BONE REGENERATION USING BIOGLASS: AN EXPERIMENTAL STUDY IN RABBIT TIBIA

Adriano Piattelli, MD, DDS
Antonio Scarano, DDS
Maurizio Piattelli, MD, DDS
Franco Coraggio, MD, DDS
Sergio Matarasso, MD, DDS

KEY WORDS

Bioglass
Bone defects
Bone regeneration

Adriano Piattelli, MD, DDS, *is Professor of Oral Medicine and Pathology, Dental School University of Chieti, Italy, Honorary Senior Lecturer, Eastman Dental Institute and Hospital for Oral Health Care Sciences, London, UK. Correspondence should be addressed to Dr. Adriano Piattelli at Via F. Sciucchi 63, 66100 Chieti, Italy.*

Antonio Scarano, DDS, *is a Research Fellow and* ***Maurizio Piattelli, MD, DDS,*** *is a Researcher, Dental School, University of Chieti.*

Franco Coraggio, MD, DDS, *is a Researcher and* ***Sergio Matarasso, MD, DDS*** *is Professor of Periodontology, Dental School, University of Naples, Naples, Italy.*

Bioglass (BG) has been shown recently to be osteoconductive and osteopromotive in different experimental and clinical conditions. The aim of the present study was to evaluate BG particles in bone defects in rabbit tibia. In control sites, bone was observed only in the peripheral areas of the defects, while in test sites, newly formed bone was found around all BG particles, even those located in the central portion of the defect. Osteoblasts were actively secreting osteoid matrix directly on the granules' surface. BG seems to be a highly osteoconductive material.

INTRODUCTION

Bioglass (BG) is a material that, when immersed in physiological fluids, produces a reaction layer consisting of a Ca–P-rich surface layer and an Si-rich subsurface layer.[1,2] BG immediately releases silicon to form a silicon-rich layer on its surface and, shortly after, both calcium and phosphate are released from BG as well as absorbed from the body fluids, and a calcium–phosphate-rich layer is formed.[3,4] Finally, hydroxyl carbonate apatite crystals form in the calcium–phosphate-rich layer, and this is stoichiometrically equivalent to bone.[3]

Exposure of a collagen to BG produces the embedding of collagen in a growing hydroxyapatite layer with the formation of a strongly bonded collagen–glass interface.[5] Osteoblasts proliferate, and collagen is formed and embedded into the silica gel layer.[3]

Recent studies show that BG is conductive and osteopromotive because it promotes migration, replication, and differentiation of osteogenic cells and their matrix production.[2,4] BG used for the treatment of periodontal defects in man showed that it was possible to obtain a 2-mm mean gain in attachment level at 3 months,[6] while in beagle dogs, a bone repair in suprabony sites was observed.[7] Moreover, an increased hemostasis, excellent handling properties, and an excellent tissue response were observed.[6] No adverse cellular response was observed.[3,8]

BG can be used also to augment bony defects at the site of dental implants,[3] and as a filler and coating material in connection with the use of implants projecting into bone cavities.[1] BG has been used in apical resection, extraction sites, cystic defects,[9] middle-ear devices, and cochlear implants.[10] The aim of the present study was to evaluate BG particles used to fill bone defects in rabbit tibia.

MATERIALS AND METHODS

BG particles (Biogran, Orthovita, Belgium) (Fig 1) and 10 white New Zealand rabbits were used in this study.

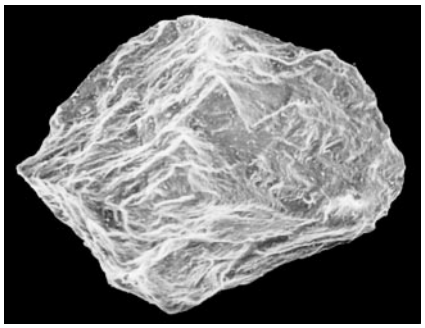

FIGURE 1. Macroscopic aspect of the bioglass particle. SEM ×100.

The animals were anesthesized with an intramuscular injection of 0.7 mg/kg body weight Hypnorm (Jansen Pharmaceuticals, Bruxelles, Belgium). A local anesthesia with 1 ml of 2% lidocaine (Astra AB, Sodentalje, Sweden) was also used. The surgical procedure was performed under sterile conditions.

After skin incision, a periosteal flap was elevated and the medial surface of proximal metaphysis of the tibia was exposed. Eight-millimeter bone defects (Fig 2) were prepared with a bur under generous saline irrigation. One defect was prepared for each rabbit tibia. A total of 20 defects were prepared. Ten defects were filled with BG, while the other 10 defects were used as controls. Two animals each were sacrificed after 1, 2, 3, 4, and 8 weeks.

The specimens were washed in saline solution and stored immediately in 10% buffered formalin and were processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy).[11] The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned longitudinally along the major axis of the implant with a high-precision diamond disk at about 150 μm and were ground down to about 30 μm. Three slides were obtained from each implant. The slides were stained with acid fuchsin and toluidine blue. A double staining with von Kossa and basic fuchsin was done to evaluate the degree of bone mineralization, and one slide per implant, after polishing, was immersed in $AgNO_3$ for 30 minutes and exposed to sunlight. The slides were then washed under tap water, dried, and immersed in basic fuchsin for 5 minutes and then washed and mounted.

