Bone Graft Substitutes: A Comparative Qualitative Histologic Review of Current Osteoconductive Grafting Materials

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This paper investigated the osteogenic potential of 6 osteoconductive grafting materials derived from human, bovine, and synthetic sources: HTR, BOP, Biogran, Laddec, Dembone, and Osteograf. Twenty-eight New Zealand rabbits were used in this study. The active group consisted of 24 animals and the control group consisted of 4 animals. The median condyle of each tibia was drilled with a 5-mm-diameter bur to form 8-mm-deep cavities. A control group included 8 osseous cavities, with 1 hole in each tibia. These cavities were washed and left unfilled. In the active group, each grafting material filled 8 osseous cavities in 8 tibiae of different animals. Half of the active and control osseous cavities were investigated with decalcified hematoxylin and eosin-stained sections. The other half were studied with scanning electron microscopy. It was concluded that Laddec bovine bone granules possessed the best potential for an osteoconductive grafting material, followed by the bioglass crystals of Biogran and the hydroxyapatite particles of Osteograf, respectively. The least potential for rapid bone formation was demonstrated by the copolymers of HTR and BOP, and Dembone allograft bone particles did not reveal active bone healing. (Int J Oral Maxillofac Implants 2001;16:105-114)

Key words: biocompatible osteoconductive polymers, bioglass, bone substitutes, histology, hydroxyapatite

Finding an ideal bone substitute material for grafting has been the goal of researchers for many years, with varying degrees of success. Several bone substitutes have been popularized during the past 10 years and used often by clinicians who deal with ridge augmentation and the reconstruction of osseous defects. These materials may be considered osteoconductive. In osteoconduction, the implanted material usually serves as a scaffold for the ingrowth of capillaries, perivascular tissue, and osteoprogenitor cells from the recipient bed. This process generally requires at least 3 walls of surrounding host bone to provide stimulation of bone ingrowth. The autogenous bone graft is the most predictable material that possesses both osteoconductive and osteoinductive properties; it stimulates non-differentiated mesenchymal cells to form bone cells and also serves as a scaffold for new bone ingrowth. However, in an attempt to avoid separate surgical procedures involving remote donor sites and reduce postsurgical pain, patient inconvenience, operating time, and cost, clinicians have increased their use of alternative grafting materials. These are derived from human, bovine, and synthetic sources.

The purpose of this study was to investigate the osteogenic potential of 6 osteoconductive grafting materials: HTR, BOP, Biogran, Laddec, Dembone, and Osteograf.

HTR (for “hard tissue replacement”; Bioplant, Norwalk, CT) is a synthetic, porous, bead-shaped, non-resorbable copolymer composed of polymethyl methacrylate resin (PMMA) sintered with polyhydroxyethyl methacrylate (PHEMA); a third layer composed of calcium hydroxide comprises its outer coating. Barium sulfate is also applied to HTR beads in very minute quantities to provide radiopacity.

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BOP (“biocompatible osteoconductive polymer”; Diversified Tech International SA, Brussels, Belgium) is another synthetic grafting material. It is a resorbable biopolymer composed of 100% copolymer methyl methacrylate (MMA) resin, 1-vinyl-2-pyrrolidone (NVP). BOP is supplied as a powder in crystal form, ranging in size between 30 and 100 µm.4 Biogran (Orthovita, Implant Innovations, Palm Beach Gardens, FL) is a third type of synthetic bone grafting material. It consists of resorbable bioactive glass granules that are approximately 300 µm in diameter. This bioceramic material comprises silicon, calcium, sodium, and phosphorus.5 Laddec (Transphyto SA, Clermont-Ferrand, France) is a resorbable natural hydroxyapatite (HA) derived from a bovine source. It consists of 600-µm mineralized cancellous bone granules and a trabecular bone matrix taken from the femoral condyle of calves under 6 months of age. It is a non-antigenic proteinized porous bone grafting material. While all raw materials and organic components are removed during processing, the treatment of this bone allows the cancellous bone matrix, essentially made of mineralized Type I collagen, to be isolated and purified without degradation while retaining the trabecular bone microstructure.6 Dembone (Pacific Coast Tissue Bank, Los Angeles, CA) is another form of resorbable natural HA grafting material but is derived from a human source. It consists of 500-µm freeze-dried demineralized ashed bone granules.7 Osteograf LD (CeraMed, Lakewood, CO) is resorbable HA prepared from a synthetic source. It is a pure, porous, non-ceramic HA manufactured to between 250 and 420 µm in size. Its chemical formula is Ca₁₀(P₁₀O₄)(OH)₂, and it contains no alpha- or betatricalcium phosphates, as are found in ceramic HAs.8

MATERIALS AND METHODS

Twenty-eight New Zealand rabbits, each weighing 3.5 kg (± 0.25 kg), were used in this experiment and were divided into an active group of 24 animals and a control group of 4 animals. Animals were fed a standard rabbit diet and maintained in separate cages at the animal research facility of King Saud University.

