A histological and histomorphometrical evaluation of the application of screw-designed calciumphosphate (Ca-P)-coated implants in the cancellous maxillary bone of the goat

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Various studies already have shown that the occurrence of oral implant failure is higher in the maxilla than in the mandible. To learn whether Ca-P coatings can improve the success rate of oral implants in the maxilla, three different plasma-sprayed, Ca-P-coated, self-tapping Brånemark® implants were inserted in the trabecular bone of the maxilla. Before the insertion of the implants, the two first upper premolars of 16 goats were bilaterally extracted. Four months later, each animal received four types of implants: three different Ca-P-coated types and one uncoated. After an endosseous period of 6 months, the implants were provided with permucosal abutments. Four months later the animals were killed. At the end of the experiment, it appeared that 10 of the 16 installed noncoated implants had failed while of the 48 Ca-P-coated implants, only 6 had failed. All successful implants were retrieved and prepared for histomorphometrical evaluation of the bone and gingiva response. The Ca-P-coated implants showed a significantly greater percentage of bone in contact with the implant surface compared with the uncoated implants. The length of the epithelium was not significantly different for the coated compared to the uncoated implants, but the connective tissue was significantly thicker for the noncoated implants than for the Ca-P-coated implants. Also, measurements revealed that all coatings showed reduction in thickness. On the basis of these findings, we concluded that the application of Ca-P coatings (1) improves the bone–implant reaction, although all coatings reduced in thickness, and (2) is of benefit during the healing period in less mineralized trabecular bone.

INTRODUCTION

The success rate for endosseous oral implants seems to be lower in the maxilla. For example, for Brånemark® implants the cumulative success rate for individual implants in the maxilla reaches only 90% after 4–5 years. In contrast, for implants in the symphyseal area, figures above 95% are reported.[1–4] This poorer performance in the maxilla may be attributed to a less mineralized and less corticalized bone, thus providing less primary stability at the implant–bone interface during the healing phase.[5]

The increasing demand for implant-supported prostheses encourages the search for new implant surface characteristics and/or geometries that will improve the success rate in the maxilla, especially for bone quality III and IV.[6] To comply with limited and poor bone quality, there may be a need for the use of modified implant designs and implants coated with a thin layer of bioactive calciumphosphate (Ca-P) ceramic. Interest in Ca-P ceramics for endosseous implants is derived from their relative similarity to the mineral phase of bone tissue, i.e. hydroxyapatite (HA) $Ca_{10}(PO4)_6(OH)_2$, octacalciumphosphate (OCP) $Ca_4H(PO4)_32.5H_2O$, and tricalciumphosphate (TCP) $Ca_3(PO4)_2$.[7,8]

According to the current literature, the most valuable characteristic of Ca-P ceramics is their ability to become coated with a microscopic layer of bone mineral after insertion into bone tissue.[9,10] In addition, normal remodeling of these deposited bone layers occurs upon implantation.[11,12] In considering these biological advantages, however, it has to be noted that

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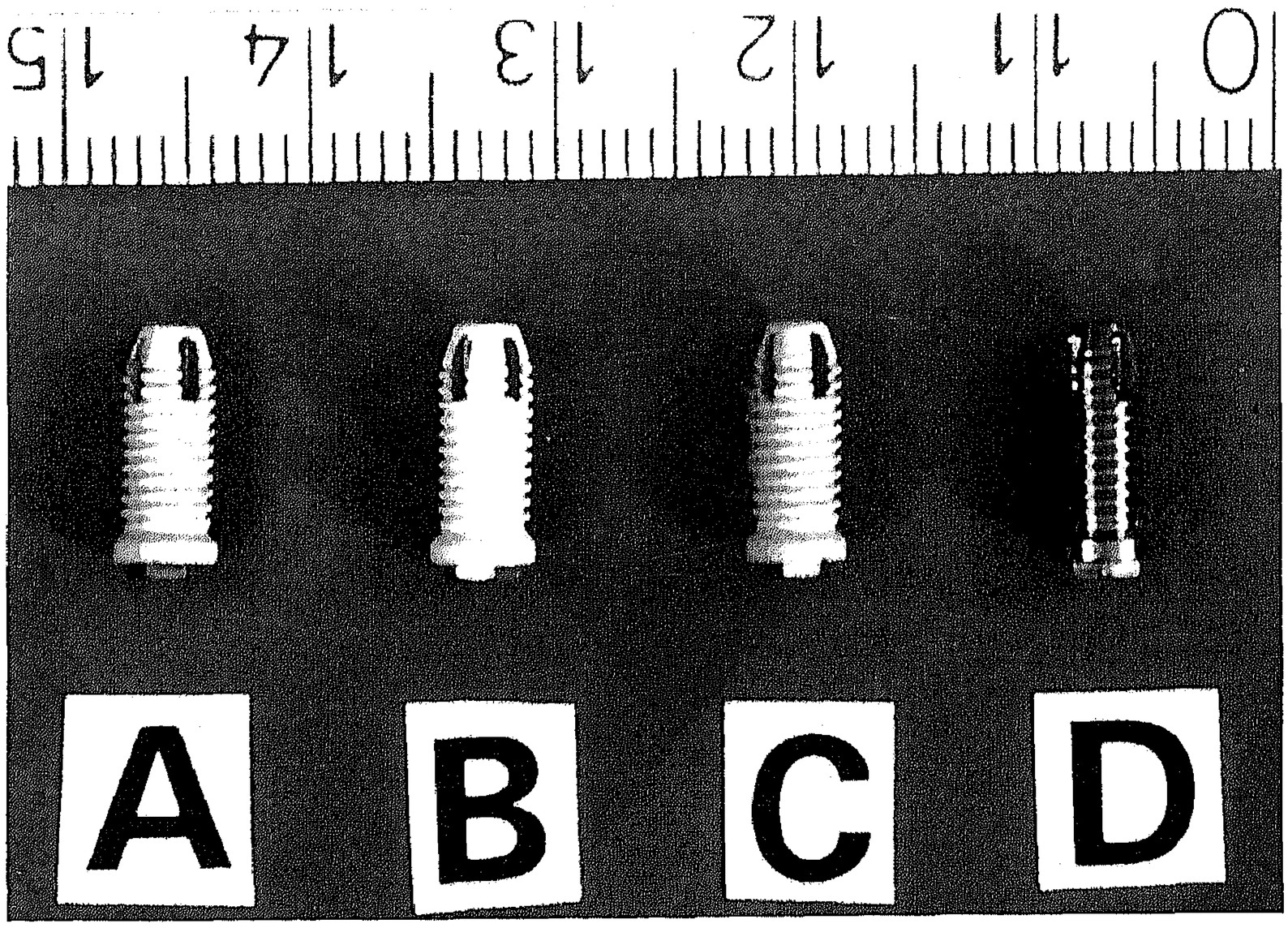


Figure 1. Photograph of the four different implant types: A, B, and C are Ca-P-coated implants; D is an uncoated titanium implant.

Ca-P ceramics can conduct bone growth only over the implant surface; they are not capable of inducing new bone formation.[13]

As a logical consequence of this recognized favorable behavior, Ca-P materials have been used in manufacturing oral implants.[14] However, bulk Ca-P ceramics demonstrate serious mechanical shortcomings. Although the materials have high resistance to compressive forces, they show low tensile and bonding strength. Therefore, implants made of bulk Ca-P cannot be used in complex loading situations. To solve this problem, it was proposed in the early 1980's to apply these materials, using a so-called plasma spray process,[15] as coating on a metallic surface.

