The effect of Ca-P plasma-sprayed coatings on the initial bone healing of oral implants: An experimental study in the goat

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The response of bone of low density to uncoated, fluorapatite (FA), hydroxyapatite (HA), and hydroxyapatite heat treated (HAHT) plasma-sprayed coated implants was investigated 3 and 6 months after installation. Forty-eight threaded implants of commercially pure titanium were inserted into the maxilla of twelve goats according to a split-plot design. One goat died shortly after installation of the implants, five goats were sacrificed 3 months after installation, and the other six goats 6 months after installation. Histological evaluation revealed no difference in bone reaction between the 3- and 6-month implantation periods. In addition, probably due to the wide inter- and intra-animal variability, no significant difference between the 3- and the 6-month periods could be observed in the histomorphometrical measurements performed. Further, no significant differences were found in bone reaction among the various implant materials. Finally, qualitatively it appeared that all coatings showed reduction in coating thickness and that such reductions were most pronounced for the HA coatings.

INTRODUCTION

The use of oral implants has grown enormously during the last 15 years because of the increased demand for dental care from patients who have lost their teeth due to trauma, disease, or neglect. This demand has resulted in the development of numerous oral implant systems. Currently, several of the available systems have a clinical success rate of 90–98%[1-4] at 3-year and more follow-up periods when the implants are installed in the symphyseal area of the mandible. Poorer results are found when implants are inserted in bone of lower density, as found in the maxilla and posterior regions of the mandible.

In addition to location, many other factors can influence the success or failure of an oral implant. Of primary importance is the surgical technique used. Surgery has to be performed in an atraumatic way, for example by the use of adequate cooling during surgery and the use of low rotational drill speed (max. 450 rpm).[5,6] Too much surgically destroyed tissue will have a negative effect on proper tissue repair.

Secondary factors that can delay the healing of implants are biomechanical in origin. Early movement of the implant before the surrounding bone is apposed to the metal surface and overload during the initial healing phase[7-9] will lead to a predominant formation of interfacial connective tissue. Therefore, implants when they are placed in bone of high mineral density, as found in the lower jaw, are usually not loaded before a healing period of 3 months. In bone of low mineral density, as found in the upper jaw, there is a waiting period of at least 6 months before loading as it is supposed that wound healing and bone repair will take longer in the latter type of bone.

In light of the above, we investigated in a previous study[10] whether calciumphosphate (Ca-P) coatings can improve the bone response to oral implants placed in low density bone of the maxilla of goats. Histomorphometrical evaluation of the retrieved implants revealed a significantly greater percentage of direct trabecular bone contact to the Ca-P-coated implants as compared to noncoated implants. In that study the surgical protocol used was the same as that used in the treatment

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of human patients, and the implants were left endosseous for 6 months to allow healing of the alveolar bone before the permucosal abutments were connected.

In addition to the overall bone response, another apparent advantage[11–13] of Ca-P coatings is the improvement of the initial bone response. If the latter hypothesis is indeed true, waiting for six months would be superfluous, and the intervening healing period could be reduced. Thus the aim of this study was to determine the effect of Ca-P coatings on the bone's response to oral implants inserted into the low-density maxillary bone of goats 3 and 6 months after installation.

MATERIALS AND METHODS

Implant materials and coating characteristics

Forty-eight threaded commercially pure titanium implants with a length of 10 mm were manufactured. Twelve of the implants were left uncoated and had a diameter of 3.75 mm. The other implants had a diameter of 3.65 mm and were subjected to a plasma-spray coating technique with Ca-P. Before coating, these implants were grit-blasted with Al_2O_3 (Ra = 4–5 μm), cleaned ultrasonically in propanol, and dried at 100°C. Three different coatings were deposited: hydroxyapatite (HA), hydroxyapatite subjected to a heat treatment (650°C for 10 min, HAHT), and fluorapatite (FA). The prepared coatings had a thickness of about 50 μm and were characterized by X-ray diffraction (XRD) and infrared spectroscopy (IR).[14] In summary, this analysis revealed that the HA coatings had a crystallinity of 60%, the HAHT coatings 65%, and the FA coatings 95%. The final diameter of all implants, coated and noncoated, was 3.75 mm.

Before surgery, all implants were cleaned ultrasonically in 100% ethanol, dried at 50°C, and then sterilized in a steam autoclave.

Experimental design and surgical procedure

Twelve healthy adult female Saane goats, with an average age of 30 months and an average weight of 65 kg, were selected for the experimental animal model. The animals were kept in quarantine for at least 4 weeks and tested for CAE/CL arthritis. For the insertion of the implants, the two first maxillary premolars were bilaterally extracted. The extraction was performed under general anesthesia induced by intravenous pentobarbital (25 mg/kg) and atropine (0.5 mg). After orotracheal intubation, anesthesia was performed by ethrane through a constant volume ventilator. The extraction wounds were closed with resorbable vicryl 2-0 sutures.

After a healing period of 4 months, the goats again were subjected to a general anesthesia for the installation of the implants. The operation field was washed with 0.1% chlorhexidine. A longitudinal incision was made on the palatal site of the edentulous region of the alveolar ridge, and a mucoperiosteal flap was raised. After exposition of the bone, two holes 7 mm apart were made with a guide drill, then the implant sites were further prepared to their final depth and width. The diameter of the last burr was 3.5 mm, which allowed a good initial stability of all the implants after installation. The bone preparation was performed with a very gentle surgical technique, and the implant placement was done under abundant irrigation with a cold saline solution. Finally, the operation site was cleaned thoroughly by rinsing before closing the mucoperiosteal flaps with vicryl 2-0 sutures.

A total of 48 implants was placed: 12 Ti, 12 coated with HA, 12 coated with HAHT, and 12 coated with FA. Each animal received all types of implants, two in the left and two in the right half of the maxilla. The implants were placed according to a balanced split-plot design in order to compensate for differences in bone quality and quantity among the implantation sites. To reduce the perioperative infection risk, prophylactic antibiotics (Albipen®) were administered for 3 days starting 1 h postoperatively.

Six of the 12 goats were killed after 3 months and the other six after 6 months by an overdose of pentobarbital (Nembutal®), after which segments of the maxilla were removed and divided into small blocks containing the implants.

The experimental protocol used for these studies was approved by the Insitutional Animal Care and Use Committee.

