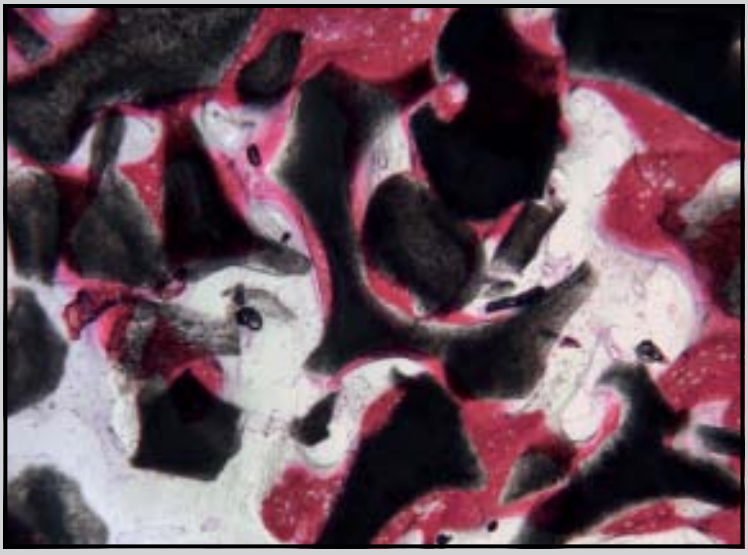
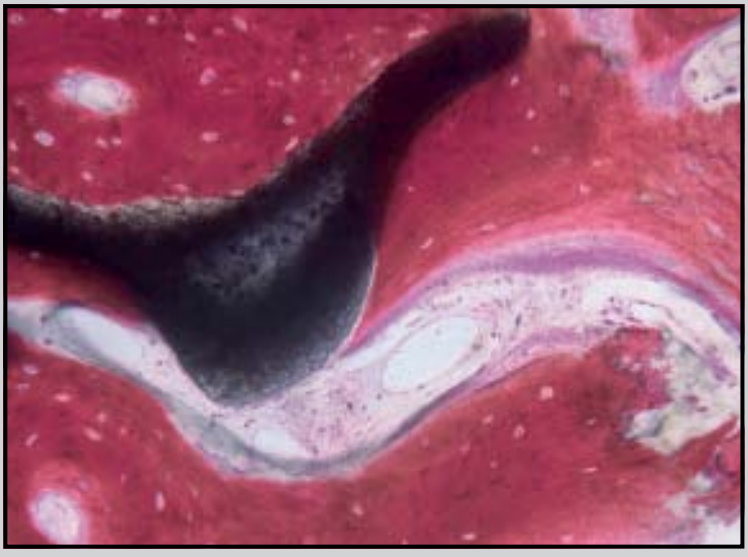




High Temperature–Treated Bovine Porous Hydroxyapatite in Sinus Augmentation Procedures: A Case Report

Tiziano Testori, MD, DDS[1]/Giovanna Iezzi, MD, PhD[2]
Licia Manzon, MD, DDS[3]/Giovanni Fratto, MD, DDS[3]
Adriano Piattelli, MD, DDS[4]/Roberto L. Weinstein, MD, DDS[5]

Among the graft materials that can be used clinically, xenografts are the most common. Xenografts are of bovine, porcine, or equine origin and require the complete removal of proteins to avoid immunologic problems and the risk of transmission of prions, viruses, etc. Protein destruction can be achieved by a chemical procedure using organic solvents and heat treatment. After this process, a carbonated hydroxyapatite similar to human bone remains. The aim of this case report is to investigate the bone formation in a sinus augmentation procedure using a high temperature–treated bovine porous hydroxyapatite. A 58-year-old woman underwent bilateral sinus augmentation using this biomaterial. After 9 months, during stage-two surgery, two core biopsy specimens were retrieved and treated to obtain thin ground undecalcified sections. Microscopically, newly formed bone was present at the interface with most particles. The major portion of the particles appeared to be completely lined and surrounded by bone. No obvious signs of resorption were present on the biomaterial surface. No gaps or connective tissue were present at the bone-biomaterial interface. No inflammatory infiltrate or fibrous encapsulation of the particles was present. Histomorphometry showed that the percentages of newly formed bone, residual grafted particles, and marrow spaces were 25.1% ± 2.3%, 37.3% ± 1.1%, and 38.5% ± 3.1%, respectively. The excellent properties demonstrated by Endobon are probably a result of its particular hydroxyapatite porous microstructure with a high percentage of interconnected micropores that promote the ingrowth of osteogenic cells and vessels, making graft integration easier and faster. (Int J Periodontics Restorative Dent 2012;32:295–301.)

