# Histomorphometric Study of Ion Implantation and Diamond-like Carbon as Dental Implant Surface Treatments in Beagle Dogs

Miguel A. De Maeztu, MD, DDS, PhD[1]/Iñigo Braceras, MEng[2]/J. Iñaki Alava, PhD[3]/
M. Angeles Sánchez-Garcés, MD, DDS[4]/Cosme Gay-Escoda, MD, DDS, PhD[5]

***Purpose:*** *Improvements in the bone-implant interface can provide clinical benefits, such as increasing the amount of bone in contact with the implant and shortening the time required to achieve sufficient bone appositioning to allow early prosthetic loading. The present study describes the results obtained with 2 new surface treatments:* (a) *CO ion implantation; and* (b) *diamond-like carbon (DLC) coating.* ***Materials and Methods:*** *Each group (ion implantation, DLC, and the control group, turned titanium) consisted of 12 samples. Beagle dogs subjected to previous partial edentulation were used. Dual histologic evaluation was made of percentage bone-implant contact (% BIC) of all samples based on conventional histomorphometric analysis and environmental scanning electron microscopy (ESEM).* ***Results:*** *The results obtained after 3 and 6 months of dental implant placement showed greater and faster bone integration in the CO ion implantation group (61% and 62% BIC, respectively) compared with the DLC group (47% and 50%); the data corresponding to the ion implanted samples were statistically significant compared with the control group (33% and 49% BIC after 3 and 6 months, respectively).* ***Conclusions:*** *The results showed improved % BIC for implants with ion-implanted surfaces in comparison to DLC coating and machined controls. Furthermore, bone integration appeared to be accelerated in the ion implantation group, since high % BIC values were recorded in the early stages after in vivo implantation.* Int J Oral Maxillofac Implants 2007;22:273–279

**Key words:** *bone integration, dental implants, diamond-like carbon, histomorphometry, ion implantation, osseointegration, periodontology, surface treatments*

Dental implant treatment success is dependent on the surface characteristics of the material used, among other factors.[1–5] These characteristics comprise both physical (macro-, micro- and nanometric roughness) and chemical factors (corrosion, contamination, ion release, bioactive surfaces).[6]

The capacity of the implant surface to retain the fibrin bound to it in the first moments of the healing phase seems to be critical. According to Davis,[7] during the blood clot retraction phase, fibrin binding to a more retentive surface facilitates osteogenic cell access. Once contact has been established, these cells undergo differentiation, giving rise to a phenomenon known as *contact osteogenesis*, or the formation of bone from the surface of the implant. This process advances in a direction opposite to the repair process, which arises from the receptor bone bed—

[1]Private Practice, Tolosa, Spain.
[2]Engineer, Lifenova Biomedical, San Sebastian, Spain.
[3]Doctor of Chemical Sciences, Inasmet Foundation Technological Center, San Sebastian, Spain.
[4]Associate Professor of Oral Surgery, University of Barcelona Dental School, Spain.
[5]Professor of Oral and Maxillofacial Surgery, University of Barcelona Dental School, Spain.

**Correspondence to:** Dr Miguel A. De Maeztu, Paseo San Francisco 43A, 20400 – Tolosa (Spain). E-mail: maeztu@cgcom.org

a phenomenon called *distance osteogenesis*. This advance of both osteogenic processes in opposite and confluent directions would explain the faster bone integration of certain implants as a result of their surface characteristics. As Davis reported,[7] the chemical composition of the surface is therefore decisive in that it can facilitate fibrin adhesion and retention.