Radiographic examination

Radiographs were taken of the block biopsies of the maxilla that contained the implants. The cone of the apparatus was oriented perpendicular to the implants. One radiograph was taken of each biopsy with a General Electric X-ray machine with exposure factors of 65 kV, 15 mA, and focal-film distance of 36 cm. All films were developed with a Dürr-Periomat (Dürr Dental-D-7120 Bietigheim-Bissingen, Germany) automatic dental film processor. The distance between the top of the neck (TN) of the implant and the bone crest (BC) was measured for each implant (Fig. 1) on the mesial and distal site, and the mean was calculated. These measurements were performed by the same investigator with vernier calipers (Mitutoyo Digimatic® 500, Japan) that had a scale up to 0.01 mm.

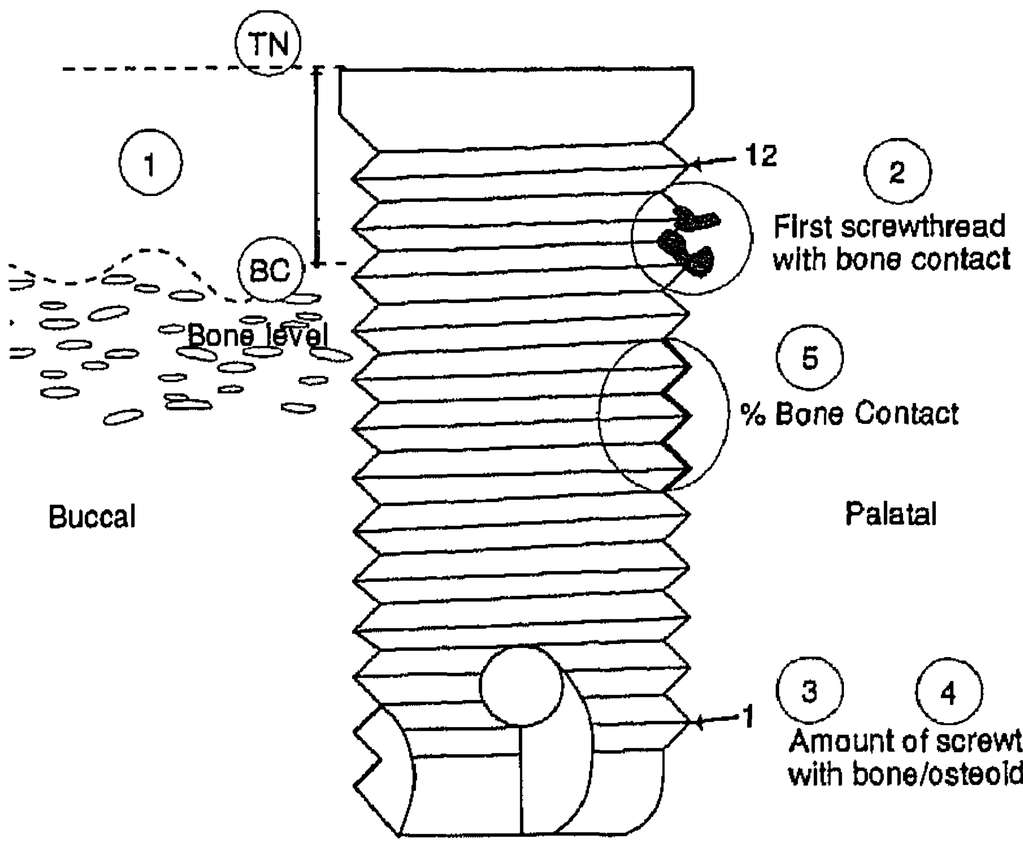


Figure 1. Schematic drawing of the parameters used for the histomorphometrical and radiological evaluations: 1, percentage of direct bone contact; 2, first buccal and palatal screwthread showing bone contact. 1 = most apical screwthread; 2 = most coronal screwthread; 3 = bone level measured as the distance from the top of the neck of the implant (TN) to the bone crest (BC) in contact with the implant surface.

Histological procedure

After radiographic examination the block biopsies were fixed in 10% buffered formalin solution, dehydrated in alcohol series, embedded in methylmethacrylate, and sectioned for light microscopical assessment with a modified inner circular saw microtome.[15,16] The sectioning technique provided 10 μm-thick serial sections in a buccopalatal direction parallel to the long axis of the implant surface. The sections were stained with methylene blue and basic fuchsin.

Histological and histomorphometrical evaluation

First, a descriptive evaluation of the trabecular bone response to the various implants was performed. Second, the bone reaction was histomorphometrically assessed using a light microscope connected to a computer equipped with a video and image analysis system (Technical Command Language image, TNO Institute of Applied Physics, Delft, The Netherlands). For this analysis, three representative sections of each implant were selected. The following parameters (Fig. 1) were evaluated: (1) the *percentage of direct bone* contact along the three best consecutive screwthreads; (2) the *first buccal and palatal screwthread* with direct bone contact, accomplished by numbering the screwthreads in ascending order from the most apical (1) to the most coronal (12) screwthread; (3) the average of the *number of buccal and palatal screwthreads* with direct bone contact; and (4) the buccal and palatal marginal *bone level*, rated as the distance from the top of the neck of the implant to the bone crest in contact with the implant surface.

RESULTS

One goat in the 3-month installation group had to be sacrificed 2 weeks after implant installation due to a broken leg. As this happened in an early period after insertion of the implants, this animal was excluded from the histological and histomorphometrical evaluations. At the end of the implantation periods, it appeared that of the remaining 44 installed implants, three implants were lost (not *in situ*) in the 3-month group and six in the 6-month group (Table I). A Chi-square test revealed that there was no significant difference in loss or maintenance between the Ca-P-coated and noncoated implants, neither for the 3-month nor for the 6-month group (respective p values of 0.7 and 0.1).

Histological description

Examination of the histological sections showed that seven of the 35 retained implants were located partially in the maxillary sinus. Apparently this position did not influence the final tissue response. In addition, we noticed no difference in bone reaction between the 3- and the 6-month groups. Around all implants abundant fibrous tissue was present no matter the installation period (Fig. 2). Mostly this fibrous tissue contained a high number of plasma cells (Fig. 3). The Ca-P-coated implants appeared to induce more bone formation than the noncoated implants (Fig. 4). The newly formed bone was observed mainly in the screwthreads. No intervening fibrous tissue layer could be observed between the implant surface and the contacting bone (Fig. 5). Occasionally, osteoid formation was seen (Fig. 6). Further, we observed that at some sites bone was closely apposed to the implant even when the implant was almost completely surrounded by inflammatory tissue. At the coronal cortical level, around all coated and noncoated implants a gap existed between the bone and the implant (Fig. 7).

