Society For Biomaterials

Osseointegration assessment of chairside argon-based nonthermal plasma-treated Ca-P coated dental implants

Gabriela Giro,[1] Nick Tovar,[2] Lukasz Witek,[2] Charles Marin,[3] Nelson R. F. Silva,[4] Estevam A. Bonfante,[5] Paulo G. Coelho[2]

[1]Department of Oral Diagnosis and Surgery, Araraquara Dental School, UNESP – Universidade Estadual Paulista, Araraquara, SP, Brazil
[2]Department of Biomaterials and Biomimetics, New York University, New York, New York
[3]Department of Dentistry, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
[4]Department of Prosthodontics, New York University, New York, New York
[5]Postgraduate Program in Dentistry, UNIGRANRIO University, School of Health Sciences, Duque de Caxias, RJ, Brazil

Received 22 January 2012; revised 6 March 2012; accepted 23 April 2012
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34304

Abstract: This study investigated the effect of an Argon-based nonthermal plasma (NTP) surface treatment-operated chairside at atmospheric pressure conditions applied immediately prior to dental implant placement in a canine model. Surfaces investigated comprised: Calcium-Phosphate (CaP) and CaP + NTP (CaP-Plasma). Surface energy was characterized by the Owens-Wendt-Rabel-Kaelble method and chemistry by X-ray photoelectron spectroscopy (XPS). Six adult beagles dogs received 2 plateau-root form implants ($n = 1$ each surface) in each radii, providing implants that remained 1 and 3 weeks *in vivo*. Histometric parameters assessed were bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO). Statistical analysis was performed by Kruskall-Wallis (95% level of significance) and Dunn's post-hoc test. The XPS analysis showed peaks of Ca, C, O, and P for the CaP and CaP-Plasma surfaces. Both surfaces presented carbon primarily as hydrocarbon (C—C, C—H) with lower levels of oxidized carbon forms. The CaP surface presented atomic percent values of 38, 42, 11, and 7 for C, O, Ca, and P, respectively, and the CaP-Plasma presented increases in O, Ca, and P atomic percent levels at 53, 12, and 13, respectively, in addition to a decrease in C content at 18 atomic percent. At 1 week no difference was found in histometric parameters between groups. At 3 weeks significantly higher BIC and BAFO were observed for CaP-Plasma treated surfaces. Surface elemental chemistry was modified by the Ar-based NTP. Ar-based NTP improved bone formation around plateau-root form implants at 3 weeks compared with CaP treatment alone. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 00A: 000–000, 2012.

Key Words: implant surface treatment, argon plasma, modified surface, osseointegration, *in vivo*

How to cite this article: Giro G, Tovar N, Witek L, Marin C, Silva NRF, Bonfante EA, Coelho PG. 2012. Osseointegration assessment of chairside argon-based nonthermal plasma-treated Ca-P coated dental implants. J Biomed Mater Res Part A 2012:00A:000–000.

INTRODUCTION

Under the prerogative of being the first part of the implant to interact with the host, implant surfaces have been thoroughly investigated in an attempt to hasten the early host-to-implant response.[1–6] The rationale for surface modification focuses upon implant interaction with biofluids positively altering the cascade of events that leads to bone healing and intimate interaction with the device.[7]

Several reviews[4,8] cover the large number of possibilities included in implant surface modifications leading to a general consensus that both rough surfaces (over smooth turned surfaces) and surface chemistry (additions of Ca-P, calcium-phosphate based bioceramics in various forms over non-coated surfaces) favor the early host-to-implant response.[2–4] From a historical perspective, dental implant surfaces evolved from the as-turned smooth surfaces towards textured rough surfaces, and recent research point towards chemistry modification of moderately rough surfaces.[2–4,8,9] However, while improvements in host-to-implant response have been experimentally demonstrated[10–12] with implant surface texture or chemistry modifications, there is no consensus concerning which surface roughness and/or chemistry combination will result in the earliest and highest quantity/quality osseointegration. In most cases, combinations of texture and chemistry known to hasten osseointegration are product-specific processes controlled by implant manufacturers during fabrication and not general-purpose processes widely available for the dental community. The ideal condition to provide the largest number of patients with improved treatment would be an economically viable,

