JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 5 (1994) 345–349

Evaluation of different preparations of plasma-spray hydroxyapatite coating on titanium alloy and duplex stainless steel in the rabbit

P. LEALI TRANQUILLI, A. MEROLLI, O. PALMACCI
Clinica Ortopedica dell'Universita' Cattolica L. go Gemelli 8, I-00168 Roma, Italy
C. GABBI, A. CACCHIOLI, G. GONIZZI
Istituto di Anatomia, Medicina Veterinaria Universita' di Parma, Italy

Many variables are involved in hydroxyapatite coating of metals by plasma-spray techniques. The authors have investigated the biological response to some of the most relevant variables in a controlled *in vivo* trial. The bone response in the rabbit towards hydroxyapatite coated cylinders was studied keeping the following variables fixed: (a) crystallinity of coating (greater than 90% and between 70% and 60%); (b) thickness of coating (50 and 100 μm); (c) metallic substrate (titanium alloy and duplex stainless steel). Analysis of the results highlights the importance of defining the crystallinity of the coating to forecast its *in vivo* behaviour: highly crystalline coating is more stable in time but can give rise to fragmented bulky particles; a less crystalline coating is subject to slow degradation in the long term but facilitates its substitution by newly formed bone. Furthermore, it has been found that no relevant differences can be ascribed to a variation in coating thickness between 50 and 100 μm. It has, also, been observed that there are no differences when duplex stainless steel is used instead of titanium alloy as metallic substrate, confirming that bone responds primarily to the coating.

1. Introduction

Hydroxyapatite coating of metallic implants for orthopaedic and dental use by plasma-spray techniques has become a common procedure for several manufacturers. It should be pointed out that many variables are involved in the accomplishment of the final result [1].

Fixation of an implant by enhanced bone growth favoured by hydroxyapatite coating requires persistence of the coating and adequate bond strength to bone and metal. Crystallinity of the coating is important in relation to its persistence. Highly crystalline and less soluble coatings are generally preferred for implant fixation [2, 3]; experiments have been made regarding this aspect [4–6] and favourable initial clinical results have been reported [7–11]. However the long-term survival of the coating seems not to be assured and, apart from hydrolytic dissolution, cellular remodelling of the coating has to be considered [12–14]. Also the mechanical competence of the metal–coating interface in the long term remains a concern [15]. Thickness is another parameter which is subject to variations among different manufacturers, and the range of coating thickness has been reduced from 200 to 50 μm in recent times. Greater thickness of highly crystalline coating will give a brittle material prone to cracking under bending or shearing forces. Also recently metallic substrates like cobalt-chromium alloys have been tested as alternatives to the classical titanium alloy.

The aim of the present study was to investigate the biological response to some of the most relevant variables in a controlled *in vivo* trial. The study was designed to evaluate the *in vivo* response in the rabbit towards hydroxyapatite-coated cylinders when the variables crystallinity and thickness of the coatings and the nature of the metallic substrate were controlled.

2. Materials and methods

Two sets of values were chosen for each variable. Crystallinity was greater than 90% in one class and between 70% and 60% in the other. Thickness was 50 μm in one class and 100 μm in the other. Metallic substrate was Ti6A14V in one class and duplex 350 stainless steel (a highly corrosion resistant steel) in the other.

Forty-four cylinders of 25 mm length and 3 mm diameter were implanted in the distal femoral canal of young adult New Zealand White rabbits weighing about 2700 g, without preference regarding their sex.

Four retrieval steps were designed at 4 weeks, 8 weeks, 26 weeks and 34 weeks. Duplex 350 stainless

0957–4530

steel cylinders were provided by SEIPI SpA, Milano (I); titanium alloy cylinders, hydroxyapatite powder and all the coatings were provided by Plasma Biotal Ltd, Tideswell (UK). Crystallinity was derived from X-ray diffraction (XRD) examination of sprayed material in comparison with the starting powder.

The implantation Schedule is described in Table I. Uncoated cylinders are present as controls for each substrate material. Un-operated controls and "sham-operated" controls (where the surgical procedure was performed but no cylinder was actually implanted) are also present.

