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Mechanical and histologic evaluation of Ca-P plasma-spray and magnetron sputter-coated implants in trabecular bone of the goat

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The aim of this study was to investigate the bone response to calcium phosphate (Ca-P) plasma-spray and radiofrequency magnetron sputter-coated implants with comparable roughness. Therefore, tapered conical screw designed implants were installed in the trabecular bone of the femurs of nine goats. They were provided with two types of coatings, a plasma-spray dual coating of fluorapatite and hydroxyapatite (FA/HA-PS) and a titanium plasma-spray coating, covered with an amorphous Ca-P magnetron sputtercoating (TPS/Ca-P-a). These implants were evaluated histologically and mechanically after 3 months of implantation. A well-controlled method to apply and measure a torsional force to load the screw-type implants to the point of failure was

introduced. All implants healed uneventful and were well fixed. No significant difference (Student *t* test, $p > 0.05$) for the torsional failure force was measured for both type of coatings. Nevertheless, SEM revealed differently situated fracture planes. Light microscopy showed intimate bone-implant contact for both types of coatings; original drill margins were still visible. A lamellar type of bone with some remodeling lacunae was shown. Histomorphometry revealed a higher percentage of bone contact for the FA/HA-PS-coated implants (student *t* test, $p < 0.05$). Measurement of the amount of bone revealed more bone mass around TPS/Ca-P-a-coated implants (analysis of variance and Tukey multiple comparison, $p < 0.05$). © 1997 John Wiley & Sons, Inc.

INTRODUCTION

Currently, the plasma-spray technique is the most frequently used method to produce calcium phosphate (Ca-P) coatings. Faster and greater bone adaptation, improved implant fixation, and faster bone healing are described advantages of plasma-spray Ca-P coatings.¹⁻⁴ Besides these benefits, concerns have been raised regarding the viable use and long-term stability of plasma-spray Ca-P coatings. Different mechanisms of coating loss, unknown long-term consequences, and confusion about the optimum substrate texture advanced the investigation of other coating techniques. Since 1991, in our laboratory radiofrequency (RF) magnetron sputtering has been extensively investigated to produce thin, adherent, and uniform Ca-P coatings for oral implants.⁵⁻⁹ Previous grit-blasting procedures for mechanical retention, as required for plasma-spray coatings, are not needed, and physico-chemically better-defined Ca-P coatings can be produced. In a previous *in vivo* experiment using rab-

bits,¹⁰ we investigated the bone behavior to these plasma-spray and RF magnetron-sputter Ca-P coatings. Implants were inserted in the trabecular femoral bone. After 6 and 9 weeks of implantation, measurements of bone contact did not reveal significant differences between the plasma-spray and RF magnetron sputter coatings. On the other hand, results of an experimental study using goats¹¹ showed better results for plasma-spray Ca-P-coated implants. In this study implants were positioned in the low-density bone of the goat maxilla. In addition, there was a difference in surface roughness between the plasma-spray and magnetron sputter-coated implants. Since both of these conditions could possibly have influenced the final bone response, the aim of this study was to evaluate the bone response to Ca-P plasma-spray and RF magnetron sputter-coated implants with comparable roughness.

MATERIALS AND METHODS

Thirty-six tapered, conical, screw-shaped dental implants (Biocomp[®] Industries, Medemblik, The Nether-

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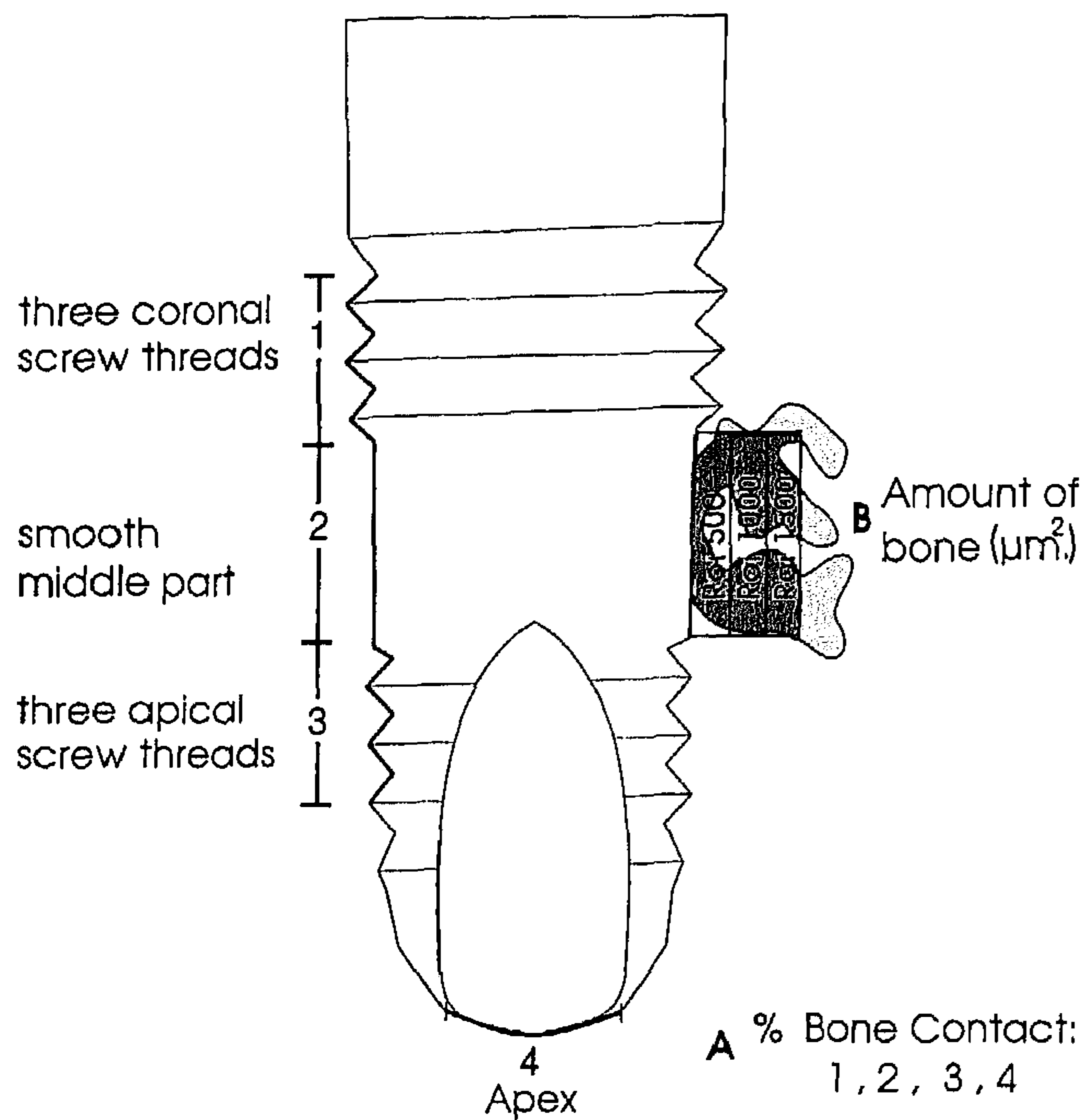