Correspondence to: P. G. Coelho; e-mail: pgcoelho@nyu.edu
Contract grant sponsors: Bicon LLC, Boston, MA

chair side, operator (dental surgeon) controlled surface treatment that would effectively enhance the host response to any implant surface. While the approach to create surface treatments that could possibly applied to any implant surface has been previously attempted during earlier days of dental implantology by utilizing large thermal, radio frequency, and glow discharge plasma devices that either operated at high temperatures or under low pressures, respectively, the equipment operational inconsistency and its economic viability led these processes into falling from favor concerning implant surface treatment.

The plasma state is often referred to as the 4th state of matter. A plasma is characterized by the presence of positive (and sometimes also negative) ions and charged electrons in a neutral background gas.[13] Plasmas can be categorized as either "thermal (or hot) plasmas" (such as those historically utilized for plasma spraying hydroxyapatite coatings on implant surfaces) or "nonthermal (or cold) plasmas" (NTPs). The main constituents of plasmas are ions, electrons and, neutrals in thermodynamic equilibrium. In NTPs, most of the energy is put into the electron component which can drive "high-temperature" chemistry allowing surface activation/modification while operating at low ambient temperatures.[14] Recently, the microplasma NTPs utilized in this work have successfully reached clinical technological significance, as it has been built in small dimensions allowing its portability in the clinical setting and have been proven to provide enough energy for increasing surface energy while presenting safe operation at atmospheric conditions (unlike previous radio-frequency technology that required low pressures).[15]

NTPs used in biomedical applications have been shown to effectively change the energy and chemistry of surfaces due to the high concentration of reactive species that are generated.[16,17] Recent work has shown that mixtures of rare gases such as Argon (Ar) with oxygen are suitable as metastable rare gas species (carriers of a significant amounts of energy), leading to the formation of reactive oxygen species via energy transfer reactions.[16,17] Thus, depending on plasma set up and chemistry, the incorporation of reactive species and further surface cleaning may result in increased levels of surface reactivity and energy that can be applied immediately prior to implant placement, presenting potential benefits to any commercially available implant surface. The objective of the present investigation was to histometrically evaluate the effect of an on site Ar-based NTP treatment onto a CaP dental implant surface performed immediately prior to implantation and compare it to a solely CaP-treated control, in a beagle dog model.

MATERIALS AND METHODS

This study utilized plateau root form endosseous Ti-6Al-4V implants of 3.5 mm in diameter by 8 mm in length (the implant plateaus resulted in healing chambers that are ~200 μm depth by 400 μm in height, total 8 chambers per implant). The investigated implant surface treatment groups comprised: calcium-phosphate (CaP) (Integra-CP™, Bicon LLC, Boston, USA-a semicrystalline HA-based calcium-phosphate coated surface that is moderately rough in texture[10])

