Effect of Plasma-Sprayed Hydroxyapatite Coating on the Osteoconductivity of Commercially Pure Titanium Implants

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Formation of a calcium phosphate layer was studied on the surfaces of plasma-sprayed hydroxyapatite (PSHA) and sandblasted commercially pure (cp) titanium in simulated body fluid with ion concentrations similar to those of human blood plasma. The PSHA surface induced the formation of calcium phosphate surface layers, while the precipitation of calcium phosphate on sandblasted cp titanium was not detected. Histologic evaluation of in vivo tests demonstrated that implants with a PSHA coating enabled the growth of bone tissue into gaps with a depth of up to 1 mm without significant formation of intermediate fibrous tissue. In comparison to sandblasted cp titanium, implants with PSHA coating exhibited greater tolerance to unfavorable conditions during healing, such as gaps at the interface or primary instability of the implant. In the case of good primary stability of the implant, filling of the gap with fibrous tissue was observed for sandblasted cp titanium implants over the greater part of the surface of gaps with a depth of 0.3 mm. Direct contact of cp titanium implants with bone was achieved only when the press-fit implantation model was used. (Int J Oral Maxillofac Implants 2000;15:483–490)

Key words: *calcium phosphates, hydroxyapatite, osteoconduction, titanium, wound healing*

It is known that, in addition to their ability to form a direct bond with living bone tissue,[1–3] bioactive materials (bioglass, apatite-wollastonite glass-ceramic, hydroxyapatite) also exhibit osteoconductive properties, in contrast to bioinert materials, such as commercially pure (cp) titanium or the titanium alloy Ti-6Al-4V.[4] Osteoconductive properties are understood to consist of the ability of the material to act as a lattice for osteoblasts and osteocytes in the interconnection of defects (gaps) during the gradual formation of new bone. It can be expected that this specific property of bioactive materials is a consequence of their ability to form a thin calcium phosphate layer on the surface of the implant during a period of minutes to days, depending on the type of material, as a consequence of reactions with body fluids.[5,6] The chemical and crystallographic properties of this calcium phosphate phase are almost identical with bone apatite.[7] It can be assumed that bioactive implants with osteoconductive ability will thus exhibit greater tolerance to unfavorable conditions during implant healing, such as micromovements[8] or gaps between the implant and the bone matrix.[9]

It was recently demonstrated in the work of Clemens et al[10,11] that there is a maximal value of gap size that still permits bone apposition on the surface of an implant with plasma-sprayed hydroxyapatite (PSHA) coating during healing, without resulting in gap filling by soft tissue. The authors demonstrated on an animal model that this limit lies between 1 and 2 mm. Gaps of 1 mm between an implant with PSHA coating and the bone were filled with new bone without gap-filling by soft tissue, in contrast to a cp titanium implant, where the bone

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Table 1 Roughness Parameters of Sandblasted and Plasma-Sprayed Hydroxyapatite-Coated Surfaces

	R_a(µm)	S_m(µm)	R_{ku}
Sandblasted samples	0.9	12.9	3.2
PSHA-coated samples	8.3	47.0	3.0

R_a = arithmetic mean of the profile departures from the mean line; S_m = mean spacing of adjacent local peaks; R_{ku} = profile sharpness.

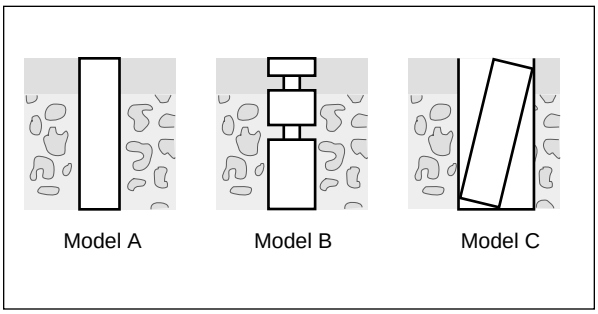


Fig 1 Schematic depiction of the manner of introduction of test implants into bones. Model A was press-fit and the diameter of the bone matrix and implant was identical (3.7 mm). Model B was press-fit and the diameter of the bone matrix and implant was identical (3.7 mm); a 0.3-mm-deep groove was present on the surface of the implant. Model C was not press-fit in the bone-matrix; the implant diameter was 3.7 mm and the bone matrix was 4.7 mm in diameter.

