In Vivo Evaluation of Hydroxyapatite Coatings of Different Crystallinities

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Purpose: *The influence of calcium phosphate (CaP) and hydroxyapatite (HA) crystallinity on bone-implant osseointegration is not well established. In this study, the effect of HA crystallinity and coating method on bone-implant osseointegration was investigated using a rat tibia model.* **Materials and Methods:** *HA coatings 1 to 5 μm thick were produced using a supersonic particle acceleration (SPA) technology. The HA crystallinities used for this study were weight ratios of 30%, 50%, 70%, and 90%. A total of 128 HA-coated implants were placed into the tibiae of 64 male Sprague-Dawley rats. Bone-implant interfaces were evaluated using histology and push-out strength testing at 3 and 9 weeks after implantation.* **Results:** *The 70% crystalline coatings exhibited significantly greater interfacial strength (5 implants/time point/treatment) than the 30%, 50%, and 90% crystalline coatings at 3 and 9 weeks following implantation. The implants with coatings of 70% crystallinity also had the greatest bone contact length. In addition, the HA coatings produced with SPA demonstrated greater interfacial strength and bone contact length than plasma-sprayed HA coatings (except for the HA coating with 30% crystallinity).* **Discussion:** *HA coatings of different crystallinities exhibited different dissolution and re-precipitation properties which may enhance early bone formation and bone bonding.* **Conclusions:** *This study suggested that coating crystallinity and coating methods can influence the bone-implant interface.*
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Key words: *animal models, crystallinity, hydroxyapatite, interfacial strength, supersonic particle acceleration*

Hydroxyapatite (HA) and calcium phosphate (CaP) coatings have been successfully used in dental and orthopedic implant therapies.[1–4] The rationale for using HA or CaP coatings is to accelerate bone formation and improve the strength of the bond between metal and bone.[5] Extensive in vitro and in vivo research has suggested that the crystallinity of CaP and HA coatings is essential to their biocompatibility and early performance when compared to noncoated titanium implants.[6–11] In a study by Overgaard and associates, HA-coated titanium implants with 50% crystallinity achieved greater mechanical fixation compared to HA-coated titanium implants with 75% crystallinity 16 weeks after implantation.[8] It was also observed that fixation of HA implants with 75% crystallinity increased from 16 weeks postimplantation to 32 weeks postimplantation, whereas fixation of HA implants with 50% crystallinity remained unchanged.[8] Thirty-two weeks following implantation, no difference between HA implants with 50% and 75% crystallinity was observed. No significant differences in extraction torque values were found between screw-type HA-coated implants with coating crystallinity of 70% and those coated with HA with a crystallinity of 40% at 4, 12, and 48 weeks.[9] However, the extraction torque values of HA-coated screw-type implants at 12 and 48 weeks were significantly increased compared to those at 4 weeks.[9]

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In a comparative investigation using a canine model involving 36 endosteal dental implants either uncoated or coated with 20-μm-, 40-μm-, or 50-μm-thick HA, bone adaptation on uncoated implants (53.9%) and on 20-μm-thick HA-coated implants (54.2%) was significantly higher compared to bone adaptation on 50-μm-thick HA-coated implants (47.5%) at 4 weeks.[12,13] However, no difference in bone adaptation was observed between uncoated implants, 20-μm-thick HA-coated implants, and 50-μm-thick HA-coated implants 12 weeks after implantation.[12,13] Since implant surfaces reported in these studies were not fully characterized, these observations have led to the suggestion that there has been a general lack of appreciation for the effects that variation in the chemical and physical characteristics of the coating may have on early bone cell activity, and how the early biologic events may influence long-term success. As a result, no consensus exists on the optimum HA coating crystallinity required for an optimum rate of development of osseointegration.

In the present study, the effect of HA coatings with different crystallinities on the interfacial strength and morphology at the bone-implant interface was investigated using a rat model. Because of the favorable biologic properties of HA and CaP, there is substantial interest in continuing the investigation of various coatings such as those produced by the low-temperature deposition process. The purpose of this investigation was to evaluate the efficacy of thin film HA coatings with different crystallinities, produced with a low-temperature deposition process, on implant-bone interfacial strength and morphology.

MATERIALS AND METHODS

Materials

Rods of grade 2 titanium (President Titanium, Hanson, MA) 3.2 mm in diameter were machined to produce cylindric implants 1.8 mm in diameter and 2 mm in length. These cylindric implants were first sandblasted using 80-grit alumina powder (BCS, Thompson, CT) using a Microblaster (model MB-1000-1, Comco, Burbank, CA), with a nozzle with a 1.17-mm-wide orifice diameter and a gauge pressure of 80 psi using pressurized nitrogen gas. The nozzle was held approximately 5 cm from the implant surface. A surface roughness (Ra) of approximately 4 μm was produced. The sandblasted implants were then ultrasonically degreased for 10 minutes in acetone, rinsed with deionized water, and degreased for 10 minutes in ethanol. Passivation was accomplished by exposing the samples to a 40% nitric acid solution at room temperature for 30 minutes (ASTM F86-76), followed by rinsing with deionized water and air drying.

