Investigation of Early Bone Formation Using Resorbable Bioactive Glass in the Rat Mandible

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Recent advances in biomaterial technology have made alloplastic bone substitutes more predictable when used with the proper clinical methodology in carefully selected patients. In this animal study, early bone formation using a novel resorbable bioactive glass in the repair of surgically created bony defects in the rat mandible was investigated. Biopsies taken from the implanted sites after 1, 2, 3, 4, 8, and 16 weeks were examined histologically by means of standard cell-staining techniques. In addition, an electron probe microanalyzer was used to determine the presence and distribution of specific elements in samples taken after 16 weeks. Results indicated the early stage of osteoconductive bone growth after approximately 4 weeks. After 16 weeks, electron probe microanalyzer scans indicated the formation of a calcium-phosphate shell formed in situ and the resorption of silica to background levels.
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Key words: localized osseous defects, osteostimulation, resorbable bioactive glass

Although autogenous bone grafts remain the most predictable and proven method for the repair and augmentation of oral bony defects or deficiencies, recent advances in biomaterials have improved the predictability of synthetic bone substitutes.[1] Hydroxyapatite, ß-tricalcium phosphate, and glass ceramics have been reported to bond directly with bony tissue.[2–5] Unlike autogenous bone grafts, alloplasts reduce the burden on patients, since bone tissue does not require harvesting from extraoral sites. This animal study investigated the osteoconductive property and efficacy of a novel resorbable bioactive amorphous glass (Biogran, Orthovita, Malvern, PA) in repairing surgically created bony defects in the rat mandible.

Materials and Methods

The material used in this study was Biogran, a resorbable bioactive glass that has a nominal composition of 45% silica oxide, 24.5% calcium oxide, 24.5% sodium oxide, and 6% phosphorus oxide (percentage by weight), and a particulate size range of 300 to 355 µm. Bony defects measuring 2.0 mm in diameter and 1 to 1.5 mm in depth were surgically created with a bur in the posterior mandibular region (masseter) of 18 adult Wistar rats (8 weeks old, average weight of 200 g). The defects were irrigated with saline solution, and bioactive glass moistened with saline solution was grafted into the prepared sites. The sites were covered with a resorbable membrane (Biomend, Colla Tec, Plainsboro, NJ, or GC membrane, GC Corp, Tokyo, Japan) before closure to prevent epithelial downgrowth and to create space for guided bone regeneration. The rats were divided into six groups of three and sacrificed at intervals of 1, 2, 3, 4, 8, and 16 weeks after grafting. Biopsies taken

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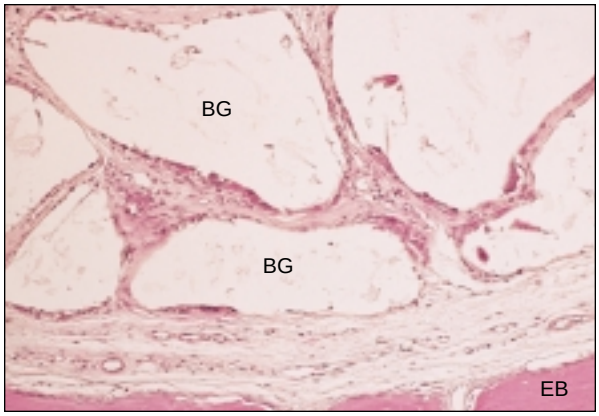

Fig 1 Erosion of the granules 1 week after implantation (BG = Biogran granule; EB = existing bone). (Hematoxylin-eosin stain; original magnification × 100.)

