Histomorphometric Analysis of a Half Hydroxyapatite-Coated Implant in Humans: A Pilot Study

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The aim of this study was to compare the characteristics of the bone-to-implant interface of hydroxyapatite-coated and non-coated commercially pure titanium threaded implants after different periods of healing in humans. To eliminate possible variations of the results from differences in bone quality and in surgical techniques used in the different test and control sites, only one half of each implant was coated with hydroxyapatite. The coated portions of the implants showed a tendency toward a higher percentage of direct bone-to-implant contact at each period of healing that was observed, although the small number of specimens does not allow definitive conclusions to be made.
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Key words: hydroxyapatite, osseointegration

Several studies have demonstrated that the treatment of partially and completely edentulous patients with osseointegrated implants represents a predictable procedure characterized by a high rate of success on a long-term basis.[1,2] Commercially pure (cp) titanium has been the most widely used material[3] because of its well-documented biocompatibility and successful long-term results. The favorable bone tissue response to cp titanium appears to depend on the oxide layer, consisting mainly of TiO_2, that covers the metal surface.[4]

In the last decade, various investigators[5–7] have proposed the use of hydroxyapatite- (HA) coated implants to increase the percentage of direct bone-to-implant contact and to reduce the time required for unloaded bone healing. As with other calcium phosphates, HA is characterized by bonelike composition and structure[8–10] and is highly biocompatible with hard and soft tissues.[8] Most investigations comparing HA-coated implants to non-coated titanium controls have demonstrated more favorable results for the HA-coated implants with regard to faster and stronger bone response.[5,11,12] However, some studies have found no significant difference between HA-coated implants and uncoated titanium controls.[13–16]

Results of the above-mentioned studies do not allow clinicians to draw definitive conclusions about differences between coated and uncoated implants in terms of bone response. Some studies have considered the HA coating to be different mixtures of calcium phosphates, others have compared HA-coated implants with titanium alloys and not cp titanium,[11,12] and most have considered only cylindric implants. Hayashi et al[6] followed HA-coated screw-shaped implants for a period of 96 weeks and by histomorphometric analysis demonstrated maximum contact to HA-coated implants after 8 weeks. Compared to the titanium nitrate–coated implants that were used as controls, there were higher percentages of direct bone contact in the HA-coated specimens. No comparison was made with cp titanium controls. In a series of comparative studies in rabbits, Gottlander and coworkers[17,18] demonstrated that HA-coated cylindric implants provided an improved bone response after 6 months in a histomorphometric evaluation. The effect of HA coating on threaded implants and after longer periods of healing has not been determined.

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Fig 1 Implant used for this study. A custom implant, half of which was coated with HA, offered the possibility of analyzing both the HA-coated and the uncoated portion in the same implant site.

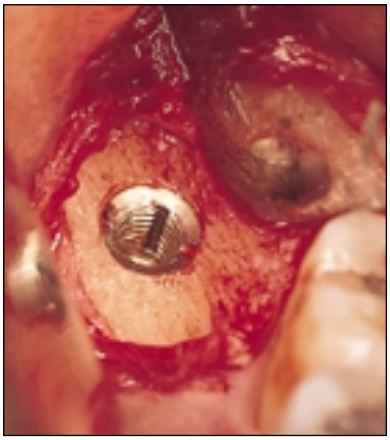


Fig 2 Surgical stage. Implants were placed in the retromolar area.

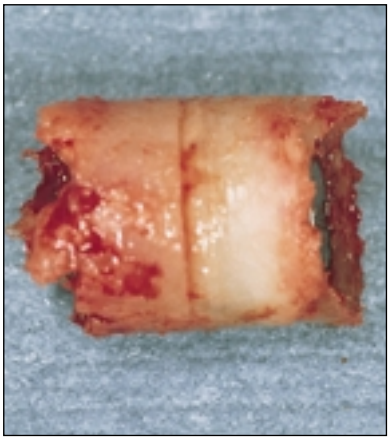


Fig 3 After the respective healing period, implants were removed in a block section that included the surrounding bone.

