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Bone ingrowth behaviour of hydroxyapatite-coated, polyethylene-intruded and uncoated, sandblasted pure titanium implants in an infected implantation site: an experimental study in miniature pigs

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We studied the dynamics of bone tissue ingrowth into the pores of hydroxyapatite-coated (plasma-spraying technique) and uncoated wire meshes of pure Ti in an infected implantation site. Samples of the test materials were implanted into the femora of 15 adult Göttingen minipigs. Just before implantation they were contaminated with *Staphylococcus aureus*. The pigs were killed after 4, 8, 12 or 24 weeks. Undecalcified ground sections of bone tissue were prepared and stained with toluidine blue for comparative histological evaluation. The hydroxyapatite-coated implants already demonstrated advanced new bone formation after 4 weeks. By 12 weeks most of the implant pores were filled with newly formed bone although all samples showed macro- as well as microscopic signs of persistent infection. Comparable reactions of the uncoated implants could be observed only after 24 weeks. Signs of degradation of the hydroxyapatite coating were seen in contact with soft tissue. This was more extensive in the infected than in the uninfected site. The results and possible clinical consequences are discussed.

1. Introduction

During the past decade hydroxyapatite-coated implant materials have been the object of numerous experiments [1–5]. Experimental studies have shown the absence of toxicity, antigenity and carcinogenity [4]. Furthermore, a direct osteogenesis on hydroxyapatite surfaces has been reported [6]. These experimental results have been confirmed by the clinical application of hydroxyapatite-coated implants [7, 8]. Nevertheless, disintegration of hydroxyapatite coatings and loosening of some of these implants occur and are not entirely understood. This is of particular interest in the case of sepsis at the implantation site, and poses a number of questions discussed below. In the course of an earlier experimental study in our department we observed that, in spite of incidental infection of hydroxyapatite-coated implants, osseointegration was only slightly delayed [9, 10]. This unexpected finding initiated the following controlled experiment using a standardized pig infection model.

2. Materials and methods

Three different modifications of a four-layer sintered pure titanium (ISO 5832-2) wire mesh (wire diameter: two outer layers 0.5 mm, two inner layers 0.3 mm) were tested (Fig. 1).

1. Uncoated Ti wire mesh.
2. Ti wire mesh coated with an hydroxyapatite-ceramic by the plasma-spraying technique (average thickness approximately 100 μm, maximum thickness approximately 200 μm, purity > 98%, high crystallinity and approximately 20% microporosity) [11].
3. Ti wire mesh (one additional layer, 0.5 mm) with a unilateral coating of ultra-high molecular weight polyethylene (UHMWPE) RCHR-1000. The polyethylene surface was characterized by the surface structure of the tool by which this material was pressed on the Ti implant, and demonstrated parallel orientated grooves. The contralateral side was identical to the uncoated implants. This implant modification was chosen because of its similarity to clinically used implants [12, 13].

Fifteen adult (mean age 28 months) female Göttingen minipigs with an average weight of 43 kg were used. Using a lateral approach to both femora, monocortical slots were prepared with a dental drill. The osteotomies were located at the proximal (hydroxyapatite left and Ti right) and distal left (UHMWPE) metaphysis.