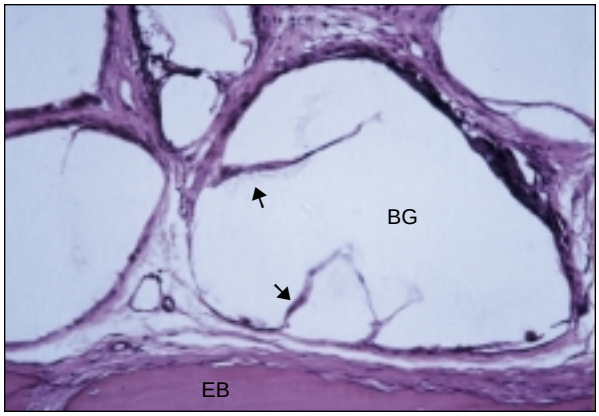

Fig 2 After 2 weeks, fissures *(arrow)* can be observed in some particles. Existing bone (EB) tissue can be seen at the bottom of the micrograph (hematoxylin-eosin stain, original magnification × 200).

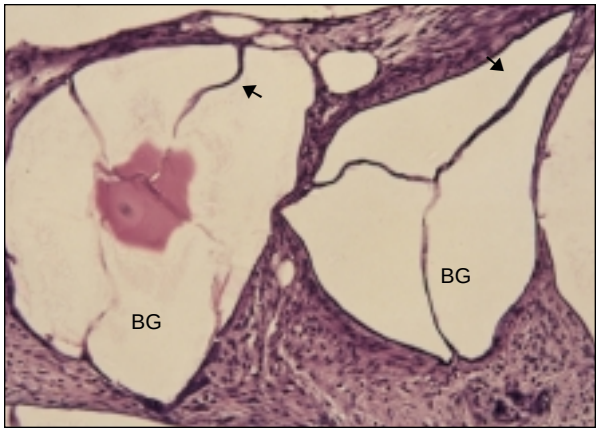

Fig 3 After 3 weeks, the fissures have penetrated to the core of the Biogran granules. Osteoprogenitor cell infiltration into these fissures *(arrow)* can be observed (hematoxylin-eosin stain, original magnification × 200).

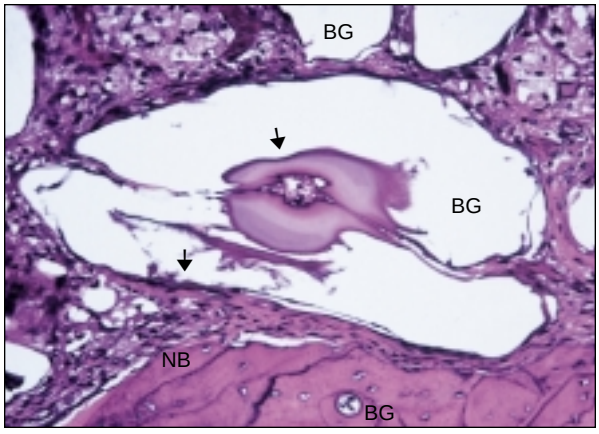

Fig 4 After 4 weeks, the core of the granule has been excavated, and osteoid formation can be seen *(arrows)* (NB = new bone). (Hematoxylin-eosin stain; original magnification × 200.)

from each of the groups were fixed with 10% formalin, decalcified, and stained with hematoxylin-eosin for histologic analysis. Additional samples taken from the 16-week group were molded with epoxy resin, polished, and analyzed for element composition and distribution using an electron probe microanalyzer (EPMA) (Shimadzu EPMA-8705L, Shimadzu Corporation, Kyoto, Japan).

Results

Examination of biopsies taken from the grafted site after 1 week showed erosion of the sharp edges of the resorbable glass granules. This initial transformation has been reported to be the result of an ionic exchange dissolution process that transforms the amorphous crystalline structure of the granules into a silica-rich gel.[6,7] Osteoprogenitor cells permeating the intergranular spaces were also observed (Fig 1). Further erosion of the granules and the appearance of fissures, believed to be associated with mechanical shrinkage, were observed after 2 weeks. Infiltration of osteoprogenitor cells into these fissures was confirmed (Fig 2). After 3 weeks, the fissures had completely penetrated to the cores of many of the granules. Progressive osteoprogenitor cell invasion into these intragranular spaces was observed (Fig 3). Excavation of the granule core by phagocytosis and early osteoid formation within the excavated chambers, as well as on the perimeter of the granules, were observed after 4 weeks (Fig 4). At 8 weeks after grafting, further phagocytic excavation of the gran-

