EFFECT OF FREEZE-DRIED BONE AND A DEXTRAN AGGLUTINANT ON THE HEALING OF DEFECTS IN THE RAT MANDIBLE

João Gualberto C. Luz, DDS, MS, DSc
Josué L. Santiago, DDS, MS, DDSc
Rogério S. Jorge, DDS
Vera C. de Araújo, DDS, PhD

The effects of inorganic, freeze-dried bone, alone or mixed with a dextran agglutinant, on the repair of experimentally created critical-size bone defects were evaluated in the rat mandible. Histological data demonstrated a progressive resorption of the freeze-dried bone particles, without osseous proliferation into the defect, and a posterior foreign-body reaction. These results indicate that the freeze-dried bone did not induce bony healing in the critical-size defect and that the addition of dextran agglutinant did not change the tissue response. Dextran may have other applications as a biocompatible, resorbable agglutinant.

KEY WORDS

Critical size mandibular defect
Freeze-dried bone
Dextran agglutinant

João Gualberto C. Luz, DDS, MS, DSc, *is associate professor at the Department of Oral and Maxillofacial Surgery, School of Dentistry, University of São Paulo. Correspondence should be addressed to Dr Luz at Rua Duarte de Azevedo 284, s.22, 02036-021 São Paulo SP, Brazil.* ***Josué L. Santiago, DDS, MS, DDSc,*** *is professor in the Department of Oral and Maxillofacial Surgery, School of Dentistry, University of São Paulo.* ***Rogério S. Jorge, DDS,*** *is a research fellow in the Department of Oral and Maxillofacial Surgery, School of Dentistry, University of São Paulo.* ***Vera C. de Araújo, DDS, PhD,*** *is professor and head of the Department of Oral Pathology, School of Dentistry, University of São Paulo.*

INTRODUCTION

A bone graft or a bone substitute is required for the repair of deformities following trauma or surgical excision. Autogenous bone grafts are a good choice, but they are associated with donor site morbidity, sparse amounts, resorption, and increased possibility of infection.[1,2] Freeze-dried bone (FDB) of bovine origin is a widely used xenograft. However, results of experimental and clinical studies involving FDB have long been a matter of controversy. It is likely that the use of a predetermined experimental model influences these results. Critical-size defect models can lead to reliable results. (The term ''critical-size'' refers to the smallest size of an intraosseous wound that will not heal spontaneously during the lifetime of the individual.[3,4])

Agglutinants have been used to improve surgical procedures when particulate or pulverized hydroxyapatite, tricalcium phosphate, or FDB are used.[5,6] Collagen is the most frequently used agglutinant, and preparations usually are solutions, gels, and pastes that are mixed with the implant material or graft.[6,7]

Dextran (DX) is a large polymer of glucose and has medical use as a plasma volume–expanding agent. It is biodegradable and is indicated in the treatment of shock and for acute posthemorrhagic anemia.[8] Recently, a DX agglutinant has become commercially available. No studies, however, have examined the effect of a DX agglutinant on the healing of critical-size bony defects in experimental animals.

The objective of the present study was to evaluate the effect of FDB, alone

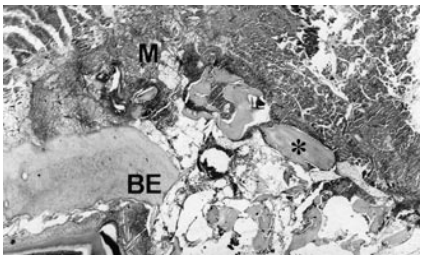

FIGURE 1. Area of implantation of freeze-dried bone particles (*) into the bony defect 24 hours after surgery. Acute inflammatory cells are also seen along the muscular fibers (M). BE indicates bone edge (H&E, magnification ×25).

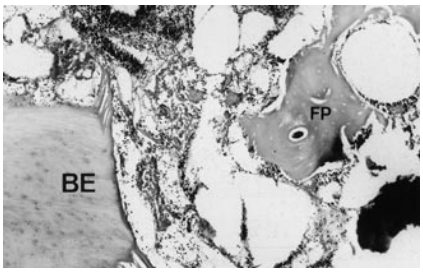

FIGURE 2. Freeze-dried bone particles (FP) and acute and serofibrinous exudates 24 hours after surgery. Note the nonvital appearance of FP. Bone edge (BE) exhibiting viable bone (H&E, magnification ×100).

