



Effect of Induced Periimplantitis on Dental Implants With and Without Ultrathin Hydroxyapatite Coating

Marwa Madi, BDS, MDS, PhD,* Osama Zakaria, BDS, MDS, PhD,† Shizuko Ichinose, PhD,‡ and Shohei Kasugai, DDS, PhD§

The use of dental implants to replace missing teeth has become a routine technique over the past 20 years.¹ The clinical success of dental implants relies on their early osseointegration; thus, there is an ongoing effort to improve the stability and integration between bone and implant.² Modifying the implant surface has been suggested to enhance osseointegration and improve its quality.²⁻⁴ Altering the oxide layer, increasing the surface roughness, and using bioactive coat and other methods have been suggested.^{5,6} Calcium phosphate (CaP) compounds (called hydroxyapatite or HA) have showed favorable osteoconductive properties, and when used as a coating material, they enhanced the bone-implant fixation. However, owing to the weak mechanical properties of CaP compounds, they are used as coatings rather than a bulk implant material.⁷⁻¹⁰ The highly osteoconductive properties of HA coat are well-documented both

Purpose: The aim of this study was to compare the effect of ligature-induced periimplantitis on dental implants with and without hydroxyapatite (HA) coat.

Methods: Thirty-two dental implants (3.3 mm wide, 13 mm long) with 4 surface treatments (8 implant/group) (M: machined, SA: sandblasted acid etched, S: sputter HA coat and P: plasma-sprayed HA coat) were inserted into canine mandibles. After 12 weeks, oral hygiene procedures were stopped and silk ligatures were placed around the implant abutments to allow plaque accumulation for the following 16 weeks. Implants with the surrounding tissues were retrieved and prepared for histological examination. Bone-to-implant contact (BIC) and implant surfaces were examined using scan-

ning electron microscopy and energy dispersive x-ray spectroscopy.

Results: Histological observation revealed marginal bone loss and large inflammatory cell infiltrates in the periimplant soft tissue. Sputter HA implants showed the largest BIC (98.1%) and machined implant showed the smallest values (70.4%). After 28 weeks, thin sputter HA coat was almost completely dissolved, whereas plasma-sprayed HA coat showed complete thickness preservation.

Conclusion: Thin sputter HA-coated implants showed more bone implant contact and less marginal bone loss than thick HA-coated implants under periimplantitis condition. (Implant Dent 2016;25:39-46)

Key Words: dental implants, hydroxyapatite, periimplantitis, bone

*Lecturer, Department of Oral Medicine, Periodontology, Diagnosis and Radiology, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.

†Lecturer, Department of Oral Surgery, Faculty of Dentistry, Pharos University, Alexandria, Egypt.

‡Professor, Research Center for Medical and Dental Science, Tokyo Medical and Dental University, Tokyo, Japan.

§Professor and Head of Department, Oral Implantology and Regenerative Dental Medicine, Tokyo Medical and Dental University, Tokyo, Japan.

Reprint requests and correspondence to: Marwa Madi, BDS, MDS, PhD, Faculty of Dentistry, Alexandria University, Champillon St, Azarita, Alexandria, Egypt, 21411, Phone: 03-5548244, Fax: 03-4858681, E-mail: marwa.madi@dent.alex.edu.eg, marwamadi95@gmail.com

ISSN 1056-6163/16/02501-039

Implant Dentistry

Volume 25 • Number 1

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

DOI: 10.1097/ID.0000000000000331

experimentally and clinically.^{11,12} HA coat enhances early bone formation while minimizing the formation of fibrous tissue resulting in better implant fixation. However, this osteoconductive property depends on the properties of the coat such as its degree of crystallinity, solubility, and coat thickness.^{13,14}

Plasma spraying is the conventional technique for depositing HA coat on a core metal substrate.^{15,16} This technique requires high temperature, and

the consequent coat is more than 50 μ m thick with very rough surface. Although the biocompatible property of plasma-sprayed HA coatings has been observed in several studies, concerns have been raised regarding the potential risk of delamination of the coat and coat degradation.^{17,18}

As an alternative, other coating deposition methods were introduced to produce more adherent thin HA film.¹⁴ Radiofrequency (RF) magnetron sputtering is a physical vapor deposition

process of depositing thin CaP films with a uniform thickness while preserving the roughness of the underlying substrate.¹⁰ Previous *in vitro* and *in vivo* studies showed that CaP deposited by sputtering technique demonstrated biocompatible property, with improved apatite formation and stimulated extracellular matrix formation as well.^{10,14,19}

However, sputtered HA coat possesses low crystallinity *in vivo*, which accelerates the dissolution of the film at an early stage after implantation. Coolsey et al²⁰ observed a complete dissolution of sputtered HA coat after 3 weeks of implant insertion. Appropriate heat treatment can crystallize the sputtered HA coat resulting in a decrease in its dissolution property without degrading the HA film. A previous study reported that after 12 weeks of implantation, the crystallized sputter HA film showed 1.6 times higher bone bonding strength than the plasma spray.²¹