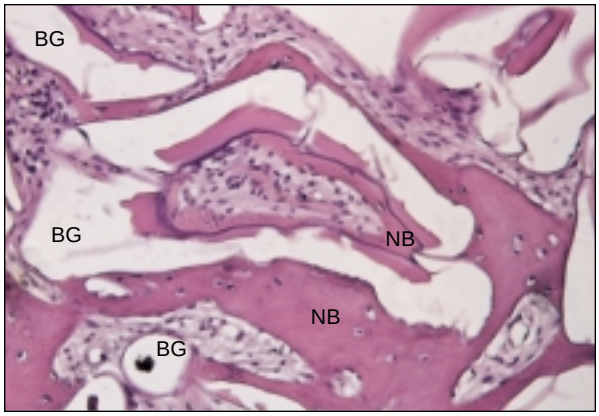

Fig 5 After 8 weeks, further calcification of the excavated core and new bone tissue deposition on the outer surface can be observed (hematoxylin-eosin stain, original magnification × 200).

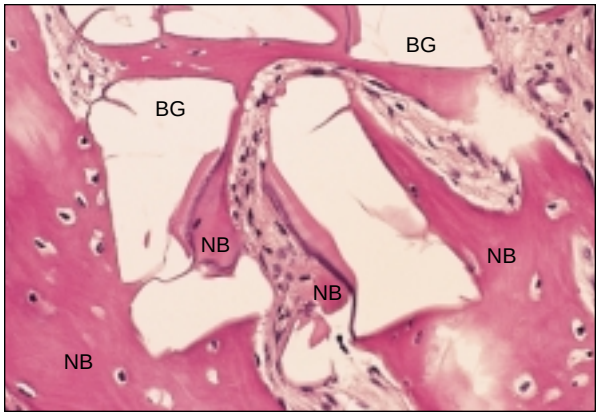

Fig 6 After 16 weeks, further bone growth can be observed inside the granules and also extending well into the surrounding intergranular spaces (hematoxylin-eosin stain, original magnification × 200).

ules with osteoprogenitor cell invasion into these spaces was observed. Staining revealed the formation of new bone tissue within the excavated chambers. New bone tissue encapsulating the resorbable bioactive glass granule was clearly evident. The presence of osteocytes within the new bone tissue was also confirmed (Fig 5). By 16 weeks after implantation, bone tissue encapsulating many of the granules was evident. The occurrence of further osteoid deposition and new bone growth was observed inside the excavated chambers (Fig 6). The results of EPMA scans on biopsies taken after 16 weeks confirmed the presence of a calcium-phosphate shell formed in situ. Silica was reduced to near background levels, confirming the completion of the primary resorptive phase (Figs 7 to 10).

Discussion

The consistent bioactivity of Biogran is largely the result of the uniformity of granules within a narrow size range (300 to 355 µm). It has been shown that when particle size is less than 200 µm, resorption occurs too rapidly and may cause inflammation.[6,7] Conversely, when particle size is greater than 400 µm, particles remain unreacted and are not resorbed, thus impeding the formation of new bone tissue throughout the particle-bone matrix.[6,7] The critical size range of 300 to 355 µm is thus a very important biologic factor.[6–8]

When Biogran is implanted into the body, the granules are transformed by an ion exchange process that instigates network dissolution.[6,7] Initially, a silica-rich gel layer is formed, upon which an in situ calcium phosphate layer is gradually precipitated, until the entire granule has reacted.[6,7] After approximately 2 weeks in the body, fissures appear in particles, which then enable phagocytic action to excavate the silica-gel core. It has been postulated that osteoprogenitor cells then enter these cracks and differentiate into osteoblasts within the protected space of the excavated particles.[6–9] Finally, although the silica gel in the core is eventually resorbed, the outer biologic calcium phosphate layer formed in situ remains, thereby acting as a protective shell to encourage further formation of bone tissue. Biogran's unique osteoconductive property has been termed "osteostimulatory" by Schepers et al.[6,7]

Histologic examination of resorbable bioactive glass grafted into surgically created bony defects of the rat mandible confirmed the appearance of numerous fissures after 2 weeks. Osteoprogenitor cells were also observed to be invading the fissures after 3 weeks. At the early stage, particles closest to the defect wall showed osteoconductive bone formation initiating on the surface of the granules and then spreading to the surrounding matrix. For those granules located away from the defect wall (ie, sections taken from the central portion of the biopsy), new bone tissue formation seemed to initiate from the core of the granules.

