Histologic investigation of the biologic behavior of different hydroxyapatite plasma-sprayed coatings in rabbits

J. A. Jansen* and J. P. C. M. van der Waerden
Department of Oral Function and Prosthetic Dentistry, Laboratory of Biomaterials, Dental School, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

J. G. C. Wolke
Department of Biomaterials, School of Medicine, University of Leiden, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands

The aim of this study was to investigate bone response to and biostability of three different hydroxyapatite (HA)-coatings. Therefore, coated and uncoated titanium implants were inserted into the tibia of rabbits. Implantation times were at 3 and 12 weeks. The histological evaluation included measurement of the amount of bone apposition to the various implant surfaces. The results demonstrated at 3 and 12 weeks no marked differences in bony reaction at the cortical level to the different implant materials. However, compared with the 3-week specimens, at 12 weeks extensive maturation of the woven bone callus had occurred. In addition, all 12-week implants induced bone formation in the medullary cavity. It was also noted that all HA-coatings showed loss of coating thickness. Quantitative determination of bone contact demonstrated that all 12-week implants showed the same high amount of bone apposition.

INTRODUCTION

The biocompatible properties of calcium phosphate ceramics, such as hydroxyapatite (HA) have been known for years.[1] Due to their favorable biological behavior, ceramic materials are used in various orthopedic and dental surgical procedures. Unfortunately, a drawback inherent with calcium phosphate ceramics is their mechanical properties: calcium phosphate ceramics are brittle and have a low strength. Therefore, they can only be used in unloaded situations. To solve this problem, the application of these materials as thin coatings on a metallic surface has been proposed.[2] In this way, the excellent biocompatible properties of calcium phosphates are combined with the mechanical properties of metal. The clinical functioning of a composite material such as an implant is requires that the physicochemical structure of the coating show no deficiencies, and that the coating adhere well to the underlying metal.

For the deposition of ceramic coatings, various techniques have been applied. One of the current methods is plasma-spraying.[3] It has recently been demonstrated that the quality of plasma-spray deposited coatings can be influenced by several parameters, such as temperature of the plasma, nature of the plasma gas, particle size of the powder, and chemical nature of the ceramic powder.[4] It is also known that variations in the material properties of calcium phosphate coatings can have an effect on their biocompatibility and biostability. For example, it has

*To whom correspondence should be addressed.

TABLE I
Particle Size Distribution of the HA75, HA125, and HAsd Apatite Powder

HA75 Apatite Powder					
%, <	10.00	25.00	50.00	75.00	100.00
size, μm	9.988	19.10	32.91	46.35	83.26
HA125 Apatite Powder					
%, <	10.00	25.00	50.00	75.00	100.00
size, μm	11.88	26.85	44.60	56.31	110.00
HAsd Apatite Powder					
%, <	10.00	25.00	50.00	75.00	100.00
size, μm	14.01	18.87	25.46	32.52	64.60

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been shown in *in vitro* dissolution experiments that changes in the particle size distribution influence the dissolution rate of a hydroxyapatite (HA) coating.[5] Whether these *in vitro* results are also true for the *in vivo* bone behavior is still undetermined. Therefore, we investigated the tissue response to, and the biological behavior of, HA coatings sprayed with various particle sizes.

MATERIALS AND METHODS

Implants

Commercially pure titanium (CP-Ti) implants (Dyna Dental Engineering) with a diameter of 3 mm and a length of 8 mm were grit-blasted to a roughness of Ra = 4–5 μm. They were cleaned ultrasonically in propanol and dried at 100°C. The implants were left uncoated or given an HA-coating, approximately 50 μm thick, using a plasma-spray technique.[6] Three different apatite powders were used in the coating process: 1) particle size between 1–75 μm (HA75); 2) particle size between 1–125 μm (HA125); or 3) spray-dried apatite powder with a particle size between 10–70 μm (HAsd).

The powders with particle sizes between 1–75 μm and 1–125 μm were crushed. The spray-dried powder consisted of spherical parts. The mean particle size of the 1–75 μm powder was 33.39 μm. The mean size of the 1–125 μm powder was 43.57 μm, and the mean size of the spray-dried powder was 26.48 μm.