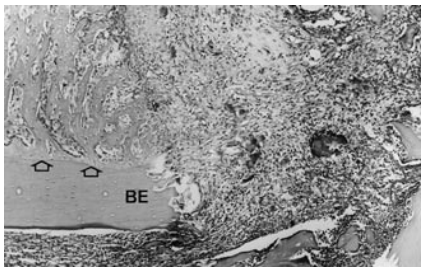

FIGURE 3. Extensive mononuclear inflammatory infiltrate is seen into the implantation area 1 week after surgery. Devitalized bone edge (BE) with osseous proliferation (arrows) presenting immature trabeculae (H&E, magnification ×100).

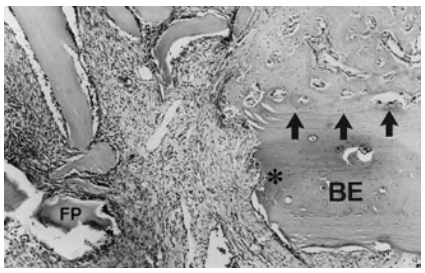

FIGURE 4. Active resorption of the freeze-dried bone particles (FP), surrounded by mononuclear inflammatory infiltrate, 2 weeks after surgery. Bone edge (BE) with signs of resorption (*) and osteoblastic activity (arrows) (H&E, magnification ×100).

or mixed with a dextran agglutinant, on the repair of critical-size defects in the rat mandible.

MATERIALS AND METHODS

The study animals were 15 adult *Rattus norvegicus albinus* rats with a mean body weight of 275 g. They were subjected to surgery under general anesthesia. Anesthesia was induced by an intraperitoneal injection of 100 mg/kg ketamine (Ketalar, Parke-Davis) after a peritoneal injection of 0.8 mg/kg xylazine hydrochloridine (Rompum, Bayer). The study was approved by the local committee on research ethics.

Submandibular and cervical areas were shaved and cleansed with povidone-iodine solution. Bilateral 10-mm submandibular incisions were made, and the mandibular angle and ramus were accessed by blunt dissection. A bicortical defect was prepared using a 3-mm trephine under generous irrigation with saline. The right-side defect was filled with particulated inorganic FDB (Osteopur, Homus, SP, Brazil); the particles were ranged from 450 to 850 μm in diameter and moistened with saline solution. The left-side defect was filled with identical particulate FDB prepared with a DX agglutinant (Aglutinante Dentoflex, Odontec, SP, Brazil). The procedure was concluded with nylon sutures (Mononylon 5-0, Ethicon). Postoperatively, the animals received a single intraperitoneal injection of 16,000 IU of benzatin benzylpenicillin (Benzetacyl, Fontoura-Wyeth). All animals were fed an ordinary diet of rodent feed and water *ad libitum.*

The animals were divided into five groups, with three rats in each. The rats were killed after 24 hours, 1 week, 2 weeks, 1 month, and 3 months. They were then decapitated and the right and left hemimandibles were removed from all the animals and fixed in 10% buffered formalin. These specimens were decalcified in 20% formic acid, and coronal and sagittal sections were obtained from all specimens. Seven-micrometer-thick semiserial sections were cut and stained with hematoxylin and eosin.

RESULTS

After the surgical procedure, the animals ate without difficulty and continued to gain weight. Histopathological findings are described according to the postoperative period. The DX agglutinant was not detected in histopathological analyses. No differences were observed between right and left sides, so the description is the same for both implantation sites.

Twenty-four hours after the operation, the bony defect was filled with FDB particles, represented by devitalized bone, lying in serofibrinous and hemorrhagic exudates. Margins of the defect presented viable bone. Numerous polymorphonucleated cells were present, particularly surrounding the FDB particles. There was evidence of acute inflammation along adjacent muscle fibers, as well as congested blood vessels (Figs 1, 2).

One week after the operation, FDB particles presented signs of resorption, as well as mononuclear inflammatory and macrophagic infiltrates, some multinucleated giant cells, and rests of fibrin. Margins of the defect were devitalized bone; there was significant newly formed bone from external corticals but no proliferation of bone into the defect. A mild inflammatory infiltrate was present along the muscular tissue (Fig. 3).

At 2 weeks, FDB particles exhibited active resorption and were surrounded by mononuclear inflammatory infiltrate, while the remaining area was filled with granulation tissue. Multinucleated giant cells were numerous, and neutrophils were rare. Margins of the defect presented signs of resorption, and the external, newly formed bone showed osteoblastic activity (Fig 4).