Surgical Procedure

Animals were anesthetized preoperatively with an intramuscular injection of ketamine hydrochloride 10 mg/kg (TEKAM, Al Hikam Pharmaceuticals, Amman, Jordan) and xylazine 0.15 mg/kg (Seton 2%, Laboratorios Calier SA, Barcelona, Spain). In addition, 1.0 mL of local anesthetic (2% lidocaine/epinephrine 1:80,000, Astra Pharmaceuticals, Wilmington, DE) was injected at the surgical sites prior to surgery. Postoperatively, all animals were given an intramuscular injection of long-acting antibiotics (0.2 mL/kg, 12,500,000 IU benzyl penicillin benzathine, and 5 g streptomycin per 100 mL; Duphapan Strep BP, Sovay, Italy) and an analgesic dose of Analgin (0.5 mL Pharmalgin, Arab Drug, Cairo, Egypt).

The tibiae on both sides were selected for the surgical procedure. The surgical field was shaved and washed with 0.2% chlorhexidine gluconate. A skin incision and subperiosteal dissection were carried out bilaterally. In the control group, the medial condyle of each tibia was drilled with a 5-mm-diameter round bur and copious saline irrigation to create an 8-mm-deep cavity. The cavities were washed with sterile saline and left unfilled. The periosteum and skin were closed in layers with resorbable 4.0 sutures (Vicryl, Ethicon, Somerville, NJ). In the active group, the medial tibial condyle of each side was drilled in the same manner. Each cavity was filled with one of the investigated materials: Biogran, Laddec, HTR, BOP, Dembone, or Osteograf. Both tibiae in each of the 24 active animals were used. Each of the 6 graft materials filled 8 bone cavities in different animals, resulting in a total of 48 active osseous cavities; the control group comprised 8 unfilled osseous cavities drilled in the tibiae of the 4 control animals.

The observation period for all animals was 8 weeks. Observation for proper wound healing occurred daily for the first postoperative week and once a week thereafter. After completion of the observation period, animals were sacrificed, and both tibial heads of each animal were dissected and sectioned en bloc. Each block was labeled and placed in a 10% neutral buffered formalin solution. Half of the grafted osseous defects with each material and control cavities were investigated using decalcified section histology; the other half were investigated with scanning electron microscopy (SEM).

Decalcified Sections

Specimens were fixed in a formalin solution for 24 hours and then placed in Cal-Ex solution (Fisher Diagnostics, Springfield, NJ) until they were fully decalcified. They were rinsed in running tap water for 5 hours to remove excess acid from the tissue and dehydrated in increasing percentages of ethyl alcohol (70% to 90%) in an automated processor. Chloroform was used for 1 hour to clear the specimens, which were then impregnated in melted paraffin wax.
Samples were then embedded in paraffin wax and sectioned in 5-µm slices in a rotatory microtome. Sections were mounted on acrylic glass slides using photopolymerizing glue. The final sections were stained with hematoxylin and eosin (H&E). The stained sections were washed with water and finally dried. A precision adhesive press affixed plain parallel acrylic glass slides with photopolymerizing glue onto the stained sections.

**Scanning Electron Microscopy Sections**

Samples were fixed with 10% glutaraldehyde for 24 hours, dehydrated with graded ethyl alcohol from 50% to 95%, and then dehydrated in absolute alcohol twice, with each stage lasting 30 minutes. Critical point drying was achieved via a Sandri PVT-3B dryer (Tousimis Research, Rochester, NY). Specimens were mounted with carbon Dotite paint. After mounting, samples were gold-coated by means of fine coat on sputter JFC-1100 (Jeol Limited, Nakagami, Akishima, Tokyo, Japan). Samples were observed and photographed through a JSMT-330 scanning electron microscope (JEOL Limited; camera: Mamiya, Tokyo, Japan).

**Histometric Analysis**

All sections were evaluated by the same examiner using the Image Analysis System (IAS). This technique was used to measure quantitative percentages of newly deposited bone; soft tissue (loose connective tissue, blood vessels, open pores, and empty spaces); and graft remnants. The system used for IAS consisted of: (1) a light optic microscope (Polyvar, Reichert-Jung, Vienna, Austria); (2) an image analysis device (Leica, Cambridge, United Kingdom); (3) a videocamera (Donphisha, Sony, Tokyo, Japan); and (4) a computer-based image processor based on the Qwin software program for histometry (Leica), a Windows-based image analysis tool that provides fully automated measurement.