The surface treatments successfully used in the last few decades are based on the application of materials such as hydroxyapatite coatings[8] or thermal plasma spraying.[9] Other surface treatment techniques have included particle blasting techniques such as sandblasting,[10] titanium oxide ($TiO_2$) blasting,[11,12] or hydroxyapatite blasting[13]; double acid-etching procedures[14,15]; surface blasting with acid-etching techniques[2]; and electrochemical anodization.[16]

A number of these treatments effectively increase the total surface area available for bone-implant contact by increasing surface roughness, thus improving bone integration of the implant. However, some authors, such as Lim and coworkers,[17] consider other parameters more important than surface roughness for ensuring biocompatibility. Specifically, they cite the angle of contact, ie, the angle formed by a standardized drop of fluid on a surface, which can be used to determine the corresponding surface free energy. These authors suggest that surface energy, represented by the angle of contact, is related to the crystalline structure of the oxide layer formed upon it, and that biocompatibility is greater in the presence of increased surface energies.[18,19]

CO ion implantation is a new surface treatment designed to improve implant bone integration by modifying the chemical structure of the implant surface at the atomic level without adding or removing material. This is a high-vacuum physical technique ($< 10^{-4}$ Pa) in which the surface of a material is bombarded with previously selected and accelerated ions that become integrated or implanted within the outer atomic layers of the surface, thereby modifying its physicochemical properties.

Following the publication of both in vivo and in vitro studies of this technique,[20–22] the present article presents the results of a study conducted in 12 beagle dogs comparing implants subjected to ion implantation with untreated control implants. In addition, dental implants subjected to a novel coating technique involving diamond-like carbon (DLC) coating were evaluated.

## MATERIALS AND METHODS

### Animals

Twelve adult male beagle dogs, aged 7 to 14 months, were used. The study was approved by the Animal Experimentation Ethics Committee of the University of Barcelona (Spain) and adhered to standards ISO-10993-2 and ISO-10993-6.

### Dental Implants

A total of 72 commercial threaded implants measuring 10 mm in length were divided into 6 groups. Five represented different surface treatments—ion implantation, DLC, double acid etching (Osseotite; 3i Implant Innovations, Palm Beach Gardens, FL), sandblasted and acid etching (SLA; Straumann, Basel, Switzerland), and anodized (TiUnite; Nobel Biocare, Göteborg, Sweden). The sixth group, the control group, comprised machined (turned) titanium implants (SplineTwist; Zimmer Dental, Carlsbad, CA).

The present study describes the results for the control group versus the ion implantation and DLC groups. These 3 groups involved a total of 36 turned SplineTwist implants measuring 3.75 × 10 mm (Zimmer Dental). Twelve were subjected to ion implantation and another 12 to DLC coating. The remaining 12 implants were used as controls and received no treatment.

### Ion Implantation

Ion implantation modifies the nano-topographic, physical, eg, surface energy, and chemical characteristics of the outermost surface layer, as reported elsewhere.[23,24] Surface energy is modified because of the metastable surface produced by the treatment, which affects properties such as contact angle, leading to increased protein adsorption at the surface and inducing a signaling pathway that promotes osteoblast differentiation and appositioning.

Twelve simple commercial machined (turned) implants were subjected to CO ion implantation surface treatment using a Danfysik Model 1090 high-current implanter (Jyllinge, Denmark) at low temperature (< 170°C) for an average of 10 minutes.

### DLC Coating

Another 12 implants were subjected to DLC coating. The DLC coating was gradually deposited by a hybrid technique that combined the use of radiofrequency plasma chemical vapor deposition (RF plasma CVD) from hydrocarbon precursors and magnetron sputtering for metal doping of the DLC. The first step of the process is the sputtering of pure titanium on the substrate in order to deposit a metal interface to improve adhesion of the DLC coating. The CVD

process is then started to produce the DLC coating from a mixture of acetylene and argon. A layer with lower metal content is thus deposited by gradually decreasing the sputtering of titanium. Finally, titanium sputtering is stopped to yield a pure DLC top layer.

### Surgical Procedure

Three mandibular premolars were removed from each side by tooth sectioning to preserve the mandibular cortical layers during the extraction maneuvers. Three months later, after tartar removal from the remaining teeth, the implants were placed. Preanesthesia consisted of acepromazine maleate 2.5 mg/10 kg (Calmo Neosan; Pfizer, Madrid, Spain) and atropine sulfate 0.05 mg/kg (Atropina Braun; B. Braun Medical, Barcelona, Spain), both administered subcutaneously. Induction was carried out with intravenous thiopental sodium 10 mg/kg (Tiobarbital Braun 0.5, B. Braun Medical), while maintenance of anesthesia was afforded by isoflurane 1.5% to 2% (Forane; Abbott, Madrid, Spain), with the animal intubated. The surgical field was subjected to local anesthesia with lidocaine plus epinephrine vasoconstrictor 1:80,000 (Xilonibsa 2%; Lab. Inibsa, Lliça de Vall, Spain).