Most of the comparative studies present in the literature[6,7,17–19] have been performed in animal models, such as rabbits or dogs. The results may be extrapolated to humans with caution, since the timing and healing pattern of bone tissue may be significantly different. Moreover, many of the existing studies involving HA-coated and uncoated cp titanium surfaces have been compared in different implant sites located in different areas of the jaws or in long bones. This could introduce some variations in results, depending on different bone quality in different locations.

The aim of the present study was to compare the characteristics of the bone-to-implant interface of HA-coated and non-coated cp titanium threaded implants after periods of 1, 3, 6, and 12 months of unloaded healing in humans. To eliminate possible variations in the results from differences in bone quality and surgical techniques used in the different test and control sites, implants that had been HA-coated on only one half of the surface were used. This approach had been used in an experimental rabbit study by Wennenberg et al using implants blasted on only one half of the surface.[20]

Materials and Methods

Subjects. Four subjects volunteered for the study. Three were investigators performing the study and the fourth was a volunteer, who was informed about the intent of the study. All subjects were in good general health. Their ages ranged between 30 and 50 years (mean 43). The period of experimentation for each subject was determined randomly. The experimental design was approved by the Ethical Committee of the University of Milan.

Implants and Surgical Technique. Each subject received 2 threaded implants, each of which had an HA coating on one half of its surface. The implants (Fig 1) had been designed especially for this histologic study (Saber Tec, Genetic Implant System Inc, Seattle, WA) using a pure titanium substrate with an HA coating that was plasma-sprayed to separate the device into 2 parts along the longitudinal axis of the screws. The screws had a diameter of 3.75 mm and a length of 7 mm. The thickness of the coating was approximately 50 to 70 μm.

Surgery was performed under aseptic conditions. The volunteers received prophylactic antibiotic treatment with amoxicillin (1 g every 12 hours for 7 days). After site preparation was carried out, using segmental drills at low rotary speed with profuse saline irrigation, following the instruction of the manufacturer, the implants were placed. Because of the experimental design, the implants had to be placed in such a way that no permanent damage would be suffered by the volunteers. In one subject, who was totally edentulous, the implants were placed in the mandibular symphysis; in the other subjects, implants were placed in the third molar area of each side of the mandible (Fig 2).

Preparation of Specimens and Histologic Evaluation. Two implants were removed from each subject simultaneously, respectively, after 1 month, 3 months, 6 months, and 12 months. The removal was carefully carried out using a 5-mm trephine drill with profuse saline cooling (Fig 3).

Table 1 Histomorphometric Results

	Mean percentage of bone-implant contact			
	1 month	3 months	6 months	12 months
Uncoated portion	43.76 (range 48.81 to 44.71)	37.37 (range 50.37 to 24.37)	56.59 (range 50.26 to 62.92)	70.70
HA-coated portion	80.97 (range 91.81 to 70.13)	91.73 (range 96.43 to 87.03)	70.62 (range 81.01 to 60.23)	95.01

The specimens were fixed in 10% neutral buffered formalin, dehydrated in an ascending series of alcohols, and embedded in an experimental hydrophilic methylmethacrylate resin (Remacryl, Istituto di Microscopia Elettronica Clinica, Sant'Orsola Hospital, Bologna, Italy). They were cut with a Micromet sawing machine (Remet, Bologna, Italy) to a thickness of 100 µm and then ground in an LS-2 grinding machine (Remet) to a thickness of approximately 30 µm. The specimens were then stained with toluidine blue–light green.

Histomorphometric analysis was performed by calculating, using a rectangular integrating grid eyepiece with horizontal and vertical lines, the length of the surface of the implant covered by bony trabeculae over the total length of the implant surface. These measurements were made with a 10× objective in all fields of each specimen, by counting the number of intersections over the implant surface.[21,22] For each biopsy, 3 sections were analyzed. Finally, the results were expressed as a percentage of the implant surface covered by bone over the respective half of the implant surface representing either HA or titanium. These measurements were made on each specimen and the results were grouped into the 4 time groups.

