Evaluation of plasma-spray and magnetron-sputter Ca-P–coated implants: An *in vivo* experiment using rabbits

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The bone response to different plasma-spray and magnetron-sputter calcium phosphate (Ca-P)–coated implants was evaluated in a rabbit animal model. Four types of Ca-P coatings have been investigated: a plasma-spray Ca-P coating (HA-PS), a heat-treated plasma-spray Ca-P coating (HA-PS/ht), an amorphous magnetron-sputter coating (Ca-P-a), and a crystalline magnetron-sputter coating (Ca-P-c). Seventy-two specially designed cylindrical implants were inserted in the lateral and medial femoral condyles of 18 New Zealand White rabbits. The four differently coated implants were positioned in one animal according to a split-plot design. After implantation periods of 3, 6, and 9 weeks, the bone–implant interface was evaluated histologically. Besides descriptive light microscopical evaluation, quantitative histomorphometrical measurements were done to determine bone contact and the amount of bone surrounding the implant–bone interface.

Light microscopical examination revealed that all types of coatings followed the same process of bone healing. Measurements of bone contact at 6 and 9 weeks did not reveal significant differences between the various coatings. For the amount of bone, in a circular region at a certain distance from the implant, the Ca-P-c–coated implants showed a significantly greater amount of bone after 6 weeks of implantation than did the other three Ca-P coatings. At 9 weeks this difference could no longer be measured.

On the basis of these findings we concluded that magnetron-sputtered Ca-P coatings show the same process of bone healing as the plasma-sprayed Ca-P coatings when inserted into the trabecular femoral bone of rabbits.

INTRODUCTION

The resulting benefits[1–5] of plasma-spray HA-coated implants in dental and medical implantology may be summarized as: faster and greater bone adaptation; improved implant fixation; absence of intervening fibrous tissue; faster bone healing, which reduces the waiting period; and improved performance in poor bone quality.

On the other hand concerns have been raised and some confusion remains regarding the viable use and prognosis of such coated implants. These concerns deal with: (1) the substrate-to-coating fracture and fatigue strength properties, and (2) the biodegradation of the coating and what will happen at the implant–bone interface when the coating has disappeared, particularly in relation to the grit-blasting procedure required to obtain mechanical retention of the coating.

Therefore other deposition methods were developed to produce thinner (100 nm–10 μm), more adherent and physiocochemically better-defined coatings. One of them, RF magnetron sputtering, has great promise, and can possibly solve some of the concerns procured with the plasma-spray techniques. The advantages of this procedure over the plasma-spray technique are: (1) more-adherent ceramic coatings can be deposited without preliminary grit-blasting of the implant surface, and (2) more-uniform ceramic coatings can be deposited, also on complex implant designs.

In view of this, experimental efforts in our laboratory have been directed to further development of this method for the deposition of thin Ca-P coatings on dental implants. Various physicochemical and biological experiments already have been performed. For example preliminary research confirmed the apatite nature of the deposited coatings,[6–9] and revealed the stabilility of sputtered coatings under cyclically loaded conditions.[10] In addition cell-culture experiments demonstrated that the sputtered Ca-P coatings are biocompatible, and induce apatite formation.[11]

The purpose of the present study is to test in experimental animals whether magnetron-sputtered Ca-P

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Journal of Biomedical Materials Research, Vol. 31, 329–337 (1996)

CCC 0021-9304/96/030329-09

coatings cause a bone behavior similar to or better than plasma-sprayed Ca-P coatings.

MATERIALS AND METHODS

Implant materials and coating characteristics

Seventy-two specially designed cylindrical, commercially pure titanium (cpTi) implants were made (Fig. 1). All implants measured 8.0 mm in length. Depending on the final coating procedure given to the implants, 36 of these implants had a diameter of 2.9 mm, and the other 36 implants had a diameter of 3.0 mm. The implants were provided with two rounded, circular grooves (height, 2.0 mm; depth, 0.5 mm). Using the plasma-spray and magnetron-sputter technique, the following coatings were deposited: (1) plasma-spray coating (HA-PS), (2) plasma-spray coating with additional heat treatment for 2 h at 600°C (HA-PS/ht), (3) magnetron-sputter coating produced on a rotating substrate holder (Ca-P-a), and (4) magnetron-sputter coating produced on substrate holder in an indexed position (Ca-P-c).

For plasma spraying, commercially available hydroxylapatite powder was used with a particle distribution of 10–70 μm. Before coating deposition, implants were grit blasted with Al_2O_3 (Ra = 4–5 μm). Magnetron-sputter coating was performed as described earlier,[7,8,11] using a commercially available unit (Edwards High Vacuum ESM 100 system). An Ha-PS copper disc served as a target for the sputter deposition. Before coating, the implants were mounted onto the rotating and water-cooled substrate holder. Coatings were applied on the as-machined surface of the implants. The thickness of the plasma-sprayed coatings was about 50 μm, while the thickness of the magnetron-sputtered coatings was 2.0–4.0 μm. Therefore the final diameter of all implants was similar (3.0 mm ± 0.004 mm). The chemical composition of all coatings was confirmed by x-ray diffraction (XRD), infrared spectroscopy, and Rutherford backscattering spectrometry (RBS).

Before surgery all implants were cleaned ultrasonically in 100% ethanol. Subsequently they were sterilized in an autoclave.