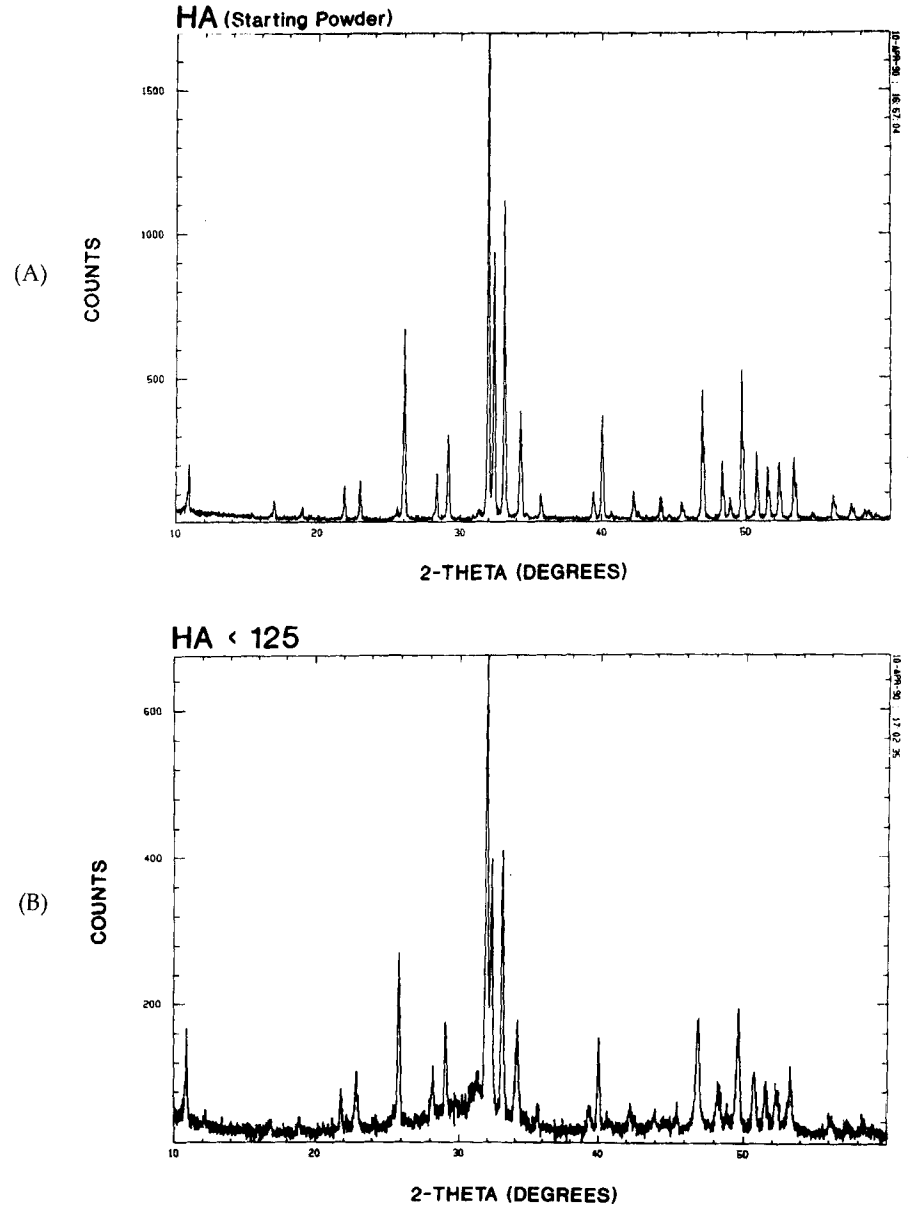

Figure 1. X-ray diffraction pattern of (A) hydroxyapatite starting powder, (B) HA125 coating, (C) HA75 coating, and (D) HAsd coating.