[1]Head, Section of Implant Dentistry and Oral Rehabilitation, Department of Odontology, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Galeazzi Institute, University of Milan, Milan, Italy.
[2]Researcher, Dental School, University of Chieti, Chieti, Italy.
[3]Full Professor, Dental School, Sapienza University, Rome, Italy.
[4]Full Professor and Chairman, Dental School, University of Chieti, Chieti, Italy.
[5]Full Professor and Chairman, Department of Odontology, IRCCS, Galeazzi Institute, University of Milan, Milan, Italy.

Correspondence to: Dr Tiziano Testori, Section of Implant Dentistry and Oral Rehabilitation, IRCCS, Galeazzi Institute, Via Riccardo Galeazzi, 4, 20161 Milano, Italy; email: info@tiziano-testori.it.

Presently, there is an increased demand for bone grafts; however, the supply of autologous bone, which is still considered the golden standard in bone regeneration procedures, is often limited clinically. Therefore, alternative bone sources with physical and chemical properties similar to human bone are needed.

Human bone consists of an organic part mainly composed of cells and blood vessels and an inorganic part mainly composed of hydroxyapatite (HA) crystals. HA may derive from human and animal bone, coral, or a synthetic process. HA, with its typical porous three-dimensional structure, offers structural support and acts as a matrix, allowing ingrowth of osteogenic cells and vessels to allow for new bone formation.[1–6] HA is available in a solid or granular form in various sizes and porosities.

An ideal graft material should be biocompatible, nontoxic, radiopaque, sterile, and inexpensive, possess excellent mechanical properties; be available in various forms to fulfill all clinical needs; and, most

importantly, stimulate bone production through three processes: osteogenesis, osteoinduction, and osteoconduction.[4–7] Blood support is very important for graft survival. Osteoconduction is the capability of a graft material to guide bone growth toward and along its surface. Bone ingrowth is promoted by the presence of pores in the structure of the graft.[2,4,5,8] A high percentage of pores will ensure that a high number of mesenchymal cells and osteoblasts will reach the site where bone regeneration is taking place. Another factor that has been shown to affect the progression of osteogenesis is the pore size.[2,5,9] Small pores promote hypoxic conditions and induce osteochondral formation before osteogenesis, while large pores, which are well vascularized, lead to direct osteogenesis without the formation of cartilage beforehand.[10] Some confusion still exists regarding the mechanisms of bone ingrowth in porous scaffolds. Some authors assert that the minimal pore size to allow bone ingrowth is approximately 100 μm,[8] while others believe that macropores up to 100 μm could be sufficient for bone ingrowth but that a larger macropore size is more suitable during the first healing period[9]; macropores of up to 300 μm have also been proposed.[11] An increase in the volume of bone ingrowth proportional to an increasing pore size has also been reported.[8,9] It is believed that pore morphology, percentage of porosity, and interconnectivity of pores are important for a faster bone ingrowth that influences[12] the primary and secondary stabilization of the implants.[13] Similar to every ceramic biomaterial, HA is brittle and can sustain high compressive forces but is easily broken by torsional or bending forces. The mechanical properties of a bone graft substitute are related to porosity, structural architecture, trabecular dimension, and thickness.[14] Faster bone ingrowth seems to be favored by a more porous interconnected structure, while denser ceramics present better mechanical properties. Many authors[2–5,14–16] have emphasized the importance of pore connectivity, which seems to be important in determining the rate of new bone formation, especially during early healing periods. A high connectivity would seem to promote the rate of osseointegration.