Following a crestal incision, a full-thickness mucoperiosteal flap was raised, and the mental foramina were identified to avoid implant placement these levels. Under abundant cooled sterile saline irrigation, bone drilling was carried out according to the instructions of the manufacturer of the implants, followed by mechanical and, finally, manual implant placement. Suturing was carried out with loose 3-0 silk sutures (Suturas Aragó, Lab. Aragó, Barcelona, Spain).

Six implants (1 from each study group) were implanted in each animal, with 3 on each side. Randomized mesiodistal distribution of the different groups was carried out.

Analgesia was provided by fentanyl 0.1 mg/kg (Fentanest; Lab. Kern Pharma, Barcelona, Spain) intraoperatively and by meloxicam 0.2 mg/kg every 24 hours (Metacam; Boehringer Ingelheim, Ingelheim, Germany) during the postoperative period.

In the 15 days after the intervention, the wound was cleaned using gauze impregnated with 0.20% chlorhexidine. The sutures were removed after 10 to 15 days.

During the bone integration period, brushing of the remaining teeth was carried out every 2 days, together with daily chlorhexidine cleaning of the exposed surfaces after the healing period. The animals were fed a soft diet in the months following implant placement surgery.

The animals were sacrificed either 3 or 6 months after implantation by means of a thiopental sodium overdose (Tiobarbital Braun, Braun Medical) following sedation with acepromazine (Calmo Neosan, Lab. Pfizer, Madrid, Spain). An Oscillow GL 2000/83 bone saw was used to extract the bone blocks containing the implants. The bone blocks were immersed in 4% formalin solution until laboratory processing.

### Histologic Preparation

Histologic preparation of the samples for light microscopic study comprised embedding in resin, sectioning longitudinally, grinding, and fine polishing down to a thickness of 50 μm according to a personalized technique,[25] followed by toluidine blue staining.

### Transmittance Histomorphometric Analysis

Evaluation of percentage of bone-implant contact (% BIC) of the histologic preparations was made using a digital microphotographic system (Nikon, Tokyo, Japan) with Adobe Photoshop 7 (Adobe, San Jose, CA) and the Omnimet image analytical system (Buehler, Lake Bluff, IL).

### Environmental Scanning Electron Microscopy

Environmental scanning electron microscopy (ESEM) (JEOL JSM-5910LV, Akishima City, Tokyo, Japan) was done directly on histologic sections embedded in resin prior to staining.

The % BIC was determined by photographing the implant and marking and measuring on the image clearly identifiable intimate BIC zones as well as the complete perimeter of the implant. The measurements were made using the Omnimet image analytical system (Buehler), with verification via digital planimetry.

### Statistical Analysis

A double-blind statistical study was made based on analysis of variance (ANOVA) and the Student *t* test for small samples (paired series) for the values obtained by both the transmittance histomorphometric analysis and the ESEM, which provided complementary information.[26]

## RESULTS

The present study evaluates the results corresponding to 3 of the implant groups considered: ion implantation, DLC, and control (untreated). The comparative analysis for the other groups of commercial implants with treated surfaces (Osseotite, SLA, and TiUnite) is presently underway and will be the subject of an upcoming publication.

