Bone Response to Plasma-Sprayed Hydroxyapatite and Radiofrequency-Sputtered Calcium Phosphate Implants in Vivo

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Purpose: *The objective of this study was to evaluate the effect of radiofrequency- (RF) sputtered calcium phosphate (CaP) coating of titanium implants on bond strength at the bone-implant interface and percent bone contact length.* ***Materials and Methods:*** *Cylindric sputtered CaP-coated and plasma-sprayed hydroxyapatite- (HA) coated implants (4.0 mm diameter and 8 mm length) were implanted in dog mandibles. Half the sputtered CaP-coated implants were heat-treated.* ***Results:*** *Twelve weeks after implant placement, no statistical differences in the mean ultimate interfacial strengths were observed between as-sputtered CaP-coated, sputtered CaP-coated heat-treated, and control plasma-sprayed HA-coated implants. Histomorphometric evaluation indicated that the percent bone contact lengths for the plasma-sprayed HA-coated implants and the as-sputtered CaP-coated implants were similar and significantly greater than that for the sputtered CaP-coated heat-treated implants. Differences in the ultimate interfacial strength and percent bone contact length between different implant sites in the mandible were not observed.* ***Discussion:*** *The results of this study, considered together with the results of previous studies, suggest that once early osseointegration is achieved, biodegradation of the thin CaP coatings is not detrimental to bone-coating-implant fixation, and does not compromise bone responses to the coated implant surfaces.* ***Conclusion:*** *The interfacial strength and histomorphometric data suggest that the CaP coatings applied using the sputtering process produce bone responses similar to those of HA coatings applied using plasma spraying.* (Int J Oral Maxillofac Implants 2002;17:581–586)

Key words: *calcium phosphate, histology, hydroxyapatites, surface properties, tensile strength*

Extensive in vivo research has indicated that plasma-sprayed hydroxyapatite- (HA) coated implants are biocompatible and may perform better than non-coated titanium (Ti) implants. It is believed that increased bone contact with implant surfaces results in more rapid osseointegration, as well as increased interfacial strength via early skeletal attachment.[1–3] Nevertheless, numerous reports indicate problems with HA- and calcium phosphate–(CaP) coated implants, including variable bond strength and poor adhesion at the coating-metal interface, non-uniform coating density, and altered HA structure.[4–6] These problems may actually reflect alterations associated with the plasma spraying process, rather than shortcomings of the coatings themselves. Crystallographic, chemical, and physical alterations occur with respect to the target CaP as a result of the plasma spraying process, but the characteristics of the plasma-sprayed HA coatings used in most studies are not usually reported.[7] The unknown quality of the coating is most likely responsible for the many conflicting animal and clinical observations reported in the literature, which have contributed to a controversy over whether or not HA coatings are actually beneficial to implant success.

The crystalline/amorphous content of the surface coating may be critical to implant success. Reports have indicated that bone responds differently to HA of different crystallinity.[8–11] It has been reported that amorphous coatings have an adverse

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effect on the establishment of an interface with bone[8,9] but are advantageous if coating longevity is desired.[10,11] Previous studies have suggested that the activity of osteoblast-like cells is dependent on the crystallinity and composition of the CaP surfaces.[8,12] Crystallinity of CaP coatings is known to greatly influence the dissolution of CaP from the surface of the implant in the human body. It is also known that the dissolution of the coatings contributes to the bioactivity of the HA surface. These studies indicate that a lack of knowledge of the characteristics of plasma-sprayed HA coatings may contribute to erroneous interpretations of the results of in vivo experiments.

Because the coating properties can be controlled, Lacefield and coworkers[13] and Cooley and associates[14] concluded that sputtering may be the method of choice for coating dental and orthopedic implants with HA or CaP. Previous studies have indicated that the tensile bond strengths of sputtered CaP coatings to the Ti substrates are in the range of 12 to 40 MPa, and these bond strengths are dependent on post-deposition heat treatments.[15] Also, studies have indicated that thin HA coatings (2 μm) have a significantly greater coating-metal interfacial strength compared to commercially available thick (70 μm) plasma-sprayed HA coatings (40 MPa versus 9 MPa).[16] Additional characterization indicates that the sputtered CaP coatings are amorphous in the absence of heat treatment.[15] With post-deposition heat treatment above 500°C, the coatings display an HA-type structure, with x-ray diffraction peaks matching the JCPDS 9-0432 standard.[17] Despite the extensive physical characterization of sputtered CaP coatings reported in the literature,[15–22] there are few data on the biologic responses to sputtered CaP coatings. In this study, the in vivo bone response to sputtered CaP coatings and plasma-sprayed HA coatings on titanium dental implants was measured.