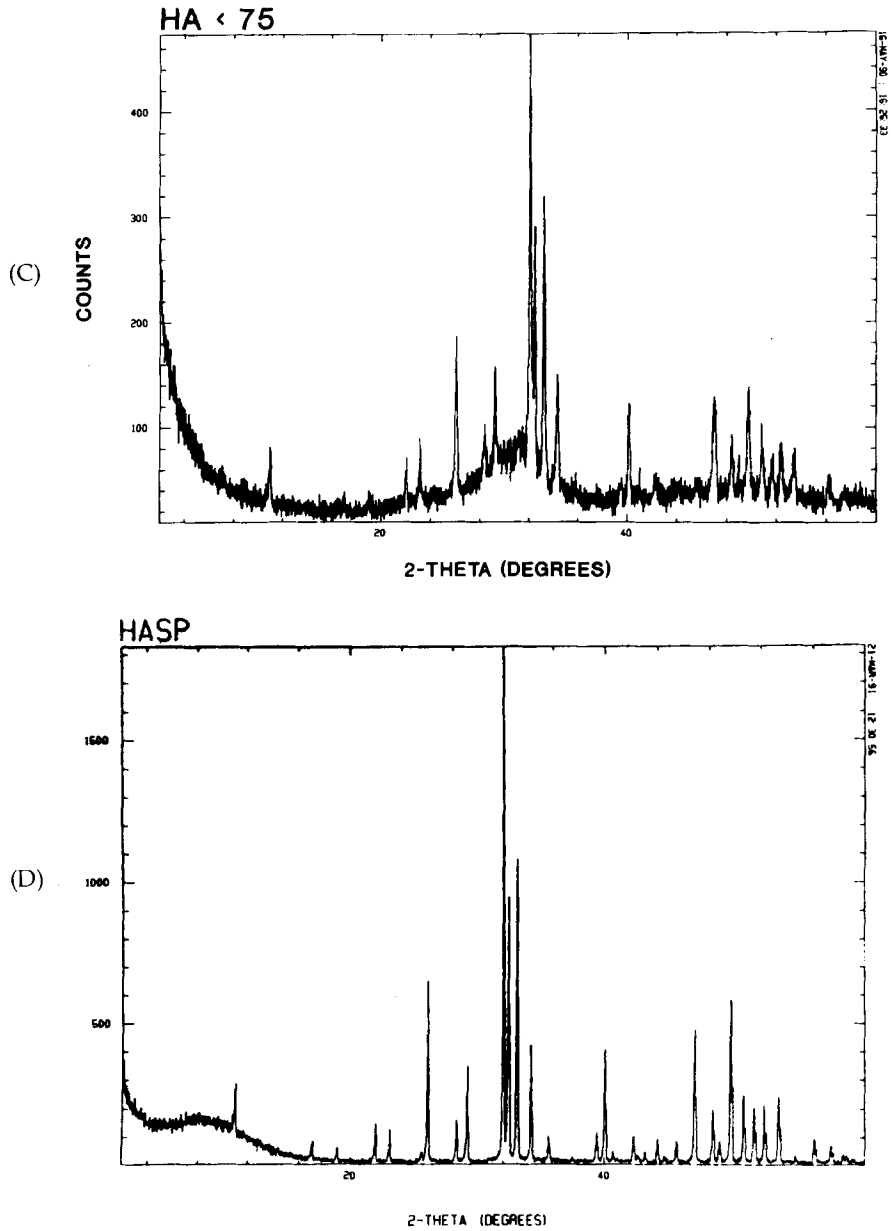

Figure 1. *(continued)*

The particle size distributions of the various powders are listed in Table I. The chemical composition and the purity of the coatings was determined by X-ray crystal-diffraction (XRD) using the following equipment and operational parameters: Philips automatic vertical X-ray diffractometer PW 1050, measuring system PW 1710, CuK α-radiation (3 kW HV generator PW 1130/90, 45 kV/33 mA; 0.018 mm nickel CuK β-filter), divergence slit 1°, receiving slit width 0.1 mm, xenon sealed proportional detector and pulse height selection. Figure 1A shows the XRD pattern of sintered HA feedstock powder. Only minimal line broadening can be seen, which indicates a well-crystallized material. Figure 1B shows the XRD pattern of HA125. It can be seen that plasma-spraying does not alter the crystallographic structure. Only a little line-broadening is visible. Figure 1C shows the XRD pattern of a plasma-sprayed HA75 coating. Compared with Figure 1B the structure of the HA-coating is changed. There is more line broadening, which indicates increased presence of amorphous phases. Figure 1D shows the XRD pattern of a HAsd-coating, which is very similar to the XRD pattern shown in Figure 1A. Further, it can be seen that the intensity of the peaks in Figure 1B and 1C is changed during the plasma-spraying. This indicates that the crystallinity of these materials is decreased to 50% and 30%, with respect to the starting material.

After plasma-spraying, the implants were cleaned ultrasonically in 100% ethanol to remove loose particles, and then dried. All implants were sterilized in an autoclave.

Animal model and implantation procedure

Eight 3-month-old female New Zealand White rabbits (weight 3 kg) were used in this experiment. During surgery the animals were sedated by intramuscular injection of fluanison/fentanylcitrate (Hypnorm, Duphar, Amsterdam). In all animals, the area of implantation was anesthetized by subcutaneous administration of Lidocaine.