was separated from the implant by fibrous tissue.[12] The same result was obtained at both 6 weeks and 6 months after implantation. It can be presumed that a maximal size of the gap for which gap-filling with soft tissue will not occur will also exist for titanium implants, with a value of less than 1 mm.[10–12]

Thus, models with gaps of 0 to 1 mm were selected for the present study to investigate the growth of bone tissue. Samples of cylindric uncoated cp titanium implants and cylindric implants with PSHA coating were implanted into the tibiae of dogs in model arrangements with different primary stability and primary contact of bone to the surface of the implant and evaluated histologically. The interactions of implants with PSHA coating and cp titanium implants with simulated body fluid (SBF) were studied by tests in vitro, using analysis of the surface of the disc implants and by determination of changes in the concentrations of ions in SBF solutions with respect to length of the exposure period.

METHODS AND MATERIALS

Sample Preparation

Samples intended for exposure in SBF (in vitro test) were prepared using cp titanium (Austenal Dentalmaterial AB, Malmö, Sweden), grade 3, in the form of discs with a diameter of 10 mm and thickness of 1 mm. Non-coated samples were roughened by sandblasting with alumina powder (grain size 100 µm). The samples were washed in ethanol in an ultrasonic cleaner and dried at 120°C. Coated samples were prepared by the plasmatic deposition of HA onto the above-mentioned sandblasted cp titanium surface. Thickness of the coating was 50 µm. Surface roughness parameters of both sandblasted and PSHA samples were then evaluated (Table 1). A Talysurf 6 profilometer (Taylor Hobson, Leicester, United Kingdom) was used. (Since discs rather than actual implants were used, this evaluation may underestimate the roughness seen on actual cylindric implants where Talysurf cannot be used. Thus, measurements made on the discs provide an estimate of roughness.)

Test implantation was carried out using cylindric implants with the above described sandblasted and PSHA-coated surfaces. Both kinds of implants were cylindric in shape (or cylinders with a 0.3-mm-deep groove; Fig 1), with a diameter of 3.7 mm and a height of 10 mm. Before plasma-spraying, the diameter of implants was machined to 3.6 mm so that an identical final diameter (3.7 ± 0.02 mm) of both PSHA-coated and sandblasted implants was reached.

Exposure and Analysis of Samples

The samples were exposed in SBF (Fig 2) with a composition similar to the composition of the inorganic part of blood plasma (Table 2). The pH was adjusted to 7.40 at 36.5°C. Exposure was carried out using 100 mL of SBF solution, where the ratio of the surface of the sample to the volume of the solution was 0.02 cm^{-1}. Changes in the concentration of sample extracts were determined spectrophotometrically (UV-1201, Shimadzu Europe Ltd, Prague, Czech Republic), by atomic absorption spectroscopy (VARIAN-Spectr AA300, Varian Inc, Palo Alto, CA), and with a pH meter (WTW-526, WTW

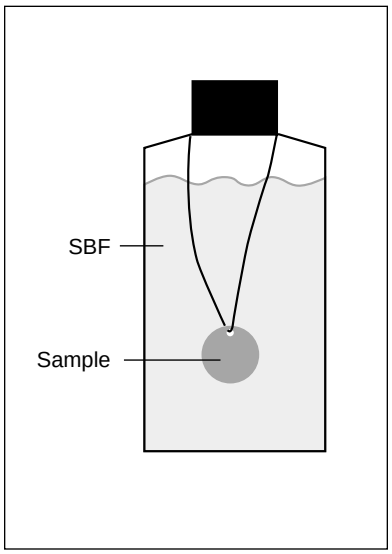


Table 2 Comparison of Composition of the Simulated Body Fluid Used and the Inorganic Part of Blood Plasma

Ion	Blood plasma (mmol/L)	Simulated body fluid (mmol/L)
Na^+	137.0–147.0	142.0
K^+	3.8–5.1	5.0
Ca^{2+}	2.25–2.75	2.5
Mg^{2+}	0.75–1.25	1.5
Cl^-	98–106	147.8
HCO_3^-	24–35	4.2
HPO_4^{2-}	0.65–1.62	1.0
SO_4^{2-}	0.5	0.5

Fig 2 *(Left)* Schematic depiction of the experimental arrangement for sample exposure in a simulated blood plasma solution (SBF).