MATERIALS AND METHODS

Implants

Thirty cylindric implants of 4 mm diameter and 8 mm length (containing apical perforations) (Friatec, Mannheim, Germany) were used in this study. Of the 30 implants, 10 were coated with HA applied using plasma spraying (by Friatec); these served as controls. The uncoated Ti implants were placed in a radiofrequency (RF) sputtering NCR 3117 system (Vacuum Technology Associates, Boulder, CO) and the chamber was pumped down to a base pressure of 6×10^{-6} torr. High-purity argon (99.999%) was backfilled into the chamber, bringing the pressure to about 10^{-4} torr. At an energy of 300 watts and a RF voltage of 1,000 V, CaP coatings were produced using a plasma-sprayed HA target (Ca/P ratio of 1.67/1). At a rate of 0.2 μm per hour, a coating thickness of 1.4 μm was achieved after 7 hours sputtering. After the coating process, the implants were rotated 120 degrees and recoated for another 7 hours. This process was repeated a third time to ensure that the entire implant surface was coated. The coatings were then divided into 2 groups: as-sputtered (not heat-treated) and heat-treated. The heat-treated CaP coatings were subjected to a post-deposition heat treatment of 700°C for 90 minutes. All sputtered CaP-coated implants were then sterilized with dry heat prior to implantation.

Extraction and Implant Placement

Five 2-year-old adult male foxhound dogs, weighing between 20 and 25 kg, were used for this study. The 5 dogs were cared for in compliance with NIH publication #86-23, *Guide for the Care and Use of Laboratory Animals.* Prior to experimentation, the protocol was evaluated and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas Health Science Center at San Antonio to ensure that the policies, standards, and guidelines for the proper use, care, handling, and treatment of animals were observed. The protocol required 2 surgical procedures: edentulation and implant placement.

Edentulation was performed 3 months prior to implant placement and was accomplished using standard oral surgical techniques by means of elevators, forceps, and a high-speed handpiece. The first through fourth mandibular premolars were removed bilaterally from each dog.

At the time of implant placement, the oral cavity was rinsed with 0.5% chlorhexidine (Sigma Chemical, St Louis, MO). A contra-angle handpiece was used with IMZ drills (Interpore International, Irvine, CA) for implant site preparation. Sixty cylindric implants, prepared as described above, were implanted. A maximum cutting drill speed of 1,700 rpm and copious irrigation were used to minimize surgical trauma to the bone. New drills were used for each animal. Six implants were placed in each mandible, with a plasma-sprayed HA implant (control), an as-sputtered CaP-coated implant (not heat-treated), and a sputtered CaP-coated heat-treated implant in each side. The position of each implant type was varied within the mandible of each dog to ensure that bone density and structure were not variables in the experiment. After placement of the implants, the screw holes on the superior surface of

the implants were sealed with cover screws. The surgical area was liberally irrigated with normal saline to remove bone fragments, and the tissue flaps were closed with continuous 3-0 Vicryl sutures (Ethicon, Somerville, NJ). All surgeries were uneventful, with no postoperative complications.

Pull-out Testing

To evaluate the ultimate interfacial strength of the implants at the bone-implant interface over time and with respect to treatment, pull-out testing was performed using an Instron Model 1125 (Instron, Canton, MA). All 5 dogs were euthanized 12 weeks after implant placement and the mandibles removed. The implants were recovered by sectioning the bone approximately 12 mm medially and distally to the implant centers. Implants from each group were placed either in saline (pull-out testing) or formalin (histology). A total of 7 implants/treatment were used to evaluate ultimate interfacial strength.

The bone-implant blocks were prepared for pull-out testing using the following procedures. An implant-pulling device was inserted through the topside of a stainless steel metal plate with a hole just large enough for the middle portion of the pulling device to tightly slip through. A bone-implant block was placed beneath the metal plate, and the implant-pulling device was screwed into the implant. The top of the implant-pulling device, which protruded through the hole in the implant, was secured in a Jacobs chuck attached to the Instron. The Instron machine was programmed at a crosshead speed of 1 mm/minute. Ultimate interfacial strength was calculated, and significant differences in ultimate interfacial strength between different groups of implants were analyzed statistically using a type of repeated-measure analysis of variance that takes into consideration data obtained from different dogs and from different implant positions within the mandible. Differences were considered statistically significant if $P < .05$.