Qualitatively, it appeared that all three coatings reduced in thickness at the end of the 3-month as well

TABLE I
Loss and Retention of the Different Implant Materials for the Different Implantation Periods

	3 Months		6 Months	
Material	Retained	Lost	Retained	Lost
FA	4	1	5	1
HAHT	4	1	5	1
HA	5	0	5	1
TI	4	1	3	3
Total	17	3	18	6

FA = fluorapatite; HAHT = hydroxyapatite heat treated; HA = hydroxyapatite; TI = titanium.

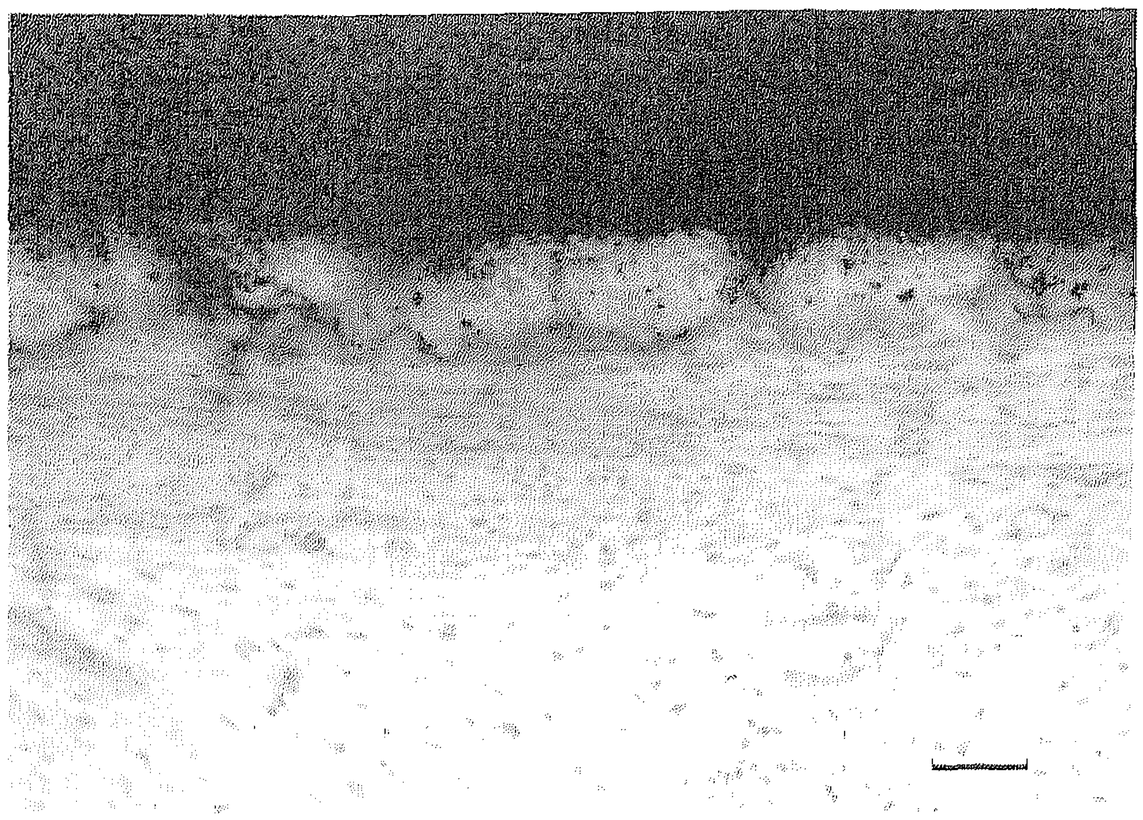


Figure 2. Light micrograph showing fibrous tissue formation around a FA-coated implant 3 months after implantation. Despite the presence of fibrous tissue, the coating is still intact. Original magnification 50X, bar = 50 μm.

as at the end of the 6-month implantation period. However, this reduction was not uniform. At some places the entire coating thickness was maintained while at other sites only a thin layer or no coating was left. This coating reduction was more severe for the HA coatings than for the HAHT and the FA coatings. Frequently, cellular activity of multinucleated cells could be observed in the vicinity of the coated implant surfaces (Fig. 8). However, these cells could not be associated with the coating reduction. In addition, no relationship could be found between coating degradation and the presence of fibrous tissue, inflammatory cells, and/or bone tissue (Fig. 9).

Histomorphometrical evaluation

Tables II and III show the results of the histomorphometrical evaluations of the 3- and 6-month specimens.

