Hot Isostatic Pressing–Processed Hydroxyapatite-Coated Titanium Implants: Light Microscopic and Scanning Electron Microscopy Investigations

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Hot isostatic pressing (HIP) was used in a new procedure to produce hydroxyapatite (HA) coatings on a commercially pure titanium (cpTi) substrate for osseous implantation. Eighteen HIP-processed HA-coated implants were placed in the inferior border of the mandibles in 2 Labrador retriever dogs and left submerged for 3 months. As control specimens, 12 sandblasted cpTi implants were placed in the same mandibles and, to compare the bone reaction, 2 additional plasma-sprayed HA-coated implants (Integral) were placed. Tissue reactions at the bony interfaces of the implants were studied in ground sections with the implants in situ, using ordinary, fluorescent, and polarized light microscopy and scanning electron microscopy (SEM). The HIP-processed HA coatings displayed an increased density in light microscopy and SEM as compared to plasma-sprayed coatings. Direct bone-implant contact was found in all 3 types of surfaces. However, the production of new bone was far more abundant for the HA-coated implants than for sandblasted cpTi implants. The presence of bone-forming and bone-resorbing cells indicated active bone remodeling in the interface area at 3 months after implant placement. The present results support the view that epitaxial bone growth may occur from the HA-coated implant surface. It was concluded that the increased density of the present HIP-processed HA material does not reduce the bioactive properties of the coatings.
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Key words: experimental study, hydroxyapatite implants, light microscopy, scanning electron microscopy, titanium implants

The term *osseointegration* has gained recognition as a description of the incorporation of a titanium implant in bone.[1] Morphologically, osseointegration has been defined as direct contact between bone tissue and the implant surface at the light microscopic level.[1] The ability to establish this direct bone contact has been reported for metallic,[1–3] polymeric,[4] and ceramic[2,5,6] biomaterials. Fundamental differences seem to exist, however, between the mode of bone attachment in titanium (Ti)[1,2] and hydroxyapatite (HA)[2,7] implants. Such differences have been observed using both light microscopy (LM) and electron microscopy.[5–8] The most intimate bonding to bone has been observed for HA, in which epitaxial growth has been reported with high-resolution transmission electron microscopy (TEM).[7] Both experimentally and clinically, HA has been found to enhance bone growth at the bone-implant interface.[5,8,9] On inert materials, including titanium dioxide on Ti implants, bone growth has been shown to generate from the bone matrix,[7,10,11] whereas some researchers have claimed that HA materials seem to show parallel growth originating from both the implant surface and the bone.[10,12–15] How this seemingly epitaxial bone growth is generated is still unknown.

Hydroxyapatite is mechanically weak, and its brittleness often results in fractures when loaded in function. Thus, the brittleness can have a negative effect on the clinical use of ceramic HA-coated implants.

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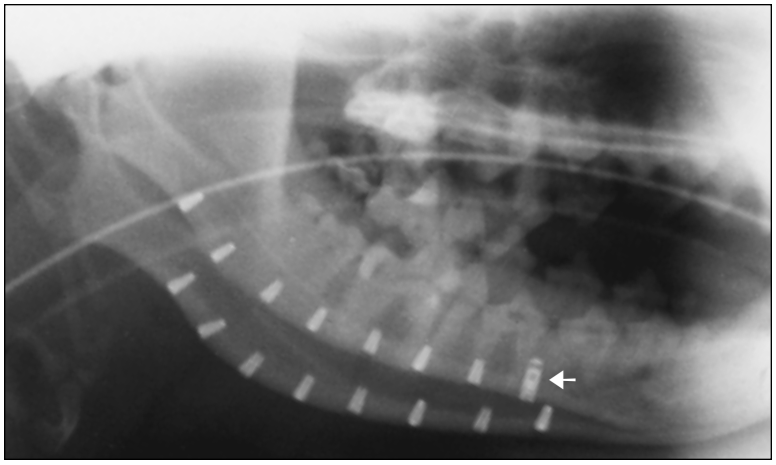


Fig 1 Radiograph showing experimental design of test implants in the inferior margin of a dog mandible with a scheduled observation period of 90 days (Multiscan GXR-60 E, Videoband X-ray recorder, and Stenoscope). The arrow indicates a plasma-sprayed implant (Integral). The other implants are either HIP-processed, HA-coated, or noncoated Ti implants.

