A New Porous Hydroxyapatite for Promotion of Bone Regeneration in Maxillary Sinus Augmentation: Clinical and Histologic Study in Humans

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Purpose: *This study was undertaken to evaluate clinically, histologically, and immunohistochemically the use of a new porous hydroxyapatite (HA) (B. Agra, Cabon, Milan, Italy) as a grafting material for maxillary sinus augmentation with simultaneous implant placement.* ***Materials and Methods:*** *A total of 28 titanium implants were placed in 12 patients with an average of 4.5 mm of bone on the sinus floor. HA granules were packed around the implants in the sinus cavity. After a healing period of 5 to 6 months, second-stage surgery was carried out. In 5 patients, bone cores were harvested from grafted areas and processed for histology and immunocytochemistry.* ***Results:*** *All implants were clinically stable at second-stage surgery and 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.* ***Discussion and Conclusion:*** *This study suggests that a new porous HA accommodated sinus floor augmentation in patients with 3 to 5 mm of bone height preoperatively. By possibly attracting circulating biocomponents at sites of tissue repair, it may promote bone regeneration.* (Int J Oral Maxillofac Implants 2003;18:23–30)

Key words: *bone matrix, dental implants, hydroxyapatites, maxillary sinus, sinus augmentation, titanium*

Placement of endosseous implants in the atrophic maxilla is often complicated by a lack of supporting bone. Numerous investigations have indicated that sinus augmentation can be clinically successful with various graft materials, including autogenous bone, freeze-dried bone allograft, hydroxyapatites (HA), and bioactive glass.[1–5] Although the results of these investigations have indicated that sinus augmentation is clinically successful with various graft materials, it has not established which of these materials, except for autogenous bone, provides the best osteogenic potential and biomechanical properties. Autogenous bone remains the material of choice currently available for bone reconstructive procedures; however, its use can be restricted by the limited amount of graft material that can be harvested intraorally and the need for general anesthesia for bone harvesting from extraoral sites in cases of major augmentation procedures.[6–8]

Because of its biocompatibility, HA is commonly used for bone augmentation and reconstruction. Ripamonti[9] reported that sintered HA implanted in the muscles of baboons induced bone formation via intrinsic osteoinductivity regulated by the geometry of the substratum.

Osteoinduction has been reported in porous HA implanted in heterotopic sites of different animal models.[9] The mechanism of osteoinduction of calcium phosphate ceramics is not clear; it appears to be both material-dependent and animal-dependent.[10,11] Tissue engineering of bone requires 3 key components: a soluble osteoinductive signal, a suitable insoluble substratum that delivers the signal and acts as a scaffold for new bone to form, and responding host cells capable of differentiation into bone cells.[12–14] The signals responsible for osteoinduction are the

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bone morphogenetic proteins (BMP-2 to BMP-14) and osteogenic proteins (OP-1 and OP-2, also known as BMP-7 and BMP-8).[15] These proteins have the capacity to induce de novo bone formation when implanted in extraskeletal sites of animals.[16] It is interesting to note that bone formation has been observed only when porous HA was implanted in block configuration.[17,18] Recently, monolithic disks of sintered HA fabricated with concavities of 400 to 1,600 µm were implanted in the rectus abdominis of the baboon *(Papio ursinus)*. Histology revealed the generation of bone exclusively within the concavities of the substratum. The same study revealed the immunolocalization of BMP-3 and OP-1/BMP-7 at the HA interface. This study demonstrated that by manipulating the geometry of the substratum, it was possible to activate osteogenesis. This phenomenon was defined as the geometric induction of bone formation.[19]

Osteoinduction implicates a series of cellular and extracellular events, such as the formation of an electron-dense layer, referred to as the lamina limitans.[20,21] This organic matrix comprises bone sialoprotein (BSP) and osteopontin (OPN), which are capable of regulating mineral deposition, and precedes active bone formation.[22,23] OPN seems to act as a bridge between bone cells and HA and is involved in bone formation.[24] This activity is indicated through its expression by pre-osteoblasts and osteoblasts, by its presence in osteoid and stimulated synthesis in the presence of hormones and cytokines, such as glucocorticosteroids and TGF-beta, which promote bone matrix formation.[25,26] Another specific activity of OPN is its capability of HA nucleator. Once mineralization has been initiated, OPN is associated with the preformed mineral and regulates the growth of HA crystals; this protein seems to have a role in the attachment of osteoblasts to osteoid during bone formation. BSP binds strongly to HA and mediates cell attachment through RGD sites.[27]

While OPN is expressed in nonmineralized tissues, BSP is restricted to mineralized connective tissues, especially in bone matrix and with directly associated cells. The role of BSP is principally the promotion of mineralization and the regulation of HA crystal growth.[28] BSP and OPN are the major constituents of the newly formed bone.

