Bone-Hydroxyapatite Interface in Retrieved Hydroxyapatite-Coated Titanium Implants: A Clinical and Histologic Report

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Two hydroxyapatite-coated implants were retrieved after 12 months of loading because of a fracture of the abutments. The specimens were treated to obtain thin ground sections, and a microprobe chemical analysis was done under a scanning electron microscope equipped with an energy-dispersive x-ray analysis and cathodoluminescence system. Under light microscopy, close contact between the bone and the hydroxyapatite coating was seen, with no gaps at the interface. In some areas of the coating a reduction of the coating thickness could be observed, along with the presence of some detached hydroxyapatite particles embedded in newly formed bone. The chemical analysis of the cathodoluminescent areas at the interface showed a reduced calcium:phosphorus ratio in this region.
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Key words: cathodoluminescence, hydroxyapatite, interface, osseointegration, titanium implants

Hydroxyapatite (HA) has a high biocompatibility with bone, connective tissue, and epithelium.[1] It usually shows a very intimate contact with bone; the bone tends to grow into the finest irregularities of the crystalline surface.[2] Long-term stability of an HA coating has been questioned, since the possibility of HA detachment and resorption exists.[3–6] An understanding of the nature of the bone-biomaterial interface has been deemed increasingly important in recent years.[7] Different results have been reported about the microscopic and ultrastructural aspects of the bone-HA interface, but the nature of the interface remains to be elucidated.[8] Many of the studies have been carried out in vitro or in experimental animals, and there are few reported histologic analyses of implants retrieved from humans.

The bone-implant interfaces of all materials comprise a so-called bonding zone composed of a calcium- and phosphorus-rich proteinaceous matrix.[8] This bonding zone varies in thickness and can measure up to 1,000 nm.[8] Its morphology resembles the lamina limitans or the cement lines of bone. The bond with HA is so strong that it is very rare to see a detachment or fracture line at this level, whereas fractures often occur inside the layers of HA.[1] The HA layer failure may be correlated to dissolution of the HA coating or to an HA coating–titanium interface fracture.[4]

Van Blitterswijk et al[9] described the formation of an electron-dense granular layer at the bone-HA interface; the presence of a calcium-phosphate salt at the level of this structure could suggest continuity between the HA crystals of the ceramic and

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those of bone. It is not known whether this layer is the result of cellular activity, physiologic processes, or both,[8] but this layer probably plays an important role in the bonding strength between bone and HA.[8,9] The presence, in some areas of the bone HA interface in implants retrieved from humans and from experimental animals, of a basophilic unmineralized tissue similar to the material present in the inner wall of the osteocytes has been described.[10] The aim of this study was to further characterize the bone-HA interface using radiographic microprobe analysis and a cathodoluminescence system in implants retrieved after a 12-month loading period because of abutment fracture and therefore with an undisturbed implant-bone interface.

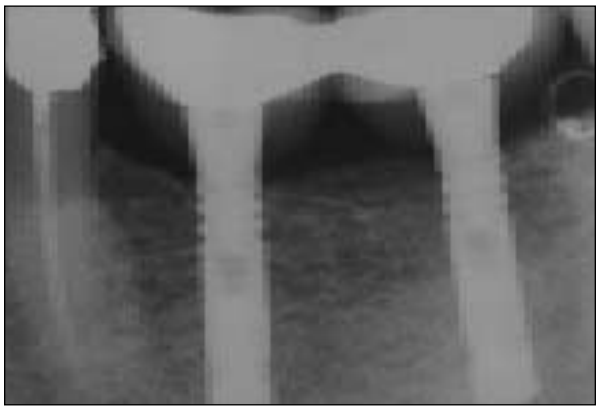


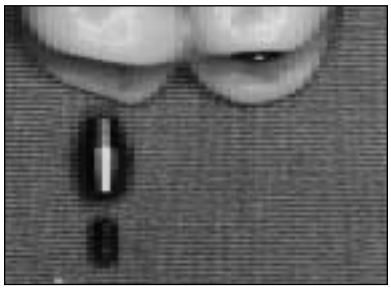
Fig 1 Periapical x-ray after 12 months of loading. No pericoronal resorption is present.

