous studies have suggested that the healing of a cortical bone

defect is determined by the local strain environment dictated by physiologic loading of the bone (9). Induction of new bone formation in the medullary space by means of bioactive glass microsphere application obviously alters the natural remodelling process of the tubular bone. It is likely that together with the mechanic support of the bioactive glass filling, the induction of new bone formation changes the strain environment of the cortical window to such an extent that the natural biomechanical signal for early bone bridging of the window delays in the tubu-

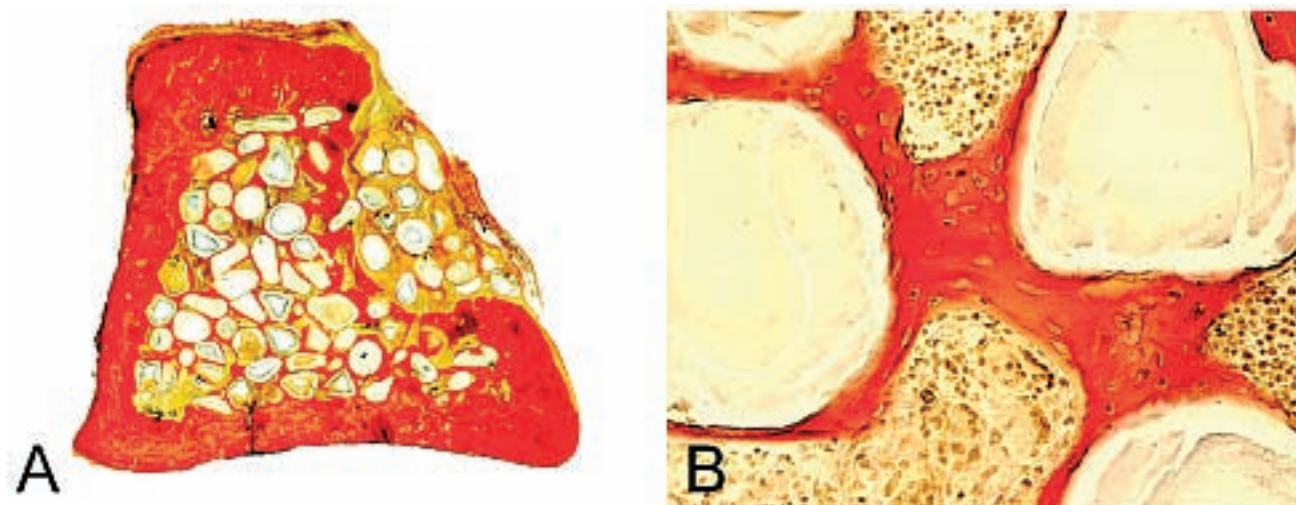


Fig. 2. Intramedullary new bone in a rat tibia at 8 weeks (A). The inter-BG microsphere spaces are filled with new mature lamellar bone (B).

lar bone. This phenomenon has also been demonstrated with the analysis of peripheral computed tomography (pQCT). At 4 weeks, the pQCT strength strain index (SSI), a mathematical parameter correlating strongly with the actual three-point bending strength, of bones filled with bioactive glass microspheres is significantly lower than the SSI of the empty controls left to heal without filling, suggesting a temporary deceleration in the mechanical adaptation of tubular bone.

MOLECULAR MECHANISM OF BIOACTIVE GLASS INCORPORATION

The chemical mechanism of the bioactive glass surface reaction is initiated after contact with body fluids, and subsequently, rapid ion exchange of Na^+ and K^+ from the bioactive glass with H^+ and H_3O^+ from the extracellular fluids takes place (Fig. 4). The network structure dissolves, Na^+ , Ca^{2+} , Mg^{2+} , P^{5+} and Si^{4+} leach, and the Si-rich layer forms through polycondensation of the hydrated silica groups (stages 1–3). The Ca^{2+} and PO_4^{3-} precipitate from the extracellular fluids onto the Si-rich layer (stages 4–5) (5).