Methods for coating metallic substrates with HA have been developed to circumvent this problem.[11,12] Plasma spraying is the only method that has so far been applied commercially. However, HA coatings produced in this way on metallic substrates are still porous, brittle, and liable to deterioration when loaded.[9] Fragments from fractured coatings have been shown to initiate cellular reactions to displaced HA particles in the interfacial bone.[13,14]

A new technique, using hot isostatic pressing (HIP) for producing stronger, less porous coatings of HA on titanium, has been developed.[13,16] The mechanically improved coatings produced by HIP have been tested in animal experiments.[13] Mechanical and histologic observations displayed a dense HA coating with a bond strength comparable to that of cortical bone (> 62 mPa)[16] and with biologic and bioactive properties maintained on approximately the same level as have been observed for plasma-sprayed coatings. The temperature and pressure cycles in the HIP chamber, including the holding time used at the different process levels, seem to be critical in obtaining the necessary strength of bonding to the substrate and shear strength of the coating.[16]

The aim of the present investigation was to study the effects of an HIP-treated HA-coated titanium implant on interfacial bone growth in a canine transcortical model using scanning electron microscopy (SEM) and histologic techniques. Noncoated Ti implants were used as controls. In addition, the tissue reactions were compared with reactions to commercially available plasma-sprayed HA-coated implants.

Materials and Methods

Titanium implants for animal experiments were designed as conical plugs, measuring 2 and 3 mm in diameter and 5 mm in length and equipped with a 1.0 mm-diameter canal through the center axis of the plugs for alignment purposes in push-out tests, as described in more detail by Herø et al[16] and Wie et al.[13] Experimental, commercially pure titanium implants (cpTi) were HIP-processed and coated with an approximately 25-µm thick HA layer at a pressure of 1000 bar and a temperature of 750°C. Chemical and physical properties have been described elsewhere.[16] The surface roughness of the HIP-processed implants was Ra = .7 µm, as measured by profilometry (Perthometer C50D, Mahr, Germany). Sandblasted cpTi implants served as controls, and commercial plasma-sprayed implants (Integral, Calcitek, San Diego, CA) were placed for comparison of the bone reaction to the HA coating. Surface roughness (Ra) of the cpTi implants was 1.6 µm, and that of the plasma-sprayed implants was 5.0 µm.

Two 1-year old, male, Labrador retriever dogs weighing 25 and 27 kg were used in the experiments. They were fed a standard pellet diet for dogs and given water as needed. Based on experience in using the inferior borders of sheep mandibles as an experimental model for implant studies,[13] these investigations were performed in the canine mandibular bone (Fig 1).

Surgery was performed under general anesthesia (Ketalar, 10 mg/ml, Park Davis, Morris Plains, NJ). Prior to surgery, local anesthesia was administered with subcutaneous injections of xylocaine (Xylocain-Adrenalin, 5 mg/ml, Astra, Södertelje, Sweden). Bilaterally, the inferior borders of the mandibles were shaved and disinfected using chlorhexidine solution after thorough irrigation of the skin with saline solution.

A 12-cm-long incision was made through the cutaneous, subcutaneous, and periosteal tissues. The facial nerve and artery were sectioned, and bleeding from major vessels was arrested using compression

and electrosurgery. The implant bone sites were prepared with special burs that conformed exactly to the size and shape of the implants. Eight implant sites were prepared vertically through the cortical bone plate into the spongious bone on each side of the mandible. Low-speed preparation and saline irrigation were used to reduce heat generation during preparation of the implant sites.