The implantation protocol consisted of the following steps:

(a) cylinders were sterilized in ethylene oxide and single-packaged in sterile envelopes;
(b) intramuscular Valium (5 mg/kg) was administered;
(c) hair cut from the area to be operated;
(d) intramuscular Ketalar (50 mg/kg) was administered;
(e) subcutaneous Xylocaine was administered.

A lateral parapatellar incision of about 15 mm was performed, the joint capsule opened and the patella dislocated medially exposing the femural inter-condylar groove. A hole was drilled into the femural canal by a special instrument specifically designed and manufactured for this operation. The integrity of the walls of the femural canal was checked by a probe. A cylinder was implanted in the distal femural canal, the patella reduced and the capsule and anatomical planes sutured. Intramascular Rifocin (250 mg) was administered.

This protocol proved to produce no artificial detachment of the coating during the insertion of the cylinder.

The retrieval protocol consisted of the following steps. The rabbit was placed in a special sealed chamber where the atmosphere was quickly saturated with CO, and left there for 3 min. The femur was carefully dissected and placed in a sealed plastic tube in 70% ethyl alcohol. X-ray films of the retrieved femur were taken in antero-posterior and latero-lateral views.

The histological protocol consists of several steps; the basic phases were:

1. The femur was squared in the distal portion, dehydrated in serial passages in alcohol and embedded in methyl-methacrylate.
2. The specimen was divided into three blocks: proximal, middle and distal.
3. Two sections of 100 μm thickness were taken with a rotating diamond-saw microtome: (a) proximal, between the proximal and middle blocks, giving a diaphyseal section; (b) distal, between the middle and distal blocks, giving a meta-epiphyseal section.
4. Sections were analysed by polarized light microscopy. Surfaces of the blocks were examined by back scattering electron microscopy (BSEM) after preliminary wet grinding and carbon coating of the block.

Morphological analysis of the biological response to implantation procedure involved bone growth towards HA coatings, possible resorption of the HA coating and possible interactions of bone with the metal substrate.

TABLE I Implantation prospect

SSU1	SKC1	SNC1	SKA1	SNA1
SSU2	SKC2	SNC2	SKA2	SNA2
SSU3	SKC3	SNC3	SKA3	SNA3
SSU4	SKC4	SNC4	SKA4	SNA4
TTU1	TKC1	TNC1	TKA1	TNA1
TTU2	TKC2	TNC2	TKA2	TNA2
TTU3	TKC3	TNC3	TKA3	TNA3
TTU4	TKC4	TNC4	TKA4	TNA4
UOP1	UOP2	SHM1	SHM2	

Code:
1st letter
S duplex 350 stainless steel
T Ti6A14V alloy
2nd letter
S uncoated duplex 350 stainless steel
T uncoated Ti6A14V alloy
K 100 μm thick HA coating
N 50 μm thin HA coating
3rd letter
U uncoated
C highly crystalline HA coating
A less crystalline HA coating
Number
1 retrieval at 4 weeks (1 month)
2 retrieval at 8 weeks (2 months)
3 retrieval at 26 weeks (6 months)
4 retrieval at 34 weeks (8 months)
Others
UOP unoperated
SHM "sham" operated

3. Results

For the sake of simplification, specimens with crystallinity higher than 90%will be referred as "highly crystalline" while specimens with crystallinity between 70% and 60% and presenting an amorphous phase will be referred as "less crystalline". For the same reason specimens with a coating thickness of 100 μm will be referred as "thick" while specimens with a coating thickness of 50 μm will be referred as "thin".

The protocol aimed to investigate the following questions:

1. What are the differences between uncoated and coated substrates?
2. What are the differences between highly crystalline coatings and less crystalline coatings, regardless of thickness?
3. What are the differences between thick and thin coatings, regardless of crystallinity?
4. What are the differences between highly crystalline coatings and less crystalline coatings of the same thickness?
5. What are the differences between thick and thin coatings of the same crystallinity?
6. What are the differences between the same classes of coating on the two different substrates?

3.1. Differences between uncoated and coated substrates

The response to uncoated samples has the morphological character of properly formed bone but growth is mostly directed to encase the implant, and, furthermore, it is more pronounced where a rough surface is present, while the largest area of metal has no direct contact with bone (Fig. 1). In contrast, the response to coated samples has the morphological character of tight apposition, and bone substituting areas of the coating, particularly in the less crystalline samples are observed. Morphological indications of physiological bone-turnover (deposition of new bone and resorption of the old one) are clearly distinguishable at the bone-coating interface.