Histologic Procedures

A total of 3 implants/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. A Buehler Isomet saw (Buehler, Lake Bluff, IL) was used to trim bone to within 4 mm of the implant surfaces. 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 (Polysciences, Warrington, PA) resins, followed by a catalyzed mixture of Osteo-Bed resin containing 1 gm of benzoyl peroxide per 100 mL. Embedding was performed using a final catalyzed resin mixture of Osteo-Bed resin solution containing 2.5 g of benzoyl peroxide per 100 mL. 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 plastic embedded specimens were removed from the vials by breaking the glass. Specimens were trimmed of excess plastic and sectioned longitudinally (80 µm each) using the Leica-1600 Microtome (Leica, Deerfield, IL). 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% hydrochloric acid), and counterstained in aqueous 1% alizarin red. Three slides representing the center of the bone-implant blocks were prepared for each of the bone-implant specimens.

The bone-implant interface was visualized using a Model SZH10 Olympus zoom stereo microscope (Melville, NY) and the image captured and digitized. Using Image Pro Plus image analysis software (Media Cybernetics, Silver Spring, MD), the length of direct contact between the bone and implant was measured over the entire implant perimeter. 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 different treatment groups were compared statistically using an analysis of variance. Differences were considered statistically significant if $P < .05$.

RESULTS

Ultimate Interfacial Strength

Twelve weeks after implant placement, there was no significant difference in mean ultimate interfacial strength between the 3 groups of implants (as-sputtered CaP-coated implants, 2.74 ± 0.31 MPa; sputtered CaP-coated heat-treated implants, 2.19 ± 0.26 MPa; and control plasma-sprayed HA-coated implants, 2.70 ± 0.28 MPa). No significant differences in the ultimate interfacial strength of similar implants placed at different implant sites were observed during the 12-week implant placement period.

Histologic Evaluations

Twelve weeks after implant placement, new bone filled the interfacial zone around the plasma-sprayed HA-coated implants, with new bone growing on preexisting cortical bone and into the apical perforations in direct contact with the implant surface (Fig 1).

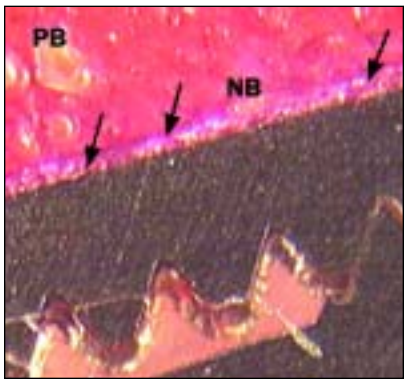

Fig 1 Histology of plasma-sprayed HA-coated implant-bone interface 12 weeks after implantation (40×) using Paragon staining. Formation of some newly formed (NB) woven bone on the implant surface on the plasma-sprayed HA coatings *(arrows)* and on preexisting cortical bone (PB) is apparent.

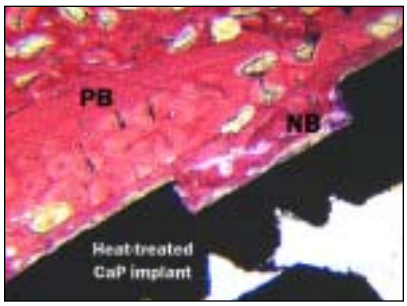

Fig 2 Histology of sputtered CaP-coated heat-treated implant-bone interface 12 weeks after implantation (40×) using Paragon staining. Intense remodeling of preexisting bone (PB) is observed. Newly formed bone (NB) is apparent on the implant surface.

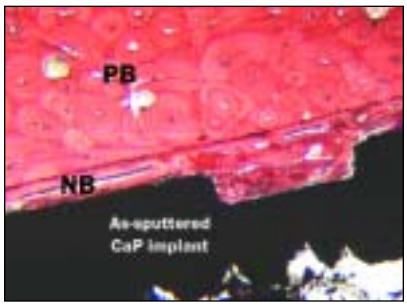

Fig 3 Histology of as-sputtered CaP-coated implant-bone interface 12 weeks after implantation (40×) using Paragon staining. Intense remodeling of preexisting bone (PB) and newly formed bone (NB) can be observed.

Table 1 Histomorphometric Evaluation at the Bone-Implant Interface

Sample	Bone contact length (%)
Plasma sprayed HA-coated implant (control)	78.6 ± 4.9
Amorphous CaP-coated implant	70.4 ± 1.6
Heat-treated CaP-coated implant	58.2 ± 4.5

Percent bone contact length represents the average percent bone contact at the implant interface ± 1 standard error.