A total of 32 implants—9 HIP-treated HA-coated implants, 1 plasma-sprayed HA-coated implant, and 6 cpTi implants—were placed in each animal using a press-fit technique to secure stable primary fixation as described in a previous publication.[13] Figure 1 provides the radiographic outline of implant placement in the mandibles. For SEM and histologic investigations, 2 plasma-sprayed, 6 HIP-treated Ti, and 6 cpTi implants were used. Additional placed implants were used for other studies and are not reported here. Prophylactic antibiotic treatment was given (Streptocillin, Boeringer Ingelheim, Ingelheim, Germany) once a day for 15 days following placement of the implants.

To trace new bone formation at the implant-bone interface in histologic examinations, vital staining[5] was performed by giving the animals intravenous injections of 20 mg calcein per kg of body weight 4 weeks prior to sacrifice. The animals were inspected daily to observe possible complications with wound healing and their general health. National guidelines for the care and use of laboratory animals were followed.

The animals were sacrificed 90 days after implant placement with an overdose of pentobarbital (50 mg Mebumal, ACO Läkemedel A/B, Solna, Sweden). The mortal weight of the animals corresponded approximately with the starting weight, indicating that the dogs had not suffered noticeably from the surgery and vital staining. Immediately after sacrifice, the mandibles were removed and blocks of bone containing the implants were cut from the mandibles using a diamond blade with water irrigation to prevent overheating the bone tissue.

Histologic Procedures. Specimens for histologic evaluation were harvested with the implants from the mandibular blocks left in situ and fixed in a 4% formaldehyde solution. The blocks were embedded in hydroxyethylmethacrylate (Technovit 7200 VLC, Kulzer, Nerheim, Germany) and undecalcified ground sections were prepared to a thickness of approximately 20 µm and stained with toluidine blue according to the procedures described by Donath and Breuner.[17] Histologic observations were performed using a light microscope (Leitz Labolux, Wetzlar, Germany) equipped for polarization and fluorescence microscopy.

SEM Procedures. Two retrieved implant samples in each of the 2 categories (cpTi and HIP-treated HA-Ti) were embedded in epoxy, sectioned with a thin diamond cutting wheel, and prepared for SEM investigations according to standard procedures using diamond paste down to 1 mm. For the plasma-sprayed HA-Ti implants, the histologic blocks were used after cutting the sections. The polished, longitudinally sectioned surfaces were covered with a thin carbon layer by sputter-coating (Balzers SCD 050, Lichtenstein). The SEM studies were carried out in a computer-controlled instrument (XL 30, Philips, Eindhoven, The Netherlands) equipped with a Philips Energy Dispersive Spectrometer (EDS) (DX-4, EDAX, Eindhoven, The Netherlands).

Results

The animals survived until scheduled sacrifice, 90 days after surgery. In 1 dog, cutaneous dehiscences were seen in portions of the wounds. However, the subcutaneous sutures were not affected, and it was assumed that these complications did not influence the healing at the implant-bone interfaces to any significant degree.

HIP-Processed HA-Coated Implants. Histologically, a homogenous, approximately 25-µm-thick layer of HA was seen coating the titanium core (Fig 2). The findings indicated a dense coating with few defects, and the surface was relatively smooth. Almost continuous coverage by cortical lamellar bone was found in direct apposition to the HA surface; this was especially evident when using polarized light (Fig 3). Fluorescent microscopy displayed green lines from calcein staining, both on bone in direct contact with the HA surface and around osteons in contact with the HA coating (Fig 4). The fluorescent lines indicated that bone had been formed at the same rate from the coated implant surface and from the bone (Fig 4).

Small HA particles were detached from the surface in some areas and could be seen engulfed in bone marrow spaces using high-power magnification (Fig 2). No inflammatory cells were seen, but in some Haversian canals, in addition to osteoblasts, multinuclear giant cells assumed to be osteoclasts and indicative of bone remodeling were observed. These cells were not associated with detached HA particles.