The cellular mechanism in which the bioactive glass attracts cells has been under extensive research. Numerous *in vitro* studies have shown that bioactive glasses stimulate the growth and maturation of osteoblasts, and promote the expression and maintenance of the osteoblastic phenotype (10–12). Moreover, it has been demonstrated that bioactive glass stimulates multipotent bone marrow stromal cell functions by both surface-mediated and solution-mediated mechanisms (13). Formation of the Si-rich layer is known to be a crucial stage in bone bonding as it acts as a template for calcium phosphate precipitation. The calcium phosphate layer then directs new bone formation together with absorbing proteins (14, 15). The extracellular proteins attract macrophages, and mesenchymal stem cells and osteoprogenitor cells

(Fig. 4, stages 6–8). It has been shown that especially fibronectin, a major glycoprotein in serum, increases the attachment of cells to the calcium phosphate layer (16). Subsequently, the osteoprogenitor cells proliferate and differentiate into matrix-producing osteoblasts (stages 9–11). In addition to the absorbing proteins, the particle size and implant porosity affect the osteoblast function, the rate of bone formation and bone ingrowth into bioactive glass material (17–19).

Studies on molecular mechanisms of bioactive glass have largely focused on osteoblasts and their gene markers *in vitro* (10, 13, 20–22). These studies have uniformly shown that bioactive glasses enhance the functions of osteoblasts. The studies in our laboratory have included the analysis of mRNAs for both synthesis and resorption markers in order to determine not only the rate of new bone formation but also the rate of bone turnover in comparison to the defects left to heal without bioactive glass filling. Besides osteoblast markers, also osteoclast markers (MMP-9, Cathepsin K and TRACP) have been analysed. This approach has proved to be useful for a better understanding of the mechanism of action of bioactive glasses. The results have shown that bioactive glass induces a high but balanced local bone turnover.

During the primary bone response (at 4 days and 2 weeks), Northern analyses have demonstrated elevated mRNA levels for both bone synthesis markers (type I, II and III collagens, osteocalcin, osteonectin and osteopontin) and resorption markers (cathepsin K and MMP-9) both in defects filled with bioactive glass microspheres and in empty controls left to heal without filling (Fig. 3). After the primary healing response, the empty control defects show normalized mRNA levels by 8 weeks, while defects filled with bioactive glass microspheres exhibit 2.1–11.6-fold higher mRNA levels. The mRNAs for TRACP and MMP-13 are also increased in bioactive glass-filled defects. Attempts have been made to manipulate this balance of high bone turnover by osteoinductive