In environments where bacterial plaque is promoted to build up, the bacteria present in the accumulated biofilm can lead to inflammatory

reaction in the adjacent periimplant mucosa (mucositis), which subsequently leads to periimplant bone loss (periimplantitis).²² Mucositis and periimplantitis were reported as contributing to failures of implants with HA-coated surfaces.²³ A controversy still exists regarding the behavior of CaP coating in periimplantitis conditions.²⁴ Martins et al²⁵ observed greater bone loss around plasma-sprayed HA-coated implants, whereas other studies showed similar periimplant bone loss around HA-coated and uncoated implants.^{23,24} The stability of the HA-coated dental implants in the oral cavity is affected by the thickness and crystallinity of the coat and the existed microorganism.²⁶

The implant surface roughness and its chemical composition surface can promote plaque formation. It has been demonstrated that more plaque accumulates on rougher surfaces and high surface free energy materials such as titanium. Irregular surfaces and hydrophilic surfaces accumulate and retain more bacteria; therefore, rough implant surfaces require more procedures to

decontaminate than machined surfaces.²⁷ *In vitro* and *in vivo* studies showed that pathogenic bacteria lead to the decrease in PH value because of high concentrations of bacterial metabolites and the dissolution of HA coat.²⁸ Gram-negative anaerobe and spirochetes were predominantly found in the periimplantitis conditions. A previous *in vitro* study reported the dissolution of HA coat after being exposed to staphylococci bacteria. Another study in dogs observed the decrease of HA coating thickness after 3 and 6 months of induced periimplantitis.²⁶

Therefore, the objective of this study was to evaluate the effect of ligature induced periimplantitis on implants with different HA coatings (plasma spraying and ultrathin sputter) using histologic, histomorphometric, scanning electron microscopy (SEM), and energy dispersive x-ray spectroscopic assessments.

MATERIAL AND METHODS

Dental Implants

Thirty-two pure titanium dental implants (3.3 mm wide and 13 mm long) with 4 different surface treatments were used in this study. The implant types were Type M: machined, Type SA: sandblasted acid etched, Type S: 1 μ m sputter HA coat, RF magnetron sputtering were performed using an SPF-410H (ANELVA Corp., Tokyo, Japan) chamber followed by a hydrothermal treatment at 120°C in an electrolyte solution containing calcium and phosphate ions for 20 hours and Type P: 50 μ m HA plasma-sprayed coat followed by pressurized hydrothermal process.

Surgical Procedure

The study protocol was approved by the regional Ethics Committee for Animal Research in Tokyo Medical and Dental University. All surgical operations were performed under general anesthesia with medetomidine hydrochloride (Dormitor; Orion Corporation, Espoo, Finland) (0.05 mL/kg) as intramuscular premedication and ketamine (Ketalaru; Daiichi-sankyo Co., Japan) (2 mL/10 kg). Infiltration anesthesia at the surgical site was performed with 2% xylocaine/epinephrine (1:80,000) (Sankin; Dentsply, Tokyo, Japan).

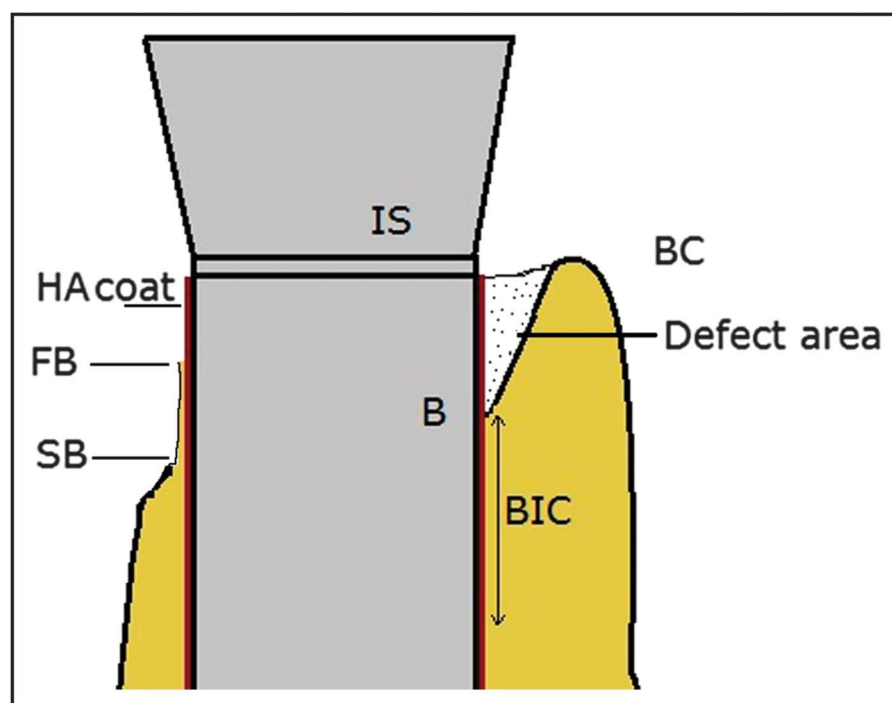


Fig. 1. Schematic drawing illustrating the landmarks used for the histometric measurements. B, coronal point of bone implant contact; BC, bone crest; BIC, bone-to-implant contact; FB, first coronal point of ultrathin bone layer; HA coat, hydroxyapatite coat; IS, implant shoulder; SB, apical point of ultrathin bone layer.