In some areas, the whole HA coating was detached from the titanium core (Fig 4). The detached coatings were often fractured. Even in these areas, new bone formation and closing of Haversian canals were seen in close apposition to the surface of the detached HA particles (Fig 4). However, where space

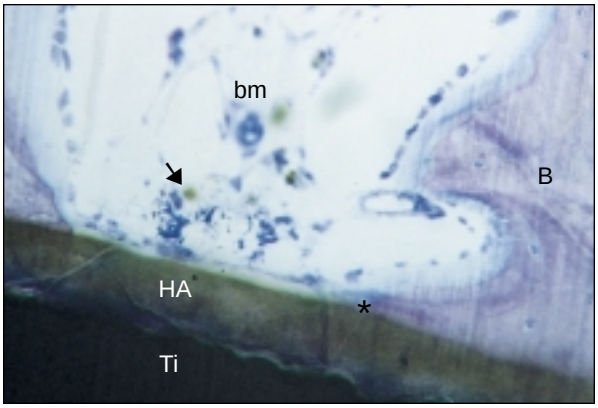
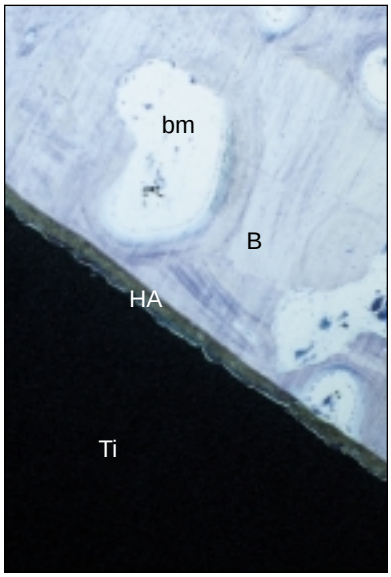


Fig 2 Photomicrograph from histologic section demonstrating interfacial bone in contact with an HIP-processed hydroxyapatite-coated (HA) cp titanium (Ti) implant in a canine mandible (observation period 90 days). A bone marrow space (bm) shows apposition of bone (B) directly on the implant surface, indicating epitaxy from the HA material (asterisk). An arrow indicates a detached HA particle in the bone marrow (ground section; objective magnification ×40; toluidine blue staining).



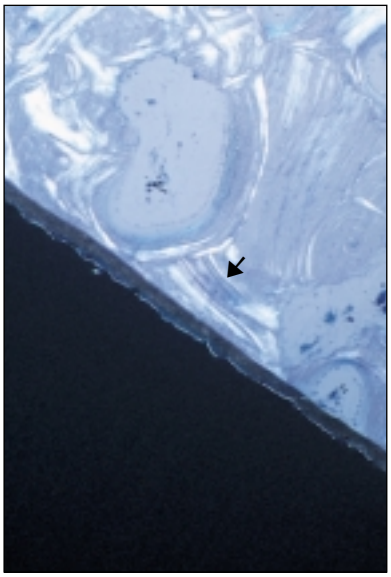
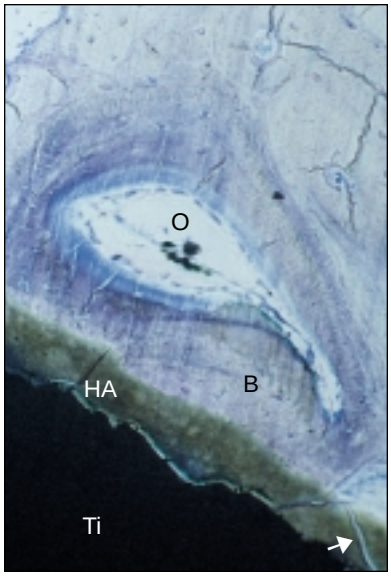


Fig 3 Photomicrographs from histologic sections demonstrating bone surrounding an HIP-processed hydroxyapatite-coated (HA) cp titanium (Ti) implant (observation period 90 days). Bone marrow (bm) indicates bone apposition directly out from the surface of the HA coating. The study of bone (B) in both transmitted light (*left*) and polarized light (*right*) show mature lamellar bone (arrow) in close apposition to the implant surface (ground section; objective magnification ×10; toluidine blue staining).