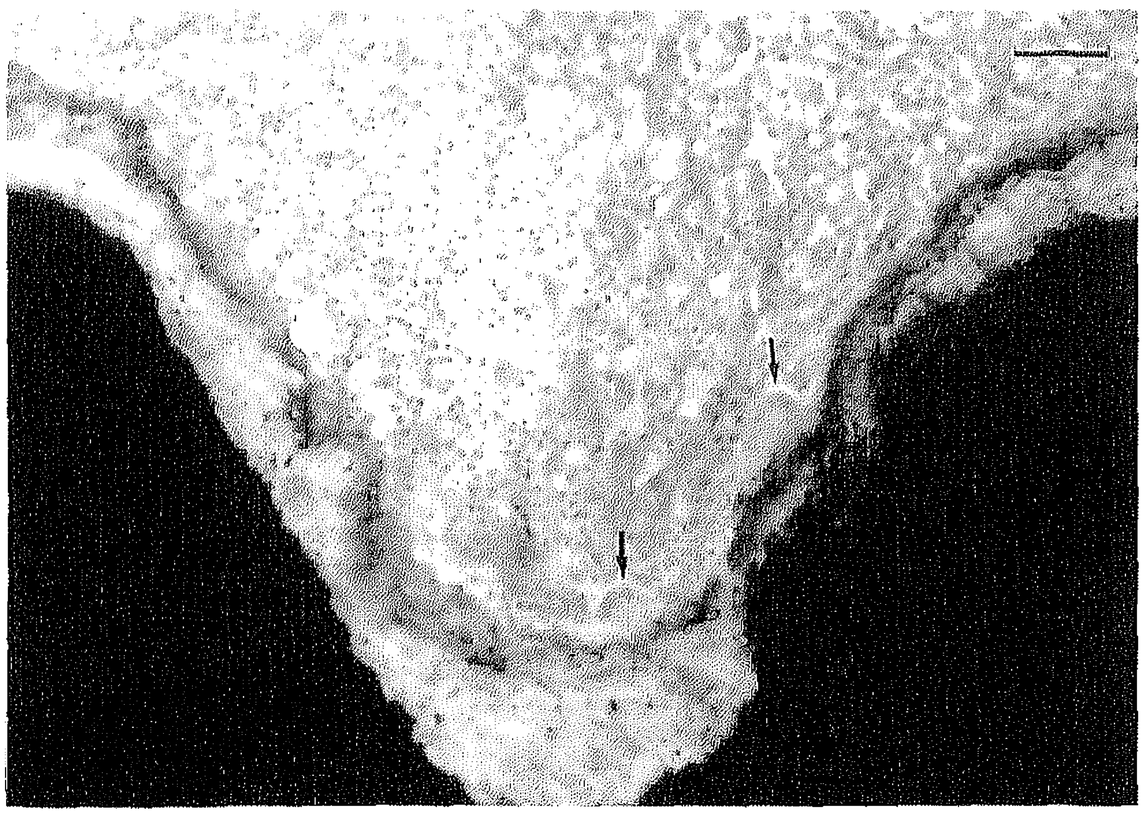
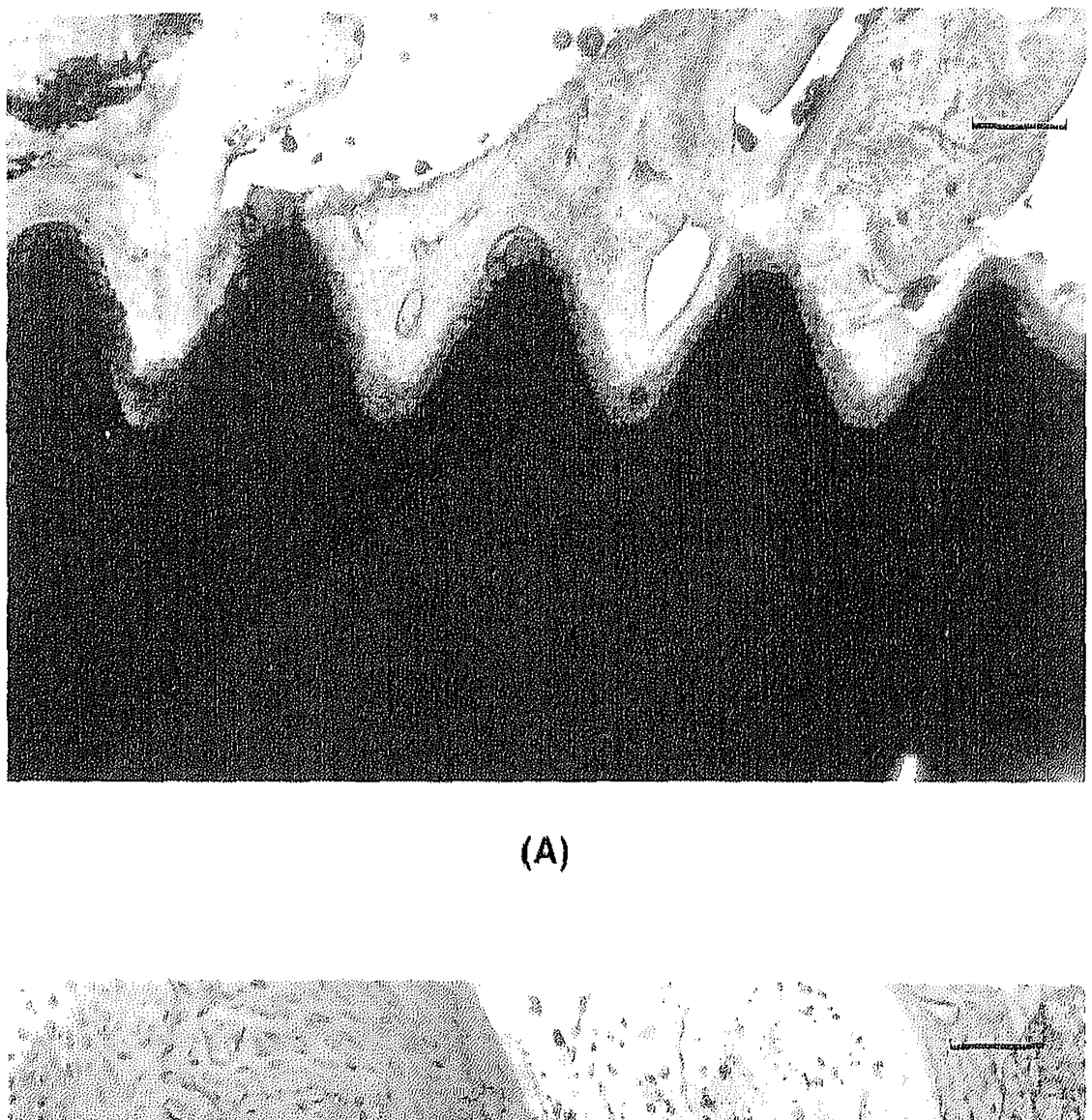


Figure 3. Light microscopial section of a FA-coated implant 3 months after implantation. Plasma cells are seen in the connective tissue surrounding the implant. On the coated surface, macrophages (arrows) are present. This inflammatory reaction did not result in coating loss. Original magnification 50X, bar = 50 μm.