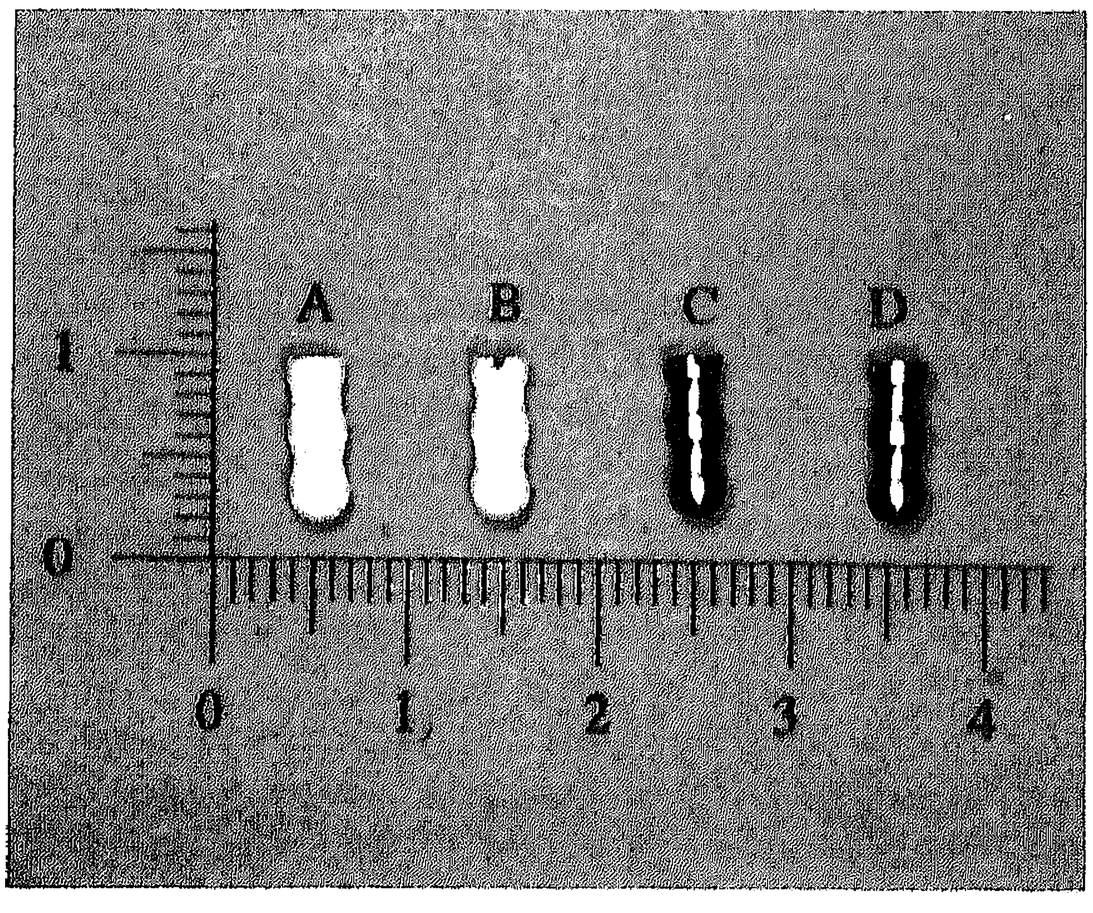

Figure 1. Implants with different Ca-P coatings. (A) HA-PS, (B) HA-PS/ht, (C) Ca-P-a, (D) Ca-P-c.

Experimental design and surgical procedure

Eighteen healthy female New Zealand white rabbits, 3–4 months old, were used in this study. Surgery was performed under general anesthesia by intravenous injection of Hypnorm (0.5 mL/kg) and atropine (0.5 mg/animal). After orotracheal intubation, anesthesia was maintained by ethrane 2–3% through a constant-volume ventilator. To reduce the risk of perioperative infection, prophylactic antibiotic (Terramicine®) was administered postoperatively by subcutaneous injection.

The implants were inserted in both condyles of each femur. Therefore the animal was immobilized on its back and the hind legs were shaved, washed, and disinfected with providone-iodine. A longitudinal incision was made on the medial side of the right and left femurs. The medial as well as the lateral condyle could be exposed by this incision. At a distance of 0.5 cm from the femoral epifyse, a pilot hole was drilled and gradually widened to the final diameter of the implants. The bone preparation was performed at a low rotational drilling speed (500 rpm) and continuous internal cooling. After press-fit insertion of the implants at the medial and lateral sides, the skin was closed by intracutaneous sutures using Vicryl 3-0.

A total of 72 implants were placed; 18 HA, 18 HAHT, 18 Ca-P-a, and 18 Ca-P-c. Each animal received four implants, one at each lateral and medial side of the right and left femoral condyle. The position of the implants was confirmed by radiographs. For the localization of the implants a balanced split-plot design was used to compensate for differences in bone quality and load characteristics between implantation sites.

Postoperatively the animals were placed in cages (five rabbits sharing one cage, 1.33 × 1.10 m). They were provided with water and rabbit chow *ad libitum*, and were allowed to move unrestricted at all times.

The animals were sacrificed after 3, 6, and 9 weeks using an overdose of pentobarbitalsodium (Nembutal®), and the femoral condyles together with the implants were excised.

Histological procedures

Excess tissue of the excised femurs was removed immediately. At room temperature the specimens

were fixed in 4% formaldehyde buffered with PBS at pH 7.2–7.4, for 1 week. Tissue blocs were then dehydrated by gradient series of ethanol. Subsequently they were embedded in methlymethacrylate and finally polymerized at 37°C. A modified diamond-blade microtome sawing technique[12] was used to prepare nondecalcified thin sections (approximately 10 μm) for examination by a light microscope. Sections were made in a horizontal plane, perpendicular to the long axis of the implant. Finally the sections were stained with methylene blue and basic fuchsin, and examined by transmittant light microscope.

Histological evaluation

Histological and histomorphometrical measurements were performed to evaluate bone response. Histological evaluation consisted of a thorough description of the observed tissue reaction. Histomorphometrical measurements were performed only on sections of the rabbits that were sacrificed after 6 and 9 weeks. For this histomorphometrical procedure a computer-based image analysis system (TCL-image) was used.

First the percentage of bone contact was determined. The amount of bone–implant contact was measured for the total implant perimeter. The percentage of bone contact was defined as the length of the interfacial area with direct bone–implant apposition.

Then the bone amount in circular regions around the implant was measured by placing the sections under a stereomicroscope which was connected to a videocamera. With use of a frame grabber with 512 × 512 pixels, 8-bit grey-level images were captured. Two circular regions of interest (r.o.i.) were marked around the implant (Fig. 2). One region was defined in direct contact with the implant, at a radial distance of 0.26 mm from the interface (circle A). The other region was determined at 0.61 mm from the implant (circle B). Finally the amount of bone in the area confined by circle A and in an area called C (C = amount of bone inside circle B substrated from the amount of bone inside circle A) were calculated by a computer-based image-analysis program. The amount of bone was quantified as bone amount per $\mu m^2/10^3$.