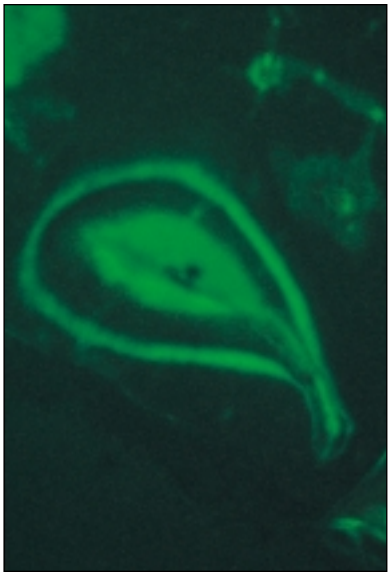


Fig 4 Photomicrographs from histologic sections demonstrating surrounding bone (B) at an HIP-processed hydroxyapatite-coated (HA) cp titanium (Ti) implant (observation period 90 days). An osteon (O) is closing at the HA surface. Transmitted light (*left, arrows point at a crack in the HA coating*) and fluorescent microscopy (*right, arrows point at HA-bone junction*) indicate simultaneous and similar growth rates, both from the old bone and from the implant surface (ground section; objective magnification ×25; toluidine blue staining).

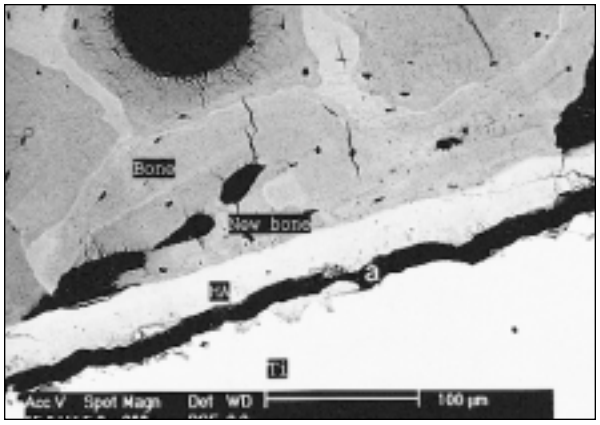


Fig 5 Scanning electron micrograph (SEM) of a ground section demonstrating surrounding bone at an HIP-processed Ti implant at the inferior border of a canine mandible (observation period 90 days). Notice bone apposition on the HIP-processed HA surface (HA). Detachment of HA coating from the titanium core is regarded as an artefact (a) (backscattered technique; magnification ×300).

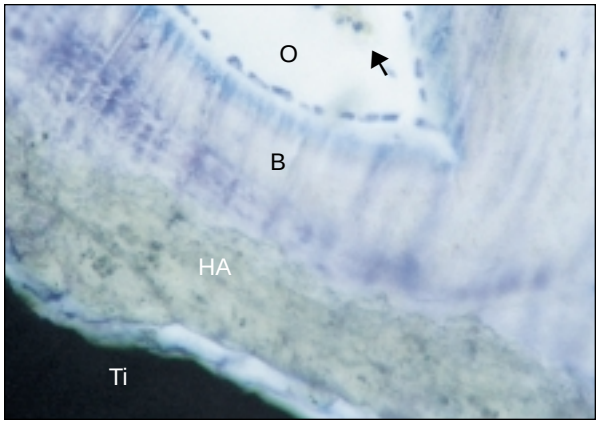


Fig 6 Photomicrograph from histologic section showing surrounding bone (B) at a commercial plasma-sprayed HA-coated metal (Ti) implant (observation period 90 days). An osteon (O) is closing at the HA surface of the implant (HA), indicating osteoblast activity from the bioactive biomaterial. The arrow indicates displaced HA particles in the osteon (ground section; objective magnification ×40; toluidine blue staining).