**Table 1 % BIC Based on Transmittance Analysis After 3 and 6 Months**

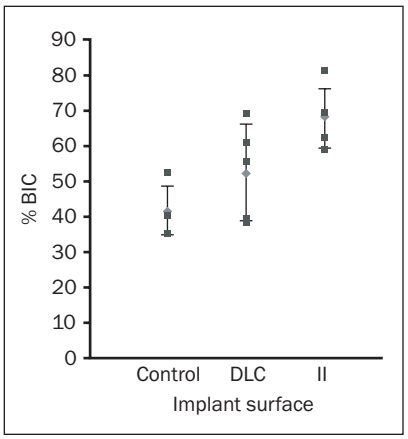
| | Mean (%) | SD | *P** | *P*† |
|---|---|---|---|---|
| 3 months | | | | |
| Ion implantation | 68 | 8 | .001 | |
| DLC | 52 | 14 | .092 | .033 |
| Control | 42 | 7 | | .001 |
| 6 months | | | | |
| Ion implantation | 61 | 8 | .031 | |
| DLC | 51 | 13 | .229 | .134 |
| Control | 44 | 14 | | .031 |

*Versus control (Student *t* test).
†Versus ion implantation group (Student *t* test).

**Table 2 % BIC Based on ESEM After 3 and 6 Months**

| | Mean (%) | SD | *P** | *P*† |
|---|---|---|---|---|
| 3 months | | | | |
| Ion implantation | 61 | 8 | .027 | |
| DLC | 47 | 10 | .130 | .017 |
| Control | 33 | 20 | | .027 |
| 6 months | | | | |
| Ion implantation | 62 | 2 | .018 | |
| DLC | 50 | 11 | .483 | .052 |
| Control | 49 | 9 | | .018 |

*Versus control (Student *t* test).
†Versus ion implantation group (Student *t* test).



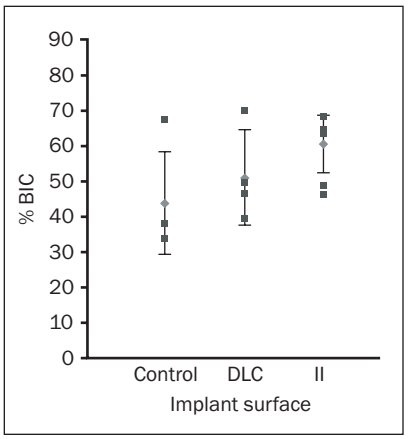
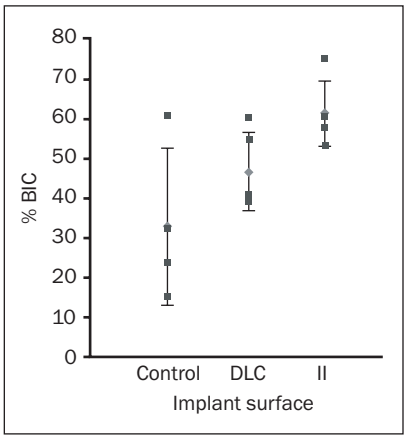




**Fig 1** *(left)* Histologic evaluation of % BIC at 3 months. DLC = diamond-like carbon; II = ion implantation.

**Fig 2** *(right)* Histologic evaluation of percentage % BIC at 6 months. DLC = diamond-like carbon; II = ion implantation.



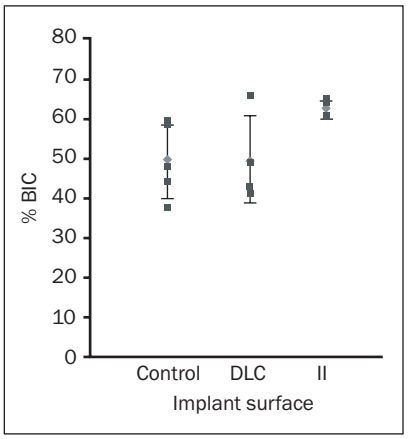




**Fig 3** *(left)* Histologic evaluation by ESEM of % BIC at 3 months. DLC = diamond-like carbon; II = ion implantation.

**Fig 4** *(right)* Histologic evaluation by ESEM of % BIC at 6 months. DLC = diamond-like carbon; II = ion implantation.

Histomorphometric evaluations were made of % BIC for each of the samples based on both light (transmittance) and ESEM.