Measurement Systems Inc, Ft. Meyers, FL). Following exposure in SBF, the surface of the samples was studied using a scanning electron microscope fitted with an energy dispersion analyzer SEM-EDS (Jeol XA-733 superprobe, Jeol USA Inc, Peabody, MA) and thin film X-ray diffraction microscopy using a Seifert XRD 3000P diffractometer (Rich Seifert & Co, Ahrensburg, Germany).

Implantation and Histologic Evaluation

The ethical board of the Teaching Hospital, Charles University, Hradec Králové, Czech Republic, approved all experimental procedures used in the study. Prior to the operation, the tested materials were sterilized with saturated water vapor at a temperature of 125°C and pressure of 140 kPa for a period of 15 minutes. Implantation was carried out in 2 dogs of both sexes of unknown breed with a weight of 12 ± 2 kg. They were premedicated with Dolsin (Biotika as, Martin, Slovakia), 10 mg/kg, 30 minutes prior to surgery. Anesthesia was carried out continuously by an intravenous infusion of a 2% solution of thiopental (VUAB, Roztoky u Prahy, Czech Republic).

Following disinfection of the operation area and toweling, an incision was made above the upper edge of the tibia. Following passage through the soft tissue and moving the periosteum to one side, holes were drilled in the cortical bone using a bur with a diameter of 3.7 or 4.7 mm.

Three implantation models were used. Cylindric implants with a diamter of 3.7 mm were introduced into holes with the same diameter, ie, they were placed inside the bone with good primary stability (press-fit; model A in Fig 1). Model B differed from model A in that the implant had a 0.3-mm-deep groove around its circumference. Once again, the press-fit principle was used. These implants exhibited good stability, and at the same time, the gap between the bone and the implants was defined exactly by the groove (Fig 1). In the third implantation model (model C), implants with a diameter of 3.7 mm were placed into a 4.7-mm opening (non press-fit). In this group, the bone did not lie immediately on the surface of the implant. Thus, the implant had low primary stability and a low area of primary contact with the bone (Fig 1).

In each implantation model, 2 pairs of implants were used. Each pair consisted of 1 PSHA implant and 1 sandblasted cp titanium implant. The first animal received 2 pairs of implants in the left tibia (models A and B) and 2 pairs of implants in the right tibia (models A and C). The other animal received 1 pair of implants in the left tibia (model C) and 1 pair of implants in the right tibia (model B). A total of 12 implants was used in the study.

In the postoperative period, antibiotics were not administered. Three months after the operation, the animals were sacrificed by an overdose of thiopental. The tibiae were removed and immersed in 10% formaldehyde, and blocks containing 1 implant each were prepared. Blocks were dehydrated using graded methanols (70% to 100%) and embedded in methylmethacrylate resin. Samples were processed undecalcified. From each implant, 2 longitudinal sections with a thickness of 5 to 50 µm were made on a saw (Struers Accutom-2, Struers, Copenhagen, Denmark). Sections were affixed onto