All of these studies suggest that a porous HA with specific geometric characteristics can act as a solid substratum for adsorption and storage of endogenously produced BMPs/OPs, which locally initiate bone formation. The purpose of the present study was to evaluate clinically, histologically, and immunohistochemically the use of a new porous HA (B. Agra, Cabon, Milan, Italy) as a grafting material for maxillary sinus augmentation procedures with simultaneous implant placement in humans.

MATERIALS AND METHODS

Hydroxyapatite

The HA was sintered starting from powder constituted of calcium phosphorous (Ca/P = 1.67 ± 0.03) to form granules with a diameter ranging from 250 to 600 µm. This HA is characterized by a very low density and crystallinity, with the grains presenting variable dimensions (0.05 to 1 µm). Another specific characteristic of the material is a high degree of bimodal porosity (ranging from nanodimension to 10 µm and from 10 to 60 µm). Chemical and physical characteristics of sintered HA were determined by x-ray diffraction (XRD) (Fig 1a) and scanning electron microscopy (Figs 1b and 1c). The HA was sterilized by gamma irradiation (25 kGy).

Patients

Twelve patients were selected on the basis of the following criteria: *(1)* maxillary partial (unilateral or bilateral) edentulism involving the premolar/molar areas and *(2)* presence of 3 to 5 mm of crestal bone between the sinus floor and alveolar ridge, as evidenced at baseline by preoperative radiographic examination. Excluded from the study were smokers, patients with systemic diseases or maxillary sinus pathology, patients with recent extractions (less than 1 year) in the involved area, and patients in whom primary stability could not be established. At the initial visit, all patients received a clinical and occlusal examination and periapical and panoramic radiographs. Informed consent was obtained from all patients. The 12 patients in this study included 4 women and 8 men ranging in age between 42 and 67 years, with a mean of 54 years. A total of 28 screw-type implants (Mac System, Cabon) were placed simultaneously with sinus augmentation. The average bone thickness of the sinus floor was 4.5 mm (Table 1).

Surgical Protocol

Immediately prior to surgery, patients rinsed with a 0.2% chlorhexidine digluconate solution for 2 minutes. Local anesthesia was obtained with articaine (Ubistesin 4%, Espe Dental, Seefeld, Germany) associated with epinephrine 1:200,000. A crestal incision was made slightly palatal supplemented by buccal releasing incisions mesially and distally. Full-thickness flaps were elevated to expose the alveolar crest and the lateral wall of the maxillary sinus. Using a round bur under cold sterile saline irrigation (4°C to

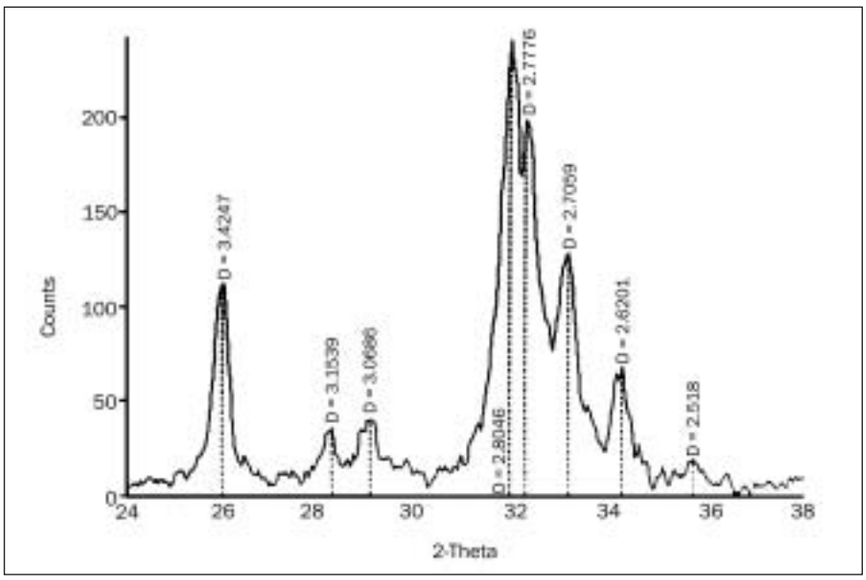

Fig 1a X-ray diffraction of hydroxyapatite (HA).

