Histomorphological study of bone response to hydroxyapatite coating on stainless steel

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Bone response to hydroxyapatite coating on stainless steel has not been so extensively tested in animals as it happened for other metallic substrate, like Ti6Al4V. For this reason, authors performed an *in vivo* histomorphological electron microscopic study of hydroxyapatite coating on duplex stainless steel cylinders, to gather further evidences on the characters of bone apposition at the interface. Sixteen HA-coated cylinders were implanted in the distal femur of New Zealand White rabbits. Comparison with uncoated controls was made. Retrieval steps were at: 4, 8, 26 and 34 weeks. Specimens were analyzed in a Jeol JSM 6301F scanning electron microscope. The response to HA-coated samples has a morphological character of tight apposition between bone and coating. Osteocytic lacunae may be found few microns close to the coating and newly formed bone is extremely interlocked with it so that even an higher magnification electron-microscopy cannot resolve any discontinuity in between. Pictures of physiological bone-turnover are distinguishable at the bone-coating interface; areas of well preserved coating may be present together with areas where local exfoliation or fragmentation has already completely exposed the metallic substrate. On the opposite in uncoated samples, despite a morphological picture of properly formed bone, the largest area of the metal has no direct apposition with it.

1. Introduction

Bone response to hydroxyapatite coating on stainless steel has not been so extensively tested in animals [1–3] as it happened for other metallic substrate, like Ti6Al4V. For this reason, authors performed an *in vivo* histomorphological electron microscopic study of hydroxyapatite coating on duplex stainless steel cylinders, to gather further evidences on the characters of bone apposition at the interface.

Recently, a clinical application of hydroxyapatite coating on stainless steel has been developed in Orthopaedic Surgery, namely the coating of threaded pins of external fixators, which are devices used to stabilize fractures of long bones [4–6]. The aim is to improve pin stability, particularly in those patients which require a prolonged fixation time and in which there is an increased risk of infection of the pin tract. The improved mechanical stability has been demonstrated *in vivo*, both in animals and in humans, by a significant rise in the pin extraction torque in comparison with uncoated pins. Hydroxyapatite coating improves the osteointegration of stainless steel pins and newly formed bone grows in tight apposition with the coating.

Stainless steel remains, by far, the most used material for implantable devices in Orthopaedic Surgery. Its characteristics like elasticity, strength and resistance to wear, depend upon several factors like composition of the alloy, treatments during production and internal structure (conformation of crystalline lattice). Commonly used stainless steel alloys are Austenitic (face-centered cubic structure): this structure gives them a good resistance against physical and chemical agents in normal atmospheric conditions. A Ferritic structure (body-centered cubic structure) is also possible: this generally provides a better resistance to electrolytic corrosion, although resistance against physical and chemical agents is worse than austenitic steels.

The property to be stainless is due to a superficial film of oxide that prevents further reaction at the surface. In the peculiar biological environment, steel is exposed to specific kinds of aggression: pitting and crevice corrosion are the most important of them. Pitting corrosion can develop at some point where it is likely that the superficial layer has been destroyed and a differential electrochemical potential develops. Crevice corrosion occurs in zones where different components are in contact (e.g. a plate and a screw) and it is greatly enhanced when steels from different alloys are used, as a differential electrochemical potential develops between them [7, 8]. To enhance mechanical characteristics and resistance to corrosion of stainless steel, ''350 duplex'' stainless steel (Cr 22, Ni 6, Mo 3, with low C, S and P

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content) has been developed (it is called ''duplex'' because both austenitic and ferritic structures are significantly represented) [9].

2. Materials and methods

Authors performed an electron microscopic analysis at the interface between newly formed bone and hydroxyapatite coating, in an experimental model in the rabbit. Samples were analyzed by scanning electron microscopy (SEM) to evaluate their morphologic characters like alignment of lamellae, location of Haversian systems, porosity of the hydroxyapatite coating. The same field was, then, compared with back-scattered electron microscopy (BSEM) analysis, which evaluate the actual material distribution on the sample.