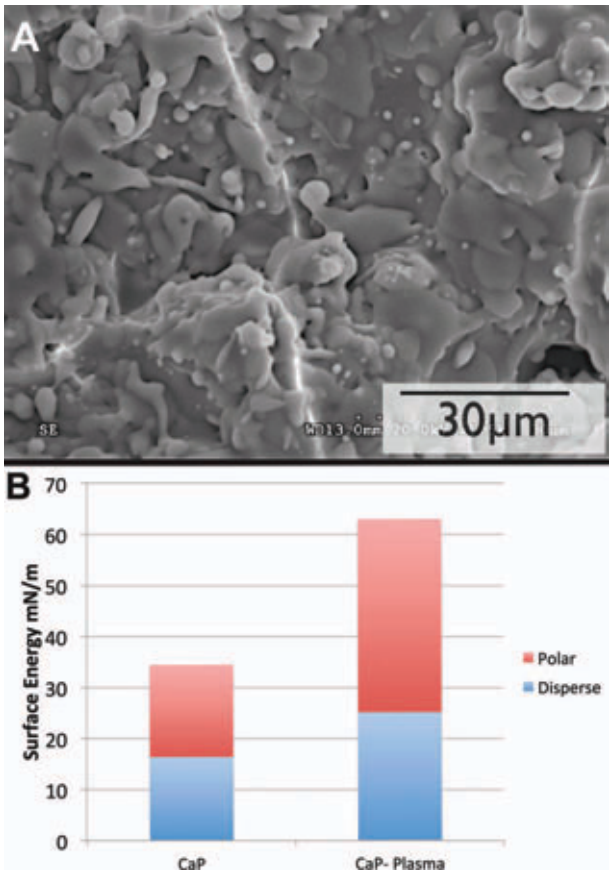

FIGURE 1. (a) Scanning electron microscopy micrograph of the Ar-based NTP treated implant surface and (b) surface energy measurements of both surfaces used in this study. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and the same CaP in addition to non thermal plasma (NTP) (CaP-Plasma) treatment with Ar gas for a period of 20 s per quadrant with a KinPen™ device (length = 155 mm, diameter = 20 mm, weight = 170 g) (INP- Greifswald, Germany). The KinPen was used for the generation of a plasma jet at atmospheric pressure connected to a high-frequency power supply (1.5 MHz, 2–6 kV peak-to-peak, system power 230 V, 65 W), and the gas supply unit was connected to a gas controller (Multi Gas Controller 647C, MKS Instruments, Andover, MA). Argon and compressed air tanks were attached to the gas controller with gas flow set at 5 standard liters per minute (slm). The surfaces were plasma treated immediately prior to implantation, and no attempt was made to prevent chemical species adsorption in the surgical setting.

A scanning electron micrograph of the Ar-based NTP treated implant surface is presented in Figure 1(a). To assess the surface energy (SE) of both surfaces, the Owens-Wendt-Rabel-Kaelble (OWRK) method was utilized.[18] Totally, 500 μL droplets of distilled water, ethylene glycol, and diiodomethane were deposited on the surface of each implant group ($n = 3$, in triplicate nested within each subject) with a micro-pipette (OCA 30, Data Physics Instruments GmbH, Filderstadt, Germany). Images were captured and analyzed using software (SCA30, version 3.4.6 build 79). The

relationship between the contact angle and SE was determined, and the SE was calculated by $\gamma_L = \gamma^D_L + \gamma^P_L$, where γ_L is the SE, γ^D_L is the disperse component and γ^P_L is the polar component. Statistical analysis was performed by one-way ANOVA at a 0.05 level of significance.

Surface specific chemical assessment was performed by X-ray photoelectron spectroscopy (XPS). The implants ($n = 3$, each group) were inserted in a vacuum transfer chamber and degassing it to 10^{-7} torr. The samples were then transferred under vacuum to a Kratos Axis 165 multitechnique XPS spectrometer (Kratos Analytical, Chestnut Ridge, NY). Survey and high-resolution spectra were obtained using a 165 mm mean radius concentric hemispherical analyzer operated at constant pass energy of 160 eV for survey and 80 eV for high resolution scans. The take off angle was 90° and a spot size of 150 μm x 150 μm was used. The implant surfaces were evaluated at various locations.

The *in vivo* study comprised of 6 adult male beagles dogs ~1.5 years of age. The experimental protocol received the approval of the École Nationale Vétérinaire d'Alfort (Maisons-Alfort, Val-de-Marne, France) and was carried out at the same location.

All surgical procedures were performed under general anesthesia. The preanesthetic procedure comprised an intramuscular (IM) administration of atropine sulfate (0.044 mg/kg) and xylasine chlorate (8 mg/kg). General anesthesia was then obtained following an IM injection of ketamine chlorate (15 mg/kg). Following hair shaving, skin exposure, and antiseptic cleaning with iodine solution at the surgical and surrounding area, a 5 cm incision at the skin level was performed. Then, a flap was reflected and the radius diaphysis exposed.