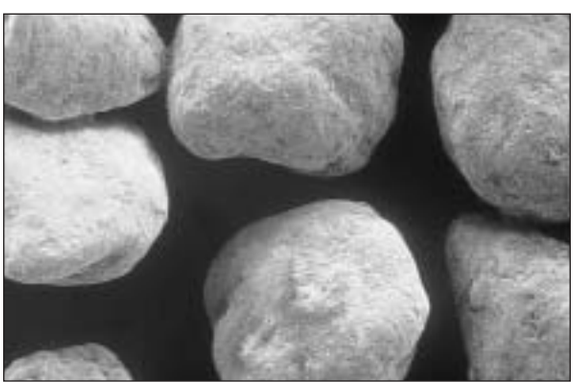

Fig 1b HA granules with a diameter ranging from 250 to 600 µm. Note the potato shape of the granules (SEM; magnification ×50).

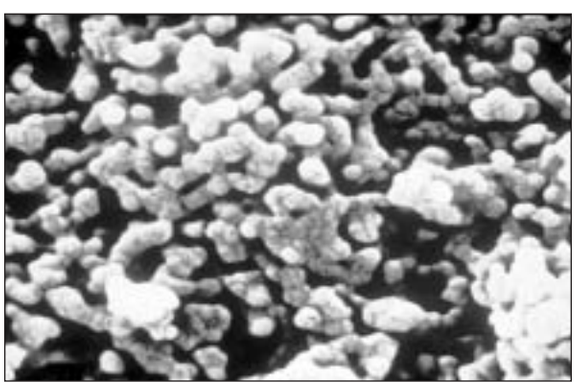

Fig 1c HA surface at higher magnification (×1,000).

5°C), a trap door was made in the lateral sinus wall (Fig 2a). The door was rotated inward and upward with a top hinge to a horizontal position. The sinus membrane was elevated with curettes of different shapes until it became completely detached from the inferior wall of the sinus. Preparation of the implant sites was undertaken according to the conventional surgical protocol.[28] The HA graft was mixed with cold (4°C to 5°C) sterile saline solution and carefully packed in the sinus cavity, especially in the posterior and in the anterior part (Fig 2b). Following graft placement, implants were placed and the remaining sinus space around the implants was completely packed with the HA graft. Care was taken to pack the graft around the apices of the implants. Finally, the flaps were sutured (Fig 2c). Antibiotics (amoxicillin 1,000 mg 2 times per day) were prescribed for 1 week

Table 1 Patient Data for Implants Placed in HA-Augmented Sinuses

Patient	Implant site(s)	Crestal bone height (mm)
1	Right first premolar–right first molar	5
2	Left first and second premolars	4
3	Left second premolar and first molar	3
4	Left first moalr	4
5	Right second molar–second premolar	5
6	Left first premolar–first molar	5
7	Right first and second premolars	4
8	Right first molar and second premolar	5
9	Left first and second molars	5
10	Left first and second premolars	4
11	Right first and second molars	5
12	Right first premolar–second molar	5

Total implants placed: 28. Mean bone height = 4.5 mm.

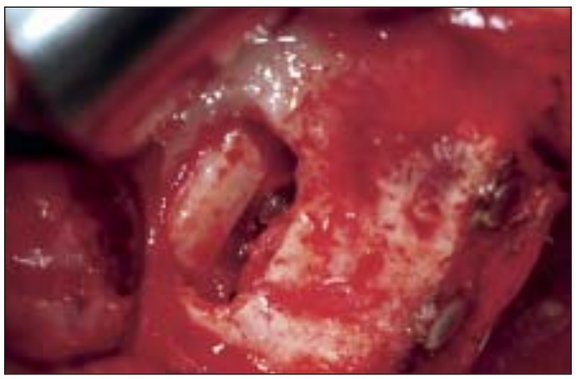

Fig 2a Lateral aspect of buccal access window after dissection and elevation of the sinus membrane.