Various studies demonstrated a faster and greater bone adaptation to such coated implants.[11,16–21] The histological results thus obtained showed significantly higher percentages of bone contact along Ca-P-coated implants compared with noncoated implants. Greater implant stability also was obtained, as confirmed by higher fixation strengths after short and prolonged implantation periods. In addition, some studies[22,23] suggested that implant surface topography, because the Ca-P coatings have a much rougher surface than as-machined titanium implants, also encourages bone formation.

Nevertheless, most evaluations of experimental oral implants apply only to high-quality cortical bone; the response of less mineralized bone, as is mostly the case in the maxilla, never has been thoroughly investigated. In order to get more insight into the response of bone with low density, in this study a goat animal model was used for installing oral implants. The implants used were selftapping, screw-shaped Brånemark® implants (Nobelpharma, AB, Gothenburg, Sweden). These implants provided both a larger surface area for bone contact and a good initial stability. The rate of clinical success of these implants in the maxilla has been documented[1,24,25] to be inferior compared to the mandible. It was hypothesized that the addition of Ca-P coatings could improve bone healing of implants inserted in low trabecular bone, as exists in the maxilla of goats.

MATERIALS AND METHODS

Implant materials

Sixty-four commercially pure titanium implants with a screw-shaped design (Nobelpharma AB, Gothenburg, Sweden) were used. The implants were selftapping, Mk II type, with a diameter of 3.75 mm and a length of 10 mm (see Fig. 1). All implants that were to be coated were grit-blasted to a roughness of Ra = 4–5 μm. Thereafter, the implants were given a calciumphosphate (Ca-P) coating, approximately 50–60 μm in thickness, using a plasma-spray process.[15] Three different types of coatings were produced. The final distribution of the experimental implants was: 16 implants coated with fluorapatite (FA); 16 implants coated with hydroxyapatite (HA); 16 implants coated

with hydroxyapatite followed by a heat treatment of 650°C for 10 min (HAHT); and 16 implants, which served as controls, left uncoated (Ti).

The coatings were characterized by X-ray diffraction (XRD) (Fig. 2) and infrared spectroscopy (IR). The analysis revealed a 95% crystallinity for the FA coating, 65% for the HAHT coating, and 60% for the HA coating.[26] After plasma spraying, the implants were cleaned ultrasonically in ethanol. Finally, all implants were sterilized in a steam autoclave.

Experimental animal design and surgery

Sixteen female adult Dutch goats, with an average weight of 50–80 kg, were selected. Only animals that had shed their deciduous teeth were used. Therefore, radiographs were taken to ensure that only permanent teeth were present. The selected animals were kept in quarantine for at least 4 weeks and tested for CAE/CL arthritis by way of blood samples.

The first and second maxillary premolars of each of

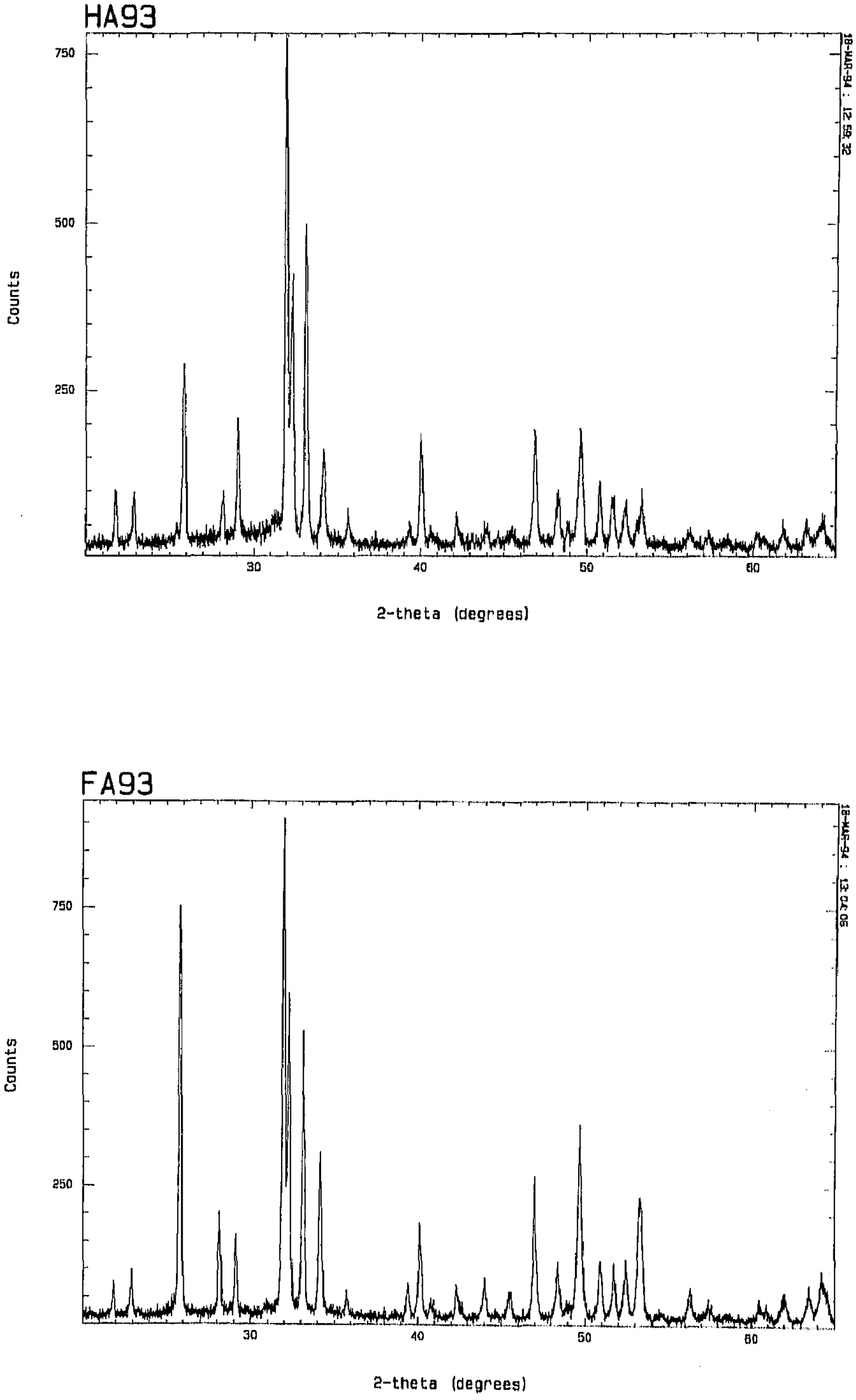


Figure 2. XRD patterns of HA (top) and FA (bottom) plasma-sprayed coatings with the 2 0 in degrees on the x axis and the total # counts on the y axis.

