Society For Biomaterials

Osseointegration of atmospheric plasma-sprayed titanium implants: Influence of the native oxide layer

Alexandre Cunha,[1] Renata Pedrolli Renz,[1] Eduardo Blando,[1] Rogério Belle de Oliveira,[2] Roberto Hübler[1,3]

[1]Research & Development in Physics, Materials and Nanostructures Laboratory, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre – RS, Brazil
[2]School of Dentistry, Oral and Maxillofacial Surgery Department, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre – RS, Brazil
[3]Physics Faculty, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre – RS, Brazil

Received 8 November 2012; accepted 11 February 2013
Published online 9 April 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34667

Abstract: The aim of this study was to evaluate *in vivo* the influence of the native oxide layer on osseointegration and new bone formation on the surface of atmospheric plasma-sprayed porous titanium coatings. Porous titanium coatings were deposited on all implant surfaces, and half of the samples were subsequently submitted to oxide layer removal treatment. Samples were implanted onto the cortical bone of sheep (tibia) and evaluated at 30 and 60 days. Implants were removed *en bloc* and the attachment of bone to implants was examined by tensile pull-out test (osseointegration assessment), light microscopy, scanning electron microscopy (histological analysis), and instrumented hardness tests (mechanical properties of mature and newly formed bone tissue). Coatings submitted to oxide layer treatment presented higher osseointegration values at both healing periods and showed more mature and mineralized bone tissue when compared with nontreated coatings. Our findings showed that the use of acid-etching in association with atmospheric plasma spraying techniques improves osseointegration of titanium implants. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 102A: 30–36, 2014.

Key Words: titanium oxide, plasma spraying, surface modification, osseointegration, biomechanics

How to cite this article: Cunha A, Renz RP, Blando E, de Oliveira RB, Hübler R. 2014. Osseointegration of atmospheric plasma-sprayed titanium implants: Influence of the native oxide layer. J Biomed Mater Res Part A 2014:102A:30–36.

INTRODUCTION

The number of patients requiring orthopedic or dental implants for the repair or replacement of damaged biological tissue has increased significantly over the last 20 years.[1–3] Titanium-based materials are considered particularly suitable for the production of such prostheses and have been widely employed in both orthopedics and dentistry. Among the attractive properties associated with titanium, its excellent biocompatibility, low Young's modulus, and good resistance to wear and corrosion stand out.[4,5]

Osseointegration, or the growth of bone on the surface of implants, is an important and desirable phenomenon whenever bone implants are used *in vivo*. Studies have shown that the behavior and growth of bone tissue are strongly affected by implant surface properties such as roughness, porosity, wettability, and chemical composition,[6–16] with an important effect on clinical success. In this sense, the use of plasma-sprayed porous titanium coatings (TC) has been a suitable option for improving osseointegration and osseoconduction.[17–20]

Porous TCs deposited by plasma spraying techniques are widely used in orthopedic implants, but are rare in dental prostheses, which are often treated by acid-etching.[21] The atmospheric plasma spraying (APS) technique tends to produce a thick oxide layer on the coatings as a result of a chemical reaction between titanium and air oxygen.[22–24] As this coating is deposited by successive liquid drops (all with high levels of oxide on its surface) oxygen concentration in the film is much higher than recommended by the ASTM F67 and ISO 5832. Although the thin native oxide is known to suppress corrosion and improved in-growth of bone cells, if this layer is very thick, it is also responsible for a poor tissue adhesion resulting in a slow osteogenesis process.[25–28] One possibility to reduce the oxygen content on the surface of plasma-sprayed implants is to submit the specimens to acid-etching, similarly to what is observed in the dental practice. Although unusual, the combination of APS and acid-etching could possibly contribute to bone growth on orthopedic implants, and could also have advantages if applied to dental prostheses. Importantly, the benefic thin native oxide layer will always be formed after the etching process once it is formed by the exposure of titanium with oxygen in the air.

Correspondence to: R. Hübler; e-mail: hubler@pucrs.br

Therefore, the aims of this study were to assess *in vivo* the influence of the native oxide layer of atmospheric plasma-sprayed titanium implants, submitted or not to etching with fluoride solution, on osseointegration and new bone formation on the implant surface.

MATERIAL AND METHODS

Preparation and characterization of titanium implants

Eighty coin-shaped titanium implants (ASTM grade 4), 4 mm thick, 6 mm in diameter, and with a 3 mm diameter center hole (NEODENT, Curitiba, Brazil and BAUMER, Mogi Mirim, Brazil) were prepared. Ti-6Al-4V screws with 1.6 mm diameter were used to ensure contact and attachment of the titanium implants to the animals' cortical bone, in accordance with previously published studies.[29–31]

The chemical composition of titanium implants and coatings was evaluated according to the ASTM F 67 standard,[32] using Shimadzu XRF 1800 wavelength dispersive X-ray fluorescence spectroscopy equipment (WXRF) with a Rhodium (Rh) target. All spectra were acquired on the titanium–uranium (Ti–U) and oxygen (O) channels using LiF and SX-14 as diffraction patterns. Corrosion was assessed according to the ASTM F 746 standard[33] by electrochemical assays carried out with an Autolab PGSTAT 302 potentiostat.

