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Microstructural analysis of implant-bone interface of hydroxyapatite-coated and uncoated Schanz screws

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The aim of the study was to compare the pin-bone interface microstructural characteristics of hydroxyapatite-coated (HAC) and stainless steel Schanz screws after 2, 4 and 6 months of implantation in a sheep model. The microstructure and composition of the hydroxyapatite coating were analyzed using scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis. Twelve coated and 12 uncoated screws were implanted into both femora of three sheep, each sheep receiving eight screws. Specimens of polished bone with screws were examined with SEM and light microscope for morphometric analyses. The HAC was approx. 40 μm thick, the grain size ranged from 5 to 40 μm, with pores less than 20 μm. The atomic ratio of Ca/P was 1.62. SEM showed that the bone-implant contact was better with HAC than with uncoated implants. The ingrowth of the bone in the HAC was clearly seen. Morphometric analysis showed good bone-implant contact in 65.1 ($\pm$24.6)% in the HAC and 32.0 ($\pm$23.3)% in the uncoated group ($p < 0.001$). Although the percentage of good contact increased with time for both groups, it was significantly higher for HAC screws. Our investigation demonstrated a time dependent improvement of implant-bone contact of the HAC compared to standard stainless steel implants in the chosen experimental conditions.

1. Introduction

Metallic implants are routinely used in orthopedic and dental surgery. Implant-bone loosening is the major concern of failure, regardless of whether the implants are used in a permanent or temporary way [1–5]. Metallic implants are fixed to the bone in different ways: using acrylic bone cement, using mechanical fixing with screws, or they are press-fitted [6]. Another concept is to combine mechanical forces with a bioactive surface, which enables ingrowth of the living bone tissue into the implant. The most important and frequently used material for bioactive coating is synthetic hydroxyapatite (HA), which is plasma-sprayed onto metallic implants [7].

Hydroxyapatite coating (HAC) on implants is used clinically mainly for orthopedic prostheses and dental implants [8–10]. Although most clinical trials showed promising results [9, 11–13], some trials have not demonstrated any significant advantage [14, 15]. The most important concerns about the use of HAC are: resorption and delamination, which can induce instability of the implant [7, 16], chemical and morphological changes of HAC caused by the plasma spraying procedure, which can influence its biological and mechanical properties [17], and HA particles producing wear of artificial joints [18]. The use of HAC for temporary implants in fracture surgery is still in the experimental phase. The most thoroughly studied are HAC pins for external fixation [19–21]) and, most recently, screws for plate fixation [22, 23]. Despite studies conducted on animals and some promising results from the first clinical trials [24, 25], HAC temporary implants are very seldom used in clinical practice. In addition to the listed concerns, fragmentation of the HAC is possible when threaded screws are inserted into the bone [19] and removal can also represent a problem [22].

The aim of the study was to analyze and compare the microstructure of the bone-implant interface of standard stainless steel and HAC Schanz screws after 2, 4 and 6 months of implantation period in a controlled experimental situation on a sheep model. The microstructural and morphologic characteristics of HAC were analyzed using scanning electron microscopy (SEM) and X-ray powder diffraction (XRD), and the microstructure of the bone-implant interface was then compared using SEM equipped with energy-dispersive X-ray

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spectroscopy (EDS) and optic microscopy with morphometric analysis.

2. Material and methods

2.1. Implants and operative procedure

After approval from the Ethical Committee of Slovenia, twelve HAC coated Schanz screws (regular Cr-based stainless steel 4.5 mm diameter screws coated with plasma-sprayed HAC, Cerasiv, Germany) and twelve standard Schanz screws (Cr-based stainless steel 4.5 mm diameter, Synthes, Switzerland) were inserted into both femora of 3 adult female sheep (aged 3 years, weight 35–40 kg). Sheep were used as the experimental animal because sheep bones are similar to the human bone in terms of inorganic and organic composition [26]. The operative procedure was performed with the use of general anesthesia according to a standard protocol. Each animal was premedicated with an intramuscular injection of Petidin 150 mg, atropine 1 mg and midazolam 40 mg, as well as with an intravenous administration of 350 mg of thiopental sodium. General anesthesia was maintained with the use of nitrous oxide and halothane under assisted ventilation and appropriate monitoring. Four implants were inserted into each femur of the hind limb. After incision of the skin, the entry point on the lateral cortex of the femur was isolated. Both cortices were predrilled with a 3.2 mm drill bit under constant cooling with 0.9% saline. The screws were inserted by hand using a standard "T" handle and cut approximately 2 cm above the outer cortex, and the skin was closed using Polisorb sutures. The positions of the screws were controlled with X-rays in both standard projections. There were no complications postoperatively and all animals returned to regular housing facilities after 24 h. The sheep were sacrificed 2, 4 and 6 months after the operation and radiographs of both femors in both projections were taken. Each radiograph was examined for the radiolucency zone as defined by Pettine *et al*. [27]. Before sacrifice, blood samples were taken for routine hematological and biochemical analysis.