The technique of back-scattered electron microscopy is particularly adequate to study the interface between bone and biomaterials because it allows the picturing of a map of material distribution where: (a) metallic substrate, (b) hydroxyapatite coating with different densities, (c) bone tissue in different stages of maturation, (d) embedding media and (e) artefactual detachments are easily discernible.

Sixteen cylinders of $25 \cdot 3\,\text{mm}(\text{length} \cdot \text{diameter})$, made of duplex stainless steel and coated with hydroxyapatite by plasma-spray technique, were implanted in the distal femural canal of young adult New Zealand White rabbits weighting about 2700 g (see Table I). Hydroxyapatite powder and all the coatings were provided by Plasma Biotal ltd (Tideswell, UK). Stainless steel cylinders were provided by CGDB s.p.a. (Milan, I). Four retrieval steps have been designed at: 4, 8, 26 and 34 weeks.

The mean crystallinity of the coating, measured from X-ray diffraction of scraped-off sprayed material in comparison with the starting powder as a reference, was 78%. The mean thickness of the coating, measured by BSEM [10], was 81 micrometers.

TABLE I Implantation prospect

4 weeks	UN-1	C-01	C-05	C-09	C-13	S1	U1
8 weeks	UN-2	C-02	C-06	C-10	C-14	S2	U2
26 weeks	UN-3	C-03	C-07	C-11	C-15	S3	U3
34 weeks	UN-4	C-04	C-08	C-12	C-16	S4	U4

letter code:
UN = uncoated stainless steel
C = HA coated stainless steel
S = ''sham'' operated
U = unoperated

Four uncoated cylinders were implanted as controls and four unoperated and four ''sham-operated'' controls (where the surgical procedure was performed but no cylinder was actually implanted) were also present in the protocol (Fig. 1). Characters of bone response to this three categories of controls have been already reported in previous studies [2].

Cylinders were sterilized by Ethylene Oxide and single-packaged in sterile envelopes. Anesthesia was obtained by administration of intramuscular diazepam (5 mg/kg), intramuscular ketamine hydrochloride (5 mg/kg) and subcutaneous lidocaine. Antibiotic prophylaxis was based on administration of intramuscular rifocin (250 mg) daily for 4 days after surgery. A hole of about 3 mm in diameter was drilled from the intercondylar groove after access has gained to articular cavity of the knee by a lateral parapatellar approach. The cylinder was inserted by press-fitting along the main axis of the femur. To retrieve and process the samples, the rabbit was placed in a specially sealed chamber where the atmo-