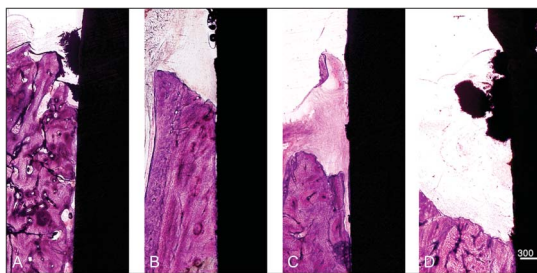


Fig. 2. Buccal-lingual undecalcified sections for implant types. Machined (A), sandblasted acid etched (B), sputter HA coated (C), and plasma-sprayed HA coated (D) (300 µm bar-toluidine blue staining).

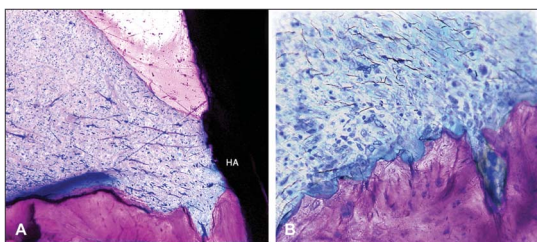


Fig. 3. Undecalcified sections showing inflammation in the sulcus (A), multinucleated and inflammatory cells in the connective tissue lateral to the osseous surface (B). Toluidine blue staining.

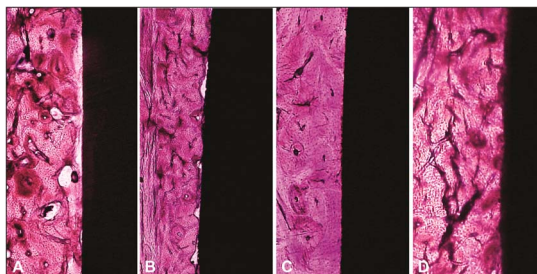


Fig. 4. Undecalcified ground section 7 months after implant placement. Machined (A), sandblasted acid etched (B), sputter HA coated (C), and plasma-sprayed HA coated (D). Toluidine blue staining.

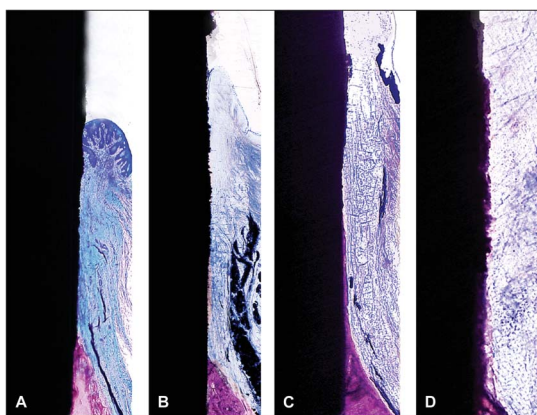


Fig. 5. Undecalcified sections showing ultrathin layer of bone attached to the implant surface (C, D). Machined (A), sandblasted acid etched (B), sputter (C), and plasma sprayed (D). Toluidine blue staining.

Prophylactic antibiotic of clindamycin (11 mg/kg body weight) was given postoperatively for 3 days.

After a 4-week accommodation period, all mandibular premolars were extracted in three 2-year-old beagle dogs. After a 3-month healing period, mucoperiosteal flaps were elevated. Four implants were inserted in each side of the mandible in a randomized order.

Implants margins were placed on the same level as the bone crest (BC). The flaps were sutured and all implants were allowed to heal in a nonsubmerged position. The sutures were removed after 2 weeks and an oral hygiene program using 2% chlorhexidine rinse (chlorhexidine gluconate 20%; ICI Pharmaceutical Group, Wilmington, DE) 3 times/week was maintained for additional 3 months. After a 3-month period, oral hygiene procedure was discontinued and periimplantitis was induced using silk ligatures placed around the abutments in submarginal position to allow plaque accumulation.

Bacterial sample was taken to confirm the presence of periodontopathic bacteria. After a four 4-month period, the dogs were euthanized with an overdose of Sodium Pentothal, and implants with the surrounding tissues were retrieved and prepared for histological examination.

Histological Examination

The specimens were dehydrated in an ascending series of alcohol and embedded in a methacrylate resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). After polymerization, ground sections were prepared containing the implant and the surrounding soft and hard tissues according to the technique of Donath and Breuner.²⁹ Each block was cut in a buccolingual plane using a cutting-grinding unit (Apparatebau; Exakts, Norderstedt, Germany). From each implant site, 3 central sections (buccal-lingual plane) were obtained and further reduced to a final thickness of approximately 50 µm using a microgrinding polishing unit (Le Cube, PRESI-Métallographie Co., Brié-et-Angonnes, France). The sections were finally stained in toluidine blue. Histological analysis was performed using optical/fluorescence microscope (Biozero-8000; Keyence,

Osaka, Japan) and the data were analyzed using BZ-analyzer software (Keyence).

SEM and EDS

Surface observation and elemental analyses were performed using SEM

(JSM-5310LV; JOEL, Tokyo, Japan) and energy dispersive x-ray spectroscopy (EDS) (EMAX-7000; Horiba Ltd., Kyoto, Japan). A bone–implant interface was selected to evaluate the residual HA coating.