2.2. XRD analysis

For X-ray powder diffraction analysis, the HAC was scraped from the screws. The X-ray diffraction pattern was recorded on Philips PW1710 powder diffractometer (Philips, Nederland) operating at 45 kV, 30 mA and 2 degrees 2Θ/min scan rate with Cu K_α radiation.

2.3. SEM analysis and optical microscopy

Polished specimens of the bone with a screw were examined with scanning electron and optical microscopy in JSM-840A scanning electron microscope (JEOL, Japan) equipped with Tracor TN 5600 EDS analytical system (Tracor, USA), JSM-5800 scanning electron microscope (JEOL, Japan) with Oxford Instruments ISIS 300 EDXS analytical system (Oxford Instruments, Great Britain) and in Olympus BX60 optical microscope (Olympus, Japan). For microscopic observations, the specimens were prepared in the following way: each bone segment containing the implant was isolated and fixed in a 10% formalin solution buffered at pH 7.2. Dehydration was performed in series of alcohols with decreasing amounts of water. Specimens were embedded in an epoxy resin and subsequently ground and polished in a medium of absolute ethanol. For SEM observations, the polished surface was sputtered by a thin layer of carbon in order to insure electrical conductivity.

2.4. Morphometric analysis

For morphometric analysis, which was performed blindly, the image-based analysis system IBAS 1000 (Contron, Germany) was used. Sixty-one interface areas (31 for coated and 30 for uncoated screws), which included the whole length of the screws in all cortices, were analyzed on photographs taken at 40 microscopic magnification. The distance between the implant and the bone, and the area of the gap between the implant and the bone per mm of the screw length were determined. The distance was measured perpendicularly to the screw surface three times at every 2 mm of the screw length. At least 30 measurements were taken and the mean distance between the cortical bone and implant was determined for each interface area. Good bone-implant contact was defined as the absence of any measurable distance between the bone and the screw, and a gap was defined as an area, in which bone and implant were not in the contact. The percentage of good contact between cortical bone and implant was determined by measuring the total length of the screw surface, and the length of the surface in which bone and implant were in contact and no gaps were present.

2.5. Statistical analysis

Statistical analyses were made with Sigma Stat Software. The interface values were compared by Student t-test and Mann-Whitney test. Probability values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Microstructural characteristics and composition of the HAC

The surface of the HAC was rough and porous and contained particles of complicated forms, mostly pancake-like or globular (Fig. 1). The pancake-like form was typical for most of the particles, which were laid down one on top of another. The size of these particles ranged from 20–40 μm, while round particles were below 10 μm. The pores that were observed in the coating ranged from a few to approximately 20 μm. In some parts of the coating, very thin fibres were observed, which were the residue of the solidification process of HA melted drops after hitting the surface during the process of plasma spraying.

The crystallinity of the HAC was established by XRD analysis. The XRD pattern of scraped HAC contained sharp lines, which corresponded to the known lattice