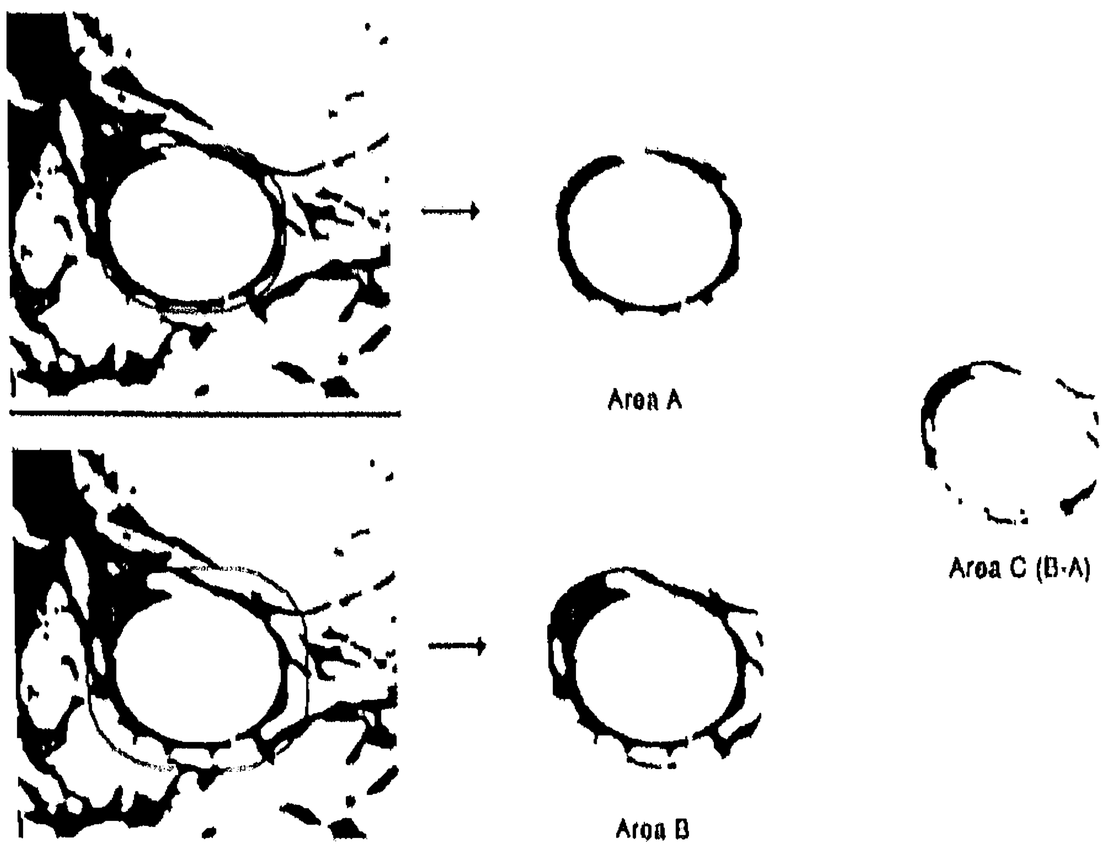

Figure 2. Illustration of the computer-based image-analysis measurements of bone amount.

For both quantitative bone evaluations, three histological sections per implant, respresentative of the bone response, were randomly chosen. Presented results are based on the average of these three measurements. For each implantation period six implants of each coating type were evaluated.

RESULTS

One of the rabbits, from the 3-week evaluation group, died of pneunomia. The other rabbits did not have any complications and appeared to be in good health during the experimental period. At sacrifice no clinical signs of inflammation or adverse tissue reaction could be seen around the implants. Radiographs, taken parallel to the long axis of the implant, showed that the implants were located in cortical as well as trabecular bone and the medular cavity of the femur.

Characterization of the Ca-P coatings

A complete description of the characteristics of the coatings has already been given elsewhere.[7,13]

In summary XRD patterns demonstrated that the HA-PS and HA-PS/ht coatings showed an amorphous/crystalline structure. After heat treatment the crystallinity was increased (crystallinity, HA-PS 60% vs. HA-PS/ht 65%). Analysis of the Ca-P-c and Ca-P-a diffractographs showed that the indexed procedure resulted in a crystalline Ca-P coating with an apatite structure. Coatings prepared with the rotating substrate holder showed an amorphous structure, without any specific reflection lines (Fig. 3).

Infrared measurements of the plasma-sprayed samples showed absorption bands characteristic of P-O and O-H bonds. In the spectra of both sputtered coatings no O-H bonds were detectable. These coatings showed a wide peak over the region from 2800 to 4000 cm^{-1} indicative of water absorption at the surface.[14]

Rutherford backscattering spectrometry (RBS) measurements of the sputtered coatings revealed for the Ca-P-c coating a Ca/P ratio of 2.8 and for the Ca-P-c coating a Ca/P ratio of 2.3 (Fig. 4).

Light microscopy

As was observed in the radiographs, histological sections of the implants confirmed that the implants