Figure 1. Schematic drawing of the tapered conical screw-type implant. (A, B) Areas of interest for histomorphometric evaluation.

lands) were used. All implants measured 10 mm in length and had a diameter of 3.9 mm (Fig. 1). These screws consisted of two threaded parts and a smooth middle part. They were grit-blasted to a roughness of R_a 4–5 μm . Using the plasma-spray method, 18 implants were provided with a 50- μm -thick, bilayered Ca-P coating, consisting of fluorapatite and hydroxyapatite (FA/HA-PS) ($R_a = 8\text{--}9 \mu\text{m}$). The other 18 implants first received a 50- μm -thick plasma-spray titanium coating. Subsequently, they were covered with

an additional thin film of RF magnetron-sputtered Ca-P (TPS/Ca-P-a) with a thickness of about 2.0 μm . The sputtering process was performed using standard conditions (background pressure: $p < 8 \cdot 10^{-6}$ mbar; argon flow: $p = 5.2 \cdot 10^{-3}$ mbar; power level: $p = 800$ W). The final roughness of these implants was $R_a = 6\text{--}7 \mu\text{m}$. The diameter of all implants was 4.0 mm, measured at the neck of the implant.

The chemical composition of the coatings was confirmed by X-ray diffraction spectrometry (XRD), Fourier transmission infrared spectroscopy (FTIR), and Rutherford backscattering spectrometry (RBS) measurements. XRD of the magnetron-sputtered Ca-P film that covered the TPS/Ca-P-a coating showed an amorphous structure (Fig. 2). FTIR spectra of the sputter Ca-P coating revealed PO-bonds and a large H_2O region. Ca-P ratio was 2.2 XRD (Fig. 2) of the plasma-sprayed FA part of the dual coating revealed >98% crystallinity, and the HA part of this coating was 60% crystalline. FTIR showed partial dehydroxylation for the HA part of the FA/HA-PS coatings. Ca-P ratio was 1.67.

Scanning electron microscopy (SEM) was used to record the surface appearance of coatings.

After the coating procedure, all implants were sonicated in ethanol 100% for 10 min to remove any loose particles. Finally, they were sterilized in an autoclave.

Experimental design and surgical procedure

Nine healthy, mature (2–4 years of age), female Saanen goats, weighing about 60 kg, were used. Blood samples of the goats were taken to ensure that the

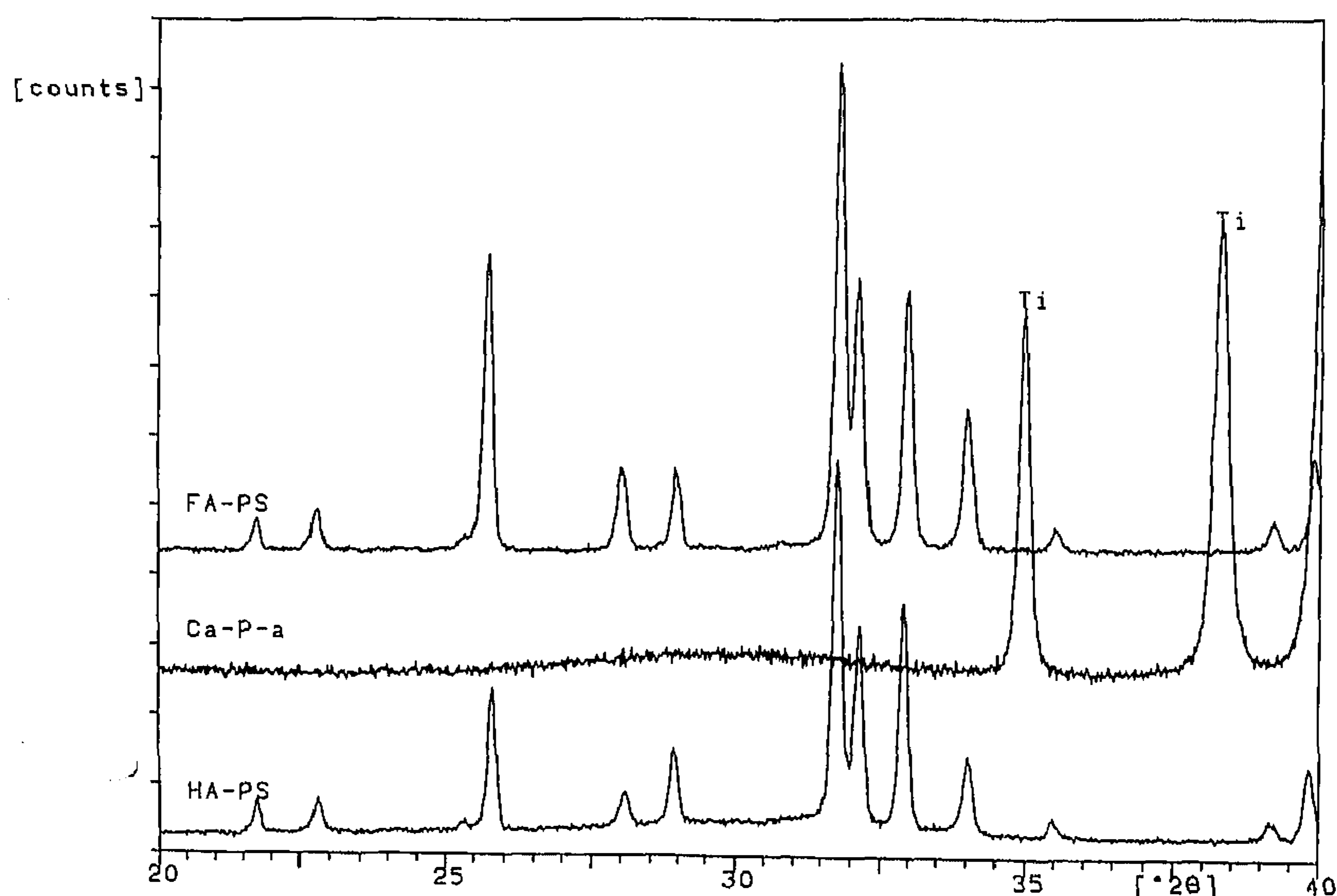


Figure 2. X-ray diffraction patterns of the different Ca-P coatings with 2θ in degrees on the x -axis and the relative counts on the y -axis.

animals are CAE/CL arthritis-free. The animals were housed in a stable. The implants were inserted into the trabecular bone of the femoral condyle. The operation was performed under general anesthesia. The anesthesia was induced by an intravenous injection of pentobarbital and maintained by ethrane 2–3% through a constant volume ventilator, administered through an endotracheal tube. The goats were connected to a heart monitor.