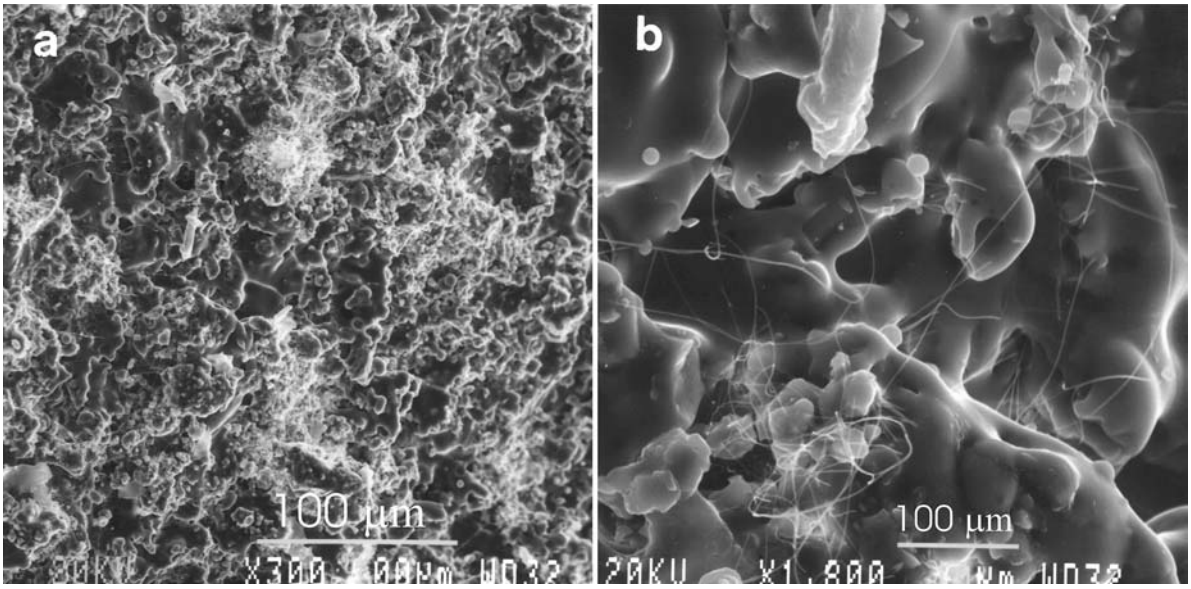

Figure 1 SEM micrographs of hydroxyapatite coating (HAC). (Magnifications: A—300, and B—1800).

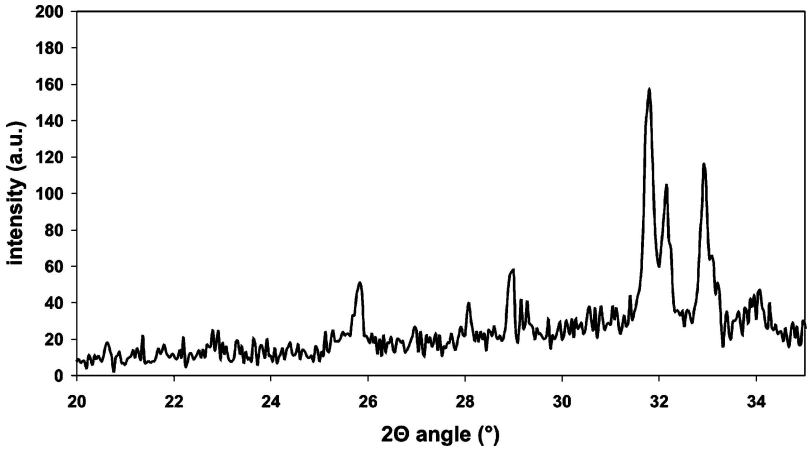

Figure 2 Part of XRD spectrum of plasma sprayed coating showing sharp diffraction peaks corresponding to well crystallized HA.

distances for $Ca_5(PO_4)_3OH$. In addition to these lines in the XRD pattern, there were a few lines of small intensities, which could not be indexed. Sharp lines and the absence of a diffuse increased background indicated that the HAC was well crystallized (Fig. 2).

The image of a polished cross-section of an HAC screw showed that the adhesion of the HAC film was very good (Fig. 3). No cracks were observed along the HA-metal interface. The thickness of the HAC was approximately 40 μm. In some regions, it was as thick as 60–70 μm and the coating was never thinner than 20 μm. The HA-metal interface was very rough due to prior sandblasting of the metal by SiC, which increased the adhesion of the plasma-sprayed HA film. EDS analysis of the HAC and the metal showed the presence of Ca and P in the HAC and Fe, Cr, Ni, Mo and Si in the metal. Quantification of the EDS spectra gave the results of the chemical composition of the HAC and the metal (Table I). The experimentally determined Ca/P ratio for HAC was 1.62, which was in very good agreement with the theoretical Ca/P ratio of 1.67 for hydroxyapatite. The metal used for the screws was a regular Cr-based stainless steel.