The implants were inserted into the tibial diaphysis of the rabbits. For the insertion of implants, the animal was immobilized on its back. Using standard sterile surgical techniques,[7] a longitudinal incision was then made on the medial surface of the tibia. After the bone had been exposed, 1-mm pilot holes were drilled through the medial cortex, the medulla, and the lateral cortex of the tibia. The holes were gradually widened with drills to the final diameter of the implants. Following insertion of the implants, the soft tissues were closed in separate layers. Finally, the position of the implants was checked radiographically. A total of 48 implants were placed: 12 uncoated CP-Ti, 12 coated HA75, 12 coated HA125, and 12 coated HAsd implants. Each animal received six implants, three in the left and three in the right diaphysal part of the tibia. Evaluation of the bone-implant interface was planned at implantation periods of 3 and 12 weeks. To allow the use of two time periods, the animals were operated in two separate surgical sessions. The bone preparation was performed with a very gentle surgical technique using very low rotational drill speeds (max. 450 rpm) and continuous internal and external cooling.

Postoperatively the animals were placed in standard rabbit cages and allowed to move unlimited all the time. Throughout the duration of the experiment at regularly time intervals radiographs were taken of each tibia.

Histological procedures

At the predetermined endpoint of the experiment the animals were killed by injecting Nembutal peritoneally; the tibiae with their surrounding tissues were excised and the excess tissue was removed immediately. Following fixation of the tibiae in a 10% buffered formalin solution, the specimens were prepared for histologic processing. They were sectioned in three pieces, each one with one implant. These tissue blocks were embedded in methylmethacrylate. After polymerization nondecalcified thin (10 μm) sections were prepared using a modified diamond blade sawing microtome technique.[8] The sections were made

