Effect of plasma sprayed hydroxyapatite coating on osteoconductivity of commercially pure titanium implants

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This study examines the formation of a calcium phosphate layer on surfaces of plasma sprayed hydroxyapatite (PSHA) and sand-blasted commercially pure (CP) titanium in simulated body fluid (SBF) with ion concentrations similar to those of human blood plasma. The surface of PSHA induced the formation of calcium phosphate surface layers, while the precipitation of calcium phosphate on sand-blasted CP titanium has not been detected. Histologic evaluation of in vivo tests has demonstrated that implants with a PSHA coating enabled the growth of the bone tissue into gaps with a depth of up to 1 mm without significant formation of intermediate fibrous tissue. In comparison to sand-blasted 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 gap-filling with fibrous tissue was observed for sand-blasted CP titanium implants over the greater part of the surface of gaps with a depth of 0.3 mm. A direct contact of CP titanium implants with bone was achieved only when press-fit implantation model was used.

Key words: *osteoconduction, titanium, plasma sprayed hydroxyapatite, calcium phosphate layer, gap-healing*

Introduction

It is known that, in addition to their ability to form a direct bond with living bone tissue (1,2,3), bioactive materials (bioglass, A-W glassceramic, Hydroxyapatite) also exhibit osteoconductive properties in contrast to bioinert materials such as CP titanium or the titanium alloy, Ti6Al4V (4). Osteoconductive properties are understood to consist of the ability of the material to act as a lattice for the osteoblast 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 micro-movements (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 maximum gap size witch still permits bone apposition on the surface of an implant with plasma sprayed hydroxyapatite (PSHA) coating during healing, without the gap filling with soft tissue. The authors demonstrated on an animal model that this limit lies between 1-2 mm. Gaps of 1 mm between an implant with PSHA coating and the bone were filled with new bone without being filled by soft tissue. This is in contrast to a CP titanium implant, where the bone was separated by fibrous tissue under the same conditions (12). The same results were achieved both 6 weeks and 6 months following the implantation. It can be assumed 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 (10,11,12).

Thus, models with gaps of 0 to 1 mm were selected for the present study to investigate the growth of bone tissue. Samples of uncoated CP titanium implants and implants with PSHA coating were implanted into the tibiae of dogs in model arrangements with different primary stability and primary contact of bones to the surface of the implant, for histologic evaluation. The interactions of implants with PSHA coating and CP titanium implants with simulated body fluid (SBF) were studied by tests in vitro. These tests involved analysis of the surface of the implants and examination of changes in the concentrations of ions in SBF solutions dependent upon the exposure period.

Tab. 1: Roughness parameters of sand-blasted and plasma sprayed hydroxyapatite (PSHA) coated surfaces.

	R_a (um)	S (um)	R_{ku}
sand-blasted	0.9	12.9	3.2
PSHA coated	8.3	47.0	3.0

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Methods and Materials

Sample preparation

Samples in simulated body fluid (in vitro test) were prepared using commercially pure (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 sand-blasting with alumina powder (grain size 100μm). The samples were washed in ethanol in an ultrasonic cleaner and dried at 120°C. Coated samples were prepared by plasmatic deposition of hydroxyapatite on the surface of the above-mentioned sand-blasted CP titanium discs. Thickness of the coating was 50μm. Surface roughness parameters of both sand-blasted and plasma sprayed hydroxyapatite (PSHA) coated samples were evaluated (Tab.2): R_a – arithmetic mean of the profile departures from the mean line, S – mean spacing of the adjacent local peaks, R_{ku}-profile sharpness. The profilometer TALYSURF 6 (Taylor Hobson, Leicester, U.K.) was used. Test implantation was undertaken using implants with the above described sand-blasted and PSHA coated surfaces. Both kinds of implants had the shape of cylinders (or cylinders with a 0.3 mm deep groove, fig 2), with a diameter of 3.7 mm and height of 10 mm. Before plasma spraying the diameter of implants was machined to 3.6 mm so that an identical final diameter (3.6mm±0.02mm) of both PSHA coated and sand-blasted implants was ensured.