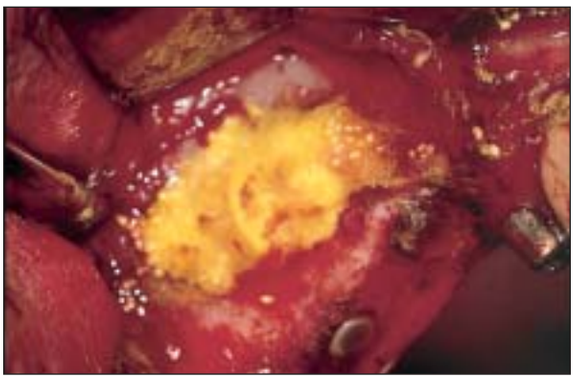

Fig 2b The HA is condensed around the implants, filling the buccal window.

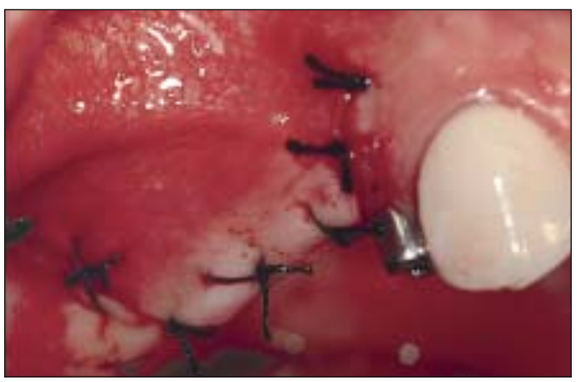

Fig 2c The mucoperiosteal flap is sutured.

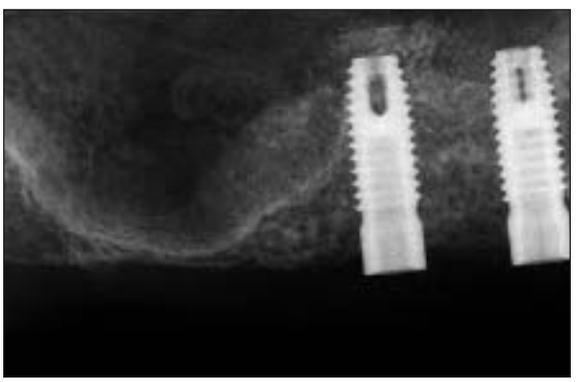

Fig 2d Postoperative radiograph.

and analgesics as required. Sutures were removed 2 weeks after surgery. Postsurgical visits were scheduled at monthly intervals to check the course of healing. After a healing period of 5 to 6 months, second-stage surgery was carried out to connect the healing abutments to the implants; at this time periapical radiographs were repeated. All implants appeared to be clinically stable and radiographically osseointegrated (Fig 2d).

Histologic Examination

In 5 patients, bone cores were harvested from the lateral wall using a 4×10-mm trephine under cold (4°C to 5°C) sterile saline irrigation. The biopsies were retrieved from areas located between the implants at about 10 mm from the alveolar ridge, at a mean depth of 8 mm. The bone cores were immediately fixed in 10% neutral buffered formaldehyde, decalcified in a formic–hydrochloric acid mixture, and double-embedded in celloidin and paraffin wax. Serial sections, 5 μm thick, were obtained. Sections were stained with Goldner's trichrome and examined with a Provis AX70 research microscope (Olympus Optical, Tokyo, Japan).

Immunohistochemical Evaluation

In a second phase of the investigation, an immunohistochemical evaluation of patients treated with the porous HA was undertaken.

The tissue was placed in 4% paraformaldehyde + 0.1% glutaraldehyde buffered with 0.1 mol/L sodium phosphate, ph 7.2, and fixed for 24 hours at 4°C. It was then washed in 0.1 mol/L phosphate buffer and decalcified for 6 hours at 4°C in Plank-Richlo's solution (12.67 g $AlCl_3$ [aluminum chloride], 8.5 mL of 10NHCl [hydrochloric acid], and 5.4 mL of 88% formic acid adjusted to 100 mL with distilled water).[29] The decalcified specimens were washed in the same buffer, dehydrated in a graded series of ethanols, and processed for embedding in LR White hard grade acrylic resin (London Resin Company, Theale, Berkshire, England). Ultrathin sections (100 nm) were cut with a diamond knife, mounted on nickel grids, and processed for postembedding colloidal gold immunocytochemistry with antibodies to human BSP (LF-6) and OPN (LF-7) (both antibodies courtesy of National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD). The grid-mounted