Deposition of porous TC and native oxide layer treatment

Porous TCs were deposited on all implant surfaces using the APS technique with titanium powder with a particle size from 100 to 200 μm (70–170 mesh) and oxygen content lower than 0.23 wt %. A mixture of pure argon (99.999%, 37.8 slpm) and pure hydrogen (99.99%, 4.7 slpm) was used as plasma gas in the APS equipment, the stabilized power source operated at 55 V and 500 A, and the distance between the plasma gun and the implant was kept at 100 mm.

After the coating deposition procedure, 40 of the titanium-coated implants were submitted to ultrasonic etching with aqueous fluoride solution (HF 5%) for 15 seconds to remove the superficial oxide layer formed during deposition. Samples were therefore divided into two distinct groups: as-deposited porous titanium coatings (TPC group) and porous titanium coatings etched with fluoride solution (TPC-HF group). The SEM micrograph of one implant of each group with magnifications of 25× and 500× are shown in Figure 1. Thirty-six samples of each group were submitted to *in vivo* tests, and four were assessed in terms of morphological and corrosion behavior. After these procedures, samples were sterilized in ethylene-oxide (ETO) and stored until implantation.

Animal model and surgical procedure

This study was approved by the Ethics Committee at Pontifical Catholic University of Rio Grande do Sul (PUCRS),

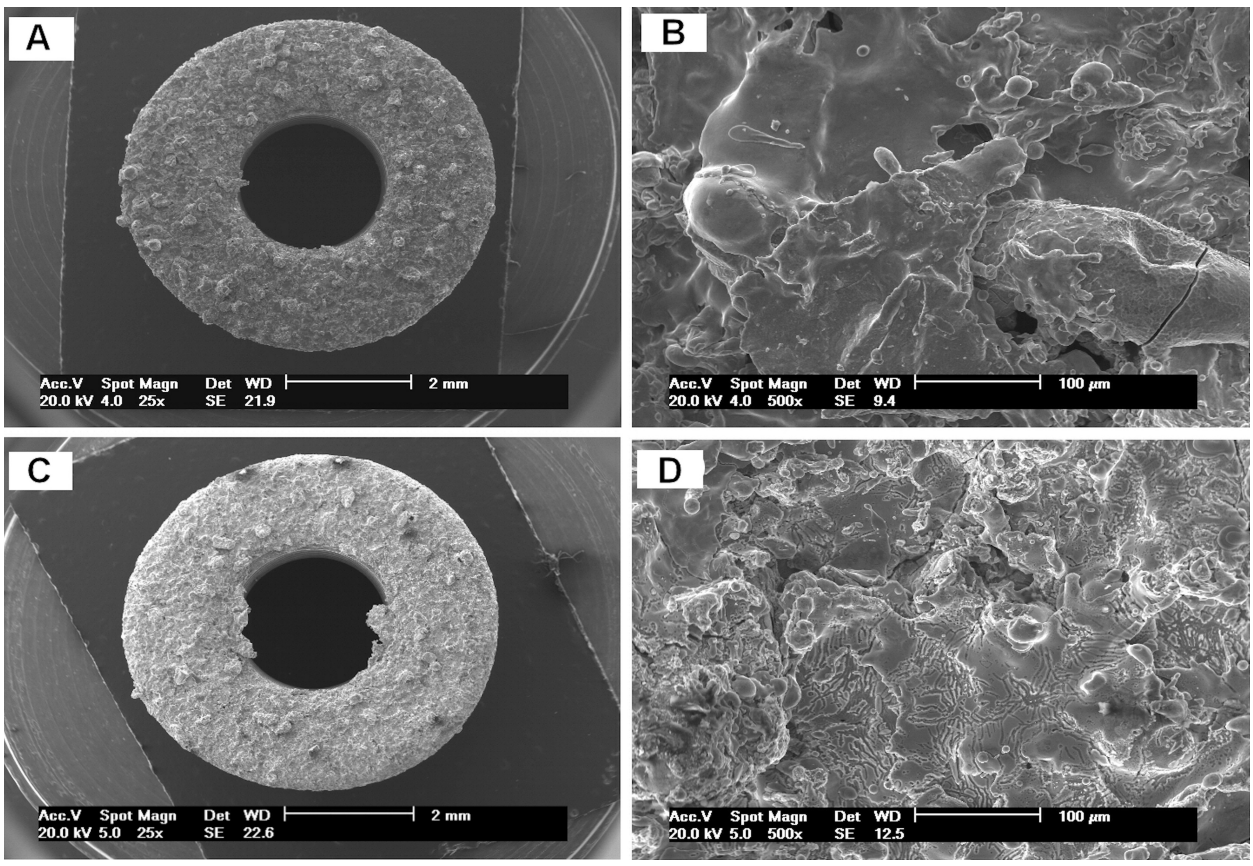

FIGURE 1. Electronic micrograph of one sample from TCP group (A) magnification 25× and (B) 500×, and from TCP-HF group (C) 25× and (D) 500×.

protocol N° 06/03548. The *in vivo* procedures were carried out in accordance with Brazilian laws and regulations, minimizing animal pain and discomfort.