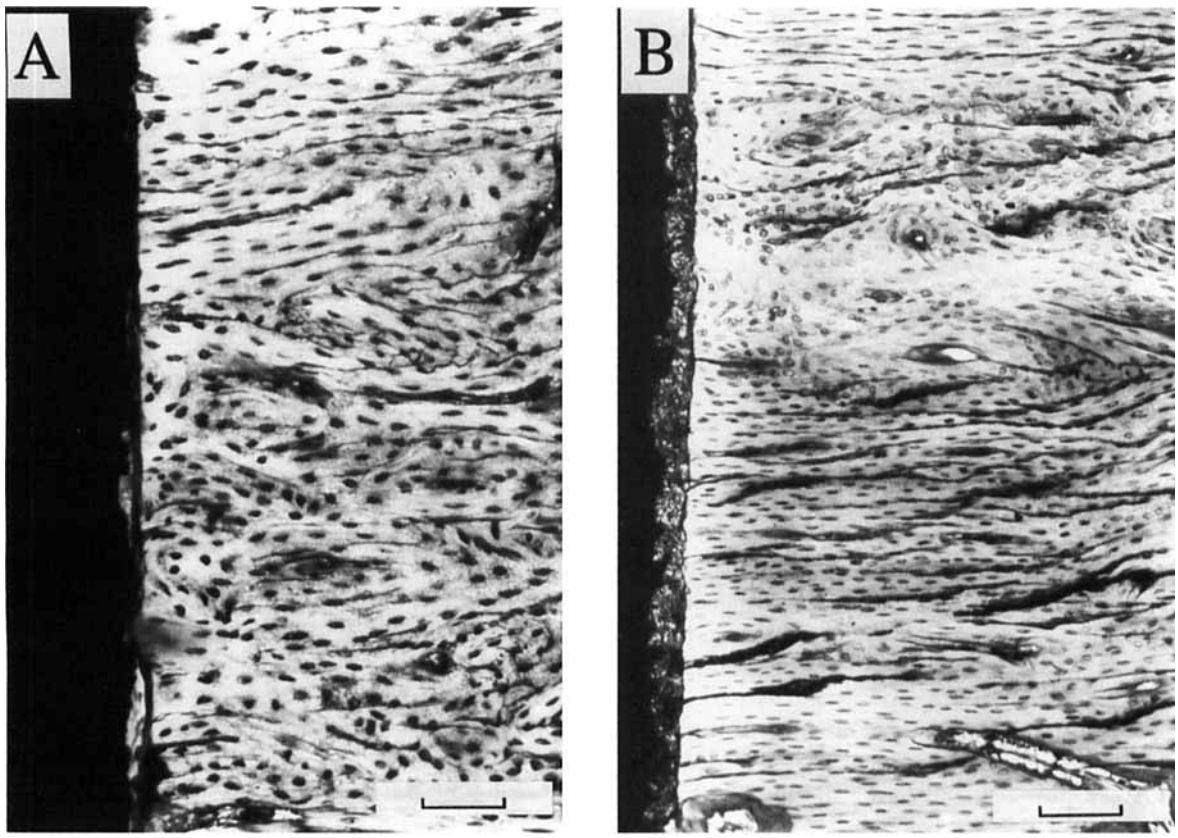

Figure 2. Histologic section showing the close contact between the implants and the surrounding cortical bone, 3 weeks after implantation. (A) uncoated titanium implant, original magnification ×124, bar = μm: (B) HAsd coated titanium implant, original magnification ×82, bar = 122 μm.

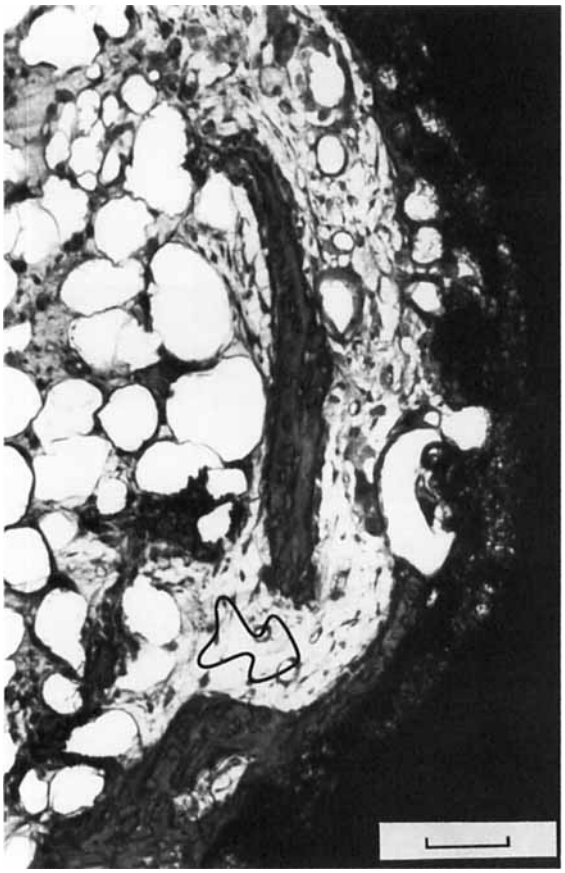

Figure 3. Light micrograph showing the cellular reaction of the bone marrow around an HAsd coated implant, 3 weeks after insertion. In addition bone formation can be observed (arrow). Original magnification × 158, bar = 63 μm.

in a transversal direction perpendicular on the axis of the implant. These sections were stained with methylene blue and basic fuchsin and the interface was examined with a light microscope. Further, for the 12-week specimens, the percentage of bone contact to the various implant materials was quantitatively assessed with a Vidas image analysis system. The percentage of bone contact was determined using the equation:

$$\% \text{ of bone contact} = \frac{\text{bone contact length}}{\text{total implant length}} \times 100\%$$

RESULTS

Histological evaluation

Three Weeks

Light microscopical analysis of the 3-week specimens demonstrated that all implants showed a close bone contact in the transcortical area without any sign of inflammatory reactions (Fig. 2). In almost all specimens a woven bone callus was found at the periosteal and endosteal tibial surfaces. This new bone formation was always restricted to the level of the cortical passage. Furthermore, around all implants the bone marrow showed a moderate cellular reaction (Fig. 3).

Twelve Weeks

At 12 weeks, no striking differences were noted in histologic reaction between the various implant surfaces. There was a tight contact without an intervening fibrous tissue layer between the cortical bone and the coated as well as uncoated implants (Fig. 4). The woven bone callus had matured and the original lattice structure of the callus was completely filled with lamellar bone.

Microscopic evaluation of the HA-coated implants revealed a reduction of coating thickness for both HA75, HA 125, and HAsd implants.

Microscopic evaluation revealed that all implants supported bone formation in the medullary region (Fig. 5). Occasionally even the entire medullar surface of the implants was covered by newly formed bone. In addition, it was noticed that the cellular infiltration in the bone marrow, as observed at 3 weeks, was decreased.

Progressive loss of HA-coating was observed for all coatings. In some areas the coating had completely disappeared. In these areas the bone was still in direct contact with the implant surface without an intervening fibrous tissue layer (Fig. 6). The coating reduction could never clearly be associated with the local presence of cellular activity as judged by the basic fuchsin and methylene blue stain.

