Case Report

Dense Hydroxyapatite Inserted Into Postextraction Sockets: A Histologic and Histomorphometric 20-Year Case Report

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Background: *The biologic behavior, i.e., the degradation of hydroxyapatite (HA) in the human body, is of relevance for clinicians. The present investigation is a long-term (20-year) histologic and histomorphometric evaluation of dense HA used in postextraction sockets.*

Methods: *Dense HA particles were used in a patient in postextraction alveolar sockets to maintain the alveolar ridge height. The patient returned after 20 years for implant treatment. A ridge remodeling was necessary during implant insertion surgery, and the HA/bone tissue was harvested with bone-cutting forceps from the canine and premolar area. The specimen was processed for histology and histomorphometry at the Implant Retrieval Centre, Dental School, University of Chieti-Pescara.*

Results: *Most of the particles (56%) were surrounded partially by bone, whereas some particles (39%) were surrounded completely. At higher magnification, bone was in close contact with the particles, and neither gaps nor fibrous tissues were present at the bone–biomaterial interface. Microscopically, the particles had a dense appearance. In only a few fields, it was possible to observe that the outer part of some particles had detached from the original particles' surface. Histomorphometry showed that bone represented 25.4% ± 3.2%, marrow spaces represented 41.3% ± 5.2%, and residual HA particles represented 38.1% ± 4.1%.*

Conclusions: *Intimate binding between bone and HA particles was present after a long-term implantation period (20 years). The fact that HA particles were surrounded closely by bone is very promising for the long-term stability of the augmentation.* J Periodontol 2008;79:929-933.

KEY WORDS

Case report; histology; hydroxyapatite; long-term study.

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Calcium phosphate (Ca-P)–based ceramics of synthetic or biologic origin can be used as an alternative to autogenous bone grafting.[1] Hydroxyapatite (HA) granules have been used for the promotion of bone formation in sinus augmentation procedures, periodontal defects, alveolar ridge augmentation, and craniofacial surgery.[2] In clinical and in vitro studies,[3-6] it was demonstrated that Ca-P materials are tolerated well, do not possess local or systemic toxicity, do not yield inflammatory responses when implanted in animals or humans, do not cause alterations of normal bone mineralization processes, and are fabricated easily into any size or shape. Moreover, they are considered biocompatible and osteoconductive and used widely in ridge preservation/augmentation procedures.[7-11] HA is generally recognized as the natural mineral component of vertebrate hard tissues, making up 60% to 70% of bone and 98% of dental enamel.[4]

There is a lack of data about HA degradation in the literature, with few long-term studies,[11] and conflicting views on the rate of resorption of this material. HA is generally believed to be non-resorbable, even over periods of many years. Therefore, it is suitable for long-term restorative and preservative clinical procedures, such as repair of periodontal defects and augmentation of deficient alveolar ridges.[12] Some researchers[13,14] reported that HA ceramics are not affected by the biodegradation processes because no change in ceramic mass could be detected when measuring the relative surface areas of bone and ceramic on histologic slides. According to Donath,[15] HA ceramics are not non-resorbable materials; they only differ in their resorption rates. Surface characteristics of the biomaterial have a relevant importance on the rate of resorption.[1] It is well known that surface area is a characteristic affecting the rate of resorption of HA substitutes. For this reason, high-density ceramics, which possess smaller surface areas than porous ones, show a lower tendency to undergo resorption.[4]

The mechanisms of HA resorption are not completely understood.[6] Some studies[16-20] observed that

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HA can be resorbed by cells or fluid-mediated processes. Osteoclasts are able to degrade Ca-P ceramics in vivo by means of simultaneous resorption and phagocytosis. In a short-term sheep study, Wenisch et al.[1] concluded that the Ca-P ceramic surface was resorbed by an acidic microenvironment beneath the ruffled borders of osteoclasts and that the resorbed Ca-P crystals were phagocytosed and degraded by osteoclasts. The osteoclast-mediated resorption depends on the method of sintering, the sintering temperature, the sintering period, the porosity, and the surface roughness.[1] HA sintered at a lower temperature and with a higher porosity tended to show greater degradation.[6]

This controversial evidence confirms the necessity of further studies to clarify the issue of HA bioabsorbability, taking into account that the biologic behavior, i.e., the degradation of HA in the human body, is of relevance for clinicians.[6] In some cases, e.g., craniofacial contour reconstructions, a non-resorbable HA may be preferable, whereas when using implants, a bioabsorbable, gradually replaced HA may be preferred.[2] Moreover, long-term histologic studies[21-23] of the interface between HA and bone in humans and of the HA resorption features are found only infrequently in the literature.