Six clinically healthy, adult female sheep weighing between 38 and 42 kg were used in this study. Three animals were sacrificed after a healing period of 30 days, supplying 18 implants of each group; the remaining animals were sacrificed after 60 days. Sheep were chosen as the animal model because of their thick cortical bone and to have the same experimental procedure as previously published works.[29–31]

Preanesthesia consisted of intramuscular administration of 0.1 mg/kg acepromazine maleate (1% Acepran) and 2 mg/kg meperidine (Dolosal). After 15 minutes, 20 mg/kg cephalothin sodium was injected intravenously. Anesthesia was induced with an intravenous injection of propofol (2–4 mg/kg) and maintained with isoflurane in 100% oxygen. The incisions were made along the longitudinal bone axis. After incision, the soft tissues were raised to expose the cortical bone area. A careful surgical technique involving bone perforation and drillings was applied under constant irrigation with saline solution to avoid heating damage to the cortical bone. After pre-threading, the implants were inserted (six implants in each tibia) 13 mm apart in a predetermined pattern as showed in Figure 2(A). Thus, each animal received three implants of each type in each tibia.

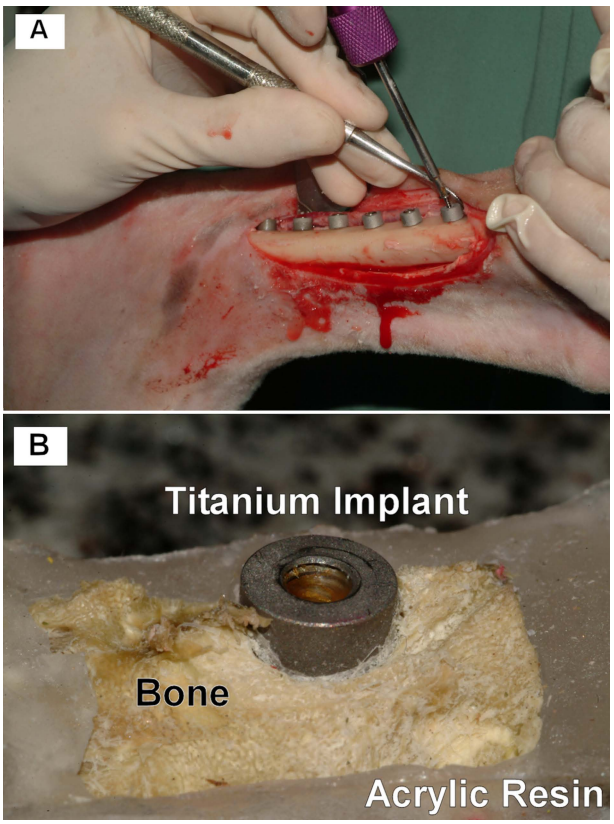

FIGURE 2. (A) Implants fixation during surgery and (B) titanium implant with surrounding bone tissue *en bloc*, embedded in acrylic resin for osseointegration assessment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Postoperatively, the animals received antibiotics at an animal dosage for 3 days. After healing times, the animals were sacrificed with a sodium thiopental injection. A detailed description of the surgical and postoperative procedures adopted has been presented previously.[30]

Sample preparation and characterization

Following euthanasia, titanium implants were removed *en bloc* with the surrounding tissue. Adhesion of bone tissue to TCs was assessed using tensile pull-out tests. The TC/bone tissue interface was submitted to histological analysis using light microscopy and scanning electron microscopy. Instrumented hardness tests (IHT) were used to measure the mechanical properties of mature and newly formed bone (NB).

For the pull-out tests, samples were first embedded in acrylic resin blocks as shown in Figure 2(B). A 3-mm titanium screw was fitted to the central thread of each implant for attachment to the top edge of the jig of the tensile testing equipment.

For the histological analysis, samples were dehydrated in graduated ethyl alcohol solutions from 10% to 100% (20 minutes at each concentration). After the last bath, samples were drained and dried in an incubator for 2 hours at about 100°C, and then stored in a low-vacuum chamber with silica gel for 2 weeks.[34,35] Samples embedded in acrylic resin were cut with a 0.3-mm-thick diamond saw. For analysis of the TC/bone tissue interface, sectioned samples were prepared using groove and polish procedures.[36] Interfaces were grooved with SiC paper with a particle size of 150–4000, polished with 9, 1, and 0.25-μm diamond paste, and finished with 50-nm SiC colloidal solution.