Exposure of samples in simulated body fluid (SBF) and analytical methods

The samples were exposed in SBF (Fig. 1) with a composition similar to that of the inorganic part of blood plasma (Tab. 2). The pH value was adjusted to 7.40 at 36.5°C. The samples were immersed in 100 ml of SBF solution, where the ratio of the surface of the sample (S) to the volume of the solution (V) had a value of S/V = 0.02 cm^{-1}. Concentration changes in sample extracts were determined spectrophotometrically (UV-1201, Shimadzu Europe, Ltd.), by atomic absorption spectroscopy (VARIAN-Spectr AA300) and using a pH meter (WTW-526). Following exposure in SBF, the surface of the samples was studied using a scanning electron fitted with an energy dispersion analyzer -SEM-EDS (Jeol XA-733-superprobe, Jeol, U.S.A., Inc.) and thin film X-ray diffraction (TF-XRD) using a Seifert XRD 3000P diffractometer.

Implantation of the tested materials and histological evaluation

The Ethical Board of the Orthopedic Cllinic of the Faculty Hospital in 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 on 2 dogs of different sex of an unknown breed with a weight of 12kg (± 2 kg).

They were premedicated with Dolsin (Biotika a.s., Slovakia), 10 mg/kg of weight, half an hour prior to the operation. Anesthesia was carried out continuously by an i.v. infusion of a 2% solution of Thiopental (Spofa a.s.,

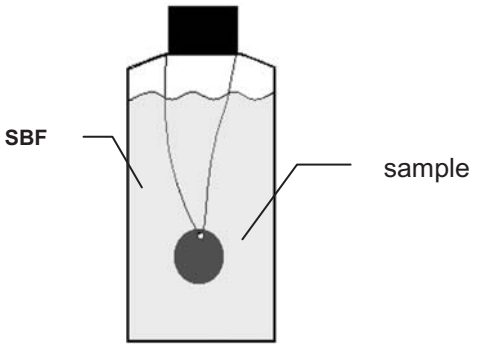


Fig. 1: Schematic depiction of the experimental arrangement for sample exposure in a simulated body fluid solution (SBF)

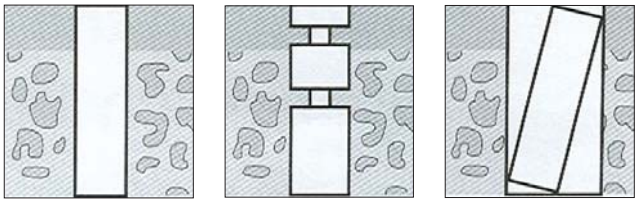
Following disinfection of the operation area and drying, an insection was made above the upper edge of the tibia. Following passage through the soft tissue and moving the periosteum to one side, holes were drilled in the corticalis using a bit with a diameter of 3.7 or 4.7 mm.

Three implantation models were used. Implants with a diameter of 3.7 mm were introduced into the holes with the same diameter, i.e. were placed inside the bone with good primary stability - press-fit (model A. Fig. 2a). The second implantation model differed from the previous one in that the implants had a groove around their circumference with a depth of 0.3 mm. Once again, the press-fit principle was used. These implants exhibited good stability and simultaneously, the gap between the bone and the implant was exactly defined by the groove (model B, Fig. 2b). In the third implantation model implants with a diameter of 3.7 mm were inserted in a bone opening with a diameter of 4.7 mm (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 of the bone with the surface of the implant (model C, Fig. 2c).

In each implantation model 2 pairs of implants were used. Each pair consisted of 1 PSHA implant and 1 sand-blasted CP titanium implant. The first animal received two pairs of implants in the left tibia -models A, B and two pairs of implants in the right tibia -models A, C. The other animal received one pair -model C in the left and one pair -model B in the right tibia. The total of 12 implants were used in the study.