Histomorphometric Analyses

The following landmarks were recognized on the histological sections and used for the linear measurements: the implant shoulder (IS), the first point of bone–implant contact (B), and the BC of the bony defect. Bone-to-implant contact (BIC) starting at the bottom of the defect and average defect area were calculated. Regarding the HA-coated implants, under high magnifications, the first ultrathin bone–implant contact (FB) and the second bone–implant contact (SB) were determined and the distance between FB–SB was assessed to evaluate the length of the ultrathin bone layer (BL). The mean residual HA coat thickness was measured for implant type S and P in the coronal, middle, and apical third of the implants (Fig. 1).

RESULTS

Data Analyses

Mean values for each variable were calculated for each implant in each animal. Differences were analyzed using analysis of variance (ANOVA) and post hoc test. The null hypothesis was rejected at $P < 0.05$.

Histological Observations

Buccal–lingual sections representing the 4 different implant types are illustrated in (Fig. 2). The periimplant tissues in all specimens showed evidence of marginal bone loss and large inflammatory cell infiltrates. The implant surfaces were occupied with varying amounts of plaque and calculus that extended from the supramarginal portion of the implant to the apical compartment of the pocket.

In all specimens, there was considerable bone–implant integration; variable degrees of marginal bone loss were observed extending to either the coronal or middle third of the implant. The mucosa surrounding the implants was characterized by the presence of a large inflammatory infiltrate (Fig. 3). The bone was in direct contact with the implant surface without intervening fibrous tissue layers (Fig. 4). The plasma-sprayed coated implants showed no signs of HA-coat degradation. Multinucleated giant cells were

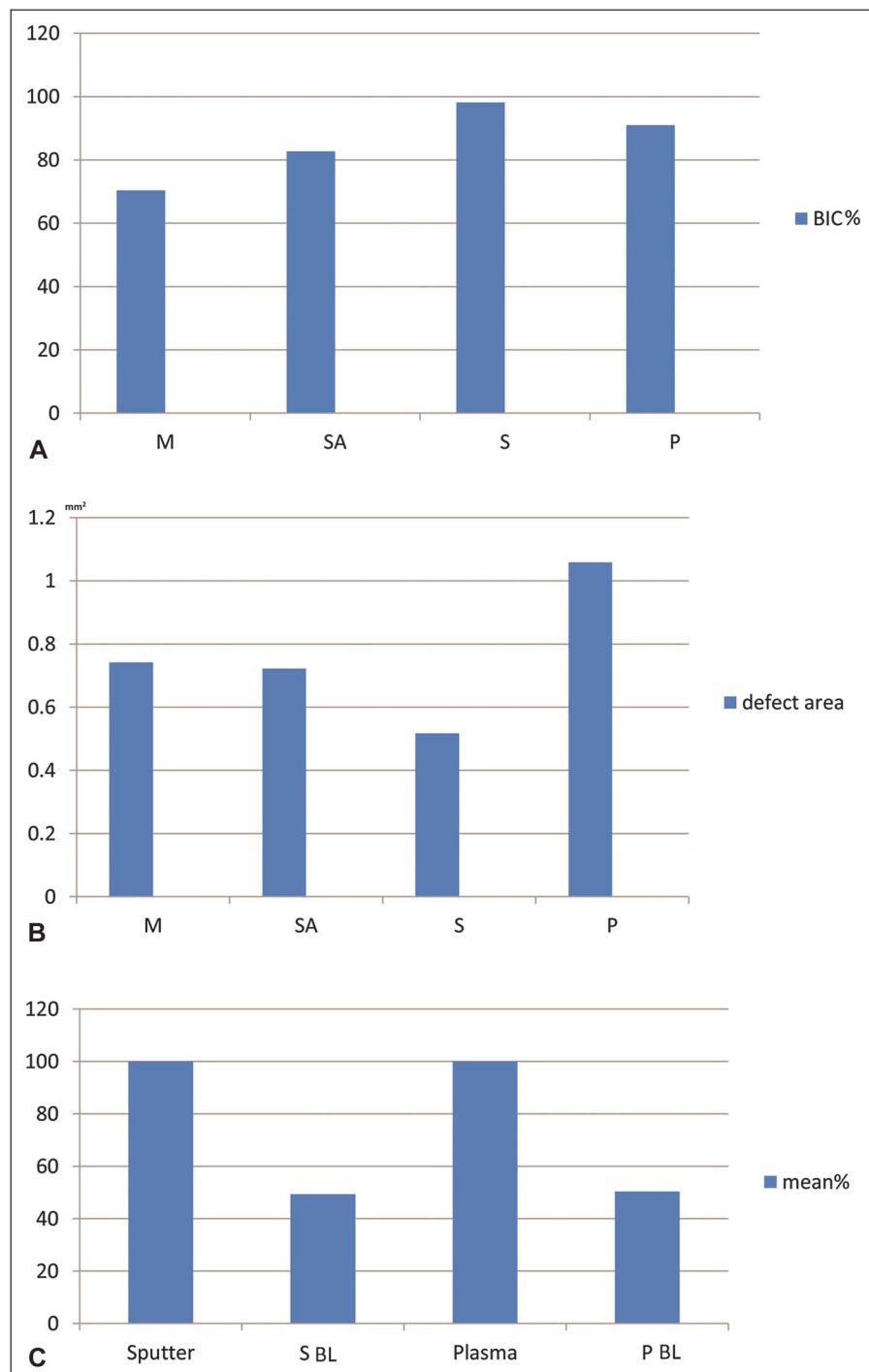


Fig. 6. (A) BIC in percent. (B) Bone defect area (in square millimeter). (C) Length of the ultrathin bone layer (BL) for sputter (S BL) and plasma (P BL) implants in relation to the whole exposed implant surface. Toluidine blue staining. M, machined; P, plasma-sprayed HA coat; S, sputter HA coat; SA, sandblasted acid etched.