Prior to light microscopy and scanning electron microscopy, samples were drained and dried again as described above. After the imaging procedure, the same samples were used to measure mechanical properties.

Tensile pull-out tests

The universal testing machine EMIC DL 2000 was used in the pull-out tests. A load cell of 500 N (50 kgf) and a constant deformation rate of 1 mm/min were employed, according to an adaptation of the ASTM C 633 standard.[37] All samples were pulled out until complete rupture of the TC/bone tissue interface.

Microscopy analyses

Images of the TC/bone tissue interface were acquired using an Olympus® BX-60 light microscope, at 100× and 200× magnifications, in bright field mode. Measurements at the interface were carried out in all samples of each group in order to observe the osseointegration and the bone tissue maturation stage. To evaluate the osteogenesis and the bone quality, the presence of Havers channels, the bone density, and the gap between the implant and NB (region without structured mineral tissue) were considered. The micrographs were analyzed using the Image J® software, calibrated in advance on the basis of the pixel-μm relationship, according to an adaptation of the ASTM 1854 standard.[38]

A Philips XL 30 scanning electron microscope, operating at 20 keV electron beam in backscattered and secondary electron mode, was used to acquire images of the implants surface before implantation for morphological analyses and after the pull-out tests in order to evaluate the remaining bone tissue on the coatings. Prior to image acquisition, all samples were covered with an approximately 30-nm-thick sputtered-gold layer.

Instrumented hardness tests

Hardness and Young's modulus of mature and NB were measured using Fischerscope HV 100, applying a maximum load of 10 mN at a load–unload cycle of 80 seconds, with a Berkovich indenter. Each bone tissue type [mature bone (MB) and NB] was submitted to 10 IHT measurements. Measurements were carried out in conformity with ISO 14577-1 standard.[39]

RESULTS

Chemical composition and native oxide layer treatment

The WXRF analysis was carried out to determine the oxygen content for the titanium powder used to form the coatings and for both groups (TPC and TPC-HF). The powder presented a low percentage of oxygen (0.23 wt %), within the limits described in the ASTM F67standard, whereas TPC samples presented the highest oxygen content (8.28 wt %), as expected, taking into consideration that the titanium films were deposited at atmospheric pressure. After etching, TPC-HF samples showed a lower oxygen content (0.10 wt %) when compared with the powder indicating that the etching procedure was appropriate for our primary objective, namely, to obtain a coated implant with an oxygen content that meets the ISO 5832-2 standard,[40] and confirming that the oxygen contamination was only in a superficial layer.

Osseointegration

Mean ultimate stress values obtained for both studied groups are shown in Figure 3. After both healing periods, the ultimate stress observed in the TPC-HF group was higher than that observed for the TPC specimens. An increasing trend in ultimate stress values was observed in relation to healing period and native oxide layer removal. At 30 days, the bone retention of TPC-HF was 117% higher than TPC group and, at 60 days, an increase of 48% was observed. One of the most significant findings was that the ultimate stress observed in the TPC-HF group at 30 days was similar to that obtained in the TPC group at 60 days, indicating that the etched surfaces, with lower oxygen content, show a better biological response than the TCP group in the early stages.

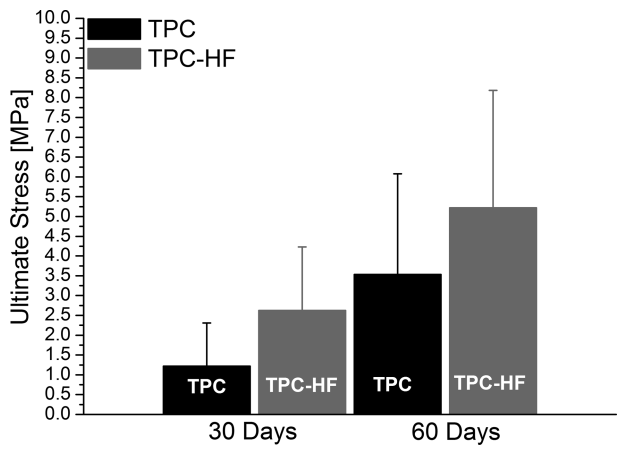

FIGURE 3. Ultimate stress values obtained for titanium coating/bone tissue interface in both groups at both healing periods.

Histological analysis

Histological analysis images are shown in Figure 4. At both 30 and 60 days, TPC-HF samples showed NB at more advanced stages of osteogenesis when compared with TPC samples on light microscopy. After 30 days, TPC samples [Fig. 4(A)] showed a newly formed region (NB) and the presence of osteocyte lacunae, whereas in the TPC-HF group [Fig. 4(B)], MB tissue was detected, with completely formed structures, as confirmed by the presence of Havers channels (arrows). In the TPC-HF group, bone tissue formation was very similar at 30 and at 60 days, that is, the same bone structures were observed at both healing periods. Furthermore, at 60 days, a region of interlocked bone tissue [Fig. 4(D)], well adhered to the TCs, was observed in the TPC-HF group. This group presents a very small gap (CBI) between the porous TC and the NB region.