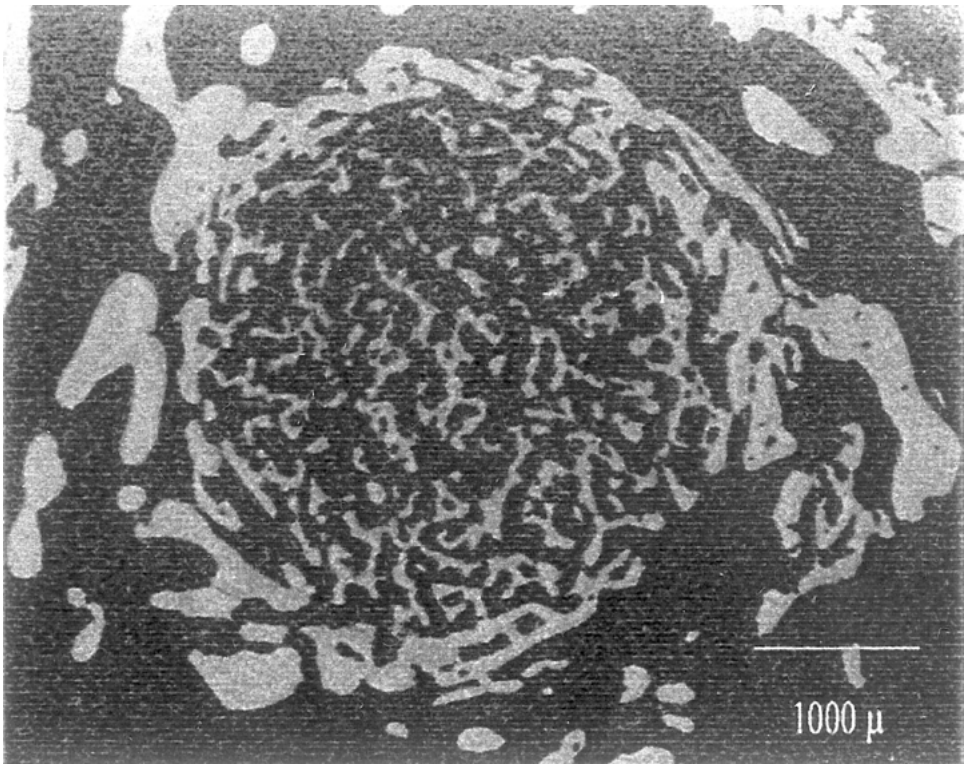

Figure 1 Transverse section which shows the reparative process occurring after the drilling of the implantation hole (at 4 weeks); tiny little trabeculae, growing in continuity with the pre-exinting trabecular bone, start to fill the hole (BSEM, magnification 15×).

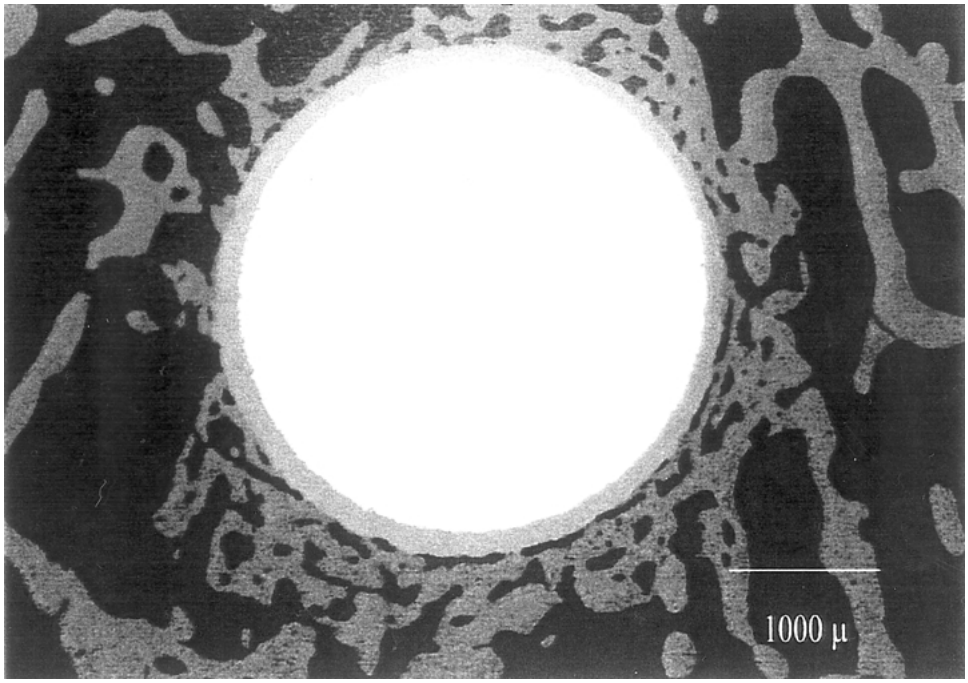

Figure 2 Transverse section which shows trabecular bone in apposition to the Hydroxyapatite coating of a stainless steel cylinder, after 4 weeks (BSEM, magnification 15×).