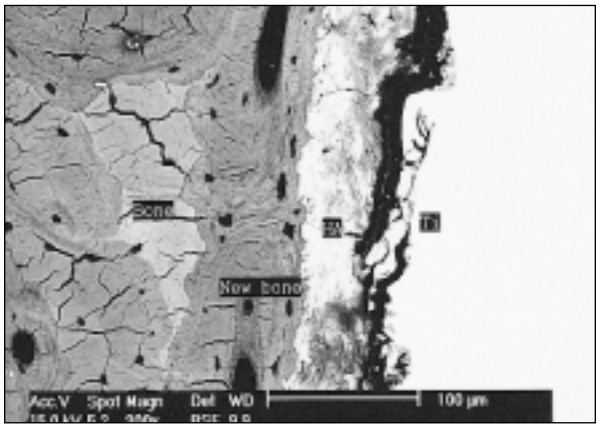


Fig 7 Scanning electron micrograph (SEM) of a ground section demonstrating the bone-implant interface at a hydroxyapatite-coated (HA) plasma-sprayed titanium (Ti) implant in the lower margin of a canine mandible (observation period 90 days). Note the sharp demarcation between old bone and new bone, indicating less bone mineral in the new bone (backscattered technique; magnification ×300).

was sufficient, a cell-rich granulation tissue containing a few lymphocytes proliferated between the coating and the Ti core.

SEM observations largely confirmed these findings. Relatively homogeneous HA coatings containing few porosities and a close relationship between the HA and surrounding bone were evident in SEM observations (Fig 5).

Plasma-Sprayed HA-Coated Implants. The plasma-sprayed coatings were about 50 µm thick and more heterogeneous (Figs 6 and 7) than the HIP-treated coatings (Fig 5). The surface was more irregular and uneven. A number of HA particles of various sizes and shapes were detached from the surface and lodged in the marrow spaces and Haversian canals even at a distance from the surface. However, close contact between the HA surface and bone tissue was observed on some of the detached particles.

Bone formation that seemed to have originated directly from the implant surface was observed. The area of direct bone-implant contact on the plasma-sprayed HA-coated implants was comparable to that on the HIP-processed implants. Gross detachments of the whole coating were less frequent than on the HIP-processed implants. No inflammatory reaction was observed.

SEM observations were in line with the above findings (Fig 7). Especially when using backscattering, a fibrous appearance of the new bone adjacent to the implant was clearly noted, consistent with that of a mature lamellar bone, and was also confirmed by polarized light microscopy (Fig 3). A "demarcation line" was observed between this bone and the more peripheral "old" bone (Fig 7).

CpTi Implants. Direct bone-implant contact was also observed in the cpTi implants (Fig 8) but in a smaller fraction of the area than in the HA-coated implants. Marrow spaces and Haversian canals along the implant surface were more numerous and tended to be more elongated than at the coated implants, where the bone coverage was more continuous. Detached Ti particles were observed along the implant surface but did not provoke any inflammatory reaction.

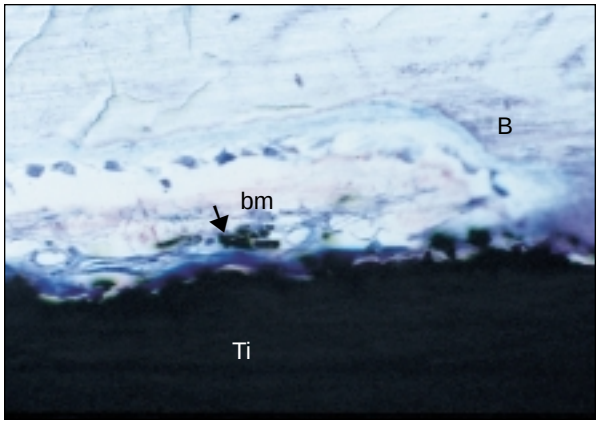


Fig 8 Photomicrograph demonstrating bone tissue (B) at cp titanium implant (Ti) in the inferior border of a canine mandible (observation period 90 days). Note apposition of new bone in the bone-implant interface by ingrowth from the neighboring bone. The *arrow* indicates detached Ti particles in a bone marrow space (bm) (ground section; objective magnification ×40; toluidine blue staining).

