Bone Healing Following the Use of Hydroxyapatite or Ionomeric Bone Substitutes Alone or Combined With a Guided Bone Regeneration Technique: An Animal Study

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The healing of standardized bone defects grafted with either particulate ionomeric or hydroxyapatite bone substitutes was compared in the mandibular ramus of 30 Sprague-Dawley rats. The possible additional response achieved when combining these materials with a guided bone regeneration (GBR) technique was also evaluated. Three groups of 10 animals received either no implant material or ionomeric or hydroxyapatite bone substitute in defects in the right ramus. The left mandibular defects received the same treatment, except that the operation site was covered by a membrane (GBR technique). Half of the animals were sacrificed at 4 and 10 weeks following surgery, and the inflammatory response at the implant site and the amount of new bone formed in the defects were determined histomorphometrically. Defects implanted with ionomeric bone substitute exhibited more bone formation (4 weeks = 3.19 ± 0.38 mm^2, 10 weeks = 5.35 ± 0.26 mm^2) than both defects that received no treatment (4 weeks = 0.88 ± 0.35 mm^2, 10 weeks = 2.1 ± 0.49 mm^2), membrane alone (4 weeks = 1.21 ± 0.05 mm^2) or hydroxyapatite bone substitute (4 weeks = 1.41 ± 0.46 mm^2, 10 weeks = 3.34 ± 0.41 mm^2) at 4 weeks ($P \leq .01$) and at 10 weeks ($P \leq .05$). The use of a GBR technique did not increase the amount of bone formed, compared to the use of bone substitutes alone. Hydroxyapatite and ionomeric bone substitutes used alone were more effective in inducing repair of the defects than was GBR membrane alone. The use of hydroxyapatite was associated with a greater inflammatory reaction ($P \leq .01$) than was ionomer in this model.
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Key words: bone regeneration, glass-ionomer cement, hydroxyapatite, ionomeric

The search for allografts and new techniques intended for either the replacement or the regeneration of bone has intensified in the last three decades. Bone substitutes, grafting, and mechanical barrier membranes that allow bone to regenerate without interfering soft tissue (guided bone regeneration) have been developed, and these techniques have been successfully applied in surgical procedures aimed at alveolar ridge augmentation, periodontal bony defect corrections, and ridge height maintenance after tooth extraction.[1–4]

The calcium phosphate ceramics, particularly hydroxyapatite (HA) because of its chemical similarities to bone, have undergone intense study. Sintered HA is used as a bone substitute material, but it is difficult to sinter unless pure and free of any ß-tricalcium phosphate, which is thought to confer the bioactive and bone-bonding properties reported with HA.[5,6] Hydroxyapatite has seen wide clinical use as a bone substitute in particulate and block forms.[7] However, it is not an ideal bone substitute for alveolar augmentation and situations where it is subject to mechanical stress. Hydroxyapatite has poor biomechanical properties (high elastic modulus [40 to 117 GPa] compared to compact bone [12 to 18 GPa]); adverse biologic responses associated with

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fibrous tissue ingrowth, encapsulation, and inflammation have been reported; and with some forms of HA, resorption is a problem.[8–13]

Alternative bone substitutes such as those based on glasses or ceramics may overcome some of the problems associated with HA.[14,15] Set defined glass (Polyalkenoate) ionomer cements[16] have been shown to be osteoconductive and have potential as biomechanically matched bone substitutes.[17,18] The ionomeric- (IM) bone interface has been reported to be similar to that observed for HA,[18–20] possibly because certain formulations of glass-ionomer cement used to produce IM implants consist of separated mullite and fluoroapatite structural units that might serve as bonding agents between the material and bone.[21,22] Particulate IM has been shown to perform well when used clinically for the prevention of alveolar bone resorption;[23] however, IM and HA bone substitutes have never been compared directly using standardized bony defects under controlled conditions.