sphere was quickly saturated with CO_2 and left there for 3 min. The retrieved sample was, then, placed in 70% ethyl alcohol, dehydrated in serial passages in ethyl alcohol and embedded in poly-methyl-metha-acrylate (PMMA). The site analyzed was the meta-epiphyseal region of the distal femur. Embedded undecalcified specimens were sectioned in the transverse plane using a toroidal rotating diamond-saw (Leitz Wetzlar, D); this special saw reduces artifacts due to blade vibrations. After sectioning, two blocks were obtained. The face of the block to be analyzed was grinded, sputter-coated with gold and analyzed in a Jeol JSM 6301F scanning electron microscope at the facilities of the center for Materials Engineering (CEMUP) and the National Institute of Biomedical Engineering in Porto, Portugal (see acknowledgments).

The experimental protocol was submitted for ethical approval which was gained prior to start.

3. Results

The response to coated samples has a morphological character of tight apposition between bone and coating (Figs. 2 and 3). Osteocytic lacunae may be found few microns close to the coating and newly formed bone is extremely interlocked with it so that even an higher magnification electron-microscopy cannot resolve any discontinuity at the interface between bone and coating (Figs. 4 and 5).

There are pictures showing bone substituting areas of coating (particularly in the less crystalline samples) and there are pictures where physiological bone-turnover (deposition of new bone and resorption of the old one) is clearly distinguishable at the bone-coating interface (Fig. 6).

Fragmentation and degradation of the coating is not an uncommon finding and true detachments have been observed in about 20% of the samples, mostly in samples with higher crystallinity coatings, even in the early stages (that is to say within 8 weeks), despite the rest and vast majority of the coating has remained intact. In longer term retrievals, mostly in samples with lower crystallinity coatings, areas of well preserved coating were present together with areas where local exfoliation or delamination had already completely exposed the metallic substrate (Fig. 7); in these coatings there was a relevant thinning of the coating after 34 weeks.

The response to uncoated control samples has a morphological character of properly formed bone but growth is mostly directed to encase the implant (Fig. 8) and the largest area of the metal has no direct contact with bone (Fig. 9).

4. Discussion

A vast literature documents that hydroxyapatite coating of metallic implants favors their fixation to bone tissue [11].

Osteocytic lacunae detectable few microns close to the coating [2, 12–15] testimony of osteoblastic production of new bone which is so tightly apposed to the coating that high magnification SEM and BSEM cannot resolve any discontinuity at the interface. This is not observed with uncoated materials, even with those which are known to promote a close apposition with bone like Ti6A14V, where large areas of metal have no direct contact with bone [2, 16].

Degradation of the hydroxyapatite coating is not simply a hydrolytic process; newly formed bone is remodeled in areas were a tight apposition with hydroxyapatite is present [2, 17–19] and, then, the coating itself is likely to be attacked by the resorptive action of multinucleated giant cells and osteoclasts [2, 20, 21]. Presence of sites of far advanced degradation close to a nearly intact coating is often observed in areas of active bone metabolism (deposition and resorption).