Results

Clinical Observations. One site (12-month volunteer) in the retromolar region developed an abscess 2 weeks after the implant surgery. After 1 day of antibiotic therapy, the site was reopened and the implant was removed. At the reopening, the implant appeared unstable and was surrounded by granulation tissue. The retrieved implant showed no evidence of coat resorption or loosening. According to the random experimental design, this site was intended to be explanted and analyzed after 1 year of healing. However, since the implant was removed, there was only one 1-year follow-up observation. In all other sites, healing was uneventful, the implants were stable, and no signs of adverse tissue reactions or inflammation were noted at the time of reentry surgery.

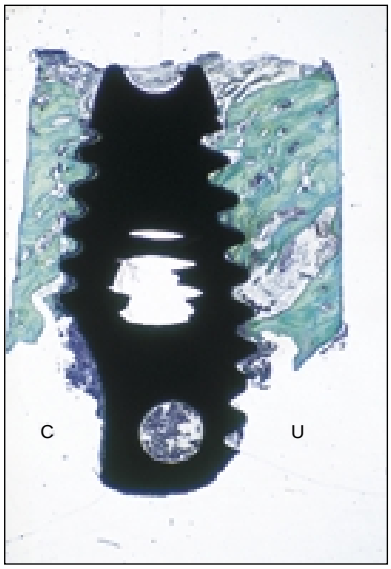
Histomorphometric Analysis. *Observation at 1 Month.* The percentage of bone-implant contact was 80.97% on the coated side of the implants and 43.76% on the cp titanium portion (Table 1, Fig 4). Because of the presence of bone debris created during the implant retrieval, an accurate evaluation of the specimens was difficult. Nevertheless, careful observation showed remarkable osteoblastic activity characterized by large portions of osteoid tissue all around the implant, most prominently on the coated portion (Fig 5). On the coated portion of the implant, a regular band of osteoid cells apposing a bone matrix could be detected. They were less regular on the uncoated portion.

On the coated side, osteoblastic activity occurred from both implant and peri-implant bone sides. This permits faster osseointegration and is a typical observation in specimens involving HA-coated implants.

On the cp titanium side, osteoblastic activity ran from the peri-implant bone side only. The implant profile differed between sections because of the apical groove in each implant. This groove is not present all around the implant apex, and thus it does not appear in every section.

Observation at 3 Months. This group of specimens showed the biggest differences between the coated and uncoated sides of the implant (Fig 6). The percentage of bone-implant contact in uncoated implants was 37.37% while HA-coated implants showed 91.73% contact.

In the cp titanium specimens, bone penetration toward the depths of the threads was nearly complete in only the cortical portion of the implant bed. The bone inside the threads was lamellar bone, and it was possible to see primary osteons (Fig 6c). In the spongeous bone, only small trabeculae were in contact with the uncoated titanium surface. The HA was in direct contact with the bone, even in areas of low bone density. Neverthe-



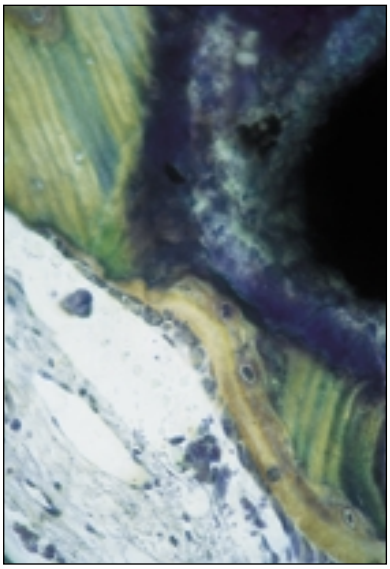


Fig 4 *(Left)* Specimen after 1 month of healing. The presence of the coating material favorably influenced the host bone response. This fact was confirmed by the histomorphometric analysis (original magnification ×6). U = uncoated portion; C = coated portion.