Bone regeneration following GBR technique was first demonstrated by Dahlin et al,[24] who prevented the impedance of osteogenesis by the ingrowth of fibrous tissue in a transosseous bony wound mechanically protected by a physical barrier. GBR techniques for covering partially exposed endosseous implants, alveolar ridge augmentation, healing of cystlike cavities in the jaw bone, and even encouragement of bone formation beyond its original contour have been extensively documented.[3,25–27] The success of GBR techniques depends on the physical support provided to the overlying soft tissue by the barrier membrane, creating a space to be filled with blood clot while excluding competing nonosteogenic cells from the defect, and possibly allowing local accumulation of growth factors under the membrane.[28]

The combination of HA or IM particulate bone substitutes with GBR techniques could be expected to lead to improved bone healing/regeneration, particularly since immobilization of bone substitutes improves their osteoconductive properties.[10,29,30] The aims of this study were to compare the relative effectiveness in healing of standardized osseous defects repaired using IM or HA particulate bone substitutes alone or in combination with a GBR procedure. This was accomplished by determining the amount of bone formed in the defects and the inflammatory response induced by these materials.

Materials and Methods

A standardized, full-thickness, nonhealing defect was created from an extraoral approach bilaterally in the mandibular rami of 30 male Sprague-Dawley rats, aged between 3 and 4 months (weight 450 to 500

g).[24] The animals were divided equally into three groups according to the treatment they received; in the right mandibular ramus, defects were left to heal unaided (no treatment [NT]) or received implantation of hydroxyapatite (Calcitite, 20 to 40 mesh size, Calcitek, Carlsbad, CA) or ionomer (Ionogran, 0.5/1.0 mm, Ionos Obberbay, Seefeld, Germany). The left mandibular defects received the same treatment, except that the operation site was covered on both sides by a GBR membrane (Goretex, W. L. Gore & Associates, Flagstaff, AZ).

Anesthesia and Surgery. Anesthesia was induced by inhalation of 5% halothane (May and Baker, UK) in 75% nitrous oxide and 25% oxygen inside a glass chamber for 2 minutes. The animals were transferred to a surgical table and anesthesia maintained with 2% halothane in 75% nitrous oxide and 25% oxygen.

All animals underwent both left and right mandibular ramus surgery. Following shaving and skin disinfection, a full-thickness, 2-cm-long skin incision was made. The lower border of the mandible was exposed by soft tissue dissection, while care was taken to preserve the facial vessels. The periosteum was cut along the lower border and raised both buccally and lingually. Using a dental bur under profuse irrigation with distilled water, a 3-mm-diameter defect was created through both cortical plates of each ramus below the inferior dental nerve, posterior to the last molar tooth.

Two sterile membranes, each 36 mm^2, were applied at the surgical site in the left ramus, one buccally, one lingually (covering the defect with an additional 3-mm overlap). The surrounding tissues were approximated, and the periosteum sutured. The muscles and skin were sutured back into place. When IM and HA particles were used, the volume of material applied to the site was determined by the amount required to fill the entire bony defect with a single layer of particles. The periosteal and muscle flaps were closed with Vicryl 5/0 sutures (Ethicon, Edinburgh, UK), while the skin flap was closed with interrupted sutures using Mersilk 4/0 (Ethicon).

The rats were allowed free access to food and water and observed daily for signs of postoperative complications or adverse reactions. At 4 and 10 weeks after surgery, five animals from each group were sacrificed. The mandibular rami and associated soft tissues overlying the surgical sites were removed en block, fixed in neutral buffered formalin, and decalcified in 4 N formic acid for 1 week. This sequence was followed by routine histologic processing and paraffin wax embedding.

Histomorphometry. Blocks were radiographed to orient the rami and sectioned to bisect the defects across their maximum diameter. Pairs of serial sec-

tions were then cut at 50-μm intervals through the defects using a rotary microtome. These sections were mounted on glass slides and stained with hematoxylin-eosin (H&E) or by van Gieson's solution.