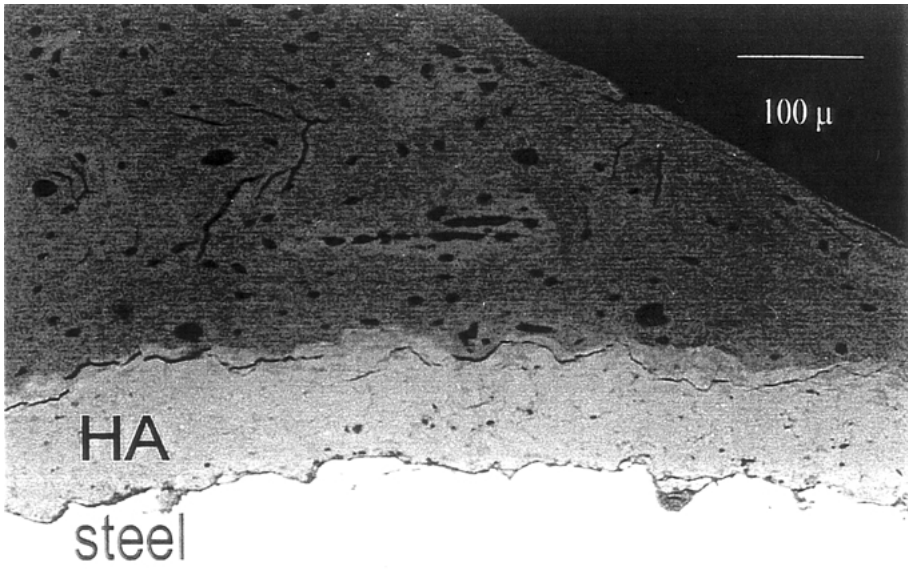

Figure 3 Transverse section which shows the apposition of bone to Hydroxyapatite coated stainless steel (at 34 weeks). Lines of fracture along the coating and at the coating-metal interface are produced in the process of sectioning (BSEM, magnification 150×).

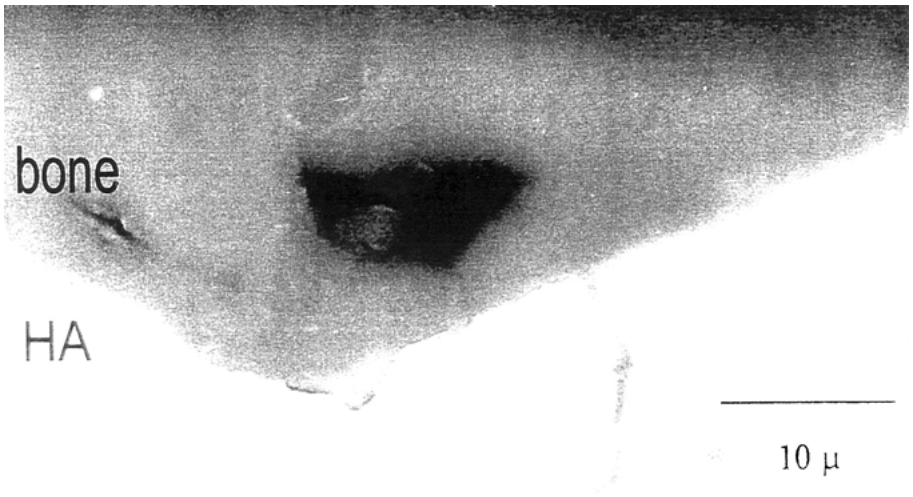

Figure 4 A high magnification back scattered electron microscopic micrograph which shows the tight apposition between newly formed bone and hydroxyapatite coating (at 4 weeks). An osteocytic lacuna is clearly visible in close proximity with the coating; it may likely belong to a former osteoblast which reached the coating surface in earlier times (BSEM, magnification 2200×). The SEM picture of this same field is given in Fig. 5.

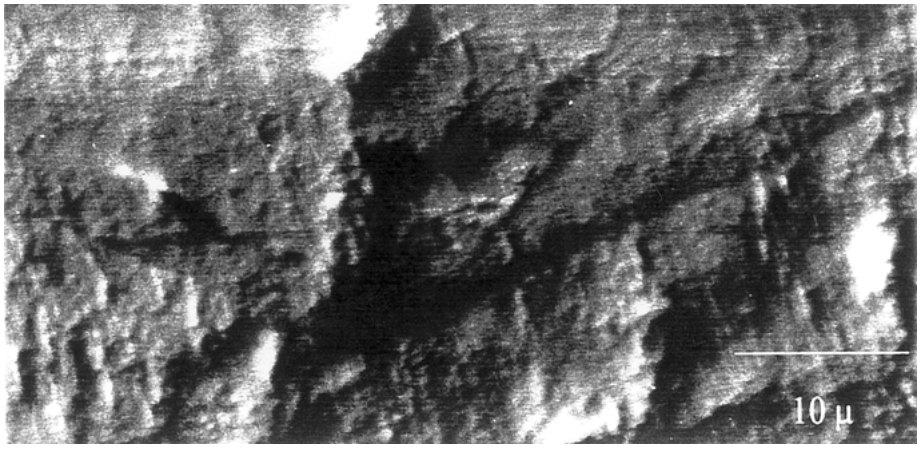

Figure 5 A scanning electron micrograph of the same field of Fig. 4 which shows the tight apposition between bone and hydroxyapatite coating (SEM, magnification 2200×).

