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A histological evaluation of the effect of hydroxyapatite coating on interfacial response

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The effect of hydroxyapatite (HA) coating on cortical bone apposition around press-fit inserted implants and implants surrounded by a gap was investigated. Uncoated and HA-coated titanium implants were inserted in burr holes with three different diameters in the tibia of rabbits. Implantation time was four months. The histological evaluation demonstrated that after four months implantation the interfacial bone reaction appeared to be identical for HA-coated and non-coated implants with various degrees of surgical fit. Although after four months the interface showed the same response, there still might be an initial advantage of the HA-interface with bony tissue.

1. Introduction

Hydroxyapatite (HA) ceramic, $Ca_{10}(PO)_4(OH)_2$, is used as implant material both in its bulk form as well as thin coating on metals. The continually increasing use of HA for surgical applications is based on its biocompatible and osteoconductive behaviour. In various *in vitro* and *in vivo* experiments it has been demonstrated that owing its close resemblance to the mineral phase of bone [1], HA ceramic surfaces can achieve direct bonding with bone [2–7]. The osteoconductive properties of HA imply that, when implanted in skeletal tissue, new bone formation is guided along the HA surface, promoting bone growth into areas that it otherwise would not occupy [8–10]. For example, the use of HA as autogenous bone graft in reconstructive surgery is based on this property. On the basis of the above-mentioned favourable tissue characteristics, it is claimed that the use of HA might allow for less-critical surgical requirements than other implant materials, especially with regard to the fit of the implant [11, 12]. It is suggested that the bone response with HA implants is enhanced, resulting in a faster bone ingrowth [13] and bridging over a gap towards an implant [14]. However, there are some inconsistencies in the literature. In other comparative studies, no proof was found for the superior behaviour of HA. It has even been suggested that titanium may have the same beneficial osteoconductive and bone bonding properties [15].

Therefore, the purpose of this study was to investigate by histological analysis the influence of implant fit on the interfacial bone response to non-coated and HA-coated titanium implants.

2. Material and methods

2.1. Implants

For the experiments, cylindrical $TiAl_5Fe_{2.5}$ alloy implants were used. The implants were left uncoated or a HA-coating was applied. All implants measured 10 mm in length. The uncoated titanium cylinders had a diameter of 2.8 mm and those to be coated initially had a 2.7 mm diameter. All implants were grit-blasted to a roughness of $R_a = 4$–5 μm. They were cleaned ultrasonically in propanol, and dried at 100 °C. The implants with a diameter of 2.7 mm were coated with an approximately 50 μm thick layer of hydroxyapatite using a plasma-spraying technique. Therefore, the final diameter of all implants was 2.8 mm. The apatite powder used in the coating process had a particle size between 1 and 125 μm. The chemical composition and purity of this coating has been described earlier [16] and is shown in Fig. 1. After plasma-spraying the implants were cleaned ultrasonically in 100% ethanol to remove loose particles, and then dried. All implants were sterilized in an autoclave.

2.2. Animal model and implantation procedure

Five three month old female New Zealand White rabbits (weight 3 kg) were used in this study. They were sedated by intramuscular injection of fluanison/fentanylcitrate (Hypnorm, Duphar, Amsterdam). Additionally, local anaesthesia was achieved by subcutaneous administration of Lidocain.

The implants were inserted into the left and right tibial diaphysis of the rabbits. For the insertion of the