The inflammatory reaction at the surgical sites in the right rami (no GBR membrane) was determined by viewing H&E sections with a transmission light microscope (Nikon, Optiphot-2, Tokyo, Japan) at 200× magnification. A semi-quantitative method was adopted to score the number of both inflammatory and multinucleated giant cells in the bony defect and surrounding soft tissues: 0 (no cells); 1 (few cells); 2 (mild infiltrate); 3 (moderate infiltrate); and 4 (severe infiltrate). The scores were assigned by a single examiner who was blinded to the materials, technique, and time period. The sections were presented in random order. The examiner was calibrated at the start of the study using predetermined calibration standards for the degree of inflammation, and repeat sections were included to confirm consistency. To ensure that the entire bony defect was inspected during evaluations, the area was systematically divided into six fields. The scored numbers from each field were used to produce a mean score for each animal.

The amount of new bone formed in the defects was estimated from three van Gieson–stained sections, one from the central portion and two from the periphery of each defect. Sections were captured from the transmission light microscope by camera/computer, and the area of new bone within the defect was measured in mm^2 using an image-analysis system (Optimas 4.1 Biosoft, Optimas UK, Milton Keynes, UK). Sections were examined in random order by an examiner blinded to the surgical technique, and repeat samples were included to check consistency.

Data were submitted to analysis of variance (ANOVA). For normally distributed samples, the Tukey test was used to establish P values, while the Kruskal-Wallis test was used to analyze abnormally distributed samples. Data are reported as the mean of three values for each histologic section ± the standard error.

Results

All 60 of the surgical sites healed uneventfully, and no postoperative complications or clinical signs of reaction to any of the materials used in these experiments were observed. In two animals in the GBR group at the 10-week assessment, the membrane had dislodged from its original position, and the entire group was thus excluded from the analysis.

Independent of the kind of treatment undertaken, the defects created in the mandibular rami of the
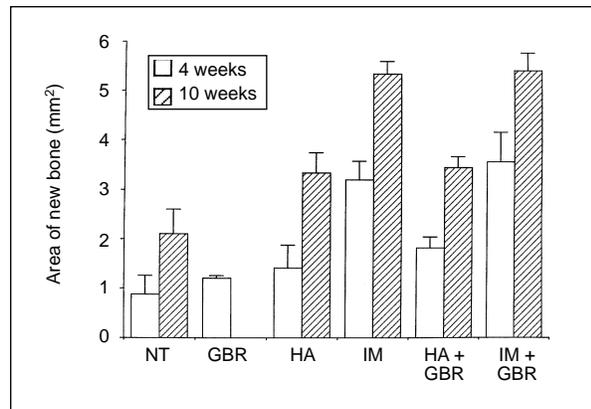

Fig 1 New bone formed in rat mandibular rami defects at 4 weeks and at 10 weeks after surgery: no treatment (NT), guided bone regeneration (GBR), hydroxyapatite implantation (HA), and ionomeric implantation (IM) (standard deviations shown).

immature rats enlarged over the time period of the experiment because of concomitant skeletal growth and the effects of the surgical intervention, so that in no case was complete healing of the bony defect observed. Defects implanted with IM (4 weeks = 3.19 ± 0.38 mm^2, 10 weeks = 5.35 ± 0.26 mm^2) exhibited more bone formation than defects receiving no treatment (4 weeks = 0.88 ± 0.35 mm^2, 10 weeks = 2.1 ± 0.49 mm^2), membrane alone (4 weeks = 1.21 ± 0.05 mm^2), or HA (4 weeks = 1.41 ± 0.46 mm^2, 10 weeks = 3.34 ± 0.41 mm^2) at both 4 weeks ($P \le .01$) and 10 weeks ($P \le .05$) (Fig 1). Defects that received no treatment showed smaller areas of new bone formation. In these defects, the bone edges were separated by soft tissue (Fig 2a), in contrast to defects implanted with IM and, to a lesser extent, HA, or protected by a membrane, which showed evidence of extensive new bone formation (Figs 2b, 2c, 3a, and 3b). The difference in bone healing was small in defects that received no treatment and in those protected by a membrane alone (Fig 1), and lacked statistical significance at 4 weeks. No comparison was possible at 10 weeks because of loss of data.