The biologic mechanism by which Biogran initiates new bone formation is not fully understood at present. However, a previous in vitro study by the authors suggests that Biogran maintains a weak alkaline condition (pH 7.8 to 8.0), which promotes the alkaline phosphatase activity of periodontal ligament cells and osteoblasts.[10] In this previous study, the suppression of peripheral blood-derived osteoclasts was also observed.[10]

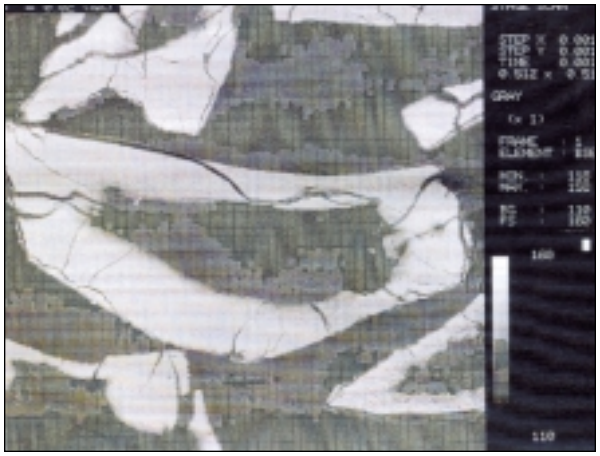

Fig 7 An electron probe microanalyzer (EPMA) base image of the Biogran sample taken at 16 weeks.

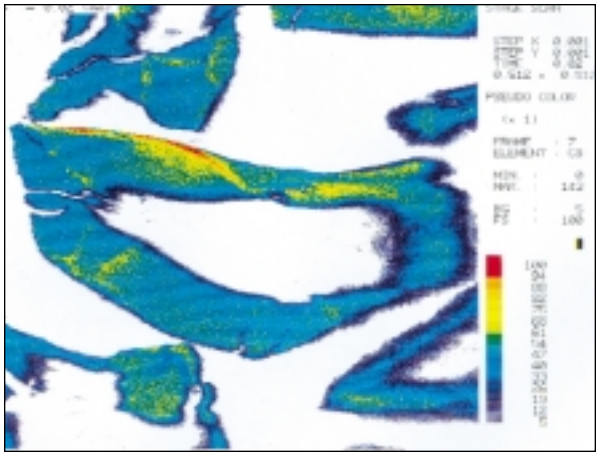

Fig 8 EPMA image of the sample at 16 weeks showing the presence of calcium.

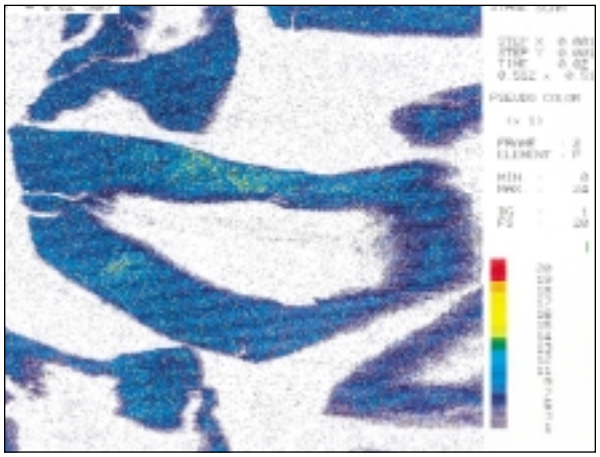

Fig 9 EPMA image of the sample at 16 weeks showing the presence of phosphorous.