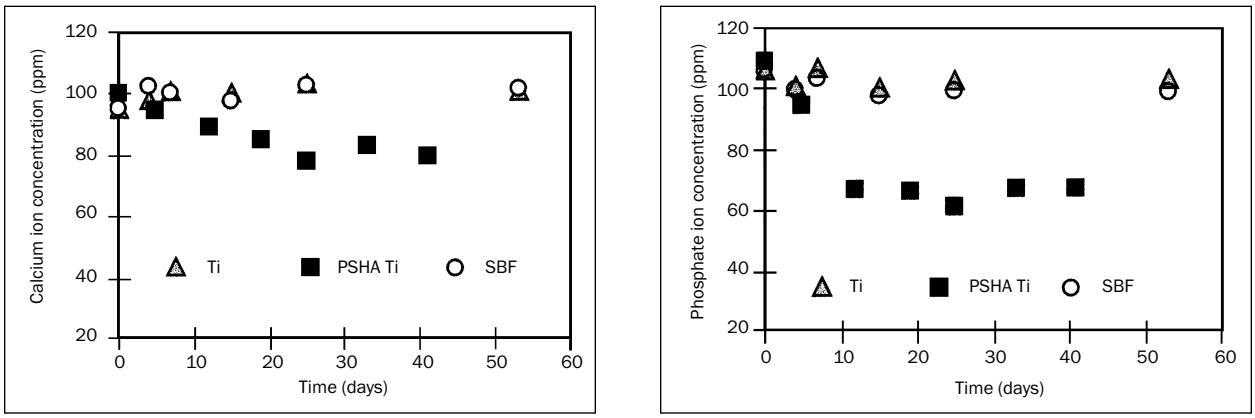
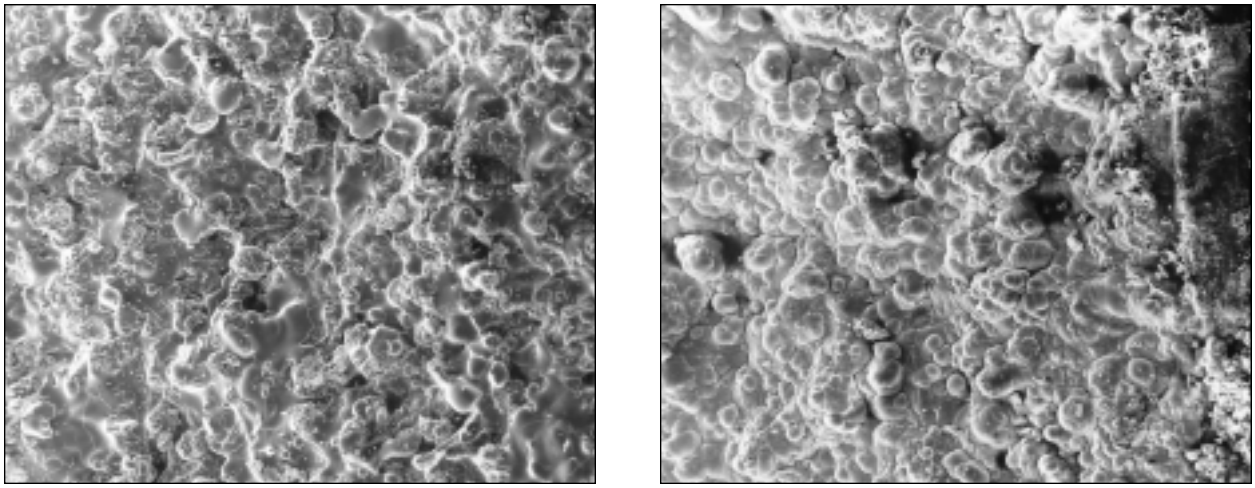


Fig 3 Changes over time in the concentration of calcium and phosphate ions in a simulated body fluid solution (SBF) for exposure of samples of pure titanium (Ti) and plasma-sprayed hydroxyapatite (PSHA Ti). The third set of values corresponds to the SBF blank (parallel experiment).