Defects implanted with either IM or HA consistently exhibited migration/spillage of particles from the bony defect into the surrounding tissues. The use of a GBR membrane, in conjunction with IM or HA, appeared to confine the particulate bone substitutes to the defect (Figs 3a and 3b), although this did not improve the performance of either material as judged by the amount of bone formed in the defect (IM + membrane at 4 weeks = 3.59 ± 0.6 mm^2, at 10 weeks = 5.44 ± 0.36 mm^2; HA + membrane at 4 weeks = 1.82 ± 0.23 mm^2, at 10 weeks = 3.45 ± 0.34 mm^2). Independent of the treatment adopted, the newly

Figs 2a to 2c Photomicrographs of defects 4 weeks after surgery (hematoxylin-eosin stain; original magnification ×10).
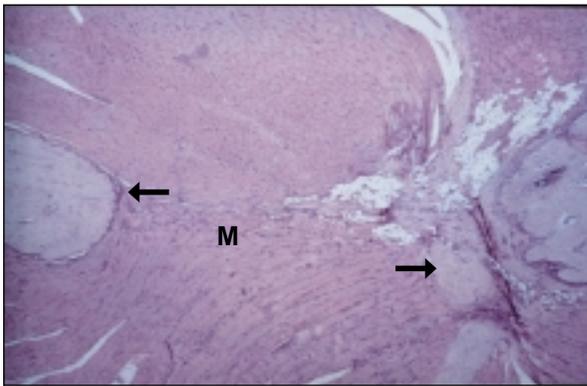

Fig 2a The defect was left to heal unaided. Bone edges *(arrows)* are separated by muscle (M).
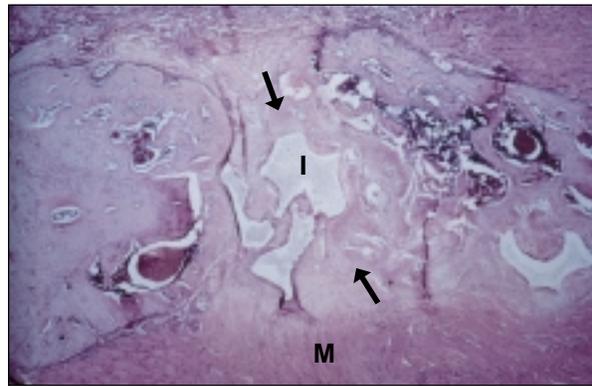

Fig 2b New bone *(arrows)* can be seen spanning the defect and closely related to the ionomeric bone substitute (I), whereas muscle (M) has been excluded from the defect. The marrow spaces are darkly stained.
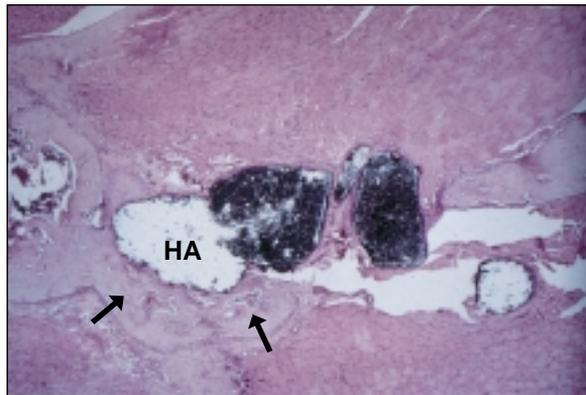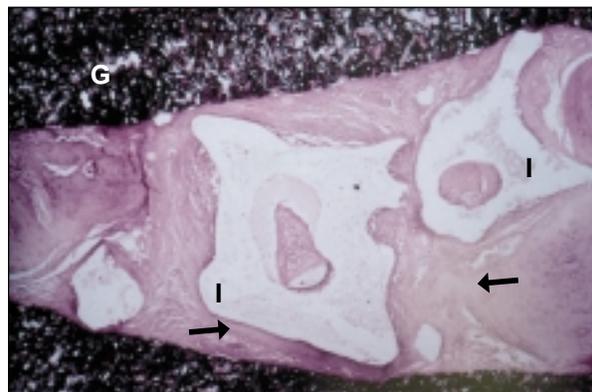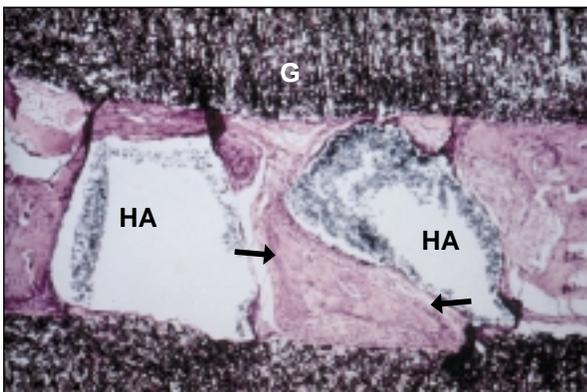