To reduce the risk of perioperative infection, the goats were treated according to the following doses of antibiotics:

- Before the operation: Albipen 15%, 3 mL/50 kg, subcutaneously (s.c.)
- 1 day after the operation: Albipen LA, 7.5 mL/50 kg, s.c.
- 3 days after the operation: Albipen LA, 7.5 mL/50 kg, s.c.

For the insertion of the implants, the animal was immobilized on its back and the hindlimbs were shaved, washed, and disinfected with povidine-iodine. A longitudinal incision was made on the medial and lateral surface of the left and right femur. After exposure of the femoral condyle a 1.6-mm pilot hole was drilled. The hole was gradually widened with different drills to the final diameter (4.0 mm) of the implant. The bone preparation was performed with a very gentle surgical technique, using low rotational drill speeds (maximum 450 rpm) and continuous internal and external cooling. Following insertion of the implant, the soft tissues were closed in separate layers using resorbable Vicryl 3–0 sutures.

A total of 36 implants were placed: 18 coated FA/HA-PS and 18 TPS/Ca-P-a implants. Each goat received four implants, medially and laterally positioned in the condyles of the right and left femur. The implants were inserted following a randomization scheme.

At the predetermined end point of the experiment, at 12 weeks, the animals were killed by an overdose of Nembutal. Subsequently, both femurs were excised. Of each goat one femur was used for histologic examinations and the other for mechanical evaluation, to perform torque measurements and subsequent SEM evaluation. In addition, of three randomly chosen goats, regional lymphnodes of both legs were excised to inspect for the presence of titanium particles.

Mechanical testing

To determine the bone-bonding strength of the implants, mechanical tests were performed. After sacrificing the goat, one femur was stored and transported on ice at a temperature of approximately 4°C. Using a

diamond saw, the femur was sectioned into two pieces, each with one implant. Of these pieces containing the implants, the bone overgrowth was removed and the cover screw of the implants was exposed and removed. This part of tissue was embedded in a mold with gypsum. A specially designed device was used to fix the specimens in the tensile bench (Fig. 3). This device always enables application of a perpendicular force (at a constant displacement speed of 0.5 mm/min) on a lever fixed in the center of the implant. This force was applied at 1.5 cm distance from the diameter of the implants. The force at the point of implant failure was registered and the test was stopped immediately afterward, to not completely fracture the interface. All samples were tested freshly.

SEM

Following the torque measurement, specimens were fixed and dehydrated by a graded series of ethanol and embedded in methylmethacrylate. After polymerization the samples were hemisectioned perpendicularly on the longitudinal axis of the implants. After polishing, they were examined with SEM in the backscatter mode to determine the fracture plane of the mechanically tested implants.

Histologic procedures

For histologic procedures 10% buffered formalin solution was used for fixation of the tissue. After fixa-