Materials and Methods

Two HA-coated titanium implants (Sustain, Lifecore, Chaska, MN) were placed in the posterior mandible. After 12 months of loading (Fig 1), both abutments fractured (Fig 2), and it was necessary to remove both implants. The implants were retrieved with a trephine, and after fixation, the specimens were sectioned in half longitudinally by the Precise 1 Automated System (Assing, Rome, Italy).[11] One half of each specimen was dehydrated in an ascending series of alcohol rinses and then infiltrated in Technovit 7200 VLC resin (Kulzer, Wehrheim, Germany) and polymerized. The blocks were then sectioned to 200 µm and then ground to approximately 20 to 30 µm. The slides were stained using basic fuchsin and toluidine blue and examined under a Laborlux light microscope (Leitz, Wetzlar, Germany). The other half of each specimen was processed for scanning electron microscopic (SEM) analysis. After the specimens were dehydrated with a critical point dryer and sputtered with a carbon film, a microprobe chemical analysis was performed using a Zeiss DSM 960 scanning electron microscope (Zeiss, Oberkochen, Germany) equipped with an energy dispersive x-ray analysis and cathodoluminescence system.

Results

The macroscopic appearance of the retrieved implants showed that the titanium core was completely covered by the HA coating (Fig 2c). At low-power magnification, a thin layer of tissue



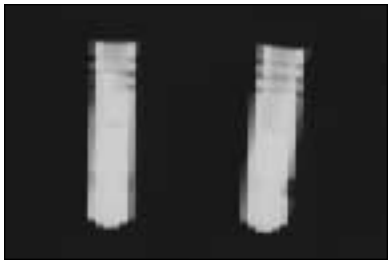


Fig 2a *(Left, top)* Fracture of the abutments.

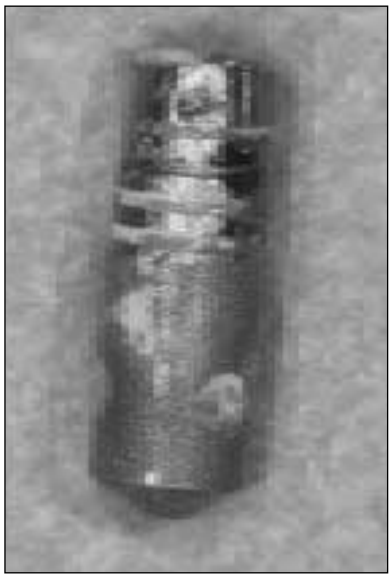


Fig 2b *(Left, bottom)* Radiograph of the retrieved implants. Part of the fractured abutment screws can be observed inside the implants.

Fig 2c *(Right)* Macroscopic aspect of one of the retrieved implants. An organic material is present on the HA coating.

could be seen on the surface of the implant (Fig 3). The fact that bone was present on only one side of the implant was the result of trephine bur action during retrieval of the implant. The incomplete adaptation of the abutment to the inner portion of the implant could be observed, and biologic tissues were interposed between the 2 structures. Intimate contact between HA and bone was seen around almost all of the implant perimeter, without the presence of a connective tissue capsule or of an inflammatory reaction (Fig 4). Macrophages and inflammatory cells were absent. Some small (20- to 100-µm) HA fragments had detached from the surface of the coating and they were completely surrounded by newly formed bone (Fig 5a). In only a few areas was it possible to observe a reduction in the thickness of the HA coating (Fig 5b).

No fractures were present inside the coating thickness. Basic fuchsin–positive material was present inside the coating; this material had a granular or lamellar appearance. Secondary electron images obtained by SEM showed a direct connection between bone and HA, and it was impossible to distinguish any boundary without backscattering (Fig 6). Chemical analysis of the cathodoluminescent areas present at the bone-HA interface was executed (Fig 7). The radiographic analysis showed a reduced calcium:phosphorus ratio in this zone (Fig 8).

Fig 3 *(Left)* Low-power magnification. Tissue is present around most of the implant (basic fuchsin and toluidine blue; original magnification ×30).

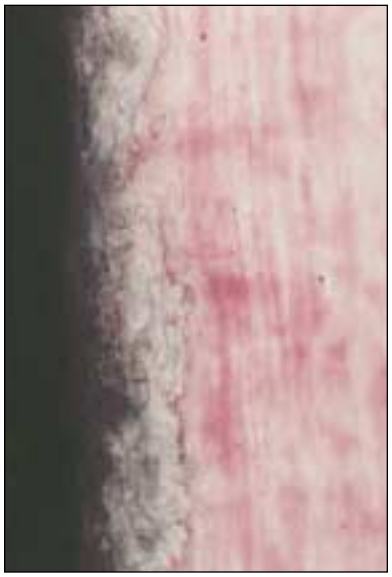


Fig 4 *(Right)* The implant is in contact with mature lamellar bone (basic fuchsin and toluidine blue; original magnification ×400).