Fig 2c Some partly decalcified hydroxyapatite granules (HA) are surrounded on one side by new bone *(arrows)*.

Figs 3a and 3b Photomicrographs of defects showing use of GBR membrane (G) in conjunction with particulate bone substitutes (H&E stains; original magnification ×20). *(Left)* Immature new bone *(arrows)* is present around the hydroxyapatite granules (HA) and on the surface of the membrane. *(Right)* Extensive repair has taken place with the new bone *(arrows)* showing evidence of maturation (I = ionomeric granules).
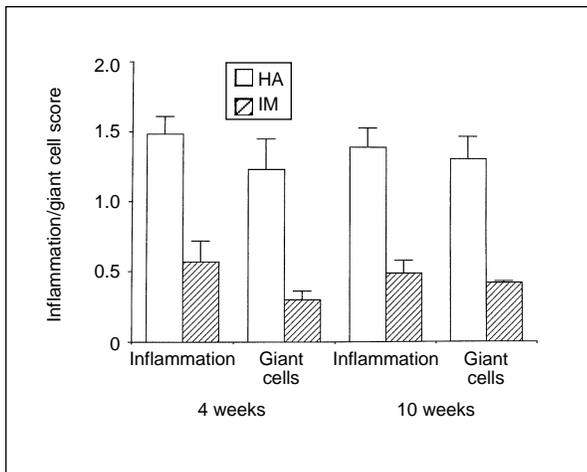
Fig 4   Inflammation and giant cell scores 4 and 10 weeks after surgery in defects containing either hydroxyapatite (HA) or ionomeric (IM) bone substitutes (standard deviations shown).

Figs 5a to 5c   Photomicrographs of bone substitutes implanted in bone defects (H&E stain; original magnification ×50).
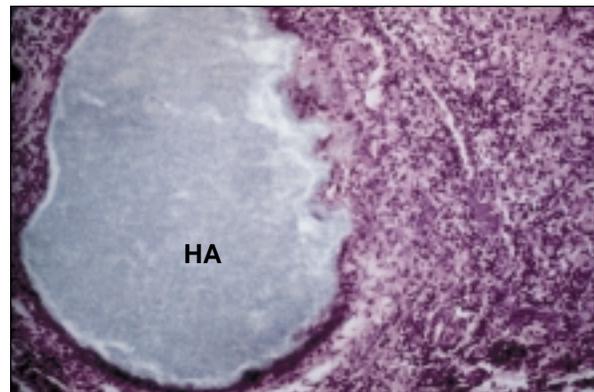

Fig 5a   Four weeks after surgery, particles of hydroxyapatite (HA) are surrounded by a dense chronic inflammatory cell infiltrate and giant cells.