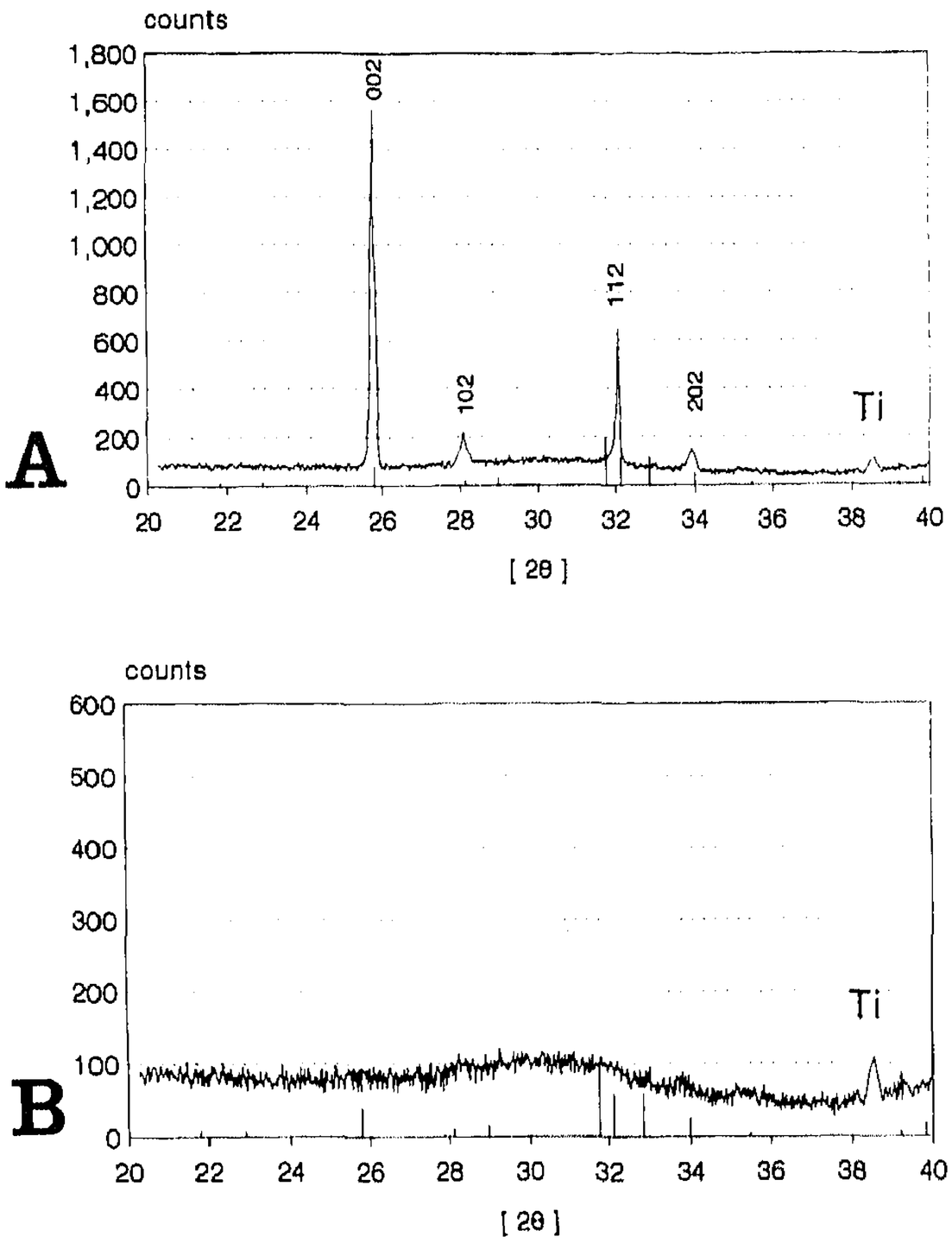

Figure 3. X-ray diffraction pattern of the magnetron-sputtered coatings. (A) Ca-P-c magnetron-sputtered coating, (B) Ca-P-a magnetron-sputtered coating.

were positioned in cortical bone as well as trabecular bone, and in the medullar cavity.

Three weeks

Three weeks after insertion around all implants a lattice of woven bone was formed, especially at those sites where the implants were positioned in the trabecular bone or medular cavity (Fig. 5). Occasionally this callus had proliferated throughout the medulla. In some sections were noted limited areas with early signs of callus remodeling. All implants had a close bone–implant contact in the cortical area. At some sites where the implants were positioned without initial bone contact, a layer of osteoid covered the implant interface. For the HA-PS coatings no signs of coating degradation were observed.

Six weeks

In these specimens the healing process had proceeded. In all histologic sections the callus was reduced in size, and had reoriented. The spaces in the lattice of the callus were filled with new bone. At some areas of the intramedullar bone–implant interface, new bone was deposited (Fig. 6). On this deposited bone active osteoblasts and a layer of osteoid could be observed. This bone was in direct contact with the implant sur-

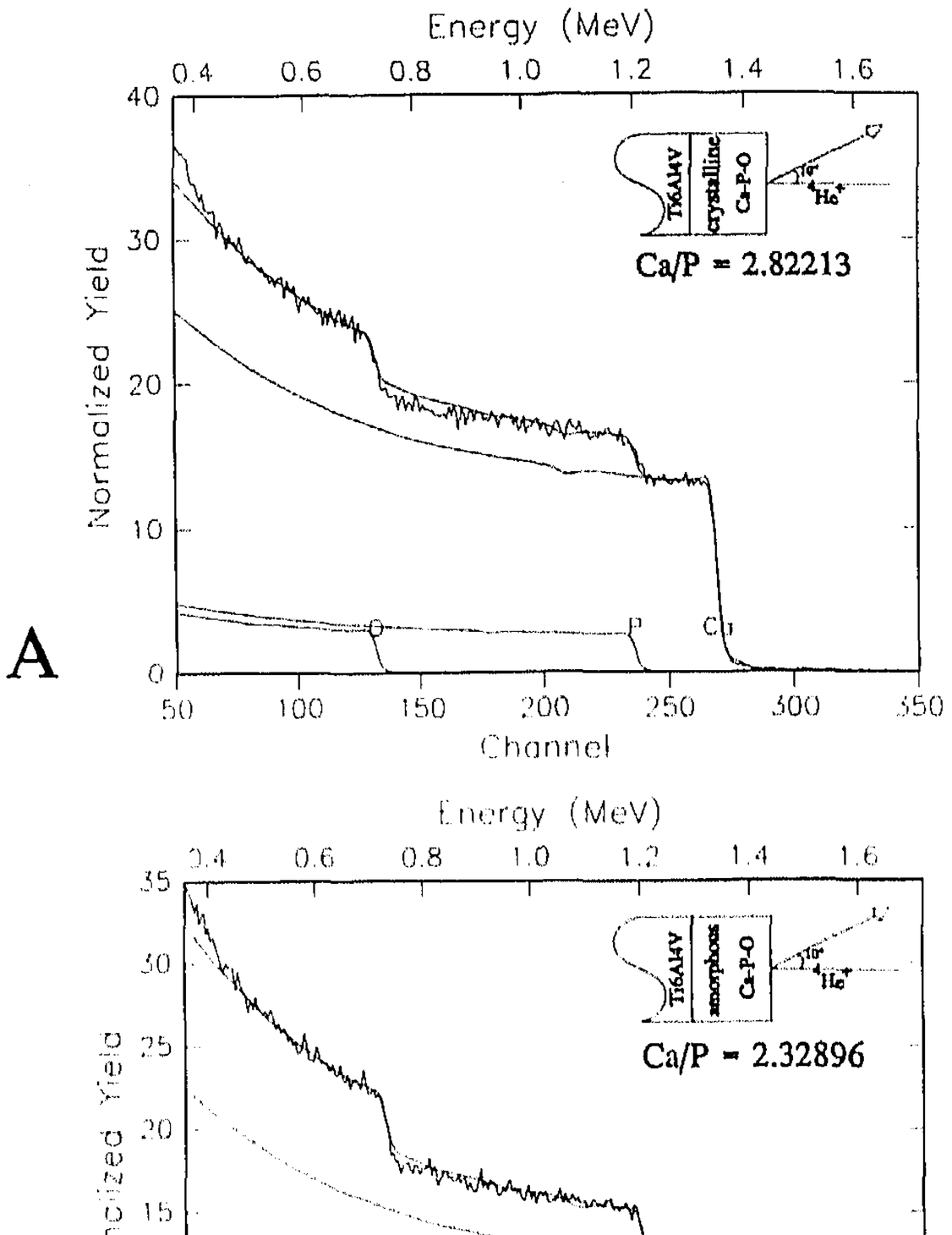

Figure 4. RBS measurements (^{4}He energy: 2.012 MeV and $\theta = 10°$) of RF magnetron-sputtered coatings. (A) Ca-P-c magnetron-sputtered coating, (B) Ca-P-a magnetron-sputtered coating.

Figure 5. Light micrograph of a HA-PS/ht coated implant after 3 weeks of implantation. The original drilling hole can be recognized, because the slide is made at height of a circular groove of the implant. This gap has been filled with a lattice of woven bone. (Original magnification ×10, bar = 300 μm).