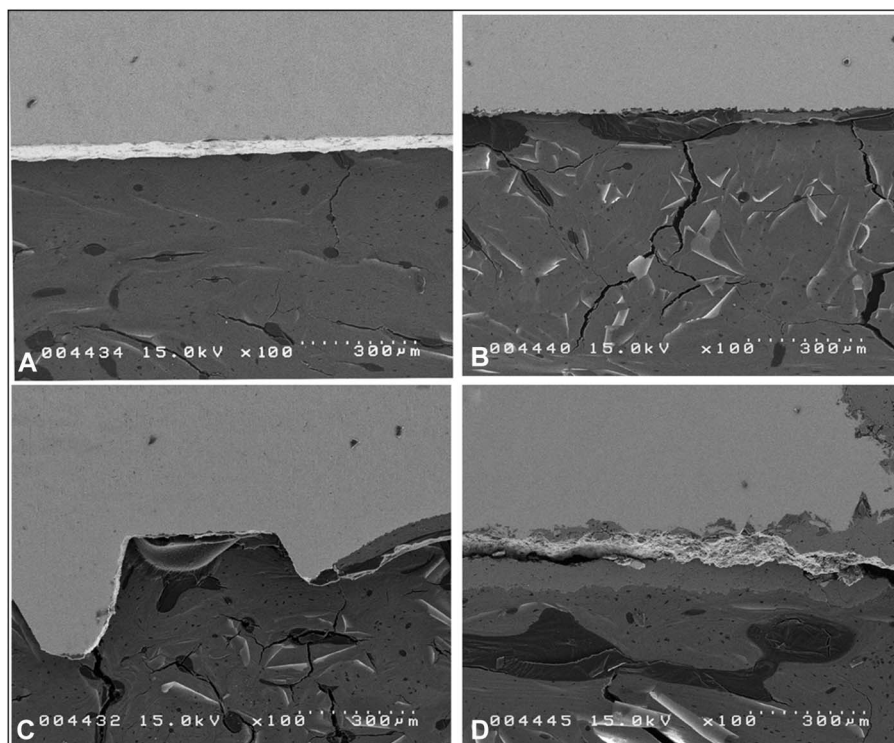


Fig. 7. SEM image of different implants after 28 weeks. Machined (A), sandblasted acid etched (B), sputter HA coated (C), and plasma-sprayed HA coated (D).

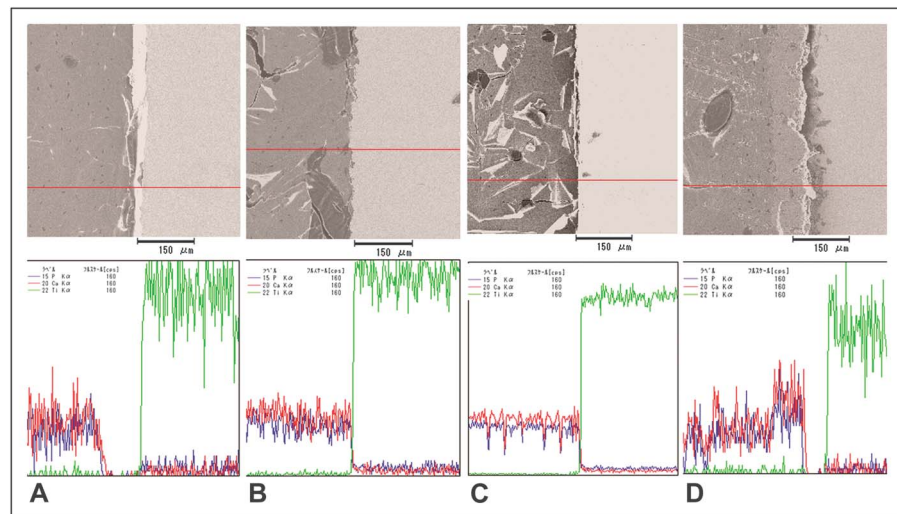


Fig. 8. EDS analysis of the implant–bone interface for different implant types. Machined (A), sandblasted acid etched (B), sputter HA coated (C), and plasma-sprayed HA coated (D).

also frequently found and the lateral bone walls that lined the lesion formed a craterlike osseous defect. Osteoclasts and multinucleated cells were found in the connective tissue adjacent to the bony defect, indicating active tissue destruction including bone resorption. The inner lateral wall of the bone defect

showed steep sloping toward the implant surface giving the characteristic saucer shape of periimplantitis bone defects. The bony surface was irregular (ruffled) and osteoclast cells was observed. The outer lateral wall of the bone defect was smooth and sloped gradually to the outer cortical bone.

Regarding the HA-coated implants, an ultrathin layer of bone in direct contact with the implant surface was observed under high magnifications along the implant surface opposite to the bone defect and extended apically until the buccal bone–implant contact. Under high magnifications, the plasma-sprayed HA coat was observed to be separated from the implant surface; these findings were confirmed under SEM observations (Fig. 5).