Fig 5b   Ten weeks after surgery, occasional giant cells (*arrows*) are associated with hydroxyapatite (HA) particles.
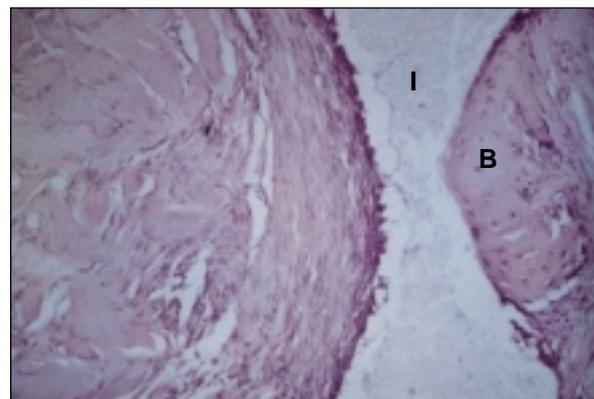
Fig 5c   Ten weeks after surgery, an ionomeric particle (I) is associated with new bone (B) and only occasional giant cells and minimal chronic inflammation.

formed bone exhibited a more mature pattern at 10 weeks than at 4 weeks, indicating that neither membrane nor bone substitutes appeared to influence the timing of bony maturation (Figs 2b and 3b). Foci of new bone were also observed outside the defect when GBR membrane was used either alone or in combination with bone substitutes.

Defects implanted with HA were associated with more inflammatory cells ($P \leq .01$) and foreign body giant cells ($P \leq .01$) than defects implanted with IM; this was consistent at both time periods (Fig 4). At both 4 and 10 weeks, the inflammatory response to HA was mainly chronic in character and comprised of lymphocytes, macrophages, and multinucleated giant cells (Figs 5a and 5b). By 10 weeks, IM particles were associated with only occasional giant cells, and there was little evidence of chronic inflammation (Fig 5c).

Discussion

In the animals studied, complete regeneration of the bony defect was not observed. The use of GBR membrane alone did not show any advantage in terms of bone regeneration over the control containing no implant material or the defects implanted with bone substitutes. Although generated over a shorter time period, these data are in sharp contrast with those obtained by Dahlin et al,[24] who, using similar methodology, reported total regeneration of membrane-covered defects after 3 weeks. Factors such as membrane mobility and instability during healing may lead to wound space collapse or may even allow penetration of soft tissues into a wound.[31–34] Unlike

the study by Dahlin et al, in this study the membrane was not secured to the bone with sutures, and thus mobility of the membranes may explain the relatively poor results obtained with GBR used alone. However, except in the few cases where a membrane was noted to have been displaced (and for this reason was excluded from analysis), the histologic evaluation did not show gaps between the intact bone surrounding the defects and the membrane.

The strict protocol of achieving immobility of membranes used in GBR is also not without its critics, since soft tissue penetration into defects protected by membranes immobilized by sutures has been reported.[35] In contrast, a high degree of bone formation has been reported when no suture was used to secure the membrane.[36] In the former studies, the membrane was applied on the mandibular lower border in the proximity of the masseter muscle insertion, whereas in the latter study the membrane was used in contact with either the lower or the upper premolar apices, and hence far away from any masticator muscle insertion. Thus, it seems reasonable to expect that membranes in which a high degree of muscle activity is expected require suturing, especially in rodents.

The collapse of unsutured membranes into the wound defect might also account, in part, for the poor bone regeneration. However, the use of particulate material to fill the defect should help to support the membrane and improve bone healing.[10,29,30] In this study, the use of GBR membrane with bone substitutes did not significantly improve the bone formation compared to the use of bone substitutes without GBR membrane (Fig 1). Indeed, the use of GBR membrane in combination with IM or HA, despite containment of the material, offered no advantage, in accordance with other studies that found no more bone formed in periodontal and peri-implant defects treated with demineralized bone combined with membrane than in defects covered by membrane only.[37–39]