The development of the coating/bone interface gap over time is given in Table I. The lowest mean gap was observed in the TPC-HF group at 60 days, whereas the highest mean gap was observed in the TPC group at 30 days. These results suggest that a longer healing period and removal of the native oxide layer contribute to reducing the coating/bone interface gap, thus promoting the formation of mature and mineralized tissue.

Scanning electron micrographs of remaining bone tissue on the coatings after interface rupture by pull-out tests are shown in Figure 5 only for the 60 days healing group. The TPC group showed a higher proportion of spherical osteoblast cells without ramifications, whereas the TPC-HF group presented the same type of cells with some visible ramifications, characterizing more mature trabecular bone tissue in comparison with the TPC group.

Mechanical properties

The mechanical properties of mature and NB measured at 60 days show a clear evidence of the surface treatment for the oxide thick layer removal. The NB tissue found in the TPC-HF group presented results that are compatible with MB ($H \sim 1000$ MPa and $Y \sim 22$ GPa): hardness of 1104.04 $\pm$ 122.79 MPa and Young's modulus of 26.10 $\pm$ 1.46 GPa. In the TPC group, NB presented hardness of 442.27 $\pm$ 48.46 MPa and Young's modulus of 9.71 $\pm$ 0.75 GPa, whereas MB had hardness of 720 $\pm$ 58.42 MPa and elastic modulus of 16.48 $\pm$ 1.46 GPa. These results show a strong influence of the surface quality of the bone formed around the implant.

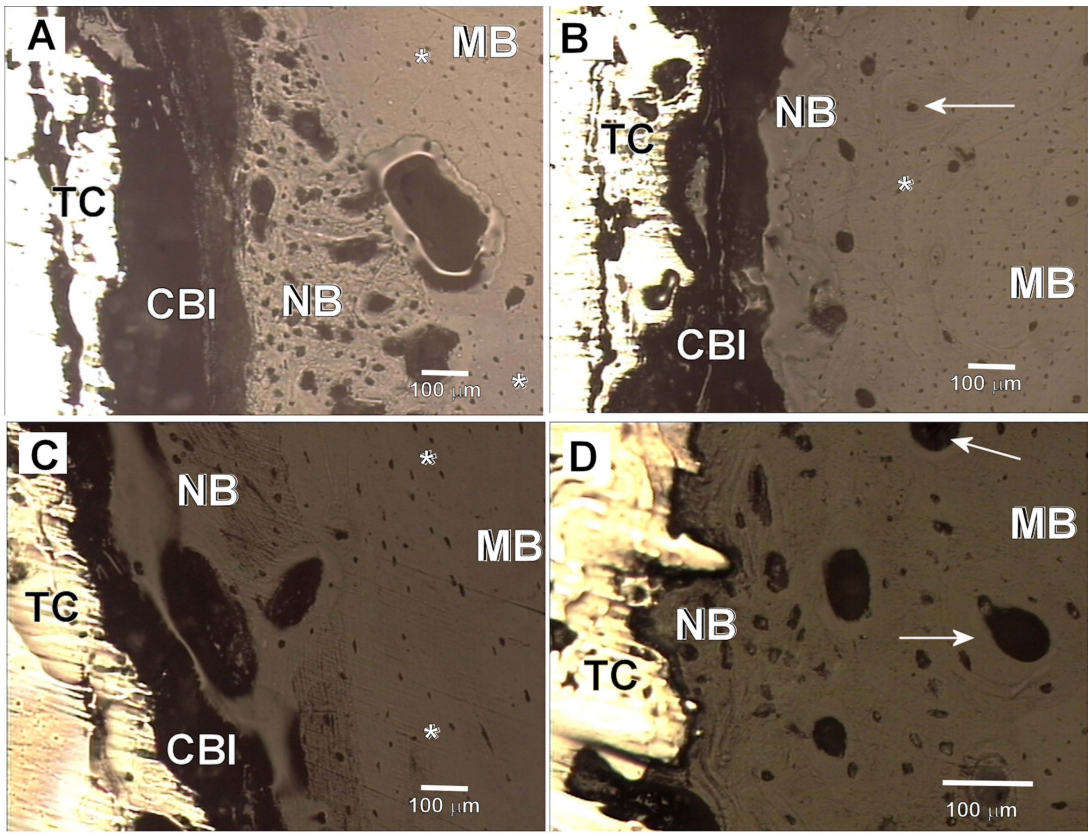

FIGURE 4. Light micrographs of the samples sectioned for histological evaluation. (A) TPC and (B) TPC-HF groups at 30 days. (C) TPC and (D) TPC-HF groups at 60 days. The images show titanium coatings (TC), the coating/bone interface (CBI), newly NB, MB, osteocyte lacunae (*), and Havers channels (→). Images from (A) to (C) were acquired at 100× magnification and image (D) with 200× (bar = 100 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

These measurements were not performed at 30 days because of the large number of voids (coating/bone interface) found in the bone tissue, which made indenter penetration very inaccurate.