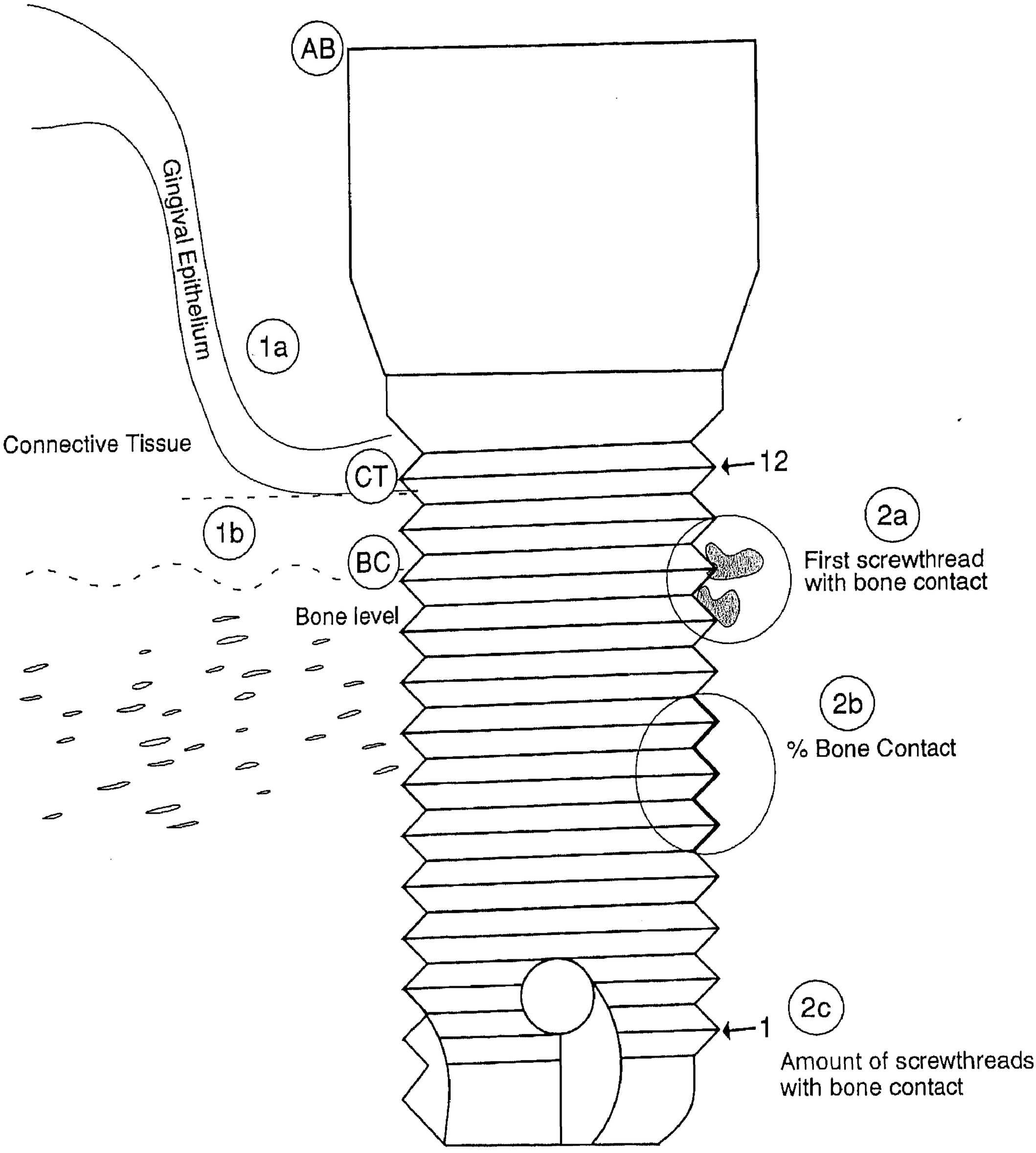


Figure 3. Schematic drawing of the histomorphometrical measurements. Gingival parameters (left): length of the gingival epithelium (1a, AB–CT); thickness of the connective tissue (1b, CT–BC), AB- top of the abutment; CT- boundary of the connective tissue; BC- marginal border of the alveolar bone crest. Bone reaction (right): 2a, first screwthread with bone contact (12 = most coronal screwthread, 1 = most apical screwthread); 2b, percentage of bone contact; 2c, number of screwthreads with bone contact.

the 16 goats were bilaterally extracted under general anesthesia. The goats were immobilized on their backs and anesthesia was induced by intravenous pentobarbital (25 mg/kg) and atropine (0.5 mg/animal) and maintained by ethrane (2–3%) given through an orotracheal tube. After careful extraction, the wounds were closed using resorbable vicryl 2–0 sutures. Twelve weeks following tooth removal, implants were inserted in the edentulous maxillary premolar regions. At that time, the mucosa was opened under general

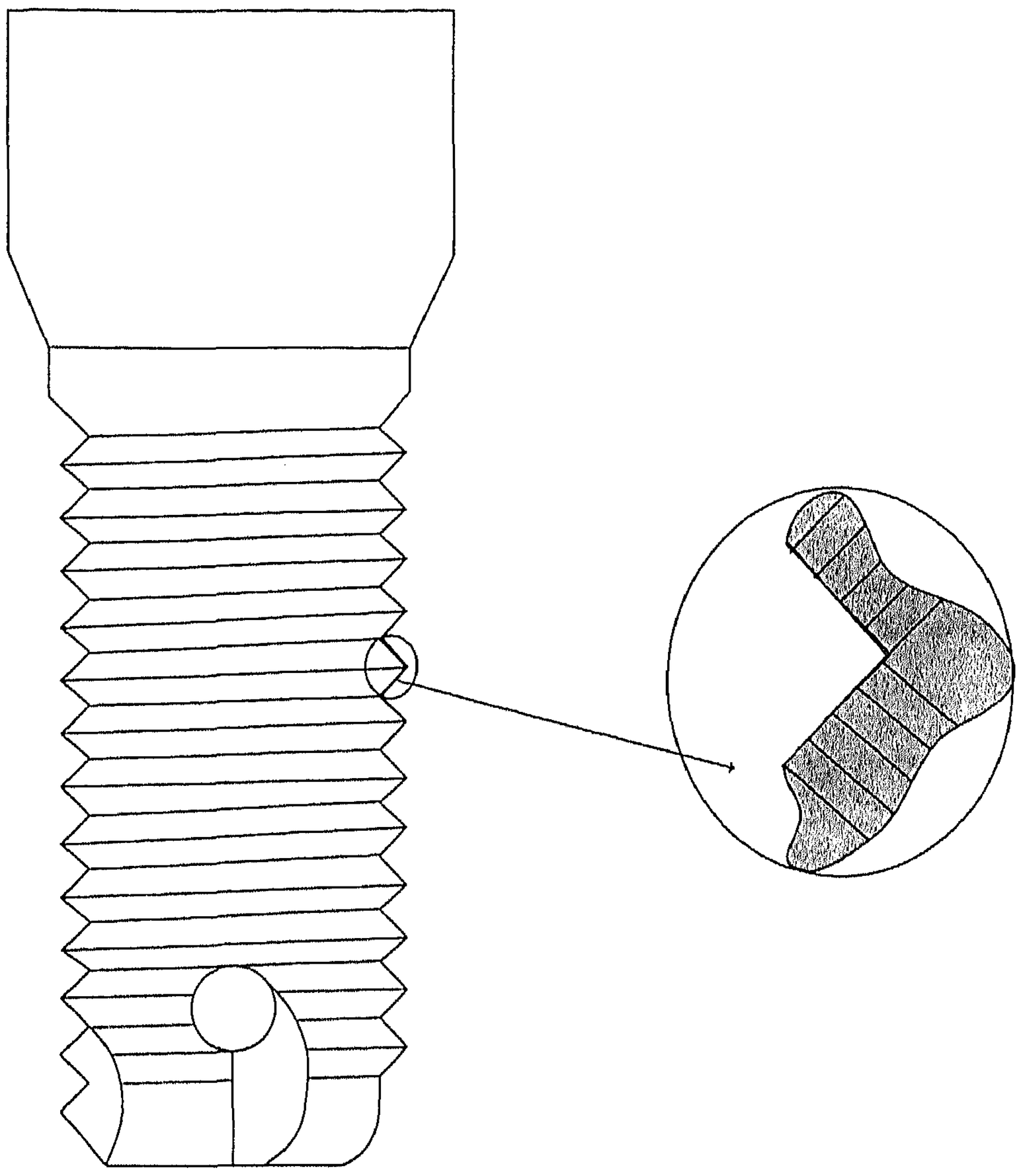


Figure 4. Schematic drawing of the measured coating thickness. On three screwthreads (buccal and palatal) 10 horizontal scan lines were drawn perpendicular to the implant surface, and the length of these lines was calculated to determine the remaining coating thickness.

anesthesia by a longitudinal incision on the palatal alveolar crest. Using the Nobelpharma drilling equipment (Nobelpharma AB, Gothenburg, Sweden), two holes were drilled bilaterally with a distance between the holes of at least 7 mm. The holes were undersized, with a final drill size of 3.5 mm, to reach a good initial stability of the implants by threading the selftapping implants (Brånemark, MK II) into the bone. The bone preparation was performed with a very gentle surgical technique and saline irrigation. Finally, the implants were covered with a cover screw of pure titanium, and the mucoperiosteal flaps were closed with vicryl 2–0 sutures. The implants were placed according to a statistically balanced split plot design in order to compensate for differences in surgical site. Each animal received all types of implants (FA, HAHT, HA, and Ti). One h postoperatively, a prophylactic antibiotic therapy (Albipen[00]) was begun and was given for 3 days to reduce the perioperative infection risk. The implants were left endosseous for 6 months to allow healing of the alveolar bone.