The cleaned implants were divided into 5 groups. One group was left uncoated. Each of the 4 remaining groups was coated with an HA coating using a unique supersonic particle acceleration (SPA) technique. HA powders of different crystallinities were accelerated and propelled under pressure through a small nozzle onto the implant surface. The particle velocity was controlled by varying the gas pressure from 40 to 200 psi. Coating thickness was in the range of 1 to 5 μm. The weight ratios of the HA crystallinities used for this study were 30% (HA1), 50% (HA2), 70% (HA3), and 90% (HA4). Crystallinities were confirmed using x-ray diffraction. Since the coatings were thin, the surface Ra of the coatings was dependent on the surface roughness of the substrate. Measurements indicated no difference in the Ra values (5 μm) after coating. Prior to implantation, tensile (ASTM F1147) and shear (ASTM F1658) tests indicated coating adhesion strengths of 35.5 and 17.3 MPa, respectively. In addition, plasma-sprayed HA-coated samples (APS Materials, Dayton, OH) were used as controls. The manufacturer reported that the crystallinity of its plasma-sprayed HA coatings ranged from 61% to 70%, with an average thickness of 50 μm.

Implantation

Sixty-four male Sprague-Dawley rats weighing about 250 to 300 g apiece were used for this study. All animal experiments were in compliance with US Department of Defense programs and US National Institutes of Health publication 86-23, *Guide for the Care and Use of Laboratory Animals.* Appropriate considerations were given to all policies, standards, and guidelines governing the proper use, care, handling, and treatment of animals. The study was approved by an institutional review board.

The rats were anesthetized with a ketamine/xylazine/acetylpromazine cocktail (8.5/1.7/0.2 mg/kg body weight) and administered intramuscularly using 0.1 mL of cocktail per 100 g body weight. Under anesthesia, the tibia bone surface was carefully exposed. After dissection of the periosteum, 2 transcortical holes were formed at intervals of 4 mm by drilling with a slow-speed (500 rpm) dental handpiece equipped with a 1.8-mm trephine bur. Profuse irrigation with physiologic saline was maintained throughout the drilling. Cylindric implants were randomly placed into each of the surgically prepared holes by tapping with a mallet until the top of the implant was flush with the cortical bone surface. A total of 128 implants were used. Three and 9 weeks following implantation, the animals were euthanized using carbon dioxide inhalation.

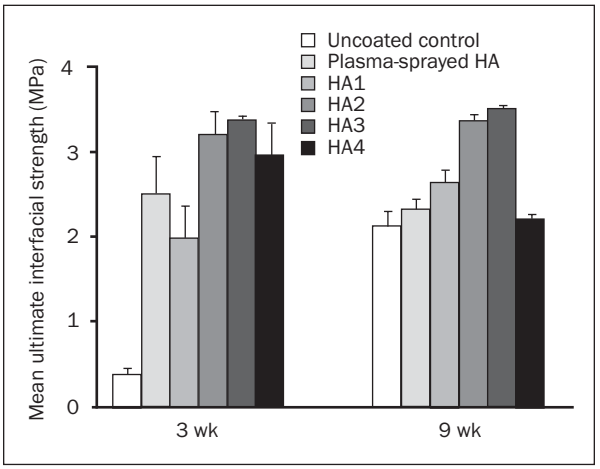

Fig 1 Mean interfacial strength of different implant surfaces after 3 and 9 weeks of implantation. Bars indicate standard error.

Push-Out Testing

The ultimate interfacial strength of the implants at the bone-implant interface over time and treatment was determined using push-out testing conducted with an Instron mechanical tester (model 1125; Instron, Canton, MA). Immediately after sacrifice, the tibiae containing implants were removed and were placed in saline. A total of 5 implants per time point per treatment were evaluated within 4 hours after sacrifice using a crosshead speed of 1 mm/min. The ultimate interfacial strength (s) was calculated using the formula $s = P/pdh$, where P was the ultimate pull-out load (N), d was the diameter of the implant (mm), and h was the length of the implant (mm) in bone. Ultimate interfacial strengths for different groups of implants were statistically analyzed using an analysis of variance (ANOVA), with Sheffé's procedure as a post-hoc test. Differences were considered significant at the $P < .05$ level.