Images of newly formed bone, soft tissue, and graft remnants were identified by different given colors in each image, digitized, and transferred to the computer software for image processing and analysis of the quantity fraction (relative percentage) for each tissue type. The mean percentage value for each tissue type was considered. All studied sections (active and control) were compared in the same manner.

**RESULTS**

**Clinical Observations**

Four rabbits died before the end of the investigation period and were excluded from the study; 2 rabbits died of aspiration pneumonia and 2 of gastrointestinal obstruction. The excluded rabbits were 2 controls, which contained 4 empty osseous cavities, and 2 active rabbits containing 4 osseous defects filled with HTR, Dembone, Laddec, and Osteograf, respectively. All other animals remained healthy during the observation period and all implantation sites healed uneventfully.

**Histologic Observations**

**H&E-Stained Sections (Table 1).** HTR. Limited rims of new bone (osteogenesis) could be seen (14%) between beads of the grafted material (56%), and most of this bone extended from the endosteal surfaces of the host cortical bone surrounding the defect. Several blood vessels (angiogenesis) in connective tissue stroma (30%) were seen between the beads, with no signs of inflammation (Fig 1a).

**BOP.** Crystals of the filling material occupied most of the osseous cavities (70%). Minimal hydrolysis of the material led to limited spaces (17%) for new bone ingrowth (13%) without inflammation (Fig 1b).

**Biogran.** Dissolution of bioglass crystals (40%) was seen, with a large amount of new bone ingrowth (28%) that filled the spaces between remnants of the material. The newly formed bone was lamellar, took place in multiple ossification sites throughout the defect, and was quite dense. This remodeled bone was incorporated well with remnants of the crystals and filled the empty spaces with a bone-implant locking feature. Several blood vessels with no signs of inflammation were apparent in active connective tissue stroma (32%) (Fig 1c).

**Laddec.** Most of the Laddec granules resorbed (21%), and their spaces were occupied by newly formed bone (33%). Organization of the newly formed bone was in a typical lamellar pattern. Implant remnants were completely integrated, with

<table>
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<th>Type of graft</th>
<th>Soft tissue/empty spaces</th>
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intimate contact to the newly formed bone. There was no sign of dissolution, but resorption was apparent. Resorption of the material was confirmed by the close contact between osteoclasts and remnants of Laddec granules. Rims of osteoblasts were also detected along the spaces of resorbed particles, indicating an active bone ingrowth process. New bone trabeculae filled the sites of the resorbed granules and capped remnants of Laddec material in a manner similar to the physiologic natural bone remodeling process. New osteons of the Haversian system were observed in several locations throughout the regenerated bone. Angiogenesis was seen in multiple locations in active connective tissue stroma (46%), with no signs of inflammation (Fig 1d).

Dembone. The field showed many remnants of graft material (52%) and diffuse areas of mononuclear cell infiltration, which indicated chronic inflammation without evidence of new bone formation. There was minimal resorption of the particles by osteoclasts. Spaces between Dembone particles were filled with connective tissue and few blood vessels (48%) (Fig 1e).

Osteograf. A moderate amount of newly formed bone (19%) was seen throughout the osseous cavities. The HA particles underwent moderate dissolution (19%). Hydrolysis of the material was similar to that seen with the Biogran material. The newly formed bone was composed of a mixture of lamellar and woven types. Incorporation of the new bone with remnants of Osteograf granules was observed.
in several sites. The remaining spaces between the particles were filled with active connective tissue stroma (62%). No signs of inflammatory cells could be seen (Fig 1f).

Control Osseous Cavities. There was a normal bone border (trabeculae) surrounding largely empty marrow spaces, fat, and bone dust (96%) (Fig 1g).

Scanning Electron Microscopy. HTR. Minimal bone deposition was seen between the beads. The matrix was filled with fibrous connective tissue and marrow spaces. Partial hydrolysis of the material and porosities were seen in the centers of some beads. Direct bone bonding to surfaces of some HTR beads and a thin layer coating of calcium hydroxide on the surfaces of HTR beads could also be seen (Fig 2a).

BOP. Partial hydrolysis of the material was seen. Thin filaments of new bone deposition and active connective tissue stroma filled the spaces between its crystals. The new bone comprised mixed woven and lamellar types. Microporosities were seen through the dissolved crystals (Fig 2b).