At the end of this time the animals were subjected to a second operation to provide the implants with permucosal abutments. The operation site was disinfected with a 0.1% solution of chlorhexidine. The location of the implants was determined by palpation of the alveolar ridge, and the mucosa was opened over the implants with a longitudinal incision. Then the coverscrew of each implant was removed and the abutments were attached. The abutments (Nobelpharma, AB, Gothenburg, Sweden) were manufactured of pure titanium with a diameter of 4.5 mm and a length of 4 mm. Finally, the abutments were provided with a plastic healing cap and the wounds were closed.

Four months later the animals were killed by an overdose of Nembutal[00]. The experimental protocol used for these studies was approved by the Institu-

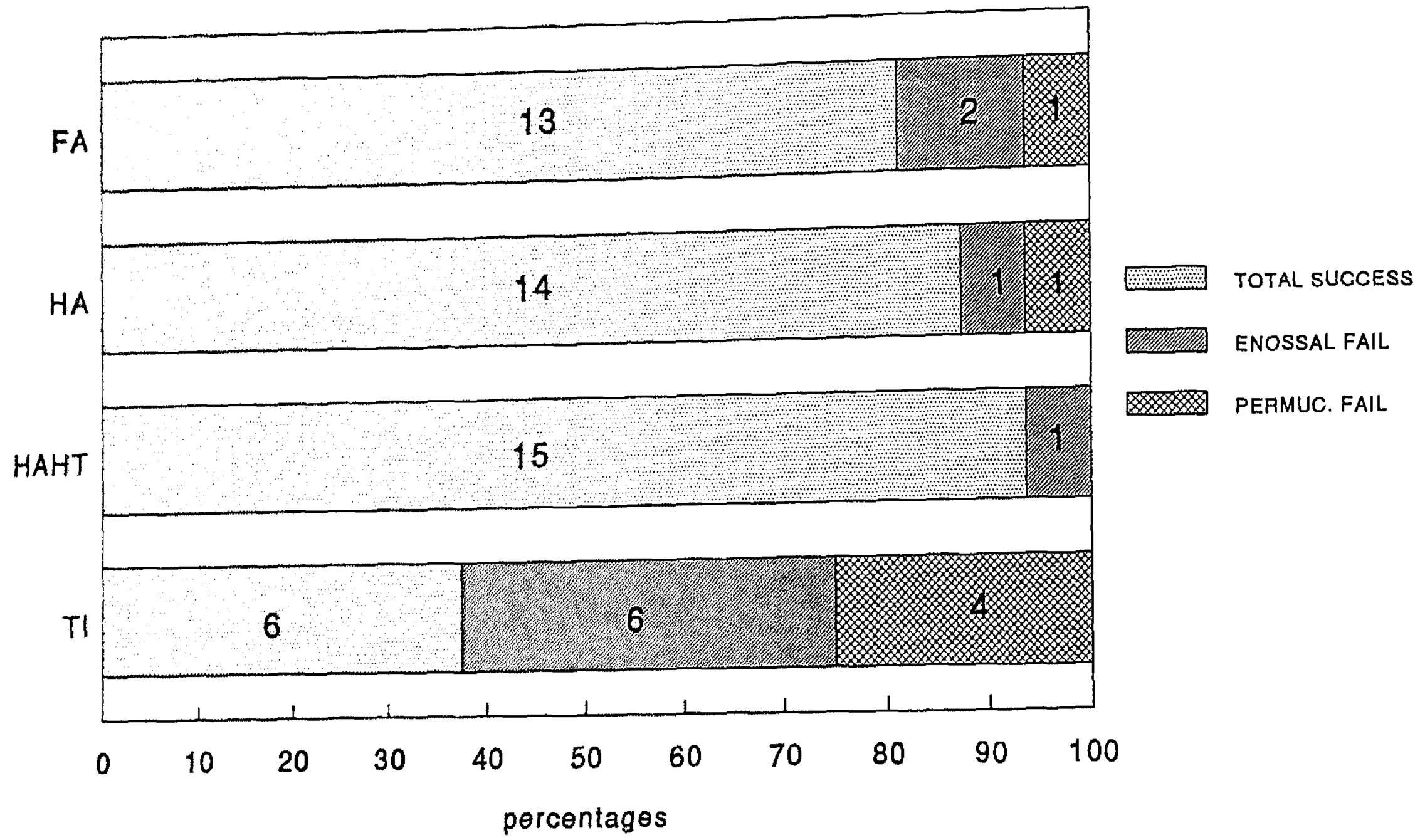


Figure 5. Clinical success rates of all the Ca-P-coated and uncoated implants. The four bars represent the percentages of total success, endosseous failures, and permucosal failures of the FA-, HA-, HAHT-coated, and uncoated titanium implants.

tional Animal Care and Use Committee and adhered to the National Institutes of Health guidelines for the use of experimental animals.

Histological procedures

Following the death of the animals, the maxillae were excised as a unit. Subsequently, the left and right implants with the surrounding tissues were removed, divided into small blocks, and fixed in 10% buffered formalin solution. After dehydration by alcohol series, the implant tissue specimens were embedded in methylmethacrylate. Nondecalcified serial sections of 10 μm thickness were prepared with a modified inner circular saw microtome.[27,28] The sections were made in a buccopalatal direction parallel to the long axis of the implant surface. The sections were stained with methylene blue and basic fuchsin for evaluation by light microscopy.

Histological and histomorphometrical evaluation

For both the histological and histomorphometrical measurements it was always the midsection of the serial section of each implant that was evaluated. All the histological sections were analyzed blind by the same operator. The *histological* evaluation was based on a description of the overall tissue reaction to the implants. The *histomorphometrical* measurements were performed using a Zeiss light microscope equipped with a video camera and connected to an Acorn computer provided with an image analysis software package (TCL-image).

In the selected sections, the following assessments were made:

Gingiva reaction (Fig. 3)

The length of the *gingival epithelium* from the top of the abutment (AB) to the coronal boundary of the connective tissue (CT) was measured [Fig. 3(1a)]; and the thickness of the *connective tissue* from the apical limitation of the gingival epithelium (CT) to the marginal

TABLE I
The Chi-Square Results of the Maintained and Lost Implants

Implant Material	Number and Percentage of Maintained Implants	Number and Percentage of Lost Implants	Total Numbers and Percentages of Inserted Implants
Ca-P coated	42	6	48
	65.6%	9.4%	75.0%
Noncoated	6	10	16
	9.4%	15.6%	25.0%

Degree of freedom = 1; chi-square = 16.0; probability < 001.

border of the alveolar bone crest (BC) was measured [Fig. 3(1b)].