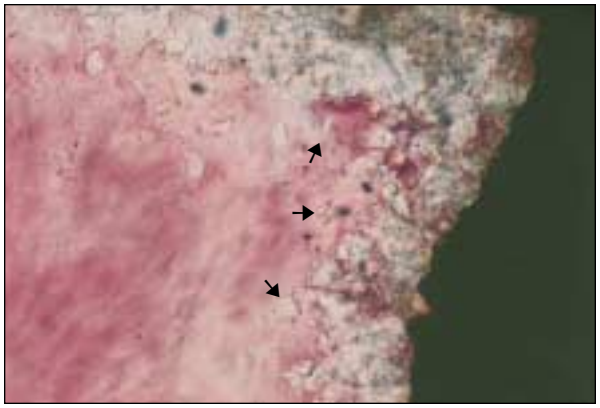


Fig 5a In some portions of the interface a resorption of the HA coating can be seen. Some detached HA particles are found embedded in newly formed bone *(arrows)*. Biologic material is present inside the coating (basic fuchsin and toluidine blue; original magnification ×400).

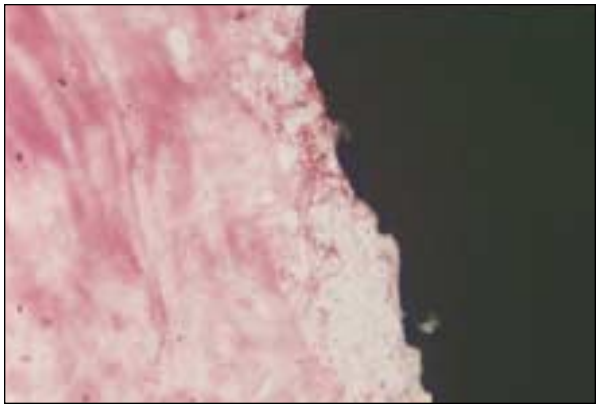


Fig 5b In some areas of the interface, a reduction of the thickness of the coating is present (basic fuchsin and toluidine blue; original magnification ×400).

Discussion

Problems with the use of HA coatings have been reported and concerns have been raised, especially about what might happen if the coatings came off.[6] Caulier et al,[12] on the other hand, found that, in an experimental study in goats, in areas where the coating was completely absent, close contact of the bone with the titanium surface was present. Bauer et al[6] found a minimal loss of HA to extracellular fluids. In contrast to the data reported by Gottlander et al,[13] who found soft tissues around loose fragments of calcium phosphate, in the specimens under consideration, all the small detached HA particles were always surrounded by newly formed bone and no untoward effects were observed.

Cathodoluminescence is the emission of light under electron beam bombardment.[14] The results of the chemical analysis of the interface of these specimens are similar to those reported by Kayser et al,[7] who, using radiographic microanalysis, found a reduced calcium:phosphorus ratio in the areas adjacent to the collagen matrix. Okumura et al[15] also found continuous levels of calcium and phosphorus at the bone-ceramic interface and a gradual decrease of these levels in the osteoid region.

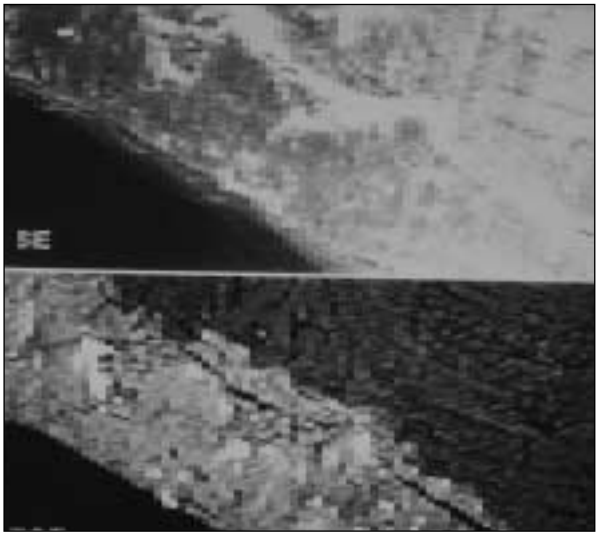


Fig 6 Backscattering revealed that no gaps were present between the bone and the coating. SE = secondary electrons.