The present investigation was a long-term (20-year) histologic and histomorphometric evaluation of dense HA used in postextraction sockets.

MATERIALS AND METHODS

A 53-year-old male patient was treated by one of the authors (CM) in February 1986 for a localized aggressive periodontitis with rapid attachment loss and bone destruction involving the mandibular cuspid and left premolars; other than that, his medical history was non-contributory. The extraction of the mandibular cuspid and left premolars was performed; it was decided to use dense HA particles in the postextraction alveolar sockets to maintain the alveolar ridge height. The site of implantation healed without complication in the following week, and a temporary, partial, removable prosthesis was manufactured. The patient was lost to follow-up and returned for implant treatment after 20 years. A panoramic radiograph revealed that the patient was completely edentulous; a radiopacity was present in the left hemimandible where the reconstruction with dense HA particles was performed 20 years earlier. A radiolucency was observed in the canine area of the right hemimandible where surgery was performed to remove a cystic neoformation associated with an unerupted third molar (Fig. 1).

Ridge remodeling was necessary during implant insertion surgery, and the HA/bone tissue was harvested with bone-cutting forceps from the canine and premolar area where the radiopacity was evident.

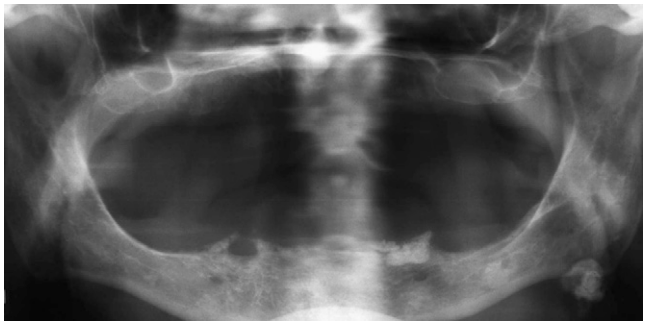

Figure 1.
Panoramic radiograph. In the canine and premolar area of the left hemimandible, it is possible to observe a radiopaque material where dense HA was grafted 20 years ago. The radiopaque area below the left angle of the mandible is an artifact of radiography. In the canine area of the right hemimandible, a radiolucency is evident where surgery was performed to remove a cystic neoformation.

Table 1.

Physical and Mechanical Characteristics of the Grafted Material

Color	Light blue
Compressive strength (MN/m^2; mean ± SD)	410 ± 75
Tensile strength (MN/m^2; mean ± SD)	39 ± 4
Vickers hardness (MN/m^2)	4,500
Density (%)	97
Modulus of elasticity (MN/m^2)	1.1 to 1.3 × 10^4
Impact strength (MN/m^2)	0.18
Bending momentum (MN/m^2; mean ± SD)	2.8 ± 0.2

The HA§ used in this case report had been manufactured as follows:[19] the starting material‖ consisted of very porous agglomerates with a mean size of 1 to 2 μm; the specific surface area as determined by Brunauer-Emmett-Teller analysis,[24] a technique used for measurements of solid surface areas, was 59 m^2/g; x-ray analysis of the powder showed rather broad peaks of the HA structure; and trace elements were determined by spectrochemical analysis, and the results showed no elements that could compromise the biologic behavior of the material. The mechanical properties of the material are given in Table 1.

Processing of Specimens

The tissues were stored immediately in 10% buffered formalin and processed for histology and histomorphometry at the Implant Retrieval Centre, Dental

§ DAC, Dense Apatite Ceramic, Novaxa, Milan, Italy.
‖ Merck, Darmstadt, Germany.

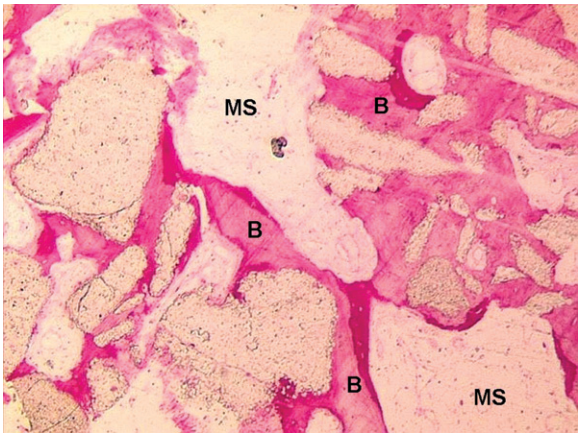

Figure 2.
At low-power magnification, it is possible to see trabecular bone (B) with many marrow spaces (MS) (acid fuchsin–toluidine blue; original magnification ×40).