3.2. Differences between highly crystalline coating and less crystalline coating regardless of thickness

The highly crystalline coating seems to be more durable over time than the less crystalline coating, where a reduction in volume is detectable. Fragmentation and degradation of the highly crystalline coating is not, anyway, an uncommon finding and true detachments of the crystalline coating have also been observed. What seems to be relevant is that few localized areas of fragmented, detached and degraded coating can be observed in the highly crystalline coating, even in the early stages (that is to say within 8 weeks) while the rest and the vast majority of the coating has remained intact. Detachments appear between the coating and the metallic substrate; they have been very seldom observed between coating and bone, the rare specimens never older than 4 weeks. When fragmentation occurs the highly crystalline coating gives rise to bulky particles whose subsequent fate is a matter of speculation: they could be swept away since they do not seem to slowly dissolve on-site (Fig. 2). In the less crystalline coatings, areas are occupied and remodelled and then substituted by newly formed bone. Even in the less crystalline coating, localized areas of far-advanced degradation can be observed in the early

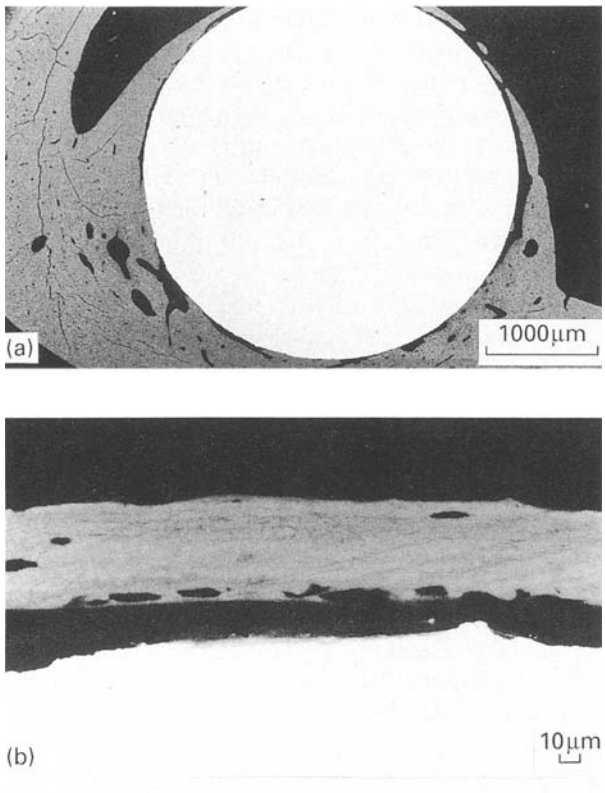

Figure 1 The response to uncoated samples has the morphological character of properly formed bone, but growth is mostly directed to encase the implant: (a) uncoated stainless steel at 26 weeks. The largest area of the metal has no direct contact with bone; (b) uncoated titanium alloy at 8 weeks.