In the post-operative period, antibiotics were not administered. Three months after the operation, the animals were put down an overdose of Thiopental. The tibiae were removed, immersed in 10% formaldehyde and blocks containing 1 implant each were prepared. The blocks were then dehydrated using graded methanols (70-100%) and embedded in methylmethacrylate. Samples were processed undecalcified. From each implant two longitudinal sections with thicknesses of 5-50μm were made on a saw (Struers Accutom-2, Struers, Copenhagen, Denmark). Sections were affixed onto a glass slides and if necessary hand-ground to a thickness of 5-10um. Sections were stained with toluidine blue. Photomicrographs for histologic analyses were taken using an OLYMPUS BX-60 microscope fitted with JAI 2040 CCD camera (JAI Corp., Yokohama, Japan). The digitized image analysis was performed using LUCIA 4.1software (Laboratory imaging, LIM, Ltd., Czech republic).



Model A Model B Model C

Fig. 2: Schematic depiction of the manner of introduction of test implants into bones: Model - A: press-fit; diameter of the bone matrix and implant is identical - 3.7 mm. Model – B: press-fit; diameter of the bone matrix and implant is identical - 3.7 mm; a 0.3 mm deep grove was present on the surface of the implant. Model - C: non-press-fit implant in the bone matrix; implant diameter - 3.7 mm. diameter of the bone matrix - 4.7 mm.

The length of the bone tissue in direct contact with implant (BC) and the total interface length (IL) were measured. The percentage of bone-implant contact is given by the ratio of the direct contact length to the total interface length (BC/IL). The presented mean values were calculated from the four results (4 sections) available for all types of the particular implantation model.

Results

Immersion of the PSHA coated implants in the simulated body fluid was accompanied by removal of calcium and phosphate ions from solution (Fig. 3a,b) and deposition of spherulitic crystals of calcium phosphate on the surface, as depicted in the electron microscope image (Fig. 4 a,b). During the first few days, there was a clear decrease of the Ca^{2+} and PO_4^{3-} concentration in the solution and the process stabilized after 10-12 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 with an electron microprobe demonstrated that the molar Ca/P ratio of 1.50 was close to that of hydroxyapatite (1,67) (Fig. 6). The 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 (Fig. 7b) indicates its diffusion character, similar to that of bone apatite (Fig. 7c), in contrast to the x-ray diffraction pattern of the original PSHA coated sample (Fig. 7a), which indicates larger crystals of the apatite phase and higher crystallinity.

Sand-blasted 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 in the time dependence in Fig. 3. The changes in the concentrations of Ca^{2+} and PO_4^{3-} ions in the solution lay within the range of the changes in concentration observed in a parallel control sample. In addition, analysis of the surface of samples using an electron microprobe did not indicate the presence of calcium or phosphorus. Microscopic examination of implants introduced by the press-fit method (model A) showed a high percentage of bone tissue apposition to the surface of the implant three months following the implantation, both for sand-blasted CP titanium implants (BC/IL=54%) and for implants with PSHA coating (BC/IL=79%) (Figs. 8 a,b). The effect of the different osteoconductive ability of the tested materials was marked for implants with a defined gap between the implant and

Tab. 2: Compositions of the simulated body fluid (SBF) used and the inorganic part of blood plasma

	blood plasma [mmol/l]	SBF [mmol/l]
Na^+	137-147.0	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

the bone with relatively good primary stability of the implant (model B). Three months after the implantation of the sand-blasted CP titanium implant, the gap (0.3 mm) 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 mm (BC/IL=2%), in contrast to the gap with the PSHA coated surface, where the newly formed bone was immediately adjacent to the surface of the implant without significant gap-filling by fibrous tissue (BC/IL=88%) (Figs. 8 c,d). Histological evaluation of the non-press-fit implants in the bone matrix with a gap of 0 - 1 mm with an average value of 0.5 mm (model C) once again demonstrated the effect of PSHA coating. The surface of the sand-blasted CP titanium implants was almost entirely coated with a layer of fibrous tissue with negligible area in direct contact with bone (BC/IL=5%). The implant coated with hydroxyapatite was in direct contact with the newly formed bone tissue over 72% of the total surface of the implant (BC/IL=72%)(Figs. 8 e, f).