The surgical region was the center of the radius diaphysis, and two implants were placed along each limb. The right and left limbs provided implants that remained for periods of 1 and 3 weeks *in vivo*. The limbs were operated 1 and 3 weeks prior to euthanization respectively (two different implant placement procedures prior to euthanasia). The different implant surfaces were alternately placed from proximal to distal at distances of 1 cm from each other along the central region of the bone, and the start surface site (CaP or CaP-Plasma) was alternated between animals. The implant distribution resulted in an equal number of implants for the 1 and 3 week comparison for both surfaces.

The initial drilling was performed by a 2 mm diameter pilot drill at 1200 rpm under saline irrigation. Then, slow speed sequential drilling with burs of 2.5, 3.0, and 3.5 mm was performed at 800 rpm under saline irrigation. The implants were then press fit into the osteotomy sites by manual pressure. Standard layered suture techniques were utilized for wound closure (4-0 vicryl- internal layers, 4-0 nylon- the skin). Post-surgical medication included antibiotics (penicillin, 20.000 UI/kg) and analgesics (ketoprophen, 1 mL/5 kg) for a period of 48 h postoperatively. The euthanasia was performed by anesthesia overdose 3 weeks after the first surgical procedure.

At necropsy, the limbs were retrieved by sharp dissection; the soft tissue was removed by surgical blades. The bone blocks were kept in 10% buffered formalin solution for 24 h, washed in running water for 24 h, and gradually dehydrated in a series of alcohol solutions ranging from 70 to 100% ethanol. Following dehydration, the samples were embedded in a methacrylate-based resin (Electron Microscopy Science, Hatfield, PA) according to the manufacturer's instructions. The blocks were then cut into slices (~300 μm thickness) aiming the center of the implant along its long axis with a precision diamond saw (Isomet 2000, Buehler, Lake Bluff, USA), glued to acrylic plates with an acrylate-based cement (Technovit 7210 VLC, Heraeus Kulzer GmbH, Wehrheim, Germany), and a 24 h setting time was allowed prior to grinding and polishing. The sections were then reduced to a final thickness of ~30 μm by means of a series of SiC abrasive papers (280, 400, 800, 1200, 1500, and 2500) (Buehler, Lake Bluff, IL) in a grinding/polishing machine (Metaserv 3000, Buehler, Lake Bluff) under water irrigation. The sections were then toluidine blue stained and referred to optical microscopy evaluation. The histologic features were evaluated at 50×–200× magnification (Leica DM2500M, Leica Microsystems GmbH, Wetzlar, Germany).

The bone-to-implant contact (BIC) was determined at 50X-200X magnification (Leica DM2500M, Leica Microsystems GmbH, Wetzlar, Germany) by means of a computer software (Leica Application Suite, Leica Microsystems GmbH, Wetzlar, Germany). The regions of bone-to-implant contact along the implant perimeter were subtracted from the total implant perimeter, and calculations were performed to determine the BIC percentage. The bone area fraction occupancy (BAFO) between plateaus was determined at 100X magnification with the same microscope and software. The percentage areas occupied by bone were calculated from the total area within the healing chambers.[19]

Statistical analysis was performed by Kruskall-Wallis at 95% level of significance and Dunn's post-hoc test. The analysis employed IBM SPSS Statistics, v. 19 (IBM, New York, NY).

RESULTS

The surface energy assessment showed a significant increase ($p < 0.01$) in both polar and disperse components occurred for the CaP-Plasma group [Fig. 1(b)] compared to the CaP group. For the CaP group, the disperse value (mean ± SD) was 16.3 ± 1.3 m*N*/m and the polar value (mean ± SD) 17.7 ± 1.4 m*N*/m. For the CaP-Plasma group, the disperse value (mean ± SD) was 25.2 ± 1.3 m*N*/m and the polar value (mean ± SD) 37.8 ± 1.4 m*N*/m.