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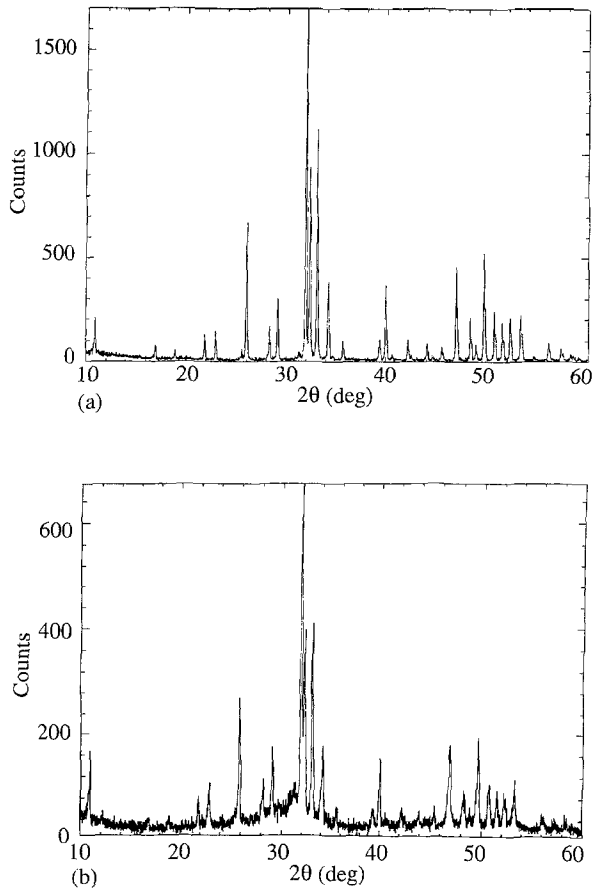

Figure 1 X-ray diffraction pattern of hydroxyapatite. (a) Starting powder, (b) coating with particle size 1–125 μm.

implants, each animal was immobilized on its back. Using standard surgical techniques [17] a longitudinal incision was made on the medial surface of the tibia and the bone was exposed by blunt dissection. Pilot holes, 1 mm, were drilled through the medial cortex, the medulla and the lateral cortex of the tibia. The holes were gradually widened with drills. On the lateral side the holes were drilled to the final diameter of the implant to ensure firm fixation of the implants. On the medial side, burr holes with three different diameters were created: diameter A 2.8 mm, diameter B 3.0 mm, and diameter C 3.3 mm (Fig. 2). The bone preparation was performed with a very gentle surgical technique using low rotational drill speeds (maximum 450 r.p.m.) and continuous internal and external cooling. After the drilling, the implants were inserted. Diameter A implants had a perfect fit in the medial cortical defect with a direct implant–bone contact. Diameter B implants showed a gap of 0.1 mm between the medial cortical bone and the implant surface, and with the diameter C implants, there was a gap of 0.25 mm. Following the instalment of the implants, the soft tissues were closed in separate layers using resorbable sutures (Dexon 3–0). Finally, the position and fit of the implants was confirmed radiographically. A total of 30 implants were placed: five HA-coated diameter A, five HA-coated diameter B, five HA-coated diameter C, five $TiAl_5Fe_{2.5}$ diameter A, five $TiAl_5Fe_{2.5}$ diameter B, and five $TiAl_5Fe_{2.5}$ diameter C implants. Each animal received six implants, three in the left and three in the right diaphyseal part of the

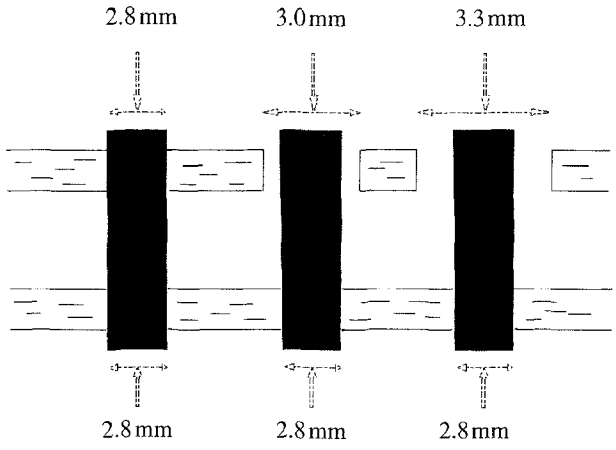

Figure 2 Schematic drawing of the surgical procedure.

tibia. Evaluation of the bone–implant interface was planned after an implantation period of four months.