Fig 5 *(Right)* Observation after 1 month of healing. A regular layer of osteoblastic cells was apposing new bone matrix directly on the implant surface. This aspect was seen only in the coated portion of the implant (original magnification ×200).

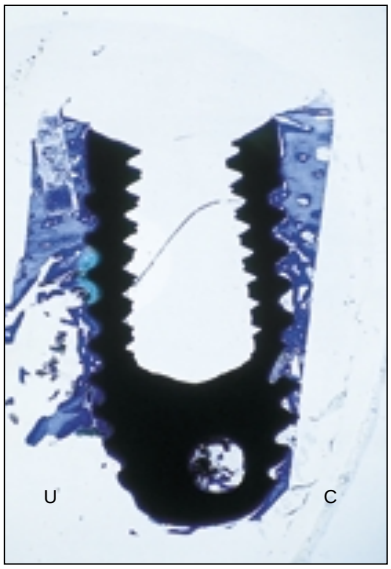


Fig 6a Specimen after 3 months. In this section, a higher percentage of direct bone-implant contact was seen in the coated portion (C) of the implant. This occurred at every time point of observation (original magnification ×6). U = uncoated portion.

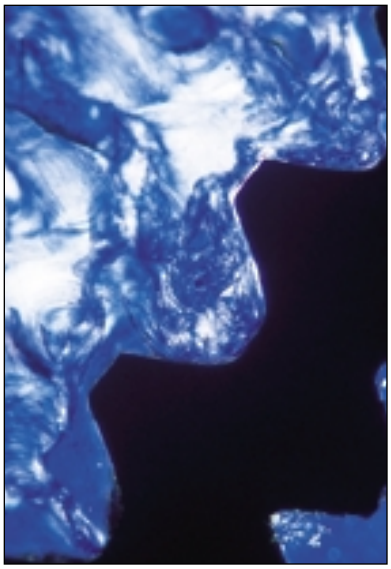


Fig 6b Observation after 3 months, coated portion of implant. The higher degree of bone disorder (ie, woven bone) was the expression of a more rapid proliferation and remodeling of the bone in contact with HA (polarized light, original magnification ×40).

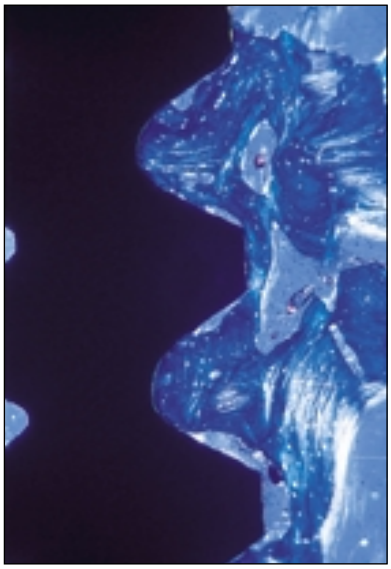


Fig 6c Observation after 3 months, uncoated portion of implant. More lamellar bone was seen than in the coated portion (polarized light, original magnification ×40).

less, the degree of bone maturation was obviously lower in the spongious bone than in the cortical portion. It was possible to notice wide areas of woven bone at the interfacial level (Fig 6b). In many of the specimens, areas of reduction in thickness or of complete resorption of the HA coating were found.