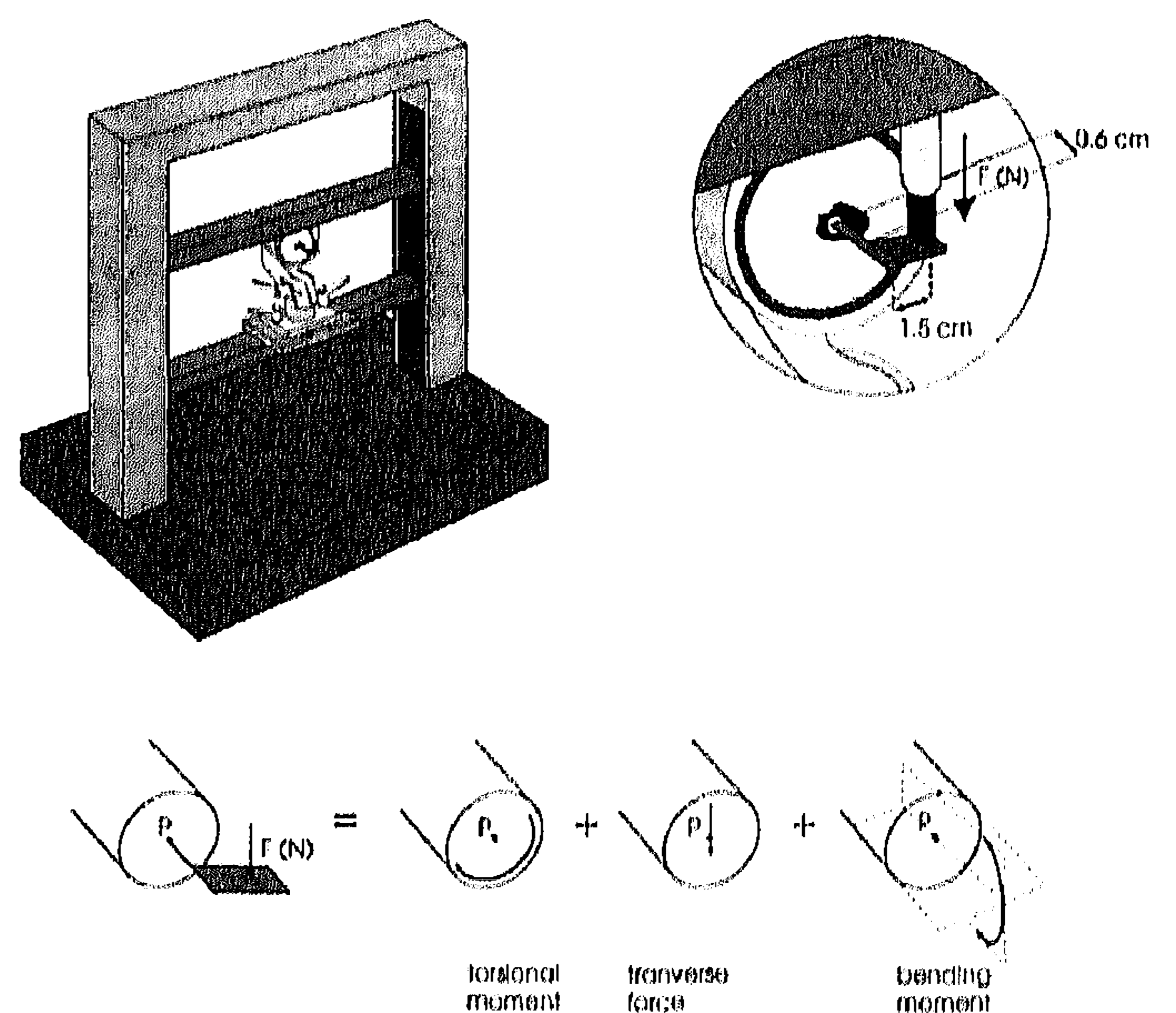


Figure 3. Schematic drawing of the torque test and mounting in tensile testing bench. The applied force resulted in a transverse force, bending moment, and torsional moment, relative to point *P*. The bending moment and the transverse force have only minor contributions to the failure of the implant.

tion, each femur was sectioned into two pieces, each with one implant. These tissue blocks were dehydrated in series of ethanol and embedded in methylmethacrylate. After polymerization nondecalcified thin (10- μm) sections were prepared in a transversal dissection perpendicular on the axis of the implant, using a modified sawing microtome technique.¹² The sections were stained with methylene blue and basic fuchsin, and the interface was investigated with a light microscope.

Excised and fixed lymph nodes were dehydrated and embedded in Paraplast. Subsequently sections were cut with a microtome ($\pm 5 \mu\text{m}$ thick), and stained with hematoxylin-eosin (HE) staining according to Mayer, and investigated for titanium particles using a light microscope.

Histomorphometric evaluation

Image analysis techniques (TLC-image; Technical Command Language, developed by TNO, Delft, The Netherlands) were used for histomorphometric evaluation. The following quantitative parameters were assessed:

Percentage of bone contact at the interface

Measurements were performed along three coronal screw threads, at the smooth middle part, along three apical screw threads of the Biocomp[®] implant, and along the apex (Fig. 1). The amount of bone contact was defined as the percentage of implant length at which there is direct bone-to-implant contact without intervening soft-tissue layers.

Amount of bone around the smooth middle part

The bone amount in rectangular regions in proximity of the smooth middle part of the implant was determined. Three regions of interest (roi); roi 500, roi 1000, and roi 1500, were marked (Fig. 1). All measured surfaces were rectangular with a width of 0.5 mm and a length of 2.0 mm, and a total area of $1 \text{ mm}^2 (=1.10^6 \mu\text{m}^2)$. Area roi 500 was defined as a rectangular surface in direct contact with the smooth middle part of the implant. Area roi 1000 was determined at 500–1000- μm distance from the implant surface, and area roi 1500 at 1000–1500- μm distance. The amount of bone was quantified as $\mu\text{m}^2 \cdot 10^3$.

All quantitative measurements were performed for two different sections per implant, at both sides of the implant. Results are presented based on the average of these measurements.

RESULTS

Mechanical testing

Table I shows the failure load \pm standard deviation for both implants. Although the TPS/Ca-P-a-coated implants appear to show a higher failure load, statistical analysis (Student *t* test) demonstrated that this difference was not significant ($p > 0.5$).

SEM

Both types of implants showed good interfacial bone contact. New bone formation was observed without intervening soft-tissue layers. Signs of degradation of the FA/HA-PS coating were visible. After 3 months of implantation on the TPS/Ca-P-a implants, no remnants of the amorphous sputter coating were shown by SEM. Apparently, the Ca-P-a coating disappeared. The fracture plane for these implants was situated at the bone-Ti-coating interface (Fig. 4). Only occasionally was a small Ti plasma-spray particle included in this fracture. For the FA/HA-PS coating the line of fracture was less predictable. In general, at the smooth middle part of the screw implant the fracture was situated at the implant-coating interface. At the threaded parts the fracture site was observed to occur at three different locations: at the implant-coating interface, inside the coating, and at the bone-coating interface (Fig. 5). For both coatings, at some locations parts of bone were included within the fracture.

Histologic evaluation

Light microscopic analysis demonstrated uneventful healing of all implants without signs of inflammation. Around both the FA/HA-PS and TPS/Ca-P-a-coated implants an intimate bone-implant contact was formed [Fig. 6(A,B)]. Remodeling lacunae with osteoblasts were clearly visible. For the FA/HA-PS-coated implants the bone around the implants appeared to have a trabecular structure (Fig. 7). Around the TPS/Ca-P-a-coated implants a denser type of bone was observed in close proximity to the interface (Fig. 8). Around all implants the original drill margins were still recognized.

TABLE I

	<i>n</i>	Failure load (N)
FA/HA-PS	9	7.71 (± 3.44)
TPS/Ca-Pa	9	10.64 (± 4.82)