The XPS survey analysis of the implant surface showed peaks of Ca, C, O, and P for the CaP and CaP-Plasma surfaces. High-resolution spectrum evaluation showed that for both surfaces carbon was observed primarily as hydrocarbon (C—C, C—H) with lower levels of oxidized carbon forms. For both groups, calcium and phosphate were detected in varied atomic concentrations. For the CaP group, the (mean ± SD) atomic percent values were 38 (±4.2), 42 (±5.2), 11 (±2.5), and 7 (±1.3) for C, O, Ca, and P, respectively. When compared to the CaP group, the CaP-Plasma group (reported as mean ± SD) presented increases in O, Ca, and P atomic

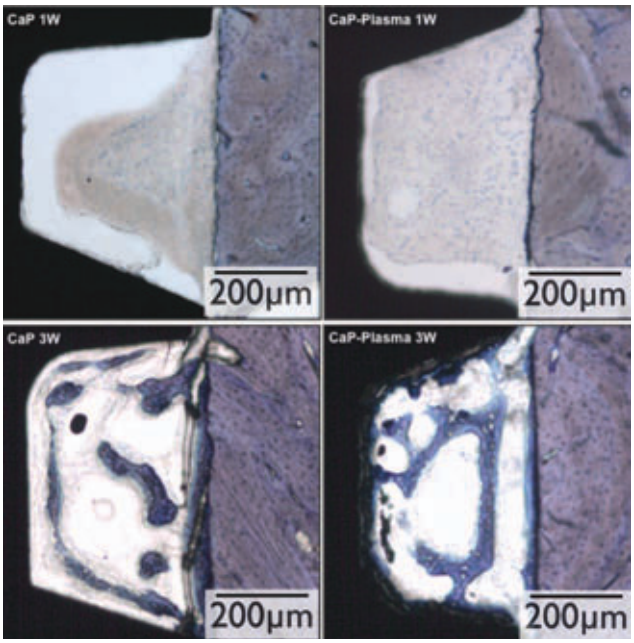

FIGURE 2. Representative overview of the histological micrographs of the plateaus at 1 and 3 weeks experimental periods at x200 magnification. At 1 week, the histologic sections of the CaP-plasma group showed initial signs of bone formation adjacent to the implant surface and the presence of layers of early connective tissue (stroma) filling the region between plateaus. In contrast, the CaP group presented the stroma collapsed to the center of the plateau. At 3 weeks, bone formation was observed throughout the healing chambers of both groups. Note the gap between tissue and implant surface was pronounced for the 1 week samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

percent levels observed at 53 (±4.1), 12 (±3.2), and 13 (±1.5), respectively. A decrease in C content was observed at 18 (±3.3) atomic percent.

The surgical procedures and follow-up demonstrated no complications or other clinical concerns, and no implant was excluded due to clinical instability (determined after euthanization).

At 1 week the histologic sections of the CaP-plasma group showed initial signs of bone formation adjacent to the implant surface and the presence of layers of early connective tissue (stroma) filling the region between plateaus (Fig. 2). In contrast, the CaP group presented the stroma collapsed to the center of the plateau (Fig. 2). At 3 weeks, bone formation was observed throughout the healing chambers of both groups (Fig. 2).

No significant difference was found for BIC and BAFO between surfaces at 1 week [Fig. 3(a,b), respectively]. At 3-weeks *in vivo*, bone formation in close contact to the implant surface (BIC) and within the plateau region (BAFO) were strongly evidenced to the CaP-Plasma group (Fig. 3). The morphologic findings for both 1 and 3 weeks were supported by the morphometric results at 3 weeks period since CaP-Plasma BIC raised over 100% [Fig. 3(a)] and an improvement of 82% was found for BAFO [Fig. 3(b)] when compared to the CaP group.