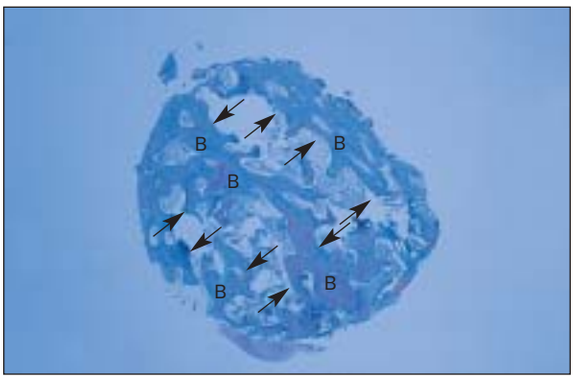

Fig 3a Photomicrograph of biopsy retrieved at 6 months postoperatively. New bone (B) is formed around HA granules (Goldner's trichrome; magnification ×6). Arrows indicate new bone.

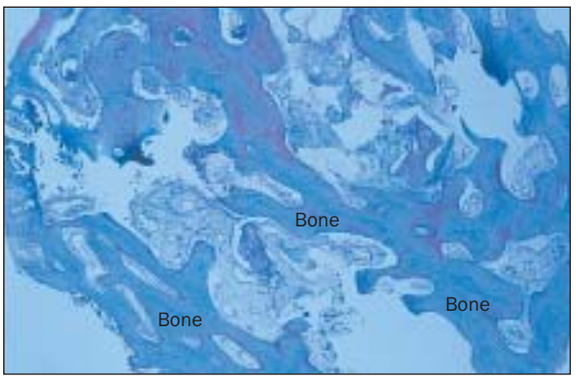

Fig 3b Higher magnification showing new lamellar bone formed around HA granules (Goldner's trichrome; magnification ×30).

sections were first blocked with 0.01 mol/L phosphate-buffered saline (PBS) containing 1% ovalbumin. They were then floated, section face down, onto a drop of antibody for 1 hour, rinsed with PBS, blocked with PBS ovalbumin, and floated onto a drop of protein A–gold complex prepared as described in Bendayan[30] using 8- to 10-nm colloidal gold particles.[31] After washing with PBS and distilled water, the grids were stained with uranyl acetate and lead citrate and examined in a Jeol JEM 1200 Ex-II transmission electron microscope operated at 60 Kv (Tokyo, Japan).

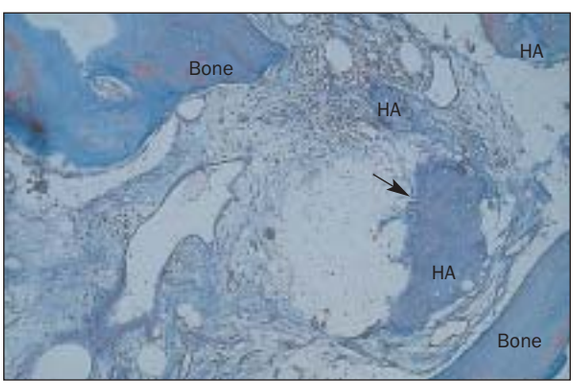

Fig 3c Higher magnification shows multinucleated cells that have resorbed some particles of a HA granule surrounded by new bone (Goldner's trichrome; magnification ×60). Arrow indicates HA resorbed particles by multinucleated cells.

RESULTS

Clinical Observations

None of the 12 patients had complications, other than normal swelling and inflammation at the surgical sites. All implants were stable at the time of the abutment connection and received provisional fixed acrylic resin prostheses for at least 6 months. After this period, all patients underwent definitive prosthetic rehabilitation with metal-ceramic fixed prostheses. All 28 implants were stable and were successfully integrated according to the criteria of Albrektsson and coworkers.[28] Ten patients (23 implants) were followed for up to 3 years after implant placement; the remaining 2 patients (5 implants) were followed for 1 year.

Periapical radiographs and orthopantomograms obtained after 12 months of prosthetic loading showed a dense mineralized material in the sinus cavities surrounding the implants.