Bone reaction (Fig. 3)

The *first screwthread* that showed direct bone contact was determined. As all the implants consisted of 12 screwthreads, we numbered the screwthreads in ascending order from the most apical (1) towards the most coronal (12) [Fig. 3(2a)]. Bone contact was defined as a *percentage of bone contact* without any soft tissue between bone and implant surface. The measurements were performed along the three best consecutive threads of the implant [Fig. 3(2b)]. The *number of screwthreads* that showed direct contact with the surrounding bone was calculated [Fig. 3(2c)].

Measurements of the first screwthread and the number of screwthreads [Fig. 3(2a,2b)] were performed independently of the amount of bone that was in direct contact with the implant surface. All measurements were performed for the buccal as well as for the palatal site of the implant.

Coating thickness (Fig. 4)

The coating thickness was measured along three threads of the buccal and the palatal surface. Ten horizontal scan lines perpendicular to the titanium surface were drawn between the respective coating boundaries. The length of each of these lines was calculated. Finally, these data were classified into 8 groups: 0–7 μm, 8–15 μm, 16–23 μm, 24–31 μm, 32–39 μm, 40–47 μm, 48–55 μm, and 56–63 μm.

RESULTS

Of the 64 installed implants, 48 implants healed uneventfully. During the endosseous phase (Fig. 5), 10 of the 64 installed implants were lost. During the permucosal phase, another six implants failed. Forty-two of the 48 Ca-P-coated implants were clinically stable (absence of mobility) while only six of the 16 uncoated titanium implants were still present at the moment of sacrifice. A Chi-square test (Table I) revealed that this difference between the coated and the uncoated implants was significant ($p < 0.001$).

Histological description

Examination of the histological sections revealed that the bone reaction to the coated implants was relatively uniform regardless of the applied Ca-P coating. Large parts of these implant surfaces showed close bone contact with mature bone, interrupted by areas of newly formed bone. All Ca-P coatings showed signs of reduction in thickness. The reduction of coating thickness did not interfere with bone contact (Fig. 6). No cellular activity of multinucleated cells could be observed in the vicinity of the implant surfaces.

The titanium uncoated implants seemed to be surrounded by less bone than the Ca-P-coated implants. Soft tissue or marrow tissue often was interposed between the implant and bone surface. At places where no fibrous layer was present, close bone apposition to the implant surface was observed (Fig. 7, left and right).

Around all the implants there was a limited downgrowth of the gingival epithelium (Fig. 8). The epithelium mostly appeared to form a stable junction with the implant surface. Two types of gingival response to the permucosal implant surface could be observed. In none of these sections were collagen fibers found that

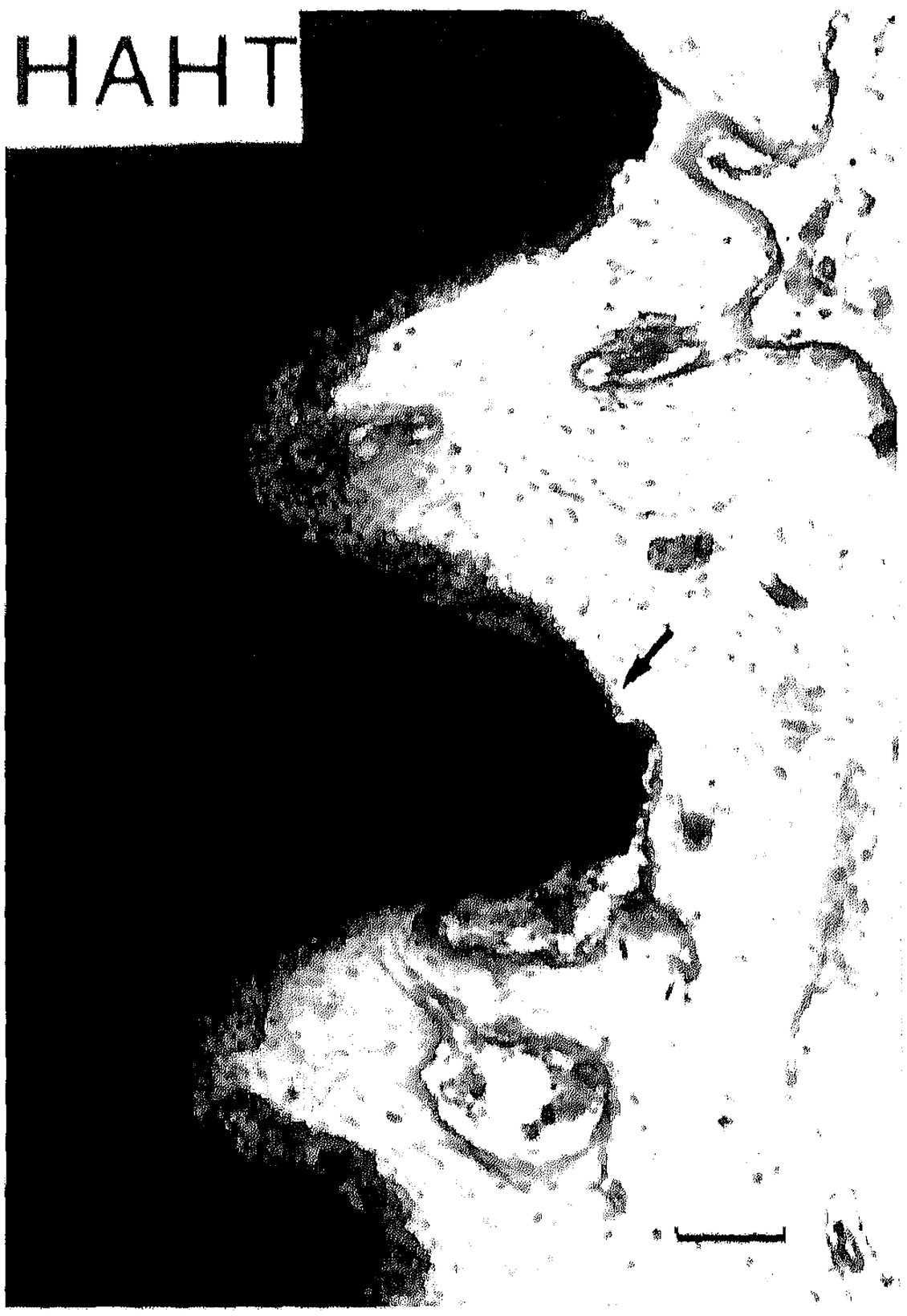
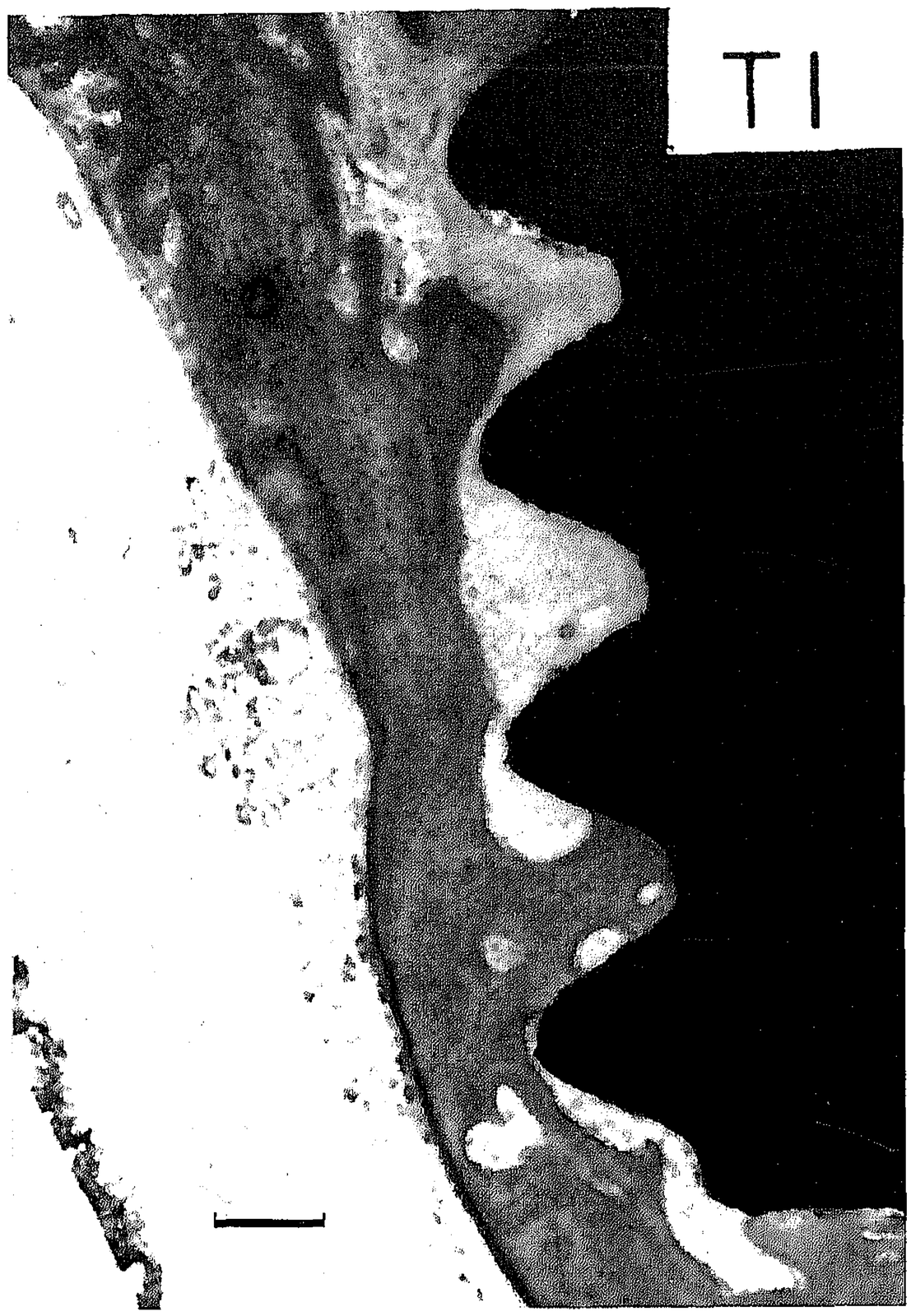