Figs 4a and 4b Electron microscopic images of (*left*) the surface of a sample with plasma-sprayed hydroxyapatite coating and (*right*) the surface of a sample with plasma-sprayed hydroxyapatite coating covered with bonelike apatite spherulitic crystals, after 7 days of soaking in SBF. Bar = 100 µm.

a glass slide and, if necessary, hand ground to a thickness of 5 to 10 µm. Sections were stained with toluidine blue. Photomicrographs for histologic analysis were taken using an Olympus BX-60 microscope (Olympus, Tokyo, Japan) fitted with a JAI 2040 CCD camera (JAI Corp, Yokohama, Japan). Digitized image analysis was performed using Lucia 4.1 software (Laboratory Imaging LIM Ltd, Prague, Czech Republic). The length of bone tissue in direct contact with the implant (BC) and the total interface length (IL) were measured. The percentage of bone-implant contact was determined by the ratio of the direct contact length to the total interface length (BC/IL). The presented mean values were calculated from the 4 results (4 sections) available for both types of implants in each particular implantation model.

RESULTS

Interaction of the PSHA-coated implants with SBF was accompanied by removal of calcium and phosphate ions from the solution (Fig 3) and deposition of spherulitic crystals of calcium phosphate on the surface, as depicted in the electron microscopic images (Figs 4a and 4b). During the first few days, there was a clear decrease of the calcium ion (Ca^{2+}) and phosphate ion (PO_4^{3-}) concentrations in solution, and the process stabilized after 10 to 12 days. Following more than 10 days of exposure, the surface of the sample was covered with a continuous layer of calcium phosphate agglomerates (Fig 5). Analysis of the surface layer by an electron microprobe demonstrated that the molar Ca/P ratio of 1.50 was close to that of HA (1.67) (Fig 6). The

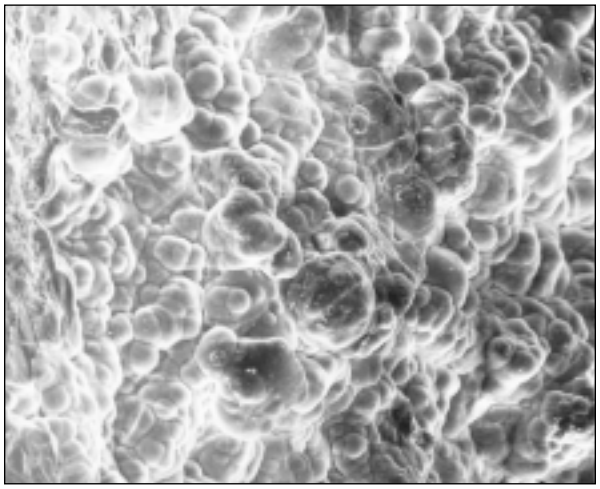


Fig 5 Electron microscopic image of a sample with plasma-sprayed hydroxyapatite coating after 14 days of soaking in SBF, showing a continuous layer of calcium phosphate agglomerates. Bar = 100 μm.

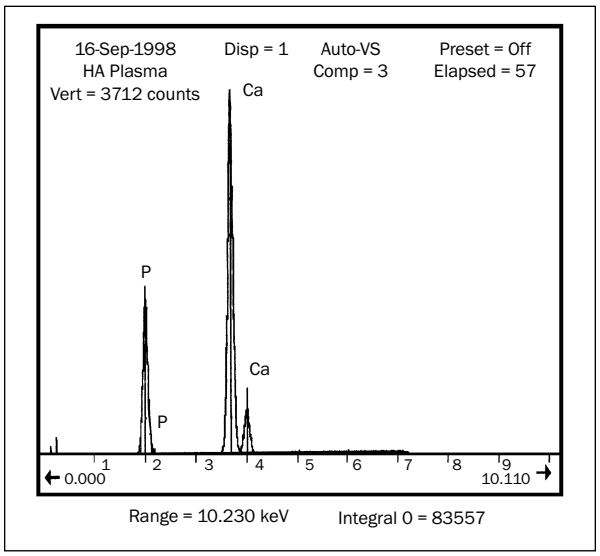


Fig 6 Energy dispersion spectroscopic analysis of layers formed on the surface of a sample with plasma-sprayed hydroxyapatite coating after 7 days of soaking in SBF.

crystalline nature of the precipitated layer was also confirmed by thin-film x-ray diffraction analysis (Fig 7). The x-ray diffraction pattern of the calcium phosphate layer indicates its diffusion character, which resembles that of bone apatite and contrasts with the x-ray diffraction pattern of the original PSHA-coated sample, which indicates larger crystals of the apatite phase and higher crystallinity.