(A)

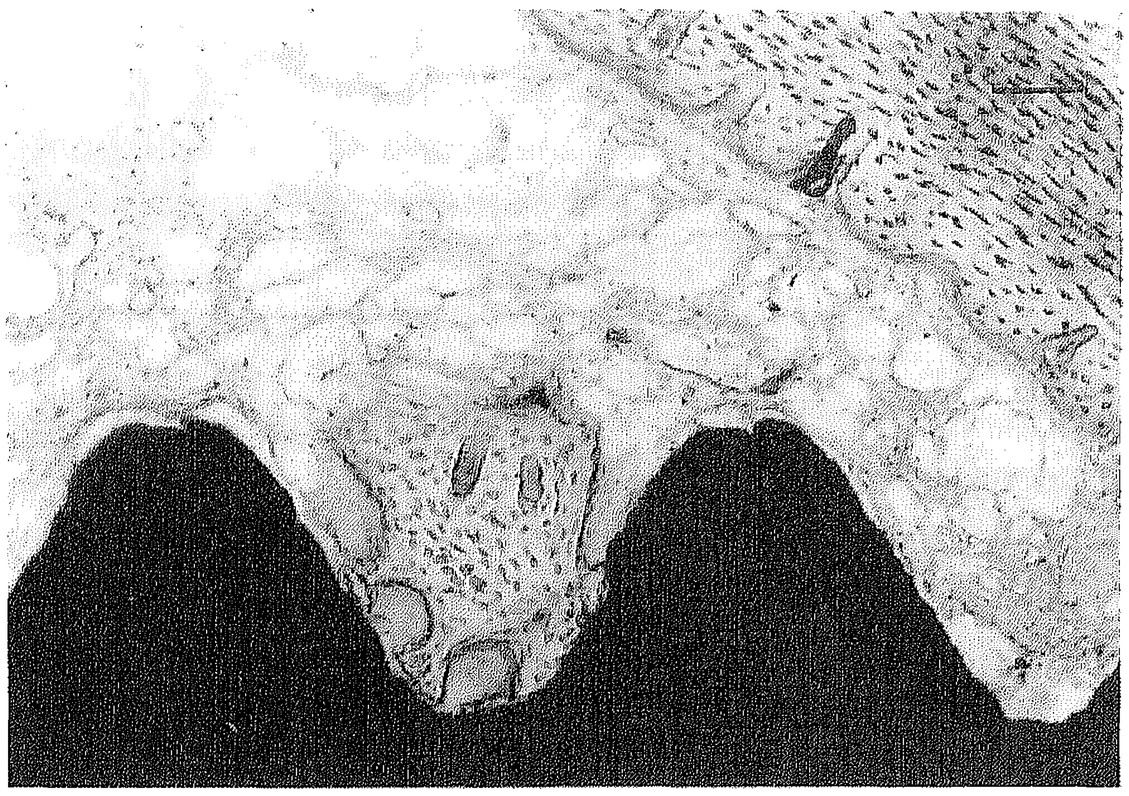
(B)

Figure 4. Histological appearance of a FA-coated implant 3 months after implantation demonstrating bone deposition on the implant surface. No intervening fibrous tissue layer can be observed in the interface. (A) Original magnification 10X, bar = 294 μm; (B) Original magnification 40X, bar = 73 μm.

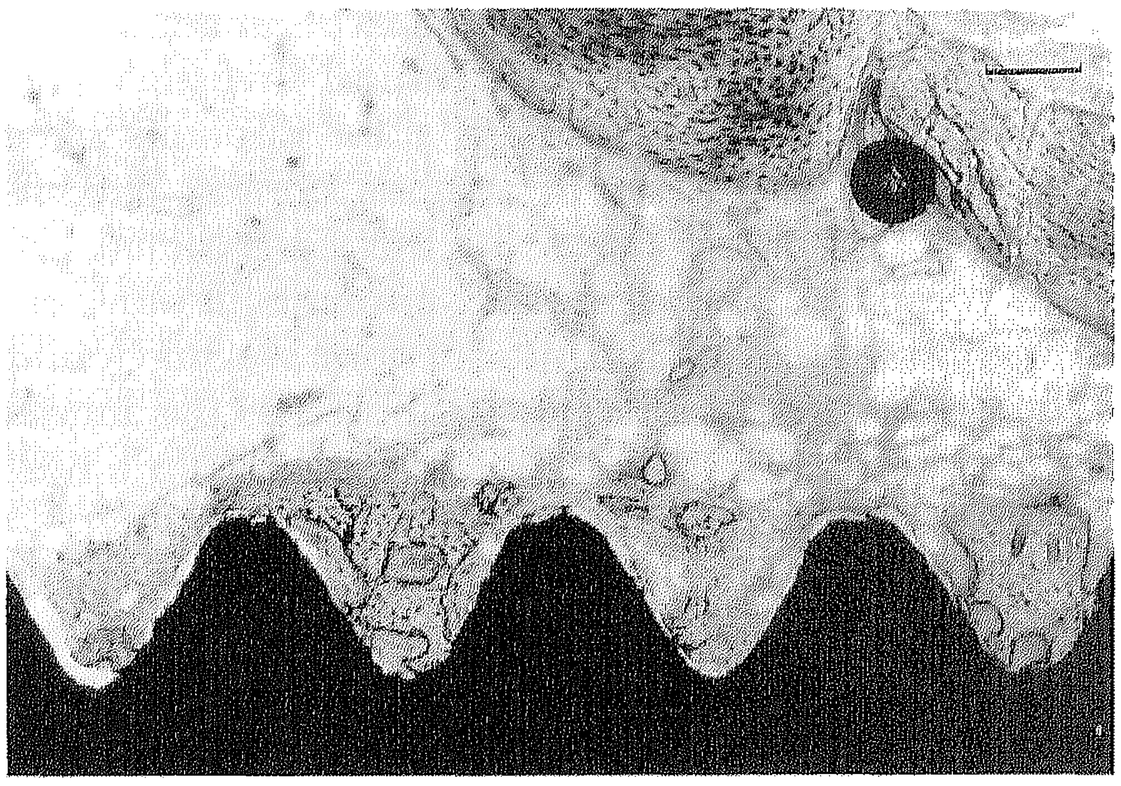
At 3 months a wide interanimal variation in bone contact percentages for FA, HAHT, and Ti implants was observed. For HA implants after 6 months of implantation, the results were more consistent. Further, the average percentage of bone contact of Ca-P-coated implants appeared to be higher than for noncoated implants. Nevertheless, statistical analysis using a one-way analysis of variance and Tukey multiple comparison procedures revealed that this difference was not significant ($p > 0.5$). In addition, no difference existed in the amount of bone contact between the 3- and the 6-month implants ($p > 0.5$).