DISCUSSION

Several previous studies have shown that the formation of NB and its properties are strongly affected by implant surface properties such as chemical composition, roughness, porosity, wettability, and others.[6–16] Some of these studies have reported that oxide formation on the surface of titanium implants may inhibit or reduce bone tissue growth, affecting the desired adhesion between implant surface and bone cells.[25–28]

TABLE I. Titanium Coating/Bone Interface Gap Measurements in Both Groups at Healing Time of 30 and 60 Days

Healing Period/ Group		Coating–Bone Interface Thickness (μm) (n = 40)		
		Minimum	Maximum	Mean ± Standard Deviation
30 days	TPC	182.94	331.86	237.36 ± 41.43
	TPC-HF	109.40	296.93	174.41 ± 51.29
60 days	TPC	51.48	238.10	147.29 ± 57.36
	TPC-HF	19.30	116.75	45.90 ± 24.85

In the present study, porous titanium implant coatings were prepared using the APS technique (TPC group), and half of the samples were subsequently submitted to etching with fluoride solution (TPC-HF group) to remove the thick oxide layer. The oxygen content deposited on the TC surface by APS resulted in the formation of a superficial oxide layer and was associated with poor osseointegration results in the TCP group, which is in accordance with the literature.[25–28] However, treatment of the TCs with fluoride solution was effective in removing the native oxide layer, as observed by the oxygen content analysis, and was associated with significantly better osseointegration results. These findings confirm that porous TC is suitable for improving osseointegration and osseoconduction phenomena when compared with other surface treatments that produce high porosity and roughness.[31,41,42] Moreover, they suggest that the etching procedure employed was appropriate for meeting the primary objective, namely, to obtain a coated implant with an oxygen content that meets the NBR-ISO 5832-2 standard.[40]

Removal of the native oxide layer showed to play a significant role in promoting osseointegration and osseoconduction in the present study. The higher osseointegration

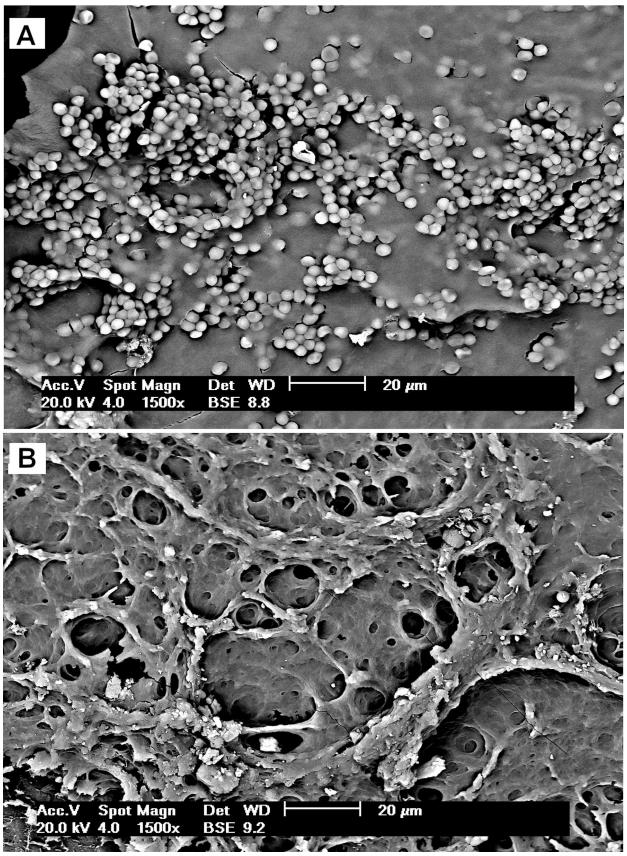

FIGURE 5. Scanning electron micrographs of the bone tissue remaining on titanium coatings after the tensile pull-out test. (A) Adherent bone at 60 days in the TPC group. (B) Adherent bone at 60 days in the TPC-HF group. Osteoblast cells can be observed in both micrographs, however, at different osteogenesis stages depending on healing period and titanium coating treatment. Micrograph B shows trabecular bone tissue, which is consistent with maturation of newly formed tissue.

values observed in the TPC-HF group were confirmed by the histological analysis and mechanical property measurements: NB tissue on these coatings was already mineralized, presented Havers channels, concentric tissue organization, and high hardness and Young modulus values. Effective osseointegration and osseoconduction were observed in the TPC-HF group already at 30 days. In this sense, one of the most significant findings of the study was that the ultimate stress values and histological characteristics observed in the TPC-HF group at 30 days were similar to those obtained in the TPC group at 60 days. At 60 days, the TPC-HF group presented rigid interlocking at the TC/bone tissue interface, confirming the formation and growth of bone tissue on the porous surface and explaining the higher ultimate stress values obtained in this group.