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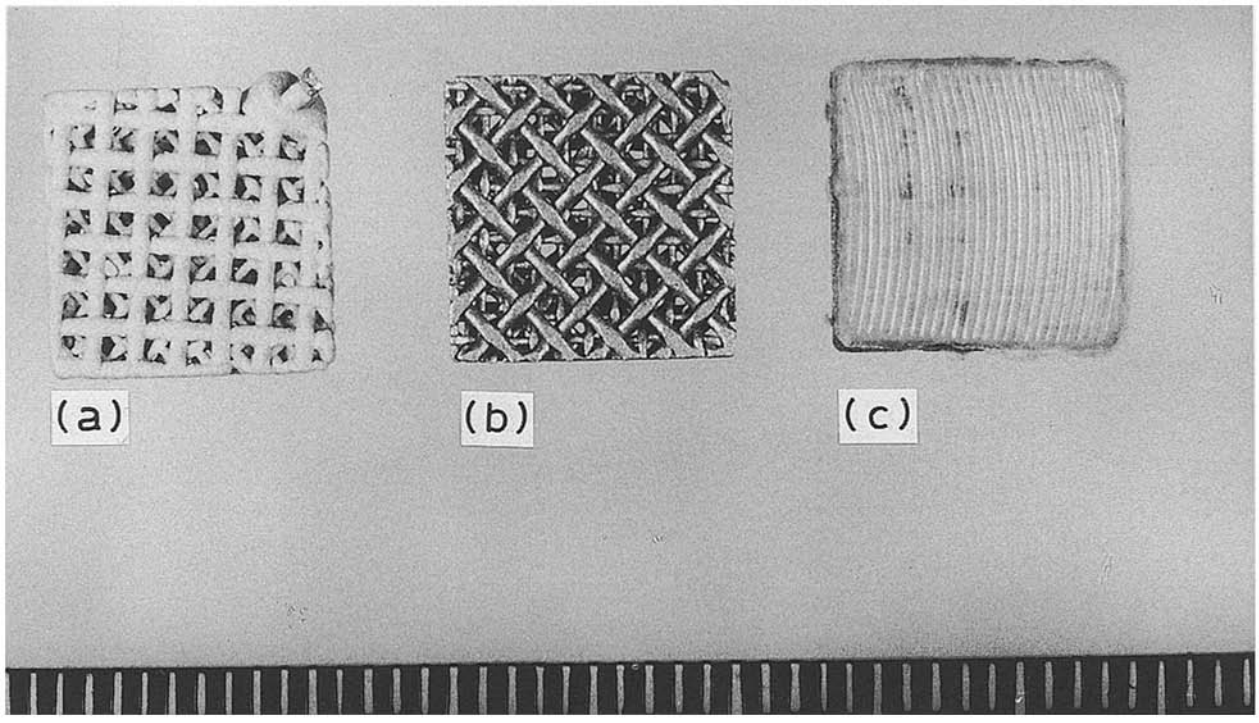

Figure 1 (a) Titanium coated with hydroxyapatite, (b) titanium and (c) UHMWPE.

Just before press-fit implantation, all samples were contaminated with 50 µl blood–brain medium with added niacin containing 10^7 or 10^3 *Staphylococcus aureus* bacteria. This bacterium, derived from pigs, was donated by the Faculty of Veterinary Medicine, University of Gießen, Germany.

The pigs were killed after 4, 8, 12 or 24 weeks. Before explantation of each femur, two bacteriological swabs of the perifemoral abscesses were taken and evaluated by the Institute of Microbiology, Philipps-University, Marburg.

The pH of the peri-implant tissue was examined with a surface microelectrode U-402-M8 (Fabrik Ingold Meßtechnik GmbH, 6374 Steinbach, Germany).

After explantation and removal of the soft tissue, contact radiographs of both femora were taken. The implant containing parts of the femora were cut in sections approximately 4 mm thick by a diamond saw and embedded in methyl methacrylate (Technovit 7200 VLC, Fabrik Kulzer, Wehrheim, Germany). Hard tissue sections (approximately 80 µm) were prepared by a sawing and grinding technique [14] and stained with toluidine blue for comparative histological investigation.

3. Results

According to the recommendation of both the Institute of Microbiology, University of Marburg, and the Faculty of Veterinary Medicine, University of Giessen, all implants of the first four pigs were infected with a blood–brain medium containing 10^7 *Staphylococcus aureus* bacteria per infection site. These four pigs died within 1 day after surgery, presenting signs of a toxic septic shock. Due to these events the experimental set-up was modified and the infection dose reduced to 10^3 bacteria. After this change no further systemic complications occurred and the remaining 11 pigs underwent further evaluation.

3.1. Macroscopic observations

All pigs that were killed after 4 or 8 weeks showed an extensive perifemoral abscess (for bacteriological details see Table I). This dramatic reaction at the infected implantation site decreased within the subsequent weeks. Twenty-four weeks after operation none of the implantation sites demonstrated macroscopic signs of infection.