Further examination showed that:

(1) The first palatal screwthread with bone contact in the 3-month specimens was located more coronally for the HA implants than for all other



(A)



(B)

Figure 5. Histological appearance of a noncoated titanium implant 6 months after implantation showing limited bone formation into the screwthreads. (A) Original magnification 12.5X, bar = 204 μm; (B) Original magnification 25X, bar = 118 μm.

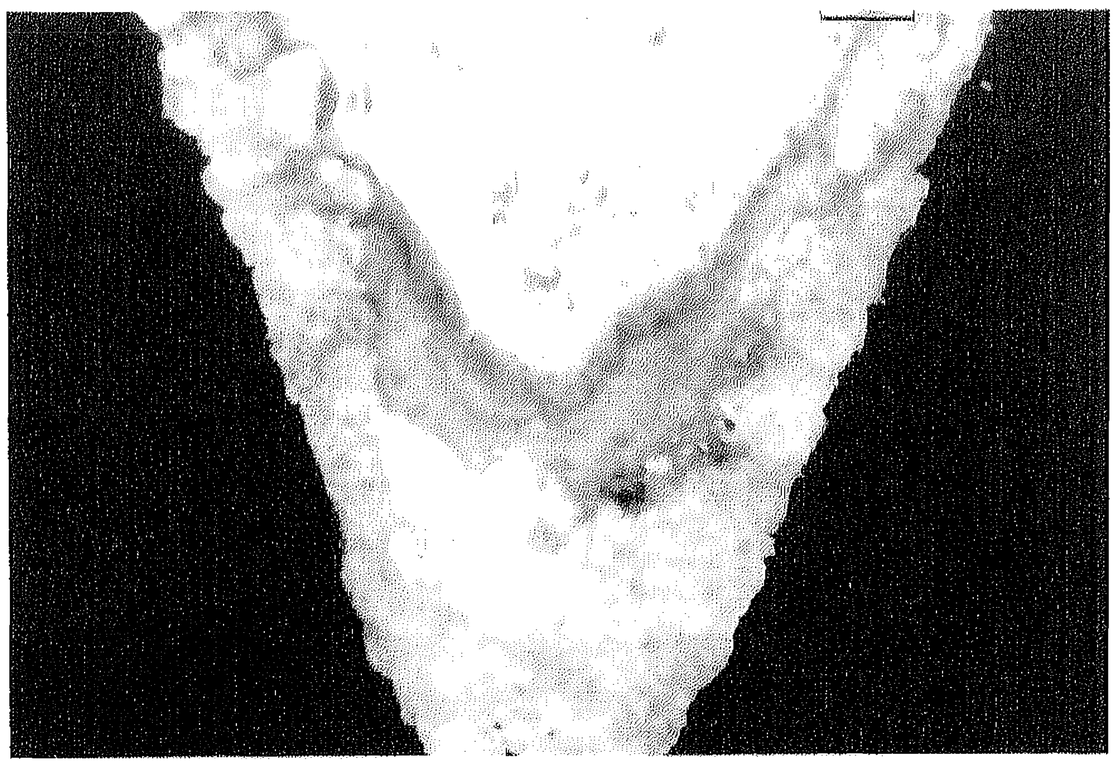


Figure 6. Osteoid formation on a FA-coated implant 3 months after insertion was observed. Osteoblasts are present on top of the osteoid. Original magnification 50X, bar = 50 μm.

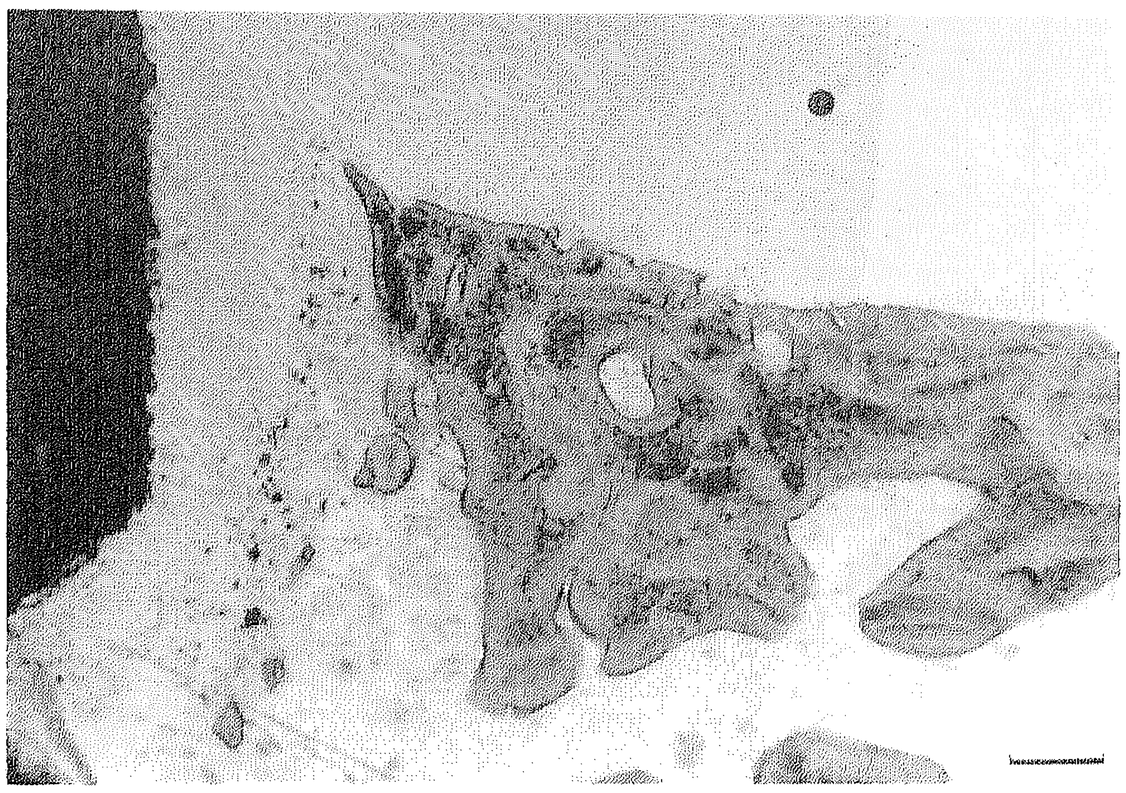


Figure 7. Bone reaction around HAHT implant 3 months after insertion. A gap is present at the coronal level. Original magnification 10X, bar = 294 μm.

implants ($p < 0.05$). On the other hand, buccally only, a difference was observed between the HA and the HAHT implants ($p < 0.05$). At 6 months a significant difference in the first thread with bone contact was observed between FA and Ti implants for the buccal side and between HA and Ti implants for the palatal side ($p < 0.05$).