The slides were observed in normal transmitted light under a Leitz Laborlux microscope (Leitz, Wetzlar, Germany). Histomorphometry of percentage of bone contact was done using a Microvid system (Leitz, Wetzlar, Germany) connected to an IBM PC.

RESULTS

Control sites

One Week

In the bone defect, a blood clot was present. Only in the most peripheral areas was it possible to observe osteoblasts actively secreting osteoid matrix.

Two Weeks

Newly formed bone was observed in the peripheral areas of the defect.

Three Weeks

Newly formed bone with wide marrow spaces was observed in the bone defect.

Four to Eight Weeks

Mature, newly formed bone was observed only in the most peripheral area of the defect.

Test sites

One Week

At low magnification, it was possible to observe the BG particles that were unstained. The particles located in the central portion of the defect were lined by osteoprogenitor cells and by blood clot. No newly formed bone was present. No inflammatory cells were present.

Two Weeks

The BG particles were not stained (Fig 3). At low magnification, small, newly formed bone trabeculae were observed in the peripheral portion of the defect. This bone was highly stained with acid fuchsin and presented wide marrow spaces and wide osteocytic lacunae. Osteoblasts, secreting osteoid matrix, were present around the BG particles. At higher magnification, some multinucleated cells were present around a few BG particles (Fig 4).

Three Weeks

Almost all particles, including those in the central area, were surrounded by the newly formed bone (Fig 5) and many osteoblasts were in close contact with the BG particles. These osteoblasts were actively secreting osteoid matrix directly on the granules' surface. With silver nitrate, it was possible to see that material not yet mineralized was present at the periphery of the granules.

Four Weeks

A higher quantity of bone was seen around the granules. No gaps were present at the granule–bone interface. In several areas, the newly formed bone bridged the gap between the BG particles. No cavitation of the central part of the particles was observed.

Eight Weeks

No differences were observed compared with the 4-weeks specimens. Mature bone was present around the BG particles, and no gaps were present at the bone–BG interface. Microfractures were present at the periphery of the granules (Figs 6, 7).

DISCUSSION

BG has been shown to be biocompatible and nontoxic and to have a direct chemical bond to bone.[1] Osteoblasts cultured on BG showed a better osteoblast-like morphology, a higher proliferation rate, and a better expression of the osteoblast phenotype when compared with cells cultured on hydroxyapatite, titanium alloy, and stainless steel.[2]