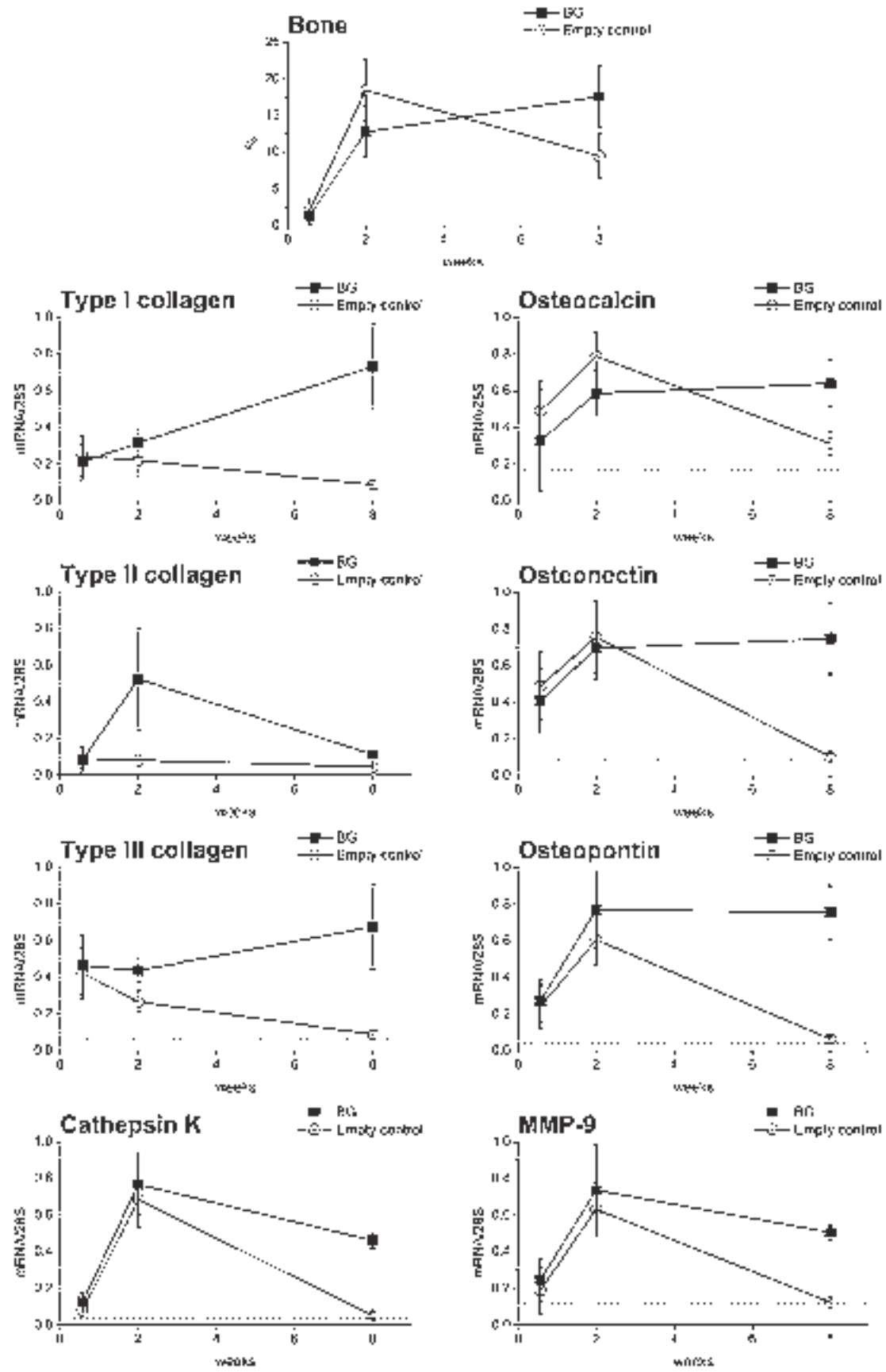


Fig. 3. The cross-sectional amount of intramedullary bone and the mRNA profiles of the bioactive-glass-filled defects and empty controls. The lines represent means \pm SD, n=5-6. The mRNA level of intact bone is marked with a dotted line.

Time	Surface reaction stage
	Bioactive glass
1 & 2	Formation of SiOH bonds
3	Polycondensation $\text{SiOH} + \text{SiOH} \rightarrow \text{Si-O-Si}$
4	Adsorption of amorphous $\text{Ca} + \text{PO}_4 + \text{CO}_3$
5	Crystallization of hydroxyl carbonate apatite (HCA)
6	Adsorption of biologic moieties in HCA layer
7	Action of macrophages
8	Attachment of mesenchymal stem cells
9	Differentiation of mesenchymal stem cells
10	Generation of matrix
11	Crystallization of matrix

Fig. 4. Sequence of interfacial reactions involved in forming a bond between bioactive glass and bone. Modified from Hench and West (5).

RAdBMP-2 treatment. On the basis of the well-demonstrated osteoinductive effects of rhBMP-2 and direct BMP-2 gene transfer, it would be reasonable to hypothesize that adjunct RAdBMP-2 gene transfer could push the balance toward bone synthesis (23, 24). However, the adjunct RAdBMP-2 therapy decelerates the bioactive-glass-induced high turnover, but does not influence the balance of synthesis and resorption.

EFFECT OF ADJUNCT BISPHOSPHONATE THERAPY IN OSTEOPOROSIS

In orthopaedic trauma surgery, osteoporosis carries an increased risk of surgical complications. Fragility of osteoporotic bones, the tendency for fracture comminution, and the presence of large fracture defects produce challenges in operative stabilization of osteoporotic fractures. There are several reasons for the use of synthetic grafts in osteoporotic patients. Traditional autogenous bone grafting may not be plausible because of bone loss and poor bone quality also at harvesting sites. Many osteoporotic fracture patients are candidates for concurrent treatment with bisphosphonates and bioceramic bone graft substitutes. Since osteopromotive silica-based bioactive glasses induce accelerated local bone turnover, adjunct antiresorptive agents, such as zoledronic acid, may affect the process.