Histologic Results

The histology showed newly formed bone in direct contact with the HA. Multinucleated cells were in contact with the HA surface and directly involved in HA resorption (Figs 3a to 3c). The newly formed bone was remodeled in lamellar-osteonic bone attached to HA particles. A large number of osteoblast cells faced the fibrovascular tissue between the HA particles. Angiogenesis was a pronounced histologic feature, and newly formed vessels were in close contact with the newly formed trabeculae that developed within and onto the HA surface. The formed bone was found protruding into the porous space of HA (Figs 4a to 4c).

Decalcification of the specimens clearly revealed that the HA granules were infiltrated with organic matrix that essentially replicated the intergrain space and outlined a network of nanopores through the granule. In many cases, the granules also exhibited an electron-dense layer of variable thickness that coated part or all of their surfaces.

Immunolabeling revealed the presence of BSP and OPN within the granules, and the surface coating was immunoreactive for BSP and OPN (Figs 5 and 6).

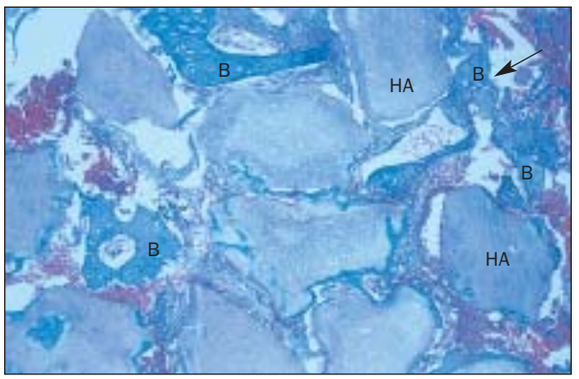

Fig 4a Another sample retrieved at 6 months postoperatively. Higher magnification suggests that a higher percentage of bone (B) volume has formed among the HA particles (Goldner's trichrome; magnification ×60). Arrow indicates new bone.

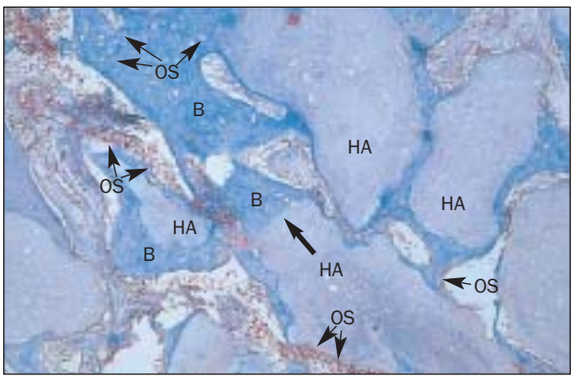

Fig 4b Bone (B) completely surrounds HA granules; several osteocytes (Oc) are present. An elongated HA granule was substituted in its triangular terminal part by new bone *(large arrow).* Osteoblast (Os) cells are apparent adjacent to new bone *(red)* (Goldner's trichrome; magnification ×30). Smaller arrows indicate osteoblasts.

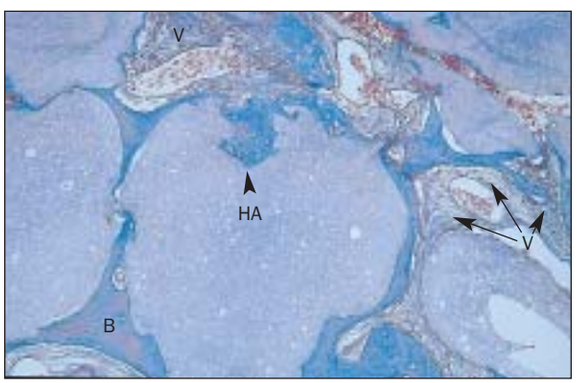

Fig 4c HA granules are surrounded by new bone (B) within microcavity of particles. Note the rich microvascular infiltrate (V) (Goldner's trichrome; magnification ×60). Arrows indicate microvascular infiltrate.