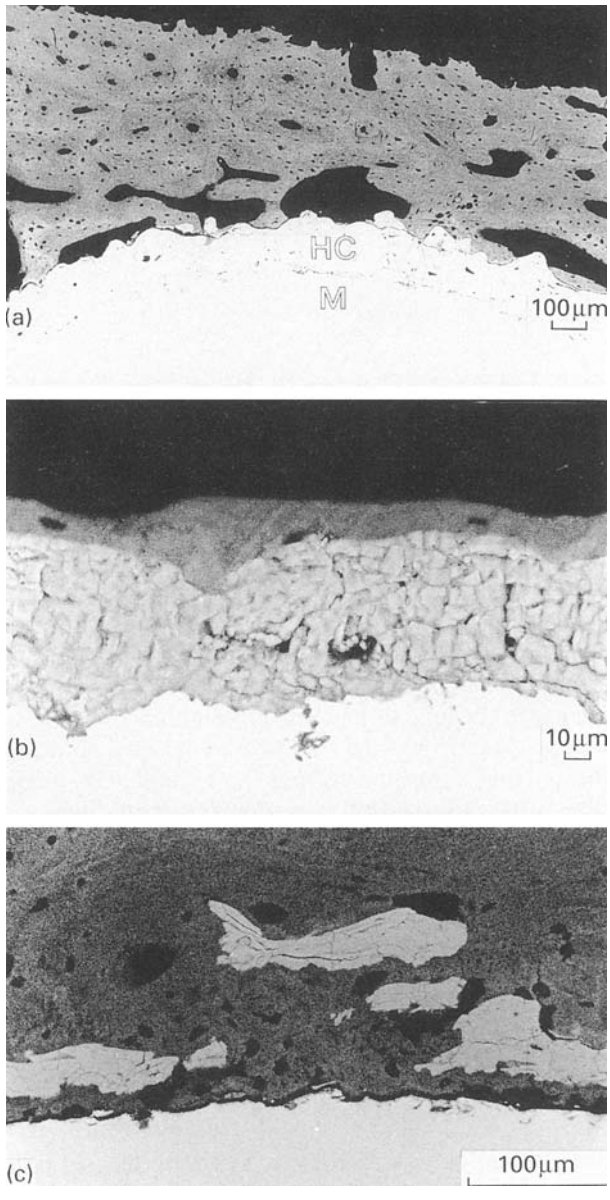

Figure 2 Highly crystalline coating (HC) seems to be durable over time and the bone response has the morphological character of remodelling, as shown in (a) with thick highly crystalline coating at 8 weeks on stainless steel (M). High apposition and a clear-cut interface between highly crystalline coating and bone are observed (b), with a thin highly crystalline coating at 26 weeks on stainless steel. Fragmentation and true detachment may occur, even if in localized areas, and give rise to bulky particles which do not seem to dissolve on site. (c) Detached particles of about 20 μm thickness which are remnants of a thick highly crystalline coating on titanium alloy after 34 weeks; bone has grown between the particles and the metal. (Bone detachment at the metal surface is an artifact of embedding).

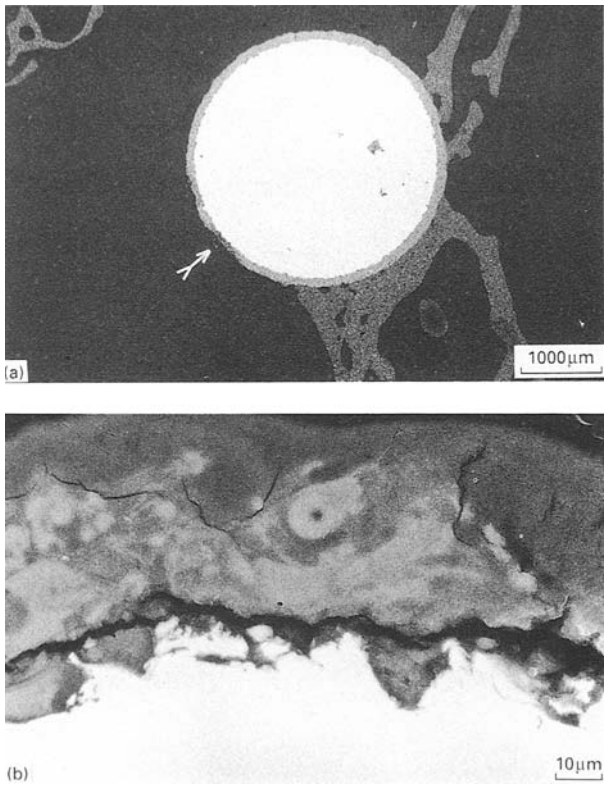

Figure 3 In less crystalline coatings localized areas of advanced degradation (arrow) can be observed despite a nearly intact coating being present all around. (a) Thick, less crystalline coating on titanium alloy at 26 weeks. In the long term, thinning of the coating and BSEM observations of the coating vanishing during integration are common. (b) Rim of bone in which vestiges of a thin, less crystalline coating on stainless steel are detectable after 34 weeks.

stages despite a nearly intact coating present all around. The less crystalline coating gives a different picture in the long term, often presenting a thinning of the coating, with some BSEM micrographs showing the coating vanishing during integration with bone (Fig. 3). To summarize, it can be stated that while a clear-cut interface is detectable (by BSEM) between the highly crystalline coating and bone, this is not often true for the less crystalline coating.