Post-operatively the animals were placed in standard rabbit cages, were provided with water and rabbit chow ad libitum and were allowed to move unrestricted at all times. Throughout the duration of the experiment, at regular time intervals, radiographs were taken of each tibia to assess the healing progress.

2.3. Histological procedure

At the predetermined endpoint of the experiment the animals were killed by injecting Nembutal peritoneally, the tibiae with their surrounding tissues were excised and the excess tissue was removed immediately. Following fixation of the tibiae in a 10% buffered formalin solution, the specimens were prepared for histological processing. They were sectioned in three pieces, each one with one implant. These tissue blocks were embedded in methylmethacrylate. After polymerization, non-decalcified thin (10 μm) sections were prepared using a modified diamond blade sawing microtome technique [18]. The sections were made in a transverse direction perpendicular to the axis of the implant. These sections were stained with methylene blue and basic fuchsin and were examined with a light microscope. In addition, the percentage of implant–bone contact at the medial cortical interface was determined. The amount of bone contact was measured with an image analysis system and was defined as the percentage of implant length at which there was direct bone-to-implant contact. The measurements between implant types and implant techniques were statistically evaluated using a one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman–Keuls).

3. Results

In all cases healing was uneventful. At the time of the sacrifice the surgical sites showed no macroscopical signs of infection. The radiographs demonstrated no signs of peri-implant radiolucensies; in fact, around most implants new bone was formed at the endosteal surface of the medial cortex (Fig. 3).

Light microscopical analysis confirmed these findings. No striking differences were observed in histo-

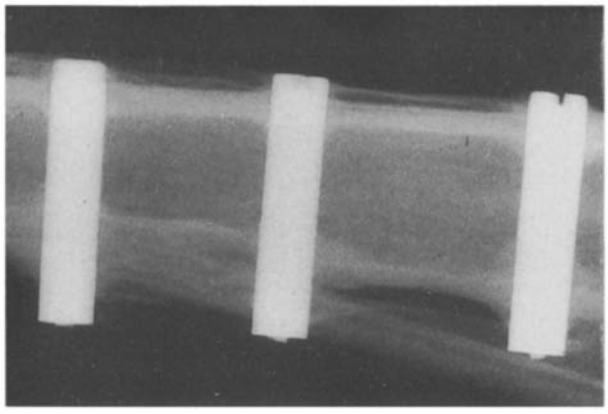

Figure 3 Radiograph taken four months after implantation of the tibia implants.

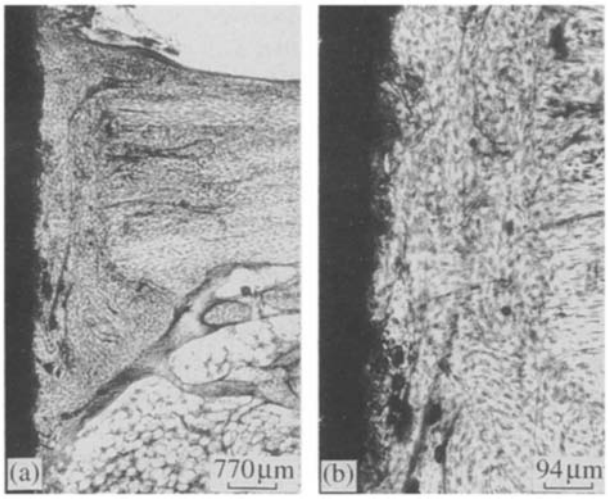

Figure 4 Histological section of a HA-coated diameter C implant, four months after implantation. Light micrographs demonstrate the direct contact between bone and implant surface. The gap around this non-interference fit implant is completely filled with newly formed bone. Original magnifications (a) × 13, (b) × 106.