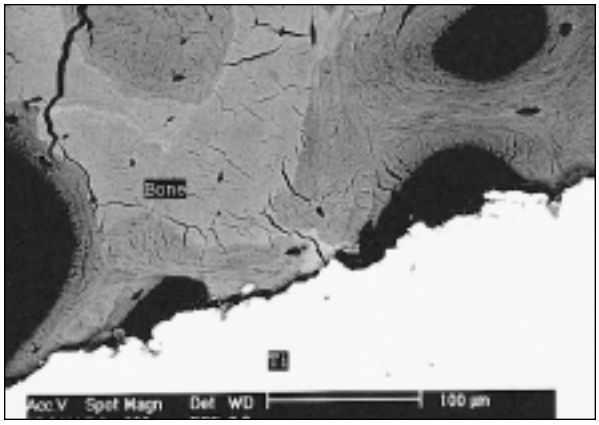


Fig 9 Scanning electron micrograph (SEM) of surrounding bone at a sandblasted cp titanium (Ti) implant in the inferior border of a canine mandible (observation period 90 days). Notice the gap between Ti and bone, possibly an artefact (backscattered technique; magnification ×300).

SEM revealed a loosening of bone contact from the Ti surface (Fig 9). This was interpreted as a possible post mortem artefact. The observation contrasted with findings on both types of HA-coated implants, however.

Discussion

Processing conditions, in which an isostatic pressure of 1000 bar and a temperature of 750°C were employed for the production of HA-coatings on titanium, yielded a weaker material than previous specimens produced at 850°C and either 1000 or 720 bar isostatic pressure.[13,16] The rationale for lowering the temperature to 750°C was the possibility that this would reduce the risk for excessive reactions between Ti and HA,[16] presumably without decreasing the strength of the coating. Unfortunately, the present material, when implanted in bone, resulted in more fragmentations than did previous HIP-processed HA-coated implants. Plasma-sprayed coatings were also fragmented, possibly a result of friction against cortical bone during implant placement.[7] The degree of fragmentation in this experiment was lower for plasma-sprayed HA implants than for HIP-processed implants. In a previous report, the HIP-treated specimens had a higher bond strength between the coating and titanium substrate than reported for plasma-sprayed specimens.[16]

A transcortical implant model using the inferior border of the canine mandible was deemed appropriate for this study. The method is described in more detail by Wie et al[13] in a previous publication. The inferior border of the mandible was chosen as the implantation site to achieve a relatively homogeneous bone model. This area of the mandible may be compared to the femoral or tibial bone models that are frequently used in implant experiments; it consists of an evenly thick cortical rim and an underlying zone of spongy bone. The area is easily accessible by a full-skin incision from underneath the mandible, and the procedure is well tolerated by the animals. The model seems appropriate for the testing of dental implant systems, since intraoral procedures that may be prone to infection can be avoided and mandibular experiments performed nonetheless. While transcortical implants of this type do not carry physiologic loads, they do represent models for healing processes associated with devices that are implanted and protected prior to actual load-bearing function. Dental implants are usually placed and remain unloaded for a healing period of 3 to 6 months, after which they are then loaded. Therefore, bone growth and interface reactions during this healing period are important for implant attachment.

Bone reactions to the 3 categories of implant surfaces were compared using SEM techniques and light microscopy with polarized light and fluorescent staining methods. The histologic sections produced with the present cutting and grinding methods[17] were only approximately 20 µm thick and much thinner than conventional ground sections, which measure 70 to 100 µm.[18] The use of light microscopy with relatively thick sections makes it impossible to observe the finest details of the bone-implant interface or cellular details, since the resolution is insuffi-

cient. The resolution in these sections, therefore, was approaching that of decalcified sections, which may be in the range of 5 to 10 μm.

With an observation time of 3 months, which is the nonfunctional period for mandibular implants in humans recommended by implant manufacturers, it was expected that new bone would have formed at the interface and that osseointegration would have taken place. In the present paper, the term osseointegration has been used to describe the direct contact between the implant surface and bone. The term seems to be generally accepted in a clinical context, though its justification in scientific work has been disputed.[8] The concept of biologic attachment has been used to describe the bonding of HA to bone tissue as being chemical in nature.[7,9]