At 1 month, FDB particles continued to be resorbed and sometimes present-

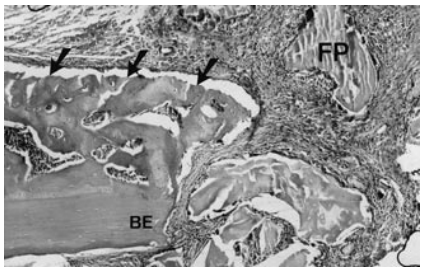

FIGURE 5. Inflammatory infiltrate is restricted to the freeze-dried bone particles (FP) 1 month after surgery. Newly formed bone on the bone edge (BE) with signs of remodeling (arrows) (H&E, magnification ×100).

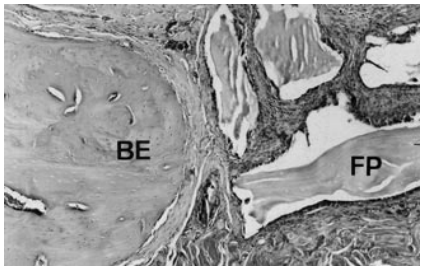

FIGURE 6. Foreign body reaction delimiting the freeze-dried bone particles (FP) 3 months after surgery. Evidence of complete remodeling of the bone edge (BE) with no bony proliferation into the defect (H&E, magnification ×100).

ed perforations. The inflammatory infiltrate was restricted to the area in which the particles had been inserted, with frequent multinucleated giant cells. The granulation tissue had been replaced by connective tissue. There were signs of remodeling of the margins of the defect, with thinning of the newly formed bone (Fig 5).

At 3 months, an inflammatory process, represented by a foreign body reaction, delimited the FDB particles, which presented signs of resorption. The number of multinucleated giant cells had diminished, while the connective tissue had matured. Margins of the defect showed remnants of newly formed bone, delimited by basophilic lines (Fig 6).

DISCUSSION

The present study has shown that healing of critical-size defects in the rat mandible with FDB and DX agglutinant occurred without osseous proliferation into the defect. A progressive resorption of the FDB particles and a late foreign-body reaction were observed. These findings indicate that FDB particles were not associated with osteoinduction or osteoconduction. Such properties have been observed when bovine bone is implanted into cavities; in general, these cases present three walls, with the implantation surrounded by the new bone tissue.[9,10]

Our results are related to the use of the critical-size defect model.[1,4,11,12] Healing of experimentally created defects is influenced by anatomical location, cortical involvement, and the presence of periosteum, dura, or both.[1] Critical-size calvarial defects have presented normal bone remodeling following bovine bone implantation.[13] Cartilage was formed when bovine bone was implanted with autogenous perichondrium of growing cricoid.[14] When local agents are evaluated using experimentally created defects, a comparison with normal biologic processes is possible. Experimentally created bone defects allow the study of the reparative process without the effect of loading mechanical forces.[4] Thus, the use of the mandible instead of long bones permits the evaluation of the response to related to clinical applications of the implanted material.[4,11]

Histological sections initially demonstrated an acute inflammatory process and serofibrinous and hemorrhagic exudates around the FDB particles as the local response to the implantation and to the bony injury.[15,16] Further steps in the process included resorption of the FDB particles, which were first surrounded by chronic inflammatory infiltrate and multinucleated giant cells while the remaining area of the defect was filled by granulation tissue, which was posteriorly replaced by connective tissue. The final result was a foreign-body reaction to the FDB particles. These results indicated that FDB was not integrated into the mandible. Similar results have been reported when bovine bone is implanted subperiosteally into sockets.[2,17,18] On the other hand, FDB particles may be used to keep a defect filled, avoiding soft tissue invagination.

The possibility of initiating immunological response is considered a disadvantage of the use of xenogenous bone grafts.[1] However, it has been demonstrated that while there is no systemic or local immune response to inorganic bovine bone, a transient macrophage infiltrate occurs.[19] Scintigraphic evaluation has revealed no allergic responses to implanted FDB in alveolar extraction cavities.[20] The use of both frontal and sagittal slices in our study leads to correct interpretation of the response to the implantation, avoiding false-positive results.

Addition of DX agglutinant did not change the results. This finding confirmed the biocompatibility of this material.[8] The ability of some polyglycans to enhance biological responses has been experimentally demonstrated. Their use, particularly in glucan therapy, can stimulate recovery of hemopoietic injury in irradiated mice.[21,22] No special properties, however, have been described with the use of DX. With respect to future trends in the treatment of bone defects, DX agglutinant may be useful as a delivery system when bone growth factors will be commercially available.[1]

REFERENCES

1. Aaboe M, Pinholt EM, Hjorting-Hansen E. Healing of experimentally created defects: a review. *Br J Oral Maxillofac Surg*. 1995;33:312–318.