(2) For the number of screwthreads with bone contact, a significant difference ($p < 0.05$) existed only between HA and Ti implants 3 months after implantation. Finally, using a Student's t test, we compared the radiologically and histologically rated marginal bone level. This test demonstrated that the radiographs overscored significantly ($p < 0.001$) the bone level (3.1 mm more coronally) compared to the histological rating of the bone level.

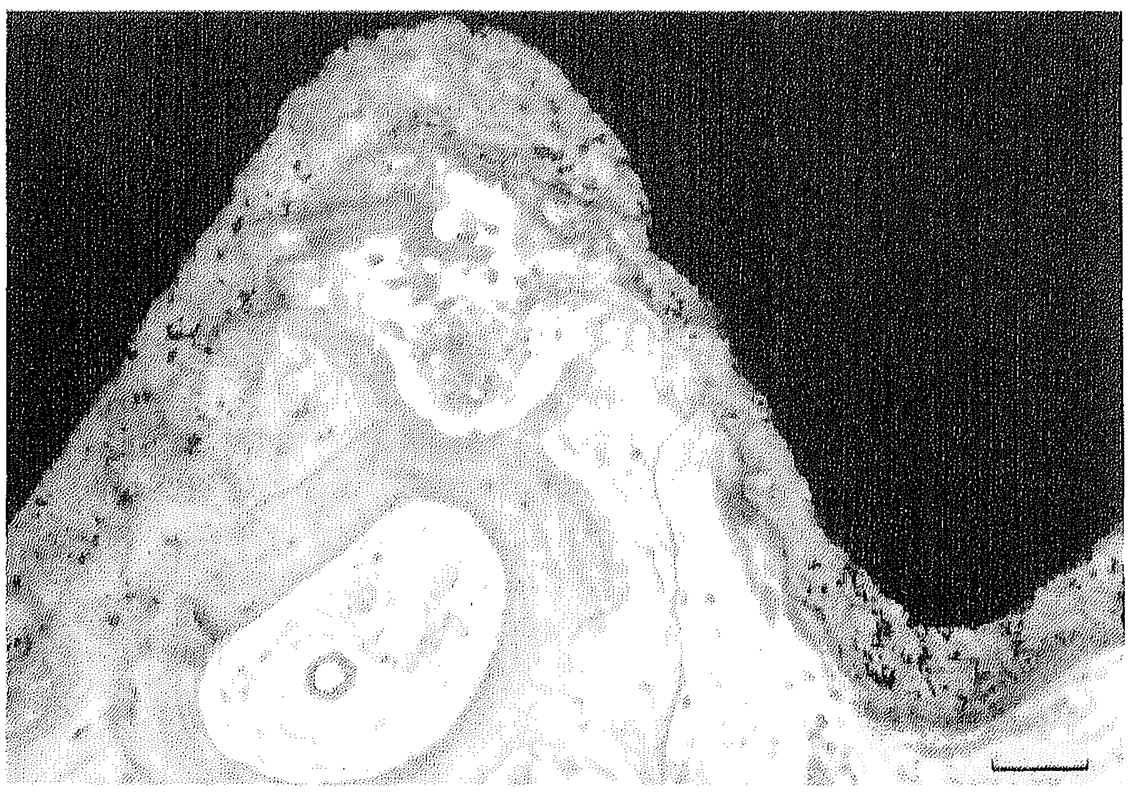


Figure 8. Histological section of a FA-coated implant 6 months after insertion. Newly formed bone can be observed into the screwthread. Despite cellular activity in the remodeling lacuna on the implant surface, there is no sign of coating degradation. Original magnification 50X, bar = 50 μm.

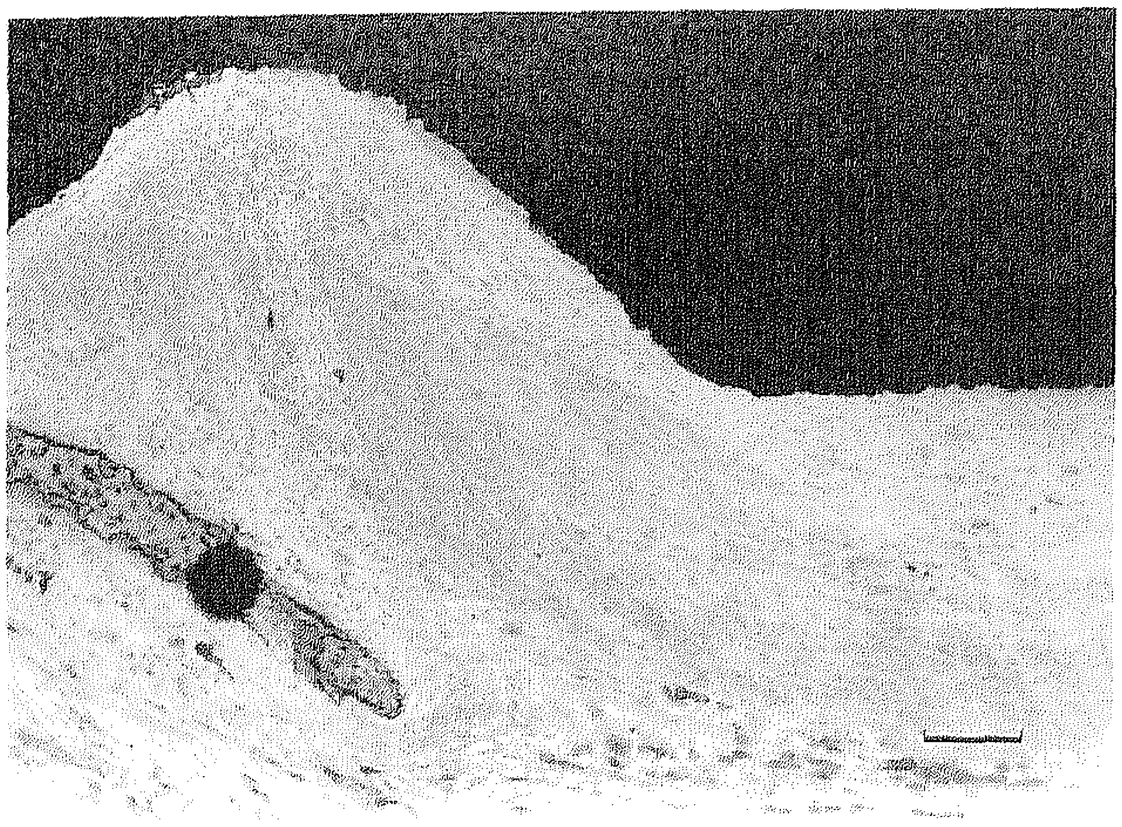


Figure 9. Histological appearance of a FA-coated implant 6 months after implantation. The coating has completely disappeared. Original magnification 25X, bar = 118 μm.