logical reaction to the various implants. After 17 weeks, almost all uncoated and HA-coated implants had a direct bone-to-implant contact at the level of the medial cortical passage. The gaps around the non-interference fit implants were filled with newly formed bone. Occasionally, the edges of the original cavity were still clearly visible (Figs 4 and 5). In the areas where new bone was formed, mature osteocytes could be observed, but also remodelling zones with osteoblasts and osteoclasts. A similar remodelling activity was seen at the bone–implant interface of the press-fit samples. Around one HA-coated diameter A, one HA-coated diameter C and one titanium diameter B implants, an intervening fibrous tissue layer was present between the edge of the cortical bone and the implant surface (Fig. 6). This gap was exclusively present on one side of the implant; at the other side there was always a direct bone-to-implant contact.

Microscopical evaluation also revealed a moderate reduction of coating thickness for all HA-coated implants. Only the implants surrounded on one side by a fibrous tissue layer showed a complete loss of coating in this non-bone contacting area.

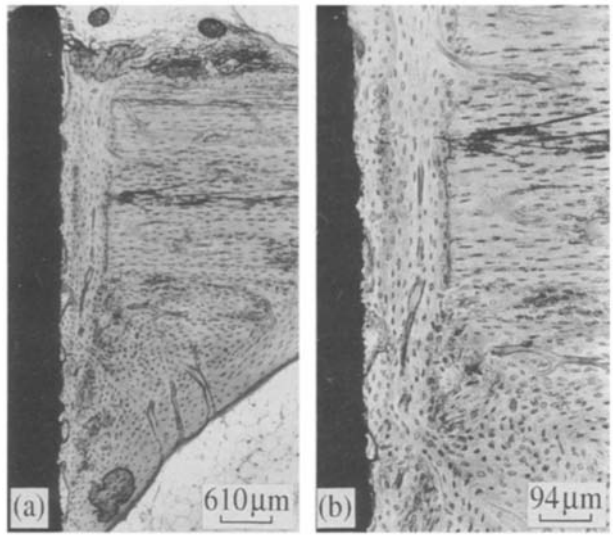

Figure 5 Titanium-diameter C implant–bone interface. Direct bone apposition is observed. Notice that the edges of the original cavity are still clearly visible. Original magnifications (a) × 16, (b) × 106.

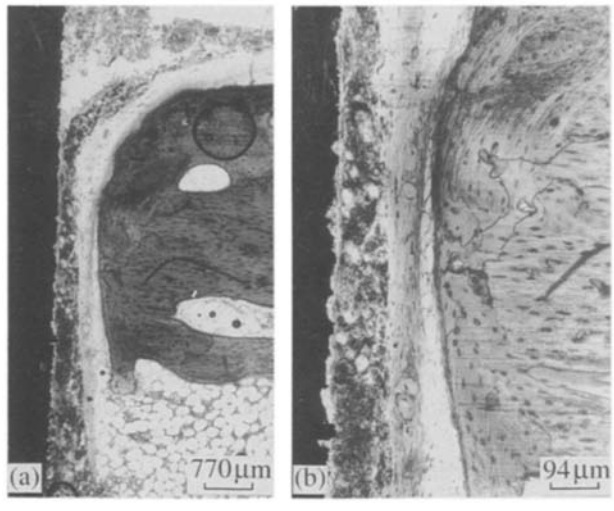

Figure 6 Histological section of a HA-coated diameter C implant, four months after implantation. A wide gap with interposed soft tissue between the cortical bone and implant surface can be seen. Original magnifications (a) × 13, (b) × 106.

The results of the quantitative determination of the percentage of bone contact to the implants are listed in Table I. Although the HA-coated implants, either inserted press-fit or non-interference fit, always showed a slightly higher bone apposition than the titanium implants, statistical testing revealed that this difference in bone apposition was never statistically significant ($P > 0.05$).

4. Discussion and conclusions

The aim of this study was to evaluate the effect of implant fit on cortical bone reaction to non-coated and HA-coated titanium implants.