Histomorphometric observations

The results of the quantitative determination of bone contact to the 12-week implants are given in Table II. One of the uncoated titanium specimens could not be evaluated because of sectioning problems. No considerable differences in bone contact between the various HA-coatings were seen. Although the HA-coated implants showed a slightly higher bone apposition than the Ti implants, statistical testing, using a one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman-Keuls), revealed that this difference in bone apposition between the coated and uncoated implants was not statistically significant ($P > .05$).

DISCUSSION AND CONCLUSIONS

The primary aim of this experimental animal study was to evaluate the bone response and biostability of

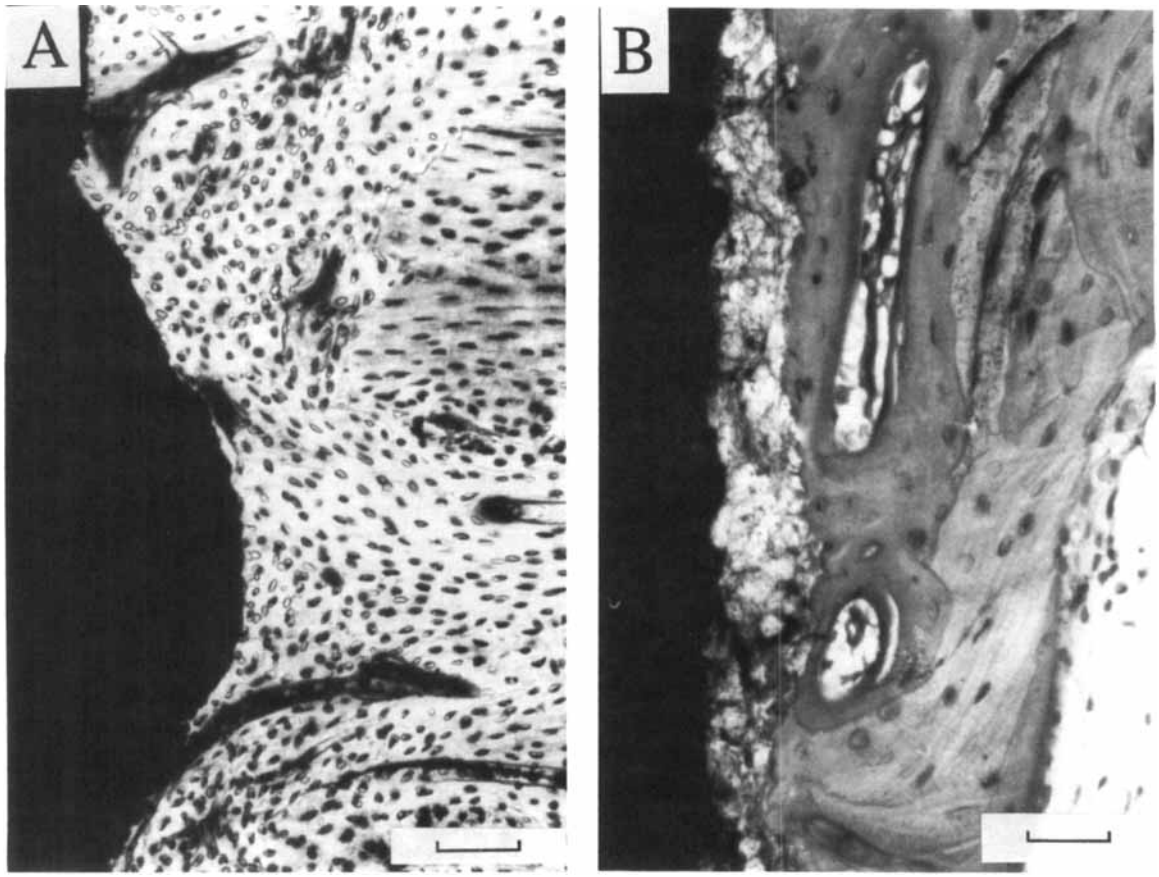

Figure 4. Nondecalcified section, showing the close contact between bone and implant surface, 12 weeks after insertion. No intervening fibrous tissue is observed. (A) uncoated titanium implant, original magnification ×124, bar = 81 μm: (B) HA75 coated titanium implant, original magnification ×264, bar = 38 μm.

three different HA-coatings. In addition, we investigated whether there were differences in bone reaction between HA-coated and uncoated Ti-implants.