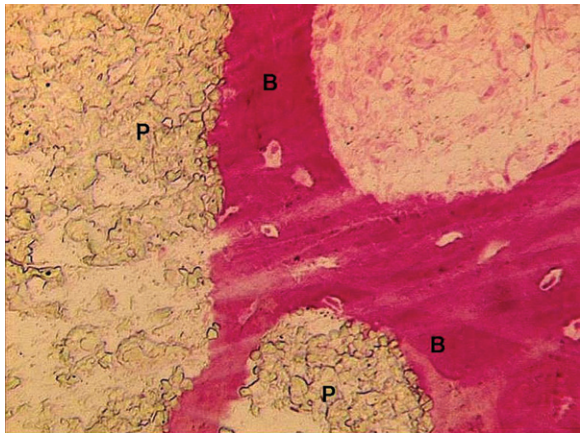

Figure 3.
At higher magnification, the biomaterial particles (P) are surrounded by bone (B). No gaps are present at the bone–biomaterial interface. (Acid fuchsin–toluidine blue; original magnification ×100.)

School, University of Chieti-Pescara, to obtain thin ground sections.[¶] The specimen was dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin.[#] After polymerization, the specimen was sectioned longitudinally along its major axis with a high-precision diamond disk at ~150 μm and ground down to ~30 μm. Three slides were obtained. The slides were stained with acid fuchsin and toluidine blue. A double staining with von Kossa and acid fuchsin was done to evaluate the degree of bone mineralization. After polishing, one slide was immersed in $AgNO_3$ for 30 minutes and exposed to sunlight. The slides were then washed under tap water, dried, immersed in acid fuchsin for 5 minutes, washed, and mounted.

The slides were observed under normal transmitted light with a microscope. Histomorphometry of the percentage of bone, marrow spaces, and residual graft particles and the percentage of residual particles partially or completely surrounded by bone was carried out using a light microscope[**] connected to a high-resolution video camera[††] and interfaced to a monitor and PC.[‡‡] This optical system was associated with a digitizing pad[§§] and a histometry software package with image-capturing capabilities.[‖‖] Three slides were evaluated; five random fields (×40 magnification) were analyzed for each specimen to obtain the histomorphometric results.

RESULTS

The particles had a dense appearance and varying sizes. Most of the particles (56%) were surrounded partially by bone. Some particles (39%) were surrounded completely by bone (Fig. 2). Only some peripheral particles (4%) were surrounded by a fibrous, connective tissue. The particles that were surrounded completely by bone were small, whereas a partial HA/bone contact was found for the larger particles. In some specimens, there were areas with newly formed bone exhibiting different degrees of maturation (Fig. 2). At higher magnification, no gaps or fibrous tissue was present at the bone–biomaterial interface (Fig. 3). The bone had grown inside the irregularities of the biomaterial surface (Fig. 4). In some areas of the specimens,

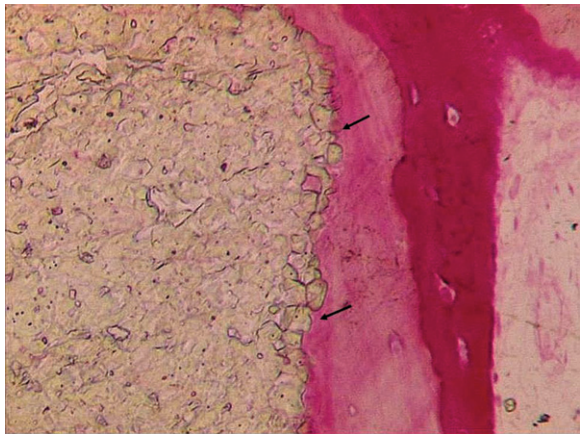

Figure 4.
Bone is found inside the surface irregularities of the graft particles. A remodeling area is present near one of the particles. Arrows denote surface irregularities. (Acid fuchsin–toluidine blue; original magnification ×40.)