Bone reactions to HIP-processed HA coatings were similar to previous findings[13] and displayed, both in SEM and histologic observation, direct bone-implant contact in areas of good adherence of the coating to the substrate. Bone tissue and the HA surface seemed to be more or less interwoven, while the bone-titanium interface looked more like a borderline between the 2 materials. In areas with detached coatings, a cell-rich loose connective tissue was found. This corroborates observations[13,14,19] that particulate HA may induce adverse reactions in the implant-bone interface. The present findings underline the importance of developing HA coating for load-bearing purposes that are strong enough to tolerate substantial stresses without deteriorating, both at the time of implant placement and in later functional use. Even though little reaction was seen in the marrow spaces, the fragments presumably represent foreign bodies, the implications of which have not been examined in this study. Dalton and Cook,[14] however, observed cell-mediated osteolysis in regions of severe coating degradation, and particle displacement was noted in regions far from the interface.

The HA coating on the metal core was intentionally made thinner on the HIP-processed specimens than the coating used on commercial plasma-sprayed implants. As the Ti core material of these implants was known to be well tolerated by bone tissue,[1] a reduction of the coating thickness was considered favorable for load-bearing considerations, and the production of a denser material would circumvent problems with porous material, which several researchers have found to fail upon implantation.[9,14] Surface dissolution of the HA layer on the implant, which increases the degree of mineralization at the interface in the first vulnerable period after implant placement, is the main purpose of the HA coatings. However, considerable uncertainty seems to exist as to the degree of dissolution sufficient to induce bone formation.[8,9,12] The similarity in crystal structure and lattice parameters of HA and bone is likely to promote epitaxy and low interfacial energies. Such circumstances tend in general to lower the energy barriers for nucleation of new crystals.[20]

The dissolution rate of the denser HIP product was anticipated to be lower than that of the plasma-sprayed material. As the establishment of a bony interface with both HIP-processed and plasma-sprayed implants was found to be similar, it was concluded that the density of HA coatings within the limits of these experiments could be increased without influencing the level of attachment to any significant degree.

The bone-forming ability of osteoblastic cells in the interface seems not to have been compromised by the smoother microtexture of the HIP-treated specimens, as compared to the coarser surface texture of the plasma-sprayed implants. The amorphous but porous superficial layer of the plasma-sprayed implants[12] and the cracks in the structure of the HIP-processed coatings may well influence cellular reactions in the interface in different ways.[16] The present experiments, however, did not uncover any significant differences in this respect. The HIP-processed coatings have been shown to be made predominantly of crystalline HA. The amount of amorphous material in these coatings is unknown.

Earlier investigations[13] with the present mandibular model have shown that bone coverage is more complete and more uniform at the interface of HA-coated implants than for noncoated cpTi implants observed for comparable periods. The difference in direct bone-implant contact was as high as 76.2% for HA, versus 28.8% for cpTi.[13] This tendency has been confirmed in the present experiments. One explanation for this observation is that bone might have been formed both from the bioactive surface of the HA implant by epitaxial growth and as apposition on the old bone matrix; this has been suggested by several authors.[10,12,15] Both the enhanced forming of osteons on the HA surface, as observed in the light microscopic sections, and the "demarcation zone" observed in the SEM sections indicated that bone formation may have begun from both sides. This zone may thus represent a fusion line between 2 directions of growth. These observations do not necessarily suggest that HA is able to induce a conversion of undifferentiated mesenchymal cells toward the osteoblast phenotype. It is possible to postulate that preosteoblasts in the tissue derived from the "old" bone, which are capable of transformation into osteoblasts, may have been introduced into the HA surface. Owing to the similarity in crystal structure, a possibly

enhanced proliferation and bone formation may have started.[6,7,10] The HA surface may thereby have acted as a scaffold for bone formation from the implant toward the bone, thus creating a seemingly epitaxial growth of bone.

Summary

The question of whether or not bone epitaxial regeneration at the surface of HA implants can be initiated directly by bone-forming cells and chemical prerequisites on the HA-coated surface through biochemical interaction has been debated.[2,7,10,21] The present findings support the view that epitaxial growth may originate from the HA implant surface. This property of HA seems to be found in both HIP-processed and plasma-sprayed HA-Ti implants.

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