DISCUSSION

The plasma state is often referred to as the 4th state of matter. A plasma is characterized by the presence of positive (and sometimes also negative) ions and negatively charged electrons in a neutral background gas.[13] Plasmas are created by supplying energy (often electrical energy) to a volume containing gases, so that a certain fraction of free electrons and ions are generated from the neutral constituents. In technical plasma devices, the plasma is generally generated using electrical discharges.[13] When generating NTPs, one aims to put most of the energy into the electron component, which can drive "high-temperature" chemistry (through electron-driven processes such as ionization, dissociation and attachment) at low ambient temperatures defined by the low neutral and ion temperatures.[14] Thus, depending on plasma set up and chemistry, a wide range of surface alterations are achievable and may be utilized during the manufacturing or at the operating room immediately prior to implant placement under atmospheric conditions (these units may be fabricated in portable sizes). The present study investigated alterations in surface energy and chemistry in a previously characterized Ca- and P-based textured surface.[10]

The surface energy assessment prior and after NTP application showed a substantial increase in surface energy (in both polar and disperse components). The disperse component of the SE characterizes the interaction between the

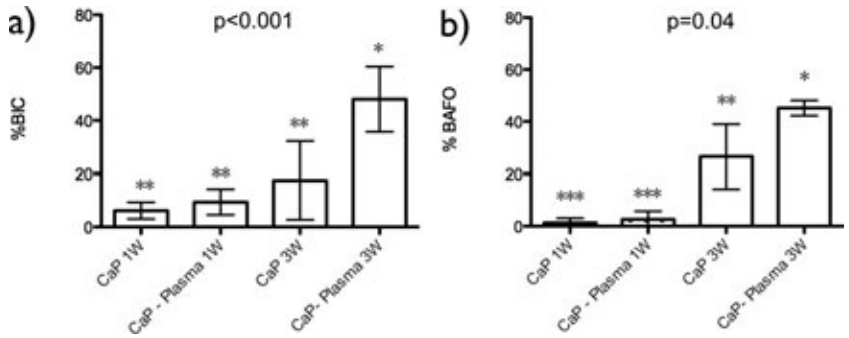

FIGURE 3. (a)Bone to implant contact and (b) bone area fraction occupancy percentages for the CaP and CaP-Plasma groups in the different experimental periods. Results shown as mean ± 95% confidence interval.

surface and the dispensed liquid in terms of the nonpolar interactions between molecules. The roughness, unevenness, and the branching level of the surface determine this component. The polar component of the SE characterizes the polar interaction between the surface of the material and the working fluid. This component is determined by the presence of polar groups, electric charges, and free radicals on the surface (Friedman, 2008).

The XPS results showed that surface elemental chemistry was modified by the 20s Ar-based NTP treatment, and that this change resulted in higher degree of exposure of the surface chemical elements mainly at the expense of the removal of adsorbed C species.[10] The higher degree of surface energy observed for the CaP-Plasma is likely related to the removal of the adsorbed C species from the surface and accounted for the increase in Ca and P in the plasma treated samples.[20–22] The XPS results support that at the energy level utilized in the present study, surface chemistry is primarily affected at the adsorbed species layers and/or few initial atomic layers (these changes may only be detected through high resolution field-emission scanning electron microscopy and may be topic of further investigation).

For the in vivo model, implants that presented healing chambers leading to the intramembranous-like ossification pathway were utilized. Such experimental model has been previously used for investigating the healing kinetics around dental implants, and such healing chambers arise from the interplay between the surgical drilling diameter that matches the outer diameter of the press fit implant circular fins,[23–28] resulting in void spaces between the implant and the osteotomy which are filled with a blood clot immediately after implant placement.[29,30]

While the experimental implant design differs from most commercially available dental implant systems (screw-shaped forms that are usually are placed in undersized drilled dimensions leaving restricted space between implant surface and bone), this model's relevance arises from the fact that in bone sites with decreased quality (types III and IV), void spaces will exist between the implant surface and bone immediately following placement.[19,30]

Our histomorphologic results are in direct agreement with previous work that demonstrated that after a few days, large blood clots filling large healing chambers[31] or the regions between bone and implant healing chamber walls[29,30] will evolve towards a provisional matrix of connective tissue presenting high content of mesenchymal cells (as observed for both groups at 1 week *in vivo*). This stroma will then serve as a scaffold for an intramembranous-like ossification, which was observed at 3 weeks *in vivo*.