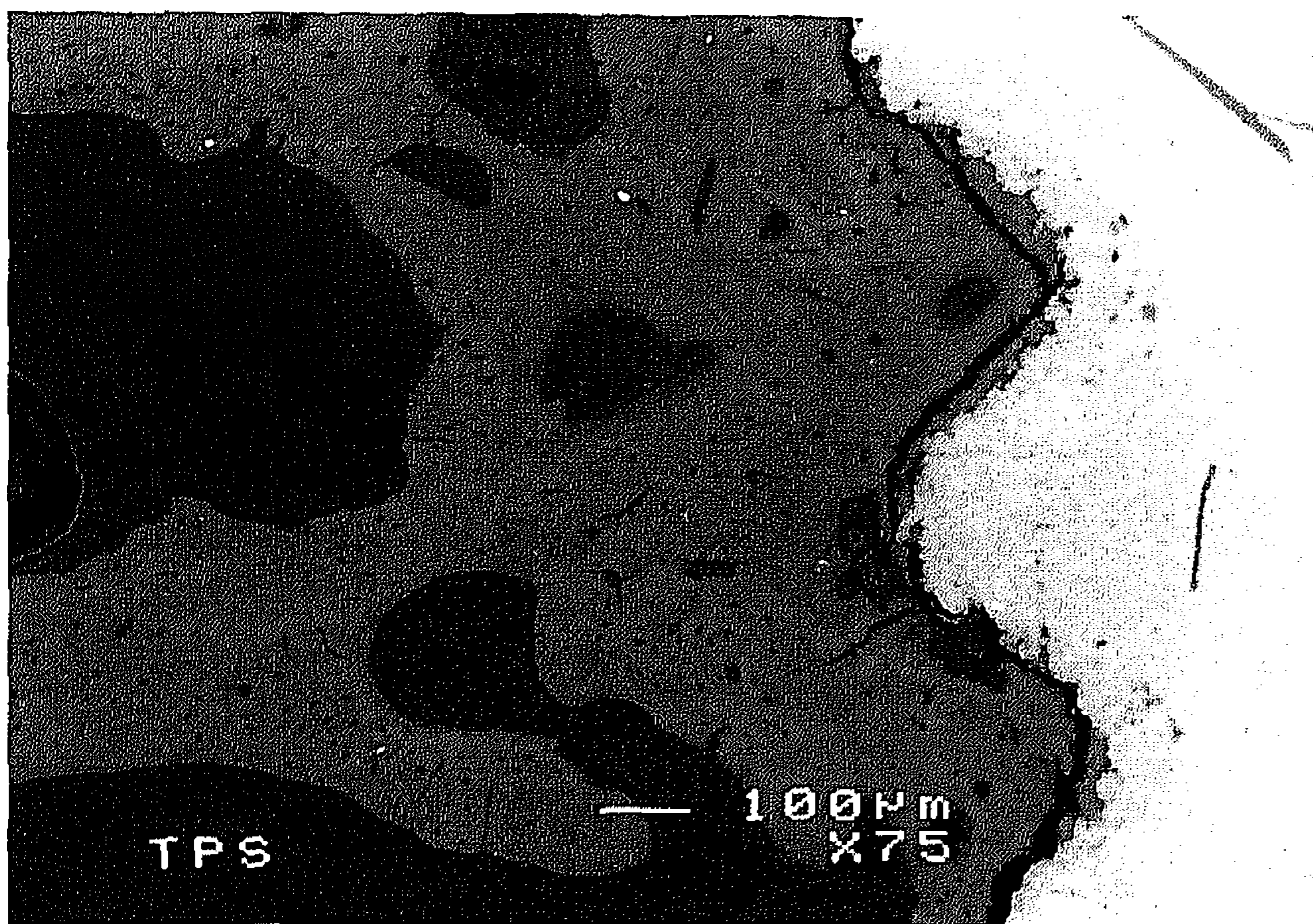


Figure 4. Scanning electron micrograph taken in backscatter mode of an implant with a TPS/Ca-P-a coating after the mechanical torque test. The fracture line is situated at the bone-coating interface. Occasionally, parts of bone are included inside the fracture. Bar represents 100 μm .

Qualitatively, FA/HA-PS coatings showed signs of degradation (Fig. 7). At some sites the complete coating had disappeared, while at other spots it was present at full thickness. Frequently, loose coating particles were observed in the tissue surrounding the implants. These particles could not be associated with cellular activity (Fig. 9). Further investigation revealed that these particles were an artifact caused by the sawing procedure.

Sections of the lymph nodes did not show any signs of titanium particles.

Histomorphometric evaluation

Table II shows the results of the measured percentages of bone-implant contact. Statistical testing (Student *t* test) revealed a significant ($p < 0.05$) percentage of bone contact for FA/HA-PS-coated implants, in regions 2, 3, and 4. For contact area 1, the coronal screw threads, no significant difference could be measured ($p > 0.05$).

Table III shows the results of the amount of bone in different regions of interest. Statistical testing [analysis of variance (ANOVA) and Tukey multiple comparison] revealed for all regions a significant ($p < 0.05$) greater amount of bone around TPS/Ca-P-a-coated implants. For both implants significant differences existed between the regions: roi 500 > roi 1000 > roi 1500.

DISCUSSION AND CONCLUSIONS

The aim of this study was to investigate the bone response to Ca-P plasma-spray and RF magnetron