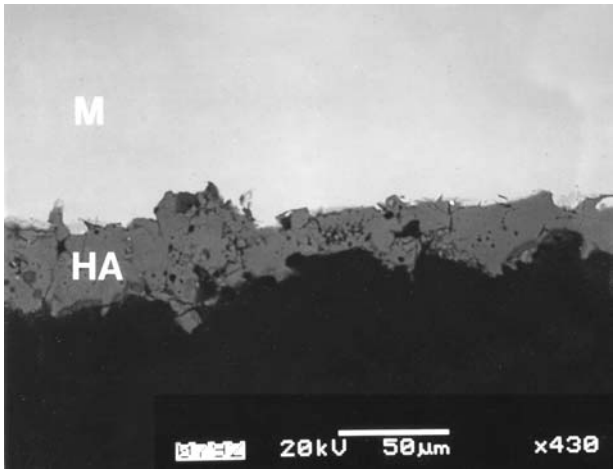

Figure 3 SEM micrograph of a polished cross-section of HAC Schanz screw. HA—hidroxyapatite, M—metal. No cracks can be observed along the interface.

3.2. Clinical and radiographic results

In the *in vivo* experiment, none of the pin tracts showed visual signs of infection as defined by soft tissue

TABLE I Results of EDXS semi-quantitative analysis

HA El.	Coating Conc. (wt%)	HA El.	Metal Conc. (wt%)
P	19.14 ± 0.02	Si	0.42 ± 0.02
Ca	40.13 ± 0.05	Cr	18.60 ± 0.03
O	40.73 ± 0.05	Fe	63.40 ± 0.15
Ca/P	1.62 ± 0.08	Ni	14.80 ± 0.03
		Mo	2.78 ± 0.04

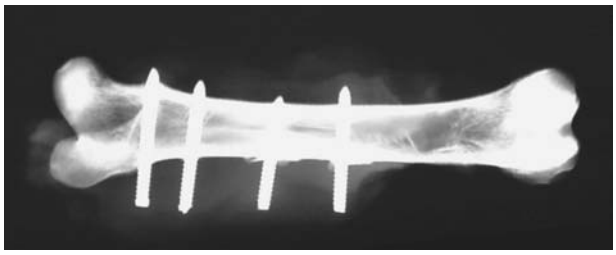

Figure 4 Radiograph of HAC screws 6 months after implantation showing modest periosteal reaction at the entry cortex of distal two screws.

swelling or exudates. After two months, no obvious sign of pin loosening were seen on the radiographs. After 4 months, no obvious signs of gross osteolisys around the screws were seen. On the radiograph taken 6 months after the implantation, a modest periostal reaction at the entry cortex of two coated screws was seen (Fig. 4) and a slight rarefaction of the entry cortex around one uncoated screw. All of the areas of rarefaction described were less than 0.5 mm wide. At the time of sacrifice, the blood and routine biochemistry values of all tested animals were within the normal range.

3.3. Microstructural characteristics and EDS analysis of the bone-implant interface

SEM micrographs of HAC implant-bone and uncoated implant-bone interfaces after 2, 4 and 6 months of implantation are shown in Fig. 5. The contact between the bone and the implant was better for the HAC implants than for the uncoated implants.

SEM and EDS analysis of the bone-uncoated implant interface 2 months after the implantation showed that the bone tissue had suffered severe osteonecrosis during the implantation (Fig. 6(a)). The EDS spectra taken from that region demonstrated the presence of bone, organic tissue and metallic debris from the screw (Fig. 6(b)), while the EDS analysis of the bone demonstrated only the presence of Ca, P and O (Fig. 6(c)). However, the bone-implant contact of uncoated screws after 4 and 6 months was better. Amorphous deposits on the uncoated screw were observed on the interface by high magnification SEM in the samples taken 6 months after implantation. EDS analysis indicated that these deposits were composed mostly of Ca and P.

SEM investigations of the bone-HAC implants interface 2 months after the implantation showed a different morphology to that of the uncoated ones. Usually, the interface was straight, with excellent contact. However, in some regions, an ingrowth of bone and HAC was clearly observed (Fig. 7). The ingrowth process was usually initiated along the grain boundaries of polycrystalline HA, which was manifested in the revelation of discrete HA grains in the coating. After 4 months, the HAC was partially resorbed and occasionally flaked off the implant. SEM of the coated implant samples after 6 months showed important changes to the morphology of the interface. The HAC between bone and implant was almost completely resorbed, but the bone-implant contact was mainly preserved (Fig. 8). Remains of the HA were mostly ingrown in the bone. High magnification SEM of these areas showed partially well preserved HA grains, which smoothly proceeded in the bone tissue (Fig. 9). The HAC was thinner but preserved in the medullary canal. A continuous thin layer of bone originating from both cortices was attached to the entire thread length of the screw in this region. HAC exposed to soft tissue was almost completely resorbed.