Biogran. Moderate dissolution occurred in some crystals and massive hydrolysis in others. There was a considerable amount of bone deposition between the Biogran crystals that was lamellar in nature. New bone trabeculae interlocked with each other in a honeycomb-like network. The absence of crystal porosities and the direct bonding of newly formed bone with implant remnants was clearly observed (Fig 2c).

Laddec. Gross resorption of the granules was apparent. Lamellar bone was deposited throughout
Figs 2a to 2h  Sections studied under scanning electron microscopy of osseous cavities filled with the tested grafting materials and the control cavities.

Fig 2a  Cavity filled with HTR shows minimal bone deposition. Note partial hydrolysis and the central porosities in some of the beads. A thin layer of calcium hydroxide coating the HTR beads was observed (original magnification ×100).

Fig 2b  BOP-filled cavity showing partial hydrolysis of the crystals. Porosities in the crystals were observed (large arrow). The newly formed bone deposited between its crystals is composed of lamellar and woven types (small arrows) (original magnification ×100).

Fig 2c  Biogran-filled cavity showing moderate dissolution of the crystals and a considerable amount of lamellar trabecular bone interlocking. Porosities and direct bone/graft bonding are absent (original magnification ×200).

Fig 2d  Cavity filled with Laddec. Gross resorption of the granules and capping of new bone to Laddec particles are apparent (arrows) (original magnification ×200).

Fig 2e  Higher magnification of the Laddec-implanted cavity shows colonies of osteoclasts (arrow) and porosities of the trabecular structure of the implanted material (asterisk) (original magnification ×1,000).

Fig 2f  Dembone-implanted cavity. The particles occupied the majority of the field with minimal resorption. Note the fibrous connective tissue deposited between its particles, without signs of bone remodeling (original magnification ×100).
the defect. Capping of the newly regenerated bone to remnants of the Laddec particles was seen. Higher magnification showed colonies of osteoclast cells consuming the graft material and trabecular pattern (porosities) of the graft particles (Figs 2d and 2e).

Dembone. Most of the field was filled with the implanted material. Porosities in the particles were seen. The predominant feature of the field was fibrous connective tissue without signs of new bone ingrowth (Fig 2f).

Osteograf. Moderate hydrolysis of the particles was seen. A mixture of lamellar bone and woven bone was deposited between the particles, and direct bone-implant bonding was observed in several locations. Microporosities of the particles were also observed (Fig 2g).

Control Cavities. The field showed predominantly bone marrow spaces and a few bundles of bone extended from cortical surfaces of the cavity (Fig 2h).

DISCUSSION

This experiment was conducted in an osteogenic environment and thus could not confirm any osteoinduction.

It is generally accepted that an optimal bone substitute is one that maintains mechanical stability and volume during the initial healing and then subsequently resorbs completely, being replaced by newly formed bone.9 The ideal bone substitute should possess the following characteristics: it should be biologically compatible, non-supportive of local pathogens or cross-infection, and osteogenic (ie, facilitate bone cell ingrowth); it should match the physical and chemical composition of natural bone trabeculae and provide scaffolding for new bone ingrowth; it should be resorbable and osteotropic (ie, enhance bone formation by its chemical or structural characteristics); it should provide calcium and phosphate sources; and it should be microporous and easy to handle. Table 2 summarizes the properties of the tested materials.

Because of differences in chemical composition and physical forms, the bioresorbability of grafting materials varies. Implants may resorb by either a solution-mediated process (ie, solubility of grafts in physiologic solution by enzymatic hydrolysis) or by a cell-mediated process (ie, physiologic bone remodeling by phagocytosis of the material with osteoclast cells).10,11 The synthetic grafting materials, such as those used in this study, underwent dissolution by the former process. The rate of solution-mediated dissolution depends on the chemical and physical compositions of the implanted material. This explained why the rate of hydrolysis and bone deposition varied among different synthetic grafts.11 On the other hand, Laddec and Dembone are resorbed by a cell-mediated process. The speed of resorption of the graft by this process depends mainly on the porosity of the particles and the surface area and purity of the material.12-16

At the end of the observation period (8 weeks), osseous defects filled with Laddec granules showed a high amount of resorption of the grafted material (remnant, 21%) and replacement by creeping, new lamellar bone ingrowth. Osteoblast rimming and intense angiogenesis, which were seen between Laddec granules, indicated an active bone deposition
process. The accompanying existence of osteoclast colonies at the margins of Laddec particles resulted in harmonious resorption of the scaffold material and simultaneous deposition of new bone, which is similar to what is seen in gradual physiologic bone remodeling. Scanning electron microscopy confirmed that bone was “capping” to remnants of the Laddec material and showed the highly porous nature of the Laddec particles.