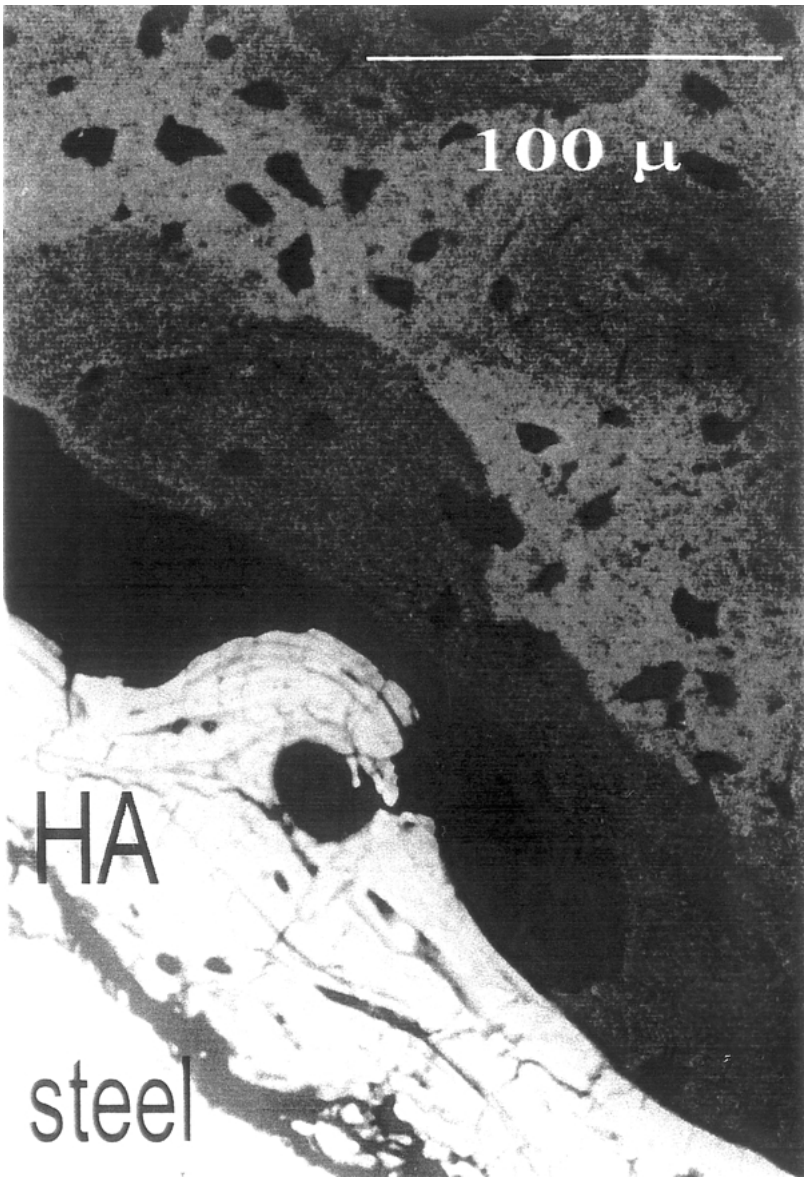

Figure 6 Transverse section which clearly shows characters of bone turn-over (newly-formed bone apposed in areas of bone resorption) in proximity and in apposition to the hydroxyapatite coating (at 26 weeks). Newly formed bone and immature (partially ossified) bone appear in darker shades of gray. Fracture along the coating-metal interface has been produced in the process of sectioning. A bleb is visible on the coating and its morphology may be related to the spraying process or to a subsequent cell-mediated remodeling (BSEM, magnification 360×).