Fractures occurred in six cases and were always located at the implantation site. Out of 33 implants, 13 were displaced into the surrounding soft tissue. It is worth noting that eight of the 13 displaced implants were samples coated with polyethylene (for details see Table II).

The stability of osseous implant fixation was roughly estimated by manual force applied to each implant in the implantation bed. At 4 weeks after implantation the hydroxyapatite-coated implants could already not be moved in their implantation bed by manual force application, whereas the uncoated implants could be moved slightly until 12 weeks after implantation. All implants with a unilateral polyethylene coating examined after 8, 12 and 24 weeks were displaced into surrounding soft tissue. Those examined after 4 weeks showed no contact with surrounding bone and were embedded in soft tissue.

3.2. Bacteriological results and pH values

Staphylococcus aureus could be cultivated in 70% of

TABLE I Bacteriological evaluation and pH value

Date of killing (weeks)	Pig no.	Bacteriological evaluation left (l): hydroxyapatite, polyethylene; right (r): titanium	pH
4	5	(l) *Streptococcus pyogenes animalis Pasteurella multocida* ssp[a].	NM[b]
		(r) *Streptococcus pyogenes animalis*	NM[b]
	6	(l) *Staphylococcus aureus*	NM[b]
		(r) *Staphylococcus aureus*	NM[b]
	7	(l) *Streptococcus pyogenes animalis Pasteurella multocida* ssp[a].	6.83
		(r) *Streptococcus pyogenes animalis Pasteurella multocida* ssp[a].	6.83
	8	(l) *Staphylococcus aureus*	7.18
		(r) *Staphylococcus aureus*	6.44
8	9	(l) *Staphylococcus aureus*	6.70
		(r) *Staphylococcus aureus*	7.30
	10	(l) *Staphylococcus aureus*	7.10
		(r) *Staphylococcus aureus Streptococcus pyogenes animalis*	6.57
	11	(l) *Staphylococcus aureus*	7.15
		(r) *Staphylococcus aureus*	6.89
12	12	(l) *Propionibacteria*	7.45
		(r) *Propionibacteria Micrococcus varians*	7.60
	13	(l) *Staphylococcus aureus*	7.40
		(r) *Staphylococcus aureus*	7.30
24	14	(l) Koag. neg. *Micrococcaceae*	6.92
		(r) Koag. neg. *Micrococcaceae*	6.95
	15	(l) *Staphylococcus aureus* Koag. neg. *Micrococcaceae*	7.00
		(r) *Staphylococcus aureus*	7.10

[a] Subspecies *Gallicida*.
[b] Not measured.

TABLE II Fractures and displacements of implants

Date of killing (weeks)	Pig no.	Fracture at the implantation site [a]				Displacement		
		HA	Ti	PE	Others	HA	Ti	PE
4	5	–	+	–	–	–	+	–
	6	–	–	–	–	–	–	–
	7	+	+	+	+	–	+	+
	8	+	–	–	+	–	–	–
8	9	–	–	–	–	–	–	+
	10	+	–	+	+	–	–	+
	11	–	–	–	–	–	–	+
12	12	–	–	–	–	–	–	+
	13	–	–	–	–	+	+	+
24	14	–	–	–	–	–	–	+
	15	–	–	–	–	–	+	+

[a] HA, Hydroxyapatite; PE, polyethylene.