DISCUSSION

In this study fewer uncoated titanium implants were lost at 3 and 6 months postimplantation compared to our previous studies,[10,17] in which the same animal model and implant materials were used. This difference can be explained by the increased skill of the surgeon as the maxilla of the goat is rather difficult to access. Further, we observed in the present study that the initial stability of the implants after installation was higher. This also can be a contributory factor to the improved survival percentage. This increased stability was obtained by omitting, because of the soft bone, the countersink drill for the adjustment of the alveolar ridge to the shape of the implant neck.

Although the clinical success was satisfying, a much lower percentage of bone contact was found for the various implant materials as compared to the previous studies (Ca-P, 60–79%; Ti, 26%).[10,17] In addition, in the present study no significant difference was observed in bone reaction among the various implant materials. It can be supposed that this lack of variation was due to the absence of mechanical stimulation of the implants. Indeed, the implants in the above-mentioned study were connected with a permucosal abutment, with a plastic healing cap on top of it that occluded with the mandibular teeth (premolars).

Nevertheless, the results of this experiment are far inferior to the results of some clinical investigations. For example, titanium screw-shaped implants have proven to be successful in various clinical circumstances.[18,19] The same holds for the Ca-P-coated implants compared to Ca-P-coated implants in other experimental studies.[20,21] The inferior bone reaction found in the present study must be attributed to the animal model used as the implants were installed in a host bed of extremely low mineralization and density.

Another interesting finding in this study is that the histological and histomorphometrical results revealed no differences in average bone reaction to the various implant surfaces between the 3- and the 6-month installation periods. This result probably is due to the wide variation in interanimal bone contact percentages seen for the FA-coated, HAHT-coated, and the noncoated implants. Such a variation was not present for the HA-coated implants. This last result appears to confirm the earlier suggested advantage of less crystalline plasma-sprayed Ca-P coatings.[22] The degradation of the amorphous component causes an increased calcium phosphate precipitation resulting in a more rapid bone bonding. Therefore, the questions arise as to (1) whether the use of very amorphous plasma-sprayed HA coatings can reduce further the intervening healing period before loading an oral implant; and (2) whether this effect is lasting, even after complete resorption of the layer and loading of the implant.

Although standard radiography is a valuable clinical tool for monitoring marginal bone-level changes over time, taking into account the maximal reduction capacity of 0.1 mm,[23] the histomorphometrical data significantly underscore the radiologically determined marginal bone level. These results are in agreement with earlier observations.[17] It has also to be remembered that the histomorphometrically determined marginal bone level was rated in the bucco–palatal plane while the radiographs revealed the bone level in the mesio–distal plane. Although in humans real differences may

TABLE II
Percentages of Bone contact for the Various Retained Implants for Both Implantation Periods

	Percentage of Bone Contact Along the Three Best Consecutive Screwthreads			
	3 Months		6 Months	
Material		Mean with SD		Mean with SD
FA	68	27 ± 33	44	26 ± 13
FA	1		20	
FA	0		34	
FA	39		10	
FA			23	
HAHT	0	16 ± 31	40	22 ± 16.4
HAHT	63		0	
HAHT	2		35	
HAHT	0		12	
HAHT			22	
HA	58	36 ± 20	54	35 ± 24
HA	12		63	
HA	49		34	
HA	18		2	
HA	44		23	
TI	30	11 ± 13	0	15 ± 26
TI	6		0	
TI	8		45	
TI	0			

FA = fluorapatite; HAHT = hydroxyapatite heat treated; HA = hydroxyapatite; TI = titanium.

TABLE III
Means and Standard Deviations of the First Screwthread* and Number of Screwthreads with Bone Contact for the Retained Implant Materials for Both Implantation Periods

	3 Months			6 Months		
Bone Contact	First Thread		Number of Threads	First Thread		Number of Threads
Material	Buccal	Palatal		Buccal	Palatal	
FA	4 (± 5)	6 (± 6)	5 (± 6)	10 (± 5)	9 (± 3)	4 (± 2)
HAHT	2 (± 4)	3 (± 4)	3 (± 5)	6 (± 5)	6 (± 6)	5 (± 5)
HA	7 (± 5)	11 (± 1)	9 (± 5)	6 (± 5)	9 (± 1)	6 (± 3)
TI	5 (± 6)	5 (± 5)	3 (± 3)	4 (± 7)	4 (± 6)	4 (± 6)

*no 1 = most apical screwthread; no 12 = most coronal screwthread; standard deviations between parentheses. FA = fluorapatite; HAHT = hydroxyapatite heat treated; HA = hydroxyapatite; TI = titanium; }, p-value < 0.05.

exist due to dehiscences, in the goat, due to the rather large bucco–palatal width of the alveolar crest, this hardly occurs.[17]

All coatings showed reduction in thickness as found in previous studies.[14,24,25] Nevertheless, the reduction was more severe for the HA-coated implants as compared to the FA- or the HAHT-coated implants. However, as already discussed in previous publications,[14,28] the reasons for and consequences of this coating loss are still not understood. We know the degradation of the coating is not related to the presence of multinucleated or inflammatory cells, fibrous tissue, and/or bone tissue. Probably the reduction of the coating thickness is due to the dissolution of the amorphous phase between the remaining crystalline coating particles. Supported by the results of this study and of earlier studies,[14,24–26] we believe that the amount of coating reduction is probably determined by the animal model (species) and woundhealing capacity of the bone (bone type and implantation site).

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