Discussion

A plasma sprayed hydroxyapatite coating exposed to a solution of simulated body fluid (SBF) induced the formation of a calcium phosphate surface layer, which was chemically and crystallographically similar to bone apatite. This was in contrast to sand-blasted CP titanium, where a calcium phosphate layer was not formed. The results indicate that the formation of calcium phosphate layers on the surface of PSHA coated implants may contribute to their osteoconductive properties. Unfavorable conditions for the healing of implants such as gaps between the bone and implant and primary instability of the implant at the time of placement were overcome better by implants with PSHA coating than sand-blasted CP titanium implants. 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 the bone was affected less by primary instability of the implant than in the case of sand-blasted CP titanium surface. The sand-blasted 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 still observed for gaps with a depth of 0.3 mm,

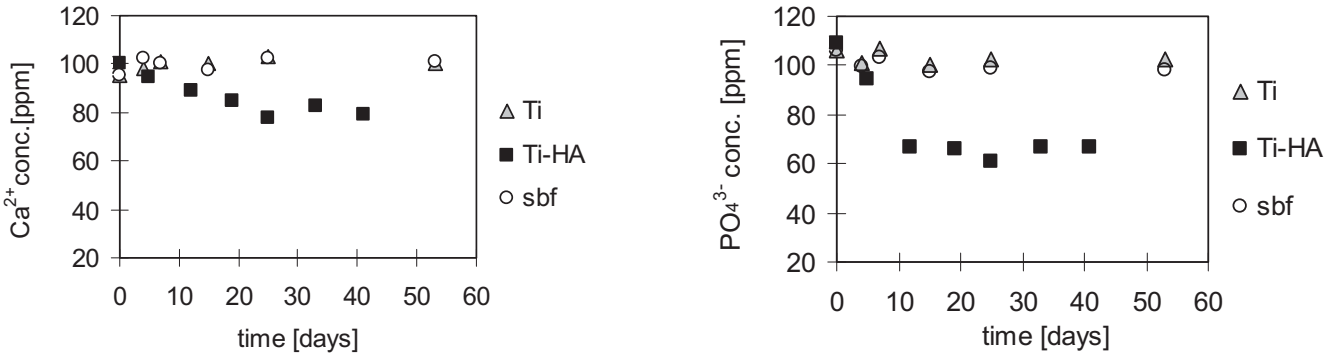


Fig. 3: Time dependence of changes in the concentration of calcium and phosphate ions in a simulated body fluid solution (SBF) for exposure of samples of pure titanium (△) and plasma sprayed hydroxyapatite (■). Values depicted as (○) correspond to the SBF blank.

Fig. 4: Electron microscope images (a) of the surface of a sample with plasma sprayed hydroxyapatite coating and (b) the surface of a sample with plasma sprayed hydroxyapatite coating covered with bone-like apatite spherulitic crystals after 7 days of soaking in SBF. Bar =100 um

with relatively good primary stability of the implant. Only the press-fit implantation model (model A) permitted osseo-integration of implants with sand-blasted CP titanium surface.

It is reasonable to assume that the observed difference in osteoconductivity of the tested surfaces is not associated with any difference in roughness, since the average roughness (R_a) of the sand-blasted surface was close to the interval of about 1-1.4 um, which is usually considered the most suitable for good metal-bone fixation (13). Unfortunately, the small number of implants used in the study was 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 histologically examined using light microscopy.