Histomorphometric Evaluation

The average BIC was greatest for implant type S (98.1%) and lowest for implant type M (70.4%). The defect area was determined to average 0.7 mm² for implant type M and SA, 1.1 mm² for implant type P, and the smallest value was 0.5 mm² for implant type S. There were no significant differences between the 2 groups ($P < 0.05$). The length of the ultrathin bone film was shown to cover up to 50% of the exposed implant surface (Fig. 6).

Coating Thickness Reduction

The HA coating appearance was not uniform. For implant type S, very thin coating (20% of the original thickness) was observed in the coronal areas, whereas complete coat loss was observed in the apical third. For implant type P, no HA coat thickness reduction was observed (Fig. 6).

SEM and EDS Analyses

SEM and EDS analyses of the bone–implant interface revealed a direct contact between thin sputter HA coated implants and bone tissue. Similar findings were observed for the SA implants. Plasma-sprayed HA coated implants demonstrated a direct bone contact with the HA coat; however, a gap was observed between the Ti substrate and the HA coat. Separation between the bone and machined implant was observed in some samples (Figs. 7 and 8).

DISCUSSION

CaP coatings have been known for decades for their osteoconductive properties and their biocompatibility. However, their clinical performance in the presence of bacteria remains controversial, particularly, coating degradation

with consequence crestal bone loss.³⁰ The advantages of a CaP coating become evident only under more severe implantation conditions. Therefore, in this study, we evaluated the effect of ligature induced periimplantitis on different implant surface treatments including 2 types of HA coat using histological, histomorphometric, SEM and EDS analyses.

Regarding the HA coat properties, our histological and SEM observations showed approximately 80% coat thickness reduction for sputter HA coated implants; however, complete thickness preservation was observed for plasma-sprayed HA implants. This could be explained by the high crystalline property of MP-1 plasma HA coat together with its relatively large thickness 50 μm compared to 1 μm sputter HA coat. Our findings conform to previously reported observations,²⁶ in which highly crystalline plasma-sprayed HA coat showed stable coat thickness after 3 and 6 months implantation in normal and periimplantitis conditions.

Similarly, Ozeki et al²¹ reported that after 12 weeks of implant insertion 80% of the sputter HA coat was resorbed. Furthermore, recent studies on biological reaction of sputtered HA coat revealed its supportive behavior to osteoclast formation and function indicating its favorable biodegradability. The degradation of the HA coat mainly in the apical part is likely due to increased blood supply in the apical area rather than the coronal part. In addition, it was observed that there was a cellular process of HA coating degradation caused by osteoclastic activity.^{17,28} As such, the replacement of HA coatings by autologous bone was suggested to occur with longer implantation time, and this replacement was the result of HA coatings taking part in the bone remodeling response similar to any other part of the skeleton.³¹ Yamamoto et al³² demonstrated that coating degradation was associated with enhanced osteoconductivity and increased BIC.

An ultrathin layer of bone attached to the implant surface adjacent to the bone defects was observed in histologic images. This bone layer was only observed in both types of HA-coated implants. Despite the absence of

osteoblast layer on the surface of this thin bone layer, the bioactivity of HA coat is confirmed. A previous study³³ demonstrated that the thin film sputtered HA coating provides a more favorable surface for osteoblastic differentiation and function than the modified titanium surface. HA coatings are bioactive and well-known for their favorable bone-bonding properties compared with noncoated titanium.^{34,35} Although this bone tissue is not vital (no osteoblast was observed), the presence of this bone tissue layer reflects the bonding of HA coating with the bone. Our EDS analysis showed the presence of calcium directly at both HA-coated implant surfaces. From this, it can be concluded that mineralized bone can grow directly on the surface of thin sputter HA-coated implants and plasma-sprayed HA surface.

Regarding the current SEM observation, the plasma HA coat showed to be separated from the Ti substrate while still being tightly bonded to the bone. This could be either attributed to the processing artifact or due to residual stresses in the HA coat. Numerous reports revealed that high-temperature coating results in residual stress at the interface between Ti and the coating layer; this is attributed to the different thermal coefficients between Ti and apatite.³⁶ Another study concluded that mechanical interlocking between plasma-sprayed HA coat and the metal substrate is the major factor determining the bond integrity. The study showed that plasma-sprayed HA coat separation was between the oxide film or the underlying substrate depending on the weakness of the bond. Simultaneously, the coating fractured into fragments but remained mechanically held in the existing substrate concavities.³⁷ A previous report explained this observation as a processing artifact.³⁸ However, another review showed that variation in bond strength between the HA coat and the metallic substrates leading to coat separation is a common problem for plasma spraying.³¹ The bonding of the plasma-sprayed HA coatings depends mainly on mechanical interaction. As evidenced in several studies, a highly roughened substrate surface exhibited a higher bond strength

compared with a smooth substrate surface.³⁷

The present SEM and EDS analyses showed separation of the bone from the machined implant surface. This observation is similar to that previously reported for machined c.p. and alloyed titanium. Numerous possible reasons for the separation and the rupture in the interface exist due to the fixation, dehydration, embedding, sawing, and grinding. Furthermore, the high vacuum in the SEM might increase the rupture.³⁹