Therefore, a recent experimental study (25) studied the effect of adjunct zoledronic acid therapy on bioactive glass incorporation in a rat bone marrow ablation model. The experimental animals received zoledronic acid (1.5 µg/kg sc, once a week, started one week before surgery). Bioactive glass incorporation and geometric bone properties were followed by sequential pQCT imaging. Bones filled with BG microspheres produced 2.5-fold more intramedullary new bone than controls with bone marrow ablation only. Adjunct therapy with zoledronic acid enhanced new bone formation on BG microspheres and partic-

ularly improved the SSI values of the BG-filled bones ($p < 0.05$). The analyses of mRNA expression confirmed high local bone turnover in all bones with BG filling. At the 9th week of zoledronic acid treatment, bones with and without BG filling showed increased the mRNA levels of bone resorption markers and decreased the mRNA levels of markers for synthesis, indicating that a corrective resorption process was already in progress in response to massive accumulation of medullary new bone at earlier stages of the therapy. Based on this study, an adjunct antiresorptive therapy seems to be beneficial for incorporation of bioactive glass microspheres. In the applied model, the therapy even resulted in favorable remodeling of the tubular bone structure.

COMPARISON WITH HA/TCP PRODUCTS

Synthetic bioresorbable bioactive ceramics, such as calcium phosphates, hydroxyapatite, and tricalcium phosphate are the most commonly used osteoconductive bone graft substitutes (26, 27). Compared with them, silica-based bioactive glasses form an own group of bioceramics with several unique properties (Table 1).

CLINICAL APPLICATIONS OF BIOACTIVE GLASS

The clinical applications of bioactive glass were initiated in the 1980s (28). Since then, bioactive glasses have been used in a variety of different shapes such as plates, bulks, granules and powder in many medical and dental applications. In head and neck surgery, bioactive glass has been applied in reconstruction of orbital floor fractures and defects of facial bones, in filling of frontal sinuses, in augmentation of the maxillary sinus floor, in grafting periodontal bone defects and in middle ear surgery (29–35). In orthopaedic applications, bioactive glass has been

TABLE 1
Comparison of synthetic HA/TCP and bioactive glasses as bone graft substitutes

	HA and HA/TCP	Bioactive glasses
Chemical composition	One or two chemical components (hydroxyapatite, tricalcium phosphate, or both)	Several components (at least four components). • Original: four-component system of SiO ₂ , Na ₂ O, CaO and P ₂ O ₅ (Hench et al. 1971) (4). • Modified systems: Na ₂ O-K ₂ O-MgO-CaO-B ₂ O ₃ -P ₂ O ₅ -SiO ₂ (Brink et al. 1997) (6)
Physical forms	Porous blocks or granules	Granules or sintered porous blocks, fibers and woven structures
Basic mechanism	Serves as osteoconductive surface	Forms chemical bonding with ongrowing new bone. Osteopromotive
Molecular mechanism of action <i>in vivo</i>	Not defined	Induce high local bone turnover
Regulation of bioactivity	Based only on HA/TCP ratio	Can be regulated by modifying the chemical composition
Resorption rate	TCP is resorbed fast (months) HA is slowly/very slowly resorbed (years, decades)	Resorption can be highly regulated (from weeks to years) by modifying the chemical composition
Mechanism of resorption	Involves chemical dissolution and osteoclastic resorption	Chemical dissolution
Antimicrobial properties	Not reported	Inhibition of bacterial growth <i>in vitro</i> , dependent on the chemical composition

tested as a filler of benign tumors in 12 patients (36). The patients showed no complications within the 15-month follow up.

At the present moment, there are no published reports on prospective randomized clinical studies of bioactive active glass as bone graft substitute. We also need cost-benefit analyses as a part of head-to-head comparisons of the different synthetic bone graft substitutes.

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Received: March 28, 2006