3.4. Morphometric analysis

Overall, the average of good contact between bone and implant was 65.1 (±24.6)% for HAC implants and 32.0 (±23.3)% for uncoated implants ($p < 0.001$). The percentage of bone-implant contact for uncoated screws was 9.5 (±21.3)% after 2 months, and increased to 36.3 (±17.5)% after 4 months and to 50.9 (±26.4)%

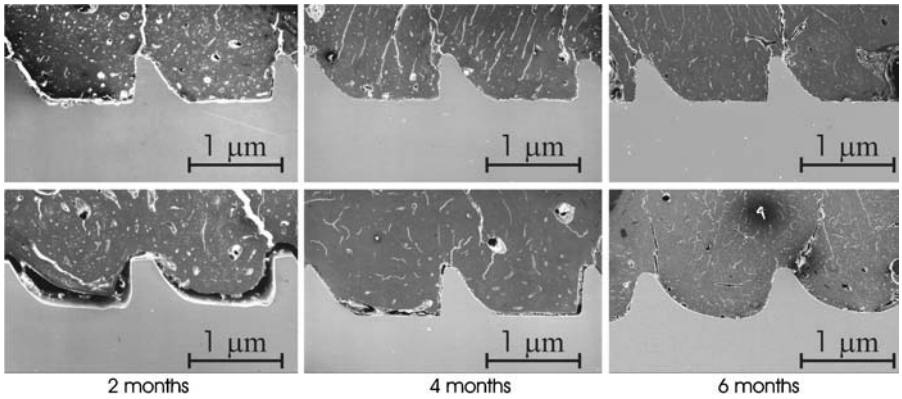

Figure 5 A polished cross-section of HA-coated and uncoated Schanz screw/bone interface after two, four and six months after implantation. Note the good contact between the bone and the implant of HAC screws (top row) compared to the uncoated (bottom row).