Histology

A total of 3 implants per time point per treatment were used for histologic evaluation of the bone-implant interface. Bone-implant specimens were recovered from the 10% buffered formalin solution in which they were fixed and trimmed to within 4 mm of the implant surface using a Buehler Isomet saw (Buehler, Lake Bluff, IL). Dehydration was accomplished using a graded series of ethyl alcohols and 3 stages of clearing fluid (xylene) in tightly capped specimen jars. Infiltration was performed using a graded series of xylene and Osteo-Bed resins (Polysciences, Warrington, PA), followed by a catalyzed mixture of Osteo-Bed resin containing 1% (wt/vol) of benzoyl peroxide. Embedding was performed using a final catalyzed resin mixture of Osteo-Bed solution containing 2.5 % (wt/vol) of benzoyl peroxide. Specimens were embedded in the final catalyzed resin mixture in the absence of air for a minimum of 48 hours. After polymerization, specimens were placed in a freezer for 24 hours.

The embedded specimens were removed from the vials by breaking the glass. Specimens were trimmed of excess plastic and sectioned using the Isomet 1000 saw. Each specimen was then stained using Paragon stain (toluidine blue O and basic fuchsin in 30% ethanol), destained in acid alcohol (30% ethanol in 1% HCl), and counterstained in aqueous 1% alizarin red. Three longitudinal sections of the bone-implant specimens were prepared and the bone-implant interface was visualized using a model SZH10 Olympus zoom stereo microscope (Olympus, Melville, NY). The image was captured and digitized, and the length of direct bone contact over the entire implant perimeter was measured using Image Pro Plus image analysis software from Media Cybernetics (Silver Spring, MD). The measured value was expressed as a percentage of the axial perimeter. The resulting measurement is referred to as the percent bone contact length. Differences in the percent bone contact length between implants from the different treatment groups were statistically compared using an ANOVA. Differences were considered statistically significant if $P < .05$.

RESULTS

The mean ultimate interfacial strengths at 3 and 9 weeks after implantation are shown in Fig 1. At 3 weeks after implantation, the mean ultimate interfacial strength of 0.37 ± 0.16 MPa for noncoated titanium implants was significantly lower than the mean ultimate interfacial strength of all HA-coated implants tested (Fig 1) ($P < .001$). Of the HA-coated implants tested, implants coated with HA2 and HA3 exhibited significantly greater interfacial strength compared to HA1-coated implants 3 weeks after implantation ($P = .03$). HA1 and plasma-sprayed HA-coated implants were not significantly different in regard to mean interfacial strength. As shown in Fig 1, the interfacial strength of HA2 implants and HA3 implants remained significantly greater than that of the HA1, HA4, plasma-sprayed HA, and titanium control implants 9 weeks after implantation. It was also observed that the interfacial strength of uncoated Ti implants increased significantly over time.

As shown in Table 1, differences in bone contact length were observed for implants 3 weeks after implantation. The HA3 implants demonstrated the highest mean bone contact length (90.8% ± 1.6%),

followed by the plasma-sprayed HA-coated implants (79.4% ± 2.3%) and the HA4 implants (76.5% ± 0.8%). The lowest mean bone contact lengths were observed for the uncoated titanium implants (56.5% ± 0.1%), the HA1 implants (56.7% ± 6.0%), and the HA2 implants (53.4% ± 2.7%). However, no differences in bone contact length were observed between groups after 9 weeks.

Figure 2 shows representative histologic images of implant-bone interfaces 3 weeks following implantation. As shown in Fig 2a, little bone-implant contact was observed for the uncoated titanium implants, whereas direct bone contact was observed for the plasma-sprayed HA implants (Fig 2b) after 3 weeks. The plasma-sprayed HA coatings remained intact and attached to the titanium substrate at 3 weeks following implantation. Bone contacts on HA1 and HA2 were similar to the titanium control implants, whereas bone contacts on HA3 and HA4 closely resembled those of plasma-sprayed HA implants after 3 weeks implantation (Fig 2c).

Representative histologic sections of implant-bone interfaces at 9 weeks following implantation are shown in Fig 3. As shown in Fig 3, direct bone contact was observed for all implants at 9 weeks following implantation. The plasma-sprayed HA coatings were found to be intact on the titanium substrate 9 weeks postimplantation.

Table 1 Percent Bone Contact Length ± SE of Different Implant Surfaces After 3 and 9 Weeks of Implantation

	3 wk	9 wk
Uncoated	56.5 ± 0.1	86.5 ± 1.8
Plasma-sprayed	79.4 ± 2.3	84.4 ± 6.1
HA1	56.7 ± 6.0	80.1 ± 1.6
HA2	53.4 ± 2.7	81.0 ± 3.5
HA3	90.8 ± 1.6	91.5 ± 6.1
HA4	76.5 ± 0.8	81.9 ± 5.5