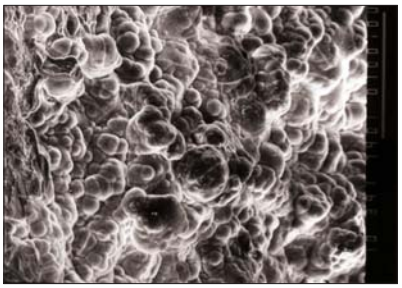


Fig. 5: Electron microscope image of a sample with plasma sprayed hydroxyapatite coating after 14 days of soaking in SBF showing a continuous layer of calcium phosphate agglomerates. Bar = 100 um

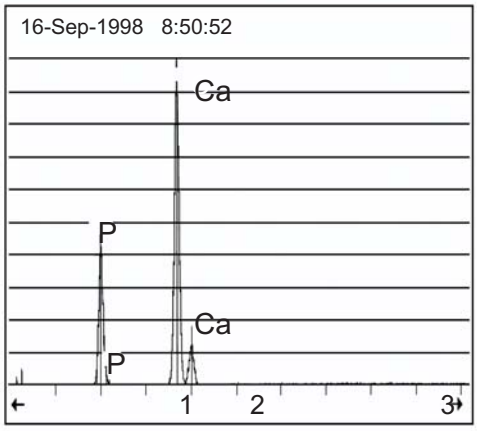


Fig. 6: SEM-EDS analysis of layers formed on the surface of a sample with plasma sprayed hydroxyapatite coating after 7 days of soaking in SBF

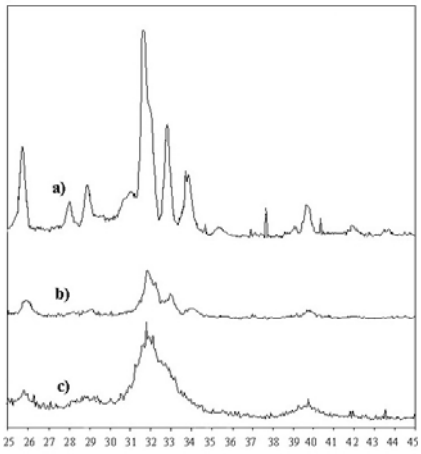
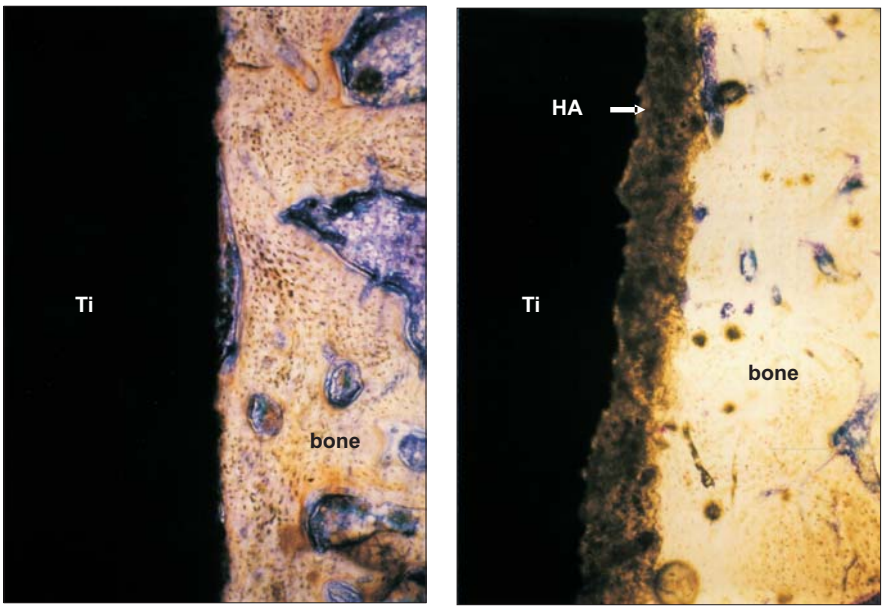