After 3 months of dental implant placement, the transmittance analysis showed a greater % BIC in the groups subjected to ion implantation (68%) and DLC (52%) versus the controls (42%). The difference between the ion implantation and control groups was statistically significant ($P = .001$). Very similar results were recorded after 6 months of dental implant placement (% BIC of 61%, 51%, and 44% for ion implantation, DLC, and controls, respectively, with statistical significance between ion implantation and the controls [$P = .031$]).

After 3 months, the ESEM analysis, in turn, yielded % BIC of 61%, 47%, and 33% for the ion implantation, DLC, and control groups, respectively; again, statistical significance was reached for the difference between the ion implantation and control groups ($P = .027$). These percentages increased after 6 months of dental implant placement (62%, 50%, and 49%, respectively). The difference between the ion implantation and control groups was statistically significant at 6 months ($P = .018$).

These results are shown in Tables 1 and 2 and in Figs 1 through 4.

## DISCUSSION

After 3 months after implantation in the canine mandible, the % BIC of the treated implants was greater than in the control group: 61% and 68% (as determined by ESEM and light microscopy, respectively) for ion implantation, 47% and 52% for the DLC group, and 33% and 42% in the controls. The differences between the ion implantation group and the control groups were statistically significant.

After 6 months, the % BIC of the treated implants was likewise greater than in the control group, although at this point in time the differences between the DLC specimens (50% and 51% as determined by ESEM and light microscopy, respectively) and controls (49% and 44%) were minimal. In contrast, the differences between the ion implantation group (62% and 61%) and control series remained statistically significant.

The % BIC in the ion implantation group changed very little in the time elapsed between 3 and 6 months in live bone, with high percentages from 3 months onward, while in the control group % BIC increased during this same period.

The high % BIC reached in the ion implantation group at 3 months was interpreted as indicative of accelerated bone integration of implants subjected to surface treatment in the form of CO ion implantation.

Despite the minor differences regarding % BIC between the 2 methods used (light microscopy and ESEM), the results were comparable. Such differences between the 2 techniques are attributable to the increased precision of ESEM in determining high calcium density areas, and in no case were they statistically significant.

The results obtained with ESEM are particularly relevant, considering the sharpness of the images, which allowed precise distinction between bone and the surrounding tissues and clear visualization of the presence or absence of intimate contact between implant and bone without the artifacts inherent to classical histomorphometric techniques (Figs 5 to 8).

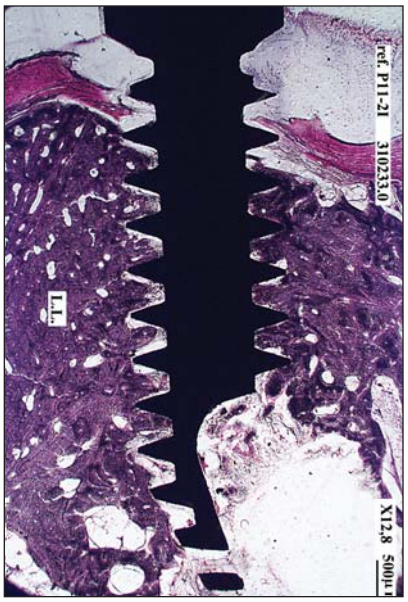
The results obtained in the present study coincide with earlier findings in New Zealand White rabbits.[22] However, no comparisons with other studies can be made, since a review of the literature has yielded no similar in vivo studies involving either CO ion implantation or DLC coating of dental implants.

## CONCLUSIONS

The results obtained show significantly improved % BIC for implants subjected to surface treatment in the form of CO ion implantation in comparison to a control group of machined (turned) dental implants not subjected to surface treatment.

Moreover, bone integration appeared to be accelerated, since unlike in the control series, the high % BIC values recorded in the ion implantation group were manifested from the early stages after in vivo implantation.

The DLC coating group, likewise, showed higher % BIC values than the controls. However, statistically significant differences were not observed, and the percentages failed to reach the levels recorded for ion implantation.