3.3. Differences between thick and thin coating, regardless of crystallinity

The thick coating is more uniformly present in the early stages (4–8 weeks) than in the thin coating. However, an equation: thickness = time of degradation, cannot be applied because areas of well-preserved coating may be present together with areas where local esfoliation/fragmentation has already completely exposed the metallic substrate, even in the early stages. As a general rule, the majority of coatings continue to be adequately preserved in time as would be expected. When degradation occurs it is more uniform along the circumference of the less crystalline coating, and then a thicker coating is preserved longer; in the highly crystalline coating the overall decrease in volume is associated with the fragmentation process already described, and then thickness seems to play a minor role.

3.4. Differences between highly crystalline coatings and less crystalline coatings of the same thickness

The same considerations as for 3.2 can be made.

3.5. Differences between thick and thin coatings of the same crystallinity

The same considerations as for 3.3 can be made.

3.6. Differences between the same classes of coating on the two-different substrates

As long as a good coating is present, a difference in bone response related to the substrate does not seem to be present. When the coating has degraded or detached, the bone response seems to be related primarily to the surface topography of the metal (rougher for steel than for titanium alloy). Observations of adequate bone growth towards the metal surface have been recorded for stainless steel and for titanium alloy.

3.7. Other results

Among other results obtained from this analysis are:

(a) The presence of significant differences between the 1 and 2 months group and the 6 and 8 months group, while differences inside these groups (between 1 and 2 months and between 6 and 8 months) are sometimes hard to detect.

(b) The assessment of the high probability that the metal substrate and any kind of substance interposed between the metal and the coating (like residues from manufacturing processes) may come into contact with biological tissues at localized sites even in the early stages (within 8 weeks).

(c) Early bone response to surgical procedure in the meta-epiphyseal region (within 8 weeks) tends to form a circumferential wall from which young tiny trabeculae will originate to fill the hole.

(d) Clinical X-ray films did not show evidence of morphological differences among coated samples; even differences between uncoated and coated samples were difficult to assess. Clinical X-ray films should be, then, considered of no value in defining the bone response to the coating, at least in this experimental model.

4. Discussion and conclusions

The response to coated samples has the morphological character of tight apposition which can be followed by substitution of areas of coating by newly formed bone. Coating, then, favours a more physiological integration of the implant, while uncoated metal produces an encasing response that confines the implant from the rest of the remodelling bone. In this respect, a coated implant should perform better from the biological point of view; however, problems may arise from the mechanical competence of the coating because detachments at the metal–coating interface are often present. They are produced, in the large majority of cases, as artifacts of embedding (due to retraction of the methacrilate). They are also observed

in vivo, and their presence, negligible in the first 4 weeks, increases in the long term (this fact discourages the explanation that they are artifacts of the implantation procedure). Both as artifacts of embedding and as produced *in vivo*, these metal-coating detachments reflect a weaker bond strength in comparison with the bone–coating interface. Detachments at the bone–coating interface have been observed only (and very seldom) during the first 4 weeks when, even if apposition between bone and coating is present, an ultramicroscopic interlocking has not always matured.

Thickness of the coating may play a role when a process of uniform degradation is expected; however, the majority of coatings continue to be adequately preserved over time. Even if a more uniform degradation can be ascribed to the less crystalline coating, while the higher crystalline coating can give rise to fragmented bulky particles [16], a far more relevant finding is that localized areas of degraded or fragmented coating can be observed regardless of crystallinity, even in the early stages (within 8 weeks). A further increase in coating thickness may lead to a reduction of these areas but, at the same time, would increase problems related to the mechanical competence of the coating [17].

When metal has been exposed, growth is more pronounced where a rough surface is present and this seems to highlight a primary bone response to topography. In our series, surface characteristics of coated duplex stainless steel cylinders differ from the uncoated controls because the latter are mirror finished, while the former have a rougher surface, suited to HA coating; this accounts in part for the good response towards duplex stainless steel once the coating was lost.