Figure 6. Light micrograph of HAPT-coated implant showing areas of close bone contact with mature bone interrupted by areas of newly formed bone. Even at places where the coating disappeared (arrow), the bone was in close contact with the implant surface. Original magnification: 25×, bar = 85 μm.



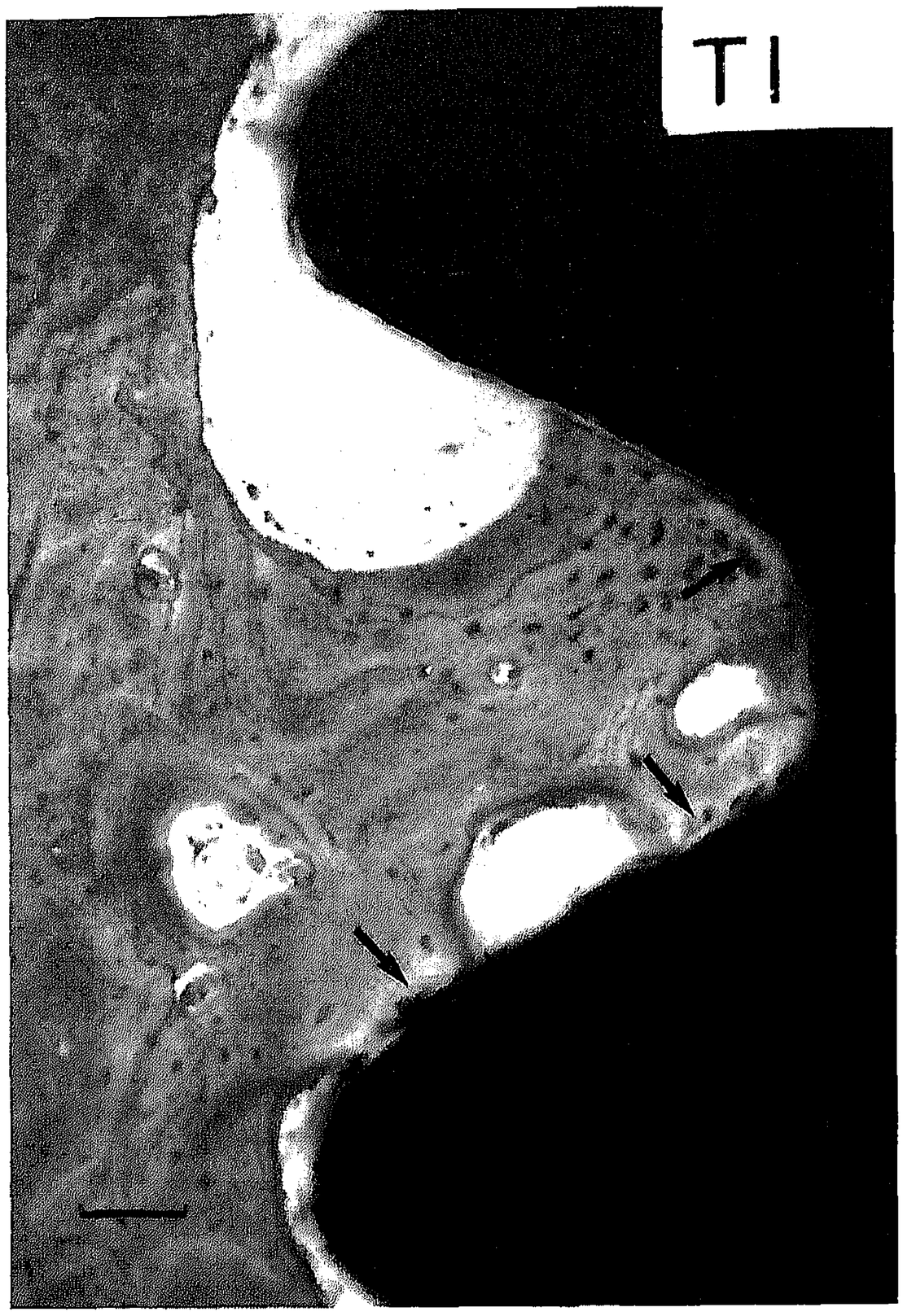


Figure 7. (left), Light micrograph of a noncoated implant showing the implant surrounded by a fibrous tissue layer. Only in the apical part of the implant is there bone contact with the implant surface. Original magnification: 10×, bar = 294 μm; (right), magnification of the 2 apical screwthreads of the left radiograph. Close bone apposition can be observed where there is no fibrous tissue present. Original magnification, 40×, bar = 73.5 μm.

were oriented perpendicular to the implant surface. The implants displayed no gross inflammatory reaction in the gingival tissue.

Histomorphometrical evaluation

Gingiva reaction, bone reaction, and coating thickness were analyzed. The outcome of these analyses are given in Tables II and III and in Figure 9. The data, as presented, are the average of palatinal and buccal measurements.

Statistical testing of the gingiva data, using a one-way analysis of variance (ANOVA) and a multiple Newman-Keuls comparison procedure, showed that a significant difference existed only in connective tissue reaction. The connective tissue layer around the noncoated implants was significantly thicker than around the Ca-P-coated implants ($p = 0.003$). Statistical testing of the bone data, using a one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman-Keuls), revealed a significant difference ($p < 0.001$) between the noncoated and the Ca-P-coated implants for the first screwthread showing bone contact. The first screwthread was located more coronally for the coated implants. No significant differences were demonstrated among the various Ca-P-coated implants.