Klinge and coworkers reported almost total resorption of bovine bone granules 14 weeks after implantation in rabbit skull defects. The trabecular pattern of Laddec granules resembles natural bone trabeculae, which allows faster ingrowth of new blood vessels and bone cells. Early resorption of the graft structure and subsequent deposition of new bone can provide for the early formation of bone lamellation of the Haversian system, as it was seen in this study (Fig 1d). Similar findings have been reported by the author and others. The weak osteogenic properties of Dembone particles may be a result of the impurity of the material. Chappard and colleagues compared the osteogenic activities of bovine bone grafted in animals using completely protein-purified bovine granules and partially purified granules. They noted a local foreign body reaction in the inadequately purified graft material, which caused an inhibition of osteoformation.

Growing awareness of disease transmission via contaminated blood and tissue has raised concerns about allograft materials. In the author's experience, this has reduced patients' acceptance of allograft materials. The combination of patient and practitioner concerns has made identification of an alternative material desirable, if optimal grafting criteria can be met.

Osteotrophy (ie, the matrix provides improvement of bone formation by its chemical and/or structural characteristics in the presence of osteogenic precursor cells) is one of the main requirements for an osteoconductive grafting material. The chemical composition of natural HA, such as the xenographic particles of the Laddec material, and synthetic HA, such as Osteograf particles, allows resorption of the material to act as a mineral reservoir. This predictably induces more bone formation than materials that do not possess these properties.

In the present study, Biogran showed the second-greatest amount of new bone ingrowth. It appeared that Biogran crystals dissolved in a slower cycle than the Laddec particles, which was demonstrated by the amount of graft remnants at the end of this experiment observation time (40% and 21%, respectively). New bone was incorporated with residual Biogran crystals, but the amount of new bone was slightly less than that seen in osseous defects filled with Laddec granules (28% and 33%, respectively).

Osteograf showed moderate bone regeneration involving a mixture of woven and lamellar bone during the observation period. Osteograf and Biogran showed a direct correlation between bone deposition and dissolution of the crystalline particles as a function of time, but with different rates of dissolution and bone ingrowth. The dissolution of Osteograf particles was slower and bone ingrowth was less than with Biogran crystals (bone ingrowth of 19% and 28%, respectively). Similarly, the new

<table>
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bone had bonded to remnants of Osteograf particles, forming a hollow bone chamber for the buildup of trabecular bone structure.5,22,25

Although microporosity plays a significant role in the effective removal and subsequent bone regeneration process,8,26 microporosity of its own accord is not adequate on its own to effect osteogenesis, as was seen in this study. The size of graft particles, their chemical and physical characteristics, and speed of resorption of the graft may play more of a role in the rate and quantity of new bone ingrowth. Density, crystal size, and material chemistry and porosity have been stressed by other researchers in comparative studies.23,25–30 It has been noted that densely sintered HA ceramic has a lower microporosity and a higher density and is prepared in relatively larger particle sizes, which leads to a slower resorption rate and often to fibrous encapsulation rather than incorporation as a viable part of the host bone.23,29 Osteograf is classified as a non-sintered, non-ceramic, resorbable, porous form of synthetic HA, which has been found to be a better osteotropic grafting material than ceramic non-resorbable HA.23,31 Different synthetic HA materials, however, show varying rates of dissolution and bone ingrowth for the above reasons.

The MMA resin implants investigated in this study (HTR and BOP) showed the least bone ingrowth between their particles (14% and 13%, respectively) within the period of this experiment. This may be the result of smaller spaces between their particles for new bone to develop, the osteotropic properties of MMA resin, and a slower dissolution rate, which retarded the speed of bone cell ingrowth as compared to Laddec, Biogran, and Osteograf (Table 1).

Donohue26 investigated 2 types of HA (Osteogen and Alveograf) and HTR. These materials filled drilled holes in the iliac bone of rats. He found that HTR was the least osteoconductive of the tested materials. The present author12 also had poor results with the BOP material, in comparison with Laddec granules in rabbit femoral osseous defects. Boyne30 studied HTR in extracted sockets of dogs. He found that this investigation found that, among the 6 graft materials reviewed, Laddec bone granules possessed optimal criteria for rapid bone regeneration in osseous defects, followed by Biogran crystals and Osteograf LD particles, respectively. The lowest potential for rapid bone formation was demonstrated by the HTR and BOP materials. Dembone particles revealed no active bone healing.

CONCLUSION

ACKNOWLEDGMENT

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