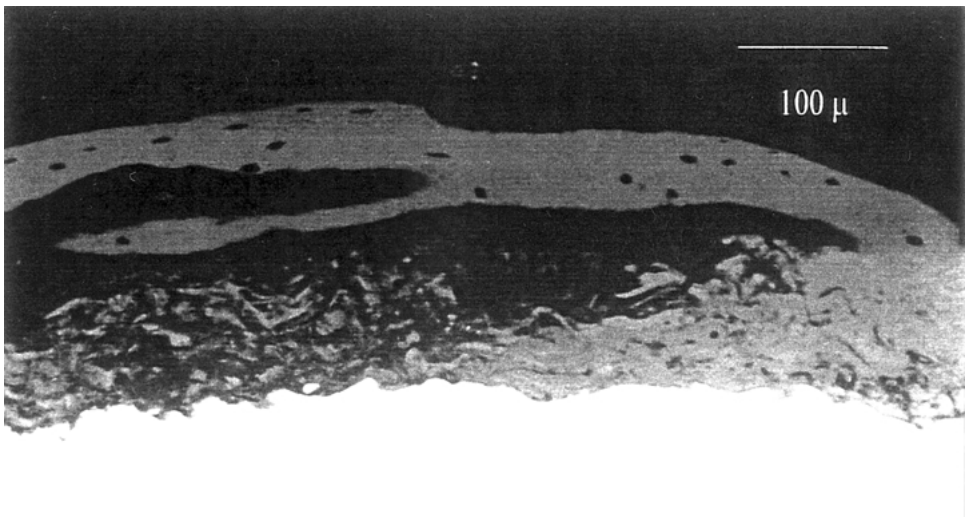

Figure 7 Fragmentation and delamination of the lower crystallinity Hydroxyapatite coating is visible in this transverse section of a specimen retrieved after 34 weeks (BSEM, magnification 150×).