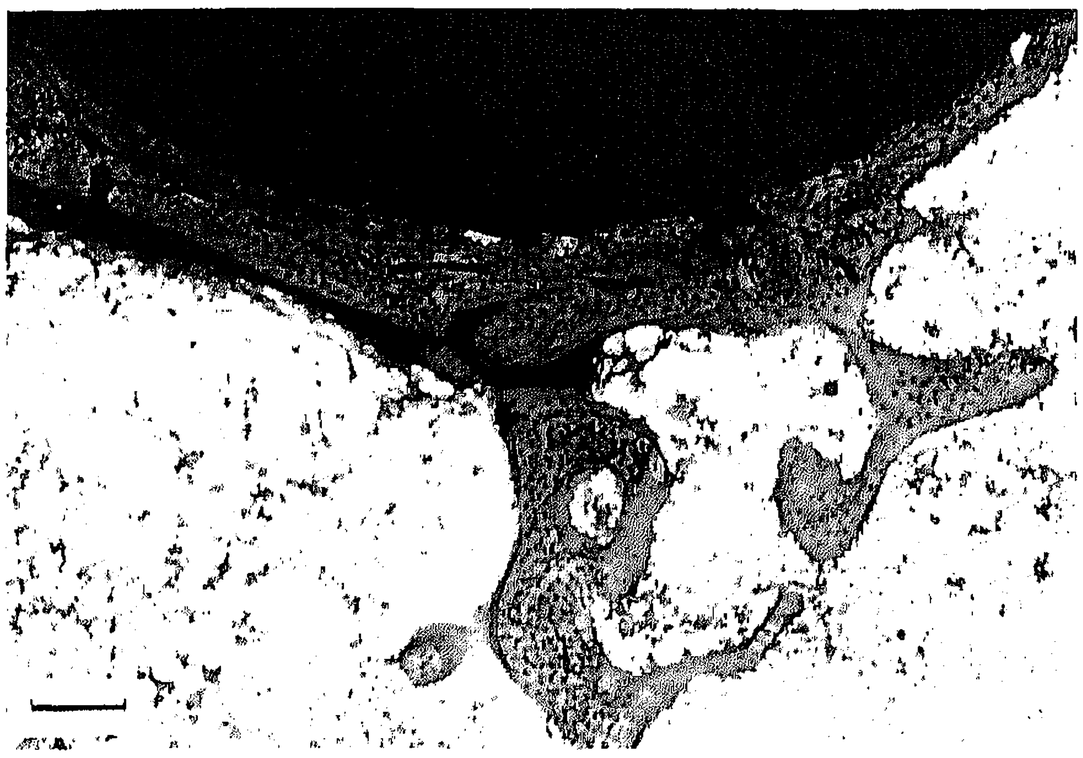

Figure 6. Light micrograph of a Ca-P-a–coated implant after 6 weeks. New bone has been deposited in the medullar cavity. (Original magnification ×10, bar = 300 μm).

face without intervening fibrous tissue layers. In some sections the entire medullar surface of the implants was surrounded by newly formed bone, or a thin layer of osteoid covered the implant (Fig. 7). Occasionally sections showed that bone was bridging the medullar cavity from the cortical walls to the implant surface. Furthermore sections made at the level of the circular grooves in the implants showed that the created gaps were completely filled and bridged with lamellar bone (Fig. 8). The HA-PS and HA-PS/ht implants showed no substantial reduction in coating thickness.

Nine weeks

At 9 weeks remodeling and compaction of the bone–implant interface was proceeding (Figs. 9 and 10). No differences in bone formation between the various coatings could be observed. The plasma-spray–coated

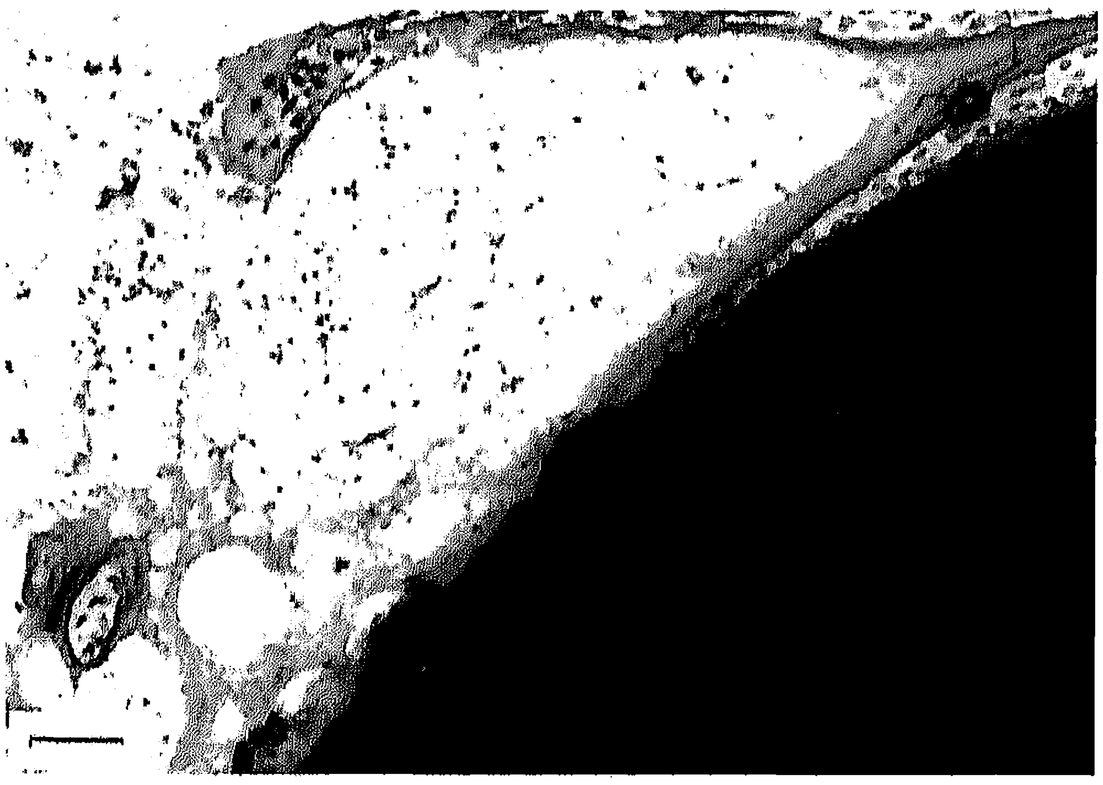

Figure 7. Histological appearence of a Ca-P-a–coated implant after 6 weeks. The implant is partially positioned in the medullar cavity. In the upper right corner, bone contact is shown. This bone is covered by a zone of osteoid (gray zone), which continues at the implant surface. (Original magnification ×32, bar = 94 μm).