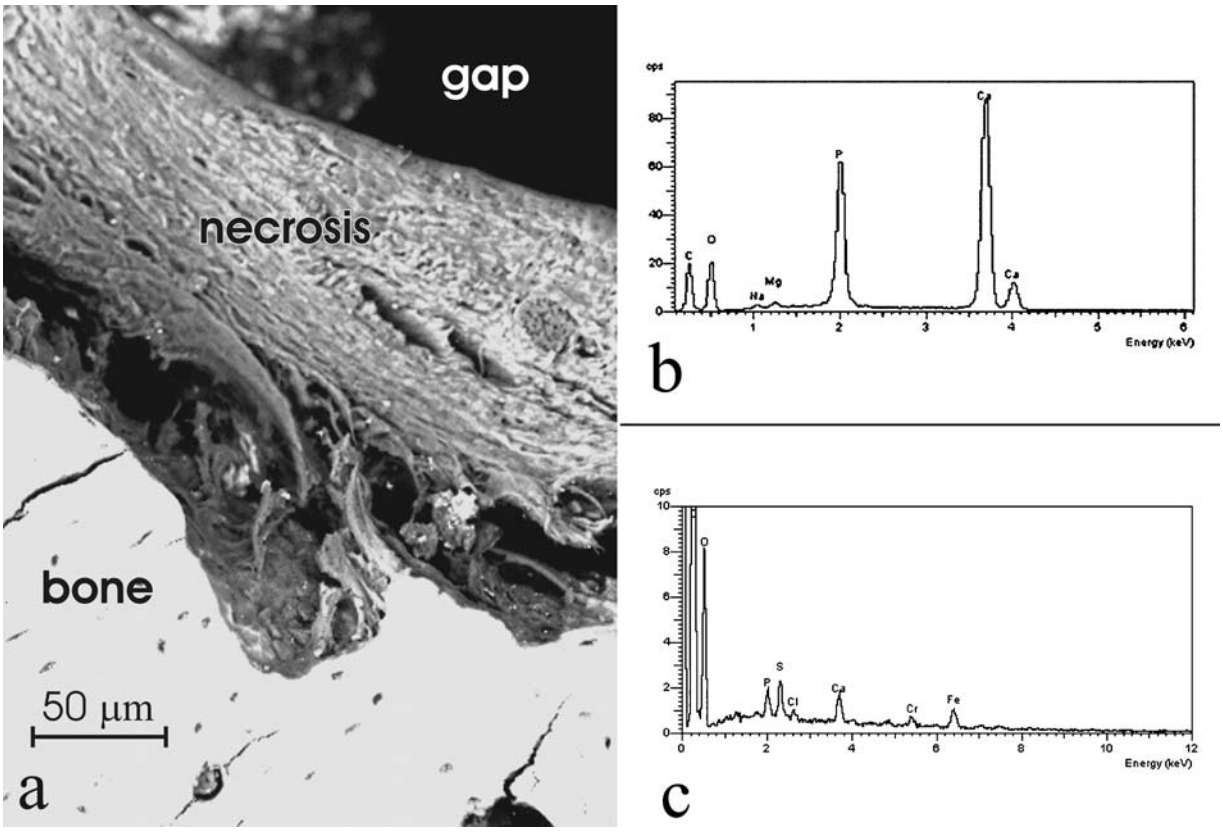

Figure 6 (a) SEM micrograph of bone adjacent to the gap (Mag.: 500). (b) EDS spectrum from bone showing major bone elements. (c) EDS spectrum acquired from osteonecrotic zone showing characteristic elements present in bone, organic tissue and metallic debris.

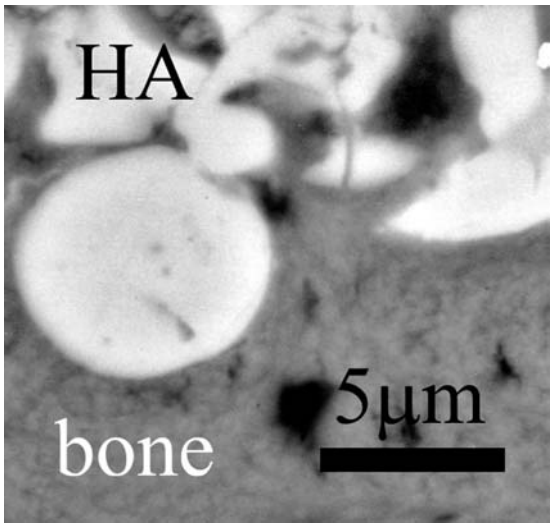

Figure 7 SEM micrograph of ingrowth of bone tissue within HA coating after 2 months.

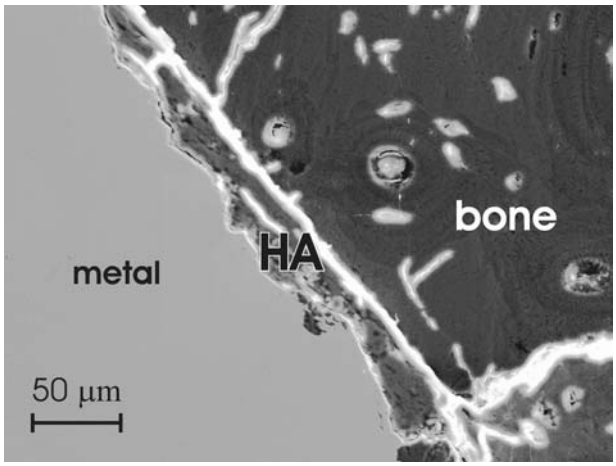

Figure 8 SEM micrograph of partially preserved HAC after 6 months. (Mag.: 430).

after 6 months. Differences between 2 and 4, and 4 and 6 months were statistically significant ($p < 0.05$). The same was also observed for the HAC group: the percentages of good contact were 42.2 (±29.9)%, 61.6 (±13.8)% and 85.5 (±7.9)% after 2, 4 and 6 months, respectively (Fig. 10). The difference between 4 and 6, and 2 and 6 months was statistically significant ($p < 0.001$). The differences of percentage of good contact between coated and uncoated screws after 2, 4 and 6 months were also statistically significant ($p < 0.008$).