Another way to assess osseointegration and osseoconduction of implants is via the gap between the implant coating and the bone. The formation of bone in the coating/bone interface gap is characterized by mineralization of the collagen matrix deposited by osteoblast cells and maturation of NB. In this process, some osteoblast cells remain isolated within the matrix (osteocytes), a process that is followed by organization of the Havers channels.

In our study, the groove and polish procedures removed the collagen fiber matrix deposited by osteoblast cells at the coating/bone tissue interface. However, interface gap measurements are known to provide reliable information about bone tissue maturation. As shown by our results, a longer healing period and removal of the native oxide layer contributed to reducing the coating/bone interface gap, which explains the best qualitative and quantitative results observed in the TPC-HF group for NB and bone maturation.

The harmful effect of the native oxide layer on bone growth (in the TPC group) was clearly demonstrated at both healing periods (30 and 60 days), particularly at 30 days. Findings included a slower osteogenesis process, characterized by low osseointegration values, and a larger coating/bone interface gap. On the other hand, coatings treated with fluoride solution were associated with adequate osseointegration and rapid, dynamic formation of bone tissue.

In sum, our findings showed that the use of acid-etching in association with APS techniques was successful and improved osseointegration and osteoconduction results in atmospheric plasma-sprayed titanium implants. From a clinical point of view, these findings are very encouraging and suggest a great potential for clinical application, as the surface treatment applied to the TCs produced better biological fixation and improved bone quality when compared with the as-deposited coatings, which are more commonly available in the biomedical industry. Further studies are necessary to test the combination of both techniques in humans and to assess its real contributions for the clinical practice.

ACKNOWLEDGMENTS

The authors are grateful to Brazilian implant manufacturers NEODENT and BAUMER, to the CEMM-IDEA PUCRS, and to the Dental Materials Research Group at the PUCRS —School of Dentistry. We also thank to Dr. A. Etges and Dr. M. G. Oliveira for skilful discussions and to Dr. D. Gaziri and Dr. G. Wantowski for help in the surgical procedure. This work was partially supported by CAPES and CNPq, Brazilian Research Agencies.

REFERENCES

1. Zhang F, Zhang Z, Zhu X, Kang E-T, Neoh K-G. Silk-functionalized titanium surfaces for enhancing osteoblast functions and reducing bacterial adhesion. Biomaterials 2008;29:4751–4759.
2. Truong VK, Lapovok R, Estrin YS, Rundell S, Wang JY, Fluke CJ, Crawford RJ, Ivanova EP. The influence of nano-scale surface roughness on bacterial adhesion to ultrafine-grained titanium. Biomaterials 2010;31:3674–3683.
3. Joos U, Wiesmann HP, Szuwart T, Meyer U. Mineralization at the interface of implants. Int J Oral Maxillofac Surg 2006;35:783–790.
4. Jones FH. Teeth and bones: applications of surface science to dental materials and related biomaterials. Surf Sci Rep 2001;42: 75–205.
5. Helsen JA, Breme HJ. Metals as Biomaterials. Boca Raton: CRC Press; 2000. p 22.
6. Pegueroles M, Aparicio C, Bosio M, Engel E, Gil FJ, Planell JA, Altankov G. Spatial organization of osteoblast fibronectin matrix on titanium surfaces: Effects of roughness, chemical heterogeneity and surface energy. Acta Biomater 2010;6:291–301.