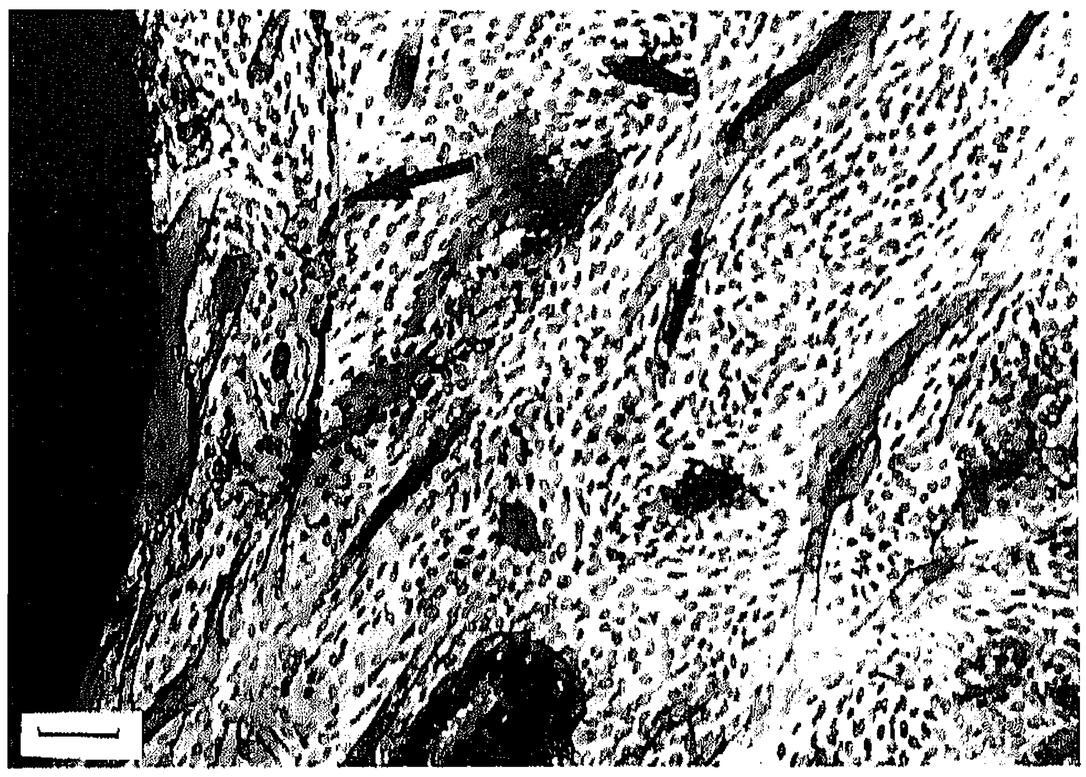

Figure 8. Histological section of a Ca-P-a–coated implant after 6 weeks. The original drilling hole (arrow) can still be recognized. The healing process continues, and lamellar compaction has started. (Original magnification ×25, bar = 96 μm).

implants still showed no signs of coating reduction. Only one of the HA-PS/ht–coated implants showed delamination of the coating. Bone tissue was found between the dislodged coating and implant surface (Fig. 11).

Histomorphometrical measurements

Results are presented in Table I. Statistical analysis of these data, using a one-way analysis of variance (ANOVA) and a multiple comparison test (Newman-Keuls), showed that no significant difference existed in bone contact between the various coatings ($p > 0.05$). In addition no significant difference was demonstrated in bone contact between 6 and 9 wk implantation time ($p > 0.05$).

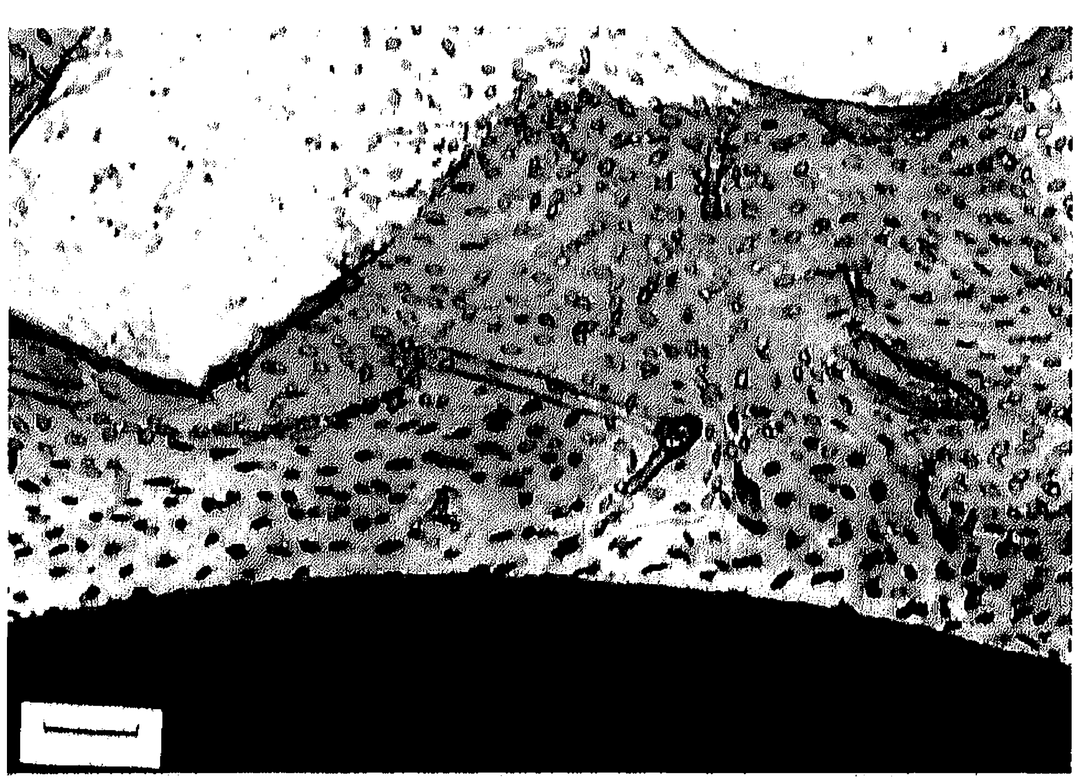

Figure 9. A Ca-P-a–coated implant after 9 weeks from a light microscopical view. Remodeling and compaction of the bone–implant interface has further proceeded. The bone is in close contact with the implant surface, without intervening fibrous tissue layers. (Original magnification ×40, bar = 75 μm).