The histological and quantitative evaluation demonstrated that there are no differences in bone behaviour and speed of gap healing around the uncoated and HA-coated implants with various degrees of surgical fit. These results are very consistent with

TABLE I Amount of bone contact (%) to the implants

Material	Press-fit	Non-interference fit	
	2.8 mm	3.0 mm	3.3 mm
HA	66.37 ± 18.78 (n = 5)	76.42 ± 20.47 (n = 5)	75.05 ± 29.64 (n = 5)
Ti	59.74 ± 16.49 (n = 5)	65.40 ± 14.14 (n = 5)	66.78 ± 15.49 (n = 5)

our previously performed studies [16, 19] using the same animal model and implantation model. Our observations also strongly corroborate with the findings of Ducheyne *et al.* [20], Cook *et al.* [13], Rivero *et al.* [21] and Oonishi *et al.* [22]. Ducheyne *et al.* [20] inserted porous HA-impregnated and non-impregnated implants for two, four, and 12 weeks into the femora of dogs. Mechanical testing of the fixation of the implants into the bone and measurement of the amount of bone ingrowth into the pores demonstrated that at 12 weeks there is no significant influence of HA-impregnation on the strength of the interfacial bond and bone apposition. Cook *et al.* [13] placed HA-coated porous titanium and uncoated porous titanium implants into the femora of dogs and evaluated after periods of three, six, and 12 weeks. After 12 weeks implantation, both implant types showed equal amounts of bone ingrowth. Rivero *et al.* [21] inserted porous uncoated and HA-coated titanium fibre implants into the humeri of dogs for periods of one, two, four, and six weeks. Quantitative measurement of the bone ingrowth did not reveal a significant difference in the volume of bone ingrowth between the implants at all time periods. Oonishi *et al.* [22] examined the bone behaviour to porous uncoated and HA-coated implants placed into the tibia of goats. The bonding strength of the implants with bone was measured at two, four, six, and 12 weeks. Twelve weeks after implantation there was no difference in bond strength between the various implants. By combining these observations with our findings, it may be assumed, according to the suggestion of Gerner *et al.* [23], that the application and benefit of HA-coatings is especially based on an initial increase of bone ingrowth.

Further, the results of this study do not support the findings of Carlsson *et al.* [24] and Soballe *et al.* [14] who performed an identical experiment. Carlsson *et al.* [24] found that gaps of 0.35 and 0.85 mm around stable, smooth titanium implants were not bridged by bone. He concluded that the critical gap between bone and implant, which prevents direct bone apposition on the implants, is close to zero. Soballe *et al.* [14] investigated the influence of HA-coating on the bone response to press-fit and non-interference fit inserted implants four weeks after implantation into the femora of dogs. The non-interference fit implants were surrounded by a gap of 1 mm. Histological evaluation demonstrated a significant increase in the amount of bone in direct contact to HA-coated implants compared with uncoated implants inserted both in gap and press-fit. He concluded that HA-coating eliminates the influence of surgical fit on the skeletal fixation of an implant. A possible explanation for this discrepancy in results between their studies and our study is that Carlsson *et al.* [24] and Soballe *et al.* [14] created two to three times larger gaps around the implants. Therefore, apparently the conclusion of Carlsson *et al.* [24] that the critical gap to be bridged approaches zero, needs to be corrected. In addition, it should be noticed that Carlsson *et al.* [24] used a smooth titanium implant. However, the use of sand-blasted instead of smooth-surfaced implants will also increase the bone contact [25].

In conclusion, the use of HA is based on its ability to form a direct bond with newly formed appositional bone. However, considering the results of this study, the histological appearance after four months implantation appears to be identical for HA-coated and non-coated implants with various degrees of surgical fit. Nevertheless, because we created only minor gaps around the implants and evaluated the bone–implant contact only after an implantation period of four months, it might still be possible that HA-coatings may have an initial effect by more rapid bone formation. Therefore, further experiments should be performed with implants inserted in a gradient series of burr holes and with variable implantation periods.

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