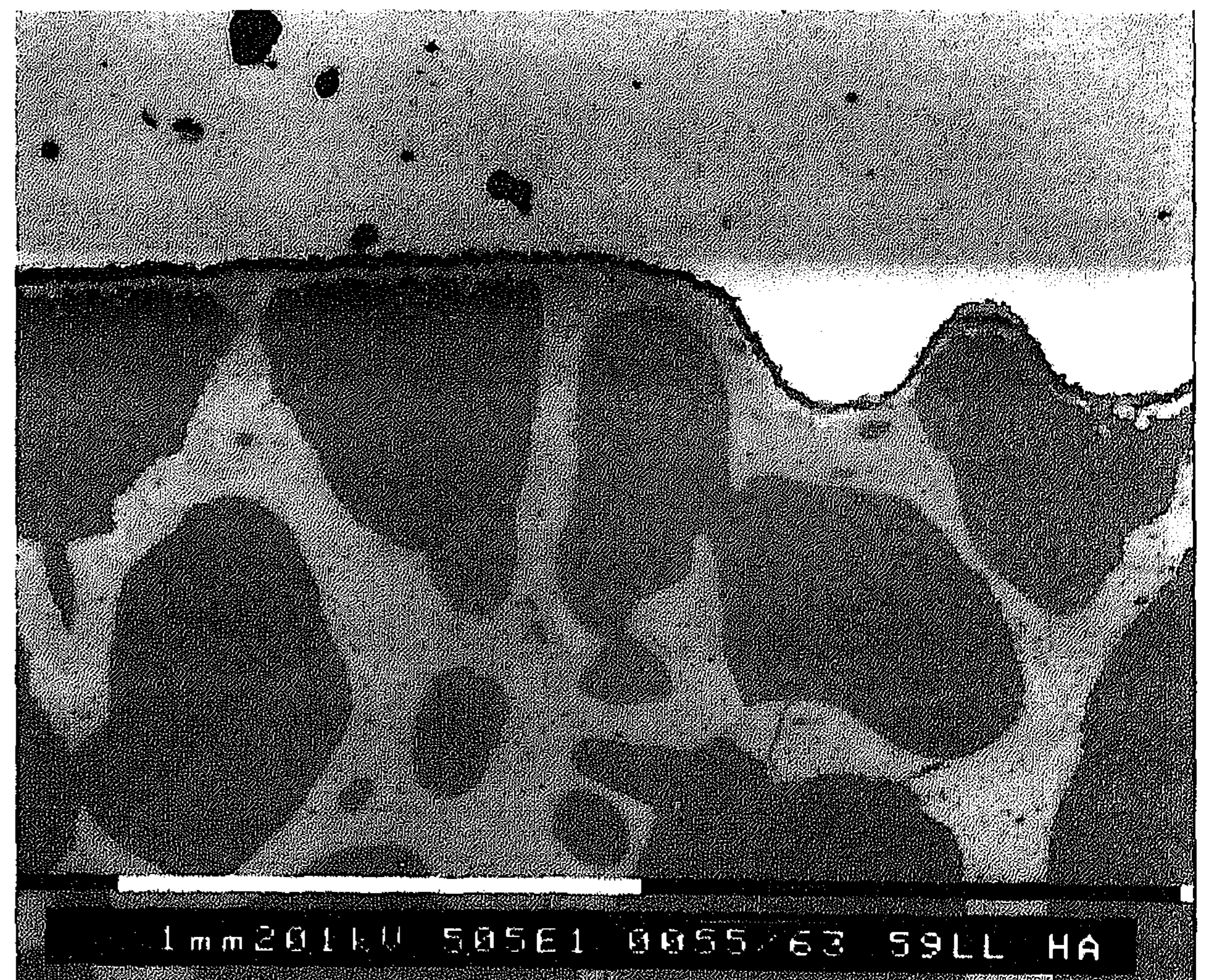


Figure 5. Backscatter SEM picture of an FA/HA-PS-coated implant. The fracture runs mainly at the implant-coating interface. Bar is 1.00 mm.

sputter-coated implants with comparable roughness. Tapered conical screw-designed implants were installed in the trabecular bone of the femurs of nine

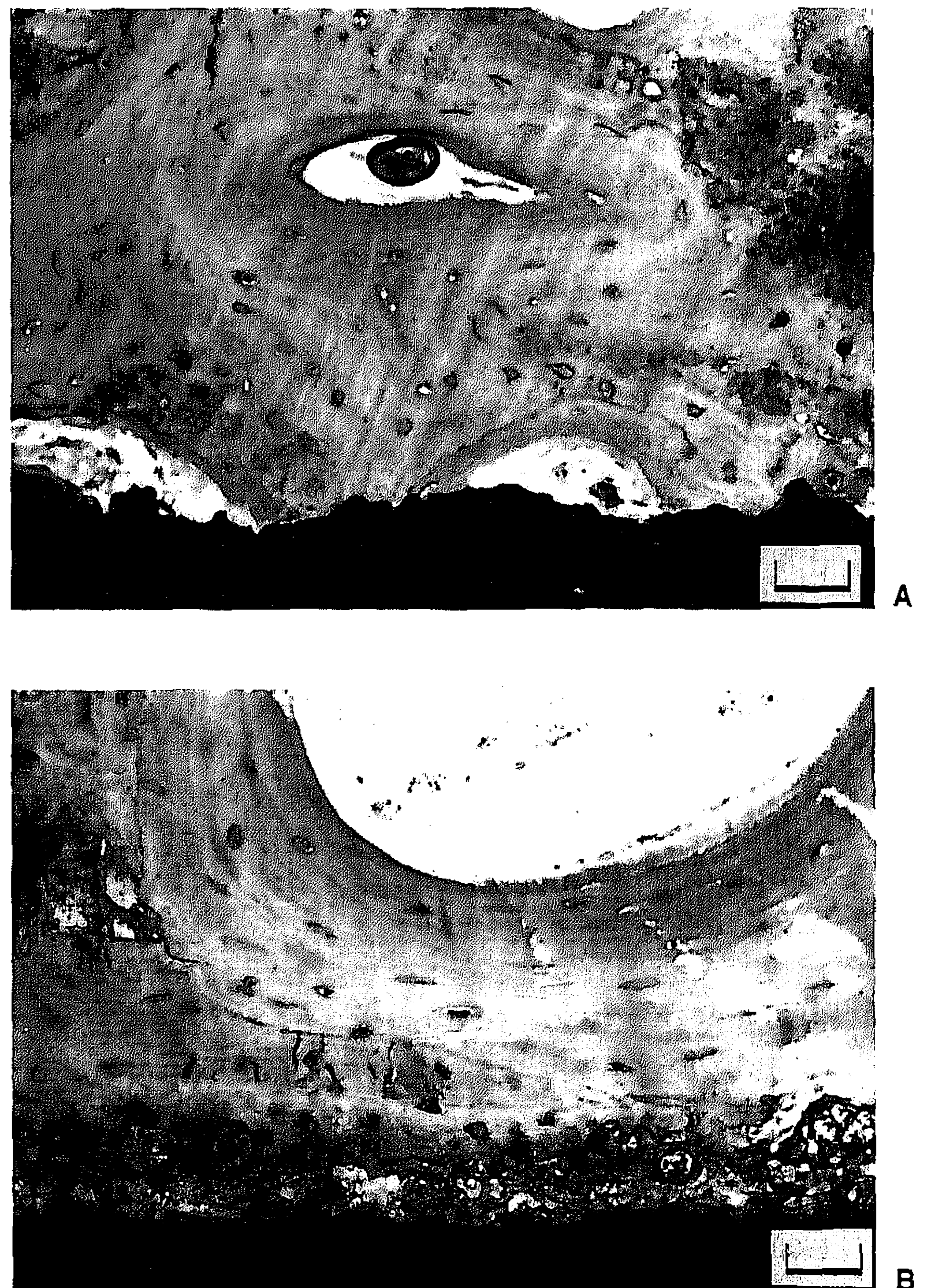


Figure 6. Light micrograph of a TPS/Ca-P-a (A) and a FA/HA-PS (B)-coated implant; close bone apposition is shown for both types of coatings. Bar = 48 μm .



Figure 7. Histologic section of an FA/HA-PS-coated implant after 3 months of implantation. In the screw threads the coating has partially disappeared. The trabecular structure of the bone around the implant can clearly be recognized. Bar = 300 μm .

goats. These implants were evaluated histologically and mechanically after 3 months of implantation.