4. Discussion

4.1. Microstructural characteristics and composition of HAC

Plasma-spraying decreases the crystallinity and purity of the HAC due to its decomposition at high temperature followed by rapid cooling [7, 17]. This can affect the biological and mechanical behaviour of the HAC in terms of its resorption, long-term stability and bone ingrowth [28]. However, our XRD results demonstrated high crystallinity of the investigated sample. Coatings with high crystallinity are more stable in the biological environment. Chang *et al*. [29] demonstrated a 16% decrease of high crytallinity HAC and 24% decrease of

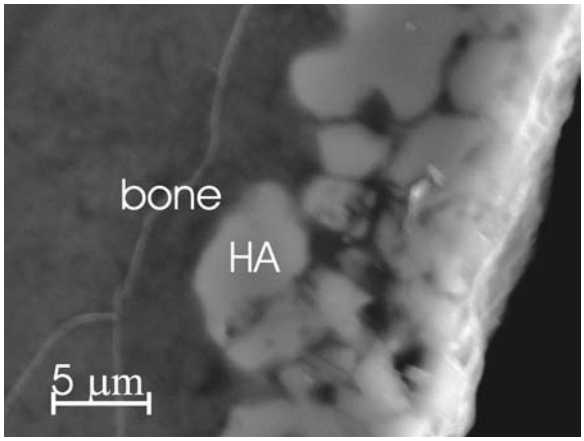

Figure 9 SEM micrograph of preserved HA grains which proceeded into the bone. (Mag.: 5000), 6 months after implantation.

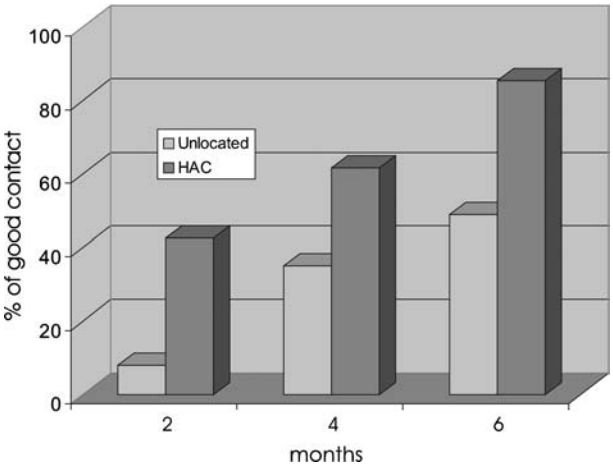

Figure 10 Percentages of good contact (%) between bone and screw in HA-coated and uncoated screws 2, 4 and 6 months after operation.

low crystallinity coating after 26 weeks in an animal model, without any significant difference in terms of bone ingrowth and pull out force. The measured Ca/P ratio of our sample was 1.62, which is very near to the theoretical ratio for HA of 1.67, indicating correct stoichiometry of the tested sample. There is also general agreement that the chemical purity of the HAC should be as high as possible [7].

In our case, the thickness of the HAC was approx. 40 μm, which is near the "optimal" thickness of 50 μm reported by de Groot *et al*. [30]. Wang *et al*. [31] proved experimentally that a 50 μm coating exhibits significantly higher shear strength than a 200 μm coating.

The surface of the examined HAC was rough and porous. With pore sizes around 20 μm, our sample was between microporous (pore size approx. 5 μm) and macroporous (pore size above 200 μm). Microporosity is important for resorbility and macroporosity for bone ingrowth [32]. In the study by Augat *et al*. [19], screws with higher porosity coating needed higher torque for extraction.

SEM microscopy of coated screws demonstrated very good contact between the HAC and the screws. The firm contact of the HAC and the screw was also demonstrated with SEM observation after 2 months. In contrast to the study by Augat *et al*. [19], no broken coating particles were found at the bone-implant interface. Quantitative EDS analysis of stainless steel demonstrated that the weight percentages of Si, Cr, Ni and Mo in the alloy corresponded to ISO-1 Composition D standard for implant stainless steel [33].

4.2. Microstructural characteristics and EDXS analysis of the bone-implant interface

SEM observation of the bone–uncoated implant interface 2 months after implantation demonstrated widely extended gaps, mainly filled with fibrous tissue and bad bone-implant contact. Fibrous tissue at the interface is usually interpreted as a sign of loosening [21, 34]. EDS analysis of the interface indicated the presence of organic tissue and metallic debris from the screw, while EDS of the same region of coated screws did not show any metallic remnants. Chang *et al*. [35] examined HAC titanium implants in an animal model and made similar observations. It seems HAC can act *in vivo* as a barrier against the release of metallic ions from the implant. Sousa and Barbosa [36] described this effect of HAC on the basis of *in vitro* experiments. SEM examination of the uncoated screws interface after 4 and 6 months showed a similar situation as after 2 month, although the contact of the bone and implant was better. After 6 months, irregular shaped deposits were observed on the threads of the screws in the gap. EDS analysis revealed that they were composed mainly of P and Ca. We were not able to elucidate the meaning of these precipitates. As far as we know there have been no reports on systematic examination of the interface of stainless steel screws by SEM and EDS.