Sandblasted cp titanium samples did not exhibit a decrease in the concentration of calcium or phosphate ions in the leaching solution following exposure in SBF, even after 50 days, as indicated by the data shown in Fig 3. The changes in the concentrations of calcium and phosphate ions in solution lay within the range of the changes in concentration in a parallel blank experiment. In addition, analysis of the surface of the samples with an electron microprobe did not prove the presence of calcium or phosphorus.

Microscopic examination of implants placed according to the press-fit method (model A) showed a high percentage of bone tissue apposition to the surface of the implant 3 months following implantation, both for sandblasted cp titanium implants (BC/IL = 54%) and for implants with PSHA coating (BC/IL = 79%) (Figs 8a and 8b). The effect of the different osteoconductive ability of the tested materials was marked for implants placed with a defined gap (0.3 mm) between the implant and the bone with relatively good primary stability of the implant (model B). Three months after implantation of the sandblasted cp titanium implant, the gap was filled with new bone tissue; however, over its entire surface it was separated from the titanium surface by fibrous tissue with a thickness of about 50 μm (BC/IL = 2%). In contrast, in the gap with the PSHA-coated surface, the newly formed bone was immediately adjacent to the surface of the implant, without significant gap-filling by fibrous tissue (BC/IL = 88%) (Figs 8c and 8d). Histologic evaluation of the non press-fit implants (model C) placed in the bone matrix with a gap of 0 to 1 mm with an average value of 0.5 mm once again demonstrated the positive effect of PSHA coating. The surface of the sandblasted cp titanium implant was coated almost entirely with a layer of fibrous tissue, with a negligible area in direct contact with the bone (BC/IL = 5%). The implant coated with HA was in direct contact with newly formed bone tissue over 72% of the total surface of the implant (BC/IL = 72%) (Figs 8e and 8f).

DISCUSSION

A PSHA coating exposed to a solution of SBF induced the formation of a calcium phosphate surface layer that was chemically and crystallographically similar to bone apatite; in contrast, no calcium phosphate layer formed on sandblasted cp titanium.

It has been shown[13–15] that bioactive materials like HA, bioactive glasses, or glass-ceramics bond directly to bone via formation of a bonelike apatite

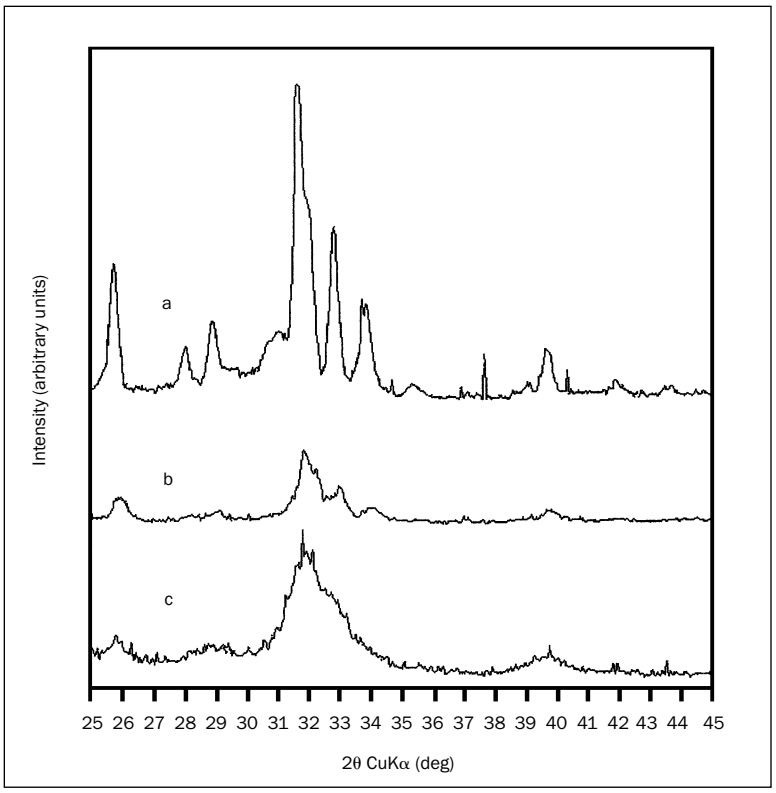


Fig 7 Thin-film x-ray diffraction patterns of (a) plasma-sprayed hydroxyapatite, (b) plasma-sprayed hydroxyapatite exposed to SBF for 14 days, and (c) bone apatite.