It was found that the results obtained in this study are very consistent with previously performed experiments[9] using the same animal model. The histologic and quantitative evaluation demonstrated that there are no differences in bone behavior and stability between the three investigated coatings. All coated implants showed the same amount of bone contact. Also, all coatings showed a reduction in coating thickness, although it was observed in *in vitro* studies that the dissolubility is dependent of the particle size of the powder.[4] Therefore, our observation confirms the previous findings of Klein,[10] that *in vitro* dissolution studies have no discernible relevance with respect to *in vivo* behavior.

The reasons for the observed decrease in thickness of the HA-coatings are still not completely understood. Already several factors have been suggested as playing a role in this process. One factor which is considered to be of influence is the Ca/P ratio. For example, Klein[5,11] and Wagner[12] showed that higher Ca/P ratios lead to more dissolution of calcium phosphate coatings. Other material related factors supposed to influence the degradation characteristics of plasma-sprayed HA are the crystallography and porosity of the coating.[13] Further, it has also been suggested that resorption of hydroxyapatite is accomplished by macrophages[14] or osteoclast-like cells.[15] Finally, it may not be overlooked that changes in the extracellular pH cause the dissolution of hydroxyapatite. However, in our study we found no clear indication that the coating reduction could be attributed to one of the above mentioned factors, since there were no accumulations of phagocytes. Therefore, in addition to the biological behavior, future studies have to focus on the exact mechanism underlying the biostability of calcium phosphate coatings.

Related to this biostability problem, there is another question that has to be answered: What will happen at the bone-implant interface if the entire coating disappears? Considering the results of this study, it may be supposed that no problems will arise if the HA-coating gradually disappears. Then as demonstrated in several of our sections, new bone will be laid in its place. Nevertheless, additional experiments have to be performed with longer implantation periods and also under loaded conditions to fully address