Assessment of the high probability that the metal substrate and any kind of substance interposed between the metal and the coating is going to come into early contact with biological tissues at localized sites, points towards a possible danger related to undesirable byproducts of the manufacturing process that may be trapped between metal and coating and, then, become candidates for local and systemic spread.

Presence of significant differences between the 1 and 2 months group and the 6 and 8 months group but not inside these groups, stresses the importance of long-term retrievals (minimum 6 months) that have to be planned in studies regarding *in vivo* behaviour of HA coatings.

The circumferential wall originated as an early response to the surgical procedure (and the young trabeculae budding into the hole) may give, in the presence of an implant, a picture of a favourable bone integration which is, in part, artificial. It is important to know the physiological response of the animal model to the implantation procedure and then avoid false positive artifacts; a lack of this morphological picture in the presence of an implant has to be considered as a true negative, signalling a somehow inhibitory action towards bone growth.

The presence of sites of far-advanced degradation close to a nearly intact coating was often observed and commonly in areas of active bone metabolism (both deposition and resorption). This stresses the importance of local cellular metabolism and seems to claim a role for a mechanism of active cellular remodelling of the coating, probably more relevant than pure hydrolythic dissolution alone.

In conclusion, the present work highlights the importance of defining the crystallinity of coatings to make forecasts on *in vivo* behaviour: a highly crystalline coating (crystallinity greater than 90%) is more stable over time but can give rise to fragmented bulky particles; a less crystalline coating (crystallinity between 70% and 60%) is subject to slow degradation in the long term but facilitates its substitution by newly formed bone. No relevant differences can be ascribed to a variation in coating thickness between 50 and 100 μm. Furthermore, it has been confirmed that bone response is related to the coating, and no differences are present regarding the *in vivo* behaviour when duplex stainless steel is used as metallic substrate instead of titanium alloy.

Acknowledgements

This work was funded by the EC BRITE/EURAM project BE3048/89 "Surface coatings for biomaterials".

BSEM was performed in collaboration with Professor F. Monteiro, Dr C. Sa' and Dr S. Sousa from the University of Porto (P).

References

1. J E. LEMONS, *Clin. Orthop.* **235** (1988) 220.
2. R. X LEGEROS, I ORLY, M GREGOIRE, Trans. Soc. *Biomater.* **10** (1987) 84.
3. C GABBI, A. MEROLLI and A. CACCHIOLI, *Biomaterials* **1/2** (1993) 17.
4. S. D. COOK, K. A THOMAS, J. F. KAY, *Clin. Orthop.* **232** (1988) 225.
5. K. DE GROOT, R. GEESINK, C. P KLEIN, *J. Biomed. Mater. Res.* **21** (1987) 1375
6. B. KOCH, J G. WOLKE and K DE GROOT, *ibid.* **24** (1990) 655.
7. T G. ABRAHAMS and O D. CROTHERS, *Invest.-Radiol.* **27** (1992) 779.
8. J. A. D'ANTONIO, W N. CAPELLO, O D CROTHERS, W L JAFFE and M. T. MANLEY, *J. Bone Joint Surg.* **74** (1992) 995.
9. R. G. GEESINK, K. DE GROOT and C. P. KLEIN, *Clin. Orthop.* **225** (1987) 147.
10. R G. GEESINK, *Orthopedics* **12** (1989) 1239.
11. R G GEESINK, *Clin. Orthop.* **261** (1990) 39.
12 K GOMI, B LOWENBERG, G. SHAPIRO and J E. DAVIES, *Biomaterials* **14** (1993) 91.
13. N. TAKESHITA, T. AKAGI, M. YAMASAKI, T OZEKI, T. NOJIMA, Y HIRAMATSU and N. NAGAI, *J. Electron Microsc.* **41** (1992) 141.
14. G. J WANG, T E BOMAN, K C. CHUNG, *Trans. Soc. Biomater.* **12** (1989) 20.
15 S. D COOK, J. F KAY, K. A. THOMAS, *Int. J. Oral Maxillofac. Implants* **2** (1987) 15
16. J. D. DE BRUIJN in "Bioceramics 5" (edited by T. Kokubo, Butterworth-Heinemann, London, 1992) p. 291.
17. J G C. WOLKE *et al.*, *J. Thermal Spray Technol.* **1** (1992) 75.