Among the graft materials that can be used clinically, xenografts are the most common. Xenografts are of bovine, porcine, or equine origin and require the complete removal of proteins to avoid immunologic problems and the risk of transmission of prions, viruses, etc. Protein destruction can be achieved by a chemical procedure using organic solvents (phenols and ethylene glycol) and heat treatment to a temperature of 300°C (unsintered materials; Bio-Oss, Geistlich) or using a heat treatment based on the calcination of the material by its exposure at temperatures between 600°C and 900°C, reaching a peak of 1,000°C to 1,200°C (sintered materials; BonAP, Endobon, Biomet 3i). After this process, a carbonated HA similar to human bone remains. No antigenic or immunogenic inflammatory response or local/systemic toxicity has been reported with the use of deproteinized bovine bone graft. However, some questions remain about the resorbability of these xenografts. Some animal studies have reported extensive resorption,[17] while other studies report only a slight[18] or no resorption.[19]

The aim of this case report is to investigate the bone formation in a sinus augmentation procedure using high temperature–treated bovine porous HA.

Method and materials

A healthy 58-year-old woman came to the School of Dentistry, IRCCS, Galeazzi Institute, Milan, Italy, seeking implant treatment. Her chief complaint was the functional and psychologic discomfort of the complete denture she wore after having recently become edentulous. The medical history of the patient was noncontributory, and her dental history revealed that the cause of the maxillary edentulism was periodontal disease combined with endodontic and restorative problems with the previous fixed full-arch restoration. A comprehensive extra- and intraoral examination was carried out; mounted study casts were created, and radiologic examinations (panoramic radiographs and computed tomography [CT] scans) were accomplished

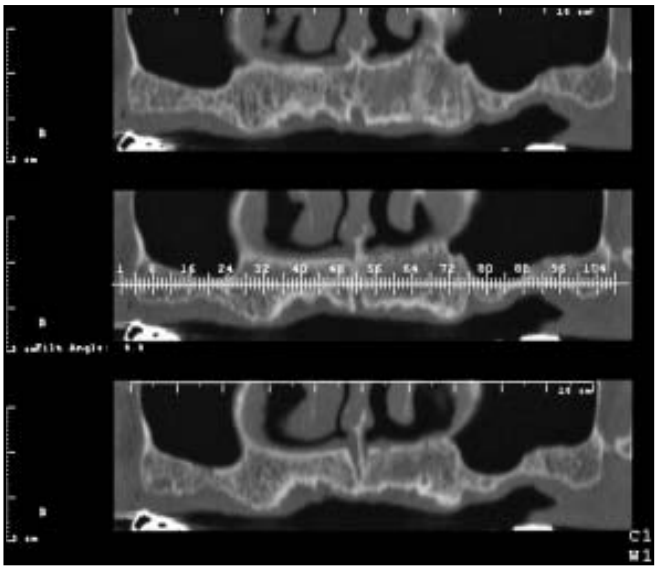


Fig 1 *CT scan showing hyperpneumatized sinuses.*

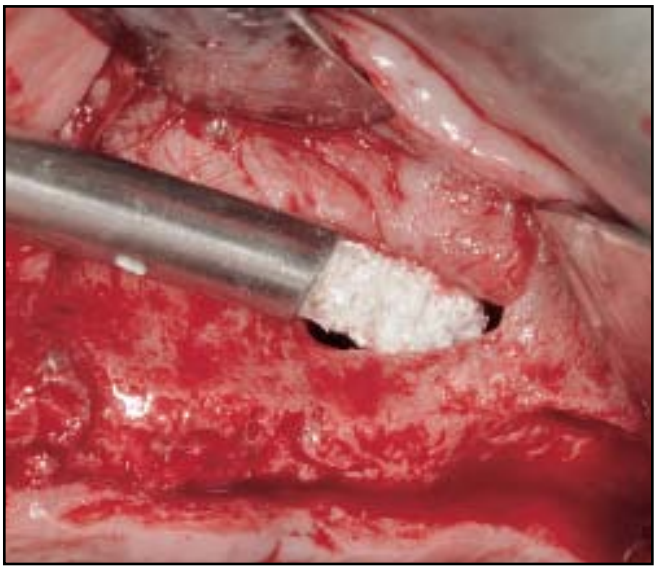
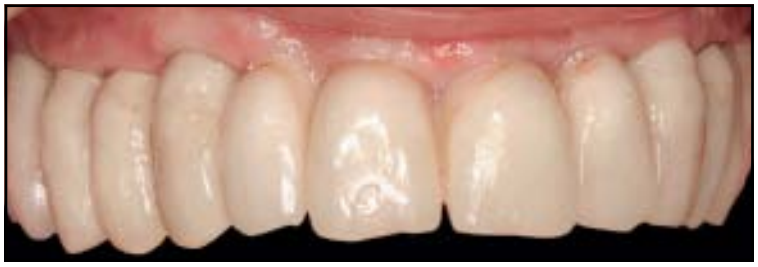
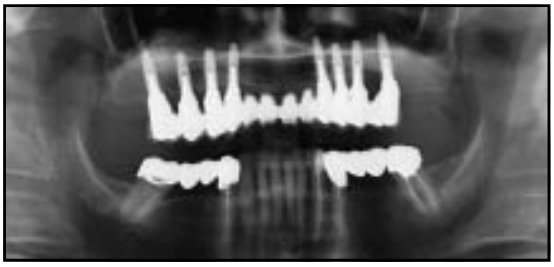