SEM of the bone-HAC implant interface after 2 and 4 months indicated good contact with the bone. Ingrowth of the bone in the HA grains was clearly seen as a narrow mineralized layer within intercrystalline spaces. This layer of ingrown bone and HAC can have better mechanical properties than the HAC itself [37]. After 4 months, SEM demonstrated some cracks and delaminations of the coating, which was also thinner. The bone-implant contact seemed well preserved. After 6 months, the HAC almost completely disappeared. Higher magnification revealed solitary areas of HAC completely ingrown within the bone, which was basically in good contact with the denudated implant. Resorption of HAC is regarded as an important problem, which can lead to deterioration of the fixation [7]. The resorption of the coating over time is hard to define. Studies, that have analyzed the HAC on coated endoprostheses retrieved *post mortem*, have demonstrated obvious resorbtion of HAC after several months to several years [34, 38, 39]. Reports on time-dependent resorption of HAC under experimental conditions are also not uniform. Moroni *et al*. [23] observed good contact and only slight resorption of HAC after six months, while in a study by Hemmerle *et al*. [6] it was only partially preserved. A likely reason for the slow dissolution of HAC is remodelling of the bone. Resorbed HAC can be replaced by bone *in vivo* [40]. Tonino *et al*. [41] analyzed the interface between HAC endoprostheses and bone retrieved

post mortem. They found separate areas of HAC that were completely incorporated in the bone. The bone was also in good contact with denudated metal parts of the implant. Our results indicated a similar situation. We also observed that the resorption of the coating was very intense in contact with soft tissue and very weak in the medullary cavity. HAC was clearly seen in the medullary canal and was entirely covered with a thin layer of bone. The same was observed by Moroni *et al*. [22].

It is not clear whether the bone layer covering the screw in the medullary canal is of any importance for the stability of the implant, although it demonstrates the bone conductivity properties of HAC. However, gradual resorption of the HAC does not present a serious problem with temporary use of implants. The strength of the bone-implant interface in the initial period, when the stability of the bone fragments is the weakest, is the most important. After healing of the bone, the implant is no longer mechanically loaded. Possible gradual and controlled weakening of the interface due to HAC resorption after complete healing of the bone, can therefore even facilitate the removal of the implant.

4.3. Morphometric analysis

Morphometric analysis of the implant-bone interface clearly demonstrated a better contact of HAC implants in all observed time periods. This has also been demonstrated by other studies irrespective of loaded or unloaded experimental conditions and substrate material [21, 23, 42, 43, 45]. The percentage of good contact 2, 4 and 6 months after implantation increased for both uncoated and coated implants. These differences were statistically significant for both groups of implants, as well as between the HAC and uncoated groups. Ingrowth progressed more or less steadily with time in both groups. Rocca *et al*. [45], who compared titanium-coated and uncoated implants, made a similar observation. They demonstrated that the ingrowth process was finished after 9 months, when the contact percentage was higher than 95%. Moroni *et al*. [21] described better contact after 1 and 3 months only for HAC implants, while the contact for uncoated titanium implants was even worse after 3 months. We consider the difference in bone contact between the two groups after 2 months (9.5% for uncoated versus 42.2% for HAC) to be very important. In clinical practice, the initial period of fracture fixation is mechanically most unstable and implants need strong contact with bone. Chang *et al*. [29] studied the interface after 1, 4, 12 and 26 weeks and observed that the percentage of good contact for HAC implants increased most intensively between 1 and 4 weeks. The percentage of good contact is in good correlation with the firmness of the bone-implant interface, which can be measured by extraction torque or pull out force [21, 27, 45].

In conclusion, our study demonstrated better contact with bone for HAC implants compared to standard stainless steel implants. The contact is essentially a time-dependent process for both groups. Plasma-sprayed HAC metal implants should be considered for clinical practice in fracture surgery.

Acknowlegments

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