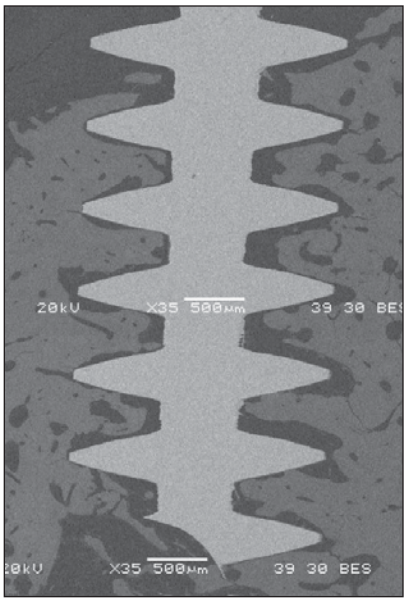
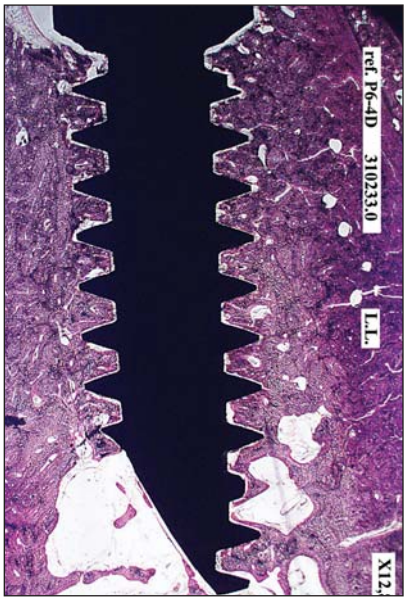




**Fig 5** *(left)* Histologic section of a control-group specimen analyzed by light microscopy (toluidine blue; original magnification ×12.8).

**Fig 6** *(right)* ESEM image of the sample in Fig 5 (original magnification ×35).



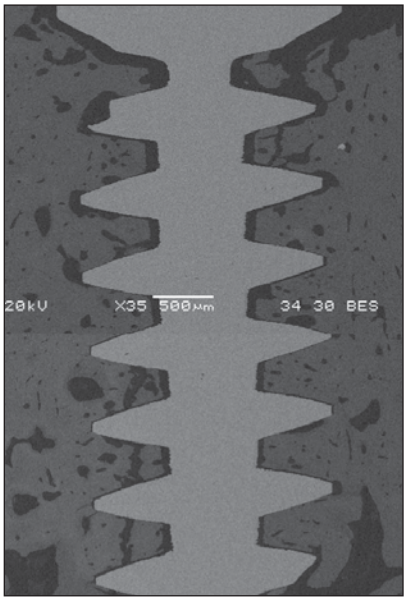




**Fig 7** *(left)* Histologic section of an ion implantation group specimen analyzed by light microscopy (toluidine blue; original magnification ×12.8).