Bioactive ceramics show both osteo-

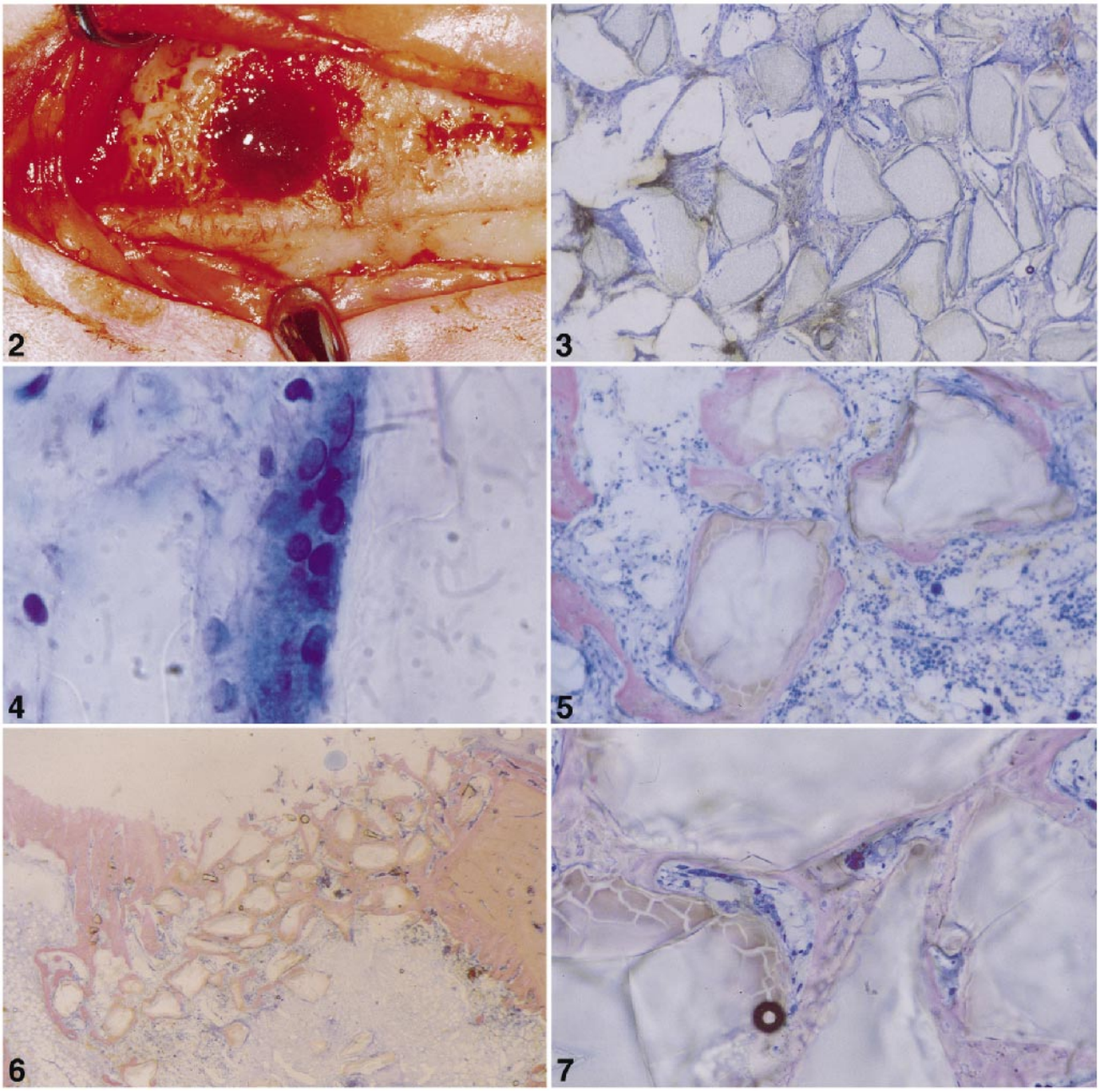

FIGURE 2. The bone defect in rabbit tibia has been prepared.

FIGURE 3. At 2 weeks, bioglass (BG) particles are surrounded by many cells. The BG particles are not stained. Toluidine blue and acid fuchsin. ×50.

FIGURE 4. At 2 weeks, it can be seen at higher magnification, that a large, multinucleated cell is in close contact with a BG particle. Toluidine blue and acid fuchsin. ×120.

FIGURE 5. At 3 weeks, newly formed bone lines the BG particles and many actively secreting osteoblasts are present. Cellular colonization of the peripheral portion of the BG particles by organic fluids is observed. Toluidine blue and acid fuchsin. ×100.

FIGURE 6. Bone defect at low magnification (at 8 weeks). Almost all BG particles are surrounded by mature bone, and no gaps between bone and Bioglass granules are observed. Toluidine blue and acid fuchsin. ×14.

FIGURE 7. At 8 weeks, it can be seen, at higher magnification, that mature bone with marrow spaces surround the BG particles. Toluidine blue and acid fuchsin. ×400.

conduction and osteoproduction, presenting a biocompatible interface for bone migration and a bioactive surface colonized by osteogenic stem cells.[6,12–14] BG particles of narrow size range determined the differentiation of osteoprogenitor cells into osteoblasts.[12] The basic reaction for bonding of BG to bone is reaction or corrosion of bioglass with tissue fluids.[1]

Several authors have reported an internal erosion of the granules, with formation of small cracks at the surface.[15,16] Osteoprogenitor cells infiltrated in these tissues,[14] and these internal zones acted as nucleation sites for enhanced bone repair.[16] In a study comparing hydroxyapatite and BG, it was found that the speed of bone growth around BG particles was faster and the new bone was denser around BG.[17] Moreover, the vascularization found in the glass-treated implant bed was more pronounced than that found in control sites, and glass-treated sites seemed to contain about twice as much bone tissue.[12] Cavities filled with BG were completely repaired by newly formed bone, and the BG particles were almost totally resorbed and substituted by bone.[18] In periodontology, BG showed significant improvement in clinical parameters compared with open flap debridement,[19] in addition to more pocket depth reduction,[20] and similar results to that of demineralized freeze-dried bone when used in moderate to deep intrabony periodontal defects.[21]

Our results show that BG is highly conductive.[1,12,14] In test sites, newly formed bone was present in the central portion of the defect, while on the contrary, in control sites, newly formed bone was present only in the most peripheral part of the defect. Moreover, BG showed high biocompatibility, and only in a few areas were some multinucleated cells found.[3,8] A tight contact between bone and BG particles was observed, with no gap at the interface or the interposition of fibrous tissue.[1] In many fields, osteoblasts were observed secreting osteoid matrix directly on the biomaterial surface. The fact that, in our specimens, no dissolution of the central part of the granules was found could be explained by the shorter time (60 days vs 180 days).[20] Present, however, were the microfractures in the external portion of the granules, and some of the BG granules had completely disappeared.[12,16] Subsequently, the particles underwent an external surface dissolution and were gradually replaced by new bone.[16]

ACKNOWLEDGMENTS

This work was partially supported by the National Research Council (C.N.R.), Rome, Italy, Finalized Project "Materials Tailored for Advanced Technologies" PF MSTA II, and by the Ministry of University, Research, Science and Technology (M.U.R.S.T.), Rome, Italy.

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