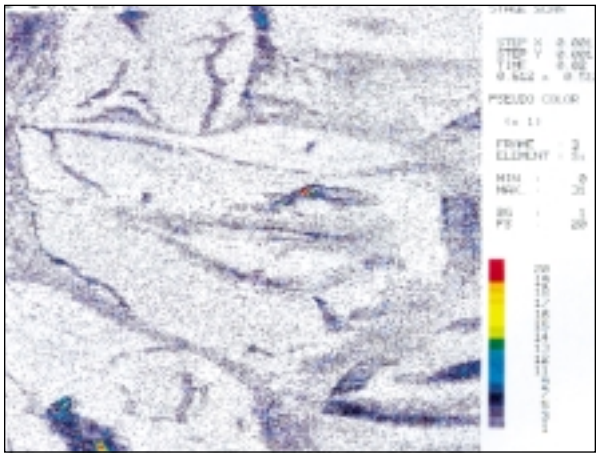

Fig 10 EPMA image of the sample at 16 weeks showing the presence of silicon. Most of the silica has been resorbed and reduced to background signal levels.

Since resorbable bioactive glass is not derived from human or animal sources, the potential transmission of viral or donor-related diseases is not a factor to be considered in its use. Other clinical benefits include elimination of the need for a patient donor site (ie, autogenous bone grafts), decrease in surgery time with accompanying reductions in anesthesia and hemorrhage, and ready availability. Another advantage of resorbable bioactive glass is the formation of a calcium-phosphate shell formed in situ, which serves to maintain the overall volume of the particle-bone matrix. This property is extremely advantageous as a bone filler and augmentation material where predictable modification of the defect or deficiency is required. In addition, histologic observations could not identify the presence of inflammatory cells, suggesting that the material has no cytotoxic effects.

Conclusion

This animal study confirmed the osteoconductive properties of resorbable bioactive glass and showed that early bone formation had begun approximately 4 weeks after implantation. Resorption of silica and formation of a calcium-phosphate shell deposited in situ were confirmed by EPMA.

This study was conducted in accordance with ethical and humane principles of animal research as approved by the Animal Experiment Committee of the Kanagawa Dental College, Yokosuka, Japan.

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References

1. Misch CE, Dietsh F. Bone-grafting materials in implant dentistry. Implant Dent 1993;2:158–167.
2. Hench LL, Splinter RJ, Allen WC. Bonding mechanisms at the interface of ceramic prosthetic materials. J Biomed Mater Res 1971;2(Part I):117–141.
3. Nery EB, Lynch KL, Hirthe WM, Mueller KH. Bioceramic implant in surgically produced infrabony defects. J Periodontol 1975;46:328–347.
4. Nery EB, Lynch KL. Preliminary clinical studies of bioceramic in periodontal osseous defects. J Periodontol 1978;49:523–527.
5. Baldock WT, Hutchens LH Jr, McFall WT Jr, Simpson DM. An evaluation of tricalcium phosphate implants in human periodontal osseous defects of two patients. J Periodontol 1985;56:1–7.
6. Schepers EJG, DeClercq M, Ducheyne P, Kempeneers R. Bioactive glass particulate as a filler for bone lesions. J Oral Rehabil 1991;18:439–452.
7. Schepers EJG, Ducheyne P, Barbier L, Scheoers S. Bioactive glass particles of narrow size range: A new material for the repair of bone defect. Implant Dent 1993;2:151–156.
8. Gatti AM, Ducheyne P, Piattelli A, Schepers E, Trisi P, Chiarini L, et al. Glass corrosion layers on bioactive glass granules of uniform size affect cellular function. Bioceramics 1993;6:396–400.
9. Furusawa T, Mizunuma K. Osteoconductive properties and efficacy of resorbable bioactive glass as a bone grafting material. Implant Dent 1997;6:93–101.
10. Shimizu Y, Mizunuma K, Yamashita S, Furusawa T. Effect of bioactive glass, hydroxylapatite and demineralized freeze dried bone in bone remodeling. J Jpn Soc Biomaterials 1997;15(3):103–112.