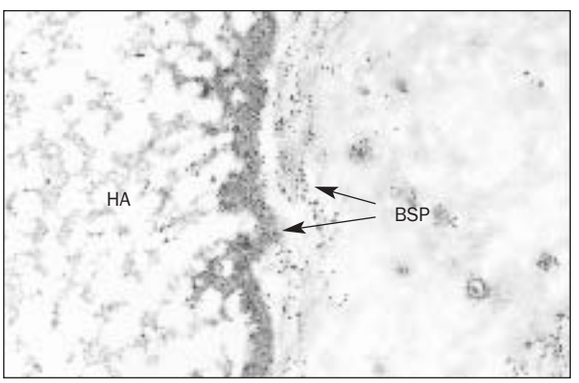

Fig 5 Immunocytochemical preparations with anti-bone sialoprotein (BSP) antibodies illustrating the presence of this non-collagenous bone matrix protein both within and at the surface of the HA granules.

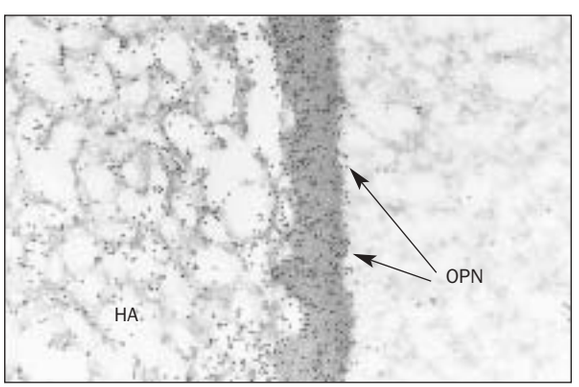

Fig 6 Colloidal gold immunocytochemistry. On the granule *(left)* an osteopontin protein layer (OPN) is deposited. Note the large quantities of gold particles deposited on the HA surface and inside the granule.

DISCUSSION

The present study evaluated the performance of a new HA material (B. Agra, Cabon) used for sinus augmentation with simultaneous implant placement in patients with 3 to 5 mm of bone height prior to grafting. The 1-step procedure offers the advantages of reducing the number of surgical steps and the time needed to complete the prosthetic restoration. Various clinical investigations have indicated that sinus augmentation can be clinically successful with various grafting materials, but autogenous bone still provides the best osteogenic potential for regenerating bone.[1,3,4,6,8] Allografts used as an alternative to autografts present several disadvantages, such as delayed resorption of particles and potential for disease transmission. HA is a synthetic material

and its biocompatibility has been demonstrated by various studies.[9,17] It was recently proposed as an ideal material to be used in tissue engineering as a delivery system.[32]

The question of placing implants simultaneously or delayed in conjunction with sinus floor augmentation procedures is controversial. If the residual bone volume is more than 5 mm in height, primary stability of the implants can usually be achieved. Therefore, simultaneous implant placement is advocated.[33] In the present study, the crestal height was between 3 and 5 mm in all cases, and primary stability could only be achieved by placing the implants so as to touch the bony door that was rotated inward and upward in the sinus and by packing the HA all around the implants.

The histology that was performed 6 months after implant placement showed close contact between the HA and the newly formed lamellar bone. The new bone developed within and onto the surface of HA particles, and angiogenesis was a prominent feature. A large number of osteoblasts were visible between the HA particles, and a number of multinucleated cells were in close contact with the material surface and apparently were involved in the resorption of some particles. It has been shown at sites of active bone deposition in the rat that implanted HA granules were infiltrated and became coated with bone matrix proteins such as BSP and OPN.[12] The present study confirms in the human that BSP and OPN infiltrate HA granules and "coat" them. Tissue engineering of bone requires 3 key components: an osteoinductive signal, a substratum that acts as a scaffold for new bone to form, and host cells capable of differentiation into bone cells.[34] The signals responsible for osteoinduction are the BMPs and OPs.[35] Bone morphogenesis in porous HA in block configuration has been reported in the absence of exogenously applied BMPs/OPs.[36] The present study demonstrated that the accumulation of bone sialoproteins, an event associated with bone modeling and remodeling, takes place not only at the surface but also within the nanopores of HA granules.

CONCLUSION

This study has indicated that a new HA, when used for sinus augmentation with simultaneous implant placement, resulted in short-term implant stability and survival in this patient population. Biopsies taken 6 months after implantation and processed for histologic and immunolabeling analysis showed formation of new bone in and around the HA particles, thus indicating that this new material may have the capacity to induce bone formation, attracting circulating biocomponents.

ACKNOWLEDGMENTS

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