Studies have shown that HA blocks covered by periosteum alone yielded bone formation at levels that resembled cases in which blocks were used in combination with a GBR technique.[38,40] The synergism between periosteum and HA in the induction of bone has previously been demonstrated.[41] Thus, it appears that the physical contact of either IM or HA with periosteum, which is prevented by the use of a membrane, produces as efficacious a combination as that of GBR membrane with bone substitute. In addition, the presence of granules in the defect may guarantee both blood clot protection and preservation of an adequate space for bone growth, as claimed for GBR procedures. These factors associated with the osteoconductive properties of both IM

and HA may explain the fact that more bone formed in defects treated with bone substitutes than in defects covered by membrane alone.

The mobility of particulate bone substitutes during the osteogenic phase of bone healing has been used to explain the formation of fibrous tissue observed around HA particles in other studies.[10,29] In the present study, the high mobility of the mandibular angle/rami and muscle pull during chewing could, to some extent, be responsible for formation of the fibrosis that was observed in the defects next to both IM and HA granules. The inflammatory process that is induced upon implantation of the materials is also implicated in fibrosis, with recruited macrophages that are unable to phagocytose the implanted particles becoming transformed into histiocytes that generate collagen fibers to encapsulate the material.[12] This feature may explain the improved bony response observed following implantation of IM particles that induced less of an inflammatory response (Figs 4 and 5).

The occurrence of macrophages, giant cells, and lymphocytes in sites implanted with HA has been previously reported in in vitro studies,[13] in animal studies,[30,42–44] and following procedures intended for ridge augmentation in humans.[33,45] It has been argued that phagocytosis takes part in the process of biodegradation of HA, as the material is progressively replaced by new bone following implantation.[46] Others have found simultaneous osteoclast-mediated absorption of HA and bone in sites infiltrated by macrophages and multinucleated giant cells.[47,48] Thus, given the fact that IM-implanted sites exhibited less inflammation than the HA-implanted sites, it might be inferred that the former were less prone to cell-mediated bone resorption than the latter, and that consequently the ratio of deposition/resorption was more favorable for IM. Alternatively, differences in the physicochemical characteristics both of the granules used in this study and the bone formed by HA and IM may play an important role in this process. It is known that HA relies on granule degradation to initiate the process of bone deposition[6,49] and since dense HA is more resistant to degradation than porous HA,[50] better results in terms of osteoconduction might be achieved when the porous form is used.[51,52] In this study, HA in its dense form has been employed, which may account for the poor osteoconduction compared to IM-implanted defects. There is evidence that the osteoconductive effect of IM is likely to benefit from fluoride in its composition, as fluoride has been shown to stimulate both the proliferation and alkaline phosphatase activity of bone-forming cells.[53–55] Although there has been no consensus in the literature concerning the quality of

bone formed with varying fluoride doses, fluoride is used in the treatment of osteoporosis.[56,57] Moreover, fluoride has been shown to aid in the osseointegration of IM materials in vivo,[18] to promote the crystallization of IM,[22] and to produce a dose-dependent fluoroapatite crystallization in contact with bone.[58] Thus, it is feasible that IM induces the formation of fluoroapatite during its process of osteoconduction. One might speculate that fluoroapatite would be more resistant to resorption than apatite and, since bone formation is a dynamic process of deposition/resorption, this also could account for the greater amount of bone associated with IM than with HA particles (Fig 1).

Conclusions

1. IM induced a better host response (superior biocompatibility) than HA as assessed by both the recruitment of inflammatory cells into the implant sites and the amount of bone formed in the surgical defects.
2. The combination of GBR technique with either IM or HA did not increase the amount of bone formation in defects in the rat mandibular rami compared to the use of bone substitutes alone.
3. GBR technique alone was not as efficient in inducing new bone formation as the use of either IM or HA bone substitutes alone.
4. The use of GBR alone gave little or no improvement in bony healing in full-thickness rat mandibular rami defects as compared with no treatment during the 10-week period of this study.

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