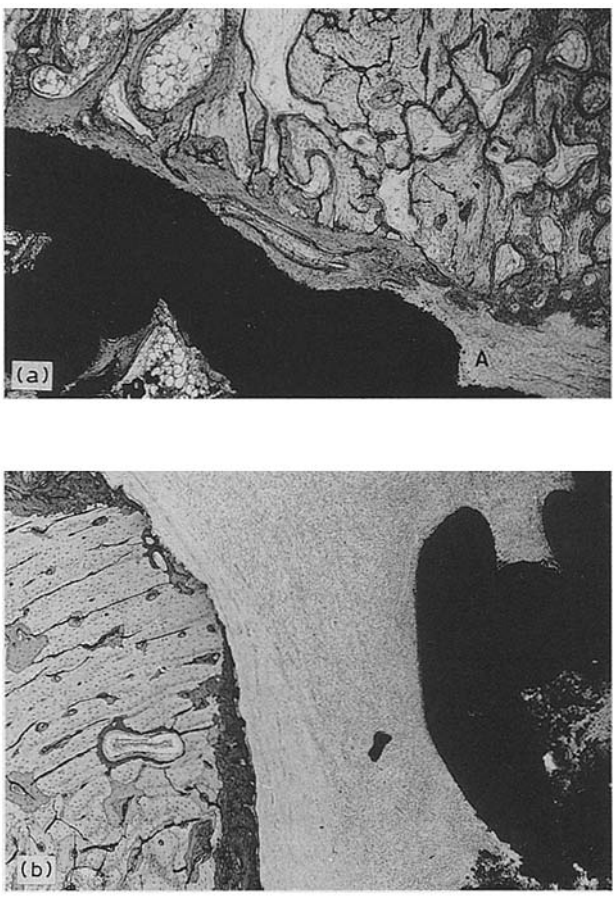

Figure 2 (a) After 4 weeks. (Lower right corner) Granulation tissue extending into the recess of the cortical implantation margin with signs of granular hydroxyapatite surface desintegration (A) (toluidine blue × 9). (b) Abscess between cortical bone and a Ti implant after 4 weeks (toluidine blue, × 9).

all bacteriological samples that were taken from each implantation site before removal of each femur. Thirty per cent of all samples showed bacteriological results different from the strain inoculated at operation.

There were no negative bacteriological tests throughout the experiment (for details see Table I). The pH values of the implantation sites, measured just before explantation, ranged from 6.44 to 7.60. No significant differences could be found after 4, 8, 12 or 24 weeks (see also Section 4).

3.3. Microscopic observations

3.3.1. 4 weeks: hydroxyapatite

The osteotomy site around the implant and the entire medullary cavity showed a vast inflammatory reaction, with numerous granulocytes and multiple fresh abscesses of various sizes. Endosteal, periosteal and medullary new bone formation were seen involving numerous osteoblasts, and appeared frequently in association with bone resorption in pre-existing cortical bone structures. A discontinuous contact of newly formed woven bone was evident between cortical bone of osteotomy and implant surface. No interposed layer of soft tissue was seen at the interface, and primary bone formation occurred directly on hydroxyapatite coatings. This bone formation was associated with a line of osteoblasts. Parts of the ceramic coating interface, which were not attached by osteoid, showed a continuous layer of mononuclear and in some cases of multinuclear cells in direct contact. Evidence for disintegrative processes at hydroxyapatite layers could not be demonstrated (Fig. 2a).

3.3.2. 4 weeks: titanium

Reactions due to induced infection were similar to those described around hydroxyapatite-coated specimens. The implants were, however, completely encapsulated by an abscess membrane that was filled with numerous granulocytes and tissue debris. Newly formed woven bone was rarely seen and terminated at the abscess membrane, separating the cortical bone and implant surface (Fig. 2b).

3.3.3. 4 weeks: polyethylene–titanium

In polyethylene–titanium implants the same reactions were basically demonstrated as in pure Ti implants.

3.3.4. 8 and 12 weeks: hydroxyapatite

The discontinuous woven bone at the interface, which was seen after 4 weeks, now formed a continuous layer between the implant and cortical bone. Pores of the hydroxyapatite-coated Ti meshwork were entirely filled with bone tissue in the cortical area. Parts of the hydroxyapatite coating, which were in contact with soft tissue in the intramedullary location, demonstrated obvious signs of disintegration. Hydroxyapatite particles were phagocytosed by macrophages. Up to 50% of the original thickness of the hydroxyapatite coating was lost. Compared with the results for 4 weeks, the inflammatory tissue reactions appeared less cellular and more organized. The number of granulocytes decreased and fibrotic granulation tissue was more prominent.