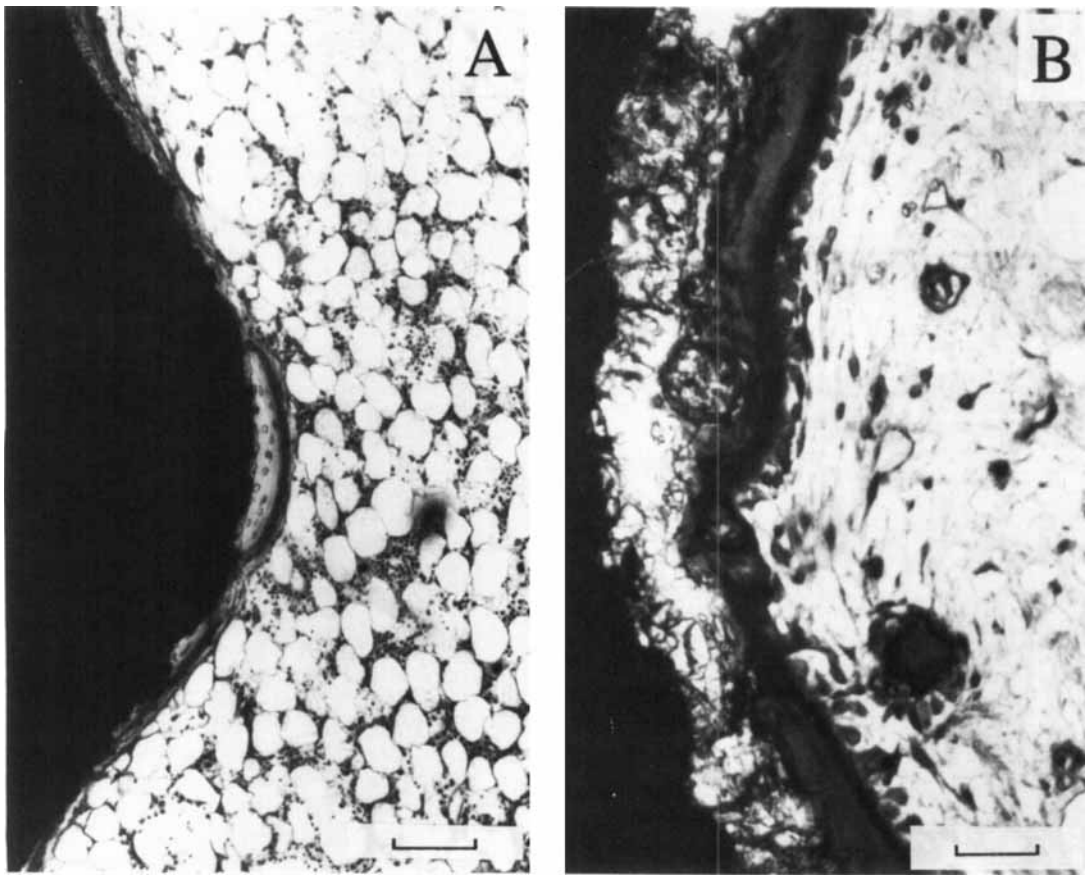

Figure 5. Histologic section showing bone formation along the medullar surface of (A) uncoated titanium implant, original magnification ×82, bar = 122 μm, and (B) HA75 coated titanium implant, original magnification ×264, bar = 38 μm.

the degradation behavior of Ca/P coatings and their postresorptive long-term reaction.

With regard to the amount of bone contact to the uncoated grit-blasted Ti-implants, it has to be mentioned that this result is in agreement with the findings of Chae et al.[13] and Dhert et al.,[16] who also compared the bone response to uncoated and calcium phosphate coated titanium implants. Chae et al. inserted unsprayed and tricalcium phosphate (TCP) sprayed titanium rods into the medullary canals of rabbit tibia. They observed that after 3 weeks the TCP-sprayed rods showed a significantly greater bone response than the unsprayed rods. However, at 12 weeks the response to sprayed and unsprayed titanium rods was comparable. Dhert et al. implanted uncoated and HA-coated titanium plugs into the femora of goats. They found that after 12 weeks HA-coated implants showed more bone apposition, but after 25 weeks the coated and uncoated Ti-implants did not differ in bone apposition. Therefore, our study supports the suggestion of Gerner et al.[17] that the application and benefit of Ca/P coatings is especially based on an initial hastening of the bone formation.

Table II
Amount of Bone Contact (%) to the Implants at 12 Weeks

Material	Bone contact (%)
CP-Ti	82.2 ± 9.7 (n = 5)
HA75	91.7 ± 2.9 (n = 6)
HA125	88.5 ± 6.9 (n = 6)
HAsd	91.8 ± 6.6 (n = 6)

In conclusion, the results of this study have indicated that the particle size of the powder does not influence the biostability and biological response, and there are only small differences in cortical bone reaction between uncoated and HA-coated titanium implants from a light microscopic point of view. Nevertheless, HA-coatings are of advantage since HA-coatings appear to induce a faster fixation of the implant into bone.

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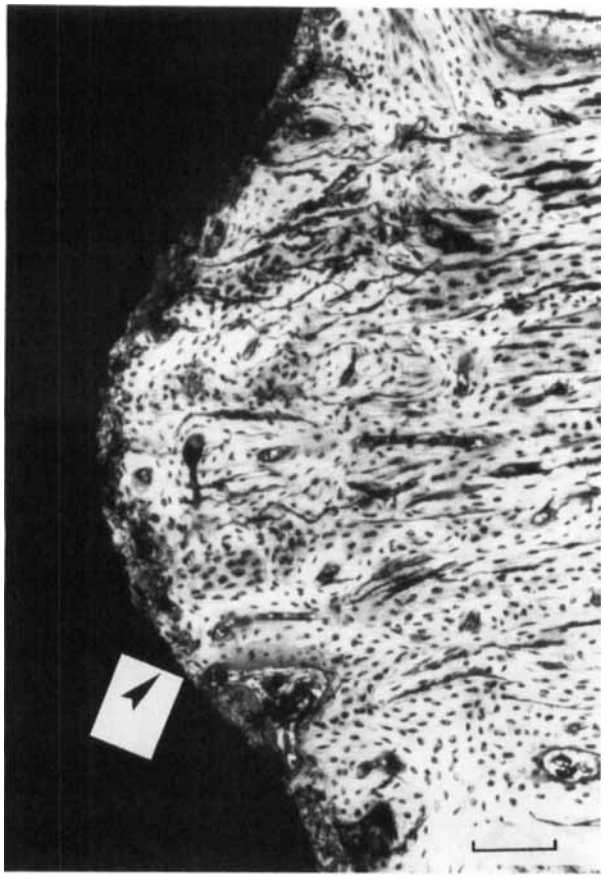

Figure 6. HA75-implant/cortical bone interface 12 weeks after implantation. Despite the substantial loss of HA-coating and even the occasional complete disappearance of the coating (arrow), there is still a direct bone-implant contact. Original magnification ×82, bar = 122 μm.

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