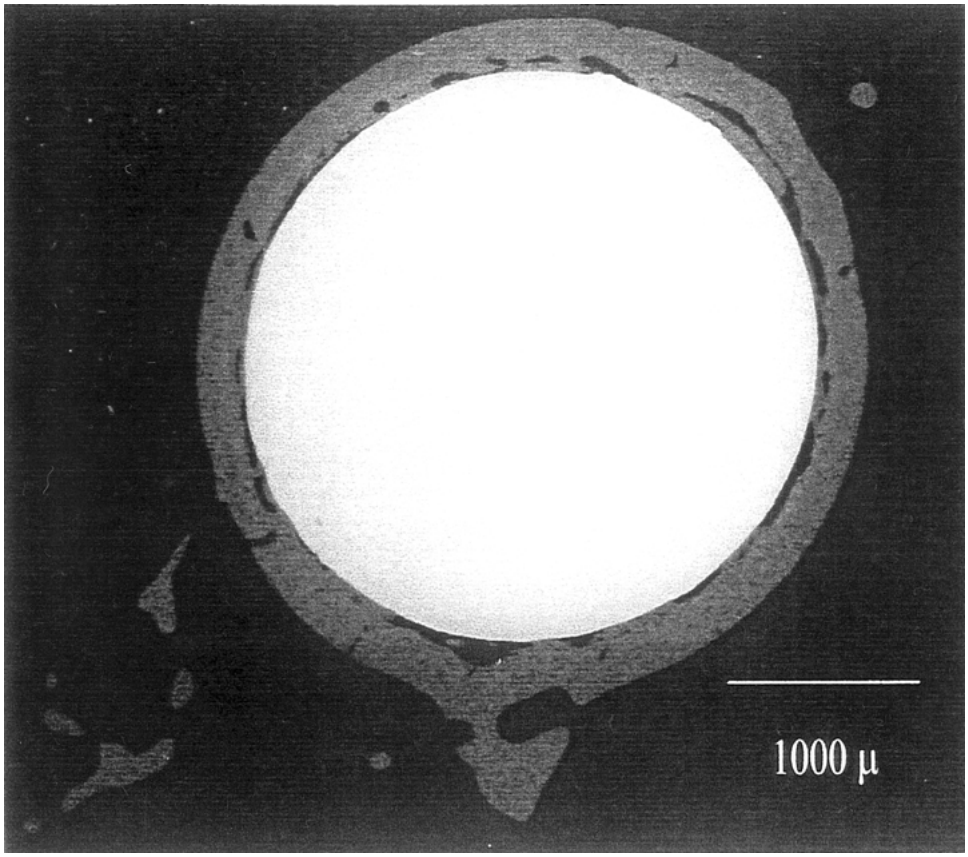

Figure 8 Transverse section which shows an annular rim of bone encircling an uncoated stainless steel cylinder after 34 weeks (BSEM, magnification 15×).

This stresses the importance of local cellular metabolism and seems to claim a role for a mechanism of active cellular remodeling of the coating, probably more relevant than pure hydrolytic dissolution alone.

Crystallinity of the coating is important in relation to its persistence; highly crystalline coatings (with a crystallinity greater than 85%) are prone to be persistent but are quite fragile and produce bulky slow-degradable particles which remain *in situ* [2, 22–24], while very low crystalline coatings may be hydrolytically degraded too fast [2, 25].

The bone response to hydroxyapatite coated samples, since the very early stages [26, 27] has a morphological character of tight apposition [2, 28–38]; this can be

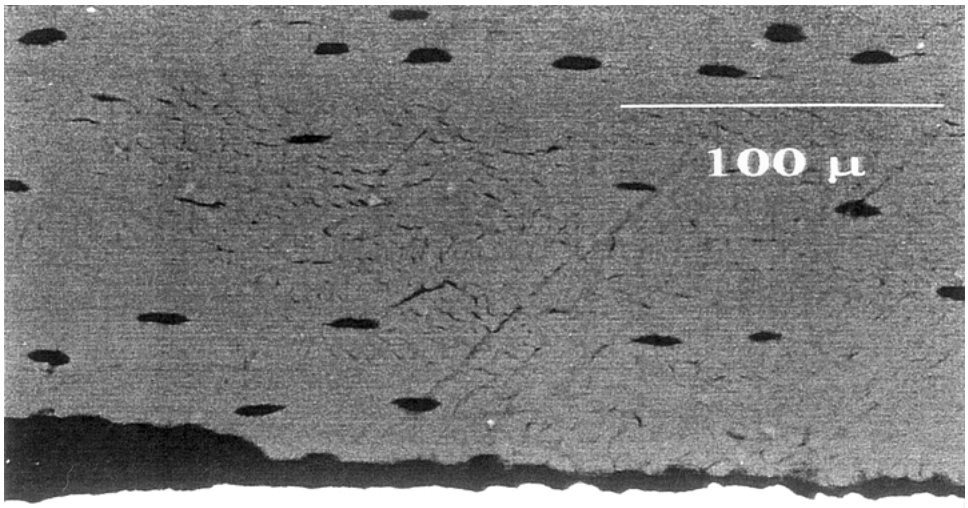

Figure 9 High magnification back scattered electron microscopy clearly shows the presence of an unossified gap between bone and stainless steel (at 26 weeks, BSEM, magnification 390×).

followed by substitution of areas of the coating by newly formed bone.

Histomorphological evidences, reported in this paper, confirm that also with stainless steel as metallic substrate, hydroxyapatite coating favors a better physiological integration of the implant with bone. In this respect it is expected that an hydroxyapatite coated stainless steel implant will perform better, from the biological point of view, in comparison with an uncoated stainless steel implant and this could find useful applications in those clinical situations where a better bone-implant interface is required for a prolonged period of time.

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