Numerous *in vivo* studies used bone-implant contact ratio as an index of osteoconductivity and to compare different apatite-coated titanium implants.³⁶ Similarly, in this study, bone-implant contact was used to evaluate the hard tissue response regarding the different implant surfaces. Both HA-coated implants showed more BIC than uncoated implants. In agreement with the previous findings, sputtered CaP coatings showed improved initial fixation and more healing response than uncoated Ti surface. Another study concluded that sputtered CaP coatings are comparable to plasma-sprayed HA coatings with respect to ultimate interfacial strength after 12 weeks of implantation.³¹

The bony defect showed irregular ruffled borders with multinucleated giant cells observed close to the bone surface reflecting active bone resorption. The soft tissue as well was infiltrated with inflammatory cells, and the pocket epithelium showed ulceration. The consequence relationship between bacterial plaque accumulation and the development of soft tissue inflammation surrounding dental implants has been reported by Pontoriero et al.⁴⁰

All implants showed marginal bone loss extended to the middle third in the buccal surface and to the coronal third in the lingual third. As for the plasma-sprayed HA-coated implants, the angular bone defect tends to be wider, whereas the other implant surfaces showed narrower defects (buccally and lingually). These results are consistent with our previous study, in which plasma-sprayed implants showed wider periimplantitis bone defect than other tested implants.⁴¹ Conceivably, the

increased surface roughness resulting from plasma spraying process allowed more bacteria plaque accumulation that can stimulate more inflammatory response in adjacent soft tissue. Therefore, wide soft tissue area became infiltrated by inflammatory cell, allowing more inflammatory cells penetration to the underlying bone. HA coat that remains for longer times after bone maturation is achieved is more likely to be susceptible to bacterial accumulation with subsequent progression of inflammation and bone loss.⁴²

CONCLUSION

In conclusion, the current research has shown some promises for thin sputter HA coat that may eliminate some of the problems associated with the plasma spraying process. Thin sputter HA-coated implants showed more favorable bone response (bone-implant contact) and less marginal bone loss than other implant surfaces despite the presence of periimplantitis.

DISCLOSURE

The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

APPROVAL

The study protocol was approved by the regional Ethics Committee for Animal Research in Tokyo Medical and Dental University.

REFERENCES

1. Le Guéhennec L, Soueidan A, Layrolle P, et al. Surface treatments of titanium dental implants for rapid osseointegration. *Dent Mater*. 2007;23:844–854.
2. Simon Z, Watson P. Biomimetic dental implants new ways to enhance osseointegration. *J Can Dent Assoc*. 2002;68:286–8.
3. Buser D, Broggini N, Wieland M, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res*. 2004;83:529–533.
4. Zhao G, Schwartz Z, Wieland M, et al. High surface energy enhances cell response to titanium substrate microstructure. *J Biomed Mater Res A*. 2005;74:49–58.
5. Cochran DL, Schenk RK, Lussi A, et al. Bone response to unloaded and loaded titanium implants with a sand-blasted and acid-etched surface: A histometric study in the canine mandible. *J Biomed Mater Res*. 1998;40:1–11.
6. Brunski J, Puleo A, Nanci A. Biomaterials and biomechanics of oral and maxillofacial implants: Current status and future developments. *Int J Oral Maxillofac Implants*. 2000;15:15–46.
7. Baker MI, Eberhardt AW, Martin DM, et al. Bone properties surrounding hydroxyapatite-coated custom osseous integrated dental implants. *J Biomed Mater Res B App Biomater*. 2010;95:218–224.
8. Renvert S, Polyzois I, Claffey N. How do implant surface characteristics influence peri-implant disease? *J Clin Periodontol*. 2011;38:214–222.
9. Wolke J, Van der Waerden J, Schaeken H, et al. In vivo dissolution behavior of various RF magnetron-sputtered Ca-P coatings on roughened titanium implants. *Biomaterials*. 2003;24:2623–2629.
10. Levine B, Sporer S, Poggie R, et al. Experimental and clinical performance of porous tantalum in orthopedic surgery. *Biomaterials*. 2006;27:4671–4681.
11. Davies J. Understanding peri-implant endosseous healing. *J Dent Educ*. 2003;67:932–949.
12. Wang X, Zakaria O, Madi M, et al. Vertical bone augmentation induced by ultrathin hydroxyapatite sputtered coated mini titanium implants in a rabbit calvaria model. *J Biomed Mater Res B Appl Biomater*. 2014. doi: 10.1002/jbm.b.33347. Epub ahead of print.
13. Daugaard H, Elmengaard B, Bechtold JE, et al. The effect on bone growth enhancement of implant coatings with hydroxyapatite and collagen deposited electrochemically and by plasma spray. *J Biomed Mater Res A*. 2010;92:913–921.
14. Hulshoff JEG, van Dijk K, van der Waerden JPCM, et al. Evaluation of plasma-spray and magnetron-sputter Ca-P-coated implants: An in vivo experiment using rabbits. *J Biomed Mater Res*. 1996;31:329–337.
15. de Groot K, Geesink R, Klein CP, et al. Plasma sprayed coatings of hydroxyapatite. *J Biomed Mater Res*. 1987;21:1375–1381.
16. Sun L, Berndt CC, Gross KA, et al. Material fundamentals and clinical performance of plasma-sprayed hydroxyapatite coatings: A review. *J Biomed Mater Res*. 2001;58:570–592.
17. Wolke J, Van der Waerden J, De Groot K, et al. Stability of radiofrequency magnetron sputtered calcium phosphate coatings under cyclically loaded conditions. *Biomaterials*. 1997;18:483–488.
18. Wolke J, De Groot K, Jansen J. In vivo dissolution behavior of various RF magnetron sputtered Ca-P coatings. *J Biomed Mater Res*. 1998;39:524–530.
19. Jansen J, Wolke J, Swann S, et al. Application of magnetron sputtering for producing ceramic coatings on implant materials. *Clin Oral Implants Res*. 1993;4:28–34.
20. Cooley DR, Van Dellen AF, Burgess JO, et al. The advantages of coated titanium implants prepared by radiofrequency sputtering from hydroxyapatite. *J Prosthet Dent*. 1992;67:93–100.
21. Ozeki K, Okuyama Y, Fukui Y, et al. Bone response to titanium implants coated with thin sputtered HA film subject to hydrothermal treatment and implanted in the canine mandible. *Biomed Mater Eng*. 2006;16:243–251.
22. Schwarz F, Herten M, Sager M, et al. Comparison of naturally occurring and ligature-induced peri-implantitis bone defects in humans and dogs. *Clin Oral Implants Res*. 2007;18:161–170.
23. Alexander F, Christian U, Stefan T, et al. Long-term effects of magnetron-sputtered calcium phosphate coating on osseointegration of dental implants in non-human primates. *Clin Oral Implants Res*. 2009;20:183–188.
24. Madi M, Zakaria O, Noritake K, et al. Peri-implantitis progression around thin sputtered hydroxyapatite-coated implants: Clinical and radiographic evaluation in dogs. *Int J Oral Maxillofac Implants*. 2012;28:701–709.
25. Martins MC, Shibli JA, Abi-Rached RS, et al. Progression of experimental chronic peri-implantitis in dogs: Clinical and radiographic evaluation. *J Periodontol*. 2005;76:1367–1373.
26. Tillmanns HW, Hermann JS, Tiffée JC, et al. Evaluation of three different dental implants in ligature-induced peri-implantitis in the beagle dog. Part II. Histology and microbiology. *Int J Oral Maxillofac Implants*. 1998;13:59–68.
27. Teughels W, Van Assche N, Sliepen I, et al. Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Implants Res*. 2006;17:68–81.
28. Mukherjee DP, Dorairaj NR, Mills DK, et al. Fatigue properties of hydroxyapatite-coated dental implants after exposure to a periodontal pathogen. *J Biomed Mater Res*. 2000;53:467–474.
29. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. *J Oral Pathol*. 1982;11:318–326.
30. Lee TM, Wang BC, Yang YC, et al. Comparison of plasma-sprayed hydroxyapatite coatings and hydroxyapatite/tricalcium phosphate composite coatings: In