DISCUSSION

Rapid bone formation is one of the most important factors in establishing osseointegration of an endosseous implant.[12–14] Some investigators have suggested that a CaP coating can provide early and firm biologic fixation through producing fluids that mediate events affecting bone cell activity, organic matrix deposition, and mineral precipitation and resorption.[15,16] As observed in this study, implants coated with HA exhibited significantly higher interfacial strength and bone contact length as compared to uncoated titanium implants.[2] HA coatings of different crystallinities produced using SPA were compared. HA coatings with 50% (HA2), 70% (HA3), and 90% (HA4) crystallinity exhibited significantly greater interfacial strength than HA coatings with 30% crystallinity (HA1) at 3 weeks following implantation, suggesting different bone responses to HA coatings of different crystallinities. In addition, a significantly lower interfacial strength was observed for plasma-sprayed HA-coated implants compared to implants coated using SPA after 3 weeks of implantation (with the exception of HA1 implants). This significantly greater ultimate interfacial strength observed for the SPA-coated HA2 and HA3 implants at 3 weeks following implantation suggests that SPA technology may be a favorable coating alternative to plasma spraying. Plasma-sprayed HA coating also peeled off in the push-out strength test. Nine weeks after implantation, the interfacial strength of SPA-coated HA2 (50% crystallinity) and HA3 (70% crystallinity) remained significantly greater than the SPA-coated HA1 (30% crystallinity), HA4 (90% crystallinity), plasma-sprayed HA-coated, and uncoated titanium implants. This difference in interfacial strength as a result of using coatings of different crystallinities is in agreement with other studies.[1–3,10] In comparing data from different studies, it must be noted that reports have indicated that aside from the type of implant surface, implant site (femur versus humerus) also affects the mechanical bonding of implants to bone.[1–3]

Significantly lower ultimate interfacial strength at 3 weeks after implantation was attributed to the observed lower bone contact. In agreement with other studies, the ultimate interfacial strength and bone contact length of titanium implants increased over time.[3,17] Similarly, the high ultimate strength of SPA-coated HA3 and HA4 at 3 weeks following implantation correlates with the high bone contact length observed. However, the ultimate interfacial strength of SPA-coated HA2 was not consistent with the histologic findings regarding percent bone contact at 3 weeks following implantation, suggesting that other factors may affect interfacial strength. Previous studies revealed that CaP coatings of different crystallinities exhibited different dissolution and reprecipitation properties, which may enhance early bone tissue formation and bone bonding.[18,19]

As a result of early bone formation and bonding, bone formation at later time points was also affected, as observed in the histomorphometric evaluations of CaP coatings (viz, HA of different crystallinities) at 9 weeks following implant placement.

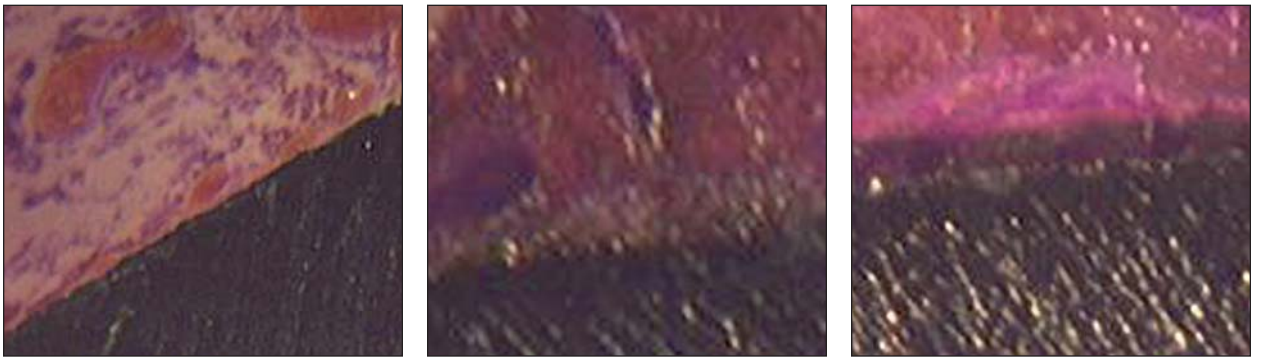

Fig 2 Histology of *(a)* titanium controls, *(b)* plasma-sprayed HA-coated implants, and *(c)* HA-coated implants created using SPA after 3 weeks of implantation (original magnification ×40).

Fig 3 Histology of *(a)* titanium controls, *(b)* plasma-sprayed HA-coated implants, and *(c)* HA-coated implants created using SPA after 9 weeks of implantation (original magnification ×40).

CONCLUSION

This study confirms that SPA technology is a viable alternative to plasma spraying for the application of CaP coatings to Ti implants. The positive effects on early osseointegration observed in this study suggest that further examination of this process may lead to further improved implant performance. The present data as well as previous reports[20–23] suggest a need for optimizing CaP crystallinity and coating processes and the necessity for additional research on the physical and chemical characteristics of CaP surfaces.

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