In addition, a significantly ($p = 0.001$) higher percentage of direct bone contact was found for the various coated implants (HAHT = 63.1%, HA = 67.4%, FA = 77.1%) compared to the titanium controls (26.5%). The differences among the coated implants were not significant. Statistical testing also showed that a significantly ($p < 0.001$) higher number of screwthreads of the Ca-P-coated implants were in contact with bone compared to the titanium uncoated implants. Again, the differences among the various Ca-P-coated implants were not significant.

Further, using a simple linear regression test, no correlation could be demonstrated ($r = 0.5$) between (1) the length of the connective tissue with the first screwthreads showing bone contact, and (2) the length of the connective tissue and the number of screwthreads showing bone contact.

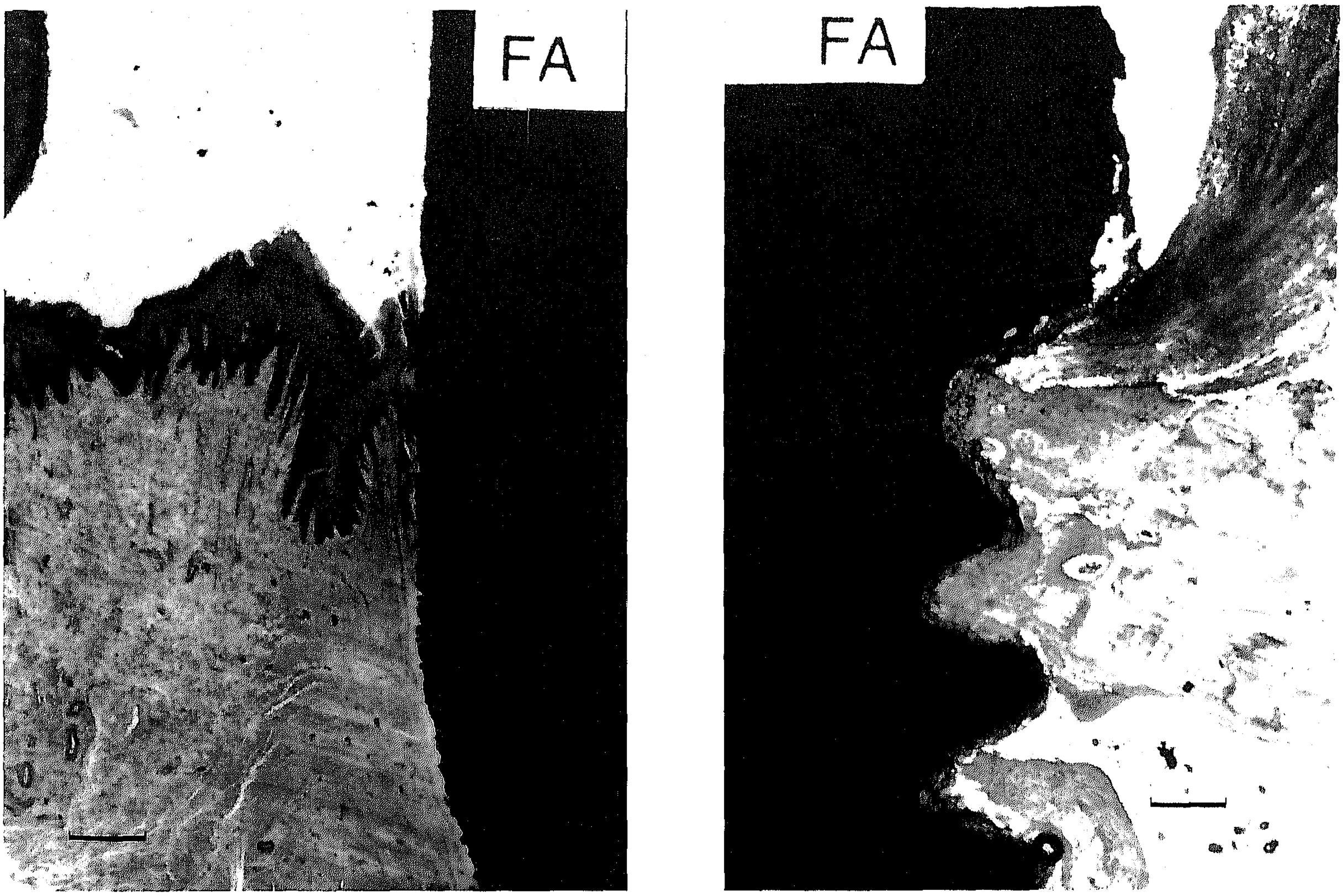


Figure 8. Two photographs of the same FA-coated implant. The length of the gingival epithelium was confined to the neck of the implant. Original magnification: 10×, bar = 294 μm. Two different types of gingival response were present: (left) gingival response characterized by the formation of a gingival epithelium followed by a thick connective tissue layer; (right) gingival epithelium followed by a thin connective tissue layer immediately followed by the bone level.

The results of the coating thickness measurements indicate that all coatings show reduction in thickness but that the reduction is nonuniform. In some areas there is no coating left while in other areas the coating did not disappear. The reduction is most severe for the HA-coated implants while the FA-coated implants seemed to be the most stable ones.

DISCUSSION

The aim of this study was to investigate the trabecular bone response to uncoated and to various types of Ca-P-coated oral implants.

The clinical results revealed that more Ca-P-coated implants were stable than were uncoated Ti implants. Besides the poorer clinical success of the uncoated Ti implants, this study also demonstrated that the biological bone reaction to the successful uncoated Ti implants was less favorable than the bone reaction to the coated implants. The coated implants showed a significantly higher percentage of bone in contact with the implants; the first screwthread with bone contact was located more coronally and the number of screwthreads showing bone contact was significantly higher than in the uncoated implants. These findings confirm the results of other studies[11,12,26,29] with Ca-

TABLE II
Histomorphometrical Data of the Gingiva Reaction to the Various Implant Materials

	Titanium	Hydroxyapatite	Hydroxyapatite Heat-Treated	Fluorapatite
Epithelial length	190 ± 131.7 (n = 6)	285 ± 134.7 (n = 14)	309.7 ± 97.2 (n =15)	300.2 ± 160 (n = 13)
Connective tissue thickness	632 ± 252.2 (n = 6)	366 ± 187.8 (n = 14)	412.6 ± 124.8 (n = 15)	324.3 ± 100.7 (n = 13)

Mean values in μm ± SD; n = number of implants.

TABLE III
Histomorphometrical Data of the Bone Reaction to the Various Implant Materials

	Titanium	Hydroxyapatite	Hydroxyapatite Heat-Treated	Fluorapatite
Percentage bone contact	26.5 ± 16.2 ($n = 6$)	67.4 ± 27 ($n = 14$)	63.1 ± 24.9 ($n = 15$)	77.1 ± 13 ($n = 13$)
First screwthread with bone contact	4.3 ± 3.3 ($n = 6$)	8.2 ± 2.4 ($n = 14$)	8.0 ± 2.6 ($n = 15$)	9.6 ± 2.2 ($n = 13$)
Number of screwthreads with bone	7 ± 4.4 ($n = 6$)	13.6 ± 5.9 ($n = 14$)	12.3 ± 5.7 ($n = 15$)	15.1 ± 5.6 ($n = 13$)

Mean values ± SD; n = number of implants.