Fig 2 *Graft placement into the sinus.*

as well (Fig 1). Different treatment plans were discussed with the patient, and it was decided that the best option to meet the patient's expectations of a fixed full-arch restoration was bilateral sinus elevations with simultaneous implant placement along with horizontal ridge expansion.

The first surgical phase was carried out. A full-thickness flap was elevated, and bilateral sinus elevations with deproteinized bovine bone in the 500 to 1,000 µm granular form (Fig 2) (Endobon), combined with treatment of the implant vestibular dehiscence using the same regenerative material covered with a resorbable membrane (Osseoguard, Biomet 3i), were performed. Endobon is an HA ceramic obtained from bovine spongiosa with a high-temperature treatment (approximately 1,200°C)[20,21] by hydrothermal defatting and calcination.[22] Endobon is composed of a partially carbonate-substituted HA containing minor ionic impurities.[12] The manufacturing consists of an oxidation of nitrogen and carbon with pyrolysis (at 450°C to 550°C for 24 hours and subsequently at 700°C to 900°C for 5 hours); an additional complete removal of all organic structures is also carried out.[22] A sintering procedure (approximately 1,200°C for 5 hours) resulted in a crystalline-like structure of > 95% of the HA.[1,22,23] The final porosity of the material is between 30% and 80%.[13] The healing process was uneventful, and 9 months later, a postsurgical CT scan was performed and the





Figs 3a and 3b *Postoperative* (left) *clinical image and* (right) *panoramic radiograph showing the definitive posts and full-arch restoration.*

patient was scheduled for stage-two surgery. A crestal palatal incision was performed to elevate a full-thickness flap to increase the amount of keratinized tissue on the vestibular aspect. The reason for using a full-thickness flap instead of a partial-thickness flap was the need to expose the vestibular bone plate to recover two biopsy specimens (with the patient's informed consent) for histologic evaluation. Two biopsy specimens at the site of the horizontal regeneration were recovered using a surgical chisel. The specimens were cleaned in saline solution, placed in a sterile vial containing 10% buffered formalin solution, and sent to the lab for histologic analysis. The bony defects were filled with deproteinized bovine bone and covered with a resorbable membrane. After 2 additional months of healing, the prosthetic phase began, and individual posts and a definitive restoration were placed (Figs 3a and 3b).

Processing of specimens

Specimens were processed to obtain thin ground sections with the Precise 1 Automated System (Assing). Specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycol methacrylate resin (Technovit 7200 VLC, Heraeus Kulzer). After polymerization, the specimens were sectioned longitudinally along their major axis with a high-precision diamond disk at approximately 150 µm and ground to approximately 30 µm. Three slides were obtained for each specimen. The slides were stained with basic fuchsin and toluidine blue. A double-staining with von Kossa and acid fuchsin was done to evaluate the degree of bone mineralization, and one slide, after polishing, was immersed in silver nitrate for 30 minutes and exposed to sunlight. The slides were then washed under tap water, dried, immersed in basic fuchsin for 5 minutes, washed, and mounted.