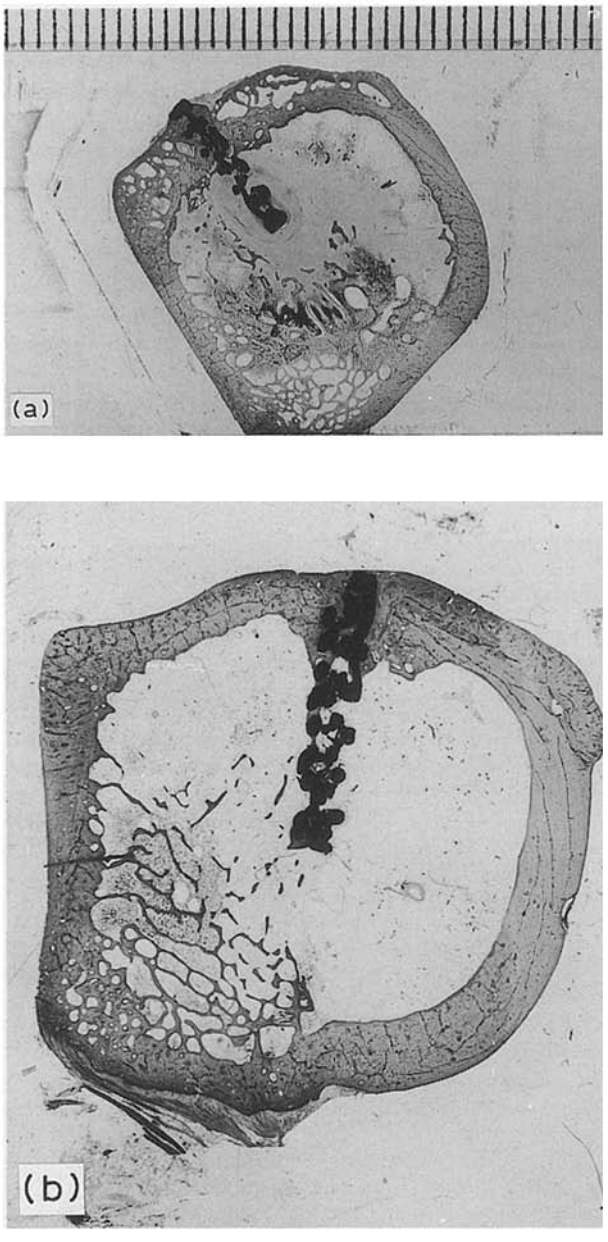

Figure 3 (a) Completely integrated hydroxyapatite implant after 24 weeks. In the medullary cavity an abscess around the implant is evident (toluidine blue, × 3). (b) Completely integrated Ti implants after 24 weeks (toluidine blue, × 3).

3.3.5. 8 and 12 weeks: titanium

The implants evaluated were still embedded in an active granulation tissue, presenting neither significant new bone formation nor bone–implant contact.

3.3.6. 8 and 12 weeks: polyethylene–titanium

All implants at this time of killing were displaced and fully encapsulated in a granulation tissue.

3.3.7. 24 weeks: hydroxyapatite

The entire osteotomy slot and the voids within the implant in the cortical area were filled with bone, which was almost completely of lamellar structure. However, the number of osteocytes per unit area had decreased. Haversian canals were seen as a sign of good vascularization of bone. In those areas where hydroxyapatite coating was in direct contact to bone no disintegrative processes of hydroxyapatite occurred. In contrast to this, in the intramedullary area no bone apposition could be observed and almost the complete hydroxyapatite coating was resorbed. Abscesses still surrounding the intramedullary part of the implants demonstrated that, even 24 weeks after implantation, the infection did not cease (Fig. 3a).

3.3.8. 24 weeks: titanium

In the cortical area the implant pores were almost totally filled with newly formed bone, demonstrating the typical ingrowth behaviour of Ti implants in non-infected conditions with direct bone–implant contacts beneath areas of thin layers of interposed soft tissue. Similar to the results after 12 weeks, only moderate signs of infection were evident (Fig. 3b).