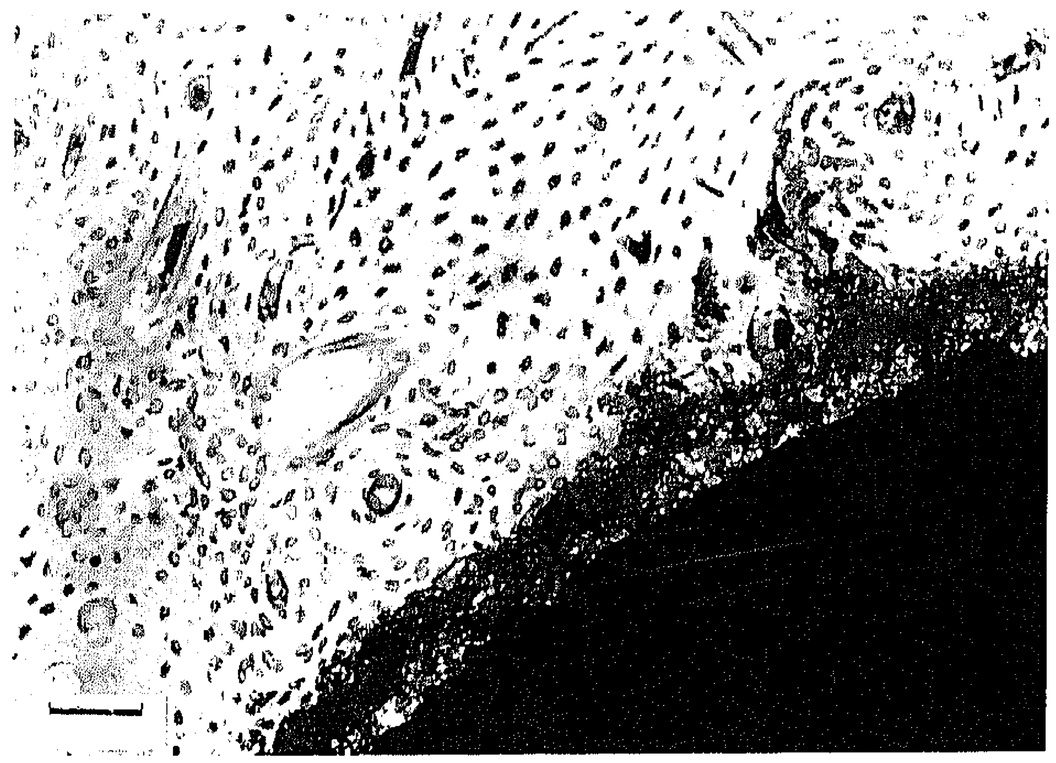

Figure 10. Light micrograph of a HA-PS–coated implant after 9 weeks. Remodeling and compaction of the bone–implant interface has further proceeded. The plasma-spray coating shows no sign of reduction in thickness. (Original magnification ×10, bar = 300 μm).

Results of the bone-amount measurements are provided in Table II. At 6 weeks ANOVA and Newman-Keuls revealed only a significant difference ($p < 0.001$) in bone amount in area C between Ca-P-c coatings and the other materials. At 9 wk no significant differences existed in bone density in all areas for all materials ($p > 0.05$ and $p > 0.05$).

DISCUSSION AND CONCLUSIONS

This study is the first to present *in vivo* results of radiofrequent sputter (RF) coatings which are prepared, fully characterized, and finally tested in the same laboratory. Results revealed that sputtered Ca-P coatings induced the same behavior as plasma-sprayed coatings. At 6 weeks, even more bone was present around the Ca-P-c coatings compared with the other materials. Furthermore we observed that bone apposition to the various coatings did not decrease with time.

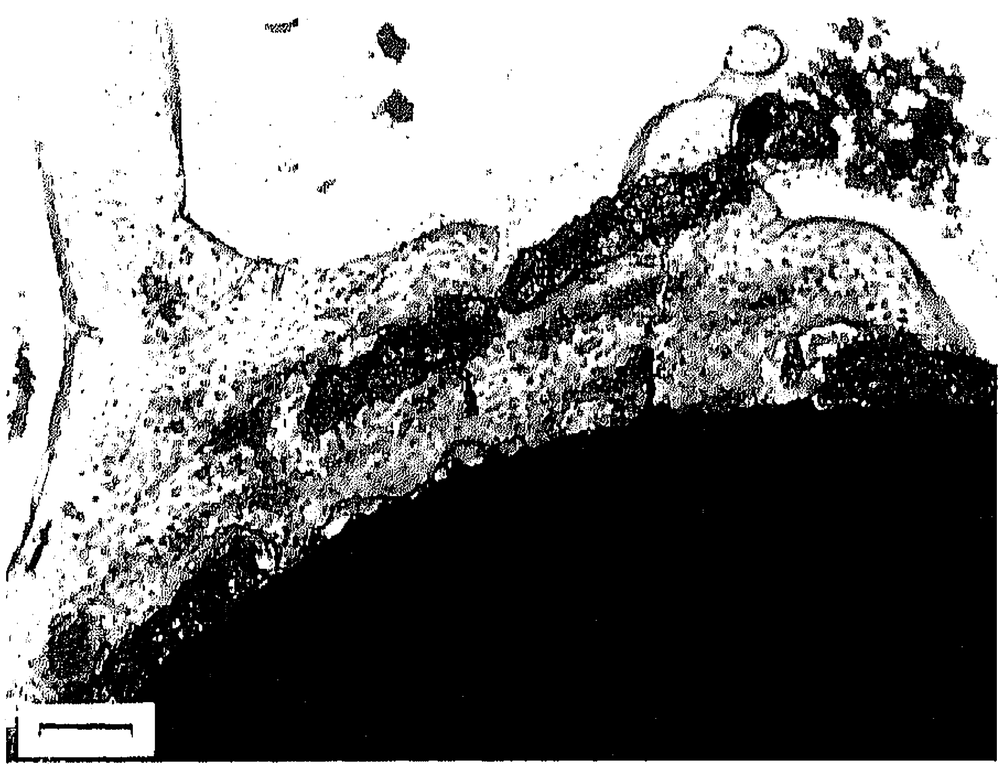

Figure 11. Detail of a HA-PS/ht–coated implant after 9 weeks. The coating appeared to be detached from the implant. Bone tissue was found growing between the coating and the implant surface. (Original magnification ×40, bar = 75 μm).