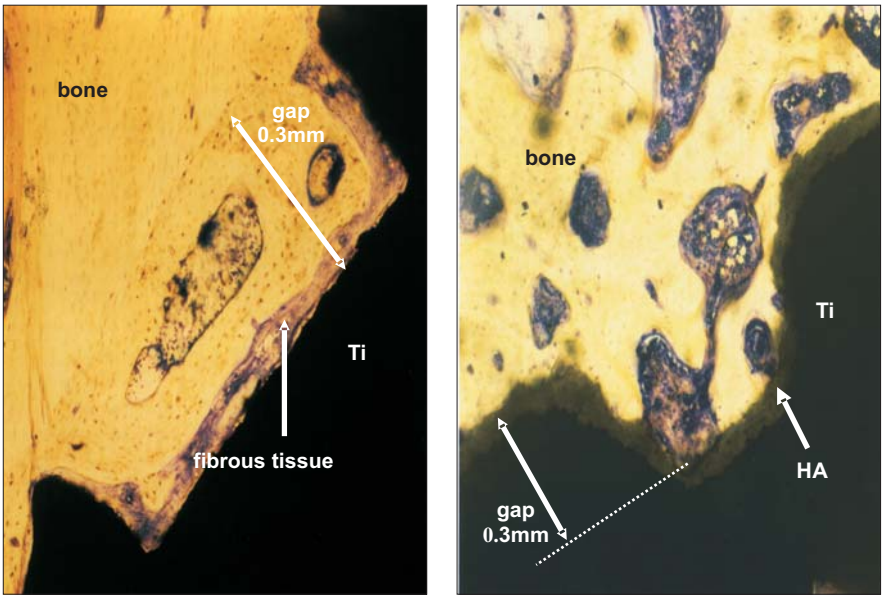
Fig. 7: Thin-film-X-ray diffraction patterns (a) of plasma sprayed hydroxyapatite. (b) plasma sprayed hydroxyapatite exposed to SBF for 14 days. (c) bone apatite

Fig. 8: Photomicrographs of the boundary between the bone tissue and

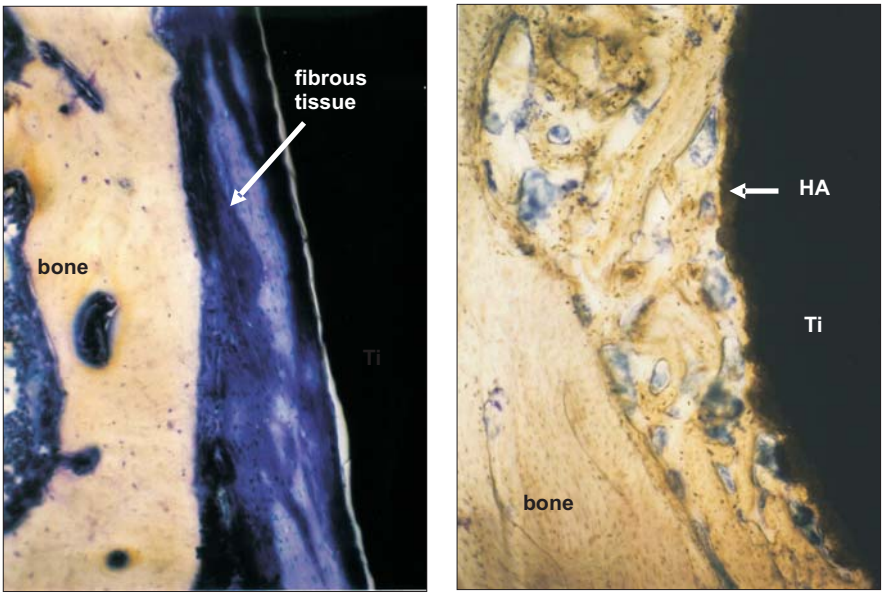
(a) a sand-blasted CP titanium implant
(b) a CP titanium implant with plasma sprayed hydroxyapatite coating - implanted by the press-fit procedure - model A (magnification 320x; toluidine blue stain)



(c) a sand-blasted CP titanium implant
(d) a CP titanium implant with plasma sprayed hydroxyapatite coating- implanted by the press-fit procedure with a 0.3 mm deep groove - model B (magnification 100x; toluidine blue)