¶ Precise 1 Automated System, Assing, Rome, Italy.
# Technovit 7200 VLC, Kulzer, Wehrheim, Germany.
** Laborlux S, Leitz, Wetzlar, Germany.
†† 3CCD, JVC KY-F55B, JVC, Yokohama, Japan.
‡‡ Intel Pentium III 1200 MMX, Intel, Santa Clara, CA.
§§ Matrix Vision, Oppenweiler, Germany.
‖‖ Image-Pro Plus 4.5, Media Cybernetics, Immagini & Computer, Milan, Italy.

osteocytes were observed near the bone–biomaterial interface, proving the biocompatibility of the material (Fig. 4). No inflammatory cell infiltrate or foreign body response was present. No macrophages or multinucleated giant cells were present. In only a few fields, it was possible to observe that the outer part of some particles had detached from the original particles' surface. Histomorphometry showed that bone represented 25.4% ± 3.2%, marrow spaces represented 41.3% ± 5.2%, and residual HA particles represented 38.1% ± 4.1%.

DISCUSSION

It is well known that ceramic materials are biocompatible and osteoconductive.[20] However, differences in the rate of resorption may play a critical role in determining the quantity of newly formed bone and, thus, the remodeling process.[20] The chemical composition, solubility, surface energy, surface morphology, crystalline structure, size, and shape of the particles influence the resorption rate of a biomaterial.[20]

Denissen and Mangano[25] described the histologic findings for dense HA (blocks), manufactured in the same way as that used in the present study and inserted in fresh extraction sites. In the early phase after insertion, the HA grafts seemed to be surrounded by fibroblasts and permeated by a variety of tissue fluids. However, 2 years after implantation, HA grafts were integrated strongly with the alveolar ridge, and no signs of inflammation were present. A layer of amorphous mineralized material, which acted as a highly efficient bonding structure, was interposed between the graft and the walls of the tooth socket, leaving no gaps at the interface. In a previous study,[21] two patterns were found at the bone–biomaterial interface under transmission electron microscopy: HA appeared to be in direct contact with the bone, and HA was separated from bone by a layer of electron-dense material. This layer is believed to represent a structure similar to the lamina limitans of bone and to play a crucial role in the strong bonding between bone and HA.

When remodeling occurs in contact with a biomaterial, such material undergoes physiologic bone turnover.[20] In the present study, intimate binding between bone and HA particles was present after a long-term implantation period (20 years); the grafted material appeared to be well incorporated into the patient's bone microstructure. Different maturation stages of bone tissue, identified by the different affinity for staining, were detected. These histologic results are similar to those reported by Oguchi et al.[21] and by Proussaefs et al.[23] after different implantation times (from 3.5 to 9 years).

However, the presence of mineralized tissue at the interface between bone and HA and the absence of an immune response or a foreign body reaction indicate a steady long-term biocompatibility of this biomaterial. This is in agreement with the data reported by Ayers et al.[22]

The slow resorption of HA can give rise to concerns for the long-term outcome of implants placed in HA-augmented sites. This issue has not been investigated extensively, especially in long-term studies. In a study by Haas et al.,[26] dental implants placed after maxillary sinus grafting with porous HA or autogenous bone were examined for their mechanical stress tolerance at 12, 16, and 26 weeks using pullout force. Mechanical tests of bone-to-implant contact in a sheep model showed that HA for one-stage sinus floor elevation significantly increased the pullout force compared to ungrafted sinuses, although it was less than that found with autogenous bone after 26 weeks. In a further study, Mangano et al.[27] evaluated the use of a porous HA, clinically, histologically, and immunohistochemically, as a grafting material for maxillary sinus augmentation with simultaneous implant placement. After a healing period of 5 to 6 months, all implants were clinically stable; they were followed for an average of 3 years. The histology showed newly formed bone in direct contact with the HA granules. Immunohistochemistry showed the presence of large quantities of bone sialoprotein and osteopontin in and around the granules of HA. The investigators concluded that HA could be considered a suitable material for sinus floor augmentation because it promoted bone regeneration, and it did not have a negative influence on the outcome of implant restorations.

HA is also used in the treatment of atrophic ridges. In a study by Mercier et al.,[28] dense HA was considered a predictable and stable biomaterial for ridge height preservation after treatment of 678 ridges with a follow-up ~5 years. El Deeb et al.[29] also reported an improvement in dentures' retention and stability 5 years after ridge augmentation with HA. To the best of our knowledge, there are no studies in the literature on implant placement in sites grafted with HA. The reported long-term stability of ridges reconstructed with HA may mean that implants might be placed in those sites because this material was demonstrated to be biocompatible and osteoconductive.

The presence of bone tissue in close contact with HA particles can be considered very promising for the long-term stability of the alveolar ridge height. Further studies are necessary for a better understanding of the resorption mechanism of HA grafts and potential implications in the field of implant dentistry.

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The authors report no conflicts of interest related to this case report.

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