Our laboratory *in vivo* model results showed key morphologic differences between groups at 1 week *in vivo* at the region within the healing chambers, and such morphologic difference likely accounted for the significantly higher results observed for the CaP-Plasma group at 3 weeks *in vivo*. The morphologic difference observed at 1 week was likely due to the higher surface wettability observed for the CaP-Plasma group, which resulted in improved blood clot adherence and closer interaction between the connective tissue matrix with the implant surface, which likely arose from the higher degrees of surface energy observed for that surface. Such closer interaction then prevented the connective tissue network from collapsing to the central area of the healing chamber (only observed for the CaP group at 1 week). The more uniform presence of osteogenic tissue throughout the chamber and closer interaction with the implant surface observed for the CaP-Plasma group at 1 week possibly resulted in the significantly higher degrees of BIC and BAFO at 3 weeks observed for the CaP-plasma group. These results are in agreement with previous work that showed that surface wettability is beneficial in hastening osseointegration in healing chambers at early times *in vivo*.[32]

In light of the findings of this study and taking into consideration that from a safety standpoint no adverse effects have been observed when NTPs were used in biomedical applications, the utilization of low temperature, atmospheric pressure Ar-plasma on surface alterations may be a promising technique in achieving osseointegration in a shorter period of time.

REFERENCES

1. Albrektsson T, Gottlow J, Meirelles L, Ostman PO, Rocci A, Sennerby L. Survival of NobelDirect implants: An analysis of 550 consecutively placed implants at 18 different clinical centers. Clin Implant Dent Relat Res 2007;9:65–70.
2. Albrektsson T, Wennerberg A. Oral implant surfaces, Part 1. Review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. Int J Prosthodont 2004;17:536–543.
3. Albrektsson T, Wennerberg A. Oral implant surfaces, Part 2. Review focusing on clinical knowledge of different surfaces. Int J Prosthodont 2004;17:544–564.
4. Coelho PG, Granjeiro JM, Romanos GE, Suzuki M, Silva NR, Cardaropoli G, Thompson VP, Lemons JE. Basic research methods and current trends of dental implant surfaces. J Biomed Mater Res B Appl Biomater 2009;88:579–596.
5. Jimbo R, Ono D, Hirakawa Y, Odatsu T, Tanaka T, Sawase T. Accelerated photo-induced hydrophilicity promotes osseointegration: An animal study. Clin Implant Dent Relat Res 2011;13:79–85.
6. Jimbo R, Sawase T, Baba K, Kurogi T, Shibata Y, Atsuta M. Enhanced initial cell responses to chemically modified anodized titanium. Clin Implant Dent Relat Res 2008;10:55–61.
7. Jimbo R, Sawase T, Shibata Y, Hirata K, Hishikawa Y, Tanaka Y, Bessho K, Ikeda T, Atsuta M. Enhanced osseointegration by the chemotactic activity of plasma fibronectin for cellular fibronectin positive cells. Biomaterials 2007;28:3469–3477.
8. Dohan Ehrenfest DM, Coelho PG, Kang BS, Sul YT, Albrektsson T. Classification of osseointegrated implant surfaces: materials, chemistry and topography. Trends Biotechnol 2010;28:198–206.
9. Kang BS, Sul YT, Oh SJ, Lee HJ, Albrektsson T. XPS, AES and SEM analysis of recent dental implants. Acta Biomater 2009;5:2222–2229.
10. Coelho PG, Lemons JE. Physico/chemical characterization and in vivo evaluation of nanothickness bioceramic depositions on alumina-blasted/acid-etched Ti-6Al-4V implant surfaces. J Biomed Mater Res A 2009;90:351–361.
11. Granato R, Marin C, Gil JN, Chuang SK, Dodson TB, Suzuki M, Coelho PG. Thin bioactive ceramic-coated alumina-blasted/acid-etched implant surface enhances biomechanical fixation of implants: An experimental study in dogs. Clin Implant Dent Relat Res 2011;13:87–94.
12. Marin C, Granato R, Suzuki M, Gil JN, Piattelli A, Coelho PG. Removal torque and histomorphometric evaluation of bioceramic grit-blasted/acid-etched and dual acid-etched implant surfaces: An experimental study in dogs. J Periodontol 2008;79:1942–1949.