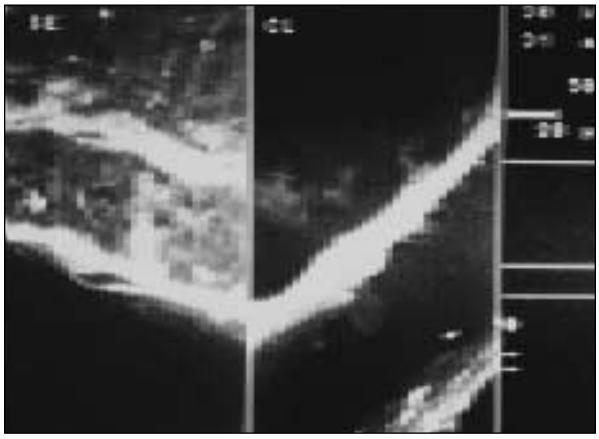
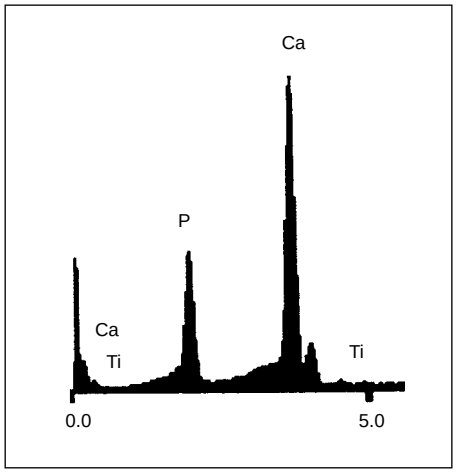
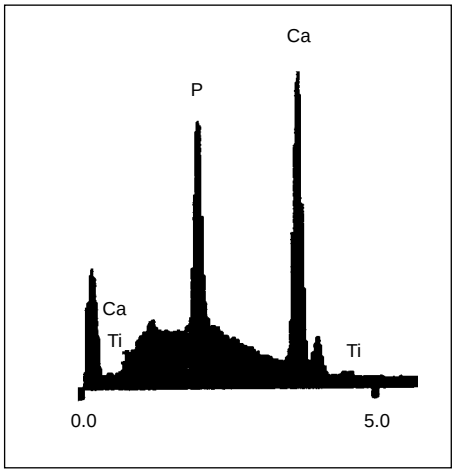


Fig 7 A cathodoluminescent line was present between the HA coating and the bone. SE = secondary electrons.





Figs 8a and 8b Radiographic analysis of the cathodoluminescent line. It is possible to see a reduced calcium:phosphorus ratio in this zone *(left)* as compared to the normal bone *(right)*.

Garcia and Doremus[16] showed a strong bone-HA bond in a retrieved human implant. This bond could possibly be the result of a coordination of negatively charged carboxylate groups on the collagen of the bone to exposed, positively charged calcium ions on the apatite surface. De Lange et al[2] reported that at the ultrastructural level they observed no direct contact between bone crystals and HA crystals, and the 2 structures were interconnected by a nonmineralized bone matrix. Collagen fibers of bone were seen within about 200 nm of the implant surface, while the bone closer to the implant showed a more amorphous aspect and no collagen fibers.[1] Tracy and Doremus[17] found that bone grew onto the ceramic surface without the interposition of an unmineralized tissue. Kawaguchi et al[18] reported the existence of a distinct mineralized zone separating the HA particles from the bone matrix. In this zone an osteopontin positivity was found using immunocytochemical and lectin-gold characterization.[18] Osteopontin could play a role in the regulation of mineralization events.[18,19]

The differences found in the various ultrastructural studies could be related to the very small area of the interface that can be examined with transmission electron microscopy. The morphology of the bone-HA interface probably differs around the perimeter of the same implant. In this respect de Bruijn et al[8] described in vitro 3 different interfacial structures: *(1)* a collagen-free amorphous zone 0.7 to 0.8 μm wide, *(2)* a glycosaminoglycan-rich, electron-dense layer 20 to 60 nm thick between HA and the mineralized matrix, and *(3)* collagen fibers directly deposited on the HA surface.

Summary

The following features were present in the specimens examined in this investigation:

1. A tight contact was present between bone and HA and no gaps were present at the interface.
2. Bone covered almost all the HA coating.
3. The HA coating was uniformly thick around most of the implant perimeter.
4. In only a few areas a reduced thickness of the coating was present, with some HA particles detached and embedded in newly formed bone.
5. A reduction in the calcium:phosphorus ratio was present at the interface.

After 12 months of loading, no degradation, resorption, detachment, or fracture of the coating was observed, but longer-term histologic studies of HA coatings are needed to evaluate their performance.[20]

Acknowledgments

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