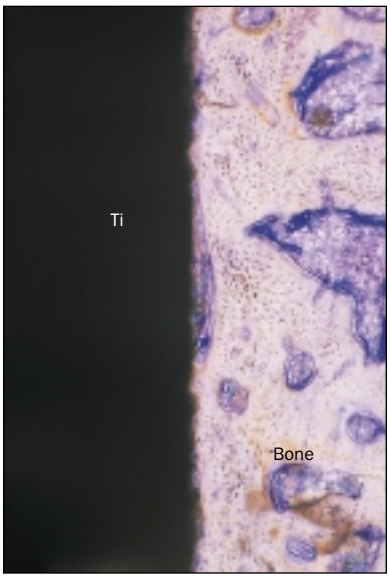
layer on their surfaces and also induce apatite formation in SBF. Thus, it may be suspected that bonelike apatite precipitation plays an important role in osseoconduction and bone-bonding formation. Provided that the apatite formation in SBF indicates osseoconductivity and the ability to bond to bone, the passive behavior of sandblasted titanium in SBF (compared to the PSHA surface) was consistent with its in vivo performance indicating lower osteoconductivity.

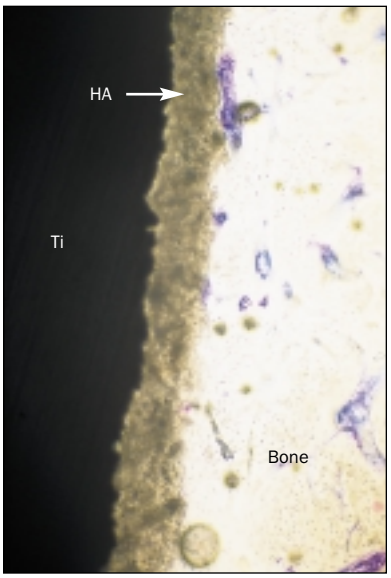
Unfavorable conditions for the healing of implants, such as gaps between the bone and the implant and primary instability of the implant at the time of placement, were better overcome by implants with PSHA coating than implants that were only sandblasted. The surface of implants with PSHA coating permitted the growth of bone tissue into gaps with a depth of 1 mm without significant gap filling by fibrous tissue. The formation of direct contact between the surface of the implant and bone was less affected by primary instability of the implant than in the case of implants with a sandblasted cp titanium surface.

The sandblasted cp titanium surface enables only a small degree of gap healing without the formation of intermedial fibrous tissue. Gap filling by soft tissue over a major part of the area of the gap was observed even for gaps with a depth of 0.3 mm, with relatively good primary stability of the implant. Only the press-fit implantation model (model A) permitted osseointegration of implants with a sandblasted cp titanium surface. Unfortunately, the small number of implants used in the study is unsuitable for statistical analysis, but clinically relevant conclusions may nevertheless be drawn from the results. It should be pointed out that these conclusions were based on unloaded implants that were histologically examined using light microscopy.