The most frequently used method to measure bone-bonding strength is a pushout test.¹³⁻¹⁷ For this test, the implant is pushed out from the enclosing bone using a tensile testing bench. From the peak force that results in movement of the implant, the interfacial shear or bonding strength can be estimated. Obviously, the pushout model is suited only for cylindrical nonthreaded implants. Consequently, in our study we performed a mechanical test in which implant loosening was due to torsion. Various studies describe the use of a Jonichi[®] torque gauge instrument to measure the moment of torsion of threaded implants.¹⁸⁻²³ This value can directly be read on the instrument. Mostly this equipment is managed manually. A disadvantage of this method is that by manual operation of the Jonichi[®] torque gauge instrument, additional shear stresses are induced. These will never be exactly the same for each measurement. Further, it is very difficult to obtain and maintain a correct perpendicular alignment of this measuring device on the implant during unscrewing. Therefore, we developed a special

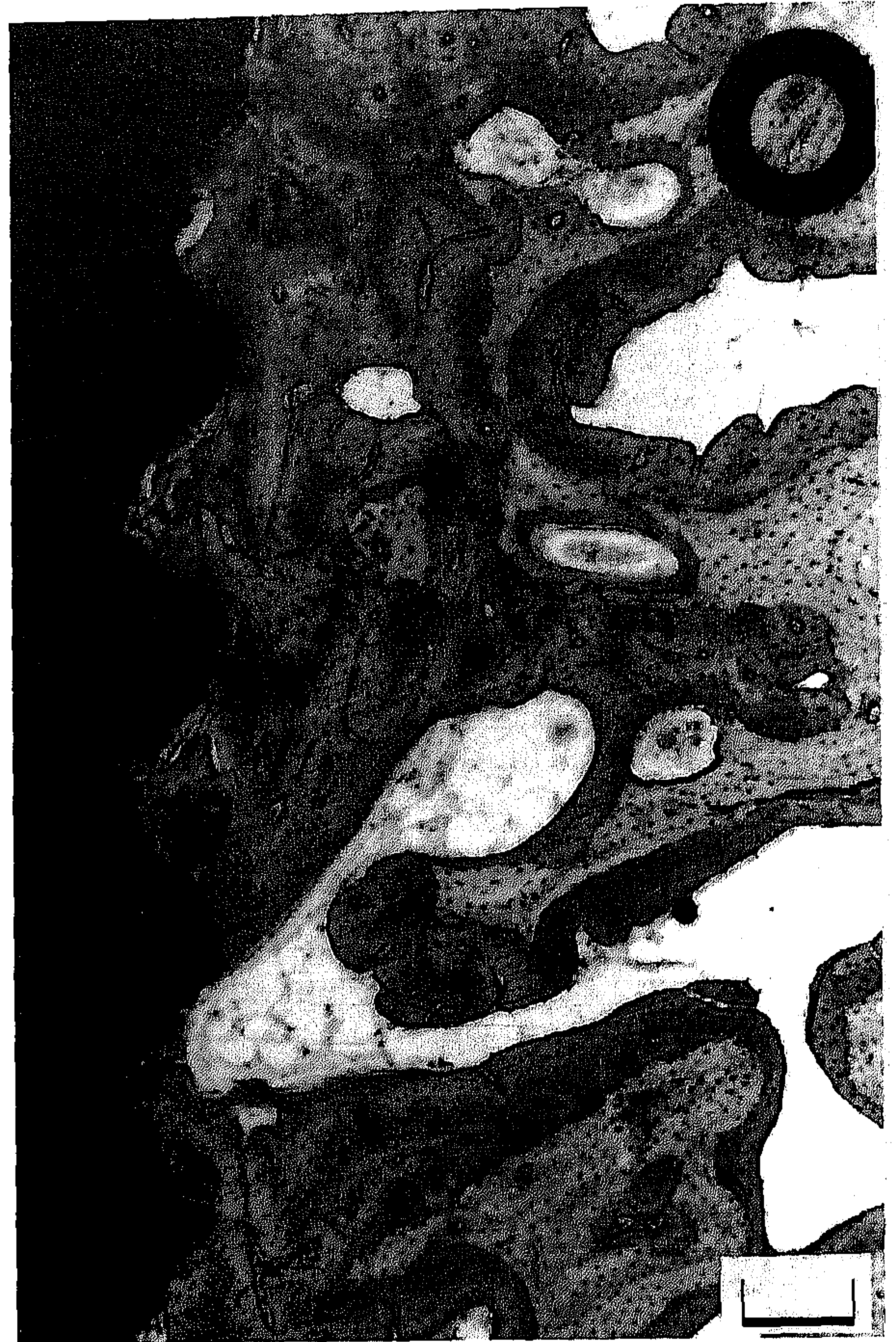


Figure 8. Light micrograph showing a denser type of bone in close proximity to a TPS/Ca-P-a-coated implant 3 months after implantation. Bar = 300 μm .

device which makes it possible to apply and maintain an exact reproducible perpendicular force on the threaded implants. This force was applied on a lever at 1.5 cm distance from the center line and 0.6 cm in front of the implant. The load results mostly in torsion at the implant, which is now well controlled by the testing apparatus. In addition, small shear and bending stresses are induced (Fig. 3), but these are also well controlled and identical for all specimens. Another advantage is that it is not necessary to unscrew the implant completely, since the force can be stopped immediately after the bone bonding is partially fractured. This allows investigation of the processes at failure by SEM. Finally, it has to be emphasized that caution must be taken to compare different studies. Measured values are relative and conclusive only for the described type and design of implant in the described type of bone.^{24,25} For correct interpretation of different studies a biomechanical evaluation by a finite element analysis of the different tests will be necessary.²⁵ However, this exceeds the aim of this study.

Using our test design, the mechanical evaluation showed that biomechanical interlocking and perhaps even chemical bonding developed between the TPS/