(e) a sand-blasted CP titanium implant (magnification 320x; toluidine blue stain)
(f) a CP titanium implant with plasma sprayed hydroxyapatite coating (magnification 200x; toluidine blue stain) - non-press-fit implanted in the bone matrix - model C



Conclusion

This study demonstrated that plasma sprayed hydroxyapatite (PSHA) coated implants exhibit greater tolerance than sand-blasted CP titanium implants to unfavorable conditions during healing such as gaps at the interface or the primary instability of the implant. PSHA coated implants showed high percentage of bone contact if gaps were smaller than 1mm even when non press-fit insertion was used. In the case of sand-blasted CP titanium implants gap-filling with fibrous tissue was observed over the greater part of the surface of gaps with a depth of 0,3mm. Direct contact of sand-blasted CP titanium implants with bone was achieved only when press-fit implantation was used. It can be concluded that more precise surgical placement is needed for CP-titanium implants than for PSHA coated ones.

Acknowledgement

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Literature

1. Hench LL, Splinter RJ, Allen WC, Greenlee TK.: Bonding mechanisms at the interface of ceramic prosthetic materials, J. Biomed. Mater. Res. Symp., 1971;2:179-149.
2. Jarcho M, Kay JF, Gumaer RH, Drobeck HP.: Tissue cellular and subcellural events at bone-ceramic hydroxyapatite interface, J. Bioeng., 1977; 2:79-92.
3. Kokubo T, Shigematsu M, Naghashima Y.: Apatite wollastonite-containing glass-ceramic for prosthetic application, Bull. Inst. Chem. Res., Kyoto Univ., 1982; 60: 260-268.
4. Claes L, Wilke HJ, Kiefer H, Maschenmoser A.: Bone defect bridging with different implant materials. In: Yamamuro T, Hench LL, Wilson J, (eds). Handbook of Bioactive Ceramics. Vol.2, Florida, CRC Press, 1990:77-86.
5. Neo M, Kotani S, Nakamura T.: A comparative study of ultrastructure of the interfaces between four kinds of surface-active ceramic and bone, J.Biomed.Mater.Res. 1992; 26:1363-1370.
6. Li P, Kangasniemi I, de Groot K, Kokubo T.: Bonelike hydroxyapatite induction by a gel-derived titania on a titanium substrate, J Am. Ceram. Soc., 1994, 77: 1307-1312
7. Soballe K, Hansen ES, B-Rasmussen H, Pedersen CM, Bünger C.: Hydroxyapatite coating enhances fixation of porous coated implants. Acta Orthop. Scand. 1990; 61:299-306.
8. Carlsson L, Rostland T, Albrektsson B, Albrektsson T.: Implant fixation improved by close fit. Cylindrical implant-bone interface studied in rabbits. Acta Orthop. Scand. 1988;59:272-275.
9. Clemens JAM, Klein CPAT, Vriesde RC, Rozing PM, de Groot K.: Healing of large (2 mm) gaps around calcium phosphate-coated bone implants: A study in goats with a follow-up of 6 months, J.Biomed. Mater.Res., 1998;40:341-349
10. Clemens JAM, Klein CPAT, Sakkers RJB, Dhert WJA., de Groot K, Rozing P M. Healing of gaps around calcium phosphate-coated implants in trabecular bone of the goat, J.Biomed.Mater.Res. 1997; 36:55-64.
11. Clemens JAM, Klein CPAT, Dhert WJA, Rozing PM, de Groot K.: Bone Apposition on Apatite Coatings Inhibited by Large Gaps, Bioceramics , Vol 7, Andersson ÖH, Yli-Urpo A, Proceedings of the International Symposium on Ceramics in Medicine, Turku, Finland, July 1994.
12. Wennerberg A, Albrektsson T, Lausmaa J.: Torgue and histomorphometric evaluation of c.p. titanium screws blasted with 25- and 75-um-sized particles of Al2O3, J. Biomed. Mater. Res., 1996;30:251-260