3.3.9. 24 weeks: polyethylene–titanium

All implants at this time of killing were displaced and fully encapsulated in infected granulation tissue.

4. Discussion

This infection model proved to be reproducible and standardized for studies in an infected implantation site in minipigs. The desired infection occurred in all

pigs and persisted up to the time of killing in all pigs. In 30% of the pigs bacterial shift could be found. Local pH values of the infected tissue were relatively constant within the range of physiological pH.

It was clearly seen that, in spite of local infection, hydroxyapatite-coated implants already demonstrated good osseous ingrowth behaviour after 4 weeks. Signs of degradation of the hydroxyapatite coating were evident after 8 weeks and increased during the entire experimental time. This resulted in a complete loss of hydroxyapatite coating after 24 weeks in the intramedullary area, where new bone formation could be observed only until 8 weeks after implantation.

Compared with earlier experiments in our department with hydroxyapatite-coated implants without induced infection [9, 10], osseointegration was delayed only slightly in the case of infection. The accelerated hydroxyapatite degradation seen in this experiment was exclusively restricted to those areas of the infected implantation site where no bone contact was seen.

This fact supports the theory that hydroxyapatite degradation is caused by cellular (macrophages and polynuclear giant cells) activity [15], which is higher in the case of infection and is finished by bone apposition, which in this experiment occurred only in the cortical area.

In an infected implantation site in the rat middle ear, van Blitterswijk *et al.* could not find significantly higher degradation rates of bulk hydroxyapatite implants after 1 year [15, 16] compared with the behaviour in an uninfected implantation site using the same animal model [17]. Only an increase in the macropore area (initial macropore area approximately 26%) 1 week after infection and 2 weeks after implantation [16] was higher (69%) and corresponded to findings in the non-infected rat middle ear after 6 and 12 months [17]. This finding was not attributed to the infection but to differences in implantation technique.

The major difference of those investigations to our experiment are implantation and infection techniques and animal species. In neither case did infection occur directly after implantation (1 week after implantation [16] and 3 weeks before killing [15]) and in neither case were the samples implanted into the bone tissue. Thus, it might have been possible that at the time of infection the implant surface was covered by tissue, which would diminish the biodegradation process. This would also explain our observation of a high degradation rate in contact with soft tissue in the intramedullary channel and only minor degradation in the cortical area, where apposed bone protected the ceramic surface.

Our experiments cannot completely answer the question whether hydroxyapatite degradation is pH-dependent. The pH values measured ranged from 6.44 to 7.60 in the infected implantation sites. Ungethüm and Fink pointed out that pH values of 3–4 or lower are obligatory for an increased degradation of bioactive materials [18]. On the other hand, Klein *et al.* demonstrated a low dissolution rate of hydroxyapatite exposed to a lactate buffer solution with a pH value of 5.2 [19], whereas no detectable bioresorption occurred in animal experiments under sterile conditions [20, 21].

The uncoated Ti implants showed a delay of bone ingrowth for approximately 20 weeks during infection in this experiment. In contrast with this, the bone ingrowth was well advanced after 4 weeks in a non-infected implantation site [9, 10].

Regarding clinical aspects of the results described here, the following conclusions can be drawn.

1. After infection the removal of hydroxyapatite-coated implants must be expected to be more difficult than for an uncoated implant because, despite infection, bone bonding seems not to be severly affected.
2. On the other hand, it is possible that the continuous direct contact of cells to the ceramic surfaces enhances the possibilities of cellular defence and can prevent infection in case of exposure to lower bacterial counts.
3. As a solid mechanical situation is one of the most important conditions in septic treatment, there is the need to discuss whether an infected hydroxyapatite-coated endoprosthesis should be removed at all, or whether conditions of conservative (antibiotics) and operative (debridement) treatment without prosthesis exchange are sufficient for osseous integration despite local infection.
4. Resorption of the hydroxyapatite coating and its dynamics in the presence of local infection are not yet fully understood. Its significance with respect to permanent implant stability cannot be validated at present.
5. More research is needed to investigate the possible benefit of implants coated with hydroxyapatite and an additional antibiotic loading [22].

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