13. Lieberman MA, Lichtenberg AJ. Principles of Plasma Discharges and Materials Processing. New York: Wiley; 1994.
14. Barker R. Introduction and overview. In: Becker UK, Schoenbach KH, Barker RJ, editors. Non-equilibrium Air Plasmas at Atmospheric Pressure. Bristol: IOP Publishing; 2005.
15. Liu F, Sun P, Bai N, Tian Y, Zhou H, Wei S, Zhou Y, Zhang J, Zhu W, Becker K, et al. Inactivation of bacteria in an aqueous environment by a direct-current, cold atmospheric-pressure air plasma microjet. Plasma Processes Polym. Forthcoming.
16. Foest R, Kindel E, Lange H, Ohl A, Stieber M. RF capillary jet-a tool for localized surface treatment. Contributions Plasma Phys 2007;47:119–128.
17. Foest R, Schmidt M, Becker K. Microplasmas, a New World of low-temperature plasmas. Int J Mass Spectrom 2005;248:87–102.
18. Owens DK, Wendt RC. Estimation of the surface free energy of polymers. J Appl Polym Sci 1969;13:1741–1747.
19. Leonard G, Coelho P, Polyzois I, Stassen L, Claffey N. A study of the bone healing kinetics of plateau versus screw root design titanium dental implants. Clin Oral Implants Res 2009;20: 232–239.
20. Baier RE. Implant dentistry forefront '85. Surface preparation. J Oral Implantol 1986;12:389–395.
21. Baier RE. Selected methods of investigation for blood-contact surfaces. Ann NY Acad Sci 1987;516:68–77.
22. Baier RE, Meyer AE. Implant surface preparation. Int J Oral Maxillofac Implants 1988;3:9–20.
23. Giro G, Marin C, Granato R, Bonfante EA, Suzuki M, Janal MN, Coelho PG. Effect of drilling technique on the early integration of plateau root form endosteal implants: An experimental study in dogs. J Oral Maxillofac Surg 2011;69:2158–2163.
24. Suzuki M, Calasans-Maia MD, Marin C, Granato R, Gil JN, Granjeiro JM, Coelho PG. Effect of surface modifications on early bone healing around plateau root form implants: An experimental study in rabbits. J Oral Maxillofac Surg 2010;68:1631–1638.
25. Coelho PG, Granato R, Marin C, Bonfante EA, Janal MN, Suzuki M. Biomechanical and bone histomorphologic evaluation of four surfaces on plateau root form implants: An experimental study in dogs. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:e39–e45.
26. Marin C, Granato R, Suzuki M, Gil JN, Janal MN, Coelho PG. Histomorphologic and histomorphometric evaluation of various endosseous implant healing chamber configurations at early implantation times: A study in dogs. Clin Oral Implants Res 2010;21:577–583.
27. Granato R, Marin C, Gil JN, Chuang SK, Dodson TB, Suzuki M, Coelho PG. Thin bioactive ceramic-coated alumina-blasted/acid-etched implant surface enhances biomechanical fixation of implants: an experimental study in dogs. Clin Implant Dent Relat Res 2011;13:87–94.
28. Coelho PG, Marin C, Granato R, Suzuki M. Histomorphologic analysis of 30 plateau root form implants retrieved after 8 to 13 years in function. A human retrieval study. J Biomed Mater Res B Appl Biomater 2009;91:975–979.
29. Berglundh T, Abrahamsson I, Albouy JP, Lindhe J. Bone healing at implants with a fluoride-modified surface: An experimental study in dogs. Clin Oral Implants Res 2007;18:147–152.
30. Berglundh T, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants. Clin Oral Implants Res 2003;14:251–262.
31. Cardaropoli G, Wennstrom JL, Lekholm U. Peri-implant bone alterations in relation to inter-unit distances. A 3-year retrospective study. Clin Oral Implants Res 2003;14:430–436.
32. Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochran DL, Hoffmann B, Lussi A, Steinemann SG. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res 2004;83:529–533.