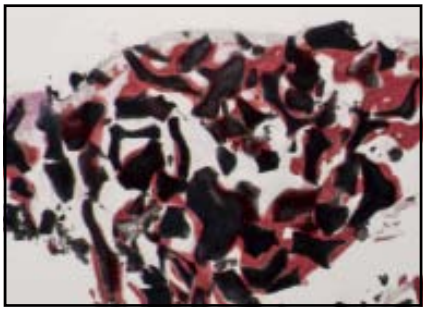


Fig 4 *Histomorphometry of the biopsy specimens. Note the presence of newly formed bone at the interface with most of the biomaterial particles (toluidine blue and acid fuchsin, magnification ×8).*

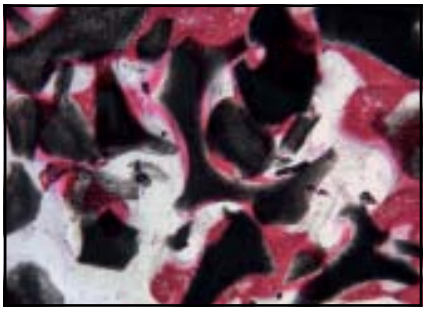


Fig 5 *Bone completely lined and surrounded the major portion of the particles. Bone trabeculae appeared to bridge the different biomaterial particles (toluidine blue and acid fuchsin, magnification ×40).*

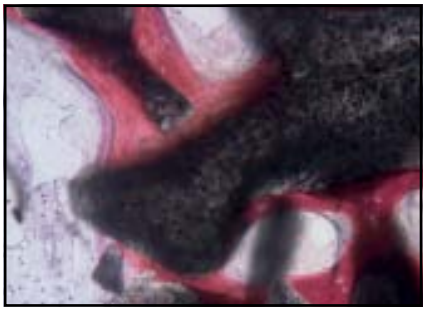


Fig 6 *No gaps were present at the newly formed bone–graft interface, and the bone was always in close contact with the biomaterial (toluidine blue and acid fuchsin, magnification ×100).*

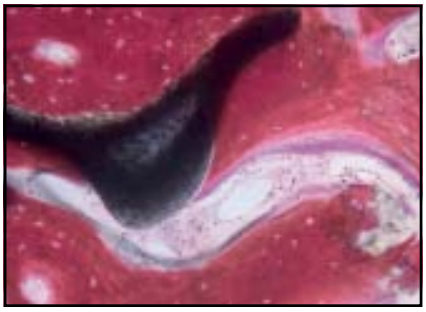
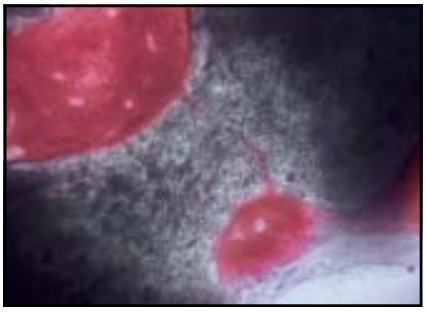


Fig 7 (left) *No gaps or connective tissue were present at the bone-biomaterial interface (toluidine blue and acid fuchsin, magnification ×100).*

Fig 8 (right) *Large osteocytic lacunae typical of recently mineralized tissue (toluidine blue and acid fuchsin, magnification ×200).*



Histomorphometry

Percentages of residual grafted biomaterials, newly formed bone, and marrow spaces were determined using a light microscope (Laborlux S, Leitz) connected to a high-resolution video camera (3CCD KY-F55B, JVC) and interfaced to a monitor and personal computer (Intel Pentium III 1200 MMX, Intel). This optical system was associated with a digitizing pad (Matrix Vision) and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics).

Results

At low-power magnification, the biomaterial particles were easily identified, and it was possible to observe the presence of newly formed bone at the interface with most of the particles (Fig 4). Particle size varied considerably. The major portion of the particles appeared to be completely lined and surrounded by bone (Fig 5). No obvious signs of resorption were present on the biomaterial surface, and bone trabeculae appeared to bridge the different biomaterial particles (Fig 6). At higher magnification, no gaps or connective tissue were present at the bone-biomaterial interface (Fig 7). Osteoblasts were present only around some particles. No macrophages or osteoclasts were visible on the bone-free portion of the graft particle surface. In most particles, osteocytes were very close to the surface of the biomaterial particle (Fig 8). No inflammatory infiltrate or fibrous encapsulation of the particles were present. Histomorphometry showed that the percentages of newly formed bone, residual grafted particles, and marrow spaces were 25.1% ± 2.3%, 37.3% ± 1.1%, and 38.5% ± 3.1%, respectively.