vivo study. *J Biomed Mater Res.* 2001;55:360–367.

31. Yang Y, Kim K-H, Ong JL. A review on calcium phosphate coatings produced using a sputtering process - An alternative to plasma spraying. *Biomaterials.* 2005;26:327–337.

32. Yamamoto H, Shibata Y, Tachikawa T, et al. In vivo performance of two different hydroxyapatite coatings on titanium prepared by discharging in electrolytes. *J Biomed Mater Res B Appl Biomater.* 2006;78:211–214.

33. Hao J, Kuroda S, Ohya K, et al. Enhanced osteoblast and osteoclast responses to a thin film sputtered hydroxyapatite coating. *J Mater Sci Mater Med.* 2011;22:1489–1499.

34. Oosterbos C, Vogely HC, Nijhof M, et al. Osseointegration of hydroxyapatite-coated and noncoated Ti6Al4V implants in the presence of local infection:

A comparative histomorphometrical study in rabbits. *J Biomed Mater Res.* 2002;60:339–347.

35. Urquia Edreira ER, Wolke JGC, Aldosari AA, et al. Effects of calcium phosphate composition in sputter coatings on in vitro and in vivo performance. *J Biomed Mater Res A.* 2015;103:300–310.

36. Mano T, Ueyama Y, Ishikawa K, et al. Initial tissue response to a titanium implant coated with apatite at room temperature using a blast coating method. *Biomaterials.* 2002;23:1931–1936.

37. Mimura K, Watanabe K, Okawa S, et al. Morphological and chemical characterizations of the interface of a hydroxyapatite-coated implant. *Dent Mater J.* 2004;23:353–60.

38. Burgess AV, Story BJ, Wagner WR, et al. Highly crystalline MP-1 hydroxyapatite coating Part II: In vivo performance on endosseous root implants in

dogs. *Clin Oral Implants Res.* 1999;10:257–266.

39. Palmquist A, Lindberg F, Emanuelsson L, et al. Biomechanical, histological, and ultrastructural analyses of laser micro-and nano-structured titanium alloy implants: A study in rabbit. *J Biomed Mater Res A.* 2010;92:1476–1486.

40. Pontoriero R, Tonelli MP, Carnevale G, et al. Experimentally induced peri-implant mucositis. A clinical study in humans. *Clin Oral Implants Res.* 1994;5:254–259.

41. Madi M, Zakaria O, S Kasugai. Coated vs uncoated Implants: Bone defect Configurations after Progressive peri-implantitis in dogs. *J Oral Implantol.* 2014;40:661–669.

42. Mombelli A, van Oosten MA, Schurch E Jr, et al. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol.* 1987;2:145–151.