Observation at 6 Months. The percentage of direct bone-implant contact was 56.59% for uncoated and 70.62% for HA-coated portions (Fig 7). The bone-implant interface was similar to the 3-month observation. On the titanium side of the implant, contact depended on the bone density, in the sense that contact occurred predomi-

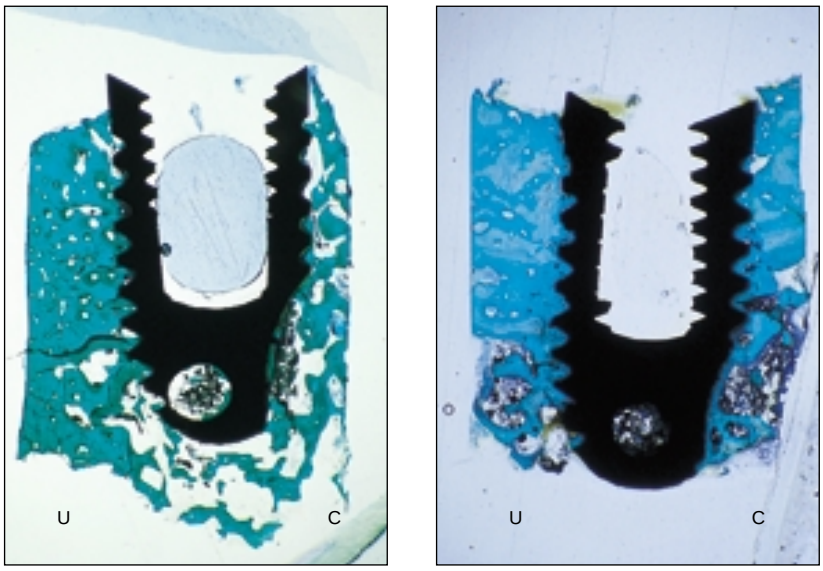


Fig 7 (*Left*) Specimen at 6 months. The difference between the coated (C) and uncoated (U) portion decreased at this time observation, although a greater percentage of bone-to-implant contact in the coated portion of the implant still existed (original magnification ×6).

Fig 8 (*Right*) Specimen after 1 year. An almost complete contact between bone and implant could be seen in both portions, even if from a quantitative point of view the coated portion (C) showed a higher percentage of direct contact to bone (original magnification ×6). U = uncoated portion.

nantly in the cortical portion of the bone. The HA side of the implant was almost completely covered by bone.

In the HA portion of the specimens, the woven bone had been completely remodeled into lamellar bone. In contrast, in the titanium portion, areas of woven bone still existed. Areas of lamellar bone could also be found in the uncoated portion, even if they were less common than in the coated portion of the implant. In this specimen, areas of resorption in the thickness of the coating could be seen. The degree of dissolution of the coating was similar to that in the 3-month specimens.

Observation at 12 Months. The percentage of direct bone-implant contact was 70.7% for uncoated portions and 95.01% for HA-coated portions (Fig 8). In the cortical portion of the bone bed it was possible to see almost total contact between the bone and the implant on both sides of the specimens, while in the cancellous portion, only the HA-coated portion showed a similar appearance. The bone demonstrated complete remodeling, since no other areas of woven bone could be seen.

The most important difference between the surfaces was the presence of small "cutting cones on the titanium side, which slightly reduced the percentage of contact. These cones were almost nonexistent at the HA bone interface, although they were present at some distance from the interface. However, in some specimens, contact was almost complete, even in the titanium portion of the implant.

Discussion

This pilot study was designed to analyze the bone-implant interface characteristics in humans of HA-coated and uncoated cp titanium implants after different healing periods. Most previous studies[6,12,13,17,18,23] have investigated the behavior of HA-coated and uncoated implants, making histomorphometric comparisons of the 2 different interfaces placed in different implant sites in animal models. The comparison of specimens from different surgical sites could introduce uncontrolled variables because of different bone quality and possible differences in surgical trauma from site to site. To diminish the influence of these conditions and to provide more direct evidence of the interface differences, a customized cp titanium implant that was HA-coated on only half of its surface was used. This implant design provided the opportunity to compare both surfaces in the same implant site.