P-coated implants. However, it has to be emphasized that the low clinical success of the uncoated implants, as found in our study, has never been observed in other studies. Indeed, the maxilla of the goat lacks a distinct cortical layer, and the implants are exclusively surrounded by cancellous bone. These findings show that Ca-P coatings apparently can play an important role during healing and bone ingrowth of oral implants in bone or poor density.

Still, it has to be emphasized that the preceding grit blasting of the implant surface and the irregular surface topography of the coatings may be responsible for the bone response rather than the Ca-P nature of the coatings themselves. This explanation is supported by

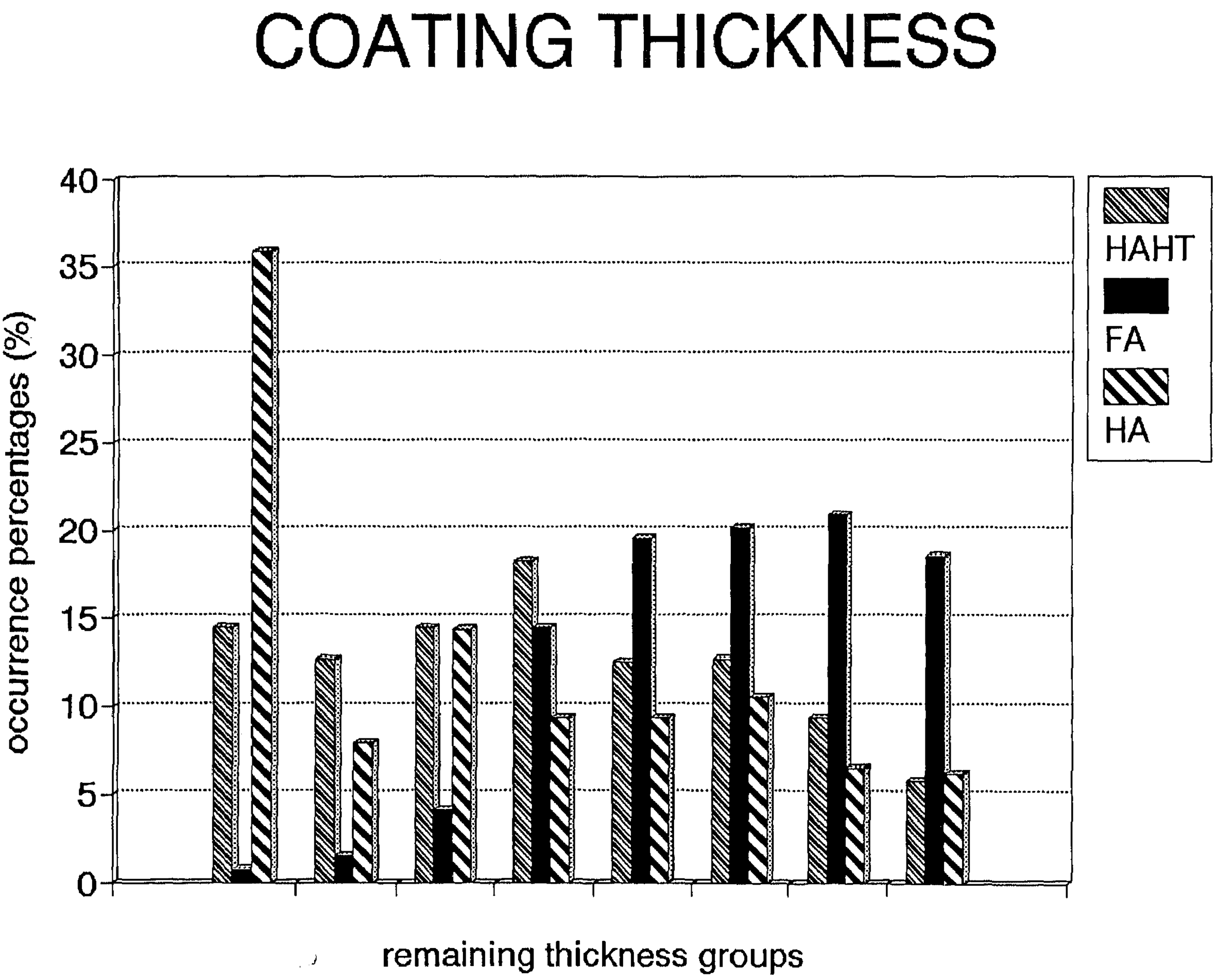


Figure 9. Bar diagram containing the results of the coating thickness measurements. The results are classified in 8 groups containing the data of the coating thickness remaining in micrometer (x axis); on the y axis the percentages of occurrence are given for the HA-, FA-, and HAHT-coated implants.

the findings of Courtney et al.,[30] who investigated extracellular matrix production on smooth and rough hydroxyapatite surfaces in cell culture, and found that matrix production was increased on rougher surfaces.

Another interesting finding in this study was the serious reduction in coating thickness for the HA and HAHT coatings. Nevertheless, in this 10-month study, this coating reduction did not influence the trabecular bone behavior. We saw no significant difference in the percentage of bone contact for the three different coatings. The lower degradation rate of fluorapatite coatings has been described earlier.[11,26] Still, the final clinical efficacy of such coatings for dental implants needs further investigation. For example, Kangasniemi et al.[31] determined in animal experiments the tensile bone-bonding strength of FA- and HA-plasma-sprayed coatings. He observed that after achievement of bone contact, all fracture failures occurred at the coating–titanium interface. On the basis of these findings, he concluded that plasma-sprayed coatings should not be used in load-bearing situations where delamination of the coating would be detrimental to the final implant lifetime.

Despite the encouraging results with the Ca-P coatings in this study and the fact that bone was still in close contact with the implant surface at places where the coating completely disappeared, the long-term effect of the biodegradation of the coating and what will happen at the bone–implant interface when the coating has disappeared remains a point of concern especially in relation to the grit-blasting procedure with Al_2O_3 particles to obtain mechanical retention of the coating. For example, remaining Al_2O_3 particle deposits can change the biocompatibility of the implants. On the other hand, as supposed by Gotfredsen et al.,[32] a possible solution for this problem could be the use of titanium dioxide particles in the blasting procedure.

In the present experiment no significant difference in the behavior of the gingival epithelium to the various implant surfaces was observed, confirming the earlier *in vitro* cell studies and *in vivo* animal studies by Jansen et al.[33–35] On the basis of various experiments, they concluded that epithelial cells always behave the same in attachment and growth, independent of the substrate surface conditions. Therefore, it can be supposed that the length of the gingival epithelium is of no importance as a parameter with predictive value for the quality of the permucosal passage and the success of permucosal devices.

On the other hand, the length of the connective tissue seems to be an important parameter. The measured thickness varied significantly between the coated and uncoated implants. A significantly thicker connective tissue was measured along the Ti implants while the differences among the various coated implants were not significant. These observations were in correlation with the percentage of bone in contact with the implant surface, as we found a significantly lower percentage of bone in contact with the Ti implants as compared to the Ca-P-coated implants. Despite this difference in connective tissue length, no inflammatory reaction could be seen around any of the implants. Since no perpendicular oriented connective tissue fibers could be demonstrated in any of these sections, we can only conclude that the connective tissue was adapted to the implant surface by fibers that run parallel or circular to the implant surface. Apparently such a connective tissue fiber adaptation is sufficient to ensure a biological seal between the contaminated environment of the oral cavity and the almost aseptic internal environment.[36]

In summary, although our experiments appear to demonstrate that plasma-sprayed Ca-P coatings deposited on oral implants are beneficial for the trabecular bone reaction, no final conclusion can be drawn due to the difference in surface roughness between the coated and noncoated implants. Consequently, the clinical efficacy of Ca-P plasma-sprayed coated implants can still be questioned. Nevertheless, the observed large loss of smooth-threaded titanium implants was most surprising and not reported earlier.

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