**Fig 8** *(right)* ESEM image of the sample in Fig 7 (original magnification ×35).

## REFERENCES


1. Thomas K, Cook S. An evaluation of variables influencing implant fixation by direct bone apposition. J Biomed Mater Res 1985;19:875–901.
2. Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. J Biomed Mater Res 1991;25:889–902.
3. Wennerberg A. On Surface Roughness and Implant Incorporation [thesis]. Göteborg, Sweden: Göteborg Univ, 1996.
4. Albrektsson T, Brånemark P-I, Hansson H-A, Lindström J. Osseointegrated titanium implants. Acta Orthop Scand 1981;52:155–170.
5. Abrahamsson I, Zitzmann NU, Berglundh T, Wennerberg A, Lindhe J. Bone and soft tissue integration to titanium implants with different surface topography: An experimental study in the dog. Int J Oral Maxillofac Implants 2001;16:323–332.
6. Shalabi MM, Gortemaker A, Van't Hof MA, Jansen JA, Creugers NH. Implant surface roughness and bone healing: A systematic review. J Dent Res 2006;85:496–500.
7. Davis JE. Mechanisms of endosseous integration. Int J Prosthodont 1998;11:391–401.
8. Block MS, Gardiner D, Kent JN, Misiek DJ, Finger IM, Guerra L. Hydroxyapatite-coated cylindrical implants in the posterior mandible: 10-year observations. Int J Maxillofac Implants 1996;11:626–633.
9. Novaes AB Jr, Souza SL, de Oliveira PT, Souza AM. Histomorphometric analysis of the bone-implant contact obtained with 4 different implant surface treatments placed side by side in the dog mandible. Int J Oral Maxillofac Implants 2002;17:377–383.
10. Kim Y-H, Koak J-Y, Chang I-T, Wennerberg A, Heo S-J. A histomorphometric analysis of the effect of various surface treatment methods on osseointegration. Int J Oral Maxillofac Implants 2003;18:349–356.
11. Gotfredsen K, Nimb L, Hjorting-Hansen E, Jensen JS, Holmen A. Histomorphometric and removal torque analysis for TiO2-blasted titanium implants. An experimental study on dogs. Clin Oral Implants Res 1992;3:77–84.
12. Ivanoff CJ, Hallgren C, Widmark G, Sennerby L, Wennerberg A. Histologic evaluation of the bone integration of $TiO_2$-blasted and turner titanium microimplants in humans. Clin Oral Implants Res 2001;12:128–134.
13. Trisi P, Marcato C, Todisco M. Bone-to-implant apposition with machined and MTX microtextured implant surfaces in human sinus grafts. Int J Periodontics Restorative Dent 2003;23:427–437.
14. Lazzara RJ, Testori T, Trisi P. A human histologic analysis of osseotite and machined surfaces using implants with two opposing surfaces. Int J Periodontics Restorative Dent 1999;19:117–128.
15. Baker D, London RM, O'Neal R. Rate of pull-out strength gain of dual attached titanium implants. A comparative study in rabbit. Int J Oral Maxillofac Implants 1999;14:722–728.
16. Gottlow J, Johansson C, Albrektsson T, Lundgren AK. Biomechanical and histologic evaluation of the TiUnite and Osseotite implant surfaces in rabbits after 6 weeks of healing. Appl Osseointegration Res 2000;1:25–27.
17. Lim YJ, Oshida Y, Andres CJ, Barco MT. Surface characterizations of variously treated titanium materials. Int J Oral Maxillofac Implants 2001;16:333–342.
18. Baier RE, Meyer AE, Natiella RR, Carter JM. Surface properties determine bioadhesive outcomes; methods and results. J Biomed Mater Res 1984;18:337–355.
19. Olefjord I, Hansson S. Surface analysis of four dental implant systems. Int J Oral Maxillofac Implants 1993;8:32–40.
20. Braceras I, Alava JI, Oñate I, et al. Improved osseointegration in ion implantation treated dental implants. Surface and Coatings Technology 2002;158-159:28–32.
21. De Maeztu MA, Alava JI, Gay-Escoda C. Ion Implantation: Surface treatment for improving the bone integration of titanium and Ti6Al4V dental implants. Clin Oral Implants Res 2003;14:57–62.
22. Braceras I, Oñate JI, Goikoetxea L, Vivente JL, Alava JI, De Maeztu MA. Bone cell adhesion on ion implanted titanium alloys. Surface and Coatings Technology 2005;196:321–326.
23. Alonso F, Arizaga A, Quaintons S, Ugarte JJ, Vivente JL, Oñate JI. Mechanical properties and structure of Ti6Al4V alloy implanted with different light ions. Surface and Coating Technology 1995;74-75:986–992.
24. Viviente JL, García A, Alonso F, Braceras I, Oñate JI. X-ray photoelectron spectroscopy characterization of high dose carbon-implanted steel and titanium alloys. Appl Surface Science 1999;144–145:249–254.
25. Alava JI, Marín F, Intxaurrandieta A, Braceras I, De Maeztu MA. Simultaneous preparation of a materialographic and histological thin section: Investigation of bone integration on dental implants. Struers J Materialography 2005;6:1–6.
26. Hedberg EL, Kroese-Deutman HC, Shih CK, et al. Methods: A comparative analysis of radiography, microcomputed tomography, and histology for bone tissue engineering. Tissue Engineering 2005;11:1356–1367.