7. Puippe JC. Surface treatments of titanium implants. Eur Cell Mater 2003;5:32–33.
8. Schneider GB, Perinpanayagam H, Clegg M, Zaharias R, Seabold D, Keller J, Stanford C. Implant surface roughness affects osteoblast gene expression. J Dent Res 2003;82:372–376.
9. Ogawa T, Ozawa S, Shih JH, Ryu KH, Sukotjo C, Yang JM, Nishimura I. Biomechanical evaluation of osseous implants having different surface topographies in rats. J Dent Res 2000;79:1857–1863.
10. Cheng Z, Zhang F, He F, Zhang L, Guo C, Zhao S, Yang G. Osseointegration of titanium implants with a roughned surface containing hydride ion a rabbit model. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:e5–e12.
11. Liu X, Chu PK, Ding C. Surface modification of titanium, titanium alloys, and related materials for biomedical applications. Mater Sci Eng R: Reports 2004;47:49–121.
12. Conforto E, Aronsson BO, Salito A, Crestou C, Caillard D. Rough surfaces of titanium and titanium alloys for implants and prostheses. Mater Sci Eng C 2004;24:611–618.
13. Vandamme K, Naert I, Geris L, Vander Sloten J, Puers R, Duyck J. The effect of micro-motion on the tissue response around immediately loaded roughened titanium implants in the rabbit. Eur J Oral Sci 2007;115:21–29.
14. Albrektsson T, Brånemark PI, Hansson HA, Lindström J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand 1981;52:155–170.
15. Ellingsen JE, Pinholt EM. Pretreatment of titanium implants with lanthanum ions alters the bone reactions. J Mater Sci Mater Med 1995;6:125–129.
16. Anselme K. Osteoblast adhesion on biomaterials. Biomaterials 2000;21:667–681.
17. Borsari V, Giavaresi G, Fini M, Torricelli P, Tschon M, Chiesa R, Chiusoli L, Salito A, Volpert A, Giardino R. Comparative in vitro study on a ultra-high roughness and dense titanium coating. Biomaterials 2005;26:4948–4955.
18. Oliveira MV, Vasconcellos LMR, Carvalho YR, Cairo CAA, Pereira LC, Campos JB. Powder processing and "*in vivo*" evaluation of a titanium porous coating implant. 7th World Biomaterials Congress 2004;783.
19. Chu PK. Enhancement of surface properties of biomaterials using plasma-based technologies. Surf Coatings Technol 2007;201: 8076–8082.
20. Chu PK. Plasma surface treatment of artificial orthopedic and cardiovascular biomaterials. Surf Coat Technol 2007;201:5601–5606.
21. Le Guéhennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 2007;23:844–854.
22. Fauchais P, Vardelle A. Heat, mass and momentum transfer in coating formation by plasma spraying. Int J Therm Sci 2000;39: 852–870.
23. American Welding Society. Thermal Spraying. Miami; 1985. p181.
24. Pawlowski L. The Science and Engineering of Thermal Spray Coatings. New York: Wiley; 1995. p 414.
25. Takemoto S, Hattori M, Yoshinari M, Kawada E, Oda Y. Corrosion behavior and surface characterization of titanium in solution containing fluoride and albumin. Biomaterials 2005;26:829–837.
26. Reclaru L, Lerf R, Eschler PY, Blatter A, Meyer JM. Evaluation of corrosion on plasma sprayed and anodized titanium implants, both with and without bone cement. Biomaterials 2003;24: 3027–3038.
27. Fernández E, Gil FJ, Aparício C, Nilsson M, Sarda S, Rodriguez D, Ginebra MP, Manero JM, Navarro M, Casals J, Planell JA. Dental Biomechanics: Materials in dental implantology. Taylor & Francis: London and New York; 2003; p 69–89.
28. Ochsenbein A, Chai F, Winter S, Traisnel M, Breme J, Hildebrand HF. Osteoblast responses to different oxide coatings produced by the sol–gel process on titanium substrates. Acta Biomater 2008;4: 1506–1517.
29. Rønold HJ, Ellingsen JE. The use of a coin shaped implant for direct *in situ* measurement of attachment strength for osseointegrating biomaterial surfaces. Biomaterials 2002;23:2201–2209.
30. Cunha A, Renz RP, Wantowski G, Oliveira RB, Blando E, Hübler R. A surgical procedure using sheep as an animal model to evaluate osseointegration. J Dent Clin Res 2007;3:59–62.
31. Renz RP, Cunha A, Wantowski G, Blando E, Hübler R. Osseointegration evaluation of treated surfaces of titanium implants applying tensile pull-out test. J Dent Clin Res 2007;3:149–157.
32. American Society for Testing and Materials. ASTM F67. Standard Specification of unalloyed Titanium, for surgical applications. 2000.
33. American Society for Testing and Materials. ASTM F746. Standard Test Method for Pitting or Crevice Corrosion of Metallic Surgical Implant Materials. 1999.
34. Alava JI, Marin F, Intxaurrandieta A, Braceras I, Maeztu M. Simultaneous preparation of a materialographic and histological thin section: investigation of bone integration on dental implants. Structure-Struers J Materialography 2006, 1–6.
35. Keogh C. Thin section preparation: histological investigation of bone ingrowth into porous coated orthopaedic implants. Structure-Struers J Materialography 1999;34:3–5.
36. Traini T, Degidi M, Iezzi G, Artese L, Piattelli A. Comparative evaluation of the peri-implant bone tissue mineral density around unloaded titanium dental implants. J Dent 2007;35:84–92.
37. American Society for Testing and Materials. ASTM C633. Standard Test Method for Adhesion or Cohesion Strength of Thermal Spray Coatings. 2001.
38. American Society for Testing and Materials. ASTM F 1854. Standard test Method for Steriological evaluation of Porous Coatings on Medical Implants. 2001.
39. International Standardization Organization. ISO 14577-1. Metallic materials-Instrumented indentation test for hardness and materials parameters, Part 1: Test Method. 2002.
40. International Standardization Organization. NBR-ISO 5832-2. Implantes para Cirurgia—Materiais metálicos, Parte 2: Titânio puro. 2001.
41. Rønold HJ, Lyngstadaas SP, Ellingsen JE. Analysing the optimal value for titanium implant roughness in bone attachment using a tensile test. Biomaterials 2003;24:4559–4564.
42. Rønold HJ, Ellingsen JE. Effect of micro-roughness produced by TiO2 blasting—tensile testing of bone attachment by using coin-shaped implants. Biomaterials 2002;23:4211–4219.