The plasma-sprayed HA coatings remained adherent to the Ti substrate 12 weeks after implantation. In the sputtered CaP-coated heat-treated implants, intense remodeling of pre-existing bone, as well as newly formed bone in contact with the surface, was observed 12 weeks after implant placement (Fig 2). Similar to the sputtered CaP-coated heat-treated implants, intense remodeling of pre-existing bone and newly formed bone were also observed for the as-sputtered CaP-coated implant group 12 weeks after implant placement (Fig 3).

Histomorphometric evaluation indicated that the percent bone contact length for as-sputtered CaP-coated implants and plasma-sprayed HA-coated implants was higher than the percent bone contact length for sputtered CaP-coated heat-treated implants 12 weeks after implant placement (Table 1). No statistical difference in percent bone contact length was observed between the as-sputtered CaP-coated implant group and the plasma-sprayed HA-coated implant group 12 weeks after implant placement. No significant differences in the percent bone contact length of similar implants placed at different implant sites were observed.

DISCUSSION

Primary fixation is one of the most important factors in establishing adequate osseointegration between bone and an implant.[23] The ultimate interfacial strength observed in the present study for sputtered CaP-coated implants (as-sputtered and heat-treated) was not significantly different from that found for plasma-sprayed HA-coated implants 12 weeks after implant placement, suggesting that the RF-sputtered CaP coatings were comparable to plasma-sprayed HA coatings with respect to ultimate interfacial strength. This was supported by the histologic findings, which indicated no statistical difference in percent bone contact length between the plasma-sprayed HA-coated implant, the as-sputtered CaP-coated implant, and the sputtered CaP-coated heat-treated implant groups 12 weeks after implant placement. Reports in the literature have indicated that implant sites (femur versus humerus), as well as the type of surface modification, affect the mechanical bonding of implants to bone.[24] In this study, differences in the ultimate interfacial strength and percent bone contact length between different implant sites in the mandible were not observed.

The percent bone contact length for plasma-sprayed HA-coated implants and as-sputtered CaP-coated implants was statistically higher than the percent bone contact length for heat-treated CaP-coated implants 12 weeks after implant placement.

The percent bone contact lengths for plasma-sprayed HA implants 12 and 24 weeks after implant placement in the femora of dogs were reported to be 50% and 75%, respectively.[25] A mean direct bone contact length between mineralized bone and Ti implants of 69% has been reported in dogs 12 weeks after implant placement.[26] In this study, the percent bone contact length observed for the as-sputtered CaP-coated implant group 12 weeks after implantation in the dog mandible was similar to that observed for plasma-sprayed HA-coated implants, whereas the percent bone contact length observed for sputtered CaP-coated heat-treated implants 12 weeks after implantation in the dog mandible was lower than that observed for plasma-sprayed HA-coated implants. However, the bone contact length observed in this study for plasma-sprayed HA-coated implants in the dog mandible was higher than the bone contact length reported for plasma-sprayed HA-coated implants in dog femora 12 weeks after implantation.[25]

Previous studies have revealed that as-sputtered CaP coatings are amorphous, whereas heat-treated CaP coatings are crystalline.[15,17] This suggests that the dissolution of the CaP coatings may play a role in the bioactivity of the coatings and that this bioactivity enhances early bone tissue formation rates and bone tissue bonding. As a result of early bone tissue formation and bone tissue bonding, bone formation at later time points is also affected, as indicated by the histomorphometric evaluations of the as-sputtered CaP coatings 12 weeks after implant placement. Other investigators have also suggested that dissolution of the CaP coating produces solution-mediated events that affect bone cell activity, organic matrix deposition, and mineral precipitation and resorption.[27,28] The results of this study, considered together with the results of previous studies, suggest that once early osseointegration is achieved, biodegradation of the thin CaP coatings is not detrimental to bone coating/implant fixation and does not compromise bone responses to the coated implant surfaces.[29] The study clearly indicates that RF sputtering is a viable alternative to plasma spraying for the application of CaP or HA coatings to implants.

CONCLUSION

This study determined that the ultimate interfacial strength of RF-sputtered CaP coatings was similar to that of plasma-sprayed HA-coated implants 12 weeks after implant placement. In addition, histomorphometric evaluation indicated that the percent of bone contact for plasma-sprayed HA-coated implants was similar to that for as-sputtered CaP-coated implants and greater than that for sputtered CaP-coated heat-treated implants. It is concluded that the RF sputtering process may provide an alternative means of coating dental and orthopedic implants with CaP, achieving equivalent early bone-implant interfacial strengths and percent bone contact at the bone-implant interface to those achieved with plasma-sprayed HA-coated Ti implants.

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