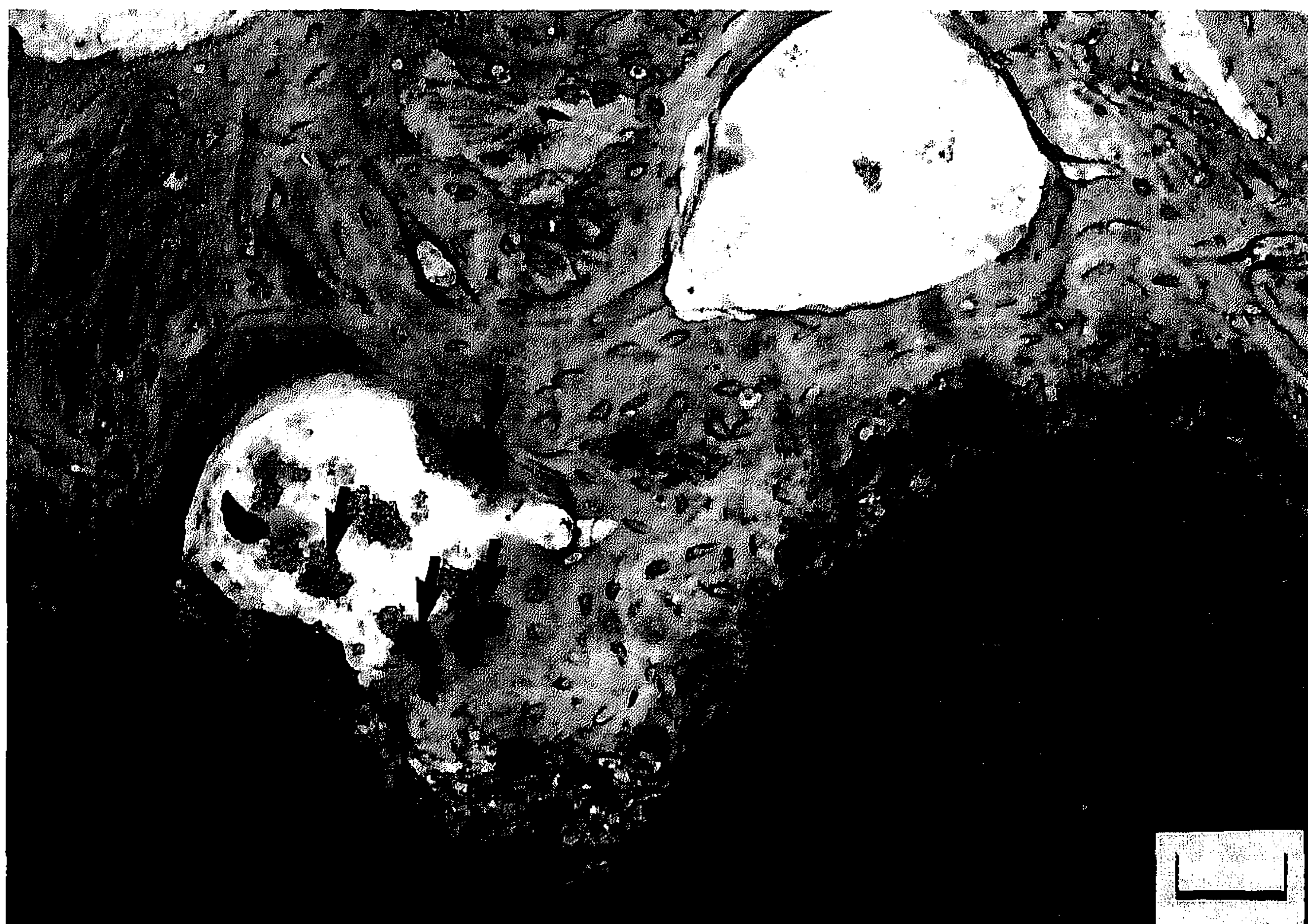


Figure 9. Microscopic picture showing FA/HA-PS particles (arrows) which are detached from the implant surface. No cellular activity is visible around the coating particles. Further examination revealed that this is an artifact caused by the histologic sectioning technique used. Bar = 75 μm .

Ca-P-a implants and surrounding bone, as depicted by the fracture in the interface bone coating. In contrast, the resistance to torsional force appeared to be inadequate for plasma-sprayed FA/HA-PS coatings, since fracture in the interface coating implant occurred. Such an inadequate attachment between Ca-P plasma-sprayed coatings and titanium has already been described earlier in pulloff tests²⁶ and *in vivo* animal experiments.²⁷ The supposed advantage of dual FA/HA-PS coatings, based on an improved bone healing response by fast dissolution of the amorphous HA component and subsequent maintenance of the bio-stable FA component, appears to be overruled by the unfavorable adhesion properties of these coatings to the implant. Therefore, we conclude that the rationale for the use of such dual coatings in loaded situations can be questioned.

After 3 months of implantation the Ca-P-a sputter coating was not detectable by SEM. This was confirmed by recent *in vitro* and *in vivo* dissolution experiments.²⁸ In these studies we demonstrated that complete dissolution of 2–4- μm -thick amorphous Ca-P

sputter coatings occurs, which is in contrast to crystalline coatings. Therefore, we think that the bioactive behavior of Ca-P sputter coatings is, among others, determined by their crystallinity.

Corten et al.²⁹ described the phenomenon of a lower percentage of bone apposition, in combination with a higher bone density for uncoated Ti implants, compared to various plasma-spray Ca-P coating. Our measurements of the amount of bone also revealed significantly more bone around TPS/Ca-P-a-coated implants in combination with less bone contact than for FA/HA-PS-coated implants. The possibility of a persisting bone reaction influenced by the implant material cannot be excluded. Consequently, a less than ideal bony integration will result in a qualitatively less stress-transferring system. This implies that the implant will act as a constant mechanical stimulus.³⁰ As a result of this persisting trauma the bone turnover around the implant will be increased, as characterized by an increased bone mass. This observation again argues for the use of well-characterized, stable Ca-P coatings, especially when degradation or mechanical properties of such coatings can be controlled or improved.

TABLE II
Percentages of Measured Bone-Implant Contact

Contact Area	FA/HA-PS	SD	TPS/Ca-P-a	SD
1	81.31	16.74	67.28	22.87
2	70.45	11.47	34.76	16.95
3	62.62	8.76	40.26	21.81
4	60.94	25.98	31.12	17.01

For each type of coating, $n = 9$. Two histologic sections/implant were measured.

TABLE III
Measured Amount of Bone ($\mu\text{m}^2 \cdot 10^3$)

	roi 500	SD	roi 1000	SD	roi 1500	SD
FA/HA-PS	518.1	118.8	438.2	86.1	395.4	92.8
TPS/Ca-P-a	685.8	45.87	532.6	128.1	466.8	117.7

For each type of coating and roi, $n = 9$. Two histologic sections/implant were measured.

Finally, Weinberg et al.³¹ described the transportation of fine titanium particles by phagocytes to regional lymph nodes. These particles were supposed to be loosened during insertion of titanium plasma-spray-coated implants. Despite this observation, our study did not reveal the presence of titanium particles in the regional lymph nodes. Although it is difficult to give an explanation for this discrepancy in observations, we suppose that differences in the manufacturing process or cleaning procedures can be a reason for the presence of loose particles. Nevertheless, concerns about loose and transported particles of titanium plasma-spray coatings appear to be justified and should always be thoroughly investigated.

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