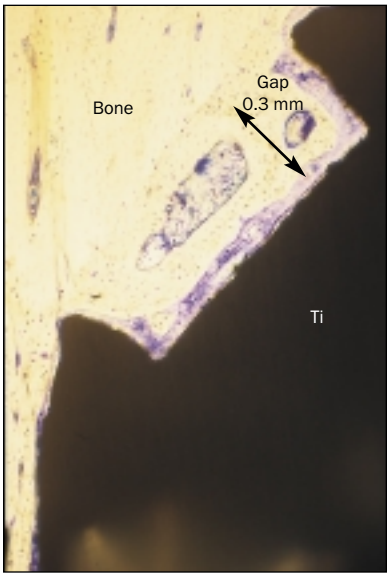
CONCLUSION

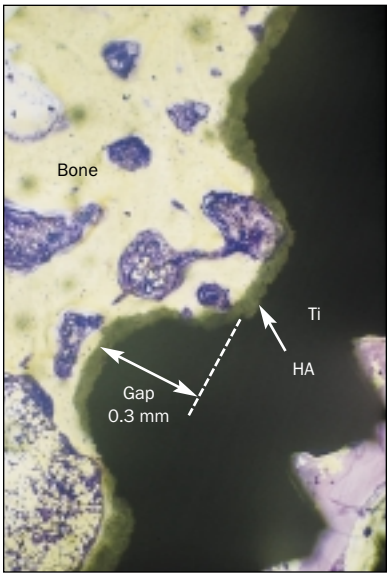
This study demonstrated that PSHA-coated implants exhibit greater tolerance than sandblasted cp titanium implants to unfavorable conditions during healing, such as gaps at the interface or primary instability of the implant. Plasma-sprayed HA-coated implants showed a high percentage of bone contacts when gaps were smaller than 1 mm, even



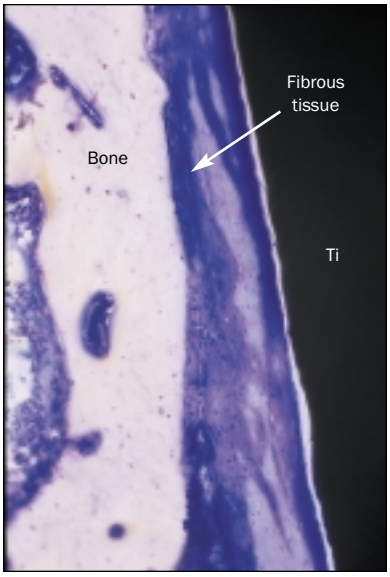


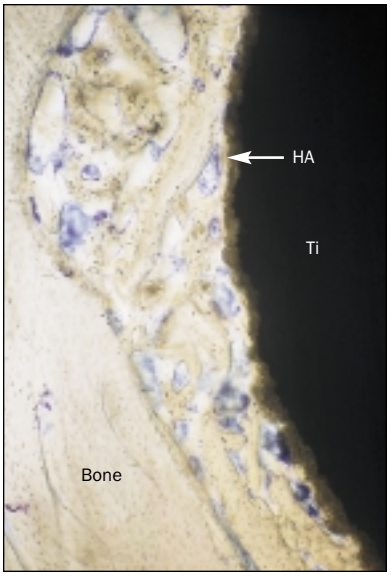
Figs 8a and 8b Photomicrographs of the boundary between the bone tissue and model A implants. (*Left*) Sandblasted cp titanium implant; (*Right*) Cp titanium implant with plasma-sprayed hydroxyapatite coating (toluidine blue stain; magnification ×320).





Figs 8c and 8d Photomicrographs of the bone-implant interface (model B samples). (*Left*) Sandblasted cp titanium implant; (*Right*) Cp titanium implant with plasma-sprayed hydroxyapatite coating (toluidine blue stain; magnification ×100).





Figs 8e and 8f Photomicrographs of the bone-implant interface (model C samples). (*Left*) Sandblasted cp titanium implant (toluidine blue stain; magnification ×320); (*Right*) Cp titanium implant with plasma-sprayed hydroxyapatite coating (toluidine blue stain; magnification ×200).

when non press-fit placement was used. In the case of sandblasted cp titanium implants, filling of gaps with fibrous tissue was observed over the greater part of the surface of gaps with a depth of 0.3 mm. Direct contact of sandblasted cp titanium implants with bone was achieved only when press-fit implantation was used. It can be concluded that more precise surgical placement may be needed for cp titanium implants than for PSHA-coated implants.

ACKNOWLEDGMENTS

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