TABLE I
Percentages of Bone Contact

	6 weeks		9 weeks	
Coating	% Contact	STD	% Contact	STD
HA-PS	63.1	10.8	71.3	11.1
HA-PS/ht	68.2	8.2	68.6	8.4
Ca-P-a	73.6	14.4	64.6	10.4
Ca-P-c	68.9	5.6	68.6	9.5

Although these results confirm our earlier *in vitro* studies,[8,15] they are in contrast with the findings of Steflik.[16] He evaluated HA plasma-spray–coated, RF-sputter–coated, and ion-beam-sputter–coated dental implants placed in the mandible of dogs. All implants were inserted using a so-called two-stage procedure, except for the RF-sputter–coated implants. These were one-stage implants. The evaluation periods were 1 and 3 months. Histomorphometrical analysis demonstrated that: (1) at 1 month postimplantation the percent of bone apposition to ion-beam–coated implants was higher compared with other implants, (2) RF-sputter–coated implants showed significantly lower bone-contact percentages, and (3) the percent of bone contact with ion-beam–coated and RF-sputter–coated implants dropped during the 3-month implantation period. The following explanation can be given for the fact that our results do not corroborate these findings. First Steflik subjected both types of sputter coating to a heat-treatment procedure to increase the crystallinity of these coatings. This heat-treatment procedure can vary the oxide properties of the titanium implant surface.[17] Subsequently when the coating dissolves during implantation, the newly formed bone will come in contact with this modified implant surface, which can result in a decreased bone response. In our study crystallinity of the RF coatings was obtained by indexing the position of the substrate holder. This makes an additional heat-treatment procedure after coating deposition unnecessary. Second, the lower bone adaptation to the RF-coated implants can be caused by the different surgical procedure. One-state implants, in contrast to two-stage implants, are directly exposed to the oral conditions. This makes their final behavior less comparable. Furthermore since Steflik positioned all experimental implants of each type and implantation period in the left or right mandible of just one dog, it cannot be ruled out that the measured results are the interanimal differences of bone healing.

Also, some critical remarks have to be made on the thickness of the prepared histological sections. As demonstrated recently[18] sections with a thickness of over

TABLE II
Results of Bone Amount Measurements

	6 weeks				9 weeks			
Coating	Area A	STD	Area C	STD	Area A	STD	Area C	STD
HA-PS	2326.67	2122.60	1859.83	417.11	2314.67	1182.14	1924.50	1034.11
HA-PS/ht	1997.50	1186.64	1755.67	551.73	1852.33	1254.32	1967.67	1169.02
Ca-P-a	2811.50	1687.79	1972.50	586.29	3242.33	2668.46	2396.17	1334.09
Ca-P-c	3736.60	1495.63	3822.80	1025.92	2540.83	912.50	2811.17	1665.90

Bone amount measurements are given in $\mu m^2/10^3$.

30 μm can lead to misleading interpretations of the true bone contact. In addition evaluation of an insufficient number of sections will result in a completely wrong conclusion about the bone reaction of an implant material. For example, in a previous study[19] we showed that evaluation of three sections taken at different levels from the same implant revealed a significant variance in bone apposition. These two prerequisites for the correct estimation of bone contact, which were met in our study, also explain the large values of standard deviations in our histomorphometrical measurements.

With regard to the sputter coatings as used in our study, another phenomenon has to be discussed. It is supposed that a Ca-P ratio of 1.67 together with an apatite structure is a prerequisite for a Ca-P ceramic implant to obtain a continuous interface with the surrounding bone.[20,21] However, despite their high Ca-P ratio, the bone reaction to the sputter coatings was at least similar to the plasma-sprayed coatings. Such reactions have been observed earlier by Donath.[22] He inserted Ca-P ceramic implants with a Ca-P ratio ranging from 1.1 to 2 in the mandible of dogs. He found that the capacity of bone bonding increases even with the calcium content. Since the bioactive behavior of Ca-P ceramics is based on the dissolution of Ca^{2+}-ions and the subsequent precipitation of CO_3-apatite crystals,[20] the enhancing effect of a high Ca content on the bone response is clear. Still it has to be emphasized that the biological properties of Ca-P ceramics, besides Ca-P ratio, also are determined by phase composition and crystallinity. RF-magnetron–created Ca-P coatings, similar to all "HA" materials, consist of several Ca-P phases, i.e., HA, tricalcium phosphate, and tetracalcium phosphate. In constrast to plasma-sprayed coatings, but similar to sintered ceramic materials, the crystallinity of sputter coatings is determined by atomic ordering and not by the size of the remaining powder particles. Therefore the dissolution process will be identical to sintered ceramics and will not result in the release of harmful coating fragments. The biological advantage of RF-magnetron-sputter coatings is that their major phase is HA in combination with an increased Ca content.

Another surprising finding was that the plasma-spray coatings showed few signs of coating reduction. This finding is in contrast with several other studies[3,5,23–25] in which plasma-sprayed coatings were reduced significantly after 12 weeks of implantation. On the other hand it confirms our earlier studies with Ca-P-plasma-spray–coated percutaneous implants.[26,27] Reasons for this difference in observation can only be hypothesized. For example the animal model used can influence the final coating behavior. After insertion, the initial coating dissolution is followed by the precipitation of CO_3-apatite. It is suggested that this precipitated layer prevents further coating reduction.[28] This process of dissolution and precipitation will be influenced by the wound-healing response of the animal model. In animals with a fast healing response, like rabbits and rats, the CO_3-apatite surface layer will be formed earlier or can be thicker than in animals with a slow healing response, like goats and dogs. Consequently in fast-recovering animals less coating reduction will occur.

Degradation of the thin magnetron-sputter coatings could not be evaluated, since the histological sectioning technique used in combination with transmittant light microscopy makes it impossible to discriminate this layer from the underlying titanium. This can be achieved only by using transmission electron microscopy (TEM). Despite some favorable publications,[29–31] which described techniques to obtain implant and bone tissue in one and the same ultrathin section, the preparation of TEM sections of solid metal and ceramic materials still is hampered by significant problems. Until a final solution has been found, no reliable information can be gained about the closeness of the contact between the implant and bone tissue *in vivo*.

In conclusion our study confirms that implants with magnetron-sputtered Ca-P coatings situated inside the trabecular femoral bone of rabbits show the same process of bone healing as do plasma-sprayed Ca-P–coated implants. However, with the magnetron-sputter procedure thin coatings ($\leq 4\mu$m) can be produced, while plasma spraying results in coatings of at least 30 μm thick. Consequently accurately machined dental implants, with geometrically complex designs like screws, do not undergo dimensional changes because of the coating procedure. In addition grit blasting

to provide mechanical retention, as required for plasma spraying, is not required.

These investigations were supported by the Netherlands Technology Foundation (STW). The authors thank Mrs. Y. C. G. J. Paquay for her surgical skills and assistance during the animal experiments, and Mrs. A. F. M. Leijdekkers-Govers for making histological sections.

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Received March 23, 1995
Accepted September 28, 1995