The results of the present study demonstrate a tendency toward a higher percentage of direct bone-to-implant contact for HA at each period of healing considered. However, the differences lessened from the 1-month to the 12-month observations. This is in accordance with previous similar studies in animals. Soballe et al[23] compared HA-coated implants with titanium plasma-sprayed cylindric implants in osteopenic-induced bone tissue in dogs. The histologic examination demonstrated better bone response in the HA-coated specimens. Similar results were seen by Gottlander et al[17] in a histomorphometric analysis in the

femurs of rabbits after 6 months of healing. However, in another study of rabbit radial metaphyses, Gottlander and Albrektsson[24] compared screw-shaped HA-coated and uncoated cp titanium implants obtaining different results. In fact, while the 6-month observation showed a higher percentage of direct bone-implant contact in the HA-coated specimens, the 12-month observation specimens showed better results for the cp titanium control group. In a subsequent similar investigation, Gottlander and Albrektsson[18] observed no substantial differences in bone-implant contact after 6 months of healing. The authors concluded that "the effect of HA coating seems to be uncertain with regard to threaded implants in contrast to unthreaded cylindrical design."

The differing results between the present and aforementioned studies could be ascribed to healing periods considered in different species. In fact, 12 months in humans could be considered to be a healing period relatively shorter than 6 or 12 months in rabbits, whose potential for healing of bone tissue is considerably higher. A longer period of observation in humans will be necessary to draw definitive conclusions. Moreover, long-term comparative studies on loaded implants should be performed. Results of the present study suggest in human beings what had been demonstrated in experimental models, namely, that there is a progression of direct bone-to-implant contact around uncoated titanium implants from the third month on. The HA coating seems to favorably influence the rapidity of bone growth toward the implants. The comparison between uncoated titanium and HA-coated surfaces was particularly interesting at the 3-month observation period, where the largest difference between the 2 groups of specimens (37% vs 80%) was seen. The maturation of the growing bone suggested important considerations. The lack of woven bone at 6 months on the HA-coated side and its presence at 3 months on the uncoated side indicated more rapid growth and differentiation of bone near the HA. This fact underlines the positive influence of an HA coating, both from quantitative and qualitative points of view.

An important modification of the implant surface created by the plasma-spraying process was the alteration of surface roughness, a fact that could alter the bone response regardless of other factors.[20,25] The roughness of the surface of an HA plasma-sprayed implant is 3 to 4 times that of machined metal implants.[26] The surface topography of a coated implant is approximately 2 to 8 times rougher than that of uncoated specimens.[6] This surface modification could partially explain the difference between the bone response to the different sides of the implant. The small number of implants placed in this study does not permit statistical analysis of the results.

From the third month observation period on, the specimens showed different degrees of coating resorption and dissolution. Similar results were published in a study by Jansen et al,[15] in which the authors demonstrated loosening of the HA coating after 6 weeks and a marked reduction in coating thickness 16 weeks after surgery. According to the manufacturer, the problems found in coating only half of the surface could be eliminated in part with full coverage of the surface, as in clinical applications.

The advanced resorption of HA coating was observed in the boundaries between the crystals of HA and was mainly the result of the quality of the plasma-spraying process. As a consequence of the plasma spraying and the high temperature reached during this process, the final coating is composed of HA granules melted together with amorphous hydroxyapatite, tricalcium phosphate, and tetracalcium phosphate. These compounds are not as resistant as HA to dissolution; this leads to a rapid disintegration of the granules and finally to a rapid resorption of the HA coating, depending on fluid-mediated dissolution and cell resorption.[27,28]

Conclusions

With all the limitations associated with the small number of implants placed, the tendency toward better results for the HA-coated half of the implants placed persists even at 1-year observations for the unloaded implants. The particular study protocol, which was carried out in human subjects and not in an experimental model, did not permit the use of a larger experimental group. The results of adding HA coating to screw-shaped implants seem to be encouraging, even if further investigations are needed to provide conclusive evidence in the long-term perspective (3 to 5 years) under loading conditions.

Acknowledgments

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