2. Merkx MAW, Maltha JC, van't Hoff M, Kuijpers-Jagtman AM, Freihofer HPM. Tooth eruption through autogenous and xenogenous bone transplants: a histological and radiographic evaluation in beagle dogs. *J Craniomaxillofac Surg*. 1997;25:212–219.

3. Hollinger JO, Kleinschmidt JC. The critical size defect as an experimental model to test bone repair materials. *J Craniofac Surg*. 1990;1:60–68.

4. Yamamoto MK, Luz JGC, Aradjo VC. Bone tissue response to granular hydroxyapatite implanted in experi-

mental defects in the rat mandible. *Rev Odontol Univ São Paulo.* 1994;8:281–286.

5. Bell R, Beirne OR. Effect of hydroxylapatite, tricalcium phosphate, and collagen on the healing of defects in the rat mandible. *J Oral Maxillofac Surg*. 1998;46:589–594.

6. Pettis GY, Kaban LB, Glowacki J. Tissue response to composite ceramic hydroxylapatite/demineralized bone implants. *J Oral Maxillofac Surg*. 1990; 48:1068–1074.

7. Tal H, Pitaru S, Moses O, Kozlovsky A. Collagen gel and membrane in guided tissue regeneration in periodontal fenestration defects in dogs. *J Clin Periodontol.* 1996;23:1–6.

8. Wyngaarden JB, Smith LH Jr, Bennett JC. *Cecil textbook of medicine,* 19th ed. Philadelphia, Pa: WB Saunders Co; 1992.

9. Guizzardi S, Di Silvestre M, Scandroglio R, Ruggeri A, Savini R. Implants of heterologous demineralized bone matrix for induction of posterior spinal fusion in rats. *Spine.* 1992;17: 701–707.

10. Wetzel AC, Stich H, Caffesse RG. Bone apposition onto oral implants in the sinus area filled with different grafting materials. *Clin Oral Implant Res.* 1995;6:155–163.

11. Kaban LB, Glowacki J. Induced osteogenesis in the repair of experimental mandibular defects in rats. *J Dent Res.* 1981;60:1356–1364.

12. Gongloff RK, Whitlow W, Montgomery CK. Use of collagen tubes for implantation of hydroxylapatite: an experimental study. *J Oral Maxillofac Surg*. 1985;43:570–573.

13. Thaller SR, Hoyt J, Dart A, Borjeson K, Tesluk H. Repair of experimental calvarial defects with Bio-Oss particles and collagen sponges in a rabbit model. *J Craniofac Surg*. 1994;5: 242–246.

14. Bean JK, Verwoerd-Verhoef HL, Meeuwis J, Verwoerd CDA. Reconstruction of the growing cricoid with a composite graft of demineralized bovine bone and autogenous perichondrium; a comparative study in rabbits. *Int J Pediatr Otorhinolaryngol.* 1993;25: 163–172.

15. Cormack D H. *Ham's histology,* 9th ed. Philadelphia, Pa: JB Lippincott; 1987.

16. Luz JGC, Jaeger RG, Araújo VC, Rezende JRV. The effect of indirect trauma on the rat temporomandibular joint. *Int J Oral Maxillofac Surg*. 1991;20: 48–52.

17. Pinholt EM, Bang G, Haanaes HR. Alveolar ridge augmentation in rats by Bio-Oss. *Scand J Dent Res.* 1991; 99:154–161.

18. Pinholt EM, Haanaes HR, Roervik M, Donath K, Bang G. Alveolar ridge augmentation by osteoinductive materials in goats. *Scand J Dent Res.* 1992;100:361–365.

19. Cohen RE, Mullarky RH, Noble B, Comeau RL, Neiders ME. Phenotypic characterization of mononuclear cells following anorganic bovine bone implantation in rats. *J Periodontol.* 1994;65: 1008–1015.

20. Gülaldi NCM, Shahlafar J, Makhsoosi M, Caner B, Araz K, Erbengi G. Scintigraphic evaluation of healing response after heterograft usage for alveolar extraction cavity. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998;85:520–525.

21. Patchen ML, DiLuzio NR, Jacques P, MacVittie TJ. Soluble polyglycans enhance recovery from Cobalt-60 induced hemopoietic injury. *J Biol Response Modif.* 1984;3:627–633.

22. Patchen ML, Brook I, Elliot TB, Jackson WE. Adverse effects of pefloxacin in irradiated C3H/HeN mice: correction with glucan therapy. *Antimicrob Agents Chemother.* 1993;37:1882–1889. ■