Discussion

Endobon undergoes a heat treatment to remove the organic component to prevent immunologic and allergic reactions. After that treatment, the HA preserves its trabecular structure and mineral matrix, physically and chemically comparable to the mineralized matrix of human bone, with a three-dimensional interconnecting network of micro- and macropores.[12] Its open pore structure[12] demonstrated two types of microporosity: micropores < 3 μm that appeared isolated and spheric, randomly distributed throughout the ceramic strut, probably formed during the sintering process, and micropores > 3 μm that were ellipsoidal in shape, probably resulting from preserved osteocyte lacunae from the original microstructure of the bone that were interconnected with one another. The first type of micropores were more numerous than the second.

The rationale behind the use of a porous structure in bone regeneration techniques is derived from the observation of a thinner fibrous capsule around porous implants resulting from mechanical interlocking due to tissue penetration.[16] Endobon has a density of 0.35 to 1.44 g/cm^3; the chemical analysis demonstrated that the natural apatite precursor of Endobon was not converted to pure HA but retained many of the ionic constituents found in the bone mineral, notably carbonate, sodium, and magnesium ions.[12] An analysis of the micro- and macrostructure of this biomaterial showed that the struts of the material had retained some traces of the osteocyte lacunae network that gave the porous structure to the material. Endobon is available on the market in two granular forms (500 to 1,000 μm and 1,000 to 2,000 μm) and in packages of different quantities (0.5, 1.0, and 2.0 mL for small granules and 2.0, 5.0, and 8.0 mL for large particles). The granules seem to facilitate a more rapid integration,[24] increasing the surface contact between the graft and human living bone. This biomaterial has been used since 1992 in orthopedics as a grafting material for many bone disorders (cysts or tumors) and various bone injuries (fractures or total joint replacements). The use of Endobon as a bone substitute in orthopedics has led to a high percentage of clinical success evaluated with either radiologic investigations,[1,24,25] magnetic resonance imaging,[21–26] or radionuclide imaging.[1] Positive results were reported up to 5 years postoperatively.[25] The time and modality of graft integration would be similar to natural bone regeneration following a bone fracture where the defect is initially filled by the blood clot and then invaded by vessels, mesenchymal cells, osteoblasts, and fibroblasts. The bone ingrowth begins at 2 weeks and continues until the sixth month. Twelve months are required for complete healing. Several authors[27] have reported that after Endobon insertion, healing was similar to that of fractured natural bone and that the majority of bone ingrowth occurred between 10 days and 5 weeks after implantation.

The present results showed that this biomaterial was biocompatible and osteoconductive. Studies[3–5] using other types of HA have shown that coralline HA (mean pore size, 230 μm) and synthetic HA (approximate pore size, 10 μm) showed retarded new bone formation and marginal osseous integration with incomplete bone penetration in the center of the implants.[4–6] In a rabbit study, the presence of macrophages and osteoclasts on the coralline porous HA implant surfaces was reported up to 6 months after implantation[5]; no osteoclasts and macrophages were observed in the present specimens. The capacity to form new bone observed in the present specimens could be a result of the higher mechanical performance of the biomaterial.[27]

Conclusions

The excellent properties demonstrated by Endobon are probably because of its particular HA porous microstructure with a high percentage of interconnected micropores that promote the ingrowth of osteogenic cells and vessels, making graft integration easier and faster. The open macroporous structure, similar to that of cancellous bone, promotes the complete infiltration of bone, bone marrow, and blood vessels.[12,14,16] Endobon HA acts as a scaffold for cells and vessels, demonstrating osteoconductive properties.[12,14,23] The degree of porosity seems to be important in the incorporation of the graft and most

likely plays an important role in the primary and secondary stabilization of the graft particles.[13] The large pore connection and high interconnectivity are very important, mainly during the early healing phase, because they promote the passage of cells and nutrients and